Sensibilité de l'espèce bioturbatrice Upogebia cf. pusilla dans un environnement littoral soumis à différents stress : infestations parasitaires et contamination métallique

Annabelle Dairain

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université de BORDEAUX

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DOCTEUR DE

L'UNIVERSITÉ DE BORDEAUX

ÉCOLE DOCTORALE SCIENCES ET ENVIRONNEMENTS

SPÉCIALITÉ : Biogéochimie et écosystèmes

Par M^elle^ Annabelle DAIRAIN

Sensibilité de l'espèce bioturbatrice *Upogebia* cf. *pusilla* dans un environnement littoral soumis à différents stress : infestations parasitaires et contamination métallique

Sous la direction de : Xavier de MONTAUDOUIN
Co-directeur : Alexia LEGEAY

Soutenue le 10 décembre 2018

Membres du jury :

Mme PERROT-MINNOT, Marie-Jeanne	Maître de conférences HDR, Université de Bourgogne	Rapporteur
M. GEFFARD, Alain	Professeur, Université de Reims Champagne-Ardenne	Rapporteur
M. GREMARE, Antoine	Professeur, Université de Bordeaux	Président
M. MERMILLOD-BLONDIN, Florian	Chargé de recherche HDR, CNRS	Examinateur
M. MAIRE, Olivier	Maître de conférences, Université de Bordeaux	Invité

Sensibilité de l'espèce bioturbatrice *Upogebia* cf. *pusilla* dans un environnement littoral soumis à différents stress : infestations parasitaires et contamination métallique

Résumé : Les processus de bioturbation constituent un exemple classique d'ingénierie de l'écosystème. Ils sont le fait d'espèces bioturbatrices qui contribuent à la structuration physique et biogéochimique des environnements sédimentaires. Dans les environnements marins meubles, les thalassinidés comptent parmi les bioturbateurs les plus influents. Ainsi, la crevette de vase *Upogebia* cf. *pusilla* est à l'origine d'un important remaniement sédimentaire et d'une large bioirrigation des sédiments dans le bassin d'Arcachon. Ces activités de bioturbation modulent les processus de minéralisation de la matière organique et les flux biogéochimiques à l'interface eau-sédiment. L'intensité de la bioturbation de *U.* cf. *pusilla* dépend de son état physiologique, celui-ci pouvant être altéré par divers facteurs de stress. Une approche couplant études de terrain et expérimentations en laboratoire a permis d'évaluer l'influence du parasitisme et de la contamination métallique sur les activités de *U.* cf. *pusilla*. Ce travail avait pour objectifs de (1) caractériser la pression parasitaire chez *U.* cf. *pusilla* à l'échelle locale, (2) déterminer les niveaux de base des éléments métalliques chez cet organisme dans le bassin d'Arcachon et d'évaluer le potentiel rôle des parasites dans les processus d'accumulation des métaux et (3) estimer l'impact du parasitisme et de la contamination métallique, seuls et en interaction, sur *U.* cf. *pusilla* et ce à deux échelles d'organisation, moléculaire et comportementale.

In situ, un suivi spatial et un suivi temporel ont permis de caractériser la dynamique spatio-temporelle de deux parasites chez *U.* cf. *pusilla*¸ un bopyre (*Gyge branchialis*), infestant la cavité branchiale, et un trématode (*Maritrema* sp.), infectant l'ensemble des organes de l'animal. Au sein de leur hôte, ces deux parasites sont négativement associés, le bopyre limitant probablement les processus d'infection par le trématode. Par ailleurs, un suivi saisonnier d'un an a permis de noter de faibles concentrations en métaux chez *U.* cf. *pusilla*, dans le bassin d'Arcachon. Ces concentrations ont été mises en parallèle avec le statut parasitaire des spécimens échantillonnés. Aucune corrélation n'a été notée entre la présence des trématodes et les quantités de métaux accumulées. A l'inverse, les observations de terrain ont permis d'envisager que le parasite bopyre puisse interférer avec les processus d'accumulation des métaux chez cet organisme. Cette dernière hypothèse a été testée en laboratoire au cours d'expérimentations visant à évaluer l'influence d'une contamination métallique (en utilisant le cadmium comme contaminant modèle) et du parasite bopyre, seuls et en interaction, sur *U.* cf. *pusilla*. A l'échelle moléculaire, le bopyre n'a été associé à aucune modification de l'expression d'une dizaine de gènes cibles. Par ailleurs, à l'échelle comportementale, ce parasite réduit peu le remaniement sédimentaire de son hôte, à l'inverse de ce qui avait été montré au cours d'une étude antérieure. En ce qui concerne l'influence du cadmium, ce métal module amplement l'expression de gènes codant notamment des protéines impliquées dans la réponse au stress oxydant et dans les mécanismes de détoxication. Ces observations suggèrent que *U.* cf. *pusilla* est capable de mettre en place des mécanismes de lutte contre le stress métallique. Au niveau comportemental, le cadmium semble stimuler le remaniement sédimentaire des organismes. Finalement, les expériences « doubles stress » ont montré un effet antagoniste du bopyre et du cadmium à la fois à l'échelle génique et à l'échelle comportementale. Ainsi, cette étude souligne la complexité des interactions entre facteurs de stress multiples et la nécessité d'effectuer de telles expérimentations. Finalement, ces travaux mettent en avant l'intérêt majeur de considérer plusieurs niveaux d'intégration au cours d'études visant à évaluer l'influence de facteurs de stress sur des organismes d'importance majeure comme les espèces ingénieures de l'écosystème.

Mots clés : crevette de vase, parasitisme, bioturbation, contamination métallique, interactions, réponse génique

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UMR CNRS 5805 EPOC
Environnements et Paléoenvironnements Océaniques et Continentaux
Allée Geoffroy Saint-Hilaire – CS50023 - 33165 Pessac Cedex - France

Sensitivity of the bioturbating species *Upogebia* cf. *pusilla* in a multi-stress littoral environment: parasitic infestations and metal contamination

Abstract: Bioturbation is a typical example of ecosystem engineering. Bioturbating species are mainly epi- or endobenthic organisms, which profoundly affect the physical structure and biogeochemical properties of sediments. In marine soft-bottom environments, thalassinidean mud shrimp are considered as one of the most prominent bioturbating organisms. Among these species is the mud shrimp *Upogebia* cf. *pusilla*, which is recognized as an important sediment reworker, also significantly contributing to the bioirrigation of sediments. *In fine*, this species greatly modulates organic matter mineralization and biogeochemical fluxes at the sediment-water interface. The influence of *U.* cf. *pusilla* in ecosystem functioning depends on the intensity of its bioturbation and thus on its fitness. Several factors can affect the physiological status of organisms, potentially resulting in behavioural changes and causing modifications of their activities. Amongst potential stressors, we evaluated the influence of parasitism and trace metal contamination on the mud shrimp *U.* cf. *pusilla* in Arcachon Bay, France. Field surveys and laboratory experiments were undertaken in order to evaluate (1) the parasite infestation levels in the mud shrimp at the local scale, (2) the metal contamination background and potential relationship between the metal accumulation and the parasitic status of organisms in the field and (3) estimate the distinct and interactive impacts of parasitism and metal contamination on two scales of organisation, by targeting a molecular (gene expression) and a behavioural (sediment reworking) endpoint.

A large spatial and temporal survey conducted in Arcachon Bay showed that at least two parasites species occur in mud shrimp: a bopyrid isopod (*Gyge branchialis*), living in one of the gill chambers of its host, and a trematode parasite (*Maritrema* sp.), infecting the whole body of mud shrimp. These two parasite species are negatively associated within their host, in which the bopyrid likely interferes with trematodes establishment. Additionally, a one year seasonal sampling demonstrated that mud shrimp displayed very low levels of metals in Arcachon Bay. The bopyrid parasite could interfere with the process of metal accumulation in mud shrimp, while such correlation was not found for the trematode parasite. Finally, complementary laboratory experiments highlighted that, at the molecular level, the bopyrid parasite did not affect the expression of the genes targeted in this study in mud shrimp. Similarly, at the behavioural level, and conversely to a previous study, the effects of the bopyrid parasite were minor, i.e. only associated to small modifications of the bioturbation activity of its host. Indeed, the intensity of the sediment reworking of mud shrimp was slightly reduced when organisms were infested with this parasite. Regarding trace metal contamination, we noticed that mud shrimp largely accumulated cadmium. This accumulation was associated to an important modulation of gene expression, especially of genes encoding proteins involved in detoxification processes, highlighting, at this organisation level, the capability of mud shrimp to deal with the deleterious effect of cadmium. At the behavioural scale, cadmium contamination positively affected the sediment reworking activity of mud shrimp. Finally, double-stress experiments evidenced an antagonistic effect of both stressors on the two targeted endpoints. This study highlights the complexity of the interactions between multiple stressors and that the response of organisms cannot be predicted from "single-stress" experiments. Moreover, this study strongly suggests to evaluate the impacts of these stressors on various levels of organisation, in particular in ecosystem engineer species such as mud shrimp, since modifications of genetic processes do not necessary prelude alterations at the behavioural level.

Keywords : mud shrimp, parasitism, bioturbation, metal contamination, interactions, gene expression

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PREAMBULE ET PLAN DE LA THESE

Le fonctionnement d'un écosystème dépend en partie des interactions entre organismes peuplant cet écosystème (interactions intra- et interspécifiques) ainsi que des interactions entre les organismes et leur environnement. Certains organismes jouent un rôle prépondérant dans le fonctionnement des écosystèmes par leur rôle dans la création, la modification et le maintien des habitats, et de ce fait influencent la disponibilité des ressources pour les autres organismes. Ce concept fut formellement défini par Jones *et al.* en 1994 sous le terme « d'ingénierie de l'écosystème » et les organismes initiant de tels processus nommés « espèces ingénieures de l'écosystème ». Il existe deux catégories d'espèces ingénieures de l'écosystème dites « autogènes », lorsque les processus d'ingénierie ne sont dus qu'à la simple présence de ces organismes, et « allogènes » lorsque ces processus sont liés à l'activité de ces espèces. De cette définition sont exclues les espèces dites « clefs » (Paine, 1969) dont l'importance dans le fonctionnement des écosystèmes est en lien avec des relations uniquement trophiques (proies, prédateurs) ainsi que les pollinisateurs.

Dans les écosystèmes côtiers, les processus de bioturbation constituent un archétype d'ingénierie de l'écosystème. Sous le terme bioturbation sont regroupés l'ensemble des processus de transport affectant de façon directe ou indirecte la matrice sédimentaire (Kristensen *et al.*, 2012). Ces processus de transport sont le fait des activités d'espèces dites bioturbatrices, constituant ainsi un exemple d'organismes ingénieurs allogènes (Jones *et al.*, 1994). Il existe toute une variété d'organismes bioturbateurs allant des gros mammifères marins jusqu'aux invertébrés benthiques, en passant par les chondrichthyens (Cadée, 2001). Classiquement, les études de bioturbation portent néanmoins sur les organismes epi- ou endobenthiques.

Le rôle joué par les espèces bioturbatrices dans le fonctionnement des écosystèmes dépend de l'intensité de leurs activités, elles-mêmes en lien avec leur bon état de santé. De nombreux paramètres environnementaux peuvent impacter le bien-être des espèces bioturbatrices. C'est dans ce contexte général que se positionne le présent travail de thèse dont l'objectif général consistait à évaluer l'influence de deux facteurs dits « de stress » sur les activités d'une espèce bioturbatrice marine. Au cours de ces travaux, nous nous sommes ainsi consacrés à l'influence du parasitisme et de la contamination métallique sur les activités de l'espèce bioturbatrice *Upogebia* cf. *pusilla*.

Ce préambule est volontairement restreint du fait de la forme revêtue par le **Chapitre I** de ce manuscrit. Ce premier chapitre fera en effet office de grande introduction générale, exposant l'état de l'art et, en conclusion, la problématique générale de ce manuscrit de thèse. La première partie du **Chapitre I** évaluera l'état des connaissances sur les liens entre espèces bioturbatrices et parasitisme et sera présentée sous la forme d'une revue soumise à publication (*Marine Ecology Progress Series,* **Chapitre I-A**). Ce sujet a pour le moment fait l'objet d'un nombre très restreint d'études et il nous est apparu nécessaire et intéressant d'en faire un état des lieux relativement exhaustif. La seconde partie de

ce premier chapitre sera consacrée aux liens entre espèces bioturbatrices et contaminants métalliques (**Chapitre I-B**). Les études portant sur ce dernier sujet sont comparativement plus nombreuses et ont déjà fait l'objet de revues ; ce sujet sera donc traité sous la forme d'un état des lieux plus « classique ».

Dans un second temps (**Chapitre II**) nous nous attacherons à décrire le modèle d'étude (*U.* cf. *pusilla*), le site d'étude choisi dans le cadre des études descriptives (i.e. le bassin d'Arcachon) ainsi que les méthodes analytiques utilisées.

Le **Chapitre III** sera consacré à la caractérisation des stress parasitaires et métalliques expérimentés par *U.* cf. *pusilla* à l'échelle locale. Ce chapitre sera divisé en deux sous-parties, la première traitant de la pression parasitaire et la seconde de la contamination métallique à l'échelle locale en lien avec le statut parasitaire des organismes. Ces travaux seront présentés sous la forme de deux articles, le premier publié (*Marine Biology*, **Chapitre III-A**) et le second en préparation (**Chapitre III-B**). Ce chapitre sera suivi d'un bilan en français permettant l'introduction des études expérimentales menées dans les **Chapitres IV** et **V**.

Le **Chapitre IV** évaluera l'influence du parasitisme sur les capacités de remobilisation d'un contaminant modèle (le cadmium) par *U.* cf. *pusilla*. Les travaux de ce chapitre seront présentés sous la forme d'un article publié (*Marine Environmental Research*). Ce chapitre sera également suivi d'un bilan en français et permettra l'introduction du chapitre suivant.

Le **Chapitre V** s'attachera, dans un premier temps, à évaluer l'influence du parasitisme et d'une contamination métallique *via* l'eau sur l'activité de bioturbation de *U.* cf. *pusilla*. La seconde partie du **Chapitre V** sera consacrée à un approfondissement des effets du parasitisme sur la physiologie de l'hôte au cours des processus de contamination métallique. Ces travaux seront également présentés sous la forme d'un article publié (*Aquatic Toxicology*, **Chapitre V-A**) et d'une note en révision (*Parasitology Research*, **Chapitre V-B**).

Une discussion générale (**Chapitre VI**) clôturera ce manuscrit et proposera des perspectives de recherche dans la continuité des travaux présentés.

CHAPITRE I

Introduction générale : parasitisme, contamination métallique et bioturbation en milieu marin

Influence of parasitism on bioturbation: from host to ecosystem functioning

DAIRAIN A., LEGEAY A. and de MONTAUDOUIN X.

Submitted in *Marine Ecology Progress Series*

Abstract

Bioturbating species represent a typical example of ecosystem engineer species in aquatic environments. These abundant endo- or epibenthic organisms modify the physical structure and geochemical properties of sediments, and at broader ecosystem scale impact nutrient flows and structure benthic communities. The ecological importance of bioturbators depends on the magnitude of their bioturbation activity. We suggest that parasitism has a substantial impact on bioturbators, and cascading effects on their role in ecosystem functioning. Reviewing 5940 papers concerning bioturbation, we identified 176 bioturbating species, with 31 % of these potentially parasitized (micro- and macroparasites). However, there is significant discrepancy in the research effort on parasites among bioturbating groups, the highest effort being devoted to molluscs with studies on annelids and arthropods rare. Furthermore, studies addressing parasites' impacts on their bioturbating hosts are still scarce, but evidence we do have indicates that parasites fundamentally impair their hosts' physiological state, fecundity, behaviour and survival. Because of impacts of parasites on phenotypic traits related to the bioturbation engineering activity of their hosts, cascading effects of parasitism could play a key role on ecosystem functioning through cascade effects. Yet, studies assessing the intricate link between parasites and their hosts' bioturbation activity, including potential effects on ecosystem functioning, are virtually non-existent.

Keys words: bioturbation, parasitism, ecosystem functioning, behaviour modifications, cascade effects

I. Introduction

The structure and dynamics of ecosystems are shaped by myriad ecological and environmental parameters, an important one of which is the activity of organisms in these ecosystems. In 1994, Jones *et al.* proposed the concept of ecosystem engineer species (EES) as an acknowledgment of the major role of some organisms in the functioning of ecosystems. The authors defined EES as species that modify their physical environment by their activity (allogenic engineers) or by their mere presence (autogenic engineers) in addition to their potential contribution to biotic interactions. EES differ from keystone species, which are defined as species occupying a significant place in a given food web (Paine, 1969). The case of beavers building dams is probably one the most striking examples of EES. This physical EES plays a key role in the structure of streams and in the functioning of their riparian zones. Indeed, dams have a marked effect on water current velocity in streams, but they also modify stream geomorphology, and retention of sediment particles and organic matter in upstream ponds, affecting biogeochemical pathways (Naiman *et al.*, 1986, 1988). As a consequence, beavers also indirectly impact other organisms and influence the structure of natural communities. For instance, beaver-modified and un-modified areas exhibit highly distinct assemblages of plant and invertebrates species, without any large dissimilarity in species richness between modified and un-modified areas (McDowell and Naiman, 1986; Wright *et al.*, 2002). Thus, by enhancing the landscape heterogeneity, beavers increase the gamma diversity of these systems (Whittaker, 1972).

There is a broad diversity of EES inhabiting various environments (Jones *et al.*, 1994). In marine benthic ecosystems, the role of bioturbating species as EES is well established (e.g., Krantzberg, 1985; Levinton, 1995; Lohrer *et al.*, 2004; Mermillod-Blondin and Rosenberg, 2006; Meysman *et al.*, 2006). The process of bioturbation is described as any modification of the sediment matrix, including interstitial waters, due to the activities of organisms living mainly in or on the substratum (Kristensen *et al.*, 2012). The locomotory, feeding and burrowing activities of bioturbators substantially displace sediment particles. These sediment reworking activities deeply affect the physical properties and geomorphology of sediments (e.g., Rhoads and Young, 1970; Jones and Jago, 1993; Volkenborn *et al.*, 2007a). Bioturbators also significantly enhance the transport of water in sediments. This water input stimulates movements of solutes between pore- and overlying waters, a process named bioirrigation (Kristensen *et al.*, 2012). In particular, sediment-dwelling organisms largely stimulate hydrological fluxes (Aller, 1988; Volkenborn *et al.*, 2012a), since biogenic structures, such as burrows, increase the surface of solute exchanges between the sediment and the overlying and porewaters. Thus, bioturbators play a key role in the biogeochemistry of sediments (e.g., Aller, 1982; Webb and Eyre, 2004a; Volkenborn *et al.*, 2007a, 2012a). The processes of sediment reworking and bioirrigation are highly intricate. Sediment reworking causes modifications of the texture of sediments (i.e. their permeability, porosity, and geomorphology), while the processes of bioirrigation rely on water penetration in sediments, which depends on their texture (Huettel and Gust, 1992; Volkenborn *et al.*, 2007b). Finally, as a consequence

of their large impact on the physical and geochemical properties of benthic habitats, bioturbators exert a major influence on other organisms and drive the composition of benthic communities (e.g., Dahlgren *et al.*, 1999; Widdicombe *et al.*, 2000; Papaspyrou *et al.*, 2005; Pillay *et al.*, 2007; Brustolin *et al.*, 2016). As an illustration, an exclusion experiment highlighted the large impact of the bioturbating mud shrimp *Callichirus (Callianassa) kraussi* on benthic communities. Mud shrimp presence was associated with lower species abundance, richness and diversity of benthic organisms compared to areas without the shrimp. However, the influence that mud shrimp had depended on the guild benthic animals belonged to, as burying infauna were less affected by mud shrimp than surfacing organisms. The effect of *C. kraussi* on the benthic community could be related to the negative influence of bioturbation on development of biofilms upon which epifauna feed (Pillay *et al.*, 2007).

The influence of bioturbators as EES depends on the magnitude of their activities and thus on the physiological state of these organisms. In natural environments, organisms are seldom in optimal environmental conditions and different abiotic and biotic factors can adversely affect the physiology and the behaviour of bioturbating organisms, altering ecosystem functioning through cascade effects. The role of environmental factors such as temperature, salinity and food availability is well established (e.g., Berkenbusch and Rowden, 1999; Ouellette *et al.*, 2004; Przeslawski *et al.*, 2009). For instance, bioturbation activity of the bivalve *Abra segmentum (ovata)* varies with temperature, organisms burrowing deeper and reworking a larger sediment surface when exposed to warm than to cold temperatures (Maire *et al.*, 2007b). Oxygen availability has also been shown to significantly affect the behaviour of organisms. Under hypoxic conditions, bioturbators burry shallower and build smaller burrows, reduce the ventilation of their burrow and even emerge at the sediment-water interface (Diaz and Rosenberg, 1995; Sturdivant *et al.*, 2012; Murray *et al.*, 2017). Such behaviour modifications affect the performance of bioturbators as EES. Biotic interactions can also have a large influence on the individual behaviour of organisms and could play a key role in bioturbation processes (Braeckman *et al.*, 2010; Maire *et al.*, 2010; Premo and Tyler, 2013; Campbell and Lindsay, 2014). For instance, interspecific interactions could change the magnitude of bioturbation activities *via* alterations of animal behaviour or injuries to bioturbating organisms (Maire *et al.*, 2010; Premo and Tyler, 2013; Campbell and Lindsay, 2014). In response to the presence of its predatory shrimp *Crangon crangon*, the clam *Limecola (Macoma) balthica* buries deeper into the sediment, thereby increasing the thickness of the bioturbated layer (Maire *et al.*, 2010). In contrast, the effect of the bioturbator *Tritia (Ilyanassa) obsoleta* on ammonium fluxes at the sediment-water interface is largely reduced when this gastropod is threatened by its predator, the mud crab *Panopeus herbstii* (Premo and Tyler, 2013).

Among biotic parameters influencing faunal EES, one major concern is the role played by parasites and associated infectious diseases. In the context of global climate change, it has been suggested that warmer conditions could increase the frequency and intensity of disease events (Marcogliese, 2001; Harvell *et al.*, 2002). For example, increase in seawater temperature enhances infection success of the trematode

Maritrema novaezealandense in the amphipod host *Paracalliope novizealandiae*. A larger parasite burden is associated with a higher mortality rate of amphipods (Studer and Poulin, 2013). Although many parasites and associated infectious diseases can produce mass mortality events in host populations (e.g., Jensen and Mouritsen, 1992; Jonsson and Andé, 1992; Fredensborg *et al.*, 2004), parasites can also have a wide range of sub-lethal effects, especially on the physiological status and behaviour of infested organisms. For example, the health of parasitized organisms is often impaired as reflected in reduced growth rate and condition index (e.g., Thieltges, 2006; Dang *et al.*, 2013; O'Connell-Milne *et al.*, 2016). This pattern could be due to parasites directly interfering with food uptake (Flye-Sainte-Marie *et al.*, 2007; Stier *et al.*, 2015) or impairing a host's metabolism (Anderson, 1977; Repetto and Griffen, 2012). In contrast, some parasites stimulate the growth of hosts, leading to cases of "gigantism" (Pearre, 1976; Sluiters *et al.*, 1980; Mouritsen and Jensen, 1994). For example, mud snails *Peringia (Hydrobia) ulvae* infected with larval trematodes grow more rapidly than unparasitized organisms. This pattern is likely due to a reallocation of energy since the faster growth rate of parasitized organisms is not correlated with increased food uptake, and locomotor activity and reproductive process are both impaired in parasitized organisms (Mouritsen and Jensen, 1994). Behaviour modifications can also result from parasitism, with infested organisms being moribund or exhibiting aberrant behaviour compared to unparasitized individuals (e.g., Thomas and Poulin, 1998; Vicente *et al.*, 2012; Pascal, 2017). Overall, it is usually assumed that parasites and associated infectious diseases have debilitating effects on their hosts, with infested organisms in poorer conditions than uninfested individuals. These effects of parasites on individual host organisms can produce broad impacts at the ecosystem level, with parasites playing a key role in structuring communities of free-living organisms for instance (Price *et al.*, 1986; Minchella and Scott, 1991; Poulin, 1999; Mouritsen and Poulin, 2002).

Regarding bioturbating species as EES, parasites and associated infectious diseases could (1) impair the physiological state of their host and thus the intensity of their bioturbation activity and/or (2) modify behaviours related to the bioturbation activity of their host. Thus, parasites could alter the influence bioturbating species have on the physical structure of sediments, in biogeochemical cycles and on the regulation of the community structure of organisms. Considering the major potential role of parasites on such important EES, it seems reasonable to investigate the influence of this stressor on bioturbators. The aim of this paper is to review current knowledge on parasites and associated infectious diseases in common bioturbators and present a general framework for future studies. We examined the relationship between parasites and bioturbators by answering four questions: (1) what are the commonly studied bioturbating species, (2) are there any parasites known to infest bioturbating species, (3) what are the effects of parasites on their host and do they modify the intensity of their bioturbation activity, and (4) do parasites infesting bioturbating species have any consequence on the functioning of ecosystems?

II. Methods

II.1. Type of bioturbators

In this study, bioturbating species were restricted to epi- or endobenthic faunal organisms influencing the physical structure (i.e. grain size, porosity, organic matter content, etc.) and/or biogeochemical properties (i.e. nutrient and solute contents, redox status, etc.) of sediments. Therefore, only studies evaluating and quantifying the influence of organisms on the sediment structure and/or in biogeochemical processes were taken into account. In addition, this literature review was limited to invertebrates occurring in coastal environments, including mangrove forests. Taking these restrictions into account, publications were searched in Scopus using the terms "bioturbation", "sediment reworking" and "bioirrigation". Because of the very high number of related papers, the search was further constrained to publications belonging to the "environmental science" subject area for the items "sediment reworking" and "bioirrigation". A list of 4912, 732 and 296 publications for the terms "bioturbation", "sediment reworking" and "bioirrigation" were gathered (May 11th, 2018), respectively. Bioturbators were taxonomically classified based on the World Register of Marine Species (WoRMS Editorial Board, 2018).

II.2. Type of parasites

Parasites were broadly defined as organisms living with benefits on (ectoparasites) or in (endo- and mesoparasites) another organism (the host), on which they have a detrimental impact (Combes, 1995). By extension, we included viruses as parasitic organisms (Combes, 1995). This study investigated both microparasites (unicellular organisms like protozoans, bacteria and viruses) and macroparasites (mainly organisms belonging to helminth and arthropod groups) of bioturbating species (Anderson and May, 1979). Helminths can also be classified as microparasites depending on which stage of their life cycle is considered (i.e. rediae and sporocysts stages). However, for simplicity, we categorized helminths as macroparasites regardless of their life stage. For each of the bioturbating species identified in our first search, a second search for publications mentioning parasites occurring in these organisms was made by using the name of the bioturbating species in combination with the terms "parasite", "infection", "bacteria" and "virus". In addition, we collected data related to prevalence, intensity and/or abundance of parasitic infections when available. In a sampled population, prevalence is defined as the percentage of infested individuals, intensity of infestation as the mean number of parasites per parasitized hosts, and abundance as the mean number of parasites per potential host (including both parasite-infested and uninfested) (Margolis *et al.*, 1982). Furthermore, the influence of parasites on the physiological state, behaviour and bioturbating activity of their host was also recorded at that time. The term infestation is used for ectoparasites and the term infection for endoparasites.

III. Results and discussion

III.1. Bioturbating species in coastal environments

Our search found a total of 176 bioturbating species to be reported in coastal environments worldwide (Figure I-A. 1; see Appendix 1 for a full list of the bioturbators). Species belonging to the phyla Arthropoda, Annelida and Mollusca were the most extensively studied, representing 37, 27 and 23 % of the total number of bioturbators, respectively (Figure I-A. 1).

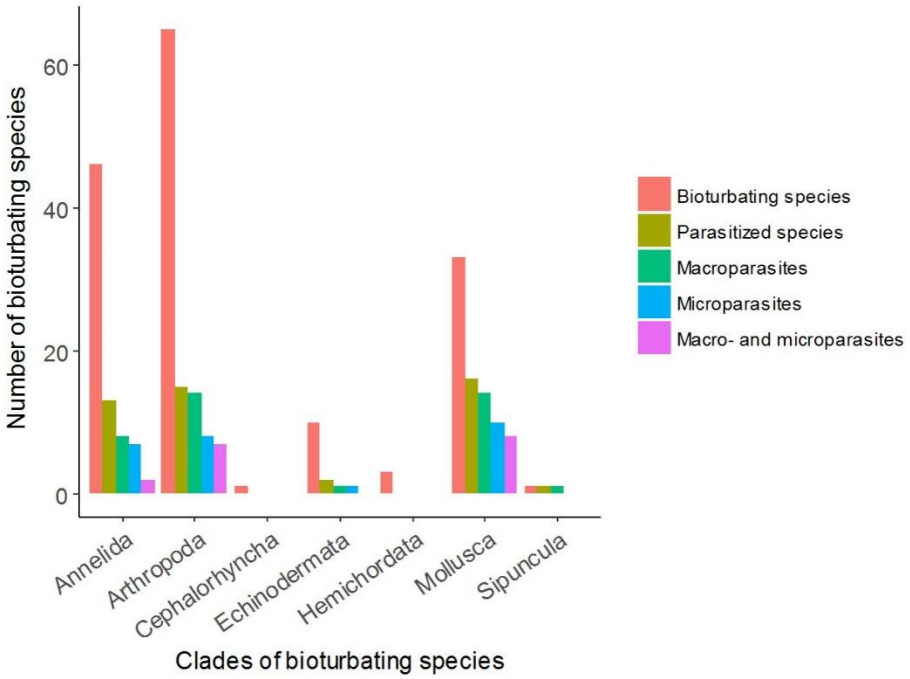

Figure I-A. 1 Number of coastal bioturbating species commonly studied and number of parasitized bioturbators per taxonomic clade. Parasitized bioturbators are categorized as being infested with macroparasites, microparasites or both groups of parasites.

III.2. Number of parasites species infesting bioturbating organisms

Of the 176 species of bioturbators identified, 55 species (i.e. 31 %) were parasitized with at least one macro- or microparasite species (Figure I-A. 1). More specifically, 44 (i.e. 25 %) and 28 (i.e. 16 %) bioturbating species are infected with a least one macro- or microparasite species, respectively and 17 bioturbators (i.e 10 %) with both macro- and microparasites (Figure I-A. 1; see Appendix 2 and Appendix 3, respectively, for a full list of macro- and microparasites of bioturbating species). There is an important disparity regarding the distribution of parasites across the phyla of bioturbators, with some known to be highly parasitized, such as molluscs (51 % of the species harboring at least one parasite species), with others less frequently documented as being parasitized, for example echinoderms (22 %) and arthropods (23 %) (Figure I-A. 1). Similarly, the number of parasite species identified per bioturbating host strongly differed among phyla (Figure I-A. 2). Numerous studies were devoted to bioturbating molluscs and highlighted they were diversely parasitized, with on average (± SE) 8.2 ± 2.9 macroparasite species (Figure I-A. 2A) and 8.6 ± 2.8 microparasite species (Figure I-A. 2B) identified

per individual species. To the contrary, only a few parasites species have been documented in annelids, with on average 1.4 ± 0.3 macroparasite species (Figure I-A. 2A) and 1.8 ± 0.4 microparasite species (Figure I-A. 2B) identified per annelid species. Finally, there was also a large variability in parasite species richness per bioturbating species within a given phylum (Figure I-A. 2). For example, the number of macro- and microparasites identified in molluscan bioturbators ranged from 1–55 and 1–26, respectively (Appendix 2 and Appendix 3).

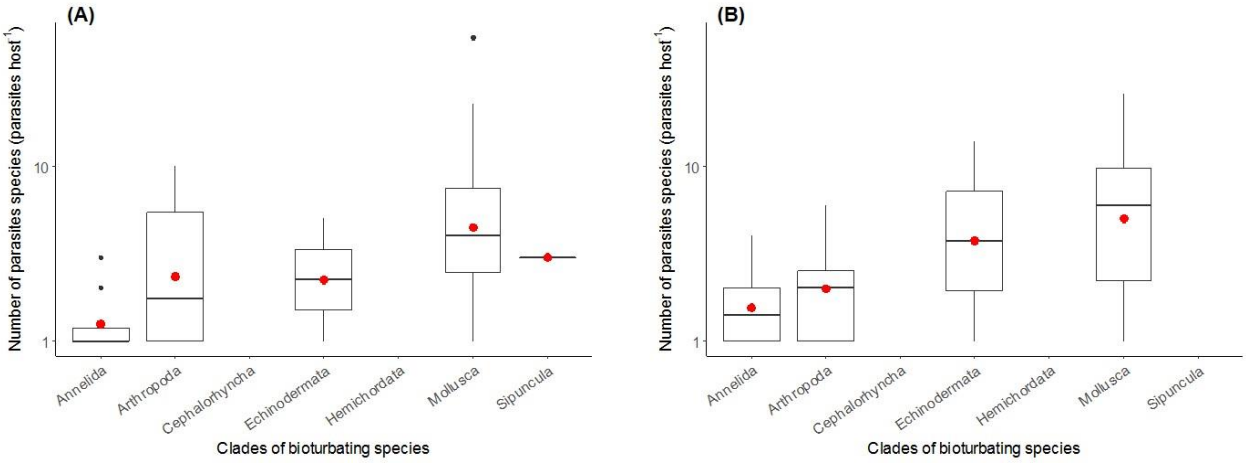

Figure I-A. 2 Number (logarithmic scale) of (A) macroparasites and (B) microparasites identified per bioturbating species. Bioturbators are classified according to their taxonomic clade. The boxes represent the interquartile range, the black lines the median and the red dots the mean number of parasites species in each clade of bioturbators.

Several factors could explain the discrepancy in parasite species richness recorded among and within the phyla of bioturbators. First, through a large meta-analysis, Kamiya *et al.* (2014) highlighted a positive association between parasite species richness and host body size, population density and geographical range. Second, difference in sensitivity and susceptibility of bioturbating species to parasites could also explain interspecific variation in parasite species richness. For instance, the common cockle *Cerastoderma edule* exhibits a more diverse trematode parasite fauna than the carpet-shell clam *Ruditapes decussatus* and the Manila clam *R. philippinarum* occurring in similar intertidal zones (Dang *et al.*, 2009). Both *R. decussartus* and *R. philippinarum* display lower parasite abundances than *C. edule* and a laboratory challenge experiment confirmed that trematode parasites more successfully infected cockles than clams. The higher resistance of clams to trematode infection could be related to stronger physical barriers presented by clam tissues (Dang *et al.*, 2009). On the other hand, we suggest that the large disparity in parasite species richness we observed among and within phyla of bioturbators is more likely related to a bias in research effort on parasites occurring in these organisms. Parasites are ubiquitous in ecosystems, with about 40 % of known species estimated to be parasitic (Dobson *et al.*, 2008), and all free-living species are likely to be parasitized with at least one parasite species. A large proportion of bioturbators, however, has not received any parasitological attention. This disparity in research on different groups of bioturbators could be related to their relative economic value. The higher the economic value of a species, the more intensive are the research efforts to identify any potential

pathogens or other stressors that could influence the sustainability of the production and/or the quality of the resource. For instance, numerous studies have been conducted in molluscs, a phylum of broad interest for fisheries and aquaculture, with the common cockle *C. edule* and the Manila clam *R. phillipinarum* being two important species. Although a small proportion of echinoderms have been described as being parasitized so far, there is a proportionally extensive literature on parasites infesting bioturbating echinoderms with a commercial value, such as the sea cucumber *Apostichopus (Stichopus) japonicus*. In contrast, annelids are poorly exploited and have been rarely studied with regard to parasites and associated infectious diseases.

III.3. Clades of parasites infesting bioturbating species

Bioturbators harbor a large diversity of macro- and microparasites species with large differences in the relative frequency among parasite phyla, some being dominant and others rarely observed (Figure I-A. 3, Appendix 2 and Appendix 3). Platyhelminthes are the most common macroparasites infecting bioturbators, with 75 % of the reported parasites species belonging to this phylum. Platyhelminthes infect molluscs, echinoderms and annelids, and to a lesser extent arthropods (Figure I-A. 3A). The phylum Platyhelminthes includes about 30,000 species (Caira and Littlewood, 2013), of which many are parasites (Dobson *et al.*, 2008), such as members of the Trematoda or Cestoda. Moreover, it is one of the most important group of marine parasites (Rohde, 2005) and which makes it unsurprising that infections with Platyhelminthes have been noted in bioturbators to such extent. On the other hand, only a few studies highlighted the occurrence of parasitic annelids or nemerteans in bioturbators. As these two groups mainly consist of free-living organisms, they represent minor parasite groups in marine environments (Rohde, 2005). Regarding microparasites, 53 % of the species identified are eukaryotes and 36 % are bacteria. Eukaryotic microparasites encompasses a large diversity of phyla, while bacteria are mainly represented by Proteobacteria (Figure I-A. 3B). Finally, a few viral syndromes and infectious diseases for which the etiological agent has not yet been fully characterized (e.g. various neoplasias, viscera ejection syndrome, brown muscle disease; Appendix 3) have been noted in bioturbating organisms as well.

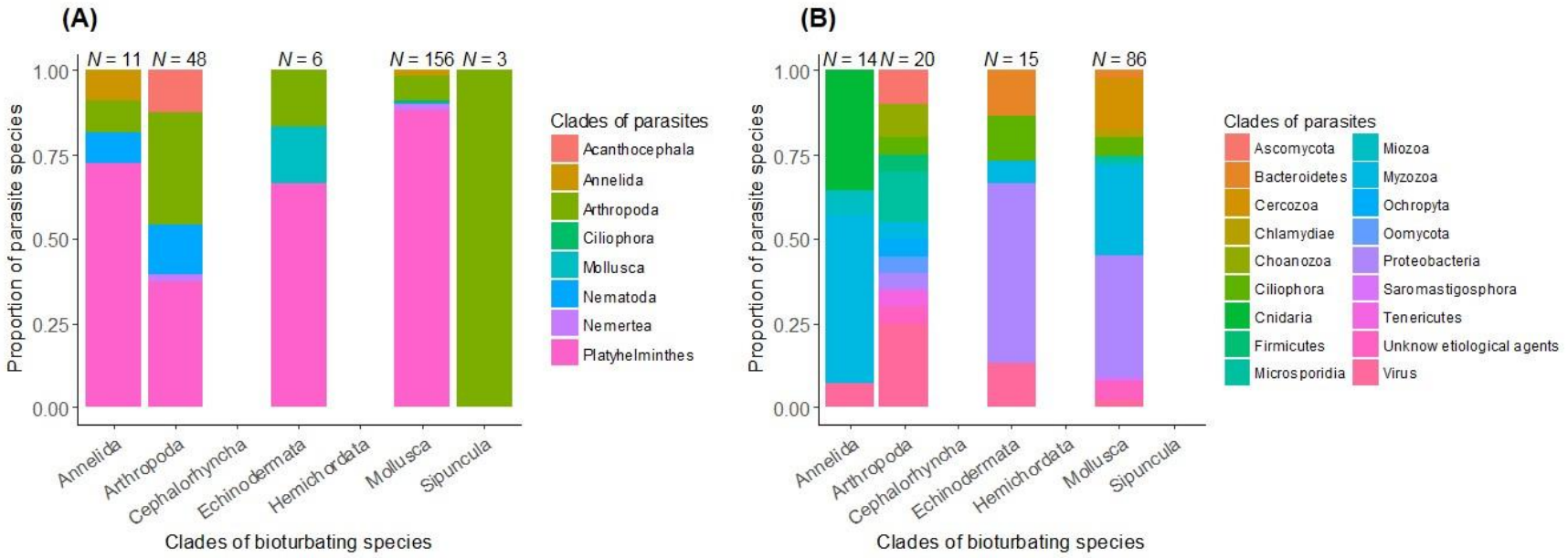

Figure I-A. 3 Taxonomic types of (A) macroparasites and (B) microparasites reported in clades of bioturbating species. The total number of parasite species identified per clade of bioturbators is given (*N*).

III.4. Effects of parasites on bioturbating species

From the literature survey, 376 macroparasites and 188 microparasites and associated infectious diseases were documented to parasitize 55 coastal bioturbating species. Among these parasites, the effects of a few were studied, with impacts of parasites on their host recognized in 32 % and 36 % of the studies highlighting the occurrence of macro- and microparasites (and associated infectious diseases) in bioturbating species, respectively. More than 82 % of the known impacts of macroparasites are due to Platyhelminthes (Appendix 2). Most studies on microparasites focused on Proteobacteria (36 %) and Myzozoa (30 %) (Appendix 3). These patterns are in accordance with those showing that Platyhelminthes, Proteobacteria and Myzozoa are the most commonly described macro- and microparasites in bioturbating species (Figure I-A. 3).

Even though the literature is thin on bioturbating species interacting with parasites, it seems that the impacts of parasites do appear to encompass various levels of organization. We roughly categorized impacts of parasites as alterations of physiological state (i.e. modulation of gene expression and cellular functions, initiation of inflammatory responses, tissue damages, etc.), modifications of reproductive functions (i.e. partial or total castration, changes in reproductive success, etc.), modifications of host behaviour (reduced activity levels, abnormal behaviours, etc.) and increase of the mortality rate of the host (Figure I-A. 4).

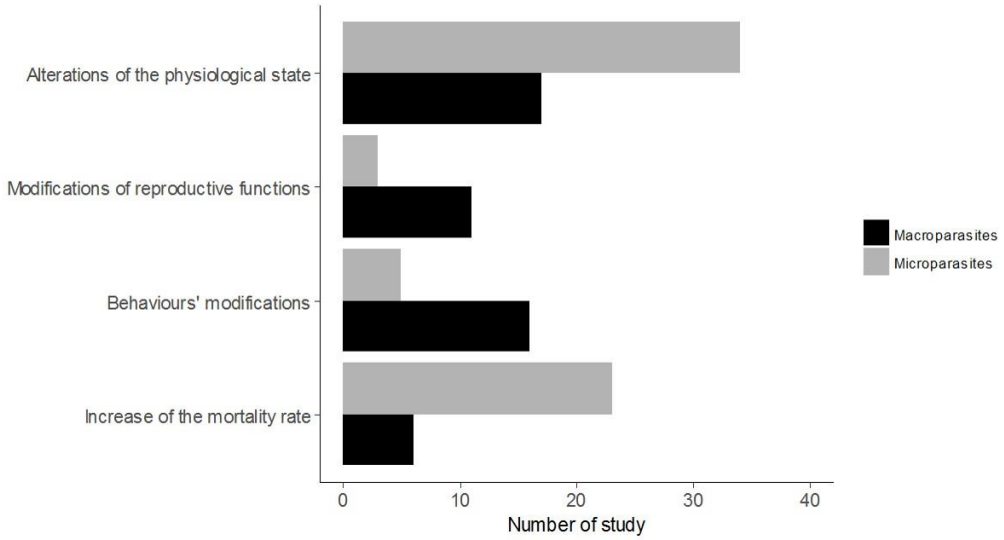

Figure I-A. 4 Impacts of macro- and microparasites on bioturbating organisms. Alterations of physiological state: parasites influencing gene expression, cellular functions, physical integrity, growth rates of the host, etc. Modifications of reproductive functions: parasites reducing or increasing their host's fecundity, causing total or partial castration, etc. Modifications of behaviour: parasites reducing their host's activity levels, inducing abnormal behaviours, etc. Total number of studies $N = 85$, of which $N = 35$ studies on macroparasites and $N = 47$ studies on microparasites.

III.4.1. Impacts of parasites on host physiology

Numerous parasites are documented to impact the physiological state of their bioturbating host (Figure I-A. 4). Modulation of biochemical and cellular processes are used as early markers of exposure to stressors, preceding potential impacts at the organism's level. Since the physiological condition of

bioturbators influences the role they will have on the community of organisms and on ecosystem functioning, studies evaluating the impact of parasites on the well-being of bioturbating species are of primary interest.

At the molecular scale, parasites interfere with gene regulation, thereby either up- or down-regulating their expression. For example, the trematode *Himasthla elongata* up-regulates the expression of several genes related to mitochondrial metabolism and oxidative responses in infected cockles *C. edule*. These modulations in gene expression are considered to be a molecular response to parasites causing energetic losses and oxidative stress in cockles (Paul-Pont *et al.*, 2010a). The Proteobacteria *Vibrio tapetis* induces a similar genic response in *C. edule*, although the induction of gene expression is stronger with the macroparasite than with the microparasite (Paul-Pont *et al.*, 2010a). It suggests that *V. tapetis* is less pathogenic for cockles than *H. elongata*. However, when cockles were experimentally infested with *V. tapetis*, the bacterium caused the death of its host, highlighting the failure of *C. edule* to deal with the microparasite infection (Paul-Pont *et al.*, 2010a).

The host's physical integrity can also be impaired by parasites since physiological alterations are concomitant with physical injuries. While developing and residing inside their host, parasites cause significant histopathological damages to host tissues (Lauckner, 1980; Robaldo *et al.*, 1999; Carballal *et al.*, 2001; Dang *et al.*, 2008). The trematode *Microphallus szidati* infects the hepatopancreas of the mud crab *Neohelice (Chasmagnathus) granulata*. This parasite causes epithelial delamination of host hepatopancreas in addition to eliciting haemocytic infiltrations by the host (Robaldo *et al.*, 1999). As the hepatopancreas plays a key role in nutrient absorption in crustaceans (Gibson and Barker, 1979), the damage caused by the parasite could impair the host energetics. Microparasites can also physically harm their host. As an illustration, *Perkinsus* spp. induce histological lesions in gills, digestive gland and gonad gland of the sand gaper clam *Mya arenaria*, in addition to eliciting host haemocytic infiltrations (McLaughlin and Faisal, 1998). Such tissues alterations are again expected to be energetically costly for the host, and may have direct repercussions on its fecundity and growth. In fact, a caging experiment conducted at two sites greatly impacted by perkinsosis along the northeast Atlantic coast of France demonstrated that growth rates of the bioturbators *R. decussatus* and *R. philippinarum* are significantly compromised as a result of *Perkinsus* spp. infections (Dang *et al.*, 2013). Macroparasites impacting the growth of their bioturbating host have also been broadly documented (e.g., Mouritsen and Jensen, 1994; Smith *et al.*, 2008; Pascal *et al.*, 2016b). The mud shrimp *Upogebia pusilla* displays reduced size when infested with the epicaridean isopod *Gyge branchialis* (Pascal *et al.*, 2016b). Contrary to the previous examples, the negative effect of the parasite is probably not related to histological lesions but to the parasite directly feeding at the expense of its host (Tucker, 1930). By doing so, the parasite strongly affects host energetics (Hughes, 1940) and consequently its physiological state (Williams and Boyko, 2012). Parasites do not always diminish their host's growth rate. The bioturbators *Peringia (Hydrobia) ulvae* infected with Platyhelminthes grow to larger sizes than their unparasitized conspecifics

(Mouritsen and Jensen, 1994). Such cases of gigantism have been reported in several host-parasite associations involving parasitic castrators (Pearre, 1976; Sluiters *et al.*, 1980; de Montaudouin *et al.*, 2003). It is assumed that energy initially devoted to the host reproduction is reallocated to somatic growth in castrated individuals, rather than the increased growth resulting from chemical stimulation of the host by the parasite (Poulin, 1998).

III.4.2. Impacts of parasites on host reproduction

We expect that if parasites modulate the reproductive functions of their hosts, as many do, there will also be consequences for host physiological state and general activity, and thus on their bioturbation activity. Evidence for alterations to host fecundity has been found in 31 % and 6 % of the studies on effects of macro- and microparasites, respectively (Figure I-A. 4). Macroparasites, and especially digenean trematodes (Platyhelminthes), are often associated with impairment of the host reproductive function (Appendix 2). Digenean trematodes have complex life cycles, with vertebrates serving as definitive hosts in which the parasites sexually reproduce. Trematode eggs are then released with feces, hatch, and develop into a miracidium larvae which usually infects gastropods and, to a lesser extent, bivalves as first intermediate hosts. In these hosts, parasites multiply asexually in sporocysts (and sometimes rediae), which produce a large number of parasitic clones, the so-called cercariae. The latter are shed into the environment and disperse through the water column before infecting a second intermediate host, either fish or invertebrates like bivalves or crustaceans, depending on the species. In these hosts the trematodes encyst as metacercariae and await ingestion by their definitive hosts. The sporocysts/rediae stage of digeneans is fundamentally damaging for the first intermediate host, in particular for its fecundity (Lauckner, 1980, 1983). Digenean trematodes infect the digestive gland and/or the gonad of their host (Probst and Kube, 1999), causing partial or complete castration *via* mechanical or chemical damages to host reproductive tissue (Hurd, 1990). For instance, infection with trematodes leads to an important reduction of the penis size of the mud snail *P. ulvae* and to almost nonexistent oviposition in females (Mouritsen and Jensen, 1994). Castration is not only caused by digenean trematodes; crustacean parasites like epicaridean isopods have also been found to be strong influences on the fecundity of their bioturbator hosts (Tucker, 1930; Dumbauld *et al.*, 2011). However, unlike to most of digenean parasites, castration of hosts by isopods is not due to a direct effect of the parasites on the gonadic tissue. Rather, it seems to be related to the parasite lowering the condition index of the host and/or interfering with the secretion of host reproductive hormones (Reinhard, 1956; Williams and Boyko, 2012).

Conversely, microsporidian parasites infecting females of the crustacean *Corophium volutator* enhance the fecundity of their host. Infected females in one study were observed to have five-fold more offsprings than unparasitized organisms (Mautner *et al.*, 2007). Moreover, microsporidian parasites are known to cause important distortions of the host sex ratios (Mautner *et al.*, 2007; Ironside and Alexander, 2015). This is due to the vertical transmission pathway of the parasite, which occurs through

female hosts only. By increasing the number of females in the host populations, microsporidian parasites enhance their own transmission success. Since the transmission of vertically transmitted Microsporidia depends on their host survival and fecundity, the virulence of these parasites is probably low (Poulin, 1998; Dunn and Smith, 2001). However, the life cycle of microsporidian parasites can involve both horizontal and vertical routes, and parasites with alternate (i.e., both vertical and horizontal) transmission routes show higher virulence than purely vertically transmitted parasites (Poulin, 1998; Dunn and Smith, 2001). Hence, predictions regarding the effects of microsporidians in bioturbating species must take the transmission route into account.

III.4.3. Impacts of parasites on host behaviour

Every biologist has heard about some terrestrial arthropods committing suicide by jumping into aquatic environments. This odd behaviour can be due to hairworm parasites infecting arthropods (Thomas *et al.*, 2002). In terms of fitness, this behaviour is entirely counterproductive for the arthropod host, but it benefits the parasites. Hairworms are meroparasites, with adults being free-living in aquatic environments while juveniles parasitized arthropods. Once arthropods have jumped into water, parasites emerge from their hosts and complete their life cycles. There is a large diversity of examples of parasite-induced phenotypic alterations (Poulin, 1994; Lafferty and Shaw, 2013), typically involving parasites with complex life cycles as host behaviour modifications are expected to enhance the transmission success of parasites (Lafferty, 1999; Combes, 2001; Moore, 2002). Concerning bioturbators, alterations of behaviours have been reported in 46 % and 11 % of the studies evaluating the influence of macro- and microparasites on their hosts, respectively (Figure I-A. 4, Appendix 2 and Appendix 3). One of the most detailed examples involves the New Zealand cockle *Austrovenus stutchburyi*, which is commonly found at the sediment surface of tidal flats, with organisms showing difficulty in burrowing (Thomas and Poulin, 1998) . Surfacing cockles (i.e., in abnormal position) are heavily infected with the trematode *Curtuteria australis*, while buried cockles (i.e., in normal position) are only lightly infected with the parasite (Thomas and Poulin, 1998). This parasite encysts in the foot of cockles and has a detrimental impact on this organ, with heavily infected organisms harboring a shorter foot as compared to lightly infected ones (Thomas and Poulin, 1998; O'Connell-Milne *et al.*, 2016). In addition, foot contractility is significantly reduced as the infection intensity increases (Mouritsen, 2002a). Therefore, the parasite *C. australis* likely reduces the growth of the foot but also deeply affects the function of this muscle in bivalve burrowing, impairing the ability of cockles to burrow (Thomas and Poulin, 1998; Mouritsen, 2002a). Surfacing organisms are also more vulnerable to avian predation than buried organisms (Thomas and Poulin, 1998). The trematode uses the New Zealand cockle as a second intermediate host and the parasite needs the cockle to be eaten by marine birds to complete its life cycle. Together, these results suggest that the parasite alters the cockle phenotype so as to it enhances its own transmission success (Thomas and Poulin, 1998; Mouritsen, 2002a). The bioturbators identified in this review host a large variety of parasites with complex life cycles (Appendix 2 and Appendix 3), many of which need to be

trophically transmitted (i.e., Platyhelminthes or Acanthocephala) to definitive hosts to reproduce. In these bioturbating species, modifications of behaviour similar to those reported for the cockle *A. stutchburyi* are thus expected and should be studied since such parasites could play a key role in the dynamics of the bioturbators' populations.

Modifications of the behaviour of the host can also be a side effect of parasitism, that is they can be phenotypic alterations with no adaptive value for either the parasite or the host (Ewald, 1980). When parasites impair the physiological status of their host, one would expect that the intensity of the host's activities would be reduced as well. However, studies that quantify how parasites causing disease in their bioturbator host influence their behaviour are still relatively rare (Figure I-A. 4; Appendix 2 and Appendix 3). The few reported examples highlighted the large impact of parasites on the magnitude of their hosts' activities. Previously, we noted that mud snails *P. ulvae* infected with trematode parasites are castrated and also exhibit increased growth rates as a result of potential energy reallocation (Mouritsen and Jensen, 1994). However, trematodes are also associated with modifications of locomotory behaviour, with parasitized snails moving slower and crawling over shorter distances as compared to uninfected organisms (Mouritsen and Jensen, 1994). The mud snail serves as the first intermediate host for the parasite and is not trophically transmitted at this stage, meaning this behavioural modification is not beneficial for the parasite lifecycle. It could, alternatively, be a side effect of parasites interfering with the host energy allocation. Locomotory activity is a highly costly behaviour and it has been suggested that parasites stimulating host growth reduce the energy available for the host to crawl (Mouritsen and Jensen, 1994). The epicaridean *G. branchialis* also deeply impacts the bevaviour of its host, the mud shrimp *U. pusilla*. Pascal (2017) monitored the influence of the parasite on the behaviour of its host through video recording. He defined four main behavioural states for the mud shrimp species as "resting", "burrowing", "ventilating" and "walking", and documented the time parasitized and unparasitized organisms spent on these activities. Unparasitized mud shrimp spent more than 75 % of their time being active, while parasitized organisms spent more than 55 % of their time at rest. "Burrowing" and "ventilating" are the two behaviours associated with bioturbation activities of the mud shrimp. Parasitized mud shrimp spent 1.8- and 2.3-fold less time to "burrowing" and "ventilating", respectively, than unparasitized mud shrimp. As for mud snails, the less active phenotype of parasitized mud shrimp could be due to the parasite presenting an energy burden for the mud shrimp (Hughes, 1940; Williams and Boyko, 2012; Pascal *et al.*, 2016b).

Regarding the impacts of microparasites on host behaviour, the literature is even more scarce, with altered behavior recognized in only 11 % of the studies documenting the occurrence of microparasites or infectious diseases in bioturbators (Figure I-A. 4; Appendix 3). Given the large influence of microparasites on the physiological status of their hosts (Appendix 3) it is likely that microparasites have side effects on host behaviour as well. For instance, brown muscle disease causes a serious atrophy of the posterior adductor muscle of the Manila clam *R. philippinarum* (Dang *et al.*, 2008). This muscle

is involved in valve opening and closing. Consequently, any alterations to this muscle could be expected to disturb clam feeding and respiration, as well as locomotion. Such modifications of locomotory activity were observed in Manila clams infected with the protozoan *Perkinus olseni*. The parasite promotes the emergence of *R. philippinaum* at the sediment surface and limits its ability to re-burrow in thermally stressful condition (Nam *et al.*, 2018).

III.4.4. Impacts on host mortality

At the population level, parasites could be important drivers of their hosts' population dynamics by modulating host breeding potential and/or by increasing host mortality rates. To assess the influence of bioturbators on the community of free-living organisms and ecosystem functioning, the effects of parasites on mortality rates of their bioturbating hosts ought to be considered, especially in cases where parasites are highly prevalent (Poulin, 1999; Mouritsen and Poulin, 2002).

An increase in mortality rate has been noticed in 17 % and 49 % of the studies evaluating the influence of macro- and microparasites, respectively (Figure I-A. 4). Regarding microparasites, the Proteobacteria *V. cyclitrophicus*, *V. harveyi*, *V. splendidus*, *V. tasmaniensis* and *Photobacterium* sp. induced 20–100 % mortality of the sea cucumber *A. japonicas* by causing a skin ulceration disease (Deng *et al.*, 2009). This disease alters the physiological state of affected sea cucumbers through skin ulcerations that can exposed underlying muscles, and probably lowers the host's weight as a result of a reduced feeding activity (Deng *et al.*, 2009). Similarly, the Galician population of the common cockle *C. edule* showed an important decline in spring 2012, with mortality up to 100 %. At this time, juvenile and adult cockles were heavily infected (up to 100 % prevalence) with the protistan parasite *Marteilia cochillia*, which is probably the cause of the population collapse (Villalba *et al.*, 2014). The parasite *M. cochillia* is an etiological agent of the marteiliosis disease. As the skin ulceration syndrome in *A. japonicus*, marteiliosis is associated with substantial physiological alterations in infected organisms. The parasite infects the digestive gland of its host and interferes with its energetic balance. For example, mussels *Mytilus galloprovincialis* harboring marteiliosis showed reduced food uptake, lower rates of nutrient absorption and a reduction in the efficiency of nutrient absorption as compared to uninfected organisms (Pérez Camacho *et al.*, 1997). Thus, marteiliosis weakened organisms such that exhibited reduced condition index and eventually died.

As previously stated, digenean trematodes play a key role in population dynamics of their host because of their significant impacts on host fecundity. However, these parasites also affect the survival of their hosts, at time causing collapses of natural populations. For instance, the trematodes *Microphallus claviformis* and *Maritrema subdolum* are considered to be the main cause of the 40 % population decline of the mud snail *P. ulvae* observed in the Danish Wadden Sea during Spring 1990 (Jensen and Mouritsen, 1992). Finally, digenean trematodes can also modulate the population size structure of their bioturbating hosts because of differential susceptibility to parasitism of small and large organisms. An extensive field survey conducted in Arcachon Bay, France, over the years 1998–1999 indicated an

important decline of the largest mud snails *P. ulvae* during the winter 1998-1999. Large mud snails exhibited high prevalences (up to 100 %) whereas small organisms were only moderately infected with digenean trematodes (prevalence less than 20 %). A laboratory experiment conducted in parallel highlighted higher mortality rates of parasitized organisms as compared to unparasitized individuals. Together, these results demonstrate that digenean parasites substantially influence the population size structure of the bioturbator *P. ulvae* (de Montaudouin *et al.*, 2003).

III.5. Influence of parasites on bioturbation activity and ecosystem functioning

III.5.1. What do we know?

Out the 215 studies evidencing the occurrence of 376 macro- and 188 microparasites in 55 bioturbating species over 176 bioturbators commonly studied in coastal environments, 82 studies showed that parasites can impair the physiological state, reproductive capacity, behaviour and/or the mortality of their bioturbating hosts. Of these studies, only 21 studies depicted effects of parasites on the behaviour of their bioturbating host. Within these 21 studies just seven focused on the link between the occurrence of parasites and their role in modulating the bioturbating activity of their hosts, with potential outputs on the community of free-living organisms and ecosystem functioning (Thomas *et al.*, 1998; Mouritsen, 2004; Mouritsen and Poulin, 2005, 2010; Mouritsen and Haun, 2008; Simão *et al.*, 2010; Pascal, 2017). Nevertheless, these few papers strongly support the idea that parasites animals that are important EES as bioturbators can act as EES themselves by modifying functional traits of their host involved in bioturbation activities (Thomas *et al.*, 1999) (Figure I-A. 5). As discussed above, Pascal (2017) demonstrated that the mud shrimp *U. pusilla* infested with the epicaridean *G. branchialis* spends significantly less time ventilating its burrow and displacing sediment particles than healthy organisms. These behavioural modifications are associated with negative impacts on the intensity of the bioturbating activity: the sediment reworking rate of parasitized organisms is 4.6-fold lower and the bioirrigation rate 2.9-fold lower compared to unparasitized organisms. As a result, biogeochemical fluxes are also strongly modified. The total oxygen and nitrate uptake are reduced and there is a diminished release of ammonium at the sediment-water interface. This suggests, therefore, that parasitized mud shrimp have a much lower influence on organic matter mineralization and nutrient turnover than uninfested organisms (Pascal, 2017). At the ecosystem level, these findings can be highly relevant (Figure I-A. 5), as epicarideans are widespread in natural populations of mud shrimp (Smith *et al.*, 2008; Pascal *et al.*, 2016b). Modifications of behaviours potentially linked to bioturbating activities have also been documented for the New Zealand cockle *A. stutchburyi.* This bivalve species harbors multiple parasites, some of which can manipulate the behaviour of cockles to facilitate transmission. However, these behaviour modifications can also change the role of cockles as EES. Heavily parasitized organisms are more likely found at the sediment surface than buried in the sediment and show reduced ability to rebury in the sediment once they occur at the sediment surface (Thomas and Poulin, 1998). Moreover, heavily parasitized cockles exhibit reduced crawling activity compared to moderately

infected conspecifics (Mouritsen and Poulin, 2005). Cockles exhibit important reworking activity in subsurface sediments, where they also deeply impact nutrients cycling and the production of primary producers (Sandwell *et al.*, 2009). The bioturbating activity of cockles likely depends on their mobility, thus it could be suggested that parasitism reduces their bioturbation potential. Mouritsen and Poulin (2005, 2010) did not evaluate the impact of parasitism on the bioturbating activity of cockles (i.e. they did not quantify sediment reworking or nutrient exchanges at the sediment-water interface, for instance) but they addressed the influence of parasitism on animal community structure. Over a long-term field experiment, they highlighted a positive relationship between macrozoobenthic species richness (and density) and the presence of parasitized cockles (Mouritsen and Poulin, 2005). A similar pattern was reported at a larger spatial scale (Mouritsen and Poulin, 2010). The authors suggest that this positive association could be related to (1) a lower disturbance of the sediment *via* parasites reducing bioturbating activities of cockles and (2) surfacing cockles acting as physical ecosystem engineers (Jones *et al.*, 1997; Thomas *et al.*, 1998).

There is no general rule regarding the influence of bioturbators on macrofaunal communities. Inhibitory and facilitative effects of bioturbators on the community diversity of free-living organisms have both been reported and depend on the bioturbating species (Posey *et al.*, 1991; Dittmann, 1996; Pillay *et al.*, 2007; Kanaya, 2014). Therefore, parasitism interfering with the activity of bioturbators can also negatively influence the community structure of free-living organisms. Mouritsen and Haun (2008) evaluated how parasitism interferes with the role of the gastropod *P. ulvae* as an EES. Parasitized snails have a negative impact on primary producers whereas unparasitized organisms enhance primary production. The authors suggest that the sediment mixing activity of parasitized snails is reduced, lowering nutrient supply to benthic primary producers. Faunal community structure is also different in sediment bioturbated by parasitized and unparasitized snails, probably because of trophic cascading effects.

III.5.2. What is expected?

Parasitism is recognized as a key biotic interaction deeply affecting host organisms *via* alterations in the physiological state, modifications of behaviours and impairment of reproductive functions (see Appendix 2 and Appendix 3 for examples). At the ecosystem scale, parasites also fundamentally influence host populations and communities (Minchella and Scott, 1991; Poulin, 1999; Mouritsen and Poulin, 2002) as well as ecosystem functioning (Thomas *et al.*, 1998, 2005; Kuris *et al.*, 2008). To quote Poulin (1999), parasites have « many roles at many levels » and we expect that parasites infesting bioturbators will play similar roles (Figure I-A. 5).

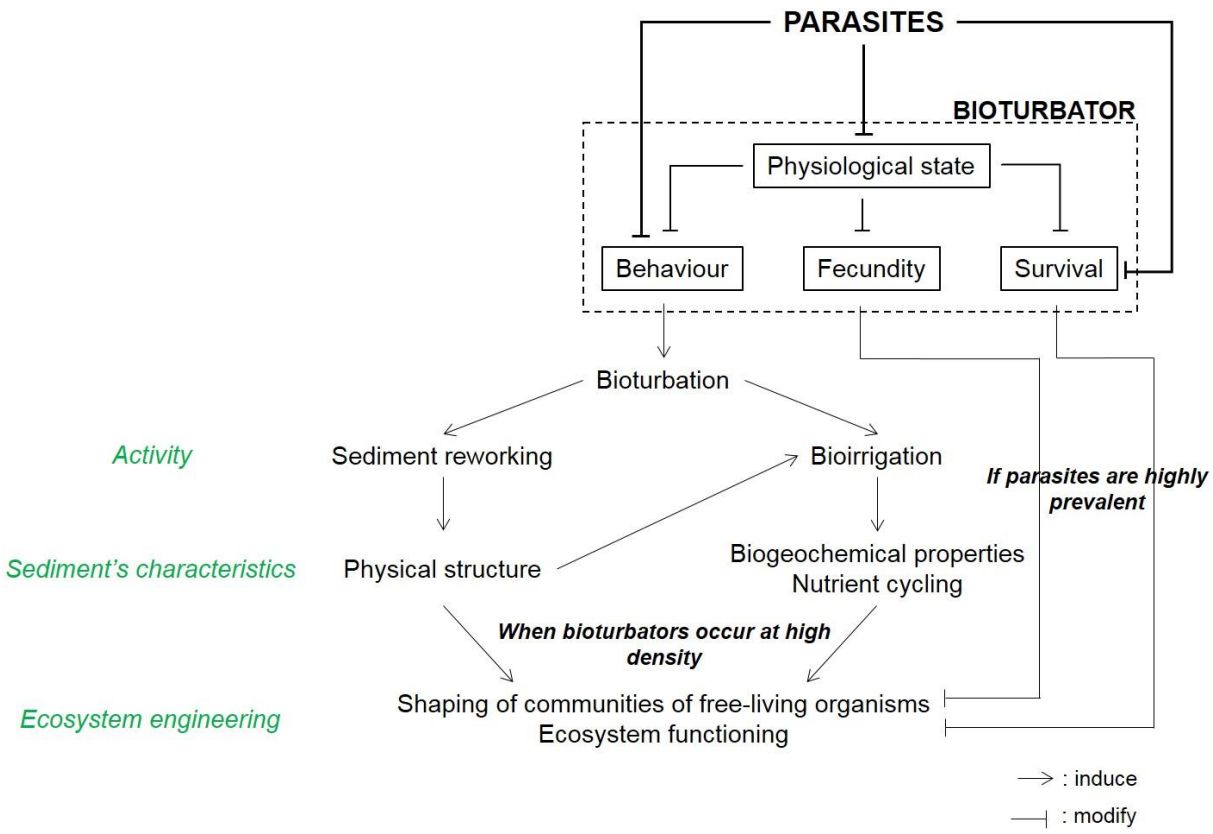

Figure I-A. 5 Impacts of parasites on bioturbating organisms and on their influence as ecosystem engineer species (EES). The behaviour of bioturbating organisms conditions their sediment reworking and bioirrigation activities. These activities play a key role on the physical structure and biogeochemical properties of sediments, and on nutrient cycling. By doing so, bioturbators can play a key role in ecosystem functioning if they are abundant. Parasites can significantly alter the activities of bioturbators by impairing their physiological state, with cascading effects on bioturbator behaviours, fecundity and survival. Finally, parasites can interfere with ecosystem functioning if they are prevalent in the populations of their bioturbating hosts.

First of all, parasites can impair the physiological state of their bioturbating hosts (Figure I-A. 4; Appendix 2 and Appendix 3). For instance, if parasites impact the energy balance of bioturbators, it is likely that the magnitude of the bioturbation activity of the host will then be reduced. Subsequently, parasites are expected to indirectly modify the behaviour of their bioturbating hosts as a side effect of their deleterious impacts on the well-being of the host (Figure I-A. 5). In addition, parasites with complex life cycle also often produce modifications of their host's behavior. Indeed, by manipulating the behaviour of their hosts, these parasites could enhance their own transmission success (Combes, 1991; Lafferty, 1999; Moore, 2002). These behavioural changes are expected to extend to modifications of the host's bioturbation activity as well. Our literature review points out that a large number of parasites infesting bioturbators have complex life cycles (e.g., Trematoda, Acanthocephala). Therefore, we think that the example of parasites modulating the burying and locomotory activities of the New Zealand cockle *A. stuchburyi* is not exceptional, and that other manipulating parasites infesting various

bioturbators would alter their host's behaviour and subsequently the magnitude of their bioturbation activity (Figure I-A. 5).

At a broader ecosystem scale, parasitism can interfere with the engineering role of their bioturbating hosts by shaping the size structure of their host populations. Several bioturbators show reduced growth rates when infested with parasites (Figure I-A. 4; Appendix 2 and Appendix 3). The magnitude of the bioturbation activities of organisms depends on their size (Bachteram *et al.*, 2005; Bosch *et al.*, 2015). In a laboratory experiment, Bosch *et al.* (2015) evaluated the influence of the nereid polychaete *Alitta (Neanthes) succinea* biomass on nitrogen cycling by manipulating the density and size of worms. They found that at similar density, large polychaetes (high biomass) stimulated nitrogen solute fluxes at the sediment-water interface to a greater extent than small worms (low biomass). Moreover, large organisms enhanced solute diffusion in deeper horizons of the sediment column compared to small worms. This pattern is probably the result of larger organisms building larger and deeper burrows than small organisms (Davey, 1994), which would boost bioirrigation processes (Mermillod-Blondin *et al.*, 2004; Bosch *et al.*, 2015). These examples suggest that parasites could theoretically interfere with the role of bioturbators in nutrient cycling and/or in shaping benthic environments (*via* reduction of sediment reworking) by modifying the size structure of the population of their bioturbating hosts (Figure I-A. 5).

Finally, parasites can affect ecosystem functioning *via* density effects on major EES (Poulin, 1999; Mouritsen and Poulin, 2002) as bioturbators (Figure I-A. 5). We reported several examples of parasites reducing the fecundity and/or the survival of their host (Appendix 2 and Appendix 3). The intensity of the bioturbation activity can vary according to population biomass and bioturbator density (e.g., Duport *et al.*, 2006; Sandwell *et al.*, 2009; Braeckman *et al.*, 2010), with potential impacts on ecosystem functioning (Figure I-A. 5). As an illustration, Lohrer *et al.* (2004) evaluated the influence of a declining density of the irregular urchin *Echinocardium* sp. on nutrient cycling. They highlighted a positive influence of density of urchins on ammonium efflux, and on oxygen, nitrite and nitrate and phosphorus influxes at the sediment-water interface. These nutrients being of fundamental importance for primary production, the microphytobenthic primary production increased with the density of *Echinocardium* sp.. Lohrer *et al.* (2004) did not record modification of chlorophyll a content in the sediment in their different density treatments and thus they suggested that the increase of microphytobenthic production could be due to the urchin modifying the microphyte community composition. A similar pattern was reported for the bivalve *A. stutchuburyi* (Sandwell *et al.*, 2009). The microphytobenthos plays a key role in primary production in shallow ecosystems (MacIntyre *et al.*, 1996) and is an important food source for benthic organisms (Miller *et al.*, 1996; Herman *et al.*, 2000). Considering the intricate link between bioturbators and microphytobenthos, one would expect that the reduction of the abundance of bioturbators related to parasitic infestation could be ecologically relevant (Figure I-A. 5).

IV. Conclusion

The structure and function of benthic environments is substantially shaped by major ecosystem engineer species (Jones *et al.*, 1994) such as bioturbators (e.g., Krantzberg, 1985; Levinton, 1995). These organisms play a key role in the physical structure of sediments, on their sediment biogeochemical properties and in nutrient cycling through intense sediment reworking and bioirrigation activities (e.g., Rhoads and Young, 1970; Jones and Jago, 1993; Webb and Eyre, 2004a; Volkenborn *et al.*, 2007b). By doing so, bioturbators deeply influence the community structure of free-living organisms (e.g., Dahlgren *et al.*, 1999; Widdicombe *et al.*, 2000; Pillay *et al.*, 2007) (Figure I-A. 5). The impact of bioturbators on ecosystem functioning is related to the intensity of their bioturbation and therefore on their physiological state. Several parameters can interfere with the role of bioturbators as EES (e.g., Duport *et al.*, 2006; Przeslawski *et al.*, 2009; Premo and Tyler, 2013), such as parasitism. Parasites are widespread in natural environments (Dobson *et al.*, 2008), but so far only a small proportions of bioturbators have been studied in this regard. We pointed out the deficit of data regarding parasites occurring in bioturbating organisms. Moreover, we showed that few studies evaluated the influence of parasites on bioturbating organisms. Among these studies, parasites have been documented to deeply impair the physiological status, fecundity, behaviour and survival of their host. By doing so, parasites could reduce the bioturbation activity of their host. Due to the impact of parasites on their bioturbating hosts and the fact that parasites can be highly prevalent in bioturbating host populations, parasites could have impacts on ecosystem functioning through cascading effects (Figure I-A. 5). In fact, parasites should be considered EES (Thomas *et al.*, 1999) just as bioturbating species are. Therefore, we strongly suggest that parasitism be taken into account when evaluating the role of bioturbators as ecosystem engineers.

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Contamination métallique en milieu aquatique, rôles de la bioturbation et impacts sur les espèces bioturbatrices

I. Contamination métallique en milieu marin

Dans les environnements naturels, deux catégories de contaminants peuvent être considérés, à savoir les contaminants inorganiques tels les métaux et les contaminants organiques. Au cours de ces travaux de thèse nous nous sommes concentrés exclusivement sur les contaminants métalliques.

I.1. Définition des éléments métalliques

Les éléments métalliques sont des éléments chimiques naturellement présents dans la croûte terrestre. Ces substances ne sont ni créées ni détruites par les activités humaines. Certains métaux sont des éléments dits majeurs (Al, Fe ou Mn), i.e. ils font partie des 12 éléments chimiques constituant 99,4 % de la masse de la croûte terrestre (par ordre décroissant d'abondance, O, Si, Al, Fe, Ca, Na, K, Mg, Ti, H, P et Mn). Néanmoins, la plupart des métaux sont classifiés en tant qu'éléments traces métalliques (ETM, anciennement « métaux lourds ») et définis comme des éléments chimiques dont la concentration dans la croûte terrestre est inférieure à 1 ppm. Il existe 68 ETM qui contribuent seulement à ca. 0,6 % de la masse de la croûte terrestre. Un certain nombre d'éléments métalliques sont essentiels pour les organismes vivants, intervenant notamment comme co-facteurs enzymatiques (Cu, Mn, Co, Cr, Se ou encore Zn) (Förstner and Wittmann, 1981). Néanmoins, à fortes concentrations ces substances peuvent s'avérer toxiques pour les organismes vivants. A l'inverse certains ETM n'ont pas de fonctions biologiques connues (Cd, Hg, Pb) ; leur toxicité dépend également de leur concentration et de leur speciation (Förstner and Wittmann, 1981).

I.2. Apports des éléments métalliques dans les zones côtières

Les éléments métalliques peuvent se retrouver de façon naturelle en milieu aquatique du fait des processus d'érosion des roches, du lessivage des sols, des éruptions volcaniques ou du fonctionnement des sources hydrothermales (Förstner and Wittmann, 1981; Mason, 2013; Bjerregaard and Andersen, 2014). Néanmoins, les activités anthropiques peuvent aussi contribuer de façon significative à leur enrichissement dans les écosystèmes (contamination) (Tableau I-B. 1; Pacyna and Pacyna, 2001), et ce, particulièrement, au niveau des zones côtières industrialisées et urbanisées (Figure I-B. 1) (Cave *et al.*, 2005; Pan and Wang, 2012). De nombreuses activités anthropiques (incinération des déchets ménagers par exemple) et notamment industrielles (en particulier l'extraction et la combustion de produits pétroliers) peuvent émettre des métaux dans l'atmosphère (Pacyna and Pacyna, 2001). Une fois dans l'atmosphère, ces éléments métalliques peuvent être transportés sur de longues distances et *in fine* contamineront les milieux aquatiques par précipitation. Ces particules peuvent également se déposer en milieu terrestre (Nicholson *et al.*, 2003). Les sols peuvent être également directement contaminés par le biais des activités agricoles (Ribolzi *et al.*, 2002; Nicholson *et al.*, 2003) et par l'urbanisation (Old *et al.*, 2004; Pan and Wang, 2012). Les processus de ruissellement permettront à ces éléments d'atteindre les cours d'eau, et finalement les écosystèmes côtiers (Ribolzi *et al.*, 2002). Enfin, les écosystèmes aquatiques constituent le réceptacle d'eaux usées, traitées ou non, qui peuvent transporter des contaminants métalliques. En effet, même lors de la mise en place de processus d'épuration, les éléments

métalliques ne sont que très variablement éliminés (de 15 à 95 % selon les procédures mises en œuvre) (Pomiès *et al.*, 2009), et il y a donc un relargage d'éléments métalliques dans les écosystèmes aquatiques (Figure I-B. 1).

Tableau I-B. 1 Comparaison entre les émissions atmosphériques anthropiques globales d'éléments métalliques et les émissions naturelles (émissions en 10^3 tonnes an^{-1}). D'après Pacyna and Pacyna (2001).

Eléments traces métalliques	Emissions anthropiques	Emissions naturelles (valeurs médianes)	Rapport émissions anthropiques/émissions naturelles
As	5,0	12,0	0,42
Cd	3,0	1,3	2,3
Cr	14,7	44,0	0,33
Cu	25,9	28,0	0,93
Mn	11,0	317,0	0,003
Ni	95,3	30,0	3,2
Pb	119,3	12,0	9,9
Se	4,6	9,3	0,49
V	240,0	28,0	8,6
Zn	57,0	45,0	1,3

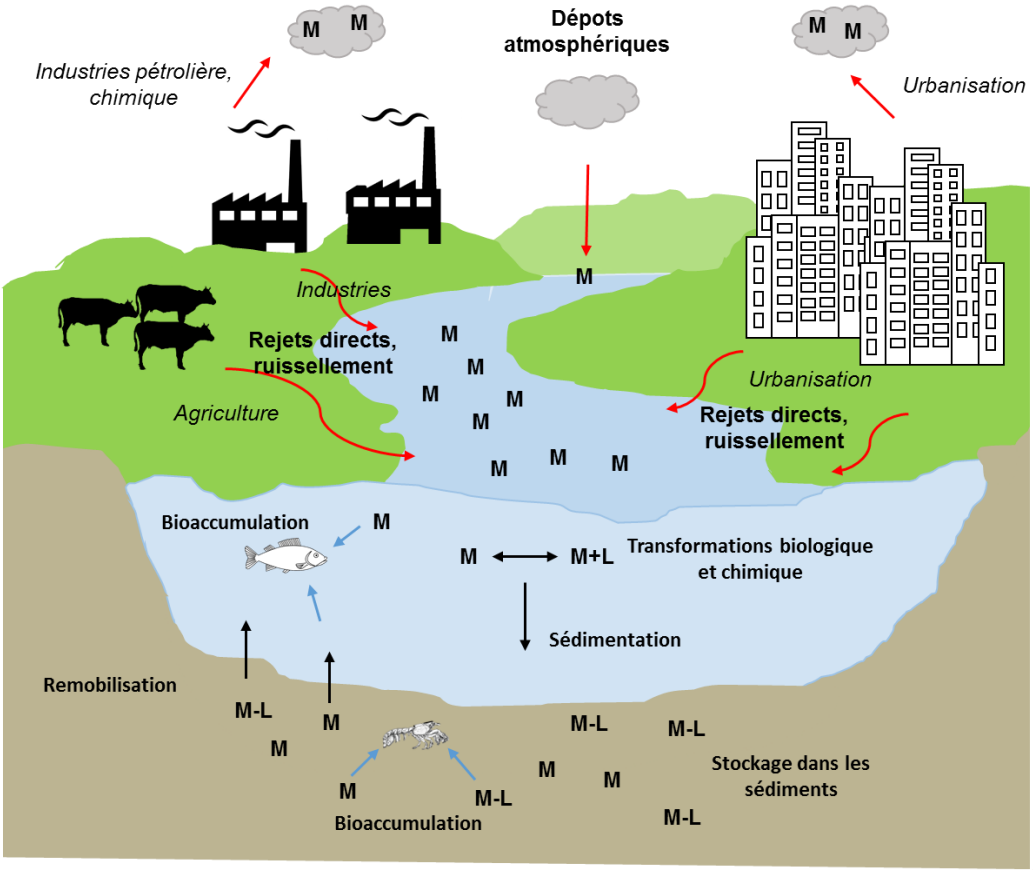

Figure I-B. 1 Apports et devenir des contaminants métalliques en milieu aquatique. M : contaminants métalliques ; L : ligands.

I.3. Devenir des éléments métalliques en milieu marin

I.3.1. Dans la colonne d'eau

Les éléments métalliques parviennent dans les zones côtières sous forme dissoute et/ou associés aux particules en suspension. Leur devenir dépend alors des propriétés physico-chimiques propres à chaque élément ainsi que de l'ensemble des variables physico-chimiques du milieu (salinité, pH, concentration en matières en suspension, etc.) qui modulent les spéciations physique et chimique des éléments métalliques. La spéciation physique distingue les différentes formes d'un métal selon leurs tailles : formes particulaires (> 220 nm), colloïdales (10–220 nm) ou dissoutes « vraies » (< 10 nm) (ces limites étant arbitraires). La spéciation chimique caractérise quant à elle la distribution des éléments métalliques parmi les différentes espèces chimiques (Figure I-B. 2). Les espèces métalliques dissoutes peuvent ainsi être retrouvées sous une forme libre ou complexée avec des composés inorganiques (Cl^-, HCO_3^-, SO_4^{2-}, etc.) ou organiques (substances humiques, acides aminés, etc.) (Figure I-B. 1, Figure I-B. 2). Les éléments métalliques peuvent également être associés à de la matière organique particulaire (restes d'organismes, pelotes fécales, etc.) et avec différentes fractions minérales (argiles, oxydes et hydroxydes de Fe, etc.), formant la fraction particulaire (Figure I-B. 2) (Gonzalez and Chiffoleau, 1999; Campbell and Couillard, 2004; Bjerregaard and Andersen, 2014). Il existe un équilibre dynamique entre les différentes formes prises par les éléments métalliques dans les environnements aquatiques, plusieurs types de réaction permettant le passage de l'une à l'autre de ces formes (réactions de complexation-dissociation, d'adsorption-désorption, d'oxydo-réduction ou encore biologique).

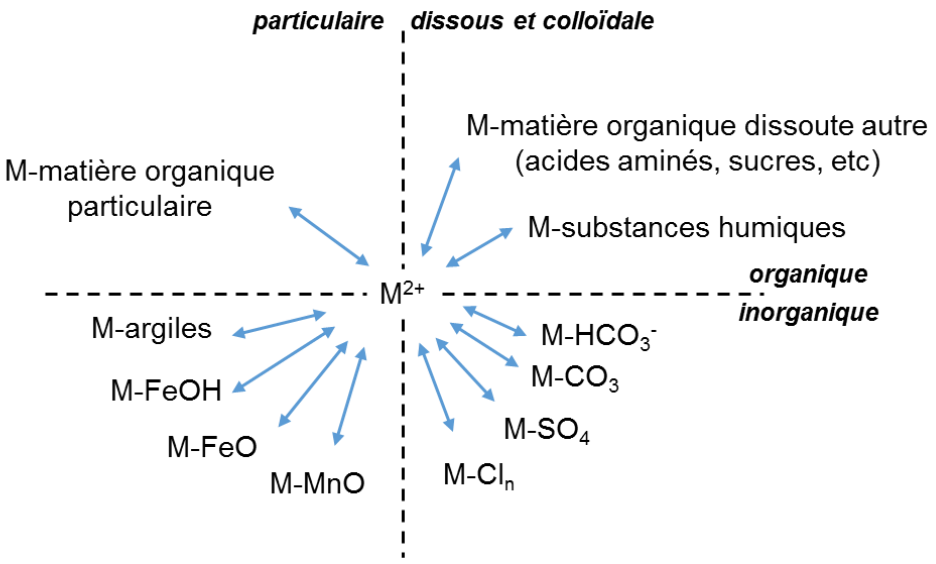

Figure I-B. 2 Spéciation des éléments métalliques en solution. M : élément métallique. D'après Bjerregaard and Andersen (2014) et Campbell and Couillard (2004).

I.3.2. Dans les sédiments

A terme, les éléments métalliques (sous formes soluble ou particulaire) seront déposés sur les sédiments marins (Figure I-B. 1), qui constituent ainsi d'importants puits pour ces éléments. Ceux-ci peuvent diffuser ou précipiter depuis la colonne d'eau vers la colonne sédimentaire mais sédimentent plus généralement sur les fonds océaniques liés aux matières en suspension.

Les sédiments constituent une matrice hétérogène, formée de trois compartiments au sein desquels les éléments métalliques se répartissent (Förstner and Wittmann, 1981) :

(1) une phase inorganique, constituée des minéraux des roches terrestres et de débris divers (coquilles, squelettes). Ces particules sont recouvertes par des (hydr)oxydes de Fe et de Mn qui présentent une importante capacité d'adsorption des éléments métalliques.

(2) une phase organique qui peut complexer les éléments métalliques, ceux-ci pouvant être libérés lors de sa dégradation. Elle joue ainsi un rôle particulièrement important dans la biodisponibilité des éléments métalliques.

(3) des eaux interstitielles, occupant l'espace entre les particules sédimentaires et dans lesquelles les éléments métalliques sont retrouvés sous forme dissoute (ions libres, complexes organiques ou inorganiques).

La distribution des éléments métalliques entre ces différents compartiments dépend des caractéristiques physico-chimiques du milieu (pH, potentiel redox, teneur en oxygène, etc.) et des propriétés propres aux métaux (Förstner and Wittmann, 1981; Calmano *et al.*, 1993; Mason, 2013). Ces caractéristiques physico-chimiques peuvent être fortement modifiées lors de la diagenèse des sédiments, notamment en lien avec les processus de dégradation et d'enfouissement de la matière organique dans les sédiments.

La diagenèse peut être définie comme l'ensemble des processus affectant un dépôt sédimentaire et qui le transforme en roche sédimentaire. Ces processus sont à la fois physiques (compaction du sédiment et expulsion de l'eau interstitielle) mais également chimiques (dissolution, précipitation, désorption, etc.). La dégradation de la matière organique est l'un des processus moteur de la diagenèse. Cette minéralisation de carbone organique est un phénomène progressif impliquant toute une séquence d'oxydo-réduction dans la colonne sédimentaire (Figure I-B. 3). Les réactions d'oxydo-réduction nécessitent la présence d'accepteurs finaux d'électrons: O_2, NO_3^-, MnO_2, Fe_2O_3, SO_4^{2-} et CO_2. Ces accepteurs d'électrons sont utilisés successivement en fonction du rendement d'énergie de la réaction redox (Froelich *et al.*, 1979). La disponibilité de ces oxydants varie dans la colonne sédimentaire. Cette dernière présente ainsi une stratification verticale chimique mais également biologique, en lien avec la présence d'organismes capables de réaliser les différentes réactions redox de minéralisation de la matière organique. De la surface vers le fond, on retrouvera tout d'abord une zone oxique dans laquelle

l'oxygène moléculaire est présent, puis une zone suboxique avec présence de nitrates, d'oxydes et d'hydroxyde de Mn et de Fe, et finalement une zone anoxique avec des sulfates.

Les échanges des éléments métalliques entre les différents compartiments du sédiment sont complexes (Förstner and Wittmann, 1981; Mason, 2013). Néanmoins, de manière générale, l'oxydation de la matière organique par l'oxygène conduit aux relargages des métaux initialement associés à ces particules dans la zone oxique. Ces éléments métalliques sont libérés dans les eaux interstitielles où ils peuvent rester dissous (Cu, Cd) ou s'associer aux (hydr)oxydes de Fe et de Mn. Dans les zones suboxiques, la réduction de ces oxydes et hydroxydes entraine la désorption des éléments métalliques et la libération du Fe et du Mn dans les eaux interstitielles sous forme dissoute. Finalement, en milieu anoxique, la réduction des sulfates libère des ions sulfures qui contribuent au piégeage des éléments métalliques, en particulier des éléments cationiques divalents qui ont une forte affinité pour ces ions (Förstner and Wittmann, 1981; Shaw *et al.*, 1990). L'ensemble de ces processus est à l'origine de gradients de concentrations des éléments métalliques dans les eaux interstitielles conduisant à la mise en place de flux biodiffusifs et d'échanges à l'interface eau-sédiment (Shaw *et al.*, 1990; Blasco *et al.*, 2000).

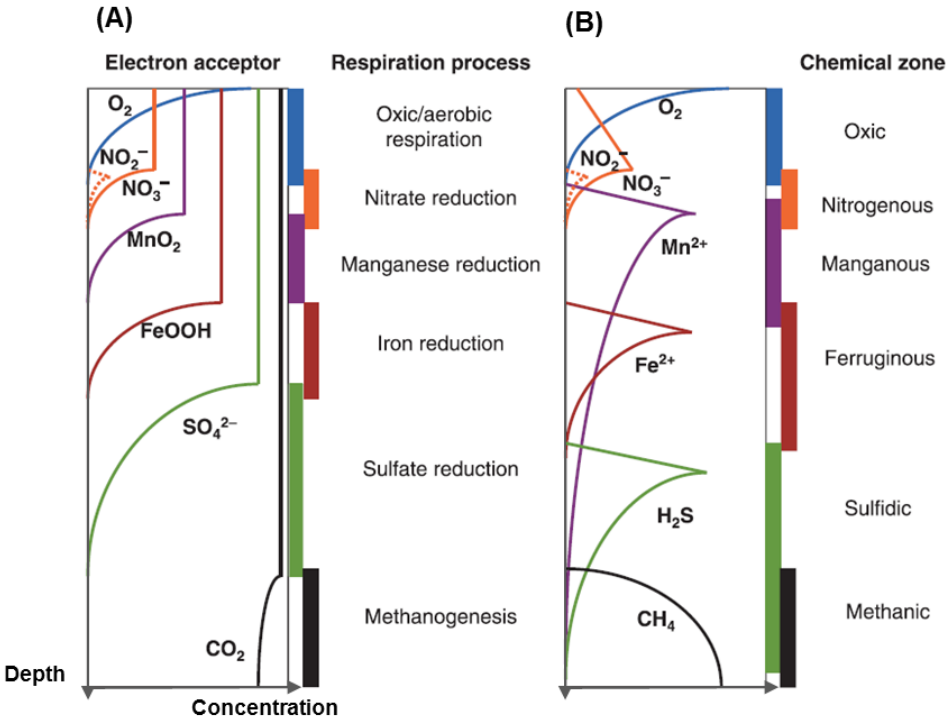

Figure I-B. 3 (A) Distribution des principaux accepteurs d'électrons et réactions d'oxydo-réduction impliquées dans les processus de minéralisation de la matière organique et (B) zonation chimique accompagnant les processus de minéralisation. D'après Canfield and Thamdrup (2009).

L'exemple du cadmium (Cd)

Le cadmium, élément n° 48 du tableau périodique, est un ETM dont les concentrations moyennes dans la croûte terrestre sont de 0,1 à 30 µmol kg^{-1} selon le type de roches considérées (ignées vs.

sédimentaires) (Mason, 2013). Cet élément est présent dans les minerais de Zn, de Pb et de Cu. Il intervient notamment dans la composition des accumulateurs (piles nickel-cadmium) et de certains alliages, il sert également de stabilisant aux matières plastiques. Le Cd n'a pas de fonction biologique connue chez les Métazoaires et est hautement toxique, ainsi considéré comme substance d'intérêt prioritaire dans le cadre de la directive cadre sur l'eau de l'Union Européenne (directive 2008/105/EC). Le cycle naturel du Cd est considéré comme l'un des plus perturbés par les activités humaines, les rejets anthropiques surpassant de loin les apports naturels (Tableau I-B. 1) (Wright and Welbourn, 1994; Gonzalez and Chiffoleau, 1999). Les apports fluviaux représentent la principale source de Cd des eaux côtières et océaniques. Le Cd est transporté sous forme particulaire (associé aux matières en suspension) et dissoute. Cet élément est dit non-conservatif, i.e. lorsque les eaux fluviales et océaniques se mélangent, la concentration du Cd ne suit pas une droite de dilution. Au contraire, on note une désorption du Cd lié aux particules en suspension à mesure que la salinité augmente, et donc une augmentation de la fraction dissoute du Cd (Figure I-B. 4). Le Cd forme alors des chlorocomplexes dissous très stables, représentant environ 97 % du Cd total dans les eaux océaniques (Figure I-B. 5) (Mantoura *et al.*, 1978; Wright and Welbourn, 1994). Le maximum de concentration de Cd est atteint pour des salinités moyennes, i.e. de 15 à 20. Au-delà de ces salinités, on observe une diminution des concentrations qui traduit un comportement conservatif (dilution avec l'eau de mer). Ainsi, les estuaires peuvent représenter des sources de Cd dissous non négligeables pour les écosystèmes côtiers (Gonzalez and Chiffoleau, 1999). La salinité est donc l'un des principaux facteurs influant sur la spéciation du Cd dans l'eau. Néanmoins, la quantité de matière en suspension (suspended particulate matter SPM) semble également jouer un rôle important dans la spéciation du Cd ; celle-ci favoriserait notamment sa solubilisation dans les milieux anoxiques (Gonzalez and Chiffoleau, 1999).

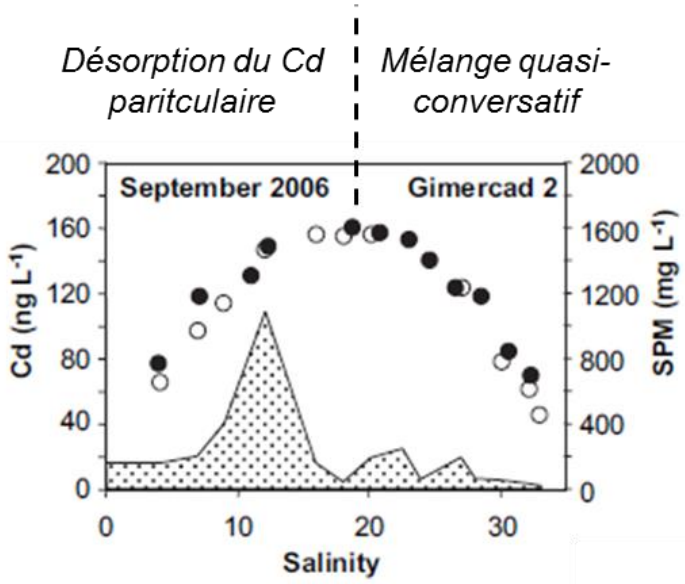

Figure I-B. 4 Concentration en cadmium dissous (Cd, ng L^{-1}) dans l'estuaire de la Gironde le long du gradient de salinité et quantité de matière en suspension (SPM, mg L^{-1}). ○ Concentration en Cd dans les eaux de surface, ● concentration en Cd dans les eaux de fond ; la zone en pointillés représente la quantité de matières en suspension. D'après Dabrin et *al.* (2009).

Les concentrations en Cd dans les eaux côtières sont relativement faibles comparativement à celles observées dans les sédiments (de l'ordre du ng–µg L^{-1} vs. mg kg^{-1}, respectivement) (voir Luo *et al.*, 2013 pour un exemple dans un environnement pollué). Ces différences d'ordre de grandeur peuvent être mises en lien avec les importants flux de contaminants métalliques depuis la colonne d'eau vers le sédiment. Il a par exemple été estimé qu'environ 69 % du Cd charrié par les eaux du Saint-Laurent était retenu dans les sédiments estuariens et côtiers (Yeats and Bewers, 1982; Mason, 2013). Dans les sédiments, la solubilité du Cd dépend notamment du pH et des ligands présents. Dans les zones oxiques, le Cd est lié à des oxydes de Fe, et dans une moindre mesure, à des oxydes de Mn et des composés organiques humiques (Wright and Welbourn, 1994). L'oxydation des hydr(oxydes) de Fe et de Mn et de la matière organique entraine la solubilisation du Cd dans les eaux interstitielles, où il peut rester sous forme dissoute. Dans les sédiments anoxiques, la réduction des sulfates entraine la libération de sulfures qui peuvent fortement lier le Cd (Di Toro *et al.*, 1990, 1996). Dans ces environnements, la quantité de Cd dans les eaux interstitielles peut être très faible dans le cas où la quantité de sulfures volatiles extractibles à l'acide (Acid-Volatile Sulfides AVS, principalement des monosulfures de Fe) excède la quantité de Cd (Di Toro *et al.*, 1990).

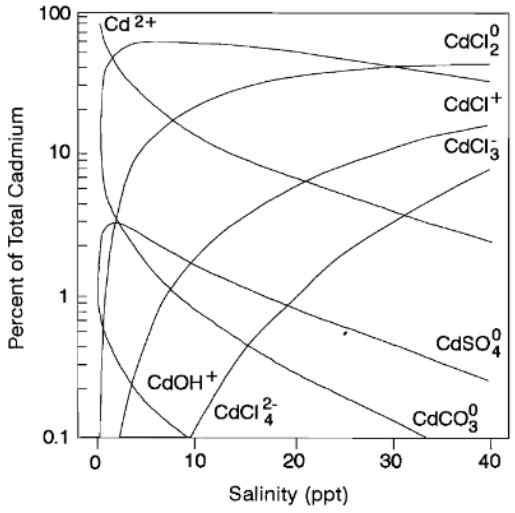

Figure I-B. 5 Spéciation chimique du cadmium en fonction de la salinité, pH = 6,8. D'après Rainbow et *al.* (1993) et Wright and Welbourn (1994).

II. Bioturbation et remobilisation des éléments métalliques

Les concentrations en éléments métalliques des sédiments marins sont souvent bien supérieures à celles notées dans la colonne d'eau et les sédiments marins sont ainsi considérés comme constituant un puits d'éléments métalliques. Néanmoins, le stockage des éléments métalliques dans les sédiments n'est pas un processus définitif, ces derniers pouvant être remobilisés depuis les phases particulaires vers l'eau interstitielle ou directement vers la colonne d'eau (Förstner and Wittmann, 1981; Salomons *et al.*, 1987; Calmano *et al.*, 1993). Les sédiments marins et les éléments métalliques qu'ils contiennent peuvent ainsi constituer une menace pour les organismes aquatiques (Roberts, 2012) ; certains auteurs utilisant même le terme de « chemical bomb » (Stigliani, 1991). La remobilisation des métaux contenus dans les

sédiments résultent des modifications des paramètres physico-chimiques du milieu (salinité, pH, potentiel redox, etc.), modifiant la spéciation des éléments métalliques. Divers processus naturels (courant, tempêtes, etc.) ou anthropiques (dragage notamment) sont à l'origine d'une remobilisation des éléments métalliques. Cette remobilisation se fait notamment *via* la remise en suspension des particules sédimentaires sur lesquelles sont fixés certains métaux (Förstner and Wittmann, 1981; Roberts, 2012; Mason, 2013). Une fois dans la colonne d'eau, des processus d'oxydo-réduction sont à l'origine de la solubilisation de certains éléments métalliques, la présence d'oxygène permettant notamment l'oxydation des complexes soufrés qui eux-mêmes lient fortement les métaux dans les zones suboxiques et anoxiques des sédiments. Dans la colonne d'eau, le taux de désorption des métaux des particules sédimentaires dépend des propriétés des sédiments remis en suspension (granulométrie, quantité de matière organique, oxyde de Fe et de Mn, etc.), mais également des propriétés physico-chimiques de la colonne d'eau elle-même (pH, salinité, concentration en oxygène, etc.) (Cantwell *et al.*, 2002; Eggleton and Thomas, 2004; Atkinson *et al.*, 2007).

Outre les processus purement physiques et chimiques, la bioturbation constitue un mécanisme biologique majeur pouvant jouer sur le devenir des métaux des sédiments. Comme évoqué au cours du **Chapitre I-A**, la bioturbation peut être définie comme l'ensemble des processus affectant la matrice sédimentaire (Kristensen *et al.*, 2012). Du fait de leur activité de fouissage, de locomotion, de nutrition ou *via* la construction et le maintien de structures biogéniques (terriers notamment), les organismes bioturbateurs sont à l'origine d'importants transports de particules sédimentaires ou remaniement sédimentaire (Rhoads and Young, 1971; Cadée, 1976; Coulon and Jangoux, 1993). Par exemple, ces processus de mélange du sédiment transportent de la matière organique labile dans les couches profondes du sédiment et stimule ainsi l'activité microbienne. De manière inverse, le remaniement sédimentaire permet un transfert des complexes réduits insolubles (FeS, FeS_2) liant les éléments métalliques vers les zones oxiques du sédiment. Par ailleurs, le déplacement des particules sédimentaires par les organismes bioturbateurs modifient la structure physique des sédiments, i.e. leur porosité ainsi que leur répartition granulométrique (e.g., Rhoads and Young, 1970; Jones and Jago, 1993; Volkenborn *et al.*, 2007a).

Chaque espèce bioturbatrice est à l'origine d'un remaniement sédimentaire qui lui est propre, dépendant de ses caractéristiques comportementales (locomotion, nutrition, etc.). Néanmoins, il existe des similitudes entre les modes de remaniement sédimentaire des bioturbateurs, permettant de les classifier en grand groupes fonctionnels (Figure I-B. 6) (Kristensen *et al.*, 2012):

(1) les biodiffuseurs sont à l'origine d'un transport des particules sédimentaires dans toutes les directions de l'espace mais sur de relativement courtes distances. Ce transport peut ainsi être comparé à un processus de diffusion moléculaire (Figure I-B. 6A).

(2) les convoyeurs sont des organismes à l'origine d'un transport orienté des sédiments. Ce transport peut s'effectuer vers l'interface eau-sédiment (convoyeurs vers le haut, Figure I-B.

6B) ou en profondeur dans la colonne sédimentaire (convoyeurs vers le bas, Figure I-B. 6C). Ces espèces sont généralement orientées verticalement dans la colonne sédimentaire ; leur orientation déterminant le sens du convoyage des particules sédimentaires.

(3) les régénérateurs sont des organismes qui construisent et maintiennent des terriers dans les sédiments. Ils sont à l'origine d'un transport de particules sédimentaires dans les différentes directions de l'espace : des particules sont excavées lors des activités de fouissage en lien avec la création et le maintien des terriers, des transports passifs de sédiments de la surface vers des horizons plus profonds sont également possibles *via* les processus de ventilation des terriers ou lorsque ceux-ci s'effondrent (Figure I-B. 6D).

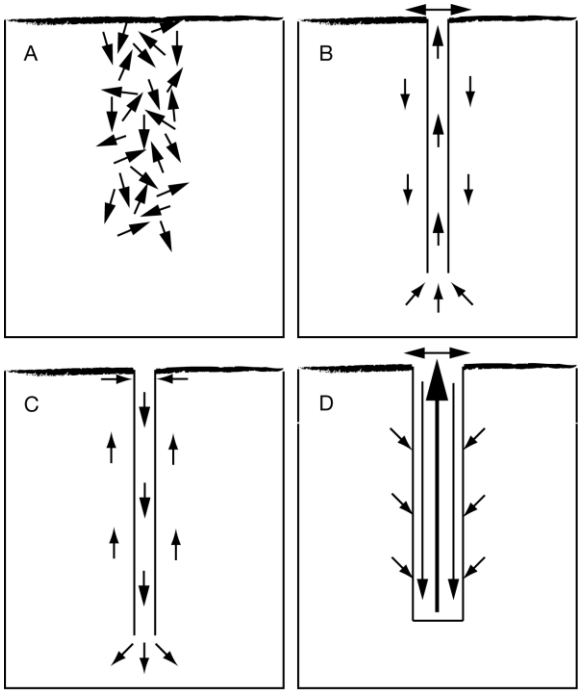

Figure I-B. 6 Principaux types de remaniement sédimentaire exercés par la faune benthique et définissant les groupes fonctionnels d'espèces bioturbatrices. (A) Biodiffuseur, (B) convoyeur vers le haut, (C) convoyeur vers le bas et (D) régénérateur. D'après Kristensen et *al.* (2012).

En plus de ces mouvements de particules sédimentaires, les espèces bioturbatrices génèrent des mouvements d'eau et de solutés dans la colonne sédimentaire et entre les eaux interstitielles et l'eau surnageante (Kristensen *et al.*, 2012). Ce phénomène de bioirrigation du sédiment est particulièrement le fait de bioturbateurs endogés construisant des structures biogéniques qu'ils doivent ventiler de manière à assurer un apport suffisant de dioxygène pour leur propre physiologie, voire de nourriture dans le cas d'organismes suspensivores. La bioirrigation du sédiment par les espèces bioturbatrices permet notamment d'étendre la profondeur de pénétration des accepteurs d'électrons, et notamment du O_2 (Figure I-B. 7) (Zorn *et al.*, 2006; Glud, 2008), et influence également les conditions d'oxydo-réduction dans les sédiments (Krantzberg, 1985). Par ailleurs, les activités intermittentes de ventilation des terriers sont à l'origine d'un apport discontinu de O_2 dans les sédiments provoquant des oscillations

des conditions d'oxydo-réduction, qui stimulent d'autant plus les processus de minéralisation de la matière organique (Aller, 1994). Ainsi, les processus de bioirrigation modulent (1) les taux d'échanges de composés chimiques dissous entre les phases particulaires et aqueuses des sédiments, (2) la répartition des espèces chimiques dans la colonne sédimentaire et (3) le cycle biogéochimique de ces espèces (Aller, 1982). *In fine*, la bioturbation interfère avec la séquence diagénétique classique présentée précédemment et altère la stratification chimique verticale théorique des sédiments (Figure I-B. 8) (Aller, 1982, 1988).

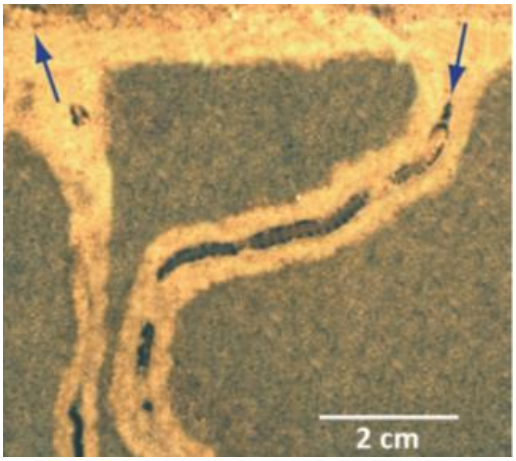

Figure I-B. 7 Influence du bioturbateur *Hediste (Nereis) diversicolor* sur les propriétés biogéochimiques d'un sédiment sableux. Les activités de ventilation du terrier entrainent l'oxydation des sédiments à proximité des parois des terriers (en marron clair) alors que le reste de la colonne sédimentaire est réduite (en marron foncé). Les flèches indiquent le sens de la ventilation du terrier. D'après Kristensen *et al.* (2012).

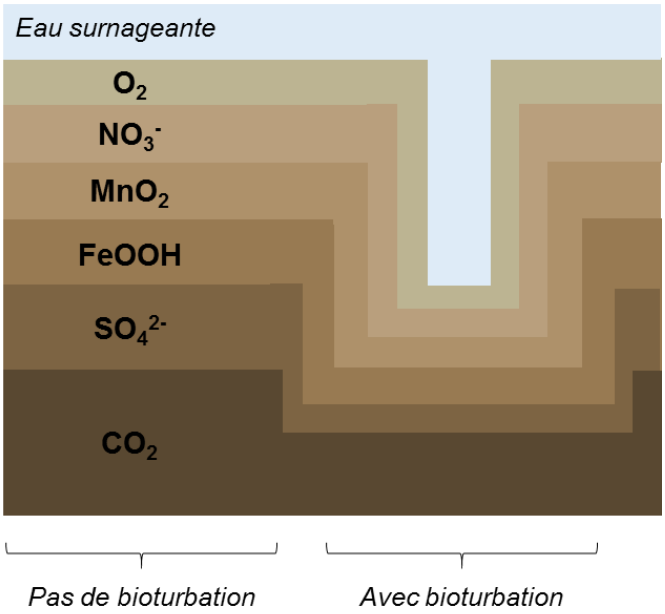

Figure I-B. 8 Modification de la stratification chimique verticale de la colonne sédimentaire par les organismes bioturbateurs. D'après Aller (1982).

Considérant que les transports de sédiments, d'eau et de solutés initiés par les espèces bioturbatrices modulent à la fois les propriétés physiques et chimiques des sédiments (Rhoads and Young, 1970; Aller, 1982; Krantzberg, 1985; Jones and Jago, 1993; Volkenborn *et al.*, 2007a), la bioturbation peut influer sur (1) la distribution des métaux au sein de la colonne sédimentaire, (2) leur répartition entre les phases particulaire et dissoute, (3) leur spéciation chimique et (4) leur transfert à l'interface eau-sédiment. L'influence des bioturbateurs sur le devenir des éléments métalliques dans les sédiments a particulièrement été étudiée dans les environnements d'eau douce (e.g., Ciutat and Boudou, 2003; Ciutat *et al.*, 2007; Lagauzère *et al.*, 2009; Schaller, 2014; Blankson and Klerks, 2016). En milieu marin la littérature est moins développée. Néanmoins, il a été montré qu'en présence d'un sédiment expérimentalement enrichi en Cd le polychète *Arenicola marina* réduit la diffusion moléculaire de ce contaminant depuis le sédiment vers la colonne d'eau. De plus, la bioturbation exercée par cet organisme modifie largement la répartition du Cd dans le sédiment, en lien avec le type de remaniement sédimentaire propre à cette espèce. En effet, *A. marina* est un convoyeur vers le haut et il a été montré que les couches sédimentaires de surface présentaient un enrichissement en Cd alors que celles plus profondes étaient appauvries en cet élément (Rasmussen *et al.*, 2000). L'influence de l'amphipode *Victoriopisa australiensis* sur le devenir et la biodisponibilité des éléments métalliques contenus dans un sédiment prélevé dans un environnement pollué a également été quantifiée. Cet amphipode module notamment les concentrations en éléments métalliques biodisponibles dans les couches superficielles de la colonne sédimentaire et stimule par ailleurs la remobilisation de certains contaminants vers la colonne d'eau surganeante (Remaili *et al.*, 2016, 2017). Finalement Atkinson *et al.* (2007) se sont également intéressés au rôle joué par le bivalve *Macomona (Tellina) deltoidalis* sur le devenir des contaminants métalliques dans les sédiments marins. Il a ainsi été mis en évidence que ce bioturbateur stimule la remobilisation du Fe et du Mn vers la colonne d'eau, mais pas celles du Pb et du Zn.

III. Sensibilité des organismes bioturbateurs à la contamination métallique

Les organismes bioturbateurs sont au contact direct des sources potentielles de contaminants métalliques, à savoir les sédiments et la colonne d'eau. Par ailleurs, et du fait de leur influence sur les propriétés physiques et biogéochimiques des sédiments, ces organismes peuvent moduler la biodisponibilité des contaminants métalliques et donc leur toxicité. L'ingestion de ces contaminants par les bioturbateurs par voie directe (contaminants dans l'eau) ou trophiques (*via* l'ingestion de sédiments ou la filtration des particules contenues dans l'eau) et leur accumulation peuvent être à l'origine d'effets délétères selon, entre autre, les quantités accumulées.

III.1. Prise en charge des éléments métalliques

Les branchies et l'épithélium intestinal sont les principales zones d'entrée des métaux chez les organismes aquatiques. L'entrée des éléments métalliques dans les cellules sous-tend le passage de la

membrane plasmique, bicouche phospholipidique dans laquelle sont enchâssées de nombreuses protéines. Bien que la membrane plasmique constitue une barrière biologique, il existe différents mécanismes permettant l'internalisation des éléments métalliques dans les cellules (Campbell and Couillard, 2004; Mason, 2013) (Figure I-B. 9):

(1) le transport passif, i.e. une simple diffusion selon un gradient de concentrations. Seuls les éléments métalliques formant des complexes lipophiles peuvent entrer dans les cellules de cette façon.

(2) le transport facilité faisant intervenir un transporteur (protéine transmembranaire) peu sélectif. Ce transport s'effectue selon un gradient électrochimique et permet l'internalisation de composés chargés.

(3) le transport actif *via* un transporteur énergie-dépendant, l'internalisation des éléments métalliques peut ainsi se faire contre un gradient électrochimique.

(4) éventuellement le transport par endocytose dans le cas d'éléments métalliques complexés, impliquant la formation de vésicules (Puckett *et al.*, 2010). Rainbow (1997) suggère néanmoins que cette voie d'entrée est probablement de mineure importance chez les crustacés.

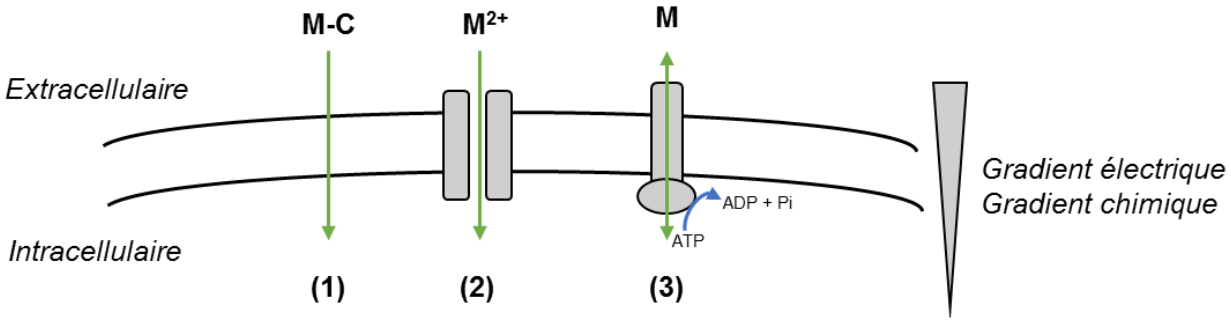

Figure I-B. 9 Principales voies d'entrée des éléments métalliques dans les cellules : (1) par simple diffusion, (2) par transport facilité, (3) par transport actif. M-C : complexe métallique lipophile, M^{2+} : cation métallique, M : complexe métallique.

Par exemple, le Cd traverse les membranes biologiques principalement sous la forme ionique Cd^{2+} (Gutknecht, 1983). L'entrée du Cd dans les cellules se fait alors notamment par transport facilité aux niveaux de canaux calciques voltage-dépendants (Roesijadi and Unger, 1993), sous forme ionique le Cd présentant un rayon et une configuration électronique proche de celle du Ca (rayon ionique Ca^{2+} = 0,97 Å et Cd^{2+} = 0,99 Å).

Précédemment, nous avons évoqué le fait que les éléments métalliques existaient sous forme dissoute ou particulaire en milieu aquatique. Sous forme dissoute, les métaux forment généralement des complexes avec de nombreux ligands inorganiques et organiques (Figure I-B. 2) et peuvent être également présents sous forme ionique (dite « libre », Figure I-B. 5). Ces phénomènes de spéciation déterminent la biodisponibilité des éléments métalliques pour les organismes marins, et *in fine* leur

toxicité. La biodisponibilité d'un élément peut être définie comme la fraction absorbable de cet élément par les organismes. Les métaux sous leur forme dissoute et, particulièrement sous leur forme libre (Cd^{2+}, Cu^{2+}, Zn^{2+}, etc.), sont considérés comme les plus biodisponibles pour les organismes vivants. Plusieurs modèles ont ainsi été développés de manière à prédire l'entrée des métaux dans les organismes en prenant en compte leur spéciation, dont le modèle du ligand biotique (Campbell, 1995; Di Toro *et al.*, 2001). Ce modèle postule que l'ion libre à l'approche de la membrane plasmique interagit avec des ligands qui constituent des sites de fixation physiologiquement inertes (pas de perturbation des fonctions cellulaires normales) ou des ligands qui sont des sites de fixation physiologiquement actifs. Dans ce dernier cas, le métal affecte le métabolisme cellulaire directement en se liant par exemple à une enzyme elle-même liée à la membrane ou indirectement, i.e. en étant transporté à l'intérieur de la cellule. Dans le cytosol, l'élément métallique interagira alors avec des ligands intracellulaires, ce qui aura des conséquences négatives ou positives sur le fonctionnement cellulaire. Ce modèle prend par ailleurs en considération le fait que l'ion libre est en compétition avec d'autres cations (Ca^{2+}, H^{+}, etc.) pour se lier aux ligands à la surface des cellules (Di Toro *et al.*, 2001). La présence de ces cations en solution peut ainsi moduler la toxicité des éléments métalliques. Le modèle du ligand biotique s'applique particulièrement aux organismes qui n'assimilent pas de matière particulaire (autotrophes pour le carbone notamment) ou à ceux pour lesquels la phase dissoute reste le vecteur principal d'assimilation (Campbell and Couillard, 2004).

En ce qui concerne les éléments métalliques sous forme particulaire, l'assimilation de ces éléments nécessite tout d'abord leur ingestion. Les particules et leurs métaux doivent ensuite être solubilisés. Cette étape peut se produire dans le tractus digestif des organismes, les particules se trouvent alors dans des conditions physico-chimiques (notamment en ce qui concerne le pH) différentes de celles de l'environnement pouvant favoriser la solubilisation des éléments métalliques liés aux particules sédimentaires. Une fois solubilisés, les métaux pourront franchir les barrières cellulaires, en principe selon les mêmes processus de transport précédemment évoqués (Figure I-B. 9) (Campbell and Couillard, 2004; Mason, 2013).

III.2. Mécanismes de séquestration et de détoxication

Une fois dans les cellules, et selon leur concentration, les éléments métalliques peuvent être à l'origine de tout un panel de dommages cellulaires. Les métaux sous forme ionique peuvent se lier à des molécules essentielles et en altérer le fonctionnement *via* des changements de conformation ou en se liant à certains groupements fonctionnels. Certains métaux peuvent également être à l'origine de la production d'espèces réactives de l'oxygène induisant un stress oxydant (Campbell and Couillard, 2004). En réponse, les organismes vivants présentent diverses stratégies de détoxication, i.e. des processus permettant de minimiser le potentiel d'un élément métallique à interagir de manière délétère avec des macromolécules essentielles (Campbell and Couillard, 2004). Chez les Invertébrés ces processus reposent notamment sur (Figure I-B. 10) (Campbell and Couillard, 2004; Ahearn *et al.*, 2004):

(1) une régulation des flux d'entrées et de sorties des éléments métalliques dans les cellules (équilibre absorption/excrétion). Les éléments métalliques importés dans la cellule peuvent être transportés dans l'hémolymphe des organismes où ils seront pris en charge par les hémocytes. Ces cellules permettent le transport des éléments métalliques vers les sites d'excrétion, notamment le système rénal.

(2) des mécanismes de séquestration intracellulaire des métaux à l'aide de ligands liant fortement les éléments métalliques, comme les métallothionéines. Les métallothionéines constituent des protéines de faibles poids moléculaires riches en cystéines et présentant une forte affinité pour les éléments métalliques. Les complexes métallothionéines/métaux seront par la suite transférés dans les lysosomes où les éléments métalliques s'associeront avec la lipofuscine (polymère insoluble résultant de la péroxydation lipidique) alors que les acides aminés des métallothionéines seront recyclés.

(3) des mécanismes de séquestration intracellulaire des métaux impliquant la formation de concrétions minérales insolubles. Ces complexes pourront être éliminés des cellules *via* des processus d'exocytose. Ils seront éliminés de l'animal par les mêmes voies d'entrée (branchiale, digestive, voire tégumentaire) ainsi que par le système rénal.

(4) des mécanismes de séquestration intracellulaire impliquant des organelles. Les métaux peuvent ainsi être stockés dans les mitochondries sous forme de complexes insolubles (granules Ca^{2+}-phosphates incorporant également des métaux comme le Cu ou le Zn) et également dans le réticulum endoplasmique.

L'ensemble des processus de transport des éléments métalliques entre les différents compartiments cellulaires et de la cellule vers le milieu extérieur fait intervenir un ensemble de transporteurs protéiques dont le fonctionnement peut nécessiter une source d'énergie (ATPase) ou reposer sur des antiports d'éléments (Ahearn *et al.*, 2004).

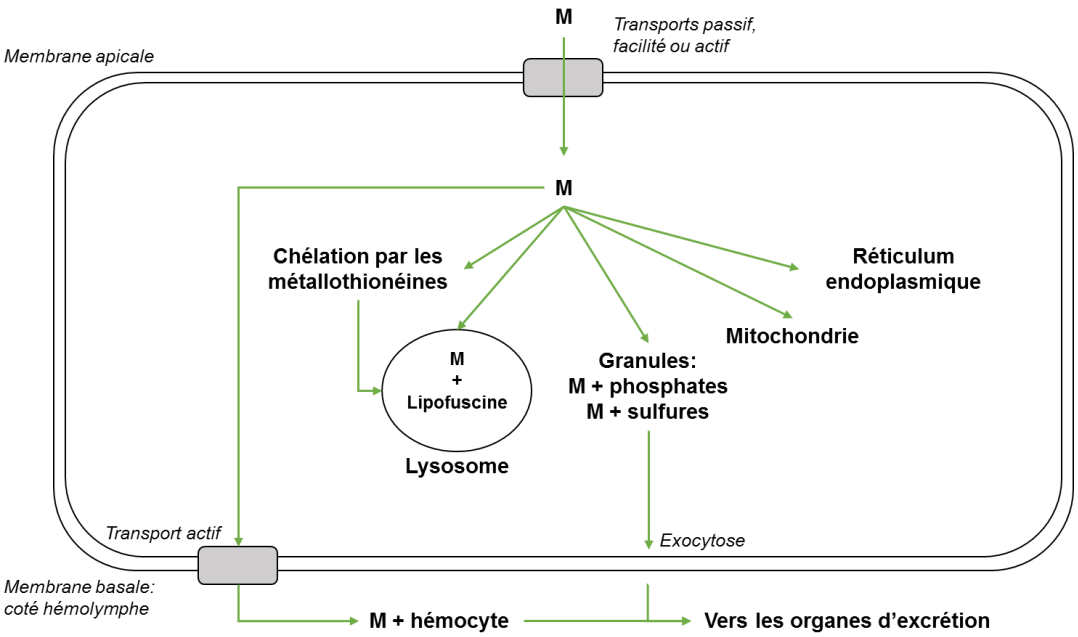

Figure I-B. 10 Principaux devenirs des éléments métalliques dans les cellules. Une fois la membrane plasmique franchie, les métaux peuvent être excrétés rapidement dans l'hémolymphe, chélatés par des protéines ayant une forte affinité pour ces éléments (comme les méthallothionéines), transportés dans divers organites (lysosome, mitochondrie ou réticulum endoplasmique) où ils seront stockés sous formes détoxiquées ou être compléxés à d'autres molécules (sulfures, phosphates) sous forme de granules qui seront exocytés. D'après Ahearn et *al.* (2004).

III.3. Effets de la contamination métallique sur les espèces bioturbatrices

La toxicité des éléments métalliques dépend de nombreux facteurs à la fois intrinsèques aux éléments métalliques eux-mêmes (spéciation, présence d'autres contaminants) mais également de caractéristiques propres aux organismes (stade de vie, état nutritionnel, etc.) (Förstner and Wittmann, 1981). Ces effets toxiques peuvent être observés à différents niveaux biologiques, i.e. génétique, biochimique, physiologique ou encore comportemental. Bien que relativement peu d'études écotoxicologiques fassent explicitement référence à des organismes bioturbateurs, de nombreuses études ont pourtant été menées sur ces organismes. En effet, plusieurs espèces bioturbatrices constituent de bonnes espèces sentinelles, utilisées dans le cadre de programmes de surveillance des niveaux des éléments métalliques en milieu marin, et une littérature extensive les concernant existe donc (cas du bivalve *Cerastoderma edule* ou du polychète *Arenicola marina*). Au vu de la diversité de la littérature, nous focaliserons ce paragraphe essentiellement sur l'effet du Cd sur les bioturbateurs marins puisque cet élément métallique a été utilisé au cours de ce travail de thèse comme contaminant métallique modèle lors d'expérimentations en laboratoire (**Chapitres IV** et **V**). Le Tableau I-B. 2 présente quelques effets du Cd sur diverses espèces bioturbatrices marines. Le Cd est considéré comme générant un important stress oxydant. En effet, cet ETM inhibe le fonctionnement des complexes II et III de la chaîne mitochondriale de transfert des électrons. De cette manière, le Cd stimule indirectement la production d'espèces réactives de l'oxygène (Reactive Oxygen Species, ROS) comme le péroxyde d'hydrogène H_2O_2 et l'anion superoxyde O^{2-}

(Wang *et al.*, 2004). La génération de ce stress oxydant a été mise en évidence de façon (1) directe (mesure des quantités de H_2O_2 dans les tissus par exemple, Lin *et al.*, 2017) chez les espèces bioturbatrices et (2) indirectement en évaluant les variations d'expression de gènes codant des enzymes intervenant dans la réponse au stress oxydant (SOD Mn, catalase, glutathione peroxydase, etc.) ou en quantifiant les concentrations de ces protéines dans les tissus (e.g., Sun and Zhou, 2008; Paul-Pont *et al.*, 2010a; Won *et al.*, 2013). En plus de ses effets oxydatifs directs, il a été mis en évidence que le Cd pouvait impacter une grande diversité de fonctions physiologiques comme le métabolisme énergétique (Paul-Pont *et al.*, 2010a; Zhang *et al.*, 2011) ou encore la capacité des organismes à réguler leur pression osmotique (Silvestre *et al.*, 2005; Thwala *et al.*, 2011). La contamination au Cd a également été associée à une altération des capacités de reproduction des organismes bioturbateurs. Cet ETM est considéré comme un perturbateur endocrinien (Rodríguez *et al.*, 2007) pouvant notamment interférer avec l'expression de gènes codant des lipoprotéines impliquées dans les processus reproductifs (Soetaert *et al.*, 2007; Zheng *et al.*, 2010; Hannas *et al.*, 2011). Chez les bioturbateurs, des retards de maturité sexuelle ou une altération de la structure des gonades ont par exemple été mis en évidence (Kogan *et al.*, 2000; Zheng *et al.*, 2010). Finalement, quelques modifications du comportement des organismes bioturbateurs ont également été notées, ceux-ci présentant notamment des difficultés à s'enfouir dans les sédiments (Olla *et al.*, 1988; Bat *et al.*, 1998; Bat and Raffaelli, 1998; Shin *et al.*, 2002). Toutefois, il convient de noter qu'en milieu marin les effets d'une contamination métallique (en particulier au Cd) sur les activités de bioturbation (remaniement sédimentaire et bioirrigation) de ces organismes n'ont été que rarement évalués. L'influence d'une contamination au Cu sur l'activité de remaniement sédimentaire du polychète *Hediste (Nereis) diversicolor* a par exemple été quantifiée par Fernandes *et al.* (2006). Ces derniers n'ont pas noté d'influence de la contamination au Cu sur le remaniement sédimentaire exercé par le polychète. A l'inverse, une littérature plus extensive existe sur les organismes d'eau douce. Ces études ont permis de mettre en évidence que l'influence des contaminants sur les activités de bioturbation des organismes dépend (1) du type de contaminant, (2) de la voie de contamination (trophique vs. directe) et (3) de l'organisme bioturbateur (Bartsch *et al.*, 1999; Ciutat *et al.*, 2005; Lagauzère *et al.*, 2009; Dedeh *et al.*, 2016; Blankson *et al.*, 2017).

Tableau I-B. 2 Exemples d'effets d'une contamination au cadmium sur quelques espèces bioturbatrices de la macrofaune benthique.

Bioturbateur	Contamination	Concentration testée	Temps d'exposition	Effets observés	Référence
GENETIQUE					
Perinereis aibuhitensis	Eau	0 à 48 mg mL^{-1}	14 jours	Induction de l'expression du gène *MP II* codant la métalloprotéine II	(Yang *et al.*, 2012)
Perinereis nuntia	Eau	50 µg L^{-1}	48 h	Induction de l'expression des gènes *SOD-Mn*, *SOD-Cu/Zn* et de plusieurs gènes *GST*	(Won *et al.*, 2013)
BIOCHIMIQUE					
Apostichopus (Stichopus) japonicus	Nourriture (pelotes de sargasses)	0 à 502 µg g^{-1} DW	30 jours	A 500 mg Cd kg^{-1}, ralentissement de la croissance, diminution des activités des protéines SOD, CAT et GPx. Augmentation des concentrations de MDA.	(Wang *et al.*, 2016)
Arenicola marina	Sédiments naturellement contaminés	< 0,1 à ca. 15 µg g^{-1} DW	7 jours	Augmentation de l'activité des protéines EROD et GST	(Ramos-Gómez *et al.*, 2011)
Cerastoderma edule	Eau	0 et 15,7 µg L^{-1}	7 jours	Stimulation de la production de MT	(Baudrimont and de Montaudouin, 2007)
Cerastoderma edule	Eau	0 et 15 µg L^{-1}	7 jours	Stimulation de la production de MT, réduction de la viabilité des hémocytes, stress oxydant, induction de l'expression génique de *COX I*, 12S et *SOD Mn*	(Paul-Pont *et al.*, 2010a)
Eriocheir sinensis	Eau	0,5 mg L^{-1}	7 jours	Altération des capacités d'osmorégulation, diminution de l'activité de la Na^+/K^+-ATPase et de la cytochrome C oxydase; altération de la structure branchiale (cellules vacuolisées, gonflement des mitochondries)	(Silvestre *et al.*, 2005)
Hediste (Nereis) diversicolor	Eau	0 à 3000 µg L^{-1}	9 jours	Augmentation de l'activité de la protéine SOD	(Sun and Zhou, 2008)
Perinereis aibuhitensis	Eau	0 à 34,4 mg L^{-1}	8 jours	Augmentation de l'activité des protéines SOD et GST. Réduction de l'activité de la protéine CAT	(Yuan *et al.*, 2010)
CELLULAIRE ET PHYSIOLOGIQUE					
Arenicola marina	Echantillons d'hémolymphe + solution de $CdCl_2$	10^{-8} à 10^{-3} M	?	Diminution de l'affinité de l'erythrocruorine pour le dioxygène et altération de l'effet Bohr à partir de C = 10^{-4} M	(Everaarts and Reichert, 1988)

Arenicola marina	Eau	0 à 10 mg L^{-1}	24 h à 30 jours	Limitation des capacités d'osmorégulation	(Rasmussen and Andersen, 2000)
Austruca (Uca) annulipes	Eau	29,2 à 57,1 mg L^{-1}	48 h	Diminution des taux de respiration	(Uma Devi and Prabhakara Rao, 1989)
Austruca (Uca) triangularis	Eau	0,028 à 92,9 mg L^{-1}	48 h	Diminution des taux de respiration	(Uma Devi and Prabhakara Rao, 1989)
Callichirus (Callianassa) kraussi	Eau	0 à 63 mg L^{-1}	96 h	Limitation des capacités d'osmorégulation	(Thwala *et al.*, 2011)
Corophium volutator	Sédiments	< 0,02 à 28,3 µg g^{-1} DW	jusqu'à 10 jours	Augmentation des taux de mortalité à partir de C = 9,2 µg g^{-1} DW	(Bat and Raffaelli, 1998)
Eriocheir sinensis	Eau	0,6 à 10,1 mg L^{-1}	6 jours	Dommages cellulaires: altération de la structure des mitochondries dans l'hépatopancréas, apoptose des cellules. Dommages à l'ADN, augmentation de l'activité des caspases 3, 8 et 9; augmentation de l'activité des protéines SOD, CAT et GPx, augmentation des concentrations en peroxyde d'hydrogène et de MDA	(Lin *et al.*, 2017)
Minuca (Uca) pugnax	Eau	0 à 2 mg L^{-1}	24 jours	Diminution des capacités de régénération après induction d'autotomies.	(Weis, 1978)
Perinereis nuntia	Eau	0 à 25 mg L^{-1}	7 jours	Organismes moribonds à partir de C = 5 mg L^{-1}, altérations de l'épiderme, forte mortalité à forte concentration (après 5 jours à 10 mg L^{-1}). Augmentation de la concentration en protéines analogues aux métallothionéines (MTLP)	(Won *et al.*, 2008)
Ruditapes philippinarum	Eau	0,05 à 2,9 µg L^{-1}	21 jours	Augmentation de la concentration en MT, altérations histo-pathologiques des branchies et de la glande digestive.	(Martín-Díaz *et al.*, 2005)
Ruditapes philippinarum	Eau	0 à 40 µg L^{-1}	96 h	Modifications du métabolome suggérant une altération des capacités de régulation osmotique et une perturbation du métabolisme énergétique	(Zhang *et al.*, 2011)
DEVELOPPEMENT ET REPRODUCTION					
Meretrix meretrix	Eau	1,1 à 10 167 µg L^{-1}	24 ou 48h	Inhibition du développement embryonnaire, diminution des capacités de nage. Diminution des taux de croissance, augmentation des taux de mortalité et diminution des taux de métamorphose.	(Wang *et al.*, 2009)

Neohelice (Chasmagnathus) granulata	Eau	0 à 0,5 mg L^{-1}	15 jours après la mue	Diminution des capacités à muer. Hypertrophie des ovaires chez les femelles contaminées ayant mué, fragmentation des noyaux des ovocytes.	(Kogan *et al.*, 2000)
Neohelice (Chasmagnathus) granulata	Eau	0 à 15 mg L^{-1}		Temps d'incubation des œufs significativement réduit. Larves avec anomalies morphologiques: hydropisie au niveau de la région céphalique, atrophie des péréiopodes et du pléon.	(Rodríguez and Medesani, 1994)
Perinereis nuntia	Eau	< 0,001 à 564 µg L^{-1}	50 jours	Retard de maturation sexuelle, diminution des taux de fertilisation des œufs et d'éclosion. Stimulation de l'expression du gène *vtg*.	(Zheng *et al.*, 2010)
Ruditapes philippinarum	Eau	0 à 40 µg L^{-1}	35 jours	Diminution des taux de clairance, de respiration et du ratio O/N ainsi que des "perspectives de croissance" (équilibre entre l'énergie acquise et celle dépensée)	(Zhao *et al.*, 2014)
COMPORTEMENT					
Arenicola marina	Sédiments	< 0,02 à 76 µg g^{-1} DW	jusqu'à 10 jours	Mortalité des organismes à partir de C = 25 µg g^{-1} DW. Emergence des polychètes à l'interface eau-sédiment et difficultés à s'enfuir de nouveau à partir de C = 48 µg g^{-1} DW	(Bat and Raffaelli, 1998)
Corophium volutator	Sédiments	2 à 102 µg g^{-1} DW	96 h	Organismes s'enfouissant préférentiellement dans un sédiment non-contaminé que contaminé.	(Bat *et al.*, 1998)
Nephtys caeca	Sédiments	< 0,2 à 38 µg g^{-1} DW	7-28 jours	Diminution des capacités à s'enfouir dans le sédiment	(Olla *et al.*, 1988)
Ruditapes philippinarum	Sédiments	0 à 7,2 µg g^{-1} DW	48 h	Diminution des capacités à s'enfouir dans le sédiment	(Shin *et al.*, 2002)

DW : poids sec (dry weight); 12S : gène 12S ; CAT : catalase ; COX I : sous-unité I de la cytochrome oxydase ; GST : glutathione S-transférase ; GPx : glutathione peroxydase ; EROD : ethoxyresorufine-O-dééthylase ; MDA : malondialdehyde ; MT : métallothionéine ; SOD Mn: superoxyde dismutase Mn ; SOD Cu/Zn: superoxyde dismutase Cu/Zn; *vtg*: gène de la vitellogénine.

BILAN DU CHAPITRE I

Le **Chapitre I** rappelle à quel point la bioturbation constitue un processus primordial dans le fonctionnement des écosystèmes benthiques du fait de l'influence significative des organismes bioturbateurs sur la structure physique et biogéochimique des environnements sédimentaires, et indirectement sur les communautés benthiques. L'état physiologique des espèces bioturbatrices conditionnent leur influence en tant qu'ingénieurs de l'écosystème et plusieurs facteurs abiotiques (température, disponibilité en O_2, etc.) ainsi que biotiques (interactions inter- et intraspécifiques) peuvent moduler les activités de ces organismes. Parmi les facteurs biotiques, le **sous-chapitre introductif I-A** a permis de mettre en avant le rôle potentiellement crucial des parasites. Malgré ce constat, un nombre particulièrement restreint d'études ont tenté d'évaluer l'influence des parasites sur les activités de bioturbation de ces espèces ingénieures.

Outre le parasitisme, les espèces bioturbatrices sont naturellement soumises à toute une variété de stress, qui agissent de concert sur ces organismes. Par exemple, et comme mis en exergue dans le **sous-chapitre I-B**, les activités anthropiques peuvent être à l'origine d'importants relargages de contaminants (notamment métalliques) en milieu côtier, impactant les organismes peuplant ces milieux. En ce qui concerne les espèces bioturbatrices, celles-ci peuvent moduler le cycle de ces éléments métalliques en milieu aquatique mais ces derniers peuvent également modifier les activités de ces espèces. Les effets des contaminants métalliques sur les organismes bioturbateurs ont d'ores et déjà été largement étudiés. Néanmoins, les études s'intéressant aux effets des contaminants sur les activités de bioturbation de ces espèces sont bien moins nombreuses que celles évaluant l'impact des contaminants aux échelles génétique ou cellulaire.

Bien que la multiplicité des facteurs de stress constitue une réalité environnementale, les études couplant parasitisme et contaminants métalliques chez les espèces bioturbatrices restent rares, et à notre connaissance, peu se sont intéressées à l'influence combinée de ces paramètres sur les espèces bioturbatrices (mais voir les travaux de I. Paul-Pont (2010)) et aucune n'a été menée en se focalisant sur les effets de ces facteurs de stress sur les processus de bioturbation eux-mêmes. Ce travail de thèse propose ainsi de coupler études descriptives (*in situ*) et expérimentales de manière à caractériser et évaluer l'influence du parasitisme et de la contamination métallique, seuls et en interaction, sur une espèce bioturbatrice modèle (*Upogebia* cf. *pusilla*).

CHAPITRE II

Modèle d'étude et bases méthodologiques

I. Sites d'étude et modèle biologique

I.1. Zone d'étude : le bassin d'Arcachon

Situé sur la côte sud Atlantique du littoral français, le bassin d'Arcachon (44° 40'N, 1° 10'W) est une lagune macrotidale semi-fermée de 180 km^2 (Figure II. 1). Le rythme tidal est de type semi-diurne avec une amplitude de marée comprise entre 0,8 et 4,6 m. Cette lagune s'ouvre sur l'océan Atlantique par deux chenaux principaux (ou « passes ») par lesquels transitent, à chaque marée, environ 150 à 400.10^6 m^3 d'eau (marée de mortes-eaux et de vives-eaux, respectivement) (Manaud *et al.*, 1997). Le bassin d'Arcachon est également alimenté en eau douce par le delta de la Leyre situé dans sa partie Est, et dans une moindre mesure par le canal du Porge situé au Nord ; ces apports représentent environ 1040.10^6 m^3 d'eau douce par an (Manaud *et al.*, 1997). Cette double influence continentale et océanique permet de distinguer trois masses d'eau dans la lagune, à savoir les eaux néritiques externes, moyennes et internes ; ces masses d'eau différant par leur salinité et leur température moyennes (Bouchet, 1968). Le domaine intertidal s'étend sur 110 km^2 ; le domaine subtidal est quant à lui composé de chenaux dont la profondeur maximale est d'environ 20 m. Environ 39 % de la zone intertidale est couverte par un herbier à zostère naine *Zostera noltei* (Plus *et al.*, 2010). Cet herbier joue un rôle prépondérant dans le fonctionnement de l'écosystème benthique, les herbiers constituant notamment d'importants lieux de production primaire (Mateo *et al.*, 2006). Ils limitent par ailleurs les contraintes hydrodynamiques (e.g., Fonseca and Cahalan, 1992), interviennent dans le cycle des nutriments (e.g., Deborde *et al.*, 2008) et sont le siège d'une importante biodiversité (Blanchet *et al.*, 2004).

Les crevettes de vase étudiées au cours de ce travail ont été récoltées sur plusieurs sites intertidaux du bassin d'Arcachon (Figure II. 1, Tableau II. 1). Il s'agit de sites majoritairement sablo-vaseux (à l'exception de « Passant »), où la densité moyenne de *U.* cf. *pusilla* varie de 0,2 ± 0,1 à 6,8 ± 0,3 ind. m^{-2} (Pascal, 2017). Au niveau de chacun de ces sites, il y a présence de zostères naines, bien que la densité de l'herbier varie d'un site à l'autre (Plus *et al.*, 2010; Pascal, 2017). L'échantillonnage des crevettes de vase été effectué à l'aide d'une pompe à vers (« yabby pump ») qui aspire l'eau du terrier, pouvant entrainer avec elle l'individu habitant le terrier ciblé.

Dix sites ont été échantillonnés au cours de l'été 2014 dans le cadre de la thèse de L. Pascal (2017). Les échantillons récoltés à cette occasion nous ont permis de caractériser la pression parasitaire chez *U.* cf. *pusilla* (**Chapitre III-B**). Au cours de cette précédente thèse, un suivi mensuel de deux ans a également été réalisé sur un des dix sites précédemment évoqués. Ce suivi initié en janvier 2013 s'est poursuivi sur les premiers mois de la présente étude. Au cours de ce travail, nous avons également réalisé un suivi saisonnier de 1 an sur deux des dix sites évoqués ci-dessus. Ces deux sites ont été choisis sur la base d'une différence d'abondance parasitaire (voir **Chapitre III-A**). Enfin, dans le cadre d'expérimentations en laboratoire (**Chapitres IV** et **V**), des échantillonnages ponctuels de *U.* cf. *pusilla* ont été réalisés au niveau du site « *Le Cla* » où les parasites sont abondants (**Chapitre III-A**).

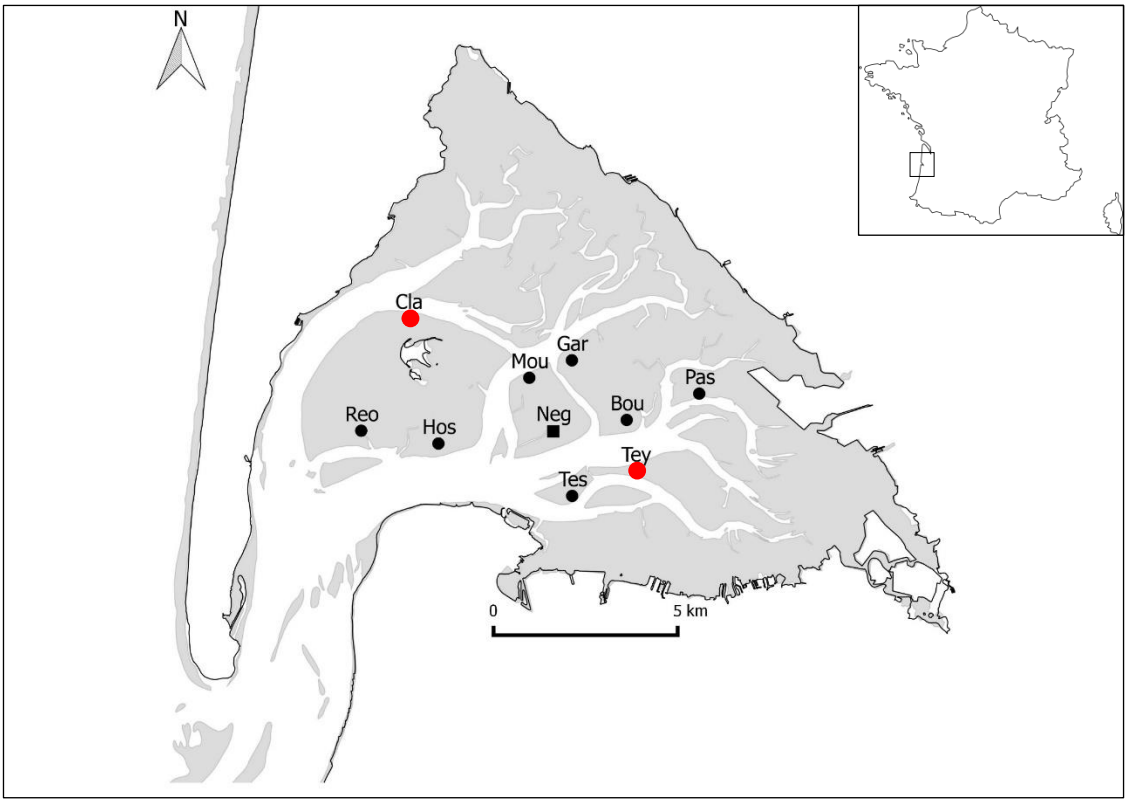

Figure II. 1 Localisation du bassin d'Arcachon sur la côte Atlantique sud française (insert) et situation des différents sites de prélèvement. ● et ●: stations échantillonnées en juin 2015 dans le cadre de la thèse de L. Pascal (2017). Les échantillons ont été utilisés dans le cadre du **Chapitre III-A**. ■ :site pour lequel un suivi mensuel de 2 ans a été initié en janvier 2014 dans le cadre de la thèse de L. Pascal. Les échantillons ont été utilisés dans le cadre du **Chapitre III-A**. ●: sites de prélèvement du suivi saisonnier (**Chapitre III-B**). Les crevettes de vase utilisées dans le cadre des manipulations expérimentales (**Chapitres IV** et **V**) provenaient du site « Le Cla » (Cla). En gris, la zone intertidale. Bou : Bourrut, Cla : Le Cla, Gar : Garrèche, Hos : Les Hosses, Mou : Les Moussettes, Neg : La Nègue, Pas : Passant, Reo : La Réousse, Tes : Le Tes, Tey : Le Teychan.

Tableau II. 1 Densités (moyennes et maximales) de *Upogebia* cf. *pusilla* et principales caractéristiques physico-chimiques des sites échantillonnés dans le bassin d'Arcachon. Le contenu en carbone inorganique des sédiments a été déterminé sur des colonnes sédimentaires de 20 cm (surface à 20 cm de profondeur). La porosité et la perméabilité ont été déterminées sur ces mêmes profondeurs. D'après Pascal (2017).

Site	Densité moyenne (± SE ; ind m^{-2})	Densité maximale (ind m^{-2})	Granulométrie (D50 en µm)	Carbone organique (CO ± SE ; %)	Porosité Φ	Perméabilité k (m^2)
Bourut	2,4 ± 0,0	15	163,1	0,67 ± 0,11	0,54 ± 0,06	$6{,}9.10^{-14}$
Garrèche	1,6 ± 0,0	9	154,5	0,77 ± 0,18	0,55 ± 0,09	$7{,}0.10^{-14}$
La Nègue	4,9 ± 0,4	23	133,7	0,53 ± 0,11	0,46 ± 0,04	$8{,}5.10^{-12}$
La Réousse	6,8 ± 0,3	39	109,5	0,93 ± 0,17	0,56 ± 0,09	$1{,}5.\ 10^{-14}$
Le Cla	5,7 ± 0,1	34	85,2	0,86 ± 0,11	0,55 ± 0,05	$2{,}9.10^{-14}$
Le Tes	2,6 ± 0,8	22	131,5	0,65 ± 0,11	0,50 ± 0,05	$1{,}3.10^{-13}$
Le Teychan	4,1 ± 1,0	19	191,9	0,48 ± 0,10	0,50 ± 0,06	$3{,}0.10^{-14}$
Les Hosses	2,9 ± 0,3	17	173,5	0,25 ± 0,03	0,40 ± 0,03	$3{,}5.10^{-13}$
Les Moussettes	2,5 ± 0,3	14	130,4	0,44 ± 0,09	0,49 ± 0,04	$6{,}5.10^{-14}$
Passant	0,2 ± 0,1	1	31,3	0,59 ± 0,05	0,54 ± 0,01	$8{,}7.10^{-14}$

I.2. Modèle d'étude : les thalassinidés et l'espèce *Upogebia pusilla*[1]

I.2.1. Généralités

Les thalassinidés sont des crustacés décapodes présentant une morphologie de type crevette et sont ainsi désignés sous le terme « crevette de vase » ou « mud shrimp » dans la littérature anglo-saxonne (on parle également de « sandprawn » ou de « ghost shrimp ») (Pillay and Branch, 2011) (Figure II. 2). Ce sont des organismes endobenthiques créant, pour la plupart, des terriers dans les sédiments meubles marins intertidaux et subtidaux. On les retrouve essentiellement au niveau des zones côtières à des profondeurs allant de 0 à 200 m, bien que quelques espèces aient été identifiées à plus de 2000 m de profondeur (Dworschak, 2000). Ces organismes sont retrouvés aussi bien au niveau des zones tropicales que tempérées, la richesse spécifique allant croissant des hautes aux basses latitudes (Dworschak, 2000). Cependant, ces crevettes de vase ne colonisent pas les régions polaires. Le groupe des thalassinidés est actuellement considéré comme paraphylétique et deux infra-ordres ont été proposés en remplacement : les Axiidea et les Gebiidea (Robles *et al.*, 2009). Néanmoins, considérant que les membres de ces deux groupes présentent des traits fonctionnels très semblables, le terme « thalassinidé » reste admis tout en reconnaissant qu'il n'a pas de valeur taxinomique (Pillay and Branch, 2011). Actuellement, les thalassinidés regroupent 556 espèces réparties en 11 familles (Dworschak, 2005).

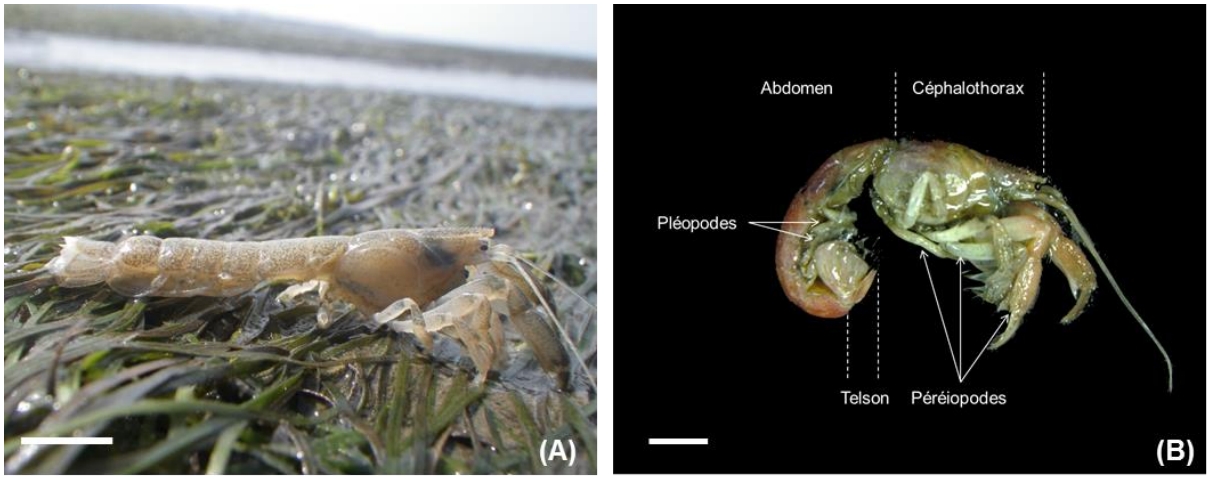

Figure II. 2 Exemple de thalassinidés: l'espèce *Upogebia* cf. *pusilla*. (A) *U.* cf. *pusilla* sur du sédiment colonisé par de l'herbier à zostère naine *Zostera noltei* (crédit: X. de Montaudouin) et (B) morphologie générale. Pour (A) et (B), échelle = 1 cm.

Cadée (2001) considère que les thalassinidés sont parmi les plus grand bioturbateurs des écosystèmes marins côtiers. Comme précédemment défini, la bioturbation constitue l'ensemble des processus affectant la matrice sédimentaire. Les thalassinidés sont à l'origine d'un important remaniement sédimentaire en lien avec la création et la maintenance de leur terrier (Branch and Pringle, 1987;

[1] **Au cours de ce chapitre nous utiliserons à la fois les termes « *Upogebia pusilla* » et « *Upogebia* cf. *pusilla* ». Le premier terme sera utilisé lorsque nous nous réfèrerons aux travaux ayant été menés hors du bassin d'Arcachon ; à l'inverse le second sera utilisé pour les organismes prélevés dans cet écosystème. En effet, de récentes analyses morphologiques et génétiques (B. Gouilleux et A. Dairain) montrent qu'il existe, à ce jour, une légère incertitude sur l'identification de l'espèce du bassin d'Arcachon.**

Berkenbusch and Rowden, 1999; Pillay and Branch, 2011). En effet, ces crustacés excavent des particules sédimentaires à la surface eau-sédiment lors de leurs activités de fouissage mais jouent également sur la répartition des particules dans la colonne sédimentaire (sédiments compactés le long des parois du terrier, stockés dans certaines galeries, etc.). Le sédiment éjecté en surface, forme de petits monticules modifiant la topographie de l'interface eau-sédiment et potentiellement le régime des courants à ce niveau (Ziebis *et al.*, 1996). En plus de leur influence sur la structure physique des sédiments, les thalassinidés jouent un rôle clé sur leur biogéochimie (D'Andrea and DeWitt, 2009; Pillay and Branch, 2011; Sasaki *et al.*, 2014; Pascal *et al.*, 2016a). Ces organismes fouisseurs vivent en effet sous la zone de pénétration de l'oxygène. Ils ventilent ainsi fréquemment leur terrier, permettant le maintien d'une concentration en oxygène nécessaire à leur respiration. La ventilation du terrier permet en outre l'élimination des déchets métaboliques. Cette ventilation entraine une bioirrigation du sédiment, stimulant les échanges de solutés (nutriments et oxygène) entre les eaux interstitielles et l'eau surnageante *via* des transports diffusifs et advectifs (Volkenborn *et al.*, 2012a; Aller, 2014). Finalement, les activités de bioturbation des thalassinidés influant sur la structure physique des sédiments ainsi que sur leur caractéristiques biogéochimiques, ces organismes impactent *in fine* la structure des communautés de micro-, meio- et macro-organismes (voir la revue de Pillay and Branch, 2011). Les thalassinidés sont ainsi considérés comme des espèces ingénieures de l'écosystème (sensu Jones *et al.*, 1994).

I.2.2. Ecologie de *Upogebia pusilla*

a) Répartition et milieu de vie

La crevette de vase *Upogebia pusilla* (anciennement *Upogebia littoralis*) appartient à l'infra-ordre des Gebiidae (Figure II. 2). La distribution géographique de cette espèce s'étend des côtes de la Mauritanie aux côtes bretonnes (de Saint Laurent and Le Loeuff, 1979). Elle a été retrouvée occasionnellement jusqu'en Norvège. Cette espèce est également commune sur le pourtour méditerranéen jusqu'en Mer Noire et en Mer Rouge (Dworschak, 1987b). En Méditerranée, *U. pusilla* occupe les zones intertidales et subtidales côtières à des densités maximales de 80 à 230 ind m^{-2} (Dworschak, 1987b). A l'inverse, *U. pusilla* n'a été retrouvée qu'en zone intertidale sur la côte Atlantique française (RESOMAR 2017). Une campagne d'échantillonnage menée dans le bassin d'Arcachon (voir ci-dessus) a montré des densités maximales de crevettes de vase plus faibles que celles rapportées en Méditerranée allant de 1 à 39 ind m^{-2} (Tableau II. 1) (Pascal, 2017). En Méditerranée comme sur la côte Atlantique, *U. pusilla* colonise les sédiments sablo-vaseux (Dworschak, 1987b; Pascal, 2017), où elle construit des terriers en forme de « U ». A cette forme générale en « U » peuvent s'ajouter différentes branches secondaires, donnant parfois l'apparence d'un « Y » au terrier. Le diamètre des branches du terrier est corrélé à la largeur de la carapace de l'organisme y habitant (Dworschak, 1983). Les terriers de *U. pusilla* peuvent s'étendre verticalement jusqu'à des profondeurs de 49 cm (Pascal, 2017). Chaque terrier n'est occupé que par un

seul individu, sauf lors de la période de reproduction, au cours de laquelle deux individus peuvent occuper le même terrier (observations personnelles).

b) Alimentation

Le crustacé *U. pusilla* est considéré comme un organisme suspensivore. La crevette initie un courant d'eau unidirectionnel dans son terrier (activité de ventilation du terrier) grâce aux battements intermittents de ces pléopodes abdominaux (Figure II. 2). La mise en place de ce courant d'eau est facilitée par l'architecture en « U » du terrier. Les particules organiques apportées par ce courant d'eau sont filtrées à l'aide de longues soies présentes sur les deux premières paires de péréiopodes des organismes. Les soies des péréiopodes s'entrelacent et forment un panier permettant de récupérer les particules en suspension. A l'aide de sa troisième paire de maxillipèdes, la crevette balaie régulièrement ce panier et transfère alors les particules alimentaires vers sa bouche (Dworschak, 1987a). En plus de ces activités de filtration, *U. pusilla* peut se nourrir directement sur les sédiments des parois de son terrier et/ou sur les sédiments de surface. Ce régime déposivore est notamment observé lors des périodes de construction du terrier (Dworschak, 1987a). Les activités de ventilation générant un flux d'eau dans le terrier permettent par ailleurs le renouvellement de la colonne d'eau et donc l'apport d'oxygène pour la respiration ainsi que l'élimination des métabolites (Dworschak, 1981).

c) Croissance et reproduction

La crevette de vase *U. pusilla* est une espèce gonochorique dont la durée de vie est estimée à 3–5 ans (Dworschak, 1988; Kevrekidis *et al.*, 1997). Il existe un profond dimorphisme sexuel entre mâles et les femelles, ces dernières atteignant en général des tailles moins importantes que les mâles et présentant notamment des chelipèdes également moins larges (Dworschak, 1988). Ainsi, les organismes peuvent mesurer jusqu'à 60 et 66 mm pour les femelles et les mâles, respectivement (longueur totale TL : de l'extrémité du rostre à la partie postérieure du telson) (Dworschak, 1988). Néanmoins, dans le bassin d'Arcachon, les tailles maximales observées sont de 54 mm pour les deux sexes. Ces tailles maximales sont atteintes en moins d'un an (Pascal *et al.*, 2016b). A ce jour, peu d'informations sont disponibles concernant la reproduction de *U. pusilla*, i.e. de la copulation au recrutement. Néanmoins, il semble que la maturité sexuelle soit atteinte au cours de la première année avec les plus petites femelles ovigères mesurant 36 mm, les mâles étant considérés comme sexuellement matures dès 15 mm TL (Dworschak, 1988; Pascal *et al.*, 2016b). Dans le bassin d'Arcachon, les femelles sont ovigères de mai à septembre. Cette espèce présente une fécondité relativement élevée, avec des femelles pouvant porter jusqu'à plus de 10 000 œufs (Dworschak, 1988). Après une période d'incubation de huit semaines, les larves sont libérées dans la colonne d'eau. Il semble que la période larvaire comprenne au moins quatre phases, suivie d'une phase post-larvaire à partir de laquelle l'animal commencerait à s'enfouir dans le sédiment. La durée de ces phases reste inconnue (Dworschak, 1988).

d) Comportement

Les différents comportements de *U.* cf. *pusilla* ont été caractérisés au cours de la thèse de L. Pascal (2017). Ce dernier a mis en évidence quatre comportements principaux:

(1) locomotion : déplacement de la crevette dans le terrier, sans transport de sédiments.

(2) fouissage : la crevette creuse et déplace les particules sédimentaires.

(3) ventilation : le battement des pléopodes de la crevette permet la mise en place d'un courant unidirectionnel d'eau dans le terrier. Ce comportement peut entrainer le transport de particules de sédiment en suspension.

(4) repos : la crevette ne se déplace pas et ne présente aucune activité visible.

Les comportements de fouissage et, dans une moindre mesure, de ventilation sont associés au processus de remaniement sédimentaire, tandis que le comportement de ventilation est à l'origine des processus de bioirrigation.

Il est à noter que la proportion de temps alloué à chacun de ces comportements varie selon la saison. Les organismes sont en effet beaucoup plus actifs en été passant environ 80 % de leur temps à ventiler leur terrier et à fouir. A l'inverse, en hiver les organismes passent plus de 50 % de leur temps inactifs. En se basant sur ces observations, nous avons ainsi choisi de réaliser nos expérimentations en laboratoire au cours de l'été (**Chapitres IV** et **V**).

I.3. Le parasitisme chez *Upogebia pusilla*

Au moins quatre espèces de macroparasites différentes sont connues pour infester *U. pusilla*. Il s'agit des isopodes Bopyridae *Gyge branchialis* et *Progebiophilus euxinicus* à l'état adulte (Bourdon, 1968), d'un trématode présent sous la forme de métacercaires (Dworschak, 1988) et d'un acanthocéphale présent sous la forme d'acanthelles (Dworschak, 1988). Au cours de cette étude, nous nous sommes focalisés sur le Bopyridae *Gyge branchialis* et sur le parasite trématode.

I.3.1. Le Bopyridae *Gyge branchialis*

a) Généralités

Le Bopyridae *G. branchialis* (Crustacea : Isopoda) est un ectoparasite de *U. pusilla* infestant la cavité branchiale de son hôte et provoquant la déformation de celle-ci (Figure II. 3A). Le parasite infeste indifféremment les branchies droite et gauche. Les infestations simultanées des deux branchies sont quant à elles très rares (Pascal *et al.*, 2016b). La plupart du temps, c'est en réalité un couple de bopyres qui infeste le crustacé (Bourdon, 1968) (Figure II. 3B). Ce couple est formé d'une très large femelle, dont la morphologie très modifiée la rend difficilement reconnaissable comme appartenant au groupe des Isopoda (Figure II. 3C). Cela est notamment dû au développement très important de la cavité incubatrice (Bonnier, 1900). La surface dorsale de la femelle est placée sur les lamelles branchiales de

son hôte; la femelle s'y accroche à l'aide de petites griffes à l'extrémité de ses péréiopodes (Bonnier, 1900). L'extrémité antérieure du parasite est toujours dirigée vers l'extrémité postérieure de son hôte (Tucker, 1930). La partie buccale de la femelle est modifiée de manière à pouvoir se nourrir des fluides internes de son hôte après perçage de sa cuticule. En ce qui concerne le mâle, il est nain et est accroché aux pléopodes de la femelle bopyre, à proximité des ouvertures génitales de cette dernière (Bonnier, 1900) (Figure II. 3B et Figure II. 3D). Bonnier (1900) suggère que, tout comme la femelle bopyre, le mâle perfore la cuticule de son hôte, y enfonçant son rostre par lequel il y aspire les fluides internes.

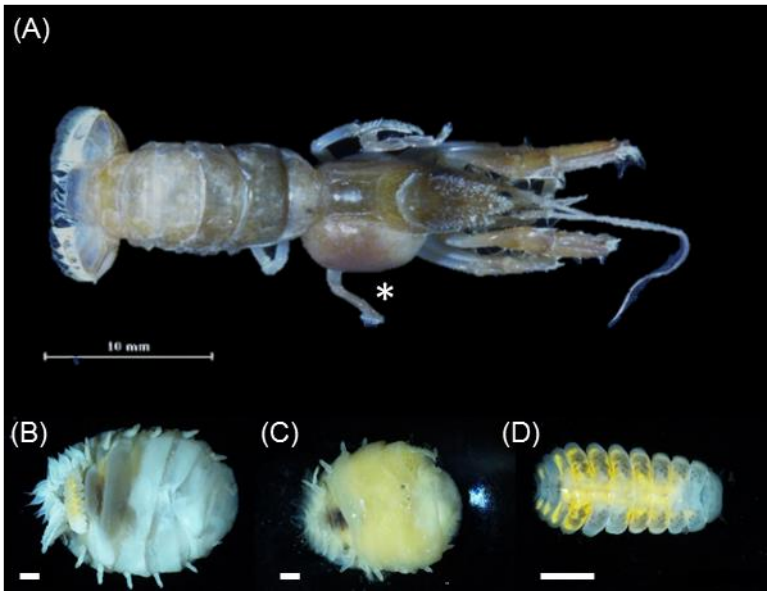

Figure II. 3 Le système hôte-parasite [*Upogebia* cf. *pusilla* + *Gyge branchialis*]. (A) *U.* cf. *pusilla* montrant un gonflement de la cavité branchiale droite (*) indiquant la présence du parasite bopyre ; (B) Couple de bopyres *G. branchialis* : la femelle est géante par rapport au mâle qui est positionné sur l'extrémité postérieure de la femelle, vue ventrale ; (C) Femelle *G. branchialis* ovigère, vue ventrale. (D) Male *G. branchialis*, vue dorsale. Pour (B), (C) et (D), échelle = 100 µm.

b) Cycle de vie

Le bopyre *G. branchialis* présente un cycle de vie complexe, i.e. qui implique différents hôtes (Figure II. 4). Le crustacé *U. pusilla* constitue l'hôte définitif, i.e. celui au sein duquel le parasite se reproduit de manière sexuée. Les femelles sont ovigères toute l'année dans le bassin d'Arcachon (Pascal *et al.*, 2016b). Les détails du cycle de vie de *G. branchialis* sont à ce jour inconnu. Néanmoins, les stades larvaires sont très semblables entre les différents genres d'isopodes et il est supposé que les Bopyridae arborent le cycle de vie typique suivant : des œufs produits par les bopyres adultes éclosent des larves épicaridium, qui quittent le marsupium de la femelle. Un fois dans la colonne d'eau, ces larves se fixent sur un copépode Calanoides et subissent une mue donnant une larve microniscus. La larve microniscus est suivie par une larve cryptoniscus adaptée à la vie pélagique. Celle-ci quitte son hôte copépode dans l'espoir de parasiter son hôte final décapode (Bonnier, 1900; Tucker, 1930; Williams and Boyko, 2012). A ce stade, il semble que les parasites soient mâles, mais les organes sexuels ne sont pas fonctionnels (Bonnier, 1900). La première larve cryptoniscus à infester la cavité branchiale d'une crevette de vase

évolue en un adulte femelle, la seconde larve microniscus deviendra un mâle fonctionnel. En ce qui concerne *U. pusilla*, l'infestation par le bopyre *G. branchialis* se fait très tôt au cours de la vie de la crevette de vase, si bien que le parasite femelle grandit en même temps que son hôte, occupant ainsi toute la cavité branchiale de ce dernier (Tucker, 1930; Pascal *et al.*, 2016b).

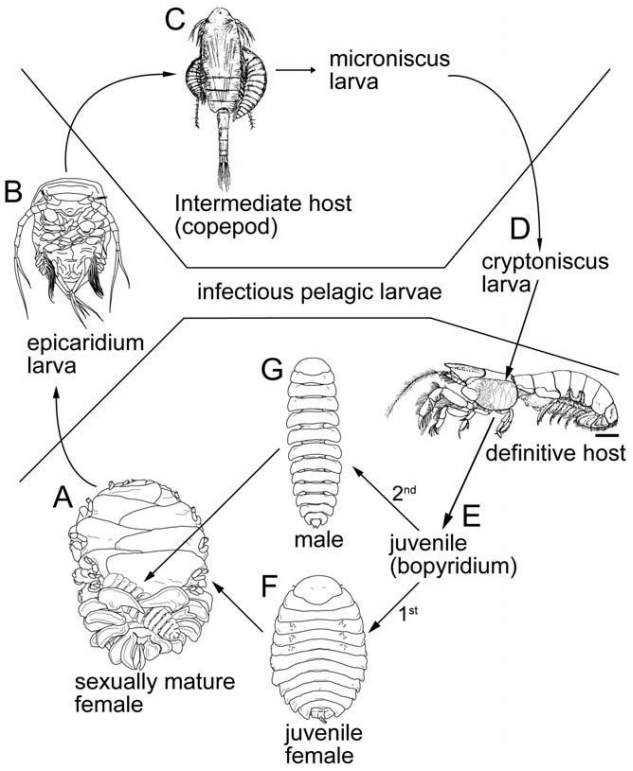

Figure II. 4 Cycle de vie typique des parasites Bopyridae. Un couple d'adultes infeste la cavité branchiale d'un crustacé. Ils forment des œufs portés par la femelle mature (A), qui après éclosion donneront des larves épicarides (B). Une fois dans la colonne d'eau, ces larves infestent un copépode Calanoides (C). Elles subissent une mue formant des larves microniscus. Une seconde mue permet la transformation en larves cryptoniscus (D), adaptées à la vie pélagique. Ces larves infestent alors l'hôte définitif et se développent en bopyres adultes (E). La première larve à infester l'hôte définitif donne une femelle bopyre (F), la seconde un mâle (G). D'après Williams and Boyko, 2012.

c) Impacts sur l'hôte

Par définition, les parasites affectent négativement leur hôte que ce soit au niveau physiologique (destruction de certains tissus, modification de l'équilibre énergétique, etc.) qu'au niveau comportemental (réduction des niveaux d'activités, comportements aberrants, etc.) (e.g., Hughes, 1940; Lauckner, 1980; Thomas *et al.*, 2002; Mouritsen, 2002b; Stier *et al.*, 2015).

(i) Reproduction

En ce qui concerne le bopyre *G. branchialis*, celui-ci entraine une castration des femelles *U. pusilla* et celles-ci n'ont jamais été observées ovigères lorsqu'infestées par ce parasite (Pascal *et al.*, 2016b). Les mâles parasités sont quant à eux féminisés : la plupart présente une paire de pléopodes sur le premier segment abdominal, qui est normalement l'apanage des femelles. Par ailleurs, les caractères sexuels

secondaires sont altérés : par exemple, les chélipèdes normalement très larges chez les mâles, présentent une morphologie similaire à celle observée chez les femelles (Bonnier, 1900; Tucker, 1930; Hughes, 1940). L'influence du parasite sur les capacités reproductrices des mâles n'est, à ce jour, pas tranchée. En effet, Tucker (1930) a noté une grande variabilité entre différents mâles parasités. Certains présentaient des gonades à peines réduites, la spermatogenèse n'étant pas affectée, alors que chez d'autres individus aucune trace des gonades n'était même détectable. Il a été suggéré que l'influence négative du parasite sur les capacités reproductives de son hôte (au moins en ce qui concerne la femelle) pourrait être due à un déséquilibre énergétique. En effet, le parasite se nourrissant sur les fluides internes de *U. pusilla*, il est possible qu'il réduise la quantité d'énergie disponible nécessaire à l'ovogenèse (et éventuellement la spermatogenèse) (Hughes, 1940; Williams and Boyko, 2012). Concernant les mâles, la féminisation pourrait être due au parasite interférant avec le fonctionnement de la glande androgénique (Reinhard, 1956; Nanri *et al.*, 2011). Cette glande produit en effet une hormone responsable de la différenciation sexuelle et de la mise en place des caractères sexuels secondaires mâles chez les crustacés.

(ii) Croissance

La croissance chez les crustacés est discontinue, s'effectuant par mues successives. Le bopyre *G. branchialis* n'empêche pas la mue de son hôte (Tucker, 1930). En revanche, les organismes parasités présentent une croissance réduite, atteignant des tailles moins importantes que leurs congénères non parasités (Pascal *et al.*, 2016b). Il peut ainsi être envisagé que la fréquence des mues soit réduite chez les individus parasités (Tucker, 1930). Par ailleurs, à taille égale, les individus parasités sont plus légers que les individus non parasités (Pascal *et al.*, 2016b), probablement en lien avec le parasite constituant un fardeau énergétique pour son hôte.

(iii) Comportement

Il a été suggéré que les parasites puissent induire des modifications indirectes du comportement de leurs hôtes, en lien avec l'influence négative sur l'état physiologique général des crustacés (Williams and Boyko, 2012). De telles modifications de comportement n'ont pas été observées par Tucker (1930) qui note que « the influence of the parasite on the general vitality of the host seems to be negligible. There is no noticeable effect on the activity of the animals or on their viability in aquaria ». A l'inverse, la thèse de L. Pascal (2017) a mis en évidence que les individus parasités étaient bien moins actifs que les individus sains. Par exemple, les organismes parasités passent en moyenne 3 fois plus de temps à se reposer que les individus sains en été. Ces modifications de comportement sont en lien avec une altération de l'intensité de la bioturbation générée par *U.* cf. *pusilla* puisque les organismes parasités montrent des taux de remaniement sédimentaire et de bioirrigation 4,5 et 2,9 fois plus faibles, respectivement, que ceux enregistrés pour les individus non parasités à cette même période.

I.3.2. Le trématode digène

Dans son travail de 1988, Dworschak fait état de la présence de métacercaires de trématodes chez *U. pusilla* en mer Adriatique. Il note qu'il s'agit d'endoparasites présents au niveau de l'hépatopancréas et de l'abdomen de *U. pusilla*. Le parasite semble prévalent (20 à 80 % des femelles parasitées), mais l'intensité des infections apparait relativement faible. A notre connaissance, il n'existe pas d'autres informations disponibles sur ce trématode, *a priori*, digène. Les trématodes digènes possèdent un cycle de vie complexe impliquant généralement trois hôtes (bien qu'un à quatre hôtes soient également possible) (Figure II. 5). Ce cycle de vie typique implique un vertébré comme hôte définitif où le parasite se reproduit de manière sexuée (exceptionnellement un invertébré peut servir d'hôte définitif dans le cas de cycles raccourcis). Les œufs formés se retrouvent dans le milieu naturel par le biais des fèces. Ils forment alors des larves miracidiums, nageuses, qui infestent un premier hôte intermédiaire (mollusque ou polychète). Ces larves se transforment d'abord en sporocystes, et éventuellement ultérieurement en rédies. Sporocystes et rédies permettent une intense multiplication asexuée du parasite. Il en résulte la formation de cercaires émises dans l'environnement. Ces larves infestent alors un second hôte intermédiaire ou sont directement ingérées par ce dernier. Dans le second hôte intermédiaire, le parasite forme des métacercaires. Le cycle est complet lorsque le second hôte intermédiaire est ingéré par l'hôte définitif dans lequel la métacercaire forme un adulte (Rohde, 2005). La crevette de vase *U. pusilla* abritant des métacercaires, elle joue le rôle de second hôte intermédiaire dans le cycle du parasite trématode dont la présence a été évoquée par Dworschak (1988).

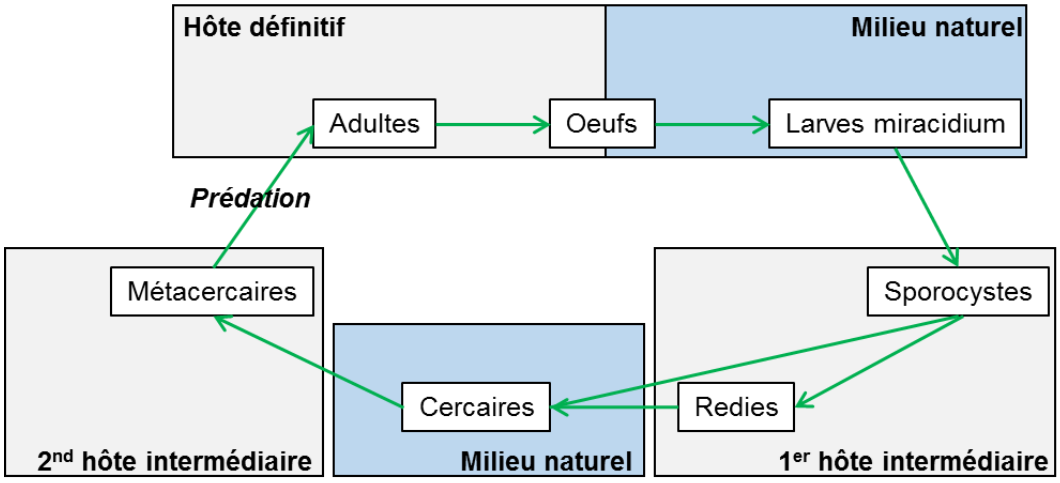

Figure II. 5 Cycle de vie typique des trématodes digènes, impliquant trois hôtes et une alternance de stades libres (boîtes bleues) et parasites (boîtes grises).

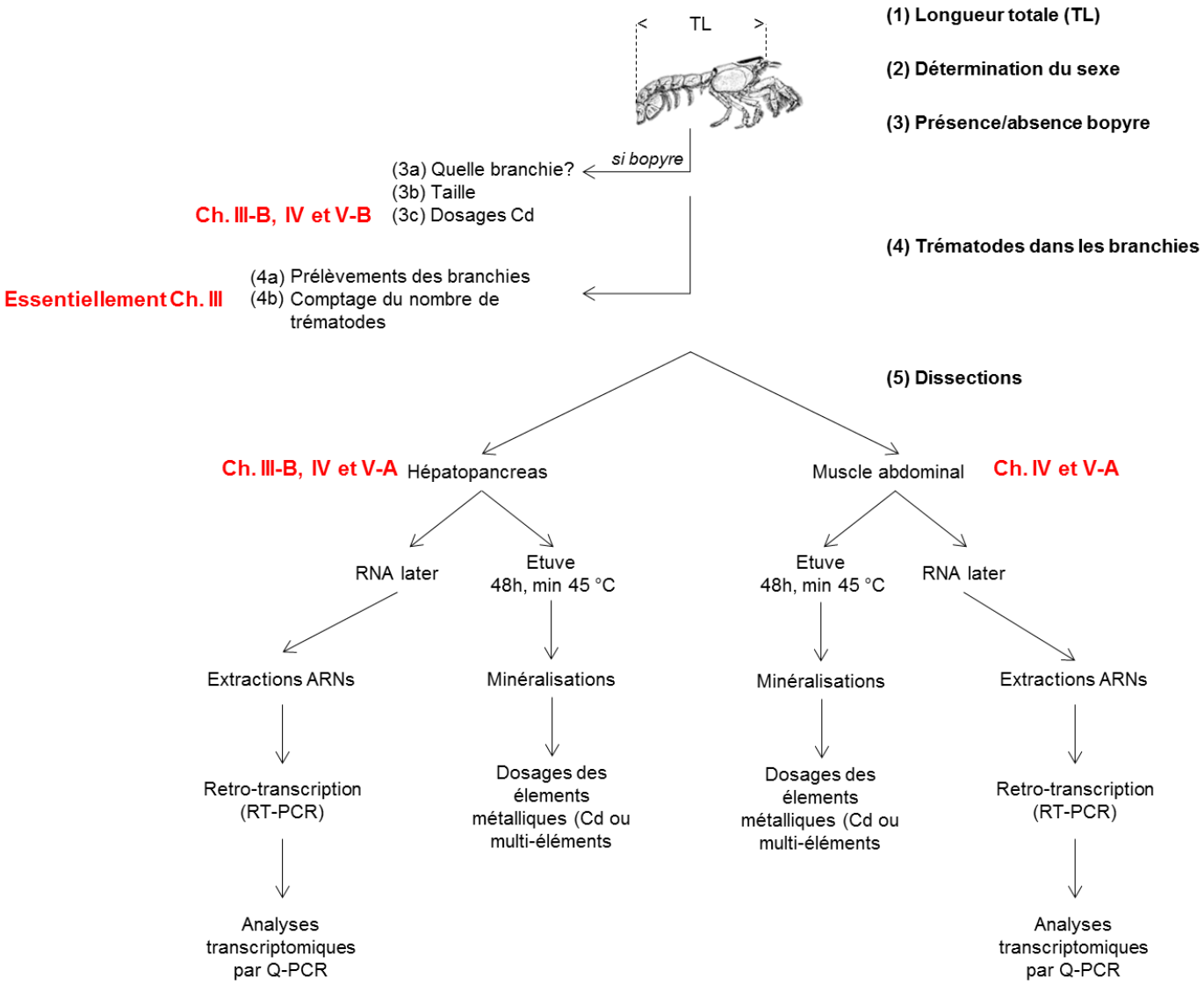

Figure II. 6 Protocole général de traitement des échantillons de *Upogebia* cf. *pusilla* récoltés au cours des suivis de terrain (**Chapitre III**) et des expériences en laboratoire (**Chapitres IV** et **V**). En ce qui concerne les échantillons des suivis spatial et temporel initiés au cours de la thèse de L. Pascal (2017) (données analysées dans le cadre du **Chapitre III-A**), on notera que le protocole s'arrête à l'étape (4) (i.e. pas de dissections de l'hépatopancréas et du muscle abdominal) et que l'étape (3c) n'a pas non plus été réalisée.

II. Protocole de traitement des échantillons et méthodologie

II.1. Protocole général de traitement des échantillons biologiques

Les échantillons biologiques, i.e. les crevettes de vase, ont subi des traitements similaires au cours des différentes expérimentations et du suivi saisonnier. Un schéma général de ce protocole de traitement est fourni de manière à faciliter la lecture de cette partie méthodologique (Figure II. 6). Ce protocole a été mis en œuvre aussitôt les organismes récoltés. De manière synthétique, les crevettes ont tout d'abord été mesurées : longueur totale (TL, au mm inférieur) de l'extrémité du rostre au telson à l'aide d'un pied à coulisse (étape (1)). Le sexe de chaque individu a ensuite été déterminé (étape (2)) en se basant sur le critère suivant : présence d'un gonopore bien visible sur le coxopodite de la troisième paire de péréiopodes chez les femelles. A l'inverse, chez les mâles cet orifice se trouve sur la cinquième paire de péréiopodes et est difficilement observable. La présence/absence du bopyre *G. branchialis* a ensuite été notée (étape (3)). Dans le cas où ce parasite était présent, nous avons repéré le côté parasité. Le bopyre a ensuite été prélevé et mesuré (longueur antéro-postérieure, au µm inférieur) à l'aide du logiciel NIS-elements 0.4.00.00 (Nikon). Dans le cadre des expérimentations en laboratoire (**Chapitres IV** et **V**), le contenu en Cd des parasites *G. branchialis* a également été déterminé (étape (3c)). Les branchies des organismes parasités et non parasités par le bopyre ont été prélevées de manière à estimer l'intensité de l'infection par les parasites trématodes (étape (4) ; essentiellement dans le cadre du **Chapitre III**). En ce qui concerne les échantillons du **Chapitre III-A**, le traitement des échantillons s'arrêtait à l'étape (4). Pour les échantillons des **Chapitres III-B** (suivi saisonnier), **IV** et **V** (expérimentations en laboratoire), des dissections ont été réalisées et visaient à récupérer le muscle abdominal et/ou l'hépatopancréas des crevettes de vase (étape (5)). Dans les deux cas, une partie de chaque organe a été conservée dans une solution de RNA later à 4 °C. Les échantillons ont été traités ultérieurement dans le cadre d'analyses de transcriptomique. Le reste des tissus a quant à lui été directement placé dans une étuve à 45 °C pendant 48 h minimum. Le contenu en éléments métalliques de ces échantillons a ensuite été déterminé (Cd seul, **Chapitres IV et V-A** et analyses multiéléments, **Chapitre III-B**) après minéralisation.

II.2. Quantification de la charge parasitaire en trématodes

Dans un premier temps, la charge parasitaire totale en trématodes d'une cinquantaine d'individus a été évaluée. Ainsi, le muscle, la masse viscérale et les branchies des crevettes ont été prélevés. Chaque organe a ensuite été écrasé entre deux lames de verre. Les parasites ont alors été comptés sous loupe binoculaire (Nikon, SMZ 1500) ou à partir de photographies lorsque le nombre de métacercaires était trop important (il pouvait excéder 1000 métacercaires par individu). Ce premier travail nous a permis de mettre en évidence une relation linéaire entre la charge parasitaire des branchies et la charge parasitaire totale (**Chapitre III-A**). De ce fait, dans le reste des manipulations, seul le nombre de métacercaires dans les branchies a été quantifié, puisqu'assimilé à un proxy de l'infection globale. Selon l'objectif, les charges parasitaires dans les branchies droite et gauche des organismes ont pu être

différenciées ainsi que celles observées dans les branchies parasitées ou non parasitées par le bopyre *G. branchialis* (**Chapitre III-A** notamment). Plusieurs variables ont été déterminées (Margolis *et al.*, 1982):

(1) Prévalence de l'infection : pourcentage d'individus parasités.

(2) abondance : nombre moyen de métacercaires par hôte parasité ou non.

(3) intensité d'infection : nombre moyen de métacercaires par hôte parasité.

II.3. Quantification des éléments métalliques

Les métaux bioaccumulés par *U.* cf. *pusilla* ont été dosés à partir de prélèvements d'hépatopancréas (**Chapitre III-B**) et de muscle abdominal (**Chapitres IV** et **V-A**) (Figure II. 6). Par ailleurs, les métaux accumulés par le bopyre ont été dosés à partir de la totalité de l'organisme (**Chapitres IV** et **V-B**) (Figure II. 6). Chez les crustacés, l'hépatopancréas constitue un organe majeur de bioaccumulation des métaux et joue également un rôle prépondérant dans les processus de détoxication de ces éléments (Marsden and Rainbow, 2004; Ahearn *et al.*, 2004; Pourang *et al.*, 2004). A l'inverse, le muscle abdominal n'est généralement pas reconnu comme bioaccumulant fortement les métaux. Néanmoins, il est possible que sur le long terme une bioaccumulation des métaux se fasse dans cet organe et a donc été investiguée chez *U.* cf. *pusilla*.

En ce qui concerne les dosages des métaux, il est à noter que l'ensemble de la verrerie utilisée (tubes à minéraliser, tubes à essais, béchers, etc.) a subi une étape de lavage à l'eau régale (acidité 5 % ; 48 h de décontamination) avant utilisation.

II.3.1. Préparation des minéralisats

Après dissection, les échantillons de tissus ont tout d'abord été mis à sécher à l'étuve à 45 °C pendant une durée minimale de 48 h. Cette étape est indispensable car elle permet de déterminer des quantités de métaux dans les échantillons par rapport à leur masse sèche (DW). De ce fait, les imprécisions dues à de potentielles différences de teneurs en eau des échantillons sont contrôlées (variabilité de l'égouttage des organes, variations dues à la congélation, etc.).

Les dosages ont été effectués par spectrométrie de masse à plasma à couplage inductif (ICP-OES) ou par spectrométrie d'absorption atomique (SAA). Ces types d'analyses ne peuvent se faire qu'à partir d'une phase liquide. Les échantillons séchés et pesés ont ainsi été digérés par une attaque à l'acide nitrique HNO_3 à 65 %. Le processus de digestion était accéléré par chauffage (plaque chauffante HotBlock; Environmental Express), s'effectuant en 3 étapes :

(1) les échantillons ont progressivement été chauffés jusqu'à une température de 100 °C pendant 1h.

(2) les échantillons ont été ensuite maintenus à 100 °C pendant 3h.

(3) la température de la plaque chauffante a été abaissée de 100 °C à une température ambiante permettant le refroidissement des échantillons sur une période de 1h.

Les minéralisats ont ensuite été dilués par ajout d'eau ultra-pure (résistivité= 18,2 MΩ ; MilliQ) afin d'obtenir une concentration finale d'acide nitrique de 11 %. Ils étaient alors gardés en chambre froide (4 °C) et à l'obscurité jusqu'à l'étape de dosage.

Remarques : (1) le dosage des métaux dans l'eau de mer ne nécessite pas d'étape de minéralisation. Les échantillons ont été acidifiés par ajout HNO_3 à 65 % de manière à obtenir une concentration finale d'acide de 11 % et placés en chambre froide jusqu'à leur dosage.

(2) dans le cadre du dosage multi-éléments des échantillons de sédiment (**Chapitre III**), la minéralisation effectuée consistait en une attaque totale, i.e. à l'aide d'acide fluorhydrique, d'acide nitrique et d'acide chlorhydrique. Cette étape a été réalisée par la plateforme de chimie analytique (plateforme M&Ms) de l'UMR EPOC sous la supervision d'A. Coynel.

II.3.2. Dosage par spectrométrie d'émission atomique à plasma couplé induit (ICP-OES)

La spectrométrie d'émission atomique à plasma couplé induit (ICP-OES) permet de quantifier les éléments métalliques d'un échantillon en analysant par spectrométrie optique des photons générés par un plasma à couplage inductif. Il s'agit d'une analyse multi-éléments. Cette méthodologie a été utilisée pour les dosages multi-éléments dans des échantillons biologiques (**Chapitre III-B**) et des échantillons de sédiment (**Chapitre III-B**) pour le dosage du Cd dans des échantillons biologiques (**Chapitre IV**) et des échantillons d'eau mer (**Chapitres IV** et **V-A**). Cette méthode repose sur le principe que les atomes soumis à une forte énergie vont absorber cette énergie, entrainant le déplacement des électrons des ions et atomes de leurs couches électroniques internes à leurs couches plus externes (théorie quantique). Les atomes et les ions passent ainsi d'un état fondamental à un état excité. Cet état excité est un état hautement instable énergétiquement (non à l'équilibre). Le retour à un état stable (i.e. de plus faible niveau énergétique) s'effectue par émission d'énergie sous forme de photons qu'il est possible de mesurer. Le nombre de photons émis est directement proportionnel à la quantité d'éléments. Chaque élément disposant d'un spectre d'émission spécifique, il est possible de convertir les mesures d'intensité d'irradiation en concentrations par comparaison avec des solutions standards.

L'appareil utilisé dans cette étude était le 700 Series ICP-OES (Agilent). La quantification des éléments métalliques contenus dans les minéralisats par ICP-OES s'effectue en quatre étapes principales. Dans un premier temps, la solution de minéralisation est introduite dans une chambre de vaporisation sous l'action d'une pompe péristaltique où elle est nébulisée en un aérosol liquide formé de micro-gouttelettes *via* l'action d'argon gazeux. Une fois cet aérosol formé, celui-ci est injecté dans une torche à plasma d'argon (mélange d'atomes isolés en équilibre entre leur état neutre et leur état ionisé). Le passage au sein de cette torche d'argon (T entre 6000 et 8000 K) permet l'atomisation puis

l'ionisation des éléments contenus dans l'échantillon. Ions et atomes vont alors émettre des photons qui sont détectés par un spectromètre à émission optique. La quantité de radiations émises est finalement convertie en une concentration par comparaison avec des solutions standards.

Il est à noter que les dosages par ICP-OES ont été réalisés par P-Y Gourves, ingénieur d'étude de l'équipe d'Ecotoxicologie Aquatique de l'UMR EPOC.

II.3.3. Dosage par spectrométrie d'absorption atomique (SAA)

La spectrométrie d'absorption atomique (SAA) est une méthode classique de dosage des éléments métalliques. Il s'agit d'une méthode de quantification relative, i.e. qu'elle nécessite la réalisation d'une courbe d'étalonnage pour déterminer la quantité de l'élément métallique d'intérêt. Cette méthode de dosage repose sur deux principes:

(1) les atomes à l'état fondamental absorbent de façon spécifique certaines longueurs d'onde du spectre lumineux qui leur sont propres.

(2) la quantité d'énergie lumineuse absorbée est fonction du nombre d'atomes présents sur le trajet optique (Loi de Beer-Lambert).

Cette méthodologie a été utilisée pour le dosage du Cd de certains échantillons biologiques (**Chapitre V-A**) et pour les échantillons de sédiment (**Chapitre IV**). L'appareil utilisé pour ces travaux était le spectromètre à absorption atomique 240Z AA (Agilent) qui présente une limite de détection de 0,075 µg L^{-1} pour le Cd. L'échantillon liquide (« minéralisat ») est tout d'abord déposé dans un four graphite (atomiseur). Plusieurs étapes de chauffage successives sont ensuite réalisées et permettront *in fine* l'atomisation de l'échantillon, i.e. la transformation de l'échantillon liquide en atomes libres absorbant l'énergie lumineuse de longueur d'onde donnée émise par une lampe spécifique (pour le cadmium λ = 228,8 nm). Les étapes de chauffage débutent par une phase initiale de désolvatation (élimination du solvant par évaporation), suivie d'une phase de pyrolyse (élimination de la matrice organique), puis une phase de minéralisation (décomposition des molécules en atomes) et finalement une phase d'atomisation (formation d'une vapeur atomique). Une mesure de l'intensité lumineuse transmise est alors réalisée et permettra de déterminer l'intensité de l'absorption lumineuse de l'échantillon (intensité lumineuse incidente – intensité lumineuse transmise) et donc la quantité d'atomes présents dans l'échantillon par comparaison avec des solutions standards. Finalement, une dernière étape de chauffage permet le nettoyage du four graphite. L'ensemble de ces étapes (à l'exception de l'étape de mesure) s'effectue sous une atmosphère d'argon, limitant les interférences avec l'oxygène (Figure II. 7).

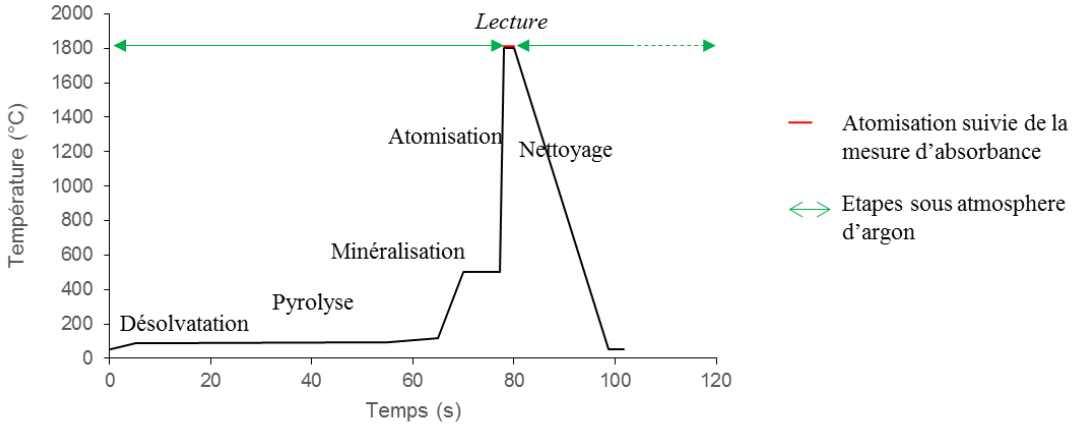

Figure II. 7 Programme thermique associé au dosage du cadmium par spectrométrie d'absorption atomique et détails des différentes étapes de chauffage.

Pour chaque échantillon, 20 µL de minéralisat étaient nécessaires, auxquels étaient ajoutés 5 µL d'une solution de palladium (Pd ; 2 g L^{-1}) et de nitrate de magnésium ($Mg(NO_3)_2$; 1 g L^{-1}). Le Pd est un modificateur d'analyte : il forme avec le Cd un complexe thermiquement plus stable que ne le serait le Cd seul. Ainsi il est possible d'appliquer des températures de pyrolyse plus élevées, permettant une élimination de la matrice plus efficace. Le nitrate de magnésium est quant à lui un modificateur de matrice, permettant la volatilisation des composés de la matrice à de plus faibles températures. Ces modificateurs sont essentiels car ils limitent l'absorption non spécifique de l'énergie lumineuse par les composés de la matrice, ce qui pourrait constituer un biais de quantification. Il est à noter qu'en plus du signal d'absorbance spécifique à l'élément atomique considéré, il est possible que des signaux d'absorbance non spécifiques (dus à des molécules non dissociées par exemple) soient enregistrés. Une correction de ces signaux non spécifiques a été réalisée en se basant sur l'effet Zeeman, dont la définition dépasse de loin le cadre de ce travail.

Remarques : (1) seul le Cd a été quantifié dans les échantillons d'eau de mer. Théoriquement, ce dosage aurait donc pu s'effectuer par SAA. Néanmoins, l'utilisation de cette méthode de mesure est conditionnée à des solutions dont la salinité est inférieure à 2 pour le dosage du Cd. En effet le NaCl a une température de volatilisation très proche de celle du Cd. De façon à limiter les risques d'interférence entre le Cd et le NaCl, il aurait fallu éliminer ce dernier en diluant fortement nos échantillons. Il est fort probable que les échantillons eussent alors présenté des concentrations en Cd inférieures à la limite de détection de l'appareil. Des dosages par ICP-OES ont donc été préférés.

(2) les dosages du Cd dans les échantillons biologiques du **chapitre IV** auraient également pu être effectués par SAA. Des problèmes techniques nous ont néanmoins amenés à doser ces échantillons par ICP-OES.

II.3.4. Validité des mesures

Afin de s'assurer de la validité des mesures, des échantillons certifiés fournis par le NRCC- CNRC (National Research Council Canada – Conseil National des Recherches Canada) ont été utilisés pour chaque série de dosages (Tableau II. 2). Des blancs de minéralisations (acide seul dilué à 11 %) ont également été réalisés afin de détecter une éventuelle contamination métallique et ont ainsi subi le même traitement que les échantillons biologiques et de sédiments. Le dosage de chaque série d'échantillons était précédé et succédé par le dosage des métaux dans le ou les certifiés et dans les blancs de minéralisation. Les échantillons certifiés permettaient de déterminer le rendement machine. Grâce à la détermination de ce rendement, il a ainsi été possible de déduire des concentrations mesurées les concentrations réelles en métaux de nos échantillons.

Tableau II. 2 Echantillons certifiés utilisés comme références au cours des différents dosages des éléments métalliques. Les certifiés sont fournis par le Conseil National des Recherches Canada (NRCC-CNRC).

Certifiés	Type d'échantillons	Utilisation
DOLT-5 : hépatopancreas de homard	Biologique	Chapitres III-B, IV et V
TORT-3 : foie de chien de mer	Biologique	Chapitre III-B
MESS-3 : sédiment marin	Sediment	Chapitre IV

Remarques : dans le cas des échantillons d'eau de mer, il n'y a pas d'échantillons certifiés (pas d'étape de minéralisation) et une gamme étalon était utilisée à la place. De la même façon, le blanc de minéralisation a été remplacé par un échantillon d'eau acidifiée à 11 %.

II.4. Techniques de biologie moléculaire

II.4.1. Identification taxinomique des trématodes à l'aide de techniques de biologie moléculaire

Les techniques de biologie moléculaire sont aujourd'hui utilisées en routine pour l'identification taxinomique des organismes vivants. L'utilisation de séquences d'acide désoxyribonucléique (ADN) dans le cadre d'une identification taxinomique peut se faire en soutien à des études morpho-anatomiques ou peut être à la base des études, selon le taxon auquel appartient l'organisme vivant considéré. Les techniques de biologie moléculaire présentent en effet de nombreux avantages et notamment, pour certaines, celui de reposer sur l'étude d'ADN, molécule universelle du vivant. De plus, ces techniques nécessitent très peu de matériel biologique et peuvent être mises en œuvre sur toutes sortes d'échantillons (organes ou tissus seuls, œufs, etc.). Par ailleurs, les séquences d'ADN utilisées sont les mêmes quel que soit le stade de vie de l'organisme ou son sexe. Finalement, ces séquences ne varient pas sous l'action de paramètres environnementaux qui peuvent à l'inverse avoir une influence non négligeable sur la morphologie d'un individu. Des séquences variées d'ADN peuvent ainsi être utilisées pour l'identification d'êtres vivants, la plus courante étant celle du gène mitochondrial codant la sous-unité I de la cytochrome C oxydase (COX I) sur laquelle repose par exemple une part importante du

projet international « Barcode of Life ». Néanmoins, quelles que soient les séquences choisies pour l'identification des organismes d'intérêt, la méthodologie reste la même et implique trois grandes étapes. Dans un premier temps, l'ADN total des échantillons est extrait et purifié. Puis vient une étape d'amplification des séquences d'intérêt. Finalement les séquences obtenues sont séquencées et comparées avec des séquences déjà disponibles et publiées dans des banques de données. Ces techniques ont été mises en œuvre dans le cadre de l'identification du parasite trématode retrouvé chez *U.* cf. *pusilla* (**Chapitre III-A**).

a) Extraction et purification de l'ADN

L'extraction et la purification des molécules d'ADN peuvent s'effectuer selon diverses techniques et de nombreux kits d'extraction sont disponibles. Les différents protocoles reposent approximativement sur le même principe général. Dans un premier temps, les échantillons sont soumis à une lyse cellulaire. L'objectif de cette première étape est de libérer le contenu cellulaire sans abimer les molécules d'ADN. Les lipides et les débris cellulaires sont ensuite éliminés, notamment à l'aide de détergents. Les protéines sont également éliminées. Finalement l'ADN est séparé des autres acides nucléiques et concentré par précipitation alcoolique.

Dans le cadre de ce travail, le kit « DNeasy Blood & Tissue Kit » (Qiagen) a été utilisé pour extraire l'ADN du parasite trématode de *U.* cf. *pusilla* selon les instructions du fournisseur. Brièvement, les trématodes ont tout d'abord été prélevés au niveau de la masse viscérale d'individus congelés. Les échantillons ont ensuite été incubés au minimum 30 min à 56 °C en présence de 20 µL d'une solution de protéinase K (20 mg mL^{-1}) et de 180 µL de tampon ATL (lyse cellulaire). Après incubation, 200 µL de tampon AL ont été ajoutés. La solution a alors été vortexée avant ajout de 200 µL d'éthanol glacé (96 %). Après avoir été de nouveau vortexée, la solution a été transférée sur une colonne collectrice qui était alors centrifugée 1 min à 3000 rpm à température ambiante (élimination des débris cellulaires et des lipides). Le filtrat a été éliminé et 500 µL de tampon AW1 ont été ajoutés sur la colonne. Une centrifugation de 5 min à 6000 rpm à température ambiante a été réalisée. Le filtrat a de nouveau été jeté avant ajout de 500 µL de tampon AW2 sur la colonne (élimination des protéines et séparation de l'ADN des autres acides nucléiques). La colonne a subi une nouvelle centrifugation de 5 min à 6000 rpm à température ambiante. Finalement, 30 µL de tampon AE ont été ajoutés à la colonne collectrice. L'ensemble a été incubé 1 min à température ambiante avant d'être centrifugé 2 min à 6000 rpm et à température ambiante, ce qui permet l'élution de l'ADN extrait.

En fin d'extraction, la quantité d'ADN extrait ainsi que la qualité de l'extraction ont été déterminées. Ces mesures ont été effectuées à l'aide d'un spectrophotomètre microplaques (Spectrophotomètre Multi-Volume Epoch ; Biotek). Cet appareil permet la mesure de l'absorbance d'une solution à une longueur d'onde donnée, i.e. le rapport entre l'intensité de la lumière incidente et l'intensité de la lumière transmise par l'échantillon (ou sortante de l'échantillon). La valeur de cette absorption est directement proportionnelle à la concentration de l'échantillon considéré (loi de Beer-Lambert). Un dispositif

monochromateur permet de générer, à partir d'une source de lumière visible ou ultraviolette, une lumière monochromatique, dont la longueur d'onde est choisie par l'utilisateur. Dans le cadre d'un dosage d'ADN, l'absorbance des échantillons est mesurée à 260 nm et à 280 nm. En effet, les acides nucléiques présentent une absorption maximale à λ = 260 nm (avec $A_{\lambda=260\ nm}$ = 1 correspondant à une concentration en ADN double brin de 50 µg mL^{-1}) et les protéines à λ = 280 nm. Pour des échantillons d'ADN pur, $A_{\lambda=260\ nm}/A_{\lambda=280\ nm}$ = 1,8. Un rapport < 1,8 indique la présence de protéines, tandis qu'un rapport > 1,8 et proche de 2 indique la présence d'ARN. L'objectif ici était donc d'obtenir des rapports $A_{\lambda=260\ nm}/A_{\lambda=280\ nm}$ le plus proche possible de 1,8.

b) Amplification des séquences d'intérêt par PCR classique

La PCR classique (Polymerase Chain Reaction ou amplification en chaîne par polymérase) est une technique usuelle en biologie moléculaire permettant une réplication ciblée *in vitro*. Cette technique permet d'obtenir de manière simple d'importantes quantités d'un fragment d'ADN d'intérêt, parfois peu abondant dans un échantillon originel. La PCR se résume à une série de réactions de réplications d'une matrice d'ADN double brin (Figure II. 8). Cette technique a pu largement se répandre grâce à l'utilisation d'une ADN polymérase spécifique : la *Taq* polymerase, enzyme thermorésistante. L'utilisation de cette enzyme identifiée chez *Thermus aquaticus*, bactérie thermophile (50-80 °C), a permis l'autonomisation de la technique de PCR et son utilisation en routine au laboratoire. En effet, un programme classique de PCR débute par une phase de dénaturation de l'ADN à 95 °C. Une ADN polymérase résistante à de telles températures (i.e. dont la structure tertiaire voire quaternaire n'est pas altérée à la température considérée) est donc nécessaire afin que la suite de la réaction puisse s'effectuer (protéine non dénaturée). Par ailleurs cette enzyme permet de synthétiser rapidement de l'ADN puisqu'elle est capable de polymériser plus de 1000 pb par minute.

Les réactions de PCR ont ainsi été réalisées en utilisant la *Taq* polymerase Go-Taq (5 U μL^{-1}, Promega). Chaque solution soumise à un programme de PCR contenait 1 µL de dNTP (mix de nucléotides, 10 mM), 3 µL de $MgCl_2$ (cofacteur de la *Taq* polymérase, 25 mM), 10 µL d'un tampon d'activité (5x PCR reaction buffer, Promega), 0,5 µL de chaque amorce (sens et anti-sens bornant la séquence à amplifier, 100 µM), 1 µL d'ADN extrait, 0,2 µL de *Taq* polymérase et 33,8 µL d'eau sans nucléases. Cette solution était alors placée dans un thermocycleur (Mastercycler, Eppendorf) réalisant le programme de PCR. L'étape initiale de dénaturation de l'ADN s'effectue généralement à 95 °C pendant 10 min. L'objectif de cette première étape est la séparation des deux brins de la molécule d'ADN ainsi que l'activation de la *Taq* polymerase. A cette première étape suit un cycle de PCR, lui-même divisible en plusieurs sous-étapes :

(1) séparation des deux brins de la molécule d'ADN : augmentation de la température jusqu'à environ 95 °C pendant 1 min permettant la dénaturation de l'ADN.

(2) hybridation des amorces bornant la séquence d'ADN à amplifier : diminution de la température jusqu'à la température optimale Tm de fixation des amorces à leurs séquences complémentaires sur la séquence à amplifier. Cette température est propre à chaque couple d'amorces et dépend de leur séquence nucléotidique (Tm = 2 (A + T) + 4 (G +C) pour une séquence nucléotidique inférieure à 30 résidus). La détermination de la Tm est cruciale, une Tm trop élevée ne permettant pas l'hybridation des amorces et une Tm trop basse favorisant une fixation non spécifique des amorces. La durée de cette étape est de 30 secs.

(3) élongation par synthèse du brin complémentaire d'ADN par la *Taq* polymérase: cette étape dure généralement 1 min (mais cela dépend de la taille du fragment à amplifier) et s'effectue à 72 °C, température optimale d'activité de la *Taq* polymérase. L'élongation est effectuée par la *Taq* polymérase à partir de l'extrémité 3'-OH des amorces préalablement hybridées sur l'ADN matriciel.

Ces trois sous-étapes constituent un cycle de PCR. Classiquement, ces cycles sont répétés entre 30 et 50 fois par PCR. Le programme de PCR s'achève par une étape effectuée à 72 °C pendant 10 min et qui permet de s'assurer que la séquence bornée par le couple d'amorces choisi a totalement été répliquée par la *Taq* polymérase.

Dans le cadre de l'identification moléculaire du trématode de *U.* cf. *pusilla*, deux séquences classiquement utilisées en taxinomie moléculaire ont été étudiées. Il s'agissait d'une séquence partielle du gène nucléaire de l'ARN ribosomique 18S (ARNr 18S) et de la séquence complète de l'espace intergénique ITS (Internal Transcibed Spacer ; ITS1, 5,8S et ITS2) située entre les gènes nucléaires 18S et 28S. Le gène de l'ARNr 18S code l'ARNr de la petite sous-unité du ribosome chez les Eucaryotes. L'intérêt majeur de ce gène, outre son universalité au sein de ce phylum, est la grande disponibilité de séquences dans les bases de données publiques telle celle du National Center for Biotechnology Information (NCBI). Néanmoins, la séquence de ce gène est très conservée au sein des Eucaryotes et ne permet donc pas de distinguer la variabilité entre organismes phylogénétiquement proches ce qui limite notamment l'identification au niveau de l'espèce. L'ITS permet de palier à ce problème car la séquence est en partie formée par deux régions non-codantes de l'ADN. Puisque non codantes, ces régions sont moins soumises à la pression de sélection donc plus polymorphes et constituent par conséquent un marqueur plus variable que l'ARNr 18S. Les amorces utilisées pour borner ces deux séquences géniques (ARNr 18 S et ITS) étaient celles identifiées par de Montaudouin *et al.* (2014) chez *Bacciger bacciger* (Trematoda : Fellodistomidae) (Tableau II. 3).

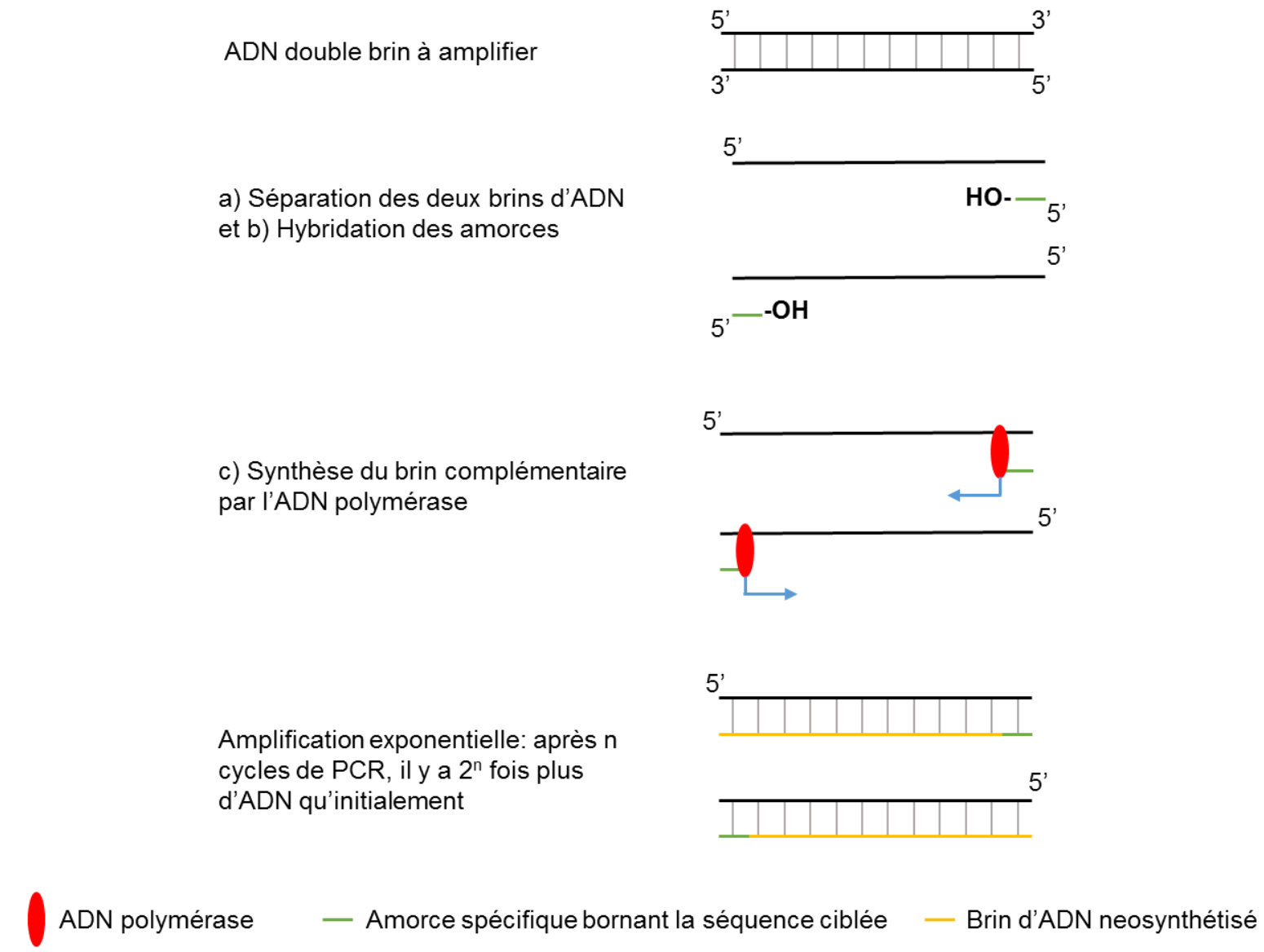

Figure II. 8 Principe de l'amplification en chaine par polymérase (Polymerase Chain Reaction, PCR).

Tableau II. 3 Séquences des amorces sens et anti-sens utilisées pour amplifier les régions 18S et ITS des parasites trématodes de *Upogebia* cf. *pusilla*. D'après de Montaudouin et *al.* (2014).

Gènes	Amorces	Séquences 5' → 3'
ARNr 18 S	Sens : Bb18S5	ACTGGAGGGCAAGTCTGGTGC
	Anti-sens : Bb18S3	CAGCTTTGCAACCATACTTCC
ITS	Sens : BbITS5	CTCCCATATGGTCGACCTGCA
	Anti-sens : BbITS3	TTGACCGAACTTGATCATTT

c) Electrophorèse

Une PCR classique est généralement suivie par une électrophorèse permettant de visualiser les amplifiats de PCR. Il s'agit de faire migrer sous l'influence d'un champ électrique des fragments d'ADN dans un gel d'agarose. En effet, la molécule d'ADN est une molécule chargée négativement du fait de la présence de groupements phosphate. En présence d'un champ électrique, la molécule d'ADN migrera ainsi en direction du pôle cathodique de ce dernier.

Des gels d'agarose à 1 % p/v ont ainsi été réalisés par mélange d'une poudre d'agarose dans un tampon TAE 1X (Tris-Acétate 40 mM + Ethylène diamine tétraacétique (EDTA) 1 mM). Ce mélange était chauffé au micro-onde ce qui permettait la dissolution de la poudre d'agarose dans le tampon. Le mélange était laissé quelques minutes à refroidir avant que quelques µL de bromure d'éthidium (BET ; 10 mg mL^{-1}) y soient ajoutés. Le BET est un agent intercalant non radioactif des acides nucléiques. Cette molécule a la propriété de fluorescer lorsqu'on l'expose à un rayonnement ultra-violet, permettant facilement la mise en évidence d'acides nucléiques. Une fois cette solution mélangée, elle était coulée dans une plaque permettant de réaliser un gel. En effet, en refroidissant, l'agarose polymérise formant un réseau de mailles plus ou moins fines selon la quantité diluée dans le tampon TAE 1X. Une fois le gel solidifié, celui-ci était placé dans une cuve électrophorétique contenant un bain de TAE 1X. Le gel était alors « chargé » : 7 µL de produit de PCR étaient prélevés et mélangés à 1 goutte de colorant de charge (DNA Gel Loading Dye (6X) ; ThermoFisher Scientific) avant d'être déposés dans un puits. De manière à estimer la taille des fragments d'ADN amplifiés, 5 µL d'un marqueur de poids moléculaires étaient également déposés dans un puits (1 Kb Plus DNA Ladder, ThermoFisher Scientific). Un champ électrique de 120 V était ensuite appliqué pendant 20 à 60 min, selon la taille du gel. Le gel solide d'agarose constitue un réseau de mailles ralentissant la migration de l'ADN. Ainsi, plus les fragments d'ADN seront petits et plus ils migreront loin dans le gel. Le gel d'agarose permet donc de séparer les fragments d'ADN selon leur taille. Une fois la migration effectuée, le gel était retiré de l'appareil électrophorétique et placé sous une lampe à UVs. La fluorescence du BET, intercalé entre les molécules d'ADN, permettait de révéler les différents fragments d'ADN amplifiés (Figure II. 9).

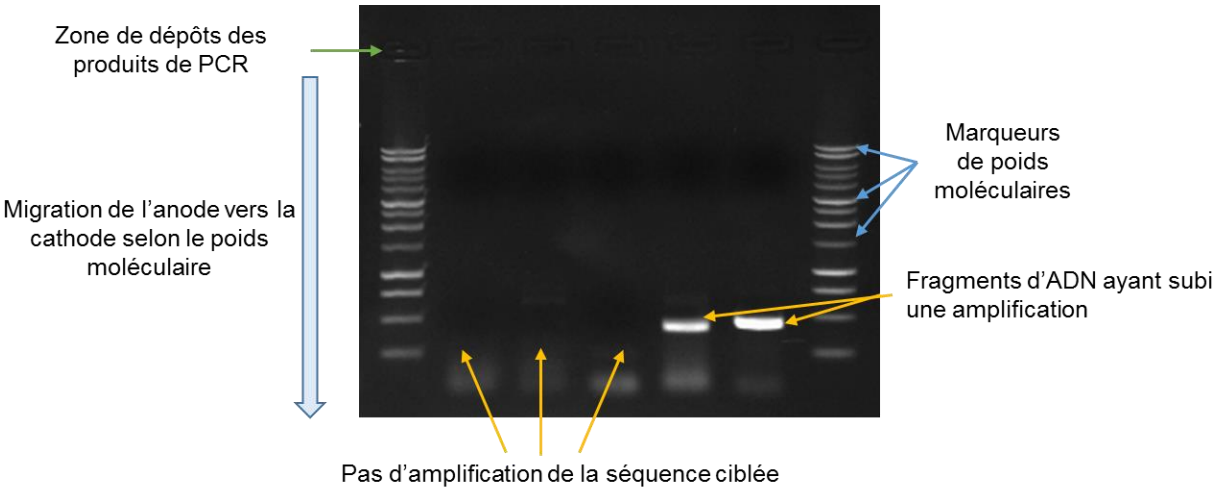

Figure II. 9 Exemple de gel d'électrophorèse obtenu après PCR. Les régions d'ADN ayant subi une amplification apparaissent sous forme d'une bande blanche sous lumière UV. La taille du fragment amplifié peut être estimée à l'aide de marqueurs de poids moléculaires.

d) Séquençage et comparaison des séquences obtenues

Grâce à l'électrophorèse, il était possible de savoir si la PCR s'était révélée concluante i.e. s'il y avait eu ou non amplification d'un fragment d'ADN de la taille attendue. Dans le cas où la PCR avait effectivement permis l'amplification d'un fragment d'ADN de la taille attendue, le produit de PCR a été séquencé. Un aliquot de 10 µL de produit de PCR dilué dans 10 µL d'eau sans nucléases, un aliquot de 2 µL d'amorces sens diluées dans 18 µL d'eau sans nucléases ainsi qu'un aliquot de 2 µL d'amorces anti-sens diluées dans 18 µL d'eau sans nucléases ont été préparés puis envoyés à une entreprise privée chargée du séquençage des fragments d'ADN d'intérêt (GATC Biotech). Les séquences sens et anti-sens obtenues ont été alignées après conversion de la séquence anti-sens en sa séquence complémentaire. Cet alignement permettait de s'assurer de la qualité du séquençage ainsi que d'identifier les erreurs de séquençage, voire de les corriger. Les séquences ainsi inspectées ont alors été soumises au programme d'alignement « Basic Local Alignment Search Tool » (BLAST) (Altschul *et al.*, 1990) sur le site web du NCBI. L'algorithme en ligne « blastn » permet la recherche des similarités entre une séquence requête nucléique et toutes les séquences nucléiques de la base de données. Une fois les résultats de l'algorithme obtenus, le premier paramètre qui a été pris en compte est la E-value qui est la probabilité que l'alignement entre la séquence soumise et la séquence provenant de la base de données soit une pure coïncidence. Plus la E-value était faible et moins il était probable que l'alignement obtenu soit uniquement dû au hasard. On s'intéressait ensuite à la valeur de « query coverage » qui correspond au pourcentage de recouvrement entre la séquence soumise et la séquence de la base de données. Plus cette valeur était élevée et plus les séquences avaient des tailles similaires et donc se recouvraient. Finalement, le pourcentage d'identité entre les deux séquences était analysé. Il s'agit de regarder l'identité de deux séquences au niveau de leurs zones de recouvrement. Ainsi une séquence requête pouvait être considérée comme homologue à une seconde séquence lorsque le résultat d'un blast fournissait une E-value très

faible (le plus proche possible de 0), une valeur de « query coverage » élevée et un pourcentage d'identité élevé (jusqu'à 100 %).

II.4.1. Quantification de l'expression génique

Chez les Eucaryotes pluricellulaires, il est possible d'observer des cellules différenciées et spécialisées dans la réalisation de fonctions spécifiques. Or le génome est identique dans chacune des cellules d'un organisme (exception de quelques cellules comme les cellules germinales). La différenciation cellulaire observée s'explique par l'expression différentielle des gènes. En effet, les gènes peuvent voir leur expression varier dans le temps (propre à un stade de développement), l'espace (expression spécifique à un type cellulaire par exemple) et/ou en réponse à un état physiologique particulier (organisme soumis à stress ou à un stimulus donné). Cette régulation de l'expression génique peut se faire à plusieurs niveaux et peut être étudiée par des études transcriptomiques et/ou protéomiques (Figure II. 10).

Le transcriptome est défini comme l'ensemble des transcrits exprimés dans un organisme à un moment donné et dans des conditions données. Le protéome quant à lui se définit comme l'ensemble des protéines actives dans une cellule à un moment donné et dans des conditions données. Transcriptome et protéome constituent par conséquent des entités dynamiques dont les compositions et quantités fluctuent en fonction des conditions intra- et extracellulaires. Dans le cadre de ce travail, nous avons choisi de réaliser une étude transcriptomique partielle, i.e. de caractériser le niveau des produits d'expression de gènes d'intérêts, à savoir les acides ribonucléiques messagers (ARNm), dans un état et à un moment donné. L'étude de la production différentielle d'ARNm chez des organismes soumis à un stress à un temps *t* permet ainsi de mettre en évidence la régulation transcriptionnelle de certains gènes d'intérêt, préalablement caractérisés, en lien avec un stress donné. Au cours de ce travail, les effets d'une infestation parasitaire et/ou d'une contamination métallique expérimentale ont ainsi été caractérisés au niveau moléculaire *via* la quantification du niveau d'expression de certains gènes d'intérêt par PCR quantitative (Q-PCR) chez *U.* cf. *pusilla* (**Chapitres III-B, IV** et **V-A**).

a) Travail préliminaire

L'étude du niveau d'expression génique par Q-PCR nécessite la connaissance des séquences codantes appartenant aux gènes d'intérêt sélectionnés. Le génome de *U.* cf. *pusilla* n'est à ce jour pas séquencé. Ainsi, les analyses réalisées en biologie moléculaire ont tout d'abord nécessité un travail de recherche et de séquençage de séquences nucléotidiques d'intérêts. Ce travail peut se décomposer en 3 étapes principales. La première étape consiste à rechercher des gènes d'intérêt et à concevoir des amorces spécifiques à ces séquences permettant leur amplification ultérieure par PCR. Dans un second temps, ces amorces sont testées en réalisant des PCR classiques sur des échantillons tests. Ces PCR sont effectuées à partir d'ADN coomplémentaires (ADNc) et nécessitent donc l'extraction d'ARN totaux présents dans certains tissus de *U.* cf. *pusilla* ainsi que leur retro-transcription en ADNc. Finalement, les produits de PCR sont séquencés et de nouvelles amorces totalement spécifiques à *U.* cf. *pusilla* sont conçues à partir de ces séquences.

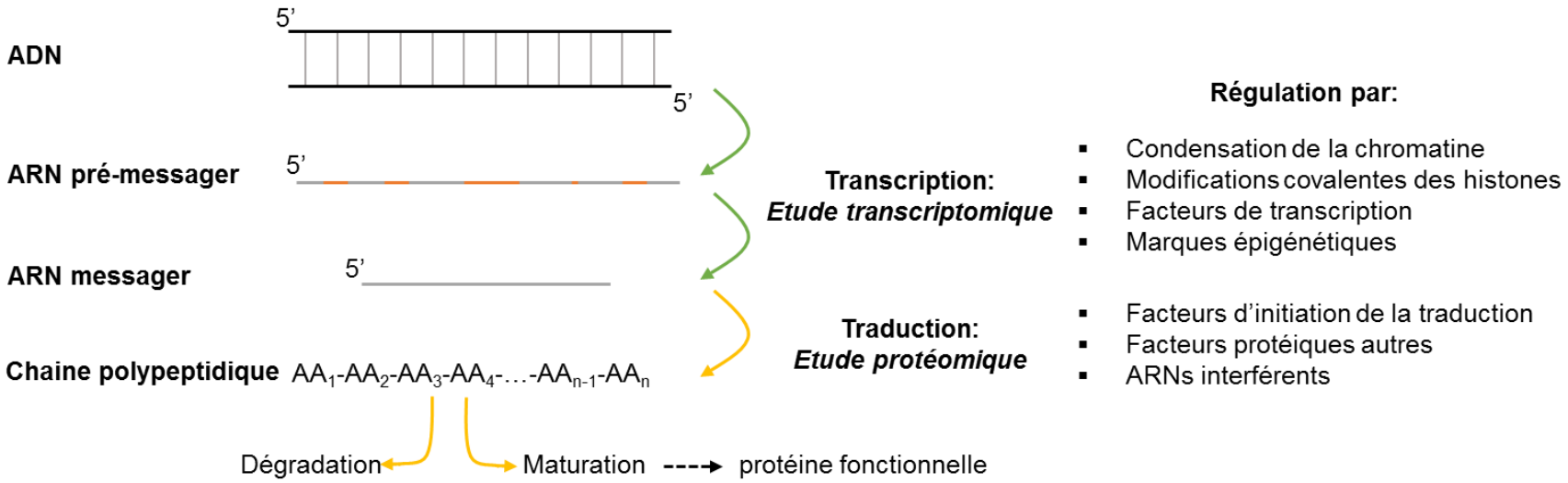

Figure II. 10 Expression du génome et positionnement des études transcriptomiques et protéomiques.

(i) Recherche de séquences nucléotidiques codantes d'intérêt

Un travail de recherche bibliographique a tout d'abord été nécessaire afin de définir des gènes d'intérêt susceptibles d'être affectés par un stress tel que la présence d'un parasite ou une contamination métallique. Ces gènes pouvaient ainsi être reliés à des fonctions physiologiques potentiellement altérées par ce type de stress : défenses immunitaires, métabolisme énergétique, métabolisme endocrinien, etc. Une fois ces gènes définis, l'étape suivante a consisté à rechercher dans la banque de séquences ADN Genbank (NCBI) les séquences nucléotidiques correspondantes chez les espèces les plus proches phylogénétiquement du modèle d'étude. Dans le cadre de ce travail, les séquences d'intérêt ont essentiellement été choisies au sein du sous-ordre des Pleocyemata. Pour chaque gène d'intérêt, plusieurs séquences ont ainsi été identifiées et rapatriées. Ces séquences ont ensuite été alignées à l'aide du programme en ligne de commande Clustal Omega. Ces alignements multiples de séquences ont permis la détermination de régions nucléotidiques conservées d'une espèce à l'autre et ont par conséquent été à l'origine de la conception de couples d'amorces (amorce sens et amorce anti-sens) spécifiques aux séquences d'intérêt. Ces amorces permettaient de borner les séquences d'intérêt dont on cherchait à étudier le niveau d'expression. Une fois les couples d'amorces choisis dans ces régions conservées, leurs séquences ont été transmises à une société privée qui les a synthétisées (Sigma-Aldrich).

(ii) Amplifications par PCR classique

Les couples d'amorces déterminés chez des espèces phylogénétiquement proches de *U.* cf. *pusilla* ont été testés, l'objectif étant l'amplification d'une séquence spécifique d'ADNc obtenue à partir d'individus tests. Le principe de ces amplifications est le même que celui mis en œuvre lors d'une PCR classique et repose donc sur l'hybridation d'un couple d'amorces spécifiques à la séquence choisie suivie de la polymérisation d'un brin complémentaire à la séquence bornée par le couple d'amorces nucléotidiques.

Une fois les amplifications réalisées, les produits de PCR ont été mis à migrer sur un gel d'agarose à 1 % p/v contenant du BET. Le gel a ensuite été révélé sous UVs. Trois cas étaient alors possibles pour chaque couple d'amorces testé (Figure II. 11):

(1) une bande de la taille attendue était observée.

(2) plusieurs bandes, dont une de la taille attendue, étaient observées.

(3) aucune bande n'était observée.

Dans le cas (1), le produit de PCR a été envoyé pour séquençage à la société GATC Biotech. La séquence obtenue a été alignée avec les séquences utilisées pour désigner les amorces. Dans le cas d'un alignement satisfaisant, cette séquence a été utilisée pour déterminer des amorces spécifiques à notre organisme d'étude à l'aide du programme de commande Primer3Plus. Dans le cadre de la réalisation

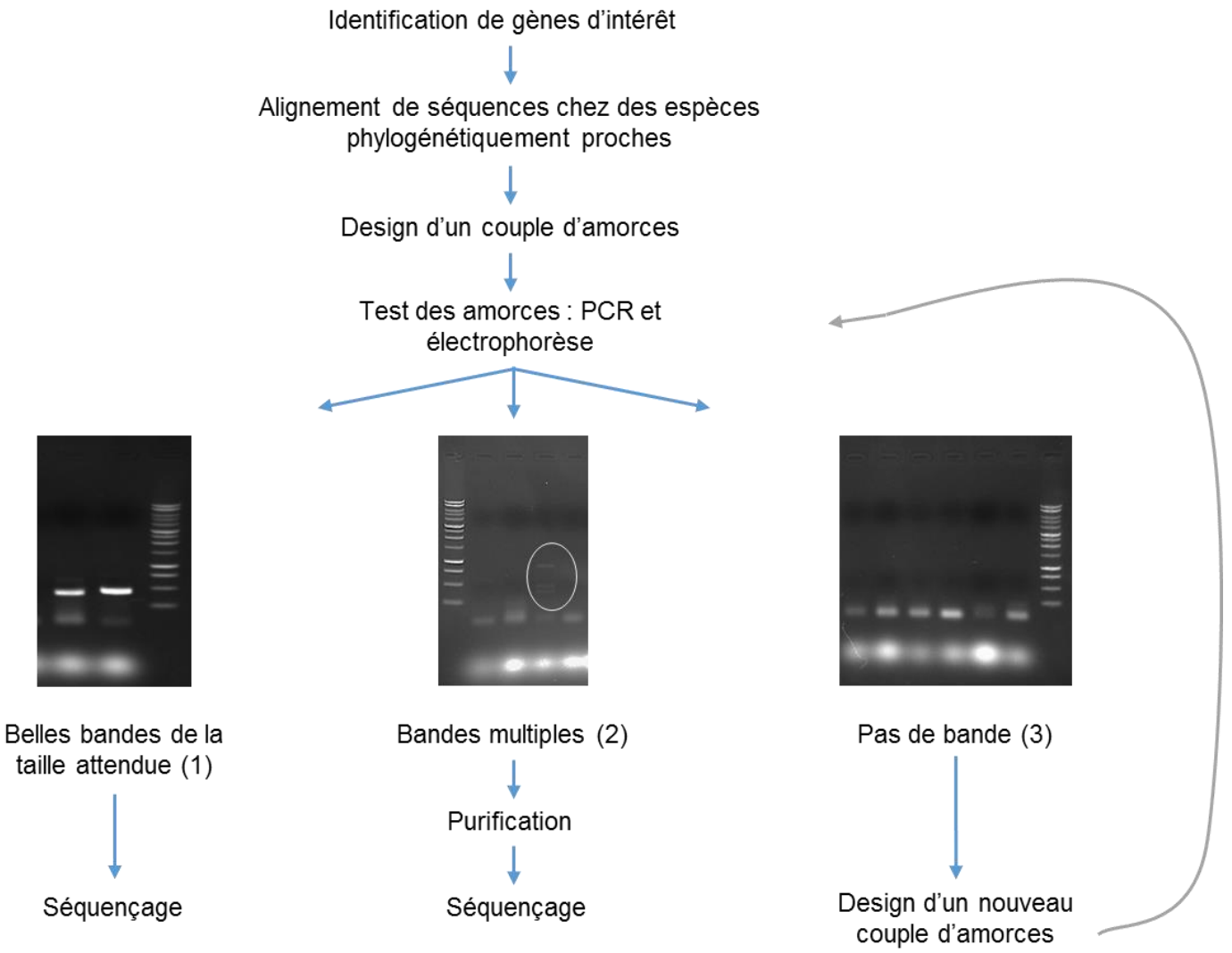

Figure II. 11 Travail préliminaire à la définition des amorces pour cibler des gènes d'intérêts en Q-PCR. (1) : les amorces testées ciblent uniquement la séquence d'intérêt et permettent son amplification spécifique; (2) : les amorces testées ciblent la séquence d'intérêt et des séquences autres, ne permettant pas une amplification spécifique; (3) : les amorces testées ne permettent pas l'amplification de la séquence d'intérêt. Il faut donc définir de nouvelles amorces.

de Q-PCR, les amorces choisies permettaient de borner une séquence d'environ 200 pb, contenant environ 50 % de GC et ayant une température d'hybridation comprise entre 60 et 63 °C.

Dans le cas (2), une migration sur gel a été relancée avec la totalité des produits de PCR. La bande d'intérêt, i.e. de la taille attendue, a été découpée dans le gel et une purification de l'ADN présent dans le gel a été réalisée à l'aide du kit Wizard SV Gel and PCR Clean-Up System (Promega). Brièvement, la bande d'intérêt a été découpée dans le gel d'électrophorèse sous exposition UVs et placée dans un tube de 1,5 mL. Puis 10 µL de membrane binding solution (acétate de potassium + thiocyanate de guanidinium) ont été ajoutés par 10 mg de gel. L'ensemble a été vortexé puis incubé dans un bain-marie à 50 °C jusqu'à dissolution complète du gel d'agarose. Une fois le gel totalement dissous, la solution a été transférée sur une colonne collectrice et incubée à température ambiante pendant 1 min. La colonne a été soumise à centrifugation pendant 1 min à 16 000 g et le filtrat a alors été éliminé. Un premier lavage a ensuite été réalisé par ajout de 700 µL d'une solution de lavage (acétate de potassium + éthanol + EDTA). La colonne collectrice a subit une nouvelle centrifugation à 16 000 g pendant 1 min puis le filtrat a été jeté. Un second lavage a été réalisé par ajout de 500 µL de solution de lavage et une nouvelle centrifugation à 16 000 g a eu lieu pendant 5 min. Le filtrat a de nouveau été jeté avant centrifugation à 16 000 g pendant 1 min ce qui permettait l'évaporation de tout résidu de la solution de lavage, et notamment d'éthanol. Finalement l'ADN purifié a été élué par ajout de 30 µL d'eau sans nucléases suivi d'une dernière centrifugation à 16 000 g pendant 1 min. De manière à vérifier l'efficacité de la purification de l'ADN, la solution d'ADN a été mise à migrer dans un gel d'agarose à 1 % p/v. Dans le cas d'une purification correcte de l'ADN, une seule bande d'électrophorèse doit être observée par solution d'ADN purifié. Si tel était le cas, l'ADN purifié a été envoyé au séquençage. Comme précédemment, la séquence obtenue a été alignée avec les séquences utilisées pour désigner les amorces. Si l'alignement était satisfaisant, des amorces pour Q-PCR ont, comme précédemment, été définies à l'aide de Primer3Plus.

Dans le cas (3), le couple d'amorces défini ne permettait pas l'amplification de la séquence ciblée. Les amorces choisies n'étaient donc pas adaptées et d'autres amorces ont dû être déterminées au niveau d'autres régions bien conservées sur les alignements précédemment réalisés.

Dans le cadre de ce travail, 10 gènes, marqueurs de quatre grandes fonctions, ont finalement pu être séquencés chez *U.* cf. *pusilla*: facteur d'élongation 1 alpha (*ef1-α*), catalase (*cat*), superoxyde dismutase Mn (*sod Mn*), ferritine (*fer*), nitric oxyde synthase (*nos*), hemocyanine (*hemo*), sous-unité 1 de la cytochrome C oxydase (*cox I*), ARNr 12S (12S), récepteur X aux rétinoïdes (*rxr*) et vitellogénine (*vtg*). Les numéros d'accession et les fonctions de ces gènes ainsi que les amorces désignées pour les analyses par Q-PCR sont reportés dans le Tableau II. 4.

Tableau II. 4 Gènes ciblés dans le cadre d'analyses par Q-PCR (**Chapitres III-B**, **IV** et **V-A**) et séquences des amorces permettant l'amplification des régions d'intérêt.

Gène	Fonction	Numéro d'accesion	Séquence des amorces 5'-3'
Factor élongation 1-alpha (*ef1-a*)	"Housekeeping gene"	KY555759	CACGTTCTGCTGTGCTTCAC[1] AGCCAGAGATGGGGAGGATT[2]
Catalase (*cat*)	Réponse au stress oxydant	KY555763	GACCCAGACTATGCCATCCG[1] TAGTTCTTGGGGTTGCGGTC[2]
Superoxyde dismutase Mn (*sod Mn*)	Réponse au stress oxydant	KY555768	TCAAGTACGCTATGGTGGCC[1] GCCCCCTCCATTGAACTTGA [2]
Ferritine (*fer*)	Réponse au stress oxidant, réponse immunitaire	K555762	GGATCACGCCCAGAAGTTCA[1] GGTCGTTGTTCTTGTTGGCC[2]
Nitric oxyde synthase (*nos*)	Réponse immunitaire	KY555765	GTGGTCCATGATGGTCACGT[1] CCTGTGTGACATCCATCGCT[2]
Hemocyanine (*hemo*)	réponse immunitaire, métabolisme mitochondrial	K555761	ATGGTGGCTACTTCCCCTCT[1] CGATCAGGTCACCCACAACA[2]
Sous-unité I de la cytochrome oxydase C (*cox I*)	Métabolisme mitochondrial	KY555767	GGAGCCGTTAATTTTATAACCACA[1] TAGGATCTCCTCCTCCAGCA[2]
ARNr 12S (12S)	Métabolisme mitochondrial	KY555758	TTAACTATTAGCTGCACCTT[1] CAAAGAACTTGGCGGTGTT[2]
Récepteur X aux retinoïdes (*rxr*)	Processus endocriniens	KY555764	AGGAGGAACGACAACGAACC[1] GGCCCATTCCACCAGTTGTA[2]
Vitellogenine (*vtg*)	Processus endocriniens	KY555760	AGACCAACGGCACCTATGTG[1] GGTCCCTGAGGATTGCTGAC[2]

[1] : amorce sens ; [2] : amorce anti-sens

b) Extraction des ARN totaux

L'extraction de l'ARN est plus délicate que celles de l'ADN du fait notamment de l'omniprésence de ribonucléases (RNAses). Des précautions particulières ont donc été prises lors de la purification de ces molécules. Les paillasses de laboratoires étaient décontaminées (éthanol et RNAse away ; Molecular BioProducts), toute la manipulation se faisait avec des gants non contaminés et l'ensemble du matériel (cônes, tubes, …) devait être certifié « RNAses free » par le fabriquant. Par ailleurs, l'eau utilisée pour la préparation des différents réactifs nécessaires aux extractions ARN était préalablement traitée contre les RNAses par le diethyl-pyrocarbonate (DEPC).

L'extraction des ARN totaux a été effectuée à partir d'hépatopancréas (**Chapitres III-B, IV** et **V-A**) et de muscle abdominal (**Chapitres IV** et **V-A**) de *U.* cf. *pusilla*, en lien avec les mesures de bioaccumulation (**II.3.**). Différentes techniques permettent l'extraction des ARN et plusieurs kits commerciaux sont disponibles. Dans le cadre de ce travail, l'extraction des ARN s'est faite par précipitation différentielle de l'ARN et de l'ADN à l'aide du kit « SV Total RNA isolation system » (Promega) selon les recommandations du fournisseur. L'extraction débutait par un broyage mécanique des muscles abdominaux et/ou des hépatopancréas à l'aide de billes de céramique (diamètre 1,4 mm ;

MP Biomedicals) dans 500 µL de RNAse lysis buffer. A ce broyat étaient ajoutés 500 µL de phénol-chloroforme-alcool isoamylique (25:24:1), l'ensemble était alors vortexé puis centrifugé 5 min à 13500 rpm et à température ambiante. Le phénol-chloroforme-alcool isoamylique est un solvant organique permettant d'éliminer les protéines hydrophobes et amphiphiles présentes dans les tissus. Une fois les tubes centrifugés, il était ainsi possible d'identifier trois phases : une phase surnageante hydrophile, une phase intermédiaire amphiphile et une phase hydrophobe dans le fond du tube. La phase surnageante hydrophile contenant les acides nucléiques était alors prélevée et déposée dans un tube à laquelle étaient ajoutés 450 µL d'éthanol à 75 %. Une fois mélangé, le contenu du tube était déposé sur une colonne collectrice, qui était centrifugée 1 min à 13500 rpm et à température ambiante. Les acides nucléiques étaient retenus sur la colonne de filtration et le filtrat éliminé. Un premier lavage était effectué par ajout de 600 µL d'une RNA wash solution. L'ajout de cette solution était suivi par une deuxième centrifugation des colonnes durant 1 min et une troisième durant 2 min, à 13500 rpm et à température ambiante; le filtrat était jeté à la fin de chaque centrifugation. De manière à dégrader les ADN fixés aux colonnes d'extractions, celles-ci étaient incubées à 37 °C durant 15 min après ajout de 49 µL d'une solution permettant l'hydrolyse des molécules d'ADN (40 µL de Yellow Core Buffer + 5 µL de $MnCl_2$ [0,09 M] + 5 µL DNAsel [1 U μL^{-1}]). Au bout de 15 min d'incubation, les réactions d'hydrolyse de l'ADN étaient stoppées par ajout de 200 µL d'une DNAse stop solution. L'incubât était alors centrifugé 1 min à 13500 rpm, puis le filtrat jeté. Un nouveau lavage était effectué par ajout de 600 µL de RNA wash solution avant centrifugation à 13500 rpm pendant 1 min à température ambiante. Un dernier lavage à l'aide de 250 µL de RNA wash solution était effectué, l'ensemble était alors centrifugé pendant 2 min à 13500 rpm et à température ambiante. La colonne de centrifugation était alors récupérée et les ARN totaux extraits élués dans 50 µL d'eau (H_2O sans nucléases). Une dernière centrifugation durant 1 min à 13500 rpm permettait de récupérer les ARN totaux purifiés. Les extraits d'ARN totaux étaient alors stockés à – 80 °C jusqu'à ce que la retro-transcription soit effectuée.

c) Retro-transcription

Les ARN étant des molécules relativement instables et fragiles, leur extraction était directement suivie par une étape de rétro-transcription permettant l'obtention de molécules d'ADN complémentaire (ADNc), plus stables. Cette étape est par ailleurs nécessaire à la réalisation de Q-PCR, la *Taq* polymérase étant incapable de réaliser une polymérisation à partir d'une matrice d'ARN. Avant d'effectuer la retro-transcription (RT) des ARN totaux extraits, ceux-ci ont été dosés de manière à connaitre la quantité d'ARN extrait ainsi que la qualité de l'extraction. Ce dosage a été effectué grâce à un spectrophotomètre microplaques (Spectrophotomètre Multi-Volume Epoch ; Biotek) dont le fonctionnement a été précisé précédemment (**II.4.1.a**).

Après avoir déterminé la qualité et la concentration de chaque extrait, la RT a été réalisée, permettant l'obtention de molécules d'ADNc à partir d'ARN totaux (Figure II. 12). L'enzyme effectuant cette réaction est une transcriptase inverse. Afin d'initier la RT, des amorces non spécifiques sont utilisées.

Elles permettent de retro-transcrire l'intégralité des ARN messagers. Il s'agit des amorces poly-T (fixation au niveau de la queue poly-A des ARN) ainsi que des amorces aléatoires de 6 nucléotides (hexaprimers se fixant sur d'autres sites des ARN longs et sur les ARN mitochondriaux, ces derniers ne disposant pas de queue poly-A). Les RT-PCR ont été réalisées à l'aide du kit « Go Script Reverse Transcription System » (Promega) selon les recommandations du fournisseur. Dans un premier temps, un mélange d'amorces (1 µL oligodT à 1 µg μL^{-1} + 1 µL d'hexaprimers à 1 µg μL^{-1}) et d'ARN totaux (1 µg d'ARN + H_2O sans nucléases qsp 10 µL pour l'hépatopancreas et 10 µL d'extrait d'ARN pour le muscle abdominal) a été incubé dans un thermocycleur 5 min à 70 °C afin de linéariser les ARN. Puis, le mélange se refroidissait progressivement jusqu'à la température de 4 °C. A ce premier mélange était alors ajouté un seconde mélange contenant un tampon de réaction (4 µL de GoScript™ 5X Reaction Buffer), du $MgCl_2$ (1,5 µL à 25 mM), des dNTPs (1 µL à 10 mM), une transcriptase inverse (GoScript™ Reverse Transcriptase, 0,5 µL) ainsi qu'une enzyme inhibitrice des ribonucléases (Recombinant RNasin Ribonuclease Inhibitor, 0,5 µL à 40 U μL^{-1}). L'ensemble était chauffé 5 min à 25 °C, ce qui permettait l'hybridation des amorces aux ARN, puis était incubé 1 h à 42 °C. Cette dernière étape permettait la synthèse d'un brin d'ADNc complémentaire à chacun des ARN extraits (Figure II. 12). Les ADNc ainsi obtenus étaient conservés à – 20 °C jusqu'aux étapes de Q-PCR.

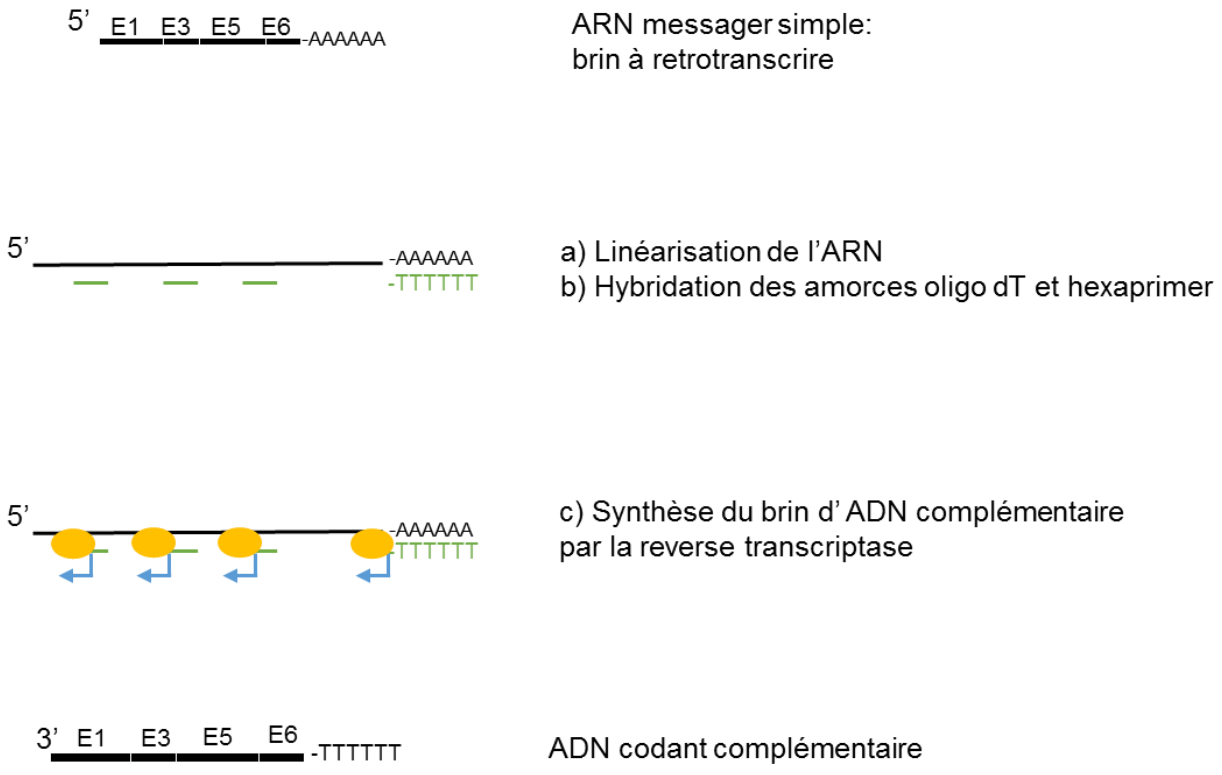

Figure II. 12 Principe général de la rétro-transcription (RT). A partir d'ARN messager simple brin il est possible de synthétiser une molécule d'ADN complémentaire (ADNc) uniquement composée de séquences codantes (i.e. sans introns). Cette molécule d'ADNc servira de base à la réalisation de Q-PCR permettant de quantifier les transcrits d'un échantillon. Ex : exon x.

d) Quantification de l'expression génique par PCR quantitative ou Q-PCR

La technique de Q-PCR ou PCR quantitative en temps réel offre la possibilité de suivre la quantité d'ADN présente dans le milieu réactionnel tout au long du processus de PCR grâce à l'utilisation d'une molécule fluorescente, le SYBR Green I. Ce fluorochrome est un intercalant de l'ADN qui possède la caractéristique de ne fluorescer que dans cette condition. Le pic de fluorescence du SYBR Green I est centré dans le vert (λ = 520 nm). L'intensité de la fluorescence émise est directement proportionnelle à la quantité d'ADN double brin présent.

Les réactions de Q-PCR ont été réalisées dans des plaques de 96 puits en polypropylène. Le milieu réactionnel comportait 10 µL d'un tampon de réaction contenant le SYBR Green I et la *Taq* polymerase (GoTaq qPCR master Mix^{TM} 2X, Promega), 1 µL de chaque amorce (2 µM), 5 µL d'ADNc dilué au dixième et 3 µL d'eau sans nucléases. Les Q-PCR ont été réalisées à l'aide de la machine Mx3000P (Stratagene ; **Chapitres III-B** et **IV**) ou du LightCycler 480 (Roche ; **Chapitre V-A**). Dans le cas du Mx3000P, les cycles de Q-PCR débutaient par une étape de dénaturation de l'ADN à 95 °C pendant 10 min. Cette étape était suivie par 45 cycles constitués de 3 phases : 95 °C pendant 30 s (dissociation des deux brins d'ADN complémentaires), puis 60 °C pendant 30 s (hybridation des amorces sur l'ADN) et 72 °C pendant 30 s (synthèse des brins d'ADN). Dans le cas du LightCycler 480, les cycles de Q-PCR débutaient par une étape de dénaturation de l'ADN à 95 °C pendant 2 min. Cette étape était suivie par 50 cycles constitués de 2 phases : 95 °C pendant 15 s, puis 60 °C pendant 1 min (hybridation des amorces sur l'ADN et synthèse). Dans les deux cas, la spécificité des réactions a été déterminée pour chaque réaction à l'aide d'une courbe de dissociation. Cette courbe a été obtenue en suivant les niveaux de fluorescence du SYBR Green I au cours d'un chauffage progressif des produits de q-PCR de 60 à 95 °C, le pic de fluorescence étant spécifique de la région ciblée (Figure II. 13A).

Typiquement, le profil d'une Q-PCR est une courbe sigmoïde (Figure II. 13B). On définit ainsi le point Ct (Cycle Threshold) comme le nombre de cycles à partir duquel le signal de fluorescence est supérieur au bruit de fond (fluorescence intrinsèque du SYBR Green I), ce qui indique que la quantité de fragment amplifié est à l'origine d'une fluorescence supérieure au bruit de fond. Le Ct marque le début de la phase exponentielle de synthèse d'ADN au cours de laquelle on a doublement du nombre de copies d'ADN à chaque cycle. Plus le nombre de molécules d'ADN cibles initialement présentes est important et plus le nombre de cycles d'amplification (et donc le Ct) nécessaire pour atteindre une fluorescence supérieure au bruit de fond sera petit. Dans le cadre de cette étude, des Q-PCR relatives ont été réalisées. La valeur de Ct des gènes ciblés au cours de ce travail a ainsi été comparée à la valeur de Ct d'un gène de référence (*ef1*-α) : $\Delta Ct = Ct_{\text{gène d'intérêt}} - Ct_{\text{gène de référence}}$. Un niveau d'expression relative a finalement été calculé selon la méthode $2^{-\Delta Ct}$ (Livak and Schmittgen, 2001). A partir de ces niveaux d'expression relative, il a été possible de définir des facteurs d'inductions (IF) en comparant l'expression relative moyenne chez des individus soumis à un traitement *x* à l'expression relative moyenne observée chez des individus témoins.

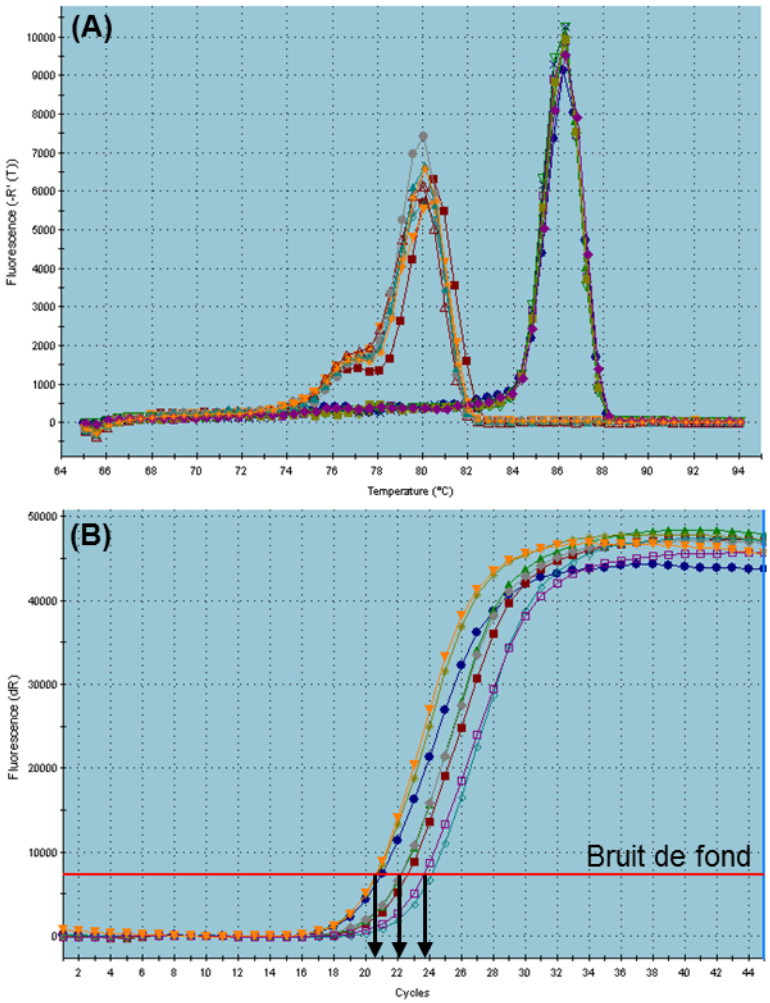

Figure II. 13 (A) Courbes de dissociation. Le pic de fluorescence est spécifique à la région d'ADN d'intérêt ciblée. Sur cet exemple, on observe deux pics de fluorescence, caractéristiques de deux régions d'intérêts distinctes et (B) Profil classique de fluorescence du SYBR Green I au cours d'une réaction de Q-PCR. La ligne rouge indique le bruit de fond de fluorescence. L'intersection entre la droite du bruit de fond et la courbe de florescence du SYBR Green I permet de déterminer un nombre de cycle de Q-PCR seuil (Ct) propre à chaque échantillon (flèches noires) correspondant à une amplification significative de l'ADNc. Cette valeur seuil est inversement proportionnelle à la quantité d'ADNc présente dans l'échantillon.

II.5. Quantification du remaniement sédimentaire

Le remaniement sédimentaire est la conséquence des activités de fouissage (pouvant mener à l'édification de structures biogéniques comme des terriers ou des tubes), de locomotion, de nutrition et de défécation des organismes benthiques. Ces activités induisent des mouvements de particules dans la colonne sédimentaire et la colonne d'eau, les bioturbateurs « mélangeant » ainsi les sédiments (Solan and Wigham, 2005; Kristensen *et al.*, 2012). Ce « mélange » n'entraine néanmoins pas forcément une homogénéisation des sédiments puisque les transports des particules peuvent être orientés ou que les organismes peuvent spécifiquement en déplacer certaines (tri des particules lors des activités de nutrition par exemple) (Kristensen *et al.*, 2012). De ce fait, les bioturbateurs peuvent contribuer à l'hétérogénéité des sédiments, créant des zones présentant des granulométries, porosités et contenus en matière organiques variés. Différentes méthodes permettant de quantifier le remaniement sédimentaire ont été passées en revue par Maire *et al.* (2008).

Dans le cadre de ce travail, une méthode de quantification indirecte utilisant des traceurs particulaires sous forme de particules fluorescentes ou « luminophores » a été utilisée (Mahaut and Graf, 1987) (**Chapitre V-A**). Cette méthode permet la quantification de la composante verticale du remaniement sédimentaire. Elle consiste en l'ajout à l'interface eau-sédiment de particules qui vont être progressivement enfouies *via* les activités de la faune benthique. La distribution verticale de ces traceurs après un temps d'incubation plus ou moins long est ensuite déterminée. Un modèle mathématique permettant de décrire les profils verticaux est ensuite appliqué sur les profils réels. Ce modèle permet d'estimer l'intensité du remaniement sédimentaire *via* la génération de coefficient(s) d'intensité du remaniement sédimentaire.

Présentement, environ 10 g de luminophores ont été déposés à l'interface eau-sédiment dans chacune des unités expérimentales contenant *U.* cf. *pusilla* 2 h avant le début des expériences. La distribution verticale de ces traceurs dans le sédiment a été étudiée après une période d'incubation de 3, 7 et 14 jours (**Chapitre V-A**). La résolution verticale était de 1 cm. Les échantillons de sédiment collectés ont d'abord été lyophilisés avant d'être broyés et passés au tamis (maille = 1 mm). Environ 1 g de sédiment (i.e. ca. 0,5 à 1 % du poids total d'un échantillon de sédiment) a ensuite été dispersé sur une boite à fond noir de façon à obtenir une couche de sédiment la moins épaisse possible. Les échantillons ont ensuite été photographiés sous lumière UV à l'aide d'un appareil photo numérique (Nikon D100) sur lequel avait été monté un filtre jaune. Une analyse semi-automatique des images obtenues a été réalisée à l'aide du logiciel Lumino développé à cette intention au laboratoire EPOC par A. Romero-Ramirez. Ce logiciel permet de compter le nombre de luminophores sur chacune des images et *in fine* de générer un profil vertical de distribution des luminophores. Les profils verticaux obtenus ont été ajustés à un modèle mathématique de manière à obtenir un taux de remaniement sédimentaire (Figure II. 14). Le modèle utilisé était un modèle biodiffusif- bioadvectif. Il permet de génèrer deux coefficients reflétant un remaniement sédimentaire aléatoire sur de petites distances (coefficient D_b dit « coefficient de

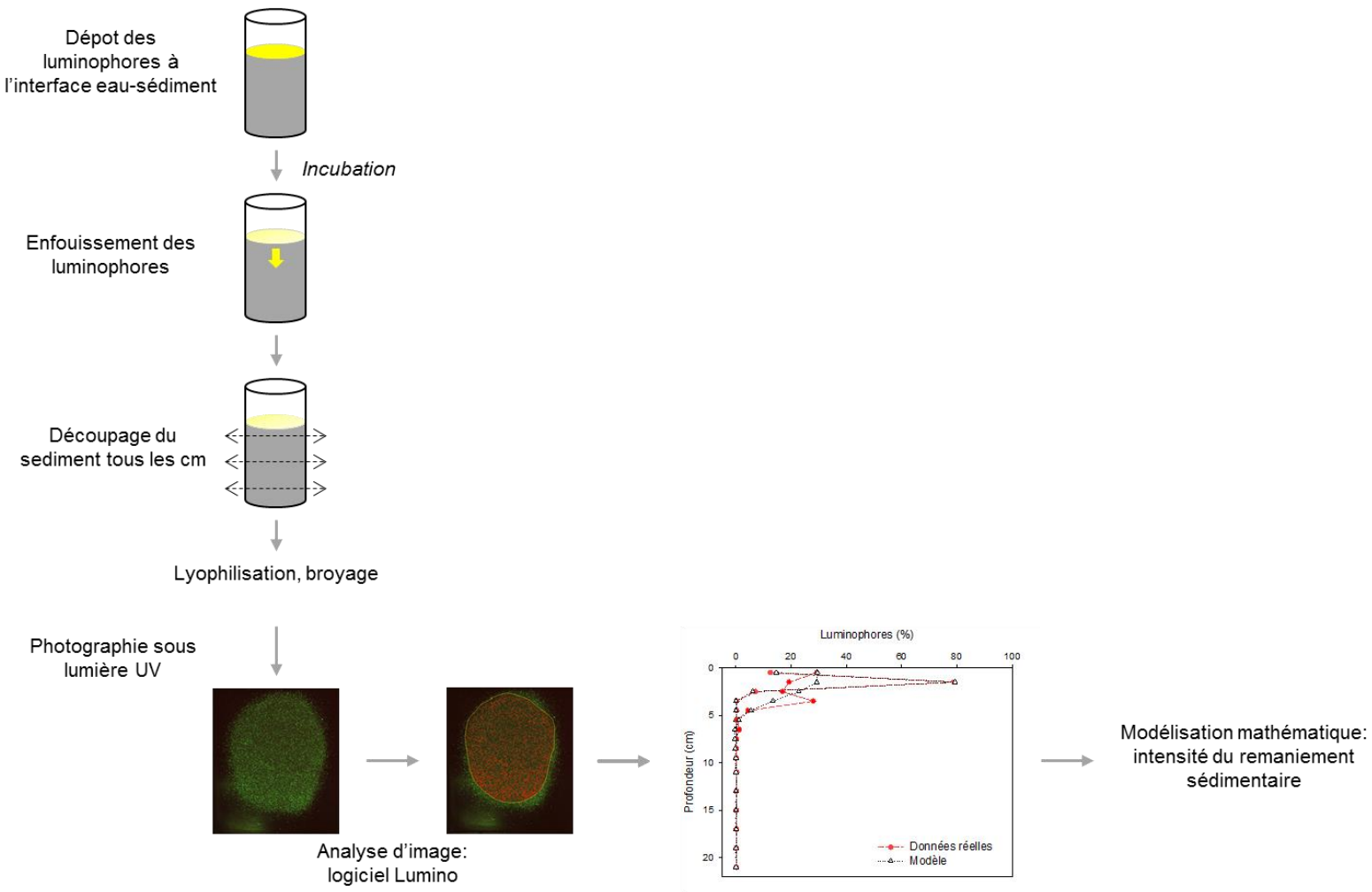

Figure II. 14 Principe de quantification du remaniement sédimentaire à l'aide de particules fluorescentes ("luminophores"). Dans cet exemple, les luminophores sont déposés à la surface d'une carotte de sédiment cylindrique. Ils sont progressivement enfouis par l'activité des organismes. Après un temps d'incubation plus ou moins long, les carottes sont découpées tous les cm, les échantillons de sédiments sont alors lyophilisés et finalement broyés. Un aliquot de chaque échantillon est photographié sous lumière UV. Le traitement d'image s'effectue avec le logiciel Lumino permettant de compter le nombre de luminophores dans une zone d'intérêt donnée pour chaque échantillon. Finalement, un profil vertical de luminophores est tracé sur lequel un modèle mathématique est appliqué, permettant *in fine* la quantification de l'intensité du remaniement sédimentaire.

biodiffusion ») et un remaniement sédimentaire non-local (coefficient v_b dit « coefficient de bioadvection ») (Meysman *et al.*, 2003; Maire *et al.*, 2008):

$$\frac{\partial^2 C}{\partial t} = D_b \frac{\partial^2 C}{\partial z^2} - v_b \frac{\partial C}{\partial z}$$

avec C la concentration en luminophores (%), t le temps (années) et z la profondeur (cm). La qualité de l'ajustement entre le profil réel et le profil modélisé a été vérifiée par inspection visuelle et par la méthode des moindres carrés.

CHAPITRE III

Pression parasitaire et contamination métallique chez *Upogebia* cf. *pusilla* : caractérisation de la population du bassin d'Arcachon

Parasite interactions in the bioturbator *Upogebia* cf. *pusilla* (Decapoda: Gebiidae): a case of amensalism?

DAIRAIN A., GONZALEZ P., LEGEAY A., MAIRE O., DAFFE G., PASCAL L. and de MONTAUDOUIN X.

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Abstract

The mud shrimp *Upogebia* cf. *pusilla* is a deep-burrowing bioturbator widespread along the Northeast Atlantic and Mediterranean coasts, where it is recognized as an ecosystem engineer. Parasitism is an important factor impacting such species' activities, and thus ecosystem functioning. At least two parasite species occur in *U.* cf. *pusilla*: the bopyrid isopod *Gyge branchialis* and an undescribed trematode. This study, carried out in Arcachon Bay, France (44°40′N, 1°10′W), over two years (2014–2015), had two goals: (1) to identify the trematode occurring in *U.* cf. *pusilla* and (2) to assess the interactions between the two parasites within their host. Using molecular techniques, the trematode was identified as *Maritrema* sp. (Microphallidae). Monthly samples taken over two years at a single site, and a ten-site spatial survey in June 2014, showed that there was a negative association in the occurrence of the two parasites in their host over time and amongst sites: bopyrid-infested mud shrimp harbored lower trematode infections compared to bopyrid-free individuals. In addition, the abundance of trematode was lower in the bopyrid-infested gill compared to the uninfested gill of bopyrid-infested individuals. It suggests that *G. branchialis* interferes with *Maritrema* sp. establishment in the mud shrimp. Conversely, the trematode appeared not to have a negative effect on bopyrid presence. The reduction of *Maritrema* sp. infection by *G. branchialis* is mainly due to the alteration of *U.* cf. *pusilla* fitness (indirect interaction) and, to a lesser extent, to overlap of parasites' niches (direct interaction). Accordingly, interactions between these two parasites can be classified as amensalism.

Key words: *Upogebia* cf. *pusilla* mud shrimp, trematode parasite, bopyrid isopod, parasite interactions, amensalism.

I. Introduction

Predation and competition are traditionally considered as the main structuring determinants of metazoan populations and communities. However, it is now well recognized that parasitism is a key biotic interaction that can fundamentally influence host populations and communities (Minchella and Scott, 1991; Poulin, 1999; Mouritsen and Poulin, 2010), In particular, parasites can regulate host populations because of significant effects on host fitness, including their reproduction (Schulte-Oehlmann *et al.*, 1997; Dumbauld *et al.*, 2011), growth (Sousa, 1983; Mouritsen and Jensen, 1994) and/or survival (Jensen *et al.*, 1996; Meißner and Bick, 1997; Ferreira *et al.*, 2005). At a broader scale, the biomass and production of parasites in estuarine systems are substantial (Thieltges *et al.*, 2008; Kuris *et al.*, 2008) and they play significant roles in ecosystem functioning (Mouritsen and Poulin, 2002, 2005; Thomas *et al.*, 2005). In particular, parasites can interfere with their host engineering functions or act themselves as ecosystem engineer species (Thomas *et al.*, 1998, 1999, 2005).

In natural communities, a one-parasite-one-host relationship is rare and concomitant infection involving multiple parasites is the rule (Cox, 2001). Consequently, parasites form infrapopulations and infracommunities inside their hosts. Therefore the outcome of a single host-parasite relationship can be influenced by interactions between the multiple parasite species themselves. Like free-living species sharing a habitat and resources, interactions between parasites occur (Lim and Heyneman, 1972; Dobson, 1985; Petney and Andrews, 1998), which may be direct or indirect in nature (Poulin, 1998). Among them, interspecific competition is the most commonly described form of direct interaction (Poulin, 1998, 1999). Indeed, it is likely that parasites belonging to the same guild and/or occupying the same niche directly compete for food, space and other resources (Poulin, 2001; Pedersen and Fenton, 2007; Mideo, 2009). A parasite species can also indirectly influence others, such as in a case of immune-mediated apparent competition where parasites compete to evade the immune responses they elicit (Cox, 2001; Pedersen and Fenton, 2007; Mideo, 2009). In addition, cases of predation of one parasite species on another have also been described. For example, in trematode species, the larval stage of one species commonly feeds on the earlier larval stage of a second species (Lie *et al.*, 1973; Basch and DiConza, 1975; Sousa, 1992). Although interspecific interactions are varied and often antagonistic (Poulin, 1998, 2001), positive interactions between parasites are also reported (Lie *et al.*, 1973; Holmes, 1987; Petney and Andrews, 1998). For instance, simultaneous exposures to diverse parasite species compromise the host immune system leading to an increase in infection success for multiple parasite species (facilitation hypothesis) (Jokela *et al.*, 2000; Krasnov *et al.*, 2005). Some parasites are also preferentially associated within a host because one species can benefit from the strategy of a second, such as in the case of hitch-hiker parasites (Thomas *et al.*, 1997). In light of the potential effects of these complex interactions, including their potential to affect disease dynamics or parasite virulence (Rigaud *et al.*, 2010; Johnson and Hoverman, 2012), they ought to be considered when studying the impacts of parasites on their host.

The gebiidean mud shrimp *Upogebia* cf. *pusilla* is a deep-burrowing bioturbator living in intertidal and upper sublittoral zones along the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff, 1979; Dworschak, 1983). In Arcachon Bay, France, *U.* cf. *pusilla* chiefly occurs in intertidal flats covered by the dwarf eelgrass *Zostera noltei*, where it plays a key role in biogeochemical exchanges at the sediment-water interface (Pascal *et al.*, 2016a). Through their bioturbation activities, gebiidean mud shrimp structure benthic habitats, impact nutrient cycling (D'Andrea and DeWitt, 2009; Pillay and Branch, 2011; Pascal *et al.*, 2016a) and influence benthic communities (Posey *et al.*, 1991; Dittmann, 1996; Papaspyrou *et al.*, 2005). Thus, they are recognized as major ecosystem engineers (Jones *et al.*, 1997).

The bopyrid isopod *Gyge branchialis* is a parasite of *U.* cf. *pusilla* (Bonnier, 1900; Tucker, 1930; Bourdon, 1968) which lives in one (very rarely both) of the two gill chambers of its host. Bopyrid parasites have substantial impacts on mud shrimp. For instance, the presence of these parasites is related to low host weight in different host/parasite systems (Smith *et al.*, 2008; Pascal *et al.*, 2016b). At a broader scale, bopyrid isopods can also be significant drivers of host population dynamics since they can castrate infected females (Dumbauld *et al.*, 2011; Pascal *et al.*, 2016b). These infertile individuals remain in the population where they still compete with bopyrid-free individuals (Dumbauld *et al.*, 2011). It is also likely that this parasite alters its host's activity, which may indirectly impact the environment through host activities involved in engineering processes (Thomas *et al.*, 1999). In addition to the bopyrid isopod, trematode parasites have also been recorded in the mud shrimp *U.* cf. *pusilla* (Dworschak, 1988). Trematodes are endoparasites found as metacercariae in this species, meaning they use *U.* cf. *pusilla* as a second intermediate host (see Ginetsinskaya, 1988 for details on trematode life cycles). There is little information available on this host-parasite association. Over three study sites in the northern Adriatic Sea, Dworschak (1988) recorded trematode prevalence of 5–80 % but only provided qualitative data regarding the abundance of the trematode (i.e. mean number of parasite larvae per host). Furthermore, although this trematode species may be very prevalent, it has not yet been identified.

To date, interactions between these two parasite species (isopod and trematode) in the host have not been studied. In general, few [*Upogebia* spp. + bopyrid] associations have thus far been examined (reviewed by Pascal *et al.*, 2016b). Multiple parasite infections of *Upogebia* spp. have not been investigated (but see Sheehan *et al.*, 2011 in a caridean shrimp). Thus, the two main objectives of this study were: (1) to provide a taxonomic identification and recognition of the trematode in *U.* cf. *pusilla*; (2) to investigate potential interactions between the isopod *G. branchialis* and trematode parasites in their host over time (two years monthly sampling) and space (ten sites) in Arcachon Bay, France.

II. Materials and Methods

II.1. Biology of *Upogebia* cf. *pusilla*

The mud shrimp *Upogebia* cf. *pusilla* is a gonochoric crustacean with a life span estimated at > 5 years (Dworschak, 1988). In Arcachon Bay, it preferentially inhabits intertidal seagrass meadows where densities can reach 39 ind. m^{-2}. It lives in a deep (up to 49 cm) and complex burrow connected to the sediment-water interface by several distant openings (Pascal, 2017). At low tide, it experiences at least 3 h of emersion. So far, *U.* cf. *pusilla* has never been collected in subtidal areas of Arcachon Bay despite extensive sampling campaigns (341 samples; RESOMAR 2017). However, it is possible that the gear used (grab, dredge) was not appropriate for capturing deep-burrowing, highly mobile invertebrates.

There is little information about *U.* cf. *pusilla* reproduction, from copulation to recruitment. For the closely related species *U. noronhensis*, the male constructs additional branches to its burrow in order to connect with a female burrow and mate (Candisani *et al.*, 2001). It is the only occasion when two adult mud shrimps have been found to occupy the same burrow. *Upogebia* cf. *pusilla* likely has the same behavior since two individuals are frequently sampled from the same burrow in the breeding period (personal observation). In Arcachon Bay, females can reproduce at a total length (TL) of > 31 mm, and are ovigerous in May–August. Larvae are released into the water column after an incubation period of eight weeks (Dworschak, 1988). Larval development and recruitment remain a "black box" (Dworschak, 1988; Pascal *et al.*, 2016b). Growth is rapid and maximum TL is soon reached so that it is difficult to identify separate cohorts using size (Pascal *et al.*, 2016b).

The effects of the bopyrid parasite *Gyge branchialis* on *U.* cf. *pusilla* have recently been documented (Pascal *et al.*, 2016b; Pascal, 2017). This ectoparasite disrupts the mud shrimp's reproduction: females are castrated (Pascal *et al.*, 2016b), males are feminized and their gonad seems to be atrophied (Tucker, 1930; Pascal *et al.*, 2016b).

II.2. Study sites and sampling procedure

Arcachon Bay (44°40' N, 1°10' W) on the French Atlantic coast is a 180-km^2 macrotidal lagoon, opening to the ocean through two narrow channels (Figure III-A. 1). The tidal cycle is semi-diurnal with an amplitude of 0.8–4.6 m. Mean sea surface temperature changes from 6 °C in winter to 22.5 °C in summer. In 2007, 39 % of the 110-km^2 intertidal area was covered by the dwarf eelgrass *Zostera noltei* (Plus *et al.*, 2010), still considered the largest intertidal seagrass meadow in Europe (Auby and Labourg, 1996).

A two-year temporal survey was performed in January 2014–December 2015 at La Nègue (Figure III-A. 1) in order to determine (1) the prevalence of the bopyrid parasite *G. branchialis* and (2) the abundance of trematode parasites in mud shrimp over time. This site was selected due to the relatively high mud shrimp density. Samples were collected monthly at low tide on this vegetated intertidal mudflat in the middle of the lagoon. In addition, in June 2014, *U.* cf. *pusilla* was sampled at ten different

sites in Arcachon Bay (Figure III-A. 1) in order to evaluate (1) the prevalence of the bopyrid parasite and (2) the abundance of trematode parasites at the scale of the whole lagoon (spatial survey). An average of 54 (40–96) and 66 (30–121) individuals were collected with a bait piston pump during individual sampling sessions for the temporal and spatial surveys, respectively. Each specimen was individually isolated in a plastic bag, to avoid fighting between mud shrimp, and associated appendage loss. In the laboratory, all mud shrimp were then rinsed of sediment with seawater, anaesthetized at low temperature (15 min at -20 °C) and preserved in 4 % formaldehyde solution.

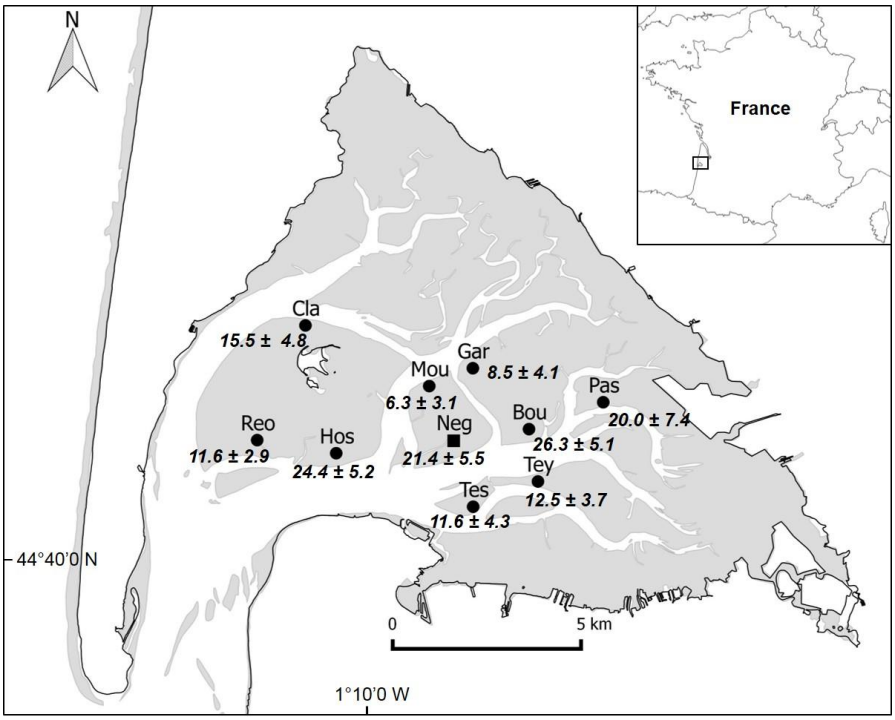

Figure III-A. 1 Location of study sites in Arcachon Bay, France. Square indicates location of temporal survey site (January 2014–December 2015). Prevalence (% ± SE) of bopyrid infestation in *Upogebia* cf. *pusilla* given for each station: Bou: Bourrut; Cla: Le Cla; Gar: Garrèche; Hos: Les Hosses; Mou: Les Moussettes; Neg: La Nègue; Pas: Passant; Reo: La Réousse; Tes: Le Tes; Tey: Le Teychan. Light grey: intertidal area. *N* = 30–121 depending on study site.

II.3. Dissection and macroscopic analysis

Prior to dissection, each *U.* cf. *pusilla* specimen was measured to the nearest mm from the rostrum tip to the telson (Total Length, TL) using a digital caliper. During dissection both gill chambers were first checked for the presence of the ectoparasite *G. branchialis* under a stereomicroscope (Nikon, SMZ1500). Next, the left and right gills were carefully removed and squeezed between two glass plates to count the respective number of trematode metacercariae. The term infestation is used for the ectoparasite (bopyrid) and the term infection for the endoparasite (trematode metacercaria). To assess the suitability of gill infection as a proxy for whole body infection, the distribution of metacercariae throughout the body of *U.* cf. *pusilla* was investigated in November 2015 (*N* = 50). For these samples, in addition to the gills, the visceral mass and abdominal muscle were dissected, removed and squeezed between two glass plates to count metacercariae under a stereomicroscope.

II.4. Trematode identification

The identification of trematode metacercaria was performed using molecular techniques (i.e DNA extraction, PCR amplification and sequencing). Encysted metacercariae were isolated from three or four *U.* cf. *pusilla* and pooled to constitute a replicate. Metacercarial DNA was extracted from three replicates using the DNeasy® Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer-recommended protocol. Partial sequences of the rRNA 18S gene and the complete internal transcribed spacers (ITS1, 5.8S and ITS2) located between the 18S and 28S genes were amplified by PCR. Consensus primer pairs Bb18S5 + Bb18S3 and BbITS5 + BbITS3, previously described for trematodes (de Montaudouin *et al.*, 2014), were used for the 18S and ITS region, respectively. The PCR reaction was carried out in a total volume of 50 µL consisting of 10 µL 5x PCR reaction buffer (Promega), 1µL of dNTPs mix (10 mM), 3 µL of $MgCl_2$ (25 mM), 0.2 µL of Go-*Taq* (5 U μL^{-1}, Promega), 0.5 µL of each primer (100 µM), 1 µL of extracted DNA and 33.8 µL of nuclease-free water. The cycling conditions were as follows: 1 cycle of initial activation at 95 °C for 10 min; followed by 40 cycles at 95 °C for 1 min, 61 °C for 30 s and 72 °C for 1 min; then a final step at 72 °C for 10 min. Amplified PCR products were analyzed by electrophoresis in a 1 % p/v agarose gel stained with ethidium bromide. Then the three different PCR products of each region were sent to GATC Biotech Company for complete double strain sequencing. Finally, the obtained sequences were submitted to GenBank using the BLAST algorithm and compared to available sequences in order to find maximal likenesses.

II.5. Data analysis

Prevalence of *G. branchialis* and abundance of metacercariae in *U.* cf. *pusilla* were calculated according to Bush *et al.* (1997). Prevalence is defined as the percentage of bopyrid-infested individuals within a sample. Trematode abundance is defined as the mean number of metacercariae $host^{-1}$, including trematode-infected and trematode-uninfected mud shrimp. Results are reported as the mean ± SE of *N* replicate measurements.

The relationship between the number of metacercariae found in the gills and the total number of metacercariae in the whole body, relative to the presence/absence of the bopyrid parasite, was estimated using a simple linear regression. Analysis of covariance (ANCOVA) was used to investigate potential differences in the relationship between these two variables with bopyrid occurrence. The distribution of metacercariae in the different organs (i.e. % of metacercariae $organ^{-1}$) was also compared between bopyrid-free and bopyrid-infested mud shrimp using chi-squared tests.

Interactions between bopyrid and trematode parasites were investigated in temporal and spatial surveys at the individual scale (i.e. by comparing bopyrid-infested and bopyrid-free mud shrimp). Data from the temporal survey were pooled and the abundance of metacercariae in gill chambers (dependent variable) was compared in mud shrimp with and without the *G. branchialis* parasite (temporal survey) by ANCOVA (with mud shrimp TL as covariate). Monthly samples consisted of mud shrimp belonging to different cohorts that could not be separated by size-class analysis (Pascal *et al.*, 2016b). Thus, we

decided to analyze the data not by date (sampling month) but by *U.* cf. *pusilla* TL, which is related to age and to time. In addition, a negative temporal relationship between the two parasites was investigated using a linear regression between prevalence of bopyrids and mean abundance of metacercariae in bopyrid-free and bopyrid-infested mud shrimp. Data from the temporal survey were also used to investigate interactions between the two parasites at the organ scale (i.e. by comparing trematode abundance in the right vs. the left gill, and in gill chambers with and without bopyrids in both bopyrid-free and bopyrid-infested mud shrimp, respectively). Abundance of metacercariae in gill chambers (right vs. left in bopyrid-free mud shrimp, and with-bopyrid vs. without-bopyrid in bopyrid-infested mud shrimp) were consequently compared using Wilcoxon signed-rank tests to determine whether one of the two gill chambers was preferentially infected with metacercariae, as well as with respect to bopyrid parasite occurrence in one of the gill chambers. Finally, for the spatial survey data, the abundance of metacercariae in gill chambers (dependent variable) was compared in mud shrimp with and without *G. branchialis* by ANCOVA (with mud shrimp TL as covariate) among sites. Prior to ANCOVAs, a Levene test was performed to assess homogeneity of variances. If necessary, data were log(x+1)-transformed. Differences were considered significant with a $p < 0.05$. All statistics were performed using the statistical software package R (R Core team 2015).

III. Results

III.1. Molecular identification of trematodes from *Upogebia* cf. *pusilla*

Trematode metacercariae collected from *Upogebia* cf. *pusilla* were colorless, encysted and displayed an oval shape with a mean length of 522 µm (381–682) and a mean width of 390 µm (296–503) (N = 100) (Appendix 4). PCR products provided two fragments of 570 bp (accession number KY555767) and 856 bp (accession number KY555766) for the 18S and the ITS sequences, respectively. No identical 18S and ITS sequences were found in GenBank for the resulting nucleotide sequences. However, the BLAST search of the 18S region obtained revealed that this sequence possesses extensive homologies (98 %) with the 18S sequence from the microphallid trematode *Maritrema oocysta* (Cribb *et al.*, 2001), accession number AJ287534.1). Concerning the ITS region, the obtained sequence showed 98 % similarity with the ITS sequences of two unidentified *Maritrema* species (Diaz and Cremonte, 2010; Gilardoni *et al.*, 2011; accession numbers KC222023.1 and KC222022.1, respectively) and from *M. madrynense* (Diaz and Cremonte, 2010; accession number KF575167.1). Thus, BLAST searches for the sequences indicate that trematodes found in *U.* cf. *pusilla* belong to the F. Microphallidae, and more precisely to the genus *Maritrema*. However, the molecular analysis did not allow identification of *U.* cf. *pusilla* trematodes to species since none of the sequences had a 100 % similarity with the 18S and ITS sequences of previously recorded *Maritrema* species.

III.2. Distribution of *Maritrema* sp. metacercariae in *Upogebia* cf. *pusilla*

The 50 dissected mud shrimp were all infected with *Maritrema* sp., with 8–1041 metacercariae host^{-1} (mean 204.1 ± 30.5). The number of metacercariae in gills was positively correlated with the total

number of metacercariae in the whole body (Linear regression, $r^2 = 0.81$, $F(1,48) = 201.10$, $p < 0.001$; Figure III-A. 2). This relationship was not affected by bopyrid presence, i.e. the number of metacercariae in the whole body was 7.7-fold the number of metacercariae in gills, independent of bopyrid presence (ANCOVA, $F = 0.10$, $p = 0.75$). The distribution of metacercariae in their host (summing the abundances of metacercariae in both gills) was similar in bopyrid-free and bopyrid-infested mud shrimp, with trematodes found mostly in the visceral mass; gills were the least-infected organs (Table III-A. 1).

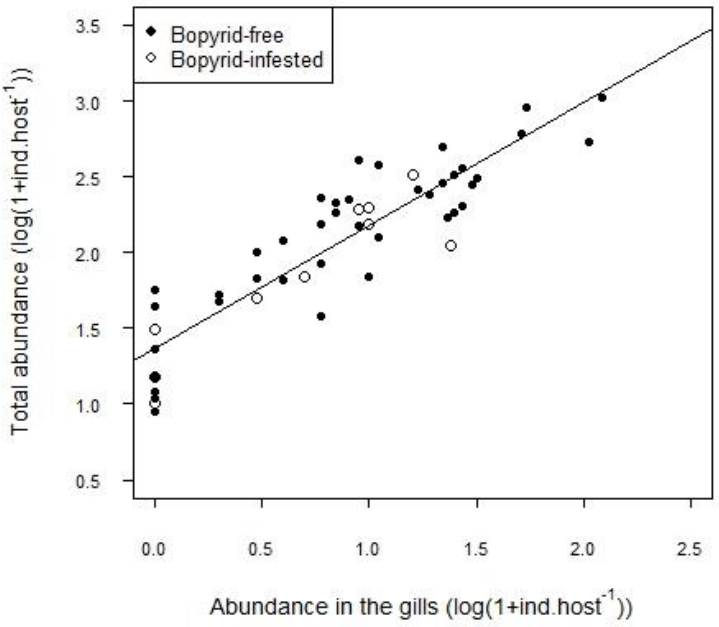

Figure III-A. 2 Total abundance of metacercariae (y, log(1+ind. host^{-1})) as a function of abundance of metacercariae in gills (x, log(1+ind. host^{-1})) of bopyrid-free and bopyrid-infested *Upogebia* cf. *pusilla* and linear model ($y = 0.81x + 3.15$; $N = 50$).

Table III-A. 1 Distribution of *Maritrema* sp. metacercariae in *Upogebia* cf. *pusilla* body (%) with respect to *Gyge branchialis* presence and results of chi-squared tests for different organ locations between bopyrid-free ($N = 40$) and bopyrid-infested ($N = 10$) mud shrimps. Mean (± SE) abundance of metacercariae in parenthesis.

Organ	Bopyrid-free mud shrimp	Bopyrid-infested mud shrimp	*p*
Abdominal muscle	24.8 % (42.2 ± 5.9)	30.6 % (23.6 ± 5.6)	0.39
Gills	5.7 % (17.1 ± 4.1)	5.0 % (7.0 ± 2.4)	0.61
Visceral mass	69.5 % (167.2 ± 28.4)	64.4 % (84.2 ± 28.6)	0.39

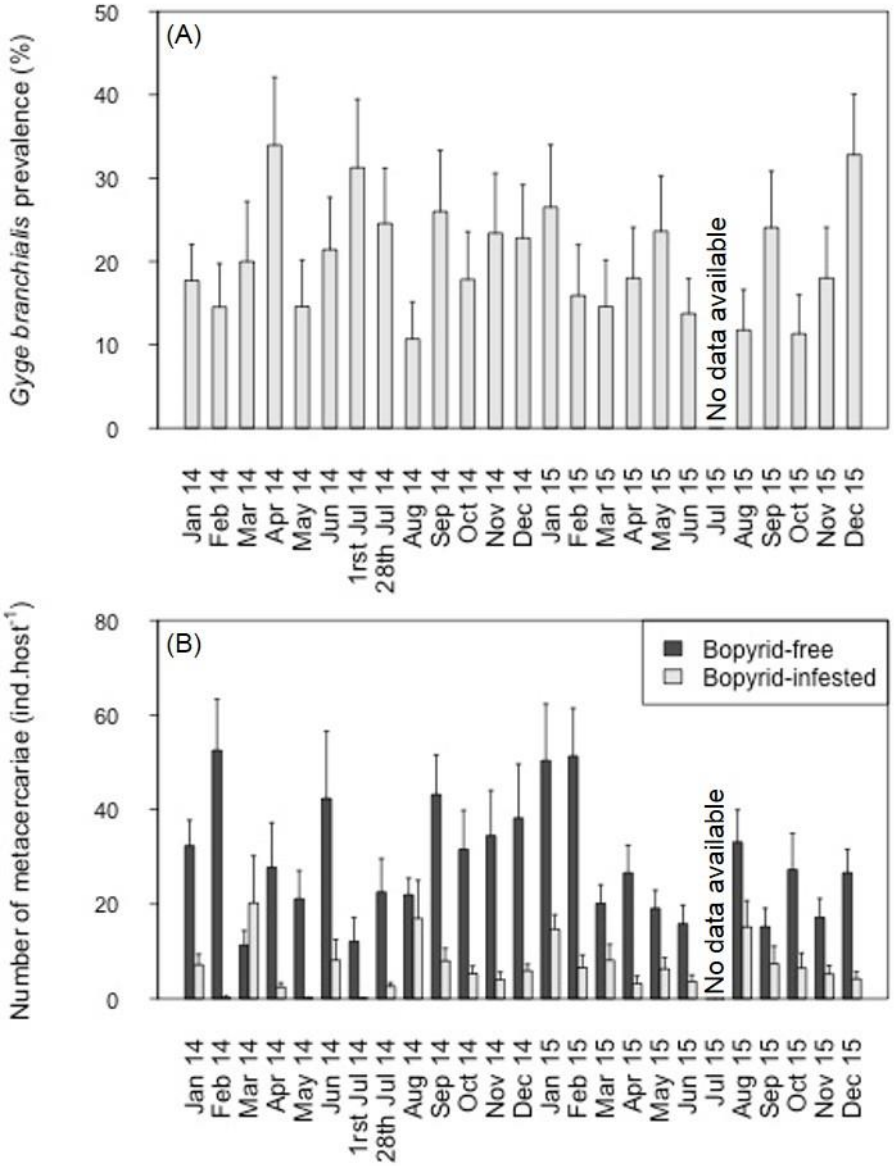

Figure III-A. 3 (A) Prevalences (% + SE) of *Gyge branchialis* in *Upogebia* cf. *pusilla* and (B) Mean abundances of *Maritrema* sp. metacercariae (+ SE) in gill chambers of bopyrid-free and bopyrid-infested *U.* cf. *pusilla* over a two-year survey (January 2014–December 2015) on a single site (La Nègue) in Arcachon Bay, France. *N* = 40–96 depending on sampling month.

III.3. Temporal survey

In a two-year survey at one site, 1317 mud shrimp were examined. Two species of parasites were recorded in the gill chambers of *U.* cf. *pusilla*: the bopyrid ectoparasite *Gyge branchialis* and the trematode endoparasite *Maritrema* sp.. Prevalence of *G. branchialis* in monthly samples was 11.3–34.0 % (October 2015, N = 53, and April 2015, N = 50, respectively) with an overall two-year mean prevalence of 20.5 ± 1.3 % (Figure III-A. 3A). Single gill infestation was predominant (97.0 % of cases) and there was no preference for the left or right gill chamber (chi-squared test, $X^2 = 0.77$, $p = 0.38$). Mean trematode abundance in gills ranged between 8.4 ± 3.5 (July 1^{rst}, 2014, N = 48) and 44.9 ± 9.5 metacercariae.host^{-1} (February 2014, N = 55) by sampling month (Figure III-A. 3B).

The smallest *U.* cf. *pusilla* (19 mm TL) infested with *G. branchialis* had no metacercariae. The smallest mud shrimp infected with at least one metacercaria was 22 mm TL (Figure III-A. 4). The number of metacercariae in gills was positively correlated with mud shrimp TL in both bopyrid-free and bopyrid-infested individuals (Figure III-A. 4, Table III-A. 2). There were on average 3.8-fold fewer metacercariae in gills of bopyrid-infested mud shrimp than in gills of bopyrid-free individuals. This pattern was not due to a negative temporal relationship between the prevalence of *G. branchialis* in the population and the mean abundance of *Maritrema* sp. in bopyrid-free (Linear regression, $r^2 < 0.001$, $F(1,22) = 0.02$, $p = 0.89$) as well as in bopyrid-infested mud shrimp (Linear regression, $r^2 = 0.06$, $F(1,22) = 1.47$, $p = 0.24$).

There was no difference in trematode abundance between left and right gill chambers in bopyrid-free mud shrimp (Wilcoxon signed-rank test, V = 170630, $p = 0.31$). In contrast, in bopyrid-infested mud shrimp the gill chamber with the bopyrid harbored on average 2.5-fold fewer metacercariae compared to the gill chamber without a bopyrid (Wilcoxon signed-rank test, V = 170630, $p < 0.001$).

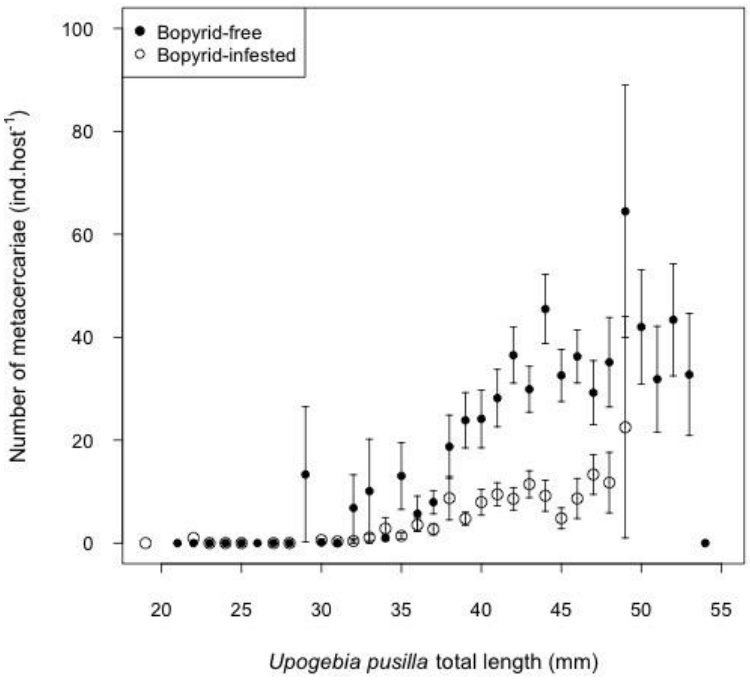

Figure III-A. 4 Relationship between mean abundance of *Maritrema* sp. metacercariae (± SE) in gill chambers and *Upogebia* cf.*pusilla* total length (mm) in bopyrid-free and bopyrid-infested individuals of a two-year survey (January 2014–December 2015) on a single site (La Nègue) in Arcachon Bay, France. *N* = 1317.

Table III-A. 2 Results of ANCOVAs on relationship of *Maritrema* sp. metacercariae abundance in *Upogebia* cf. *pusilla* gill chambers (dependent variable) and its total length (covariate) with respect to *Gyge branchialis* presence (temporal survey, *N* = 1317) and depending on sampling sites (spatial survey, *N* = 659) (independent variables). P-values in bold indicate significant factor effects.

	Factors	**Df**	**F**	***p***
Temporal survey	Total length	1	409.55	**< 0.001**
	Bopyrid occurrence	1	34.53	**< 0.001**
Spatial survey	Total length	1	144.18	**< 0.001**
	Site	9	39.04	**< 0.001**
	Bopyrid occurrence	1	10.63	**1.17×10^{-3}**
	Site * Bopyrid occurrence	9	0.72	0.69

III.4. Spatial survey

A total of 659 *U.* cf. *pusilla*, collected from ten sites in June 2014 (Figure III-A. 1), were examined in the spatial survey. Prevalence of *G. branchialis* over the ten sites was 6.3–26.3 % (Les Moussettes, *N* = 64, and Bourrut, *N* = 76, respectively) with a mean prevalence of 15.8 ± 2.2 % (Figure III-A. 1). Mean trematode abundance in gills ranged from 0.7 ± 0.3 (Passant, *N* = 30) to 90.1 ± 16.7 (Le Cla, *N* = 58) metacercariae.host^{-1} (Figure III-A. 5). Bopyrid-infested mud shrimp were on average 6.4-fold less infected with trematodes than bopyrid-free mud shrimp (Figure III-A. 5). The relationship between trematode abundance and TL also differed among sampling sites with no significant interaction between bopyrid presence and site (Table III-A. 2).

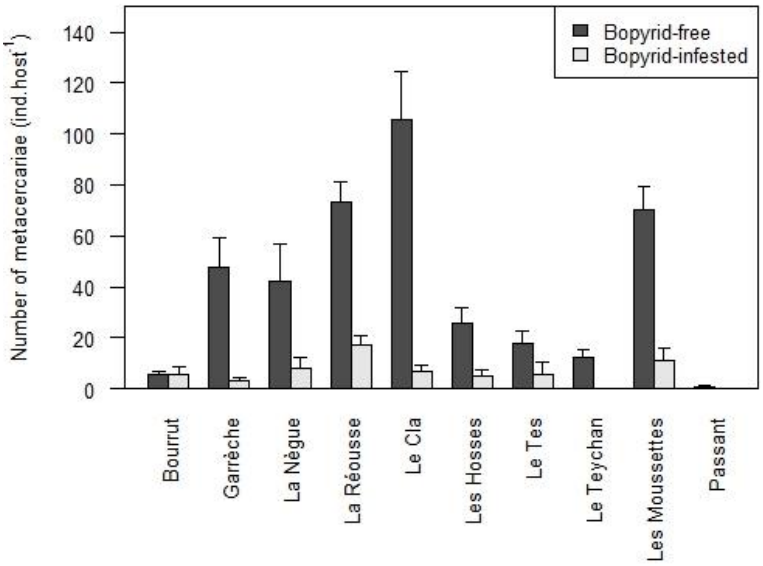

Figure III-A. 5 Mean abundances of *Maritrema* sp. metacercariae (+ SE) in gill chambers of bopyrid-free and bopyrid-infested *Upogebia* cf. *pusilla* from a ten-site survey in Arcachon Bay, France. *N* = 30–121 depending on study site.

IV. Discussion

Parasites of Gebiidae species have received little attention. In 1988, trematode parasites were first reported from *Upogebia* cf. *pusilla* (Dworschak, 1988), but never described however. Here we have molecularly identified a trematode parasite occurring in *U.* cf. *pusilla* in Arcachon Bay, France, and we have examined its interaction with the isopod parasite *Gyge branchialis*. The trematode parasite was identified as belonging to the genus *Maritrema*. This genus encompasses at least 65 species, typically with a three-host life cycle (Presswell *et al.*, 2014). Several common species of gastropods act as first intermediate hosts of *Maritrema* spp. (Martorelli *et al.*, 2004; Studer and Poulin, 2012; Presswell *et al.*, 2014) such as the common mud snail *Peringia* (*Hydrobia*) *ulvae*. *Peringia ulvae* is widespread and abundant in Arcachon Bay's *Zostera noltei* meadows (Blanchet *et al.*, 2004) where *U.* cf. *pusilla* lives. It has already been identified as the first intermediate host for Microphallidae species in this area (de Montaudouin *et al.*, 2003) and could be a potential first intermediate host in the present case. In order to complete their life cycle, these parasites have to be ingested by their definitive hosts. Most *Maritrema* species complete their life cycle in a bird's intestine (Dawes, 1946; Deblock, 2008). Along European western coasts, common definitive host species include the European herring gull *Larus argentatus* and the oystercatcher *Haematopus ostralegus* (Dawes, 1946). These coastal birds commonly feed on crustaceans such as the green shore crab *Carcinus maenas* (Crothers, 1968), which harbors a variety of trematode species (see Torchin *et al.*, 2001 for a review), including *Maritrema* spp. (Pina *et al.*, 2011). Conversely, it seems unlikely that *U.* cf. *pusilla* is preyed on by marine birds due to its cryptic life style. Indeed, *U.* cf. *pusilla* is a deep-burrower (Dworschak, 1983) out of reach of most avian predators (Astall

et al., 1997). Thus, we suggest that the mud shrimp *U.* cf. *pusilla* is an accidental second intermediate host and a dead-end for the parasite *Maritrema* sp..

In natural environments, host organisms are exposed to multiple parasite species that form a dynamic infracommunity within the host (Cox, 2001; Pedersen and Fenton, 2007; Mideo, 2009). These parasites experience their host as a habitat with limited resources (such as space and/or food) (Poulin, 2001; Pedersen and Fenton, 2007; Mideo, 2009), thus direct and indirect interactions between them are expected. In co-infections, there are usually two types of observable consequences of parasite interactions (Thomson, 1980; Poulin, 2001): (1) a numerical response (i.e. a change in the infrapopulation size of one parasite species in response to the other); or (2) a functional response (i.e. a change in resource utilization by one or more parasite species).

In Arcachon Bay, *U.* cf. *pusilla* infestation with the bopyrid isopod *Gyge branchialis* is related to a 3.8-fold lower infection with metacercariae, but with no differences in their distribution among the gills, visceral mass and abdominal muscle in bopyrid-infested and bopyrid-free mud shrimp. In bopyrid-infested mud shrimp, the bopyrid-free gill, as well as the rest of the body, are consistently less infected with trematodes compared to bopyrid-free individuals. It shows that the bopyrid not only prevents cercariae from penetrating the gill it inhabits, it impacts the infection process in the shrimp as a whole. Free-living cercariae encounter mud shrimp because of the water current the shrimp initiates through its burrow and, upon contact, they can actively penetrate the host's cuticle. The bopyrid isopod could reduce the water circulation initiated by the mud shrimp and/or the time the shrimp spends ventilating its burrow. Consequently, the chance of an encounter between trematode parasites and mud shrimp would be reduced by the presence of bopyrid parasites (partially closed encounter filter, sensu Combes, 2001). Previous studies examining the impacts of bopyrid parasites in mud shrimp confirm that parasites belonging to the F. Bopyridae significantly alter the physiological status (Smith *et al.*, 2008; Dumbauld *et al.*, 2011; Pascal *et al.*, 2016b) and reduce activity levels of their host (McGrew and Hultgren, 2011). In so doing, *G. branchialis* could reduce ventilation of the burrow of *U.* cf. *pusilla*, and thereby its overall filtering capacity (Pascal *et al.*, 2016b). Thus, the bopyrid indirectly interferes with the trematode infection process.

Although the impact of *G. branchialis* on trematode infection is mostly indirect, the low metacercaria infection in bopyrid-infested mud shrimp is even lower (2.5-fold lower) in the infested gill chamber compared to the uninfested gill chamber. Here we hypothesize that the bopyrid settles in *U.* cf. *pusilla* before trematodes do, or at least before the mud shrimp develops a strong trematode infection. Since, there is an overlap in the spatial niche of *Maritrema* sp. and *G. branchialis*, these two species compete for space in the mud shrimp gill chamber. The bopyrid isopod settles early in the mud shrimp's life, grows with its host and finally occupies most of the space in the gill chamber (Tucker, 1930; Pascal *et al.*, 2016b). Thus, we suggest that the bopyrid isopod *G. branchialis* greatly impacts the chance of

encountering trematode cercariae but does not impact the compatibility of the host-parasite [*U.* cf. *pusilla* + *Maritrema* sp.] association (encounter filter vs. compatibility filter, Combes, 2001).

At the lagoon-wide scale, bopyrid parasites negatively affect trematode establishment in the mud shrimp *U.* cf. *pusilla*. Indeed, the spatial survey showed that (1) trematode and bopyrid parasites occurred at each of the ten sites and (2) mean trematode abundance was always higher in bopyrid-free mud shrimp compared to bopyrid-uninfested mud shrimp. Although the average trematode abundance differed amongst sites, the positive relationship between trematode abundance and mud shrimp size remains and depends on bopyrid occurrence with no interaction between the two factors. Other local factors may affect trematode density and/or infectivity, but the presence of *G. branchialis* drastically reduces the number of trematodes settling in the host and seems to have a primary impact on the encounter filter (Combes, 2001) between trematodes and mud shrimp in Arcachon Bay.

In conclusion, both direct and indirect interactions occur between the bopyrid isopod *G. branchialis* and the trematode *Maritrema* sp. in the mud shrimp *U.* cf. *pusilla*, with the bopyrid negatively impacting trematode abundance at both the host individual and host organ scale. At the individual scale, the bopyrid isopod strongly interferes with trematode infection, probably by affecting its host fitness. In addition, direct interaction between the two parasites occurs at the organ scale. If we assume that *G. branchialis* infestation occurs before metacercaria infection (or very early in the life of *U.* cf. *pusilla* when the abundance of metacercariae is still low) and that eventually *G. branchialis* grows simultaneously with its host (Pascal *et al.*, 2016b), then it suggests that the interaction between these parasite species is asymmetrical: trematodes are less abundant because of the presence of the bopyrid, while the bopyrid seems unaffected by the trematodes. Thus, interactions between *G. branchialis* and *Maritrema* sp. could be described as amensalism (0/- interaction) (Chaneton and Bonsall, 2000). Finally, due to the negative association in the presence of these two parasites in their mud shrimp host, we suggest that the impacts they have on their host be investigated separately in bopyrid-infested, in trematode-infected, and, to a lesser extent, in doubly parasitized mud shrimp. Such studies will shed further light on how different parasite species may impair the mud shrimp's bioturbation, and therefore its influence as an ecosystem engineer.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no conflict of interest.

Animal rights: All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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BILAN DU CHAPITRE III-A ET PERSPECTIVES

L'étude présentée dans le **Chapitre III-A** consistait en une caractérisation spatiale (à l'échelle du bassin d'Arcachon, 10 sites) et temporelle (suivi mensuel sur deux ans au niveau d'un site) de la pression parasitaire chez la crevette de vase *Upogebia* cf. *pusilla*. A l'échelle locale, *U.* cf. *pusilla* apparait communément parasitée par l'isopode bopyridé *Gyge branchialis* ainsi que par un trématode digène, non identifié jusqu'à présent.

La présence du bopyre *G. branchialis* a été notée sur l'ensemble des sites échantillonnés dans le bassin d'Arcachon et tout au long de l'année au niveau du site échantillonné mensuellement pendant deux ans (« La Nègue »). La prévalence du parasite varie de 6,3 à 26,3 % dans le bassin d'Arcachon.

En ce qui concerne le parasite trématode, l'utilisation de techniques de biologie moléculaire a permis de l'identifier comme *Maritrema* sp. (Microphallidae). Ce trématode infecte les branchies, la cavité viscérale et le muscle abdominal de *U.* cf. *pusilla*. Par ailleurs, ce parasite est retrouvé sous la forme de métacercaires chez *U.* cf. *pusilla* indiquant que la crevette est le (l'un des ?) second hôte intermédiaire de ce parasite. Au vu de la littérature sur les Microphallidea (Dawes, 1946; Deblock, 2008), l'hôte définitif du trématode est probablement un oiseau, la transmission du second hôte à l'hôte définitif se faisant par prédation. Peu de données sont disponibles sur les prédateurs de *U.* cf. *pusilla.* Nonobstant, considérant la vie endogée de cette crevette de vase, il est peu probable qu'elle subisse une forte pression de prédation aviaire. Il est ainsi suggéré que la crevette de vase *U.* cf. *pusilla* constitue un cul-de-sac pour ce trématode. Tout comme le bopyre *G. branchialis*, *Maritrema* sp. a été retrouvé chez les crevettes de vase au niveau de chacune des stations d'étude ainsi que tout au long de l'année. Le calcul de la prévalence d'infection avec *Maritrema* sp. ne constitue pas un bon descripteur de la pression parasitaire dans le cas des trématodes au stade métacercaire car, contrairement au bopyre, le nombre de trématodes peut fortement varier d'un individu à l'autre. Dans le cas de *Maritrema* sp., l'intensité moyenne d'infection (nombre moyen de parasites par hôte infecté dans la population) ou l'abondance des parasites (nombre moyen de parasites par hôte, prenant ainsi en compte la prévalence) sont de ce fait déterminées (Bush *et al.*, 1997). Les intensités moyennes d'infection avec *Maritrema* sp. sont très variables entre les différents sites étudiés, de 0,8 ± 0,4 à 105,3 ± 19,0 métacercaires par individu (moyenne ± erreur standard). De même, sur deux ans, les intensités moyennes d'infection ont varié de 11,3 ± 3,1 à 52,3 ± 10,8 métacercaires par individu.

Bien que présents aux mêmes sites et en même temps, *G. branchialis* et *Maritrema* sp. apparaissent négativement associés chez leur hôte *U.* cf. *pusilla.* En effet, les individus non parasités avec le bopyre *G. branchialis* montrent une charge moyenne en trématodes quasiment quatre fois supérieure à celle notée chez les crevettes parasitées par le bopyre. Nous proposons que cette association négative soit en grande partie du fait du bopyre *G. branchialis* qui, en réduisant les activités de filtration de son hôte, limiterait le nombre de larves de trématodes arrivant au contact de *U.* cf. *pusilla* et *de facto* les niveaux

d'infection avec ce parasite trématode.

Le bopyre *G. branchialis* et le trématode *Maritrema* sp. sont donc négativement associés au sein de leur hôte *U.* cf. *pusilla*, et il semble alors que des expériences en laboratoire visant à étudier l'influence de ces parasites sur la crevette de vase doivent se concentrer sur ces deux parasites pris séparément. Contrairement à l'infestation avec le bopyre, l'infection avec les parasites trématodes n'est pas visible sans dissection (et donc sacrifice) de l'animal. De manière à étudier l'influence du trématode sur *U.* cf. *pusilla*, des infections contrôlées devraient donc être mises en œuvre. Le cycle de vie du trématode *Maritrema* sp. étant à ce jour inconnu, il n'est pas possible d'obtenir des larves de parasites qui infecteraient les crevettes de vase. Par ailleurs, à l'échelle du bassin d'Arcachon, nous ne disposons pas de populations naturellement vierges de trématodes, nécessaires à la réalisation d'infections contrôlées en laboratoire. Finalement, en supposant que *U.* cf. *pusilla* constitue un cul-de-sac dans le cycle de vie de *Maritrema* sp., il est très peu probable que ce parasite soit associé à des stratégies évolutives favorisant sa transmission à l'hôte final comme des modifications du comportement de l'hôte. Nos études expérimentales se sont donc concentrées sur l'influence du bopyre *G. branchialis* sur la crevette de vase *U.* cf. *pusilla* (**Chapitres IV** et **V**).

Relationships between metal content and parasitic pressure in the bioturbating mud shrimp *Upogebia* cf. *pusilla*: a field survey

DAIRAIN A., LEGEAY A., GONZALEZ P., BAUDRIMONT M., GOURVES P.-Y. and de MONTAUDOUIN X.

En préparation

Abstract

In marine environments, pollutant monitoring programs usually focus on bivalve species because these organisms are recognized as relevant biological indicators. Consequently, the environmental contaminant levels in a vast majority of other species are ignored. Especially, only a few studies have been characterized the environmental levels of pollutants in bioturbating species. Bioturbators are epi- or endobenthic species, which shape the physical structure of sediments and influence their biogeochemical properties. Bioturbators are considered as ecosystem engineer species and in order to better understand the functioning of ecosystems, a key point would be to characterize stressors, such as contaminants, which could impact these influential organisms.

In natural environment, bioturbating species undergo a wide range of stress which can influence their physiological status and, by a cascade effect, their ability to accumulate pollutants. Among them, parasitism is of major concern. Parasites are widespread in natural environments and deeply influence the fitness of their host. Moreover, in the context of global climate change, it is suggested that warmer conditions could promote the frequency of parasitic infections.

This study aims to assess the relationship between parasitism and metal accumulation in the bioturbating mud shrimp *Upogebia* cf. *pusilla*. A one-year seasonal survey was conducted in Arcachon Bay, France, characterizing the levels of metals in the mud shrimp in regard of the occurrence of two parasite species (a bopyrid isopod and a trematode). In addition, the influence of parasites and levels of metals on the mud shrimp well-being was evaluated by transcriptomic analyses. In general, mud shrimp in Arcachon Bay displayed very low values of metals. Bopyrid-infested mud shrimp exhibited higher levels of metals as compared to bopyrid-uninfested organisms. Conversely, there was no correlation between the infection with the trematode parasite and the metal levels in mud shrimp. The expression of several genes was modulated according to climatic conditions, whereas metals and parasites were weakly correlated to genes' expressions.

Key words: mud shrimp; parasites; metals; field survey; seasonal fluctuations; transcriptomic analyses

I. Introduction

Despite their relative small size and low visibility, parasitic organisms are widespread in natural environments, representing ca. 40 % of the known species (Dobson *et al.*, 2008). These abundant organisms play a key role on their host fitness, modifying their physiological status (e.g., McLaughlin and Faisal, 1998; Stier *et al.*, 2015), fecundity (e.g., Lauckner, 1980; Mautner *et al.*, 2007), behaviour (e.g., Thomas and Poulin, 1998; Pascal, 2017) and survival (e.g., Jensen and Mouritsen, 1992; de Montaudouin *et al.*, 2003). At the broader ecosystem scale, the biomass and production of parasites in estuarine systems are substantial (Thieltges *et al.*, 2008; Kuris *et al.*, 2008). This key biotic interaction can also shape the community structure of free-living and parasitic organisms (Minchella and Scott, 1991; Poulin, 1999; Mouritsen and Poulin, 2002). For instance, by infesting important ecosystem engineer species (sensu Jones *et al.*, 1994), parasites can interfere with functional traits of their host involved in their engineering functions. In this way, parasites act themselves as ecosystem engineer species (Thomas *et al.*, 1999).

In marine benthic ecosystems, bioturbation is a typical example of ecosystem engineering (Levinton, 1995; Mermillod-Blondin and Rosenberg, 2006; Meysman *et al.*, 2006). The process of bioturbation is described as any modification of the sediment matrix, including interstitial waters, due to the activities of organisms mainly living in or on the substratum (Kristensen *et al.*, 2012). The locomotory, feeding and burrowing activities of bioturbators are the source of important displacements of sediment particles (= sediment reworking), which deeply affect the physical properties and the geomorphology of sediments (Rhoads and Young, 1970; Swinbanks, 1981; Jones and Jago, 1993). Bioturbators also significantly enhance the transport of water in sediments, stimulating solutes' movements between pore- and overlying waters (= bioirrigation) (Aller, 1988; Webb and Eyre, 2004a; Volkenborn *et al.*, 2012a). As a consequence of their large impact on the physical and geochemical properties of benthic habitats, bioturbators exert a major influence on other organisms and drive the composition of benthic communities (Dahlgren *et al.*, 1999; Widdicombe *et al.*, 2000; Pillay *et al.*, 2007). The influence of bioturbators as ecosystem engineer species depends on the magnitude of their bioturbation activities, and inherently on their well-being. Parasites could impair the bioturbators' physiological status or modify their behaviour, and, by cascade effects, interfere with the roles of bioturbators in ecosystem functioning (Thomas et al., 1999).

In natural environments, bioturbators are seldom in optimal environmental conditions and, in addition to parasites, they have to cope with several others biotic (Duport *et al.*, 2006; Premo and Tyler, 2013) and abiotic stressors (Diaz and Rosenberg, 1995; Berkenbusch and Rowden, 1999; Ouellette *et al.*, 2004) influencing the intensity of their bioturbation activities. For example, increase in human populations densities and associated activities have led to important releases of metals and organic pollutants in the environment. Pollutants emitted in the atmosphere or contained in terrestrial runoffs are carried into marine environments, especially in coastal and estuarine waters, leading locally to high levels of

contamination (Tueros *et al.*, 2009; Pan and Wang, 2012). Once in coastal environments, pollutants precipitate as solids or are adsorbed to suspended particles on the seafloor bottom. Hence, coastal environments are an important sink for a wide variety of contaminants, where they have a large range of detrimental effects on bioturbating organisms (e.g., Chapman and Fink, 1984; Bat *et al.*, 1998; Moreira *et al.*, 2006). As an illustration, the bioturbating polychaete *Capitella teleta* exposed to sediments contaminated with trace metals and PCBs experiences oxidative stress and neurological impairment. The behaviour of *C. teleta* is also impacted by pollutants, contaminated worms showing reduced egestion rates (Gomes *et al.*, 2014).

Over the last two decades, an increase number of studies has taken interest in the link between parasitism and pollution in aquatic organisms since both stressors likely act together with additive, synergistic or antagonistic effects on the health of organisms (Sures, 2008; Marcogliese and Pietrock, 2011). For instance, parasites can interfere with pollutants accumulation processes, enhancing or decreasing contaminants burden in organisms. The common cockle *Cerastoderma edule* infected with the trematode parasites *Himasthla elongata* or *Bucephalus minimus (Labratrema minimum)* bioaccumulated more Cd than unparasitized conspecifics over a 7-day contamination experiment (Baudrimont and de Montaudouin, 2007). On the opposite, the bopyrid parasite *Gyge branchialis* was associated to a reduction of the Cd burden in the mud shrimp *Upogebia* cf. *pusilla* when exposed to Cd-contaminated sediments (Dairain *et al.*, 2018a). In addition to their roles in bioaccumulation processes, parasites can modulate the antitoxic responses of their host to pollutants (Sures and Radszuweit, 2007; Gismondi *et al.*, 2012b). As an illustration, the amphipod *Gammarus roeseli* infected with the microsporidian parasites *Dictyocoela roeselum* or *D. muelleri* exhibited reduced ability to cope with Cd contamination as evidenced by the weak increase in metallothionein concentrations (proteins involved in metal chelating process) and the unchanged concentrations of the reduced glutathione (a peptide involved in neutralization of reactive oxygen species) in parasitized organisms (Gismondi *et al.*, 2012a). Finally, parasites are able to largely accumulate contaminants (Siddall and Sures, 1998; Bergey *et al.*, 2002) and one would argue that contaminants could have toxic effects on parasites themselves. For instance, the free-living stages of several parasites show reduced infectivity or increased mortality rates when exposed to pollutants (Morley *et al.*, 2003; Pietrock and Marcogliese, 2003). Similar toxic effects of contaminants on established parasites could be at the benefice of parasitized organisms if the negative impact of contaminants on the host does not overcome the negative impact of the parasites.

Although there is an increase concern in the interactive effects of pollutants and parasites on aquatic organisms, most of these studies deals with experimental work. In sharp contrast, only a few environmental surveys have been conducted in the aim to characterize levels of contaminants in the light of parasitism (Kim *et al.*, 1998, 2008; de Montaudouin *et al.*, 2010) and almost none of these studies took interest in bioturbating species. Because such environmental surveys are primary steps before conducting relevant laboratory experiments, we propose to describe the background environmental

levels of metals in the bioturbating mud shrimp *Upogebia* cf. *pusilla* in respect to its parasitic status. This species harbors two parasite species, the bopyrid isopod *Gyge branchialis* and the trematode *Maritrema* sp.. A one-year seasonal survey was initiated in Fall 2016 in Arcachon Bay, France, in two sites with contrasting parasite pressure (Dairain *et al.*, 2017), assessing the influence of parasitism and seasonal conditions, as their interactive effects, on the accumulation of metals in mud shrimp. In parallel, we investigated the impact of parasites and metals on the mud shrimp well-being by evaluating the transcriptional responses of several genes involved in the response to metal contamination and/or parasite infestation.

II. Materials and methods

II.1. Biology of *Upogebia* cf. *pusilla* and its parasites

The gebiidean mud shrimp *Upogebia* cf. *pusilla* is a gonochoric crustacean decapod with a life span estimated at > 5 years (Dworschak, 1988). It occurs in intertidal and upper sublittoral zones along the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff, 1979; Dworschak, 1983). In Arcachon Bay, France, *U.* cf. *pusilla* preferentially inhabits intertidal seagrass meadows where densities can reach 39 ind. m^{-2}. It lives in a deep (up to 49 cm) and complex burrow connected to the sediment-water interface by several distant openings (Pascal, 2017). Only one individual inhabits a burrow, except during the breeding period when two adult mud shrimps have been found to occupy the same burrow (personal observation).

The crustacean bopyrid *Gyge branchialis* is a parasite of *U.* cf. *pusilla* (Bonnier, 1900; Tucker, 1930; Bourdon, 1965) which lives in one (very rarely both) of the two gill chambers of its host (Pascal *et al.*, 2016b). The bopyrid settles early in the mud shrimp's life, grows with its host and finally occupies most of the space in the gill chamber (Tucker, 1930; Pascal *et al.*, 2016b). The effects of this parasite species on *U.* cf. *pusilla* have recently been documented (Pascal *et al.*, 2016b; Pascal, 2017). This ectoparasite disrupts the mud shrimp's reproduction: females are castrated (Pascal *et al.*, 2016b), males are feminized and their gonad seems to be atrophied (Tucker, 1930; Pascal *et al.*, 2016b). The parasite also reduced the host activity levels (Pascal, 2017).

The mud shrimp *U. pusilla* is also parasitized by the digenean trematode *Maritrema* sp.. This parasite infected the gills, the visceral cavity as well as the abdominal muscle of its host (Dairain *et al.*, 2017). The trematode occurs as metacercariae in the mud shrimp, meaning the mud shrimp is the second intermediate host of the parasite. The effects of this parasite on *U.* cf. *pusilla* have never been documented. Bopyrid and trematode parasites are negatively associated in their host. This pattern is likely due to the bopyrid parasite interfering with trematode infection *via* alteration of the host fitness (Pascal, 2017; Dairain *et al.*, 2017).

II.2. Study sites and sampling strategy

Located on the French Atlantic coast, Arcachon Bay (44°42' N, 1°11' W) is a 180-km^2 macrotidal lagoon opening to the ocean through two narrow channels (Figure III-B. 1). The tidal cycle is semi-diurnal with an amplitude of 0.8–4.6 m. Mean sea surface temperature changes from 6 °C in winter to 22.5 °C in summer. In 2007, 39 % of the 110-km^2 intertidal area was covered by the dwarf eelgrass *Zostera noltei* (Plus *et al.*, 2010).

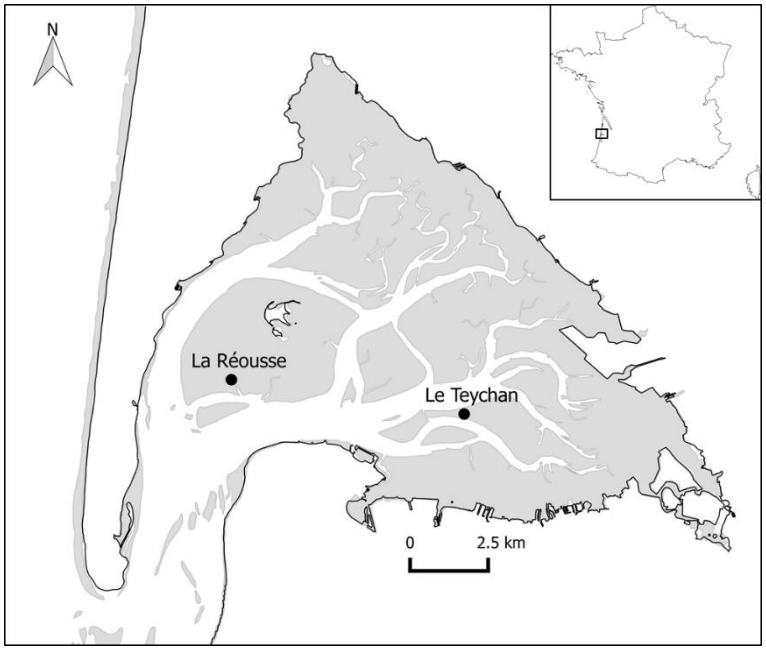

Figure III-B. 1 Location of the two intertidal sampling sites in Arcachon Bay, France. Light grey = intertidal area.

A one year seasonal sampling survey was initiated in Fall 2016 on two intertidal sites ("La Réousse" and "Le Teychan") in Arcachon Bay (Figure III-B. 1, Table III-B. 1). Both sites harbour a relatively dense population of *U.* cf. *pusilla*, with a significant proportion infested with the bopyrid isopod *G. branchialis* (Pascal *et al.*, 2016b). These two sites were selected based on differences in trematode infection; mud shrimp inhabiting La Réousse being heavily infected with *Maritrema* sp. while at Le Teychan organisms are on average 6-fold less infected with trematodes (Dairain *et al.*, 2017). For each site and at each season, 10 bopyrid-infested and 10 bopyrid-uninfested males and females were sampled by using a bait piston pump. The mud shrimp's sex was determined as follow: females show a gonopore on each pereiopod 3 coxae and have a first pair of pleopods; males show none of those two characters. Once collected, each specimen was individually isolated in a plastic bag and kept on ice until be back to the laboratory, where mud shrimp were immediately processed (see below). In parallel, sediments samples were collected for metals quantification (Ag, As, Cd, Cu, Fe, Mn, Ni, and Zn), except during Fall 2016. Sediments cores were sampled with 30-cm Plexiglas tubes. Three cores per site were taken; the three samples were mixed together and considered to be representative of the sampling site. Surface sediments (ca. 0–2 cm depth) and deeper horizons (ca. 18–20 cm depth) were differentiated. Samples were placed in plastic bags and frozen (- 20 °C) once back to the laboratory.

Table III-B. 1 Characteristics of the two sampling sites in Arcachon Bay, France.

	La Réousse	Le Teychan
Position	44°41'17.1"N 1°12'5.5"W	44°40'18.9"N 1°6'26.6"W
Zonation	Intertidal	Intertidal
D_{50} (µm)	200.0	133.0
Gravel (%)	0.4	2.3
Sand (%)	95.7	70.7
Mud (%)	3.9	27.0
Density of mud shrimp (ind. m^{-2}, mean ± SD)	6.8 ± 0.7	4.1 ± 1.7
Prevalence of *Gyge branchialis* (%)	11.6	12.5
Prevalence of *Maritrema* sp. (%)	93.4	58.9

II.3. Dissection

Each *U.* cf. *pusilla* was firstly measured from the rostrum tip to the extremity of the telson (Total Length, TL) using a digital caliper. The measured size was rounded down to the last mm. Then, the two gill chambers of each individual were checked for the ectoparasite *G. branchialis* presence. Regarding the trematode parasite, the gill infection is a proxy for the whole body infection (Dairain *et al.*, 2017). Thereby, the abundance of trematodes was determined by counting parasites only in the gills of mud shrimp. Gills were taken out, squeezed between two glass plates and metacercariae were counted under a stereomicroscope (Nikon, SMZ1500). The term infestation is used for the ectoparasite (bopyrid) and the term infection for the endoparasite (trematode). Finally, the mud shrimp's hepatopancreas was sampled for genetic and metal analyses. Since the mass tissue was small, the hepatopancreas of two mud shrimp of same sex and size were pooled. Thus, for each season and each site, the genetic and trace metal analyses were performed on five replicates for male and female bopyrid-infested and bopyrid-uninfested mud shrimp. Concerning genetic analyses, a small part of the two pooled hepatopancreas was placed in a RNA later buffer and kept at – 20 °C pending RNA extractions. The remaining tissue was used for metals quantification. Thus, the survey consisted in 160 samples: 2 sites (La Réousse and Le Teychan) x 4 seasons x 2 parasitic status (bopyrid-uninfested vs. bopyrid-infested) x 2 sexes (males and females) x 5 replicates.

II.4. Quantification of metals in biological samples

After being dried (at least 48 h at 45 °C), biological samples were placed in polypropylene tubes and digested with nitric acid (1 to 3 mL depending on samples dry weight DW, HNO3 65 %; Carlo Erba Reagents) at 100 °C for 3 h (HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11 %. The metals contents of the mud shrimp's hepatopancreas were then measured by ICP-OES (700 Series ICP-OES, Agilent). Method blanks and certified reference materials (DOLT-5, dogfish liver, and TORT-3, lobster hepatopancreas; NRCC-CNRC) were included in each analytical batch and consequently treated and

analysed in the same way as biological samples. Metals concentrations in biological samples were expressed in µg g^{-1} DW.

II.5. Quantification of metals in sediments samples

Sediments samples were firstly frizzed-dried before ca. 30 mg DW were placed in polypropylene tubes and digested with 1.5 mL HCl (12 M, Merck), 0.75 mL HNO_3 (14 M, Merck) and 2 mL HF (22 M, Merck) at 110 °C for 2h (DigiPREP, SCP Sciences). After evaporation to dryness, the residues were completely re-dissolved in 0.25 mL HNO_3 (14M, Merck) and 5 mL of ultrapure water (Milli-Q), heat to 65 °C for 15 min and brought to 10 mL in volumetric flasks using ultrapure water (Milli-Q) after cooling. Metals concentrations were determined using ICP-MS (XSeries, Thermo Scientific). Certified reference materials (RM 8704 and BCR-277R, NIST) were used in order to check the quality of the analytical method.

II.6. Gene expression

II.6.1. Total RNA extraction and reverse transcription

Total RNAs were extracted from the hepatopancreas of mud shrimp using the "SV Total RNA Isolation System" kit (Promega; Fitchburg, WI, USA) according to the manufacturer-recommended protocol. The quality of RNA extractions as well as RNA concentrations were assessed by spectrophotometry (Spectro Multivolume Epoch; BioTek). Immediately following total RNA extractions, reverse transcriptions of RNA (RT) were realized. First-strand cDNA were synthesized using the "GoScript Reverse Transcription System" kit (Promega) according to the manufacturer's instructions. The RT reaction was performed from 1 µg of total RNA. The cDNA mixture was then stored at - 20 °C pending real-time PCR reactions.

II.6.2. Real-time PCR

For genetic analyses, ten genes of interest, related to four main cellular functions which may be impaired by parasitism and/or trace metal contamination, were targeted (Table III-B. 2). For each gene, specific primer pairs were designed using Primer3Plus probe design software (version 2.4.1; Untergasser et *al.*, 2012) (Table III-B. 2). The amplification of cDNA was monitored using the DNA intercaling dye SybrGreen I. Real-time PCR reactions were carried out in a total volume of 20 µL consisting of 10 µL GoTaq® qPCR Master Mix (2X; Promega), 2 µL of a mix of the gene-specific primer pairs at a final concentration of 2 µM for each primer, 5 µL of a ten-time diluted cDNA mixture and 3 µL of nuclease-free water. Real-time PCR were performed in a Mx3000P (Stratagene). The cycling conditions were as follow: 1 cycle of initial activation at 95 °C for 10 min; followed by 45 cycles at 95 °C for 30 s, 60 °C for 30s and 72 °C for 30 s. Reaction specificity was determined for each reaction from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SybrGreen I fluorescence level during a gradual heating of the PCR products from 65 to 95 °C. Relative quantification of each gene expression was normalized according to the elongation factor 1-alpha (*ef1-*

α) gene expression. This reference gene had a constant level of expression during all season and between sites, bopyrid-infested and uninfested mud shrimp as well as between males and females. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to generate relative mRNA expression.

Table III-B. 2 Accession numbers and specific primer pairs for the ten genes from *Upogebia* cf. *pusilla* used in quantitative PCR analyses.

Gene	Function	Accession numbers	Primer sequence 5'-3'
Elongation factor 1-alpha (*ef1-a*)	Housekeeping gene	KY555759	CACGTTCTGCTGTGCTTCAC[1] AGCCAGAGATGGGGAGGATT[2]
Catalase (*cat*)	Oxidative stress response	KY555763	GACCCAGACTATGCCATCCG[1] TAGTTCTTGGGGTTGCGGTC[2]
Superoxide dismutase Mn (*sod Mn*)	Oxidative stress response	KY555768	TCAAGTACGCTATGGTGGCC[1] GCCCCCTCCATTGAACTTGA [2]
Ferritin (*fer*)	Oxidative stress response, immune response	KY555762	GGATCACGCCCAGAAGTTCA[1] GGTCGTTGTTCTTGTTGGCC[2]
Nitric oxide synthase (*nos)*	Immune response	KY555765	GTGGTCCATGATGGTCACGT[1] CCTGTGTGACATCCATCGCT[2]
Hemocyanin (*hemo*)	Immune response, respiratory metabolism	KY555761	ATGGTGGCTACTTCCCCTCT[1] CGATCAGGTCACCCACAACA[2]
Subunit 1 of cytochrome C oxidase (*cox I*)	Mitochondrial metabolism	KY555767	GGAGCCGTTAATTTTATAACCACA[1] TAGGATCTCCTCCTCCAGCA[2]
rRNA 12S (12S)	Mitochondrial metabolism	KY555758	TTAACTATTAGCTGCACCTT[1] CAAAGAACTTGGCGGTGTT[2]
Retinoid X receptor (*rxr*)	Endocrine processes	KY555764	AGGAGGAACGACAACGAACC[1] GGCCCATTCCACCAGTTGTA[2]
Vitellogenin (*vtg*)	Reproductive processes	KY555760	AGACCAACGGCACCTATGTG[1] GGTCCCTGAGGATTGCTGAC[2]

Abbreviations: [1]Forward primer; [2]Reverse primer.

II.7. Statistical analysis

The abundance of trematodes was defined as the mean number of metacercariae in the population taking into account both trematode-infected and uninfected mud shrimp (Bush *et al.*, 1997). The influence of the bopyrid presence and season on the abundance of trematodes in mud shrimp from both sampling sites was evaluated using a two-way ANOVA or a two-way ANOVA on ranks (Sokal and Rohlf, 1995).

The metals content in sediments was compared between the two sampling sites using a Wilcoxon test.

In order to obtain average annual values, the metals content in mud shrimp's hepatopancreas were firstly compared between organisms from both sites using a t-test (parametric) or a Wilcoxon test (unparametric).

The relationship between the abundance of trematodes and concentrations of metals in mud shrimp was evaluated in bopyrid-uninfested organisms by using a Kendall rank correlation test. Statistical analyses were performed on mud shrimp of both sex and from the two sampling sites separately for each of the sampling season. The relationship between the abundance of trematodes and gene expression levels was evaluated in a similar way.

The influence of the bopyrid infestation and season on the accumulation of metals in the mud shrimp's hepatopancreas and on gene expression was assessed using a two-way ANOVA or a two-way ANOVA on ranks (Sokal and Rohlf, 1995). To this end, both sites were differentiated as well as sex of mud shrimp.

Finally, a factor analysis for mixed data (FAMD) was conducted to get an overview of the *in situ* monitoring results. The analysis was performed with data related to the metal content in the mud shrimp' hepatopancreas and gene expression in combination. The influence of mud shrimp' sex, bopyrid presence, season and sampling site were evaluated. The qualitative variables "occurrence of the bopyrid parasite" and "sex of the mud shrimp" were included in the analysis, while "sampling site" and "sampling season" were considered as supplementary variables.

Results are reported as the mean ± SE of N replicate measurements. A Levene test was performed to assess homogeneity of variances. If necessary, data were log-transformed or unparametric analyses performed (ANOVA on rank or Wilcoxon test). Differences were considered significant for $p < 0.05$.

III. Results

Female and male mud shrimp collected at La Réousse and Le Teychan had similar size, with TL = 45 ± 0.5 mm for females and males from La Réousse and TL = 46.3 ± 0.5 mm and 46.3 ± 0.6 mm for females and males from Le Teychan, respectively.

III.1. Trematode infection and the influence of the bopyrid parasite

The number of trematodes in the gills of *Upogebia* cf. *pusilla* ranged 0–305 and 0–13 metacercariae per mud shrimp at La Réousse and Le Teychan, respectively (Figure III-B. 2). On average, mud shrimp from La Réousse harbored 35-fold more metacercariae in their gills than organisms from Le Teychan (Wilcoxon test; $W = 23394$, $p < 0.001$). There was no significant influence of the bopyrid parasite on the abundance of trematodes in gills of female (ANOVA, $F = 2.98$, $p = 0.089$) and male (ANOVA, $F = 1.53$, $p = 0.22$) mud shrimp from La Réousse, even though bopyrid-uninfested mud shrimp tend to accumulate more metacercariae than bopyrid-infested organisms. Moreover, there was no influence of the sampling season (ANOVA, $F = 0.39$, $p = 0.76$ and $F = 0.81$, $p = 0.49$ for females and males, respectively) and no interaction between the sampling season and bopyrid occurrence (ANOVA, $F = 1.03$, $p = 0.38$ and $F = 0.72$, $p = 0.55$ for females and males, respectively) on the trematode abundance in gills of mud shrimp from La Réousse.

In contrast, bopyrid-uninfested female and male mud shrimp harbored 2.5 and 5.2-fold more trematodes in their gills than their bopyrid-infested conspecifics (ANOVA, $F = 6.08$, $p < 0.05$ and ANOVA on ranks, $H = 91.87$, $p < 0.05$ for females and males, respectively). We also noticed a significant influence of the sampling season (ANOVA, $F = 3.40$, $p < 0.05$ and ANOVA on ranks, $H = 16.64$, $p < 0.05$ for females and males, respectively) and no interaction between the sampling season and bopyrid occurrence (ANOVA, $F = 1.07$, $p = 0.37$ and ANOVA on ranks, $H = 2.27$, $p = 0.52$ for females and males, respectively) on the trematode abundance in gills of mud shrimp sampled at Le Teychan.

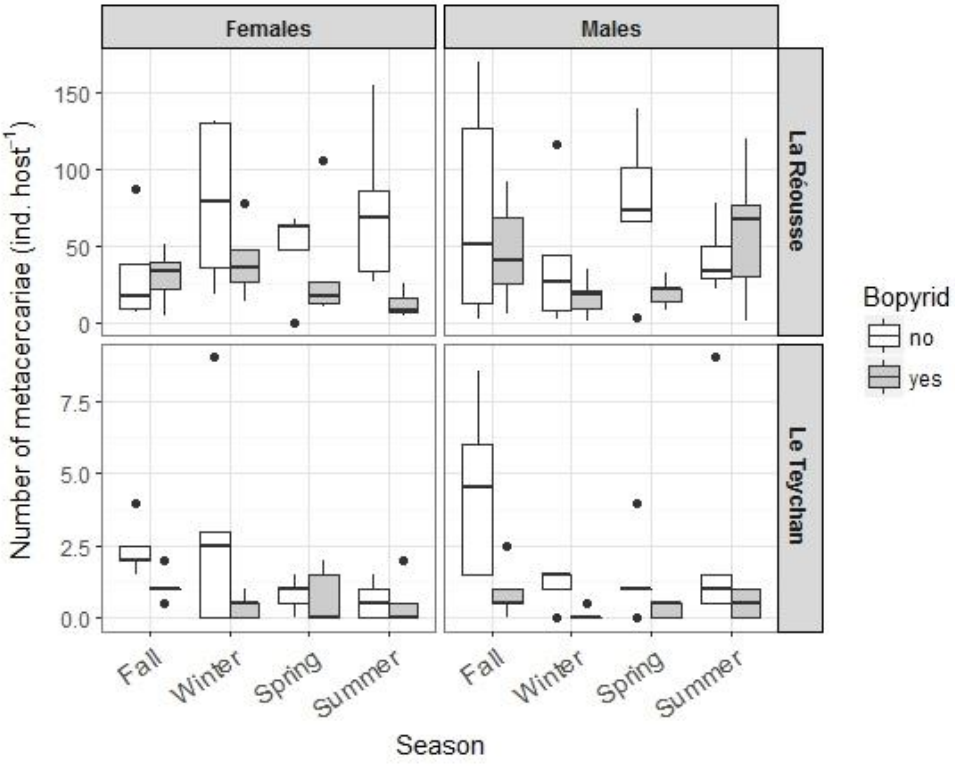

Figure III-B. 2 Number of metacercariae (ind. host^{-1}; median, first and third quartiles) in the gills of female (“F”) and male (“M”) mud shrimp *Upogebia* cf. *pusilla* uninfested (“no”) and infested (“yes”) with the bopyrid parasite *Gyge branchialis* over a one-year seasonal survey initiated in Fall 2016 in two intertidal sites ("La Reousse" and "Le Teychan") in Arcachon Bay, France.

III.2. Concentrations of metals in the sediment

Metal contents in the sediment are presented in Appendix 5. There was no significant influence of the season or depth on metal concentrations in sediments, except regarding Ag and Mn in La Réousse, where concentrations are higher in the upper part of the sediment column as compared to deeper horizons (Appendix 5). Thus, concentrations of metals in sediments at La Réousse and Le Teychan were compared independently of the sampling season or the depth in the sediment column (Table III-B. 3). At Le Teychan, sediments were 1.6 to 4.5-fold more contaminated than at La Réousse.

Table III-B. 3 Concentrations of metals in sediments (µg g^{-1} DW; mean ± SE) in two sampling sites in Arcachon Bay, France (La Réousse and Le Teychan) and results of Wilcoxon tests. P-values in bold indicate statistically significant effect.

	La Réousse	Le Teychan	*p*
Ag	0.15 ± 0.01	0.30 ±0.00	< **0.05**
As	4.11 ± 0.047	18.35 ± 0.24	< **0.05**
Cd	0.092 ± 0.00	0.23 ±0.00	< **0.05**
Cu	3.36 ± 0.05	10.51 ± 0.12	< **0.05**
Fe	6837.5 ± 96.38	24817.83 ± 223.02	< **0.05**
Mn	140.33 ± 4.75	221.5 ± 2.06	< **0.05**
Ni	5.20 ± 0.08	16.07 ± 0.16	< **0.05**
Zn	31.02 ± 0.65	74.33 ± 1.26	< **0.05**

III.3. Concentrations of metals in mud shrimp and influence of parasitism

To get an overview of the metal content in mud shrimp, we first compared the levels of metals between mud shrimp sampled in the two sites independently of the sampling season, the bopyrid presence or the sex of organisms (Table III-B. 4). The levels of Ag, Cd and Cu were significantly higher in mud shrimp from Le Teychan compared to the values reported in organisms from La Réousse. Conversely, the levels of As, Mn and Ni were significantly higher in mud shrimp from La Réousse compared to the values reported in organisms from Le Teychan. The levels of Fe and Zn in the mud shrimp's hepatopancreas were similar between organisms of both sampling sites.

Table III-B. 4 Concentrations of metals in mud shrimp *Upogebia* cf. *pusilla* (µg g^{-1} DW; mean ± SE) sampled on two sites in Arcachon Bay, France (La Réousse and Le Teychan) and results of Wilcoxon tests. P-values in bold indicate statistically significant effect.

	La Réousse	Le Teychan	*p*
Ag	3.42 ± 0.15	4.19 ± 0.14	< **0.05**
As	54.84 ± 1.65	42.69 ± 1.18	< **0.05**
Cd	0.43 ± 0.02	0.50 ± 0.02	< **0.05**
Cu	446.12 ± 30.52	682.63 ± 28.68	< **0.05**
Fe	242.49 ± 19.51	259.37 ± 18.21	0.53
Mn	32.85 ± 1.18	24.05 ± 0.77	< **0.05**
Ni	1.98 ± 0.1	1.27 ± 0.07	< **0.05**
Zn	88.19 ± 1.92	85.88 ± 1.58	0.35

III.3.1. Influence of the trematode parasite *Maritrema* sp.

There was no significant correlation between the abundance of trematode parasites and levels of metals in mud shrimp (Appendix 6).

III.3.2. Influence of the bopyrid parasite *Gyge branchialis*

The relationships between bopyrid parasitism and sampling season and the metal content in mud shrimp's hepatopancreas are presented in Figure III-B. 3 and results of statistical tests in Table III-B. 5.

Silver concentrations ranged between 1.1 and 8.2 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested organisms from La Réousse showed higher content of Ag than uninfested mud shrimp,

except during summer when the reverse pattern was observed. Bopyrid-infested mud shrimp from Le Teychan exhibited higher levels of Ag than uninfested organisms (but $p = 0.054$ and 0.073 for females and males, respectively). There was no influence of the sampling season on the Ag content in the mud shrimp's hepatopancreas.

Arsenic concentrations ranged between 21.7 and 109.4 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested males from La Réousse showed higher levels of As than bopyrid-uninfested individuals. A similar pattern was noticed in females from La Réousse (but $p = 0.0076$). During summer, the opposite pattern was noticed. The As content in mud shrimp from Le Teychan varied according to the sampling season, with the highest As concentrations noticed in winter.

Cadmium concentrations ranged between 0.15 and 0.95 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested males showed higher levels of Cd than uninfested ones in both sites. However, in summer, bopyrid-uninfested and infested males from both sites showed similar Cd content. The Cd content in mud shrimp of both sexes and from both sites varied according to the sampling season, with the highest Cd concentrations noticed in spring and summer.

Copper concentrations ranged between 69.9 and 1461.1 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested female and male organisms showed higher levels of Cu than uninfested ones in both sites. However, in summer, the opposite pattern was observed. The Cu content in female mud shrimp from Le Teychan varied according to the sampling season, with the highest concentrations of Cu noticed in summer.

Iron concentrations ranged between 62.2 and 1171.7 µg g^{-1} DW over the one-year seasonal survey. There were no influence of bopyrid presence and sampling season on the Fe content in mud shrimp's hepatopancreas.

Manganese concentrations ranged between 9.4 and 73.6 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested female and male mud shrimp from La Réousse showed higher levels of Mn than bopyrid-uninfested individuals. However, in summer, the opposite pattern was observed in females, while bopyrid-uninfested and infested males showed similar Mn content. The Mn content in mud shrimp of both sexes and from both sites varied according to the sampling season, with the highest Mn concentrations noticed in fall and summer for mud shrimp from La Réousse and Le Teychan, respectively.

Nickel concentrations ranged between 0.06 and 4.0 µg g^{-1} DW over the one-year seasonal survey, with concentrations under the limit of detection for 13 samples. At La Réousse, bopyrid-infested females showed higher levels of Ni than bopyrid-uninfested individuals. However, in summer, the opposite pattern was observed. The Ni content in mud shrimp from La Réousse varied according to the sampling season, with the highest Ni concentration noticed in summer and fall for females and males, respectively.

Zinc concentrations ranged between 56.2 and 143.6 µg g^{-1} DW over the one-year seasonal survey. Bopyrid-infested males from La Réousse and bopyrid-infested females from Le Teychan showed higher levels of Zn than their bopyrid-uninfested conspecifics. However, in summer, the opposite pattern was noticed. There was no influence of the sampling season on the Zn content in the mud shrimp's hepatopancreas.

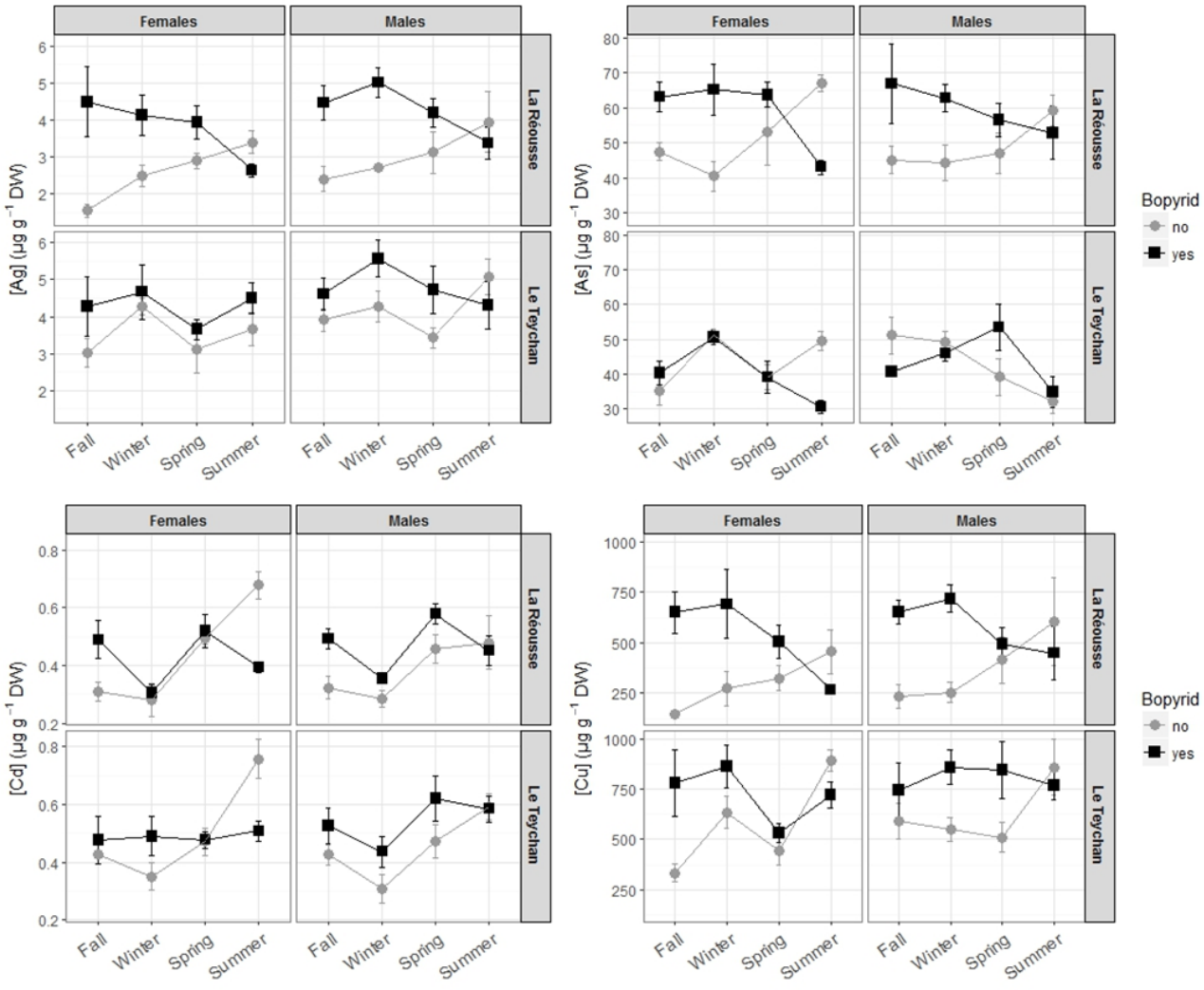

Figure III-B. 3 Concentrations (µg g^{-1} DW) of silver (Ag), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) in the hepatopancreas of female and male mud shrimp *Upogebia* cf. *pusilla* uninfested ("no") and infested ("yes") with the bopyrid parasite *Gyge branchialis* over a one-year seasonal survey initiated in Fall 2016 in two intertidal sites ("La Reousse" and "Le Teychan") in Arcachon Bay, France.

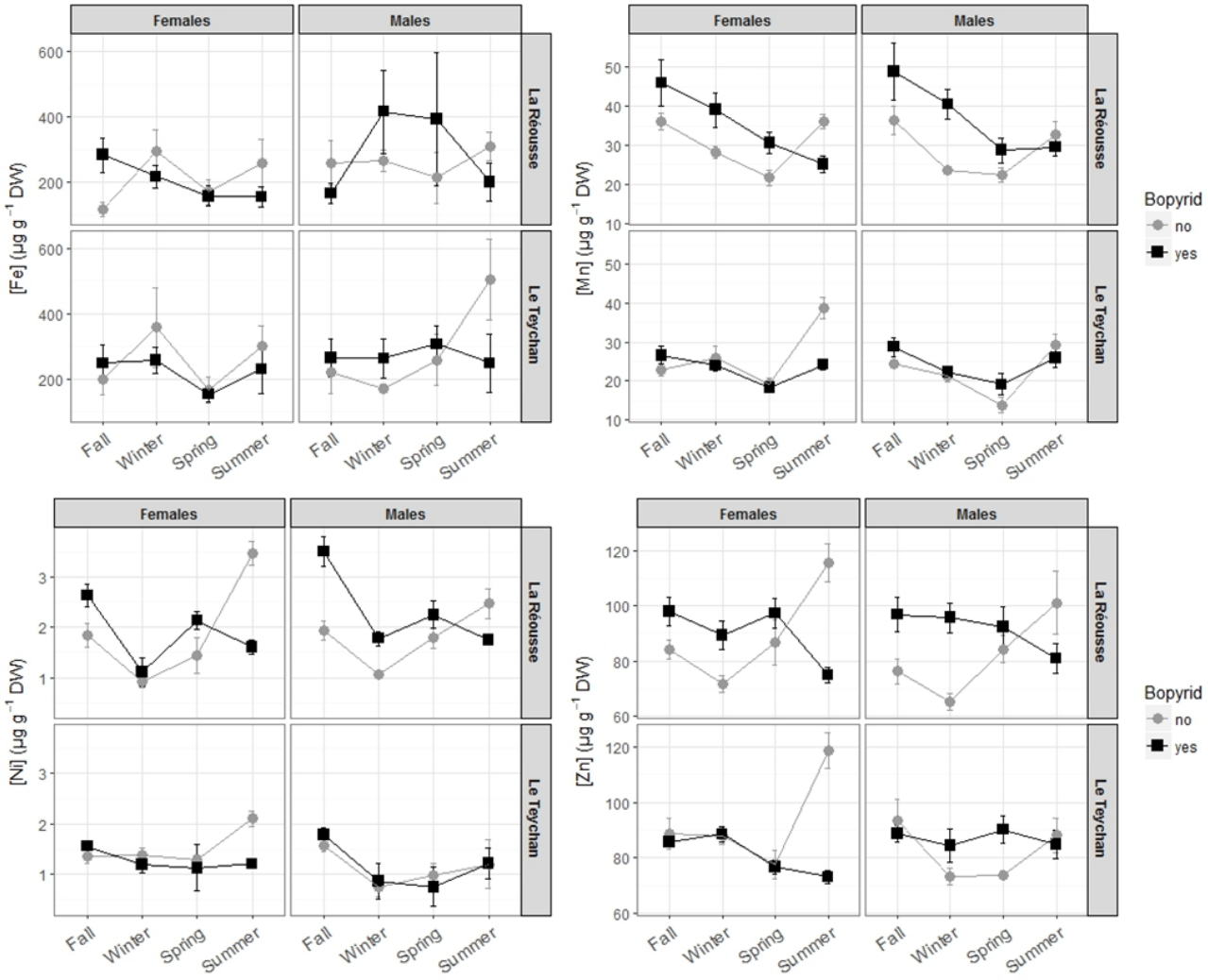

Figure III-B. 3 Suite.

Table III-B. 5 Results of two-way ANOVA comparing the effect of the presence of the bopyrid parasite *Gyge branchialis* and the sampling season (fixed factors) on metals content in the hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla*. Mud shrimp were separated according to the sampling site in Arcachon Bay, France (La Réousse and Le Teychan) and their sex. P-values in bold indicate statistically significant effect.

		La Réousse						**Le Teychan**					
		Females			**Males**			**Females**			**Males**		
Variable	**Factor**	df	*F-value*	*p*	df	*F-value*	*p*	df	*F-value*	*p*	df	*F-value*	*p*
Ag	Bopyrid (1)	1	13.94	**< 0.05**	1	12.88	**< 0.05**	1	4.02	0.054	1	3.44	0.073
	Season (2)	3	0.39	0.76	3	0.28	0.84	3	1.6	0.21	3	1.27	0.30
	(1) x (2)	3	5.6	**< 0.05**	3	3.7	**< 0.05**	3	0.25	0.86	3	2.05	0.13
	Residuals	32			32			32			32		
As	Bopyrid (1)	1	3.44	0.073	1	5.98	**< 0.05**	1	3.16	0.075	1	0.35	0.55
	Season (2)	3	0.41	0.75	3	0.21	0.89	3	14.34	**< 0.05**	3	11.6	**< 0.05**
	(1) x (2)	3	8.76	**< 0.05**	3	1.98	0.14	3	10.07	**< 0.05**	3	7.94	**< 0.05**
	Residuals	32			32			32			32		
Cd	Bopyrid (1)	1	0.2	0.66	1	5.84	**< 0.05**	1	0.12	0.73	1	5.68	**< 0.05**
	Season (2)	3	12.27	**< 0.05**	3	6.15	**< 0.05**	3	6.08	**< 0.05**	3	5.92	**< 0.05**
	(1) x (2)	3	9.46	**< 0.05**	3	1.54	0.22	3	4.71	**< 0.05**	3	0.80	0.51
	Residuals	32			32			32			32		
Cu	Bopyrid (1)	1	12.07	**< 0.05**	1	6.5	**< 0.05**	1	5.71	**< 0.05**	1	5.66	**< 0.05**
	Season (2)	3	0.59	0.63	3	0.23	0.88	3	5.95	**< 0.05**	3	0.83	0.49
	(1) x (2)	3	5.55	**< 0.05**	3	3.48	**< 0.05**	3	4.30	**< 0.05**	3	1.72	0.18
	Residuals	32			32			32			32		
Fe	Bopyrid (1)	1	0.04	0.84	1	0.21	0.65	1	0.54	0.47	1	0.11	0.74
	Season (2)	3	1.32	0.28	3	0.67	0.58	3	1.86	0.16	3	1.76	0.17
	(1) x (2)	3	3.42	**< 0.05**	3	1.25	0.31	3	0.52	0.67	3	2.31	0.095
	Residuals	32			32			32			32		
Mn	Bopyrid (1)	1	4.34	**< 0.05**	1	9.65	**< 0.05**	1	3.60	0.067	1	1.43	0.24
	Season (2)	3	7.76	**< 0.05**	3	7.27	**< 0.05**	3	13.16	**< 0.05**	3	11.40	**< 0.05**
	(1) x (2)	3	5.31	**< 0.05**	3	2.78	0.057	3	5.35	**< 0.05**	3	1.74	0.18
	Residuals	32			32			32			32		
Ni	Bopyrid (1)	1	0.11	0.74	1	11.16	**< 0.05**	1	3.62	0.066	1	0.019	0.89

	Season (2)	3	4.93	**< 0.05**	3	12.45	**< 0.05**	3	2.39	0.087	3	3.61	0.24
	(1) x (2)	3	2.57	0.072	3	9.92	**< 0.05**	3	4.22	**< 0.05**	3	0.19	0.90
	Residuals	32			32			32			32		
Zn	Bopyrid (1)	1	0.15	0.70	1	4.58	**< 0.05**	1	16.8	**< 0.05**	1	1.90	0.18
	Season (2)	3	2.76	0.058	3	0.97	0.42	3	7.16	0.067	3	2.21	0.11
	(1) x (2)	3	13.08	**< 0.05**	3	5.85	**< 0.05**	3	15.19	**< 0.05**	3	2.16	0.11
	Residuals	32			32			32			32		

III.4. Gene expression

The seasonal expression pattern of the genes 12S, *cat*, *cox I*, *fer*, *hemo*, *nos*, *rxr*, *sod Mn* and *vtg* was studied in bopyrid-infested and uninfested mud shrimp of both sexes and from both sampling site (Figure III-B 4, Table III-B. 6, Appendix 7).

III.4.1. Influence of the trematode parasite *Maritrema* sp.

The relationships between the trematode abundance and gene expression in mud shrimp were evaluated on bopyrid-uninfested organisms using Kendall rank correlation tests. Almost none of the correlations were significant (Appendix 7). Significant correlations were only reported for mud shrimp sampled at La Réousse: (1) in winter, there was a negative correlation between the abundance of trematodes and the expression of the gene *sod Mn* in female mud shrimp; (2) in spring, there was a positive correlation between the abundance of trematodes and the expression of the genes 12S and *nos* in male mud shrimp; (3) in summer we reported a positive correlation between the abundance of trematodes and the expression of the genes *fer* in male mud shrimp.

III.4.2. Influence of the bopyrid parasite *Gyge branchialis*

The relationships between bopyrid parasitism and sampling season and gene expression in mud shrimp's hepatopancreas are presented in Figure III-B. 4 and results of statistical tests in Table III-B. 6.

The expression of the gene 12S was significantly enhanced in bopyrid-uninfested females from Le Teychan as compared to their bopyrid-uninfested conspecifics. The expression of 12S also showed seasonal fluctuations in mud shrimp of both sexes at the two sampling sites, with the highest levels of expression observed in winter in females from La Réousse and in females and males from Le Teychan, and in fall in males from La Réousse.

The expression of the gene *cat* was significantly enhanced in bopyrid-infested females from Le Teychan. Seasonal fluctuations of the expression of *cat* were also noticed in females mud shrimp from La Réousse, with the highest level of expression reported in summer.

The expression of the gene *cox I* did not vary according to the bopyrid presence. Conversely, its expression was significantly enhanced in spring and in summer in female and male mud shrimp from Le Teychan, respectively.

The expression of the gene *fer* did not vary according to the bopyrid presence. Conversely, its expression was significantly enhanced in mud shrimp of both sexes and from the two sampling sites in winter.

The expression of the gene *hemo* was significantly enhanced in bopyrid-infested female mud shrimp from both sampling sites. On the opposite, its expression did not display any significant seasonal variation.

The expression of the gene *nos* was significantly enhanced in bopyrid-infested females from La Réousse and in males from Le Teychan while it was downregulated in bopyrid-infested males from La Réousse. The expression of *nos* was significantly enhanced in spring and in summer in male mud shrimp from La Réousse and Le Teychan, respectively.

The expression of the gene *rxr* did not vary according to the bopyrid presence. Conversely, its expression was significantly enhanced in all mud shrimp in summer and winter.

The expression of the gene *sod Mn* did not vary according to the bopyrid presence. Conversely, its expression was significantly enhanced in female mud shrimp from La Réousse in summer.

The expression of the gene *vtg* was significantly down-regulated in bopyrid-infested female mud shrimp from both sites. The expression of *vtg* was also significantly enhanced in females in spring and winter in females and males from Le Teychan, respectively.

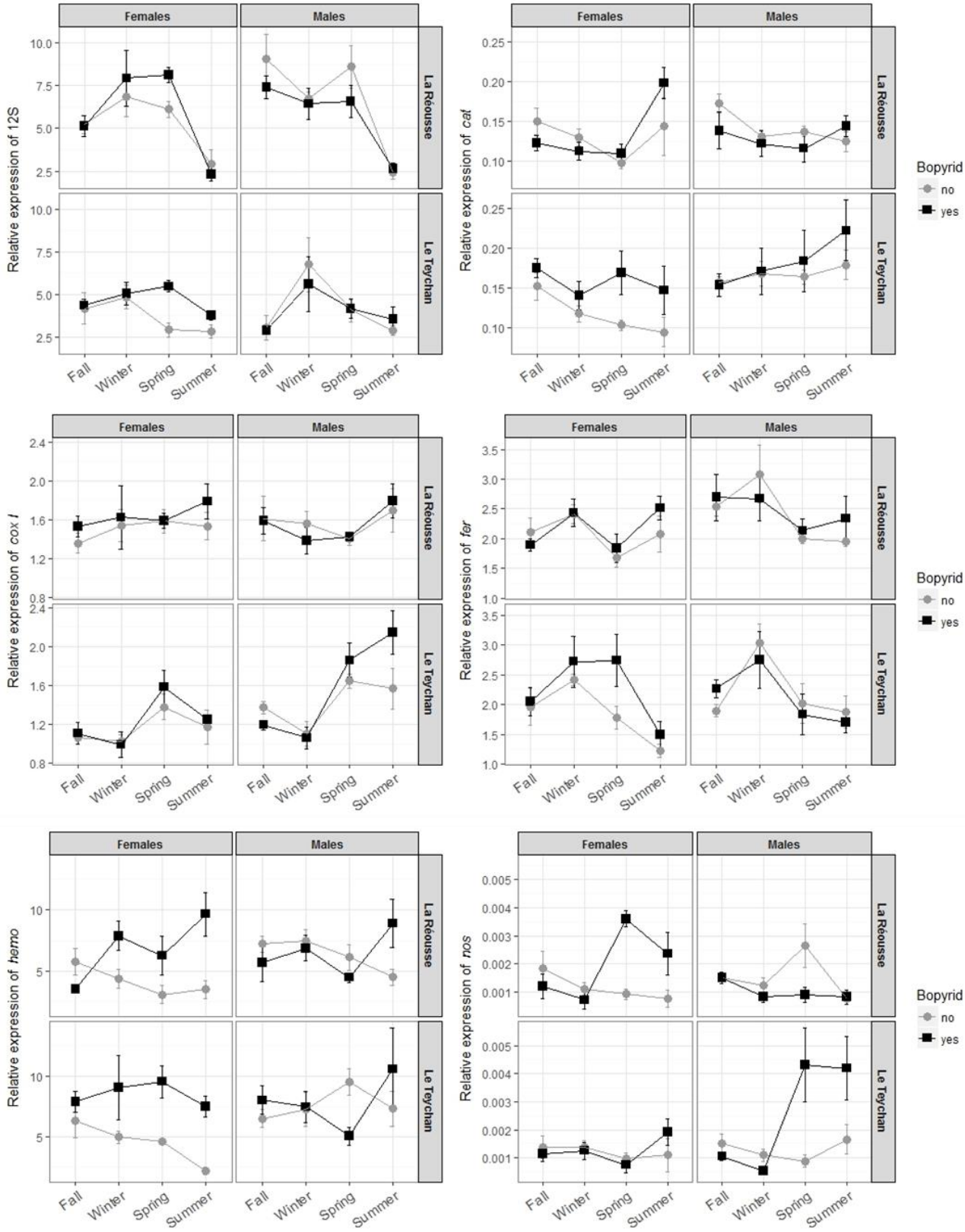

Figure III-B. 2 Relative expression of the genes 12S, *cat*, *cox I*, *fer*, *hemo*, *nos*, *rxr*, *sod Mn* and *vtg* in the hepatopancreas of female ("F") and male ("M") mud shrimp *Upogebia* cf. *pusilla* uninfested ("no") and infested ("yes") with the bopyrid parasite *Gyge branchialis* over a one-year seasonal survey initiated in Fall 2016 in two intertidal sites ("La Reousse" and "Le Teychan") in Arcachon Bay, France.

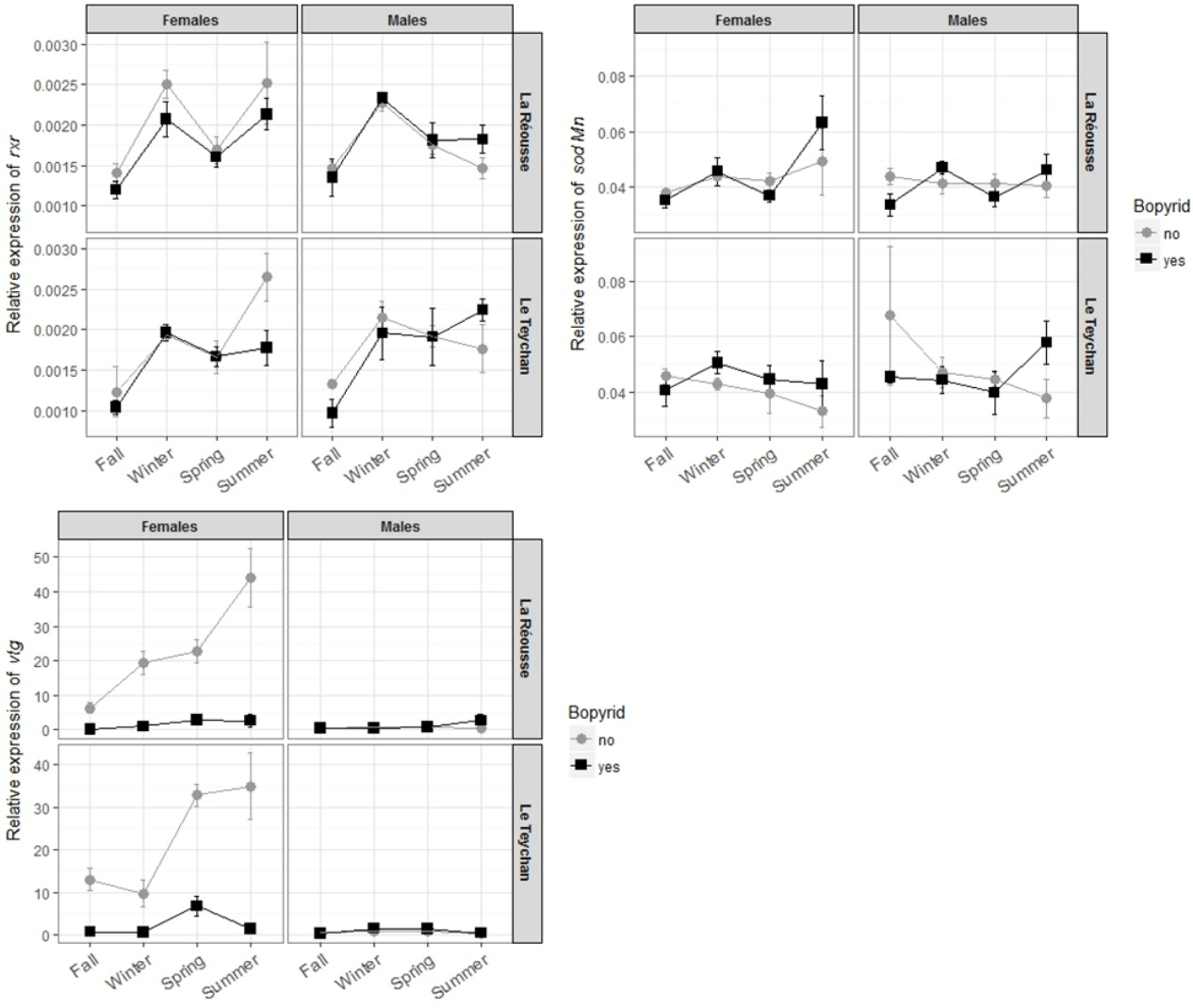

Figure III-B. 4 Suite.

Table III-B. 6 Results of two-way ANOVA comparing the effect of the presence of the bopyrid parasite *Gyge branchialis* and the sampling season (fixed factors) on gene relative expression in the hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla*. Mud shrimp were separated according to the sampling site in Arcachon Bay, France (La Réousse and Le Teychan) and their sex. P-values in bold indicate statistically significant effect.

		La Réousse						**Le Teychan**					
		Females			**Males**			**Females**			**Males**		
Variable	**Factor**	df	*F-value*	*p*	df	*F-value*	*p*	df	*F-value*	*p*	df	*F-value*	*p*
12S	Bopyrid (1)	1	0.23	0.64	1	2.13	0.16	1	5.87	**< 0.05**	1	0.0025	0.96
	Season (2)	3	20.36	**< 0.05**	3	16.58	**< 0.05**	3	2.99	**< 0.05**	3	4.36	**< 0.05**
	(1) x (2)	3	0.77	0.52	3	0.73	0.54	3	1.85	0.16	3	0.33	0.80
	Residuals	32			30			31			32		
cat	Bopyrid (1)	1	0.26	0.61	1	1.23	0.28	1	9.38	**< 0.05**	1	0.022	0.88
	Season (2)	3	4.28	**< 0.05**	3	1.82	0.16	3	1.89	0.15	3	0.43	0.93
	(1) x (2)	3	2.55	0.073	3	1.31	0.29	3	0.65	0.59	3	7.58	0.056
	Residuals	32			32			32			32		
cox I	Bopyrid (1)	1	1.18	0.29	1	1.13	0.29	1	0.69	0.41	1	0.5	0.46
	Season (2)	3	0.56	0.64	3	2.44	0.49	3	5.80	**< 0.05**	3	21.91	**< 0.05**
	(1) x (2)	3	0.21	0.89	3	0.65	0.89	3	0.33	0.80	3	6.54	0.088
	Residuals	32			31			31			32		
fer	Bopyrid (1)	1	0.44	0.51	1	0.10	0.75	1	4.10	0.051	1	0.15	0.70
	Season (2)	3	3.96	**< 0.05**	3	3.23	**< 0.05**	3	6.61	**< 0.05**	3	5.24	**< 0.05**
	(1) x (2)	3	0.83	0.49	3	0.62	0.61	3	0.90	0.45	3	0.60	0.62
	Residuals	32			32			32			32		
hemo	Bopyrid (1)	1	12.01	**< 0.05**	1	0.031	0.86	1	17.56	**< 0.05**	1	0.011	0.92
	Season (2)	3	1.83	0.16	3	0.88	0.46	3	1.20	0.33	3	0.56	0.65
	(1) x (2)	3	5.58	**< 0.05**	3	3.02	**0.045**	3	0.81	0.50	3	2.05	0.13
	Residuals	30			31			30			29		
nos	Bopyrid (1)	1	4.2	**< 0.05**	1	6.72	**< 0.05**	1	0.022	0.88	1	6.1	**< 0.05**
	Season (2)	3	7.11	0.069	3	10.11	**< 0.05**	3	0.43	0.94	3	8.82	**< 0.05**
	(1) x (2)	3	11.08	**< 0.05**	3	3.27	0.35	3	7.58	0.056	3	3.74	0.29
	Residuals	31			32			32			32		
rxr	Bopyrid (1)	1	2.86	0.10	1	0.72	0.40	1	3.49	0.071	1	0.022	0.88
	Season (2)	3	9.05	**< 0.05**	3	12.99	**< 0.05**	3	11.03	**< 0.05**	3	6.82	**< 0.05**
	(1) x (2)	3	0.24	0.87	3	0.86	0.48	3	2.34	0.092	3	1.26	0.31
	Residuals	32			32			32			32		
sod Mn	Bopyrid (1)	1	0.35	0.55	1	0.13	0.72	1	1.35	0.26	1	0.14	0.71
	Season (2)	3	7.86	**< 0.05**	3	1.08	0.37	3	0.90	0.45	3	0.88	0.46
	(1) x (2)	3	4.25	0.24	3	2.02	0.13	3	0.75	0.53	3	1.73	0.18

	Residuals	32			32			32			30		
vtg	Bopyrid (1)	1	72.18	**< 0.05**	1	0.94	0.34	1	200.53	**< 0.05**	1	0.63	0.44
	Season (2)	3	10.9	**< 0.05**	3	0.88	0.46	3	19.54	**< 0.05**	3	3.36	**< 0.05**
	(1) x (2)	3	8.45	**< 0.05**	3	1.84	0.16	3	3.36	**< 0.05**	3	0.57	0.64
	Residuals	32			32			31			31		

III.5. Factor analysis for mixed data

The two first axis of the FAMD explained 34.6 % of the total variance (Figure III-B. 5). The first axis separated "stress factors", i.e. metals, and "response factors", i.e. gene expression (Figure III-B. 5A). The second axis discriminated mud shrimp from the two sampling site, and also discriminated mud shrimp based on their sex (Figure III-B. 5B). In contrast, mud shrimp sampled at the different season were quite similar. Overall, mud shrimp of both sites differed according to their levels of metals and their gene expression levels. However, mud shrimp of La Réousse and Le Teychan were not separated based on the level of one specific metal nor the expression level of one gene. Bopyrid-infested mud shrimp were characterized by higher levels of metals as compared to bopyrid-uninfested mud shrimp. Female and male mud shrimp differed according to their level of gene expression, with females mainly characterized by the expression of the gene *vtg*.

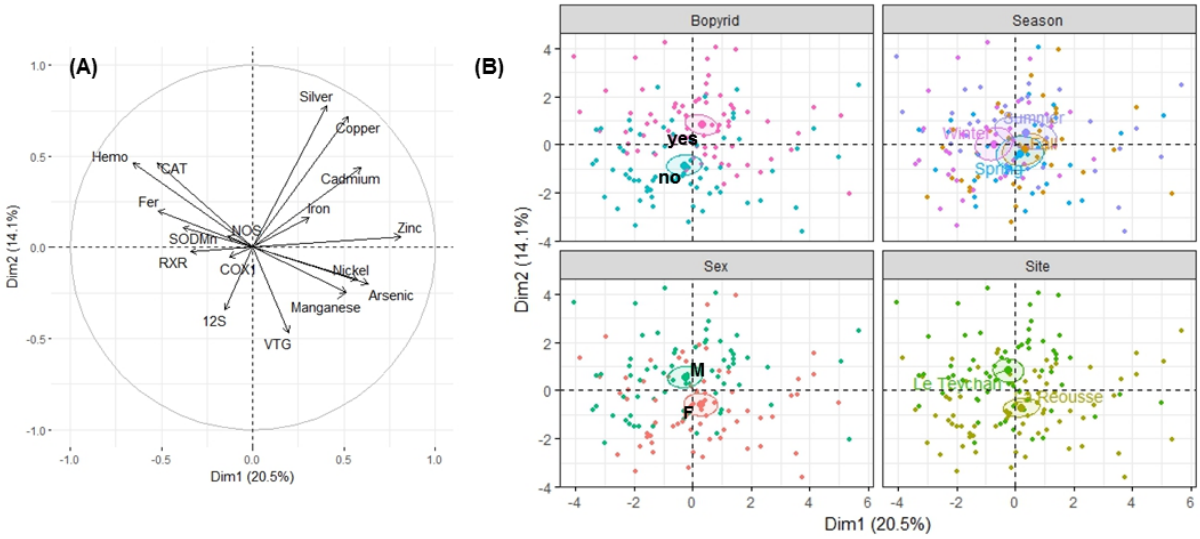

Figure III-B. 3 Factorial analysis for mixed data (FAMD) on metals concentrations and gene expression in female ("F") and male ("M") mud shrimp *Upogebia* cf. *pusilla* uninfested ("no") and infested ("yes") with the bopyrid isopod *Gyge branchialis* and sampled in two intertidal sites ("La Réousse" and "Le Teychan") in Arcachon Bay, France over four seasons. (A) Correlation circle and (B) discrimination of the investigated factors on a factorial plan.

IV. Discussion

IV.1. Trematode infection and influence of the bopyrid isopod

The two sampling sites were chosen based on previous results showing that mud shrimp from La Réousse are heavily infected with the trematode parasite *Maritrema* sp. while organisms from Le Teychan exhibit low level of infection (Dairain *et al.*, 2017). A similar pattern was noticed in this study. We reported that the abundance of trematodes depends on the bopyrid parasite occurrence in mud shrimp from Le Teychan. Bopyrid-infested organisms harbour less trematode parasites than bopyrid-uninfested specimens. Although not statistically significant, a similar tendency was reported in mud shrimp from La Réousse. This result is in accordance with a previous study suggesting that the bopyrid parasite

interferes with the trematode infestation process, probably by reducing the ventilation activity of its mud shrimp host (Pascal, 2017; Dairain *et al.*, 2017).

IV.2. Concentrations of metals in sediments

Over the field survey, metals in sediments displayed relatively low concentrations at both sites, values being significantly inferior to the effect low range thresholds determined by Long *et al.* (1995). The only exception was As, for which the concentration in sediments from Le Teychan was 2.2-fold the effect low range threshold (Long *et al.*, 1995). The same tendency was documented by de Montaudouin *et al.* (2016), who conducted a larger sampling survey in Arcachon Bay in 2010–2012. This small As-enrichment of sediments is difficult to explain since Arcachon Bay is not an industrialized area. Nonetheless, the increase in human population densities in this area as well as the intense anthropic pressure (agriculture, tourism and oyster farming) have been associated with an increasing release of pollutants in the waters of the lagoon over the last years (Tapie and Budzinski, 2018) and an increase of the levels of various contaminants in benthic organisms (Oger-Jeanneret *et al.*, 2016).

We also noticed that sediments from Le Teychan displayed higher metal concentrations than those from La Réousse. Le Teychan is in closer vicinity to the main harbor of Arcachon Bay and also to the Leyre River, the main input of freshwater in the lagoon (Manaud *et al.*, 1997). Although industrial and urban wastes had not been dumped in the lagoon since the 70s', inputs of metals from freshwater sources are still possible because the Leyre River drains a watershed dominated by urban and agriculture areas. Particle size of sediments also affects the distribution of metals, with coarse sediments harboring reduced metals concentrations as compared to finer grain size sediments (Horowitz, 1985). Indeed, sediments at Le Teychan are muddier than at La Réousse, which could contribute to their higher levels of metals.

IV.3. Metals in mud shrimp

While sediments from Le Teychan showed higher levels of metals than at La Réousse, mud shrimp did not exhibit the same tendency. Overall, mud shrimp from the two sites exhibited different metals burden (except regarding Fe and Zn), but the levels of metals were not systematically higher in mud shrimp from Le Teychan than in organisms from La Réousse. Indeed, the quantity of As, Mn and Ni in the hepatopancreas of mud shrimp from La Réousse were globally superior to the values reported in organisms from Le Teychan. Although mud shrimp in the two sampling sites displayed different concentrations of metals, these concentrations remained low at both sites. In our opinion, it is very unlikely that the difference of metal accumulation noticed between mud shrimp from the two sampling sites are biologically (toxically) relevant.

The reported levels of metals in mud shrimp were similar to those documented in other field surveys conducted in Arcachon Bay and targeting the Manila clam *Ruditapes philippinarum* (de Montaudouin *et al.*, 2016), the common cockle *Cerastoderma edule* (de Montaudouin *et al.*, 2010) or the blue mussel

Mytilus sp. (Devier *et al.*, 2005). Nonetheless, there are some discrepancies between these bivalves and the mud shrimp. For instance, the concentrations of Cu in mud shrimp are up to ca. 100-fold superior to those reported for the bivalves. Copper is an essential element which, by definition, can be toxic to organisms at a relatively high concentration. For instance, Cu causes the production of reactive oxygen species, cellular and DNA damages (Hebel *et al.*, 1997; Rhee *et al.*, 2013). As an essential element, Cu acts as a protein cofactor for several enzymes or can be a key element in proteins without any enzymatic function, such as hemocyanins. Hemocyanins are the copper-dependent oxygen-carrying proteins of crustaceans. Thus, it is not surprising to notice high levels of Cu in mud shrimp. However, hemocyanins have also been evidenced in bivalves (Morse *et al.*, 1986). While it is not clear if all bivalves have hemocyanins, it might be suggested that the large difference of contamination with Cu between mud shrimp and bivalves is related to diverse processes of Cu detoxification in the two groups of organisms.

IV.3.1. Seasonal fluctuations

While there were no seasonal fluctuations of the concentrations of metals in sediment, we reported an effect of the sampling season on the concentrations of several metals (As, Cd, Mn and Ni) in mud shrimp. There was no obvious general seasonal pattern since the maximal level of contamination was noticed in winter for some metals, while it was reported in summer for others. Nonetheless, we reported a decrease of the concentrations of several metals (Ag, As, Cu, Mn, Ni) in mud shrimp during warmer seasons, especially in organisms sampled at La Réousse. Mud shrimp are highly active during summer, where they spend a considerable amount of time to transport sediments and to ventilate their burrow. Conversely, their activity level is reduced during winter, mud shrimp allocating a significant proportion of their time to rest (Pascal, 2017). The higher activity level noticed during summer is likely associated to an increase of the energetic demand and consequently to an increase of the mud shrimp feeding rate. The mud shrimp *U.* cf. *pusilla* is a suspension feeder, which can also directly feed on sediments (Dworschak, 1987a). Therefore, we would expect that mud shrimp show a higher accumulation of metals in summer than in winter, conversely to the pattern presently noticed.

Seasonal fluctuations of metals concentrations have also been noticed in the blue mussel *Mytilus* sp. in Arcachon Bay. Nonetheless, maximal concentrations of metals in bivalves were reported in autumn-winter and minimal values in spring-summer (Devier *et al.*, 2005). A similar pattern has also been documented for mussels *M. galloprovincialis* and oysters *Magallana (Crassostrea) gigas* from Spain and Italy (Franco *et al.*, 2002; Fattorini *et al.*, 2008; Solaun *et al.*, 2013). In sharp contrast, a peak of accumulation of Cu and Fe occurred in spring in the periwinkle *Littorina littorea* sampled in Northern Ireland (Cross *et al.*, 2003). The reasons behind these patterns are still unclear, but it may be related to the reproductive cycle of bivalves (Páez-Osuna *et al.*, 1995; Fattorini *et al.*, 2008) as well as climatological conditions (Fowler and Oregioni, 1976) and similar hypotheses could be raised to explain the lower metal burdens noticed in spring and summer in mud shrimp as compared to fall and winter.

IV.3.2. Influence of parasitism

The main point of this study was to investigate the relationship between parasite pressure and the levels of metals in the mud shrimp *U.* cf. *pusilla* over time. In Arcachon Bay, mud shrimp host two parasite species: the bopyrid isopod *Gyge branchialis* and the trematode *Maritrema* sp.. Both types of parasites have a substantial influence in the process of contamination of their host, but their impacts had mainly been investigated over laboratory experiments (Bergey *et al.*, 2002; Williamson *et al.*, 2009; Paul-Pont *et al.*, 2010a; 2010c). For instance, the bopyrid isopod *Probopyrus pandalicola* enhances the sensitivity of the grass shrimp *Palaemonetes pugio* to the pesticide resmethrin as evidenced by the reduced LC_{10} of parasitized organisms (Williamson *et al.*, 2009). However, the effect of the bopyrid parasite appears to depend on the nature of the contaminant since bopyrid-infested grass shrimp accumulate far less Hg than unparasitized organisms (Bergey *et al.*, 2002). Similarly, the Manila clam *R. phillipinarum* and the common cockle *C. edule* infected with the trematode parasite *Himasthla elongata* display lower Cd values than trematode-uninfected bivalves (Paul-Pont *et al.*, 2010a; 2010c). To our knowledge, field surveys assessing the relationships between bopyrid parasites and the metal burden of their host have never been conducted yet. Regarding trematode parasites, such monitoring are still relatively scarce and have mainly targeted mollusc species (Kim *et al.*, 1998; Powell *et al.*, 1999; de Montaudouin *et al.*, 2010, 2016). These studies evidenced various relationships between parasites and the host contaminants burden depending on the type of pollutants (organic contaminants vs. metals), the type of parasites (micro vs. macroparasites) and the locality where the study has been conducted.

a) Trematode parasites

Our study did not report any significant correlations between the metals burden in the mud shrimp *U.* cf. *pusilla* and the abundance of the trematode *Maritrema* sp.. These results are in accordance with those of Kim et *al.* (1998) in the US. Over a large sampling survey (125 sites along the East and West coast of the US), they studied the association between trematode parasites in the bivalves *Crassostrea virginica, Mytilus* spp. and *Dreissena polymorpha* and their pollutants burden (organic contaminants and metals). Six metals were studied (Ag, Cd, Cu, Hg, Ni and Se). The authors only reported a positive correlation between the levels of Hg in Mytilid mussels from the US East coast and the relative abundance of the trematode parasite *Bucephalus* spp.. None of the other metals correlated with the abundances of trematode parasites. Kim *et al.* (1998) also evaluated the relationships between contaminants burden and the occurrence (presence vs. absence) of parasites. Although they highlighted that significant correlations were more frequent by taking into account the prevalence of parasites instead of their abundances, none of these relationships involved trematode parasites but other types of parasites, i.e. cestodes, ciliates or nematodes. In contrast, the surveys initiated by Cross *et al.* (2003) and Evans *et al.* (2001) in Northern Ireland recognized a strong link between the occurrence of the trematode parasites *Renicola roscovita* and *Cryptocotyle lingua* and the levels of metals in the periwinkle *Littorina littorea*. Indeed, the concentrations of Cu, Fe and Ni were lower in parasitized periwinkles than in

unparasitized organisms. The trematodes *R. roscovita and C. lingua* both use the periwinkles as a first intermediate host. Thereby, Cross *et al.* (2003) and Evans *et al.* (2001) focused on the relationship between the occurrence of parasites and the contaminants burden of their host, and did not take into account the intensity or abundance of infections. We focused on the relationship between the abundance of the trematode parasite and the contents of metals in the host for each sampling site. It would have been possible to investigate the influence of a weak and heavy trematode infection by comparing the levels of metals between mud shrimp from Le Teychan (low abundance of trematodes) and La Réousse (high abundance of trematodes). But in our opinion, such comparisons were not relevant because of several confounding factors. Indeed, both sampling sites are well different, especially regarding the levels of metals in the sediments.

b) Bopyrid parasites

Conversely to the trematode parasite, we documented a positive relationship between the occurrence of the bopyrid parasite *G. branchialis* and the levels of metals in mud shrimp for the majority of the metals targeted in this study, i.e. Ag, As, Cd, Cu, Mn, Ni and Zn. Such as for the influence of the sampling season, these significant associations were reported for mud shrimp sampled at La Réousse, the influence of the bopyrid parasite on organisms from Le Teychan being less obvious. Overall, bopyrid-infested organisms exhibited higher values of metals as compared to uninfested mud shrimp. This pattern was modified during summer, where bopyrid-infested mud shrimp showed lower or similar values of contaminants than bopyrid-uninfested organisms.

The lower concentrations of metals largely noticed in bopyrid-uninfested organisms are surprising since a previous lab experiment highlighted that the occurrence of the bopyrid parasite significantly reduced the Cd burden in mud shrimp (Dairain *et al.*, 2018a). Bergey *et al.* (2002) also reported that bopyrid-infested grass shrimp *P. pugio* accumulated less Hg than their bopyrid-uninfested conspecific. The higher levels of metals in bopyrid-infested mud shrimp presently documented might be related to a modification of the "metal dilution" effect (Rainbow *et al.*, 1990). In relatively low polluted areas, negative correlations between the weight of organisms and their metals content have been reported (Páez-Osuna *et al.*, 1995; Bijoux, 2014). It has been suggested that organisms grow faster than they accumulate contaminants (Rainbow *et al.*, 1990). The effect of bopyrid parasites on the growth rates of their host are still unclear (O'Brien and Van Wyk, 1985), but bopyrid-infested mud shrimp *U.* cf. *pusilla* are lighter than bopyrid-uninfested organisms, when standardize for TL (Pascal *et al.*, 2016b). By lowering the mud shrimp's weight, the bopyrid might reduce the "metal dilution" effect, explaining the higher levels of metals in the host. However, this hypothesis does not clarify the higher levels of metals noticed in bopyrid-uninfested organisms as compared to the levels reported in bopyrid-infested mud shrimp during the warmer sampling periods. As previously stated, season is an important factor modulating the intensity of the activity of *U.* cf. *pusilla*, with organisms being more active during summer than winter (Pascal, 2017). As a consequence, the feeding rate of mud shrimp might be

enhanced at that time, and therefore their trophic metal accumulation more intense. The bopyrid parasite *G. branchialis* also diminishes the activity levels of its mud shrimp host (Pascal, 2017). In addition, there is an interactive effect between the seasonal fluctuations and the bopyrid parasite occurrence on the levels of activity of the mud shrimp, the differences of levels of activity between bopyrid-infested and uninfested mud shrimp being more pronounced during summer than winter (Pascal, 2017). We propose that the higher levels of metals noticed in bopyrid-uninfested mud shrimp compared to bopyrid-infested organisms in summer are the result of an increase in activity levels, which counterbalances the larger "metal dilution" effect noticed in bopyrid-uninfested mud shrimp over the other sampling seasons. Nonetheless, mud shrimp displayed lower values of metals when they are supposed to be the most active (i.e. during summer). It highlights that others factors overwhelm the potential influence of the variations of mud shrimp's activity levels on metal accumulation processes.

The bopyrid parasite was not associated to modifications of Fe accumulation in mud shrimp. This result is not surprising considering that the effect of parasites on the host's contaminant contents differs according to the type of pollutants (Kim *et al.*, 1998; Cross *et al.*, 2003). However, the reasons behind this pattern are unclear.

IV.4. Gene expression: responses to stressors

IV.4.1. Seasonal fluctuations

The expression of the genes 12S, *fer*, *rxr* and *vtg* varied according to seasons in mud shrimp from both sites. The gene 12S is taken for a proxy of the number of mitochondria in cells (Al Kaddissi *et al.*, 2014; Arini *et al.*, 2015) since it has been evidenced that the number of rRNA copies per mitochondria was constant (Cantatore *et al.*, 1984; Gadaleta *et al.*, 1990). The expression of the gene 12S was reduced in summer, especially in mud shrimp from La Réousse, suggesting a lower energetic demand at that time. These results are in accordance with those showing reduced levels of metals in mud shrimp in summer, and probably a lower energetic cost of mechanisms involved in the detoxification of metals. Similarly, the expression of the gene *fer* was globally reduced in spring and summer. This gene is encoding the ferritin metal binding protein, which is involved in the storage and scavenging of metals, especially iron (Chasteen and Harrison, 1999). The concentration of Fe did not vary in mud shrimp according to the sampling season. Nonetheless, the lower expression of the gene *fer* in the warmer period may also be paralleled to the globally lower metal burden in mud shrimp at that time. Concerning the genes *rxr* and *vtg*, there are both involved in endocrine processes. Especially, *vtg* is encoding the vitellogenin, the major egg yolk protein. Several organic contaminants and metals act as endocrine disrupting factors, modulating endocrine processes (see Rodríguez *et al.*, 2007 for a review) and the expression of genes related to these processes (Hannas *et al.*, 2011; Park *et al.*, 2014). For instance, *vtg* was significantly up-regulated in the crustacean *Daphnia magna* after 72 h of exposition to water-borne Cd (Hannas *et al.*, 2011). Considering the low values of contaminants displayed by mud shrimp over the field survey, we hypothesize that the up-regulation of *vtg* in female mud shrimp in spring and summer is unlikely related

to the variation of their metal content. Rather, it is probably in close link with the reproductive cycle of organisms, female *U.* cf. *pusilla* being ovigerous in May–August in Arcachon Bay (Pascal *et al.*, 2016b). On the opposite, the seasonal expression pattern of *rxr* is less obvious. The gene *rxr* is encoding the retinoid family receptor, a protein involved in the molting process. Mud shrimp in Arcachon Bay molt at the end of spring (personal observation). Nonetheless, no significant increase of the expression of *rxr* in spring was noticed, while higher expression of this gene have been reported for the Chinese shrimp *Penaeus* (*Fenneropenaeus) chinensis* during a late pre-molting stage (Priya *et al.*, 2009). The modifications of the expression of *rxr* over the molting cycle have been noticed based on whole body samples. On the opposite, we only evaluated the expression of this gene in the mud shrimp's hepatopancreas. Besides, the expression of *rxr* varies between the different organs of shrimp (Priya *et al.*, 2009) and it might be that hepatopancreas was presently not the most relevant organ to evaluate seasonal fluctuations of the expression of *rxr*.

IV.4.2. Influence of parasitism

Such as for the concentrations of metals, trematodes had barely no influence on the expression of the targeted genes. Indeed, the broad majority of the Kendall correlations evaluating the association between the trematode abundance and the relative expression of the targeted genes are non-significant, with no difference between the two sampling sites. Magalhães *et al.* (2017) highlighted that the trematode parasite *B. minimus* induced a significant expression of the gene *cat* in spring, summer and fall in common cockles *C. edule* collected in Arcachon Bay. The genes *cox I* and 12S were also up-regulated in summer. Finally, the authors highlighted a significant repression of the gene *sod Mn* in cockles sampled in fall. The trematode *B. minumus* use the common cockle as a first intermediate host, where it largely asexually multiplies, finally invading the entire organism. This parasite is very deleterious for it host causing castration, starvation and autolysis of the digestive tract (see Magalhães *et al.*, 2015 for a review). In contrast, the trematode *Maritrema* sp. uses the mud shrimp as a second intermediate host, where it encysts awaiting to attain its final host through trophic transmission. The impact of *Maritrema* sp. on its mud shrimp host is unknown. Nonetheless, trematode parasites globally impair the physiological status of their second intermediate host to a lesser extent than what is usually observed in the first intermediate (Lauckner, 1980, 1983). Rather, they modify the behaviour of their host, enhancing their transmission success to their final host (e.g., Thomas and Poulin, 1998). Thus, it is not surprising that the trematode parasite *Maritrema* sp. has a low impact on gene expression in its mud shrimp host.

In a similar manner, the presence of the bopyrid parasite was related to minor fluctuations of the gene expression over this seasonal survey with only a few genes showing a modified pattern of expression. For instance, the gene *nos* was significantly up-regulated during spring and summer in bopyrid-infested females from La Réousse and males from Le Teychan. The gene *nos* is encoding the nitric oxide synthase, a protein involved in the production of nitric oxide (NO), a key effector in the immune system of invertebrates (Rivero, 2006). The gene was highly up-regulated in the hepatopancreas of the Pacific

white shrimp *Penaeus (Litopenaeus) vannamei* challenged with the bacteria *Vibrio harveyi* (Chen *et al.*, 2015). In addition, the concentration of NO increased with the induction of *nos* in the hepatopancreas of the shrimp, allowing to inhibit the growth of bacteria and to kill them. The induction of *nos* in the Pacific white shrimp was documented after organisms have been experimentally infected with parasites. Conversely, the *G. branchialis*/*U.* cf. *pusilla* association is a long term relationship with the bopyrid parasite settling early in the mud shrimp' life and growing with its host (Tucker, 1930; Pascal *et al.*, 2016b). The modification of gene expression is a time dependent process with genes playing a role in immune response up-regulated early during the infection process while others can show delayed modifications of expression (Clark *et al.*, 2013; Li *et al.*, 2016). However, the induction of *nos* was inconsistently reported in female and male mud shrimp infested with the bopyrid parasite over time. In our opinion, it is unlikely that the variations of the expression of *nos* presently documented were due to the bopyrid parasite, but instead might be related to other sporadic infections with microparasites. Finally, we also noticed a strong association between the presence of the bopyrid parasite and the expression of *vtg.* This gene was largely down-regulated in bopyrid-infested females from both site during the reproductive period (spring–summer). Bopyrid-infested females were never found ovigerous (Pascal *et al.*, 2016b) and it has been documented that the ovaries of bopyrid-infested females are reduced, sometimes totally disappearing (Tucker, 1930). Our results confirm at the molecular scale the negative influence of the bopyrid parasite on the reproduction of females. Regarding males, the bopyrid parasite induces their feminization, i.e. males show altered secondary sexual characters (Tucker, 1930; Pascal *et al.*, 2016b). However, the influence of the parasite on the reproductive functions of male hosts are still unclear. Indeed, Tucker (1930) reported that in some specimens the structure of testes as their functioning were not impaired by the bopyrid presence, while in others testes were not detectable. In addition, he documented the presence of an important number of egg-cells in the testes of several bopyrid-infested males. In this study, the gene *vtg* was not up-regulated in bopyrid-infested males, suggesting that the bopyrid did not stimulate oogenesis in infested males. Therefore, one might suggest that the bopyrid parasite *G. branchialis* "un-masculinizes" the male mud shrimp *U.* cf. *pusilla*, rather than it feminizes it *sensu stricto*.

V. Conclusion

While parasites have been evidenced to deeply modulate the physiological status (e.g., Lauckner, 1980; McLaughlin and Faisal, 1998), reproductive functions (e.g., Tucker, 1930; Lauckner, 1980) and activities (e.g., Stier *et al.*, 2015) of their host, surprisingly there have been only a few studies assessing their influence on the metal body burden of their host over field surveys (Kim *et al.*, 1998; de Montaudouin *et al.*, 2010). Moreover, these studies usually target bivalve species, recognized as relevant and sensitive biomonitoring organisms. In this study, we focused on the mud shrimp *Upogebia* cf. *pusilla.* Crustaceans are not considered as relevant sentinels species because their molting status or their sex deeply influence their ability to accumulate pollutants. We do not suggest that *U.* cf. *pusilla* would

be an interesting sentinel organisms. Rather, we focused on this organism because of its key role in ecosystem functioning. Indeed, *U.* cf. *pusilla* is a bioturbating species which influences the physical and biogeochemical properties of sediments (Pascal, 2017). In the aim to better understand the functioning of benthic ecosystems, we argue that it is necessary to characterize potential stressors, such as pollutants, which might modify the activity of such organisms. Thus, we conducted a field survey characterizing parasitism and metal accumulation in *U.* cf. *pusilla.* In Arcachon Bay, France, levels of metals in mud shrimp are relatively low, suggesting a low impairment of the physiological status of organisms. Overall, the levels of metals in mud shrimp varied according to seasons and the parasitic status of organisms. Regarding parasites, it should be noticed that the influence they play on their host metal burden depends on the type of parasites studied, as already reported by Kim et *al.* (1998). At the gene level, we did not notice obvious correlations between gene expression and parasites. Rather, seasons and the life cycle state of organisms influence this response parameter. Finally, this study highlights the relative good status of the Arcachon Bay mud shrimp population in regard of metal contamination. Nonetheless, considering that we noticed significant correlations between the occurrence of parasites and the levels of metals in mud shrimp exposed to such low contamination pressure, it strongly suggests that parasites need to be taken into account during eco-toxicological studies targeting mud shrimp species.

Compliance with ethical standards

Declarations of interest: none.

Animal rights: All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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BILAN DU CHAPITRE III-B ET PERSPECTIVES

Les travaux présentés dans le **Chapitre III-B** consistent en une caractérisation des concentrations des éléments métalliques chez la crevette de vase *Upogebia* cf. *pusilla* au regard de son statut parasitaire dans le bassin d'Arcachon. Les relations entre parasitisme (presence/absence du bopyre *Gyge branchialis* et intensité d'infection avec le trématode *Maritrema* sp.) et les concentrations des éléments métalliques ont ainsi été évaluées au cours d'un suivi saisonnier d'un an effectué sur deux sites dans le bassin d'Arcachon. Ces deux sites ont été choisis sur la base d'une différence de pression parasitaire avec le trématode *Maritrema* sp., les organismes d'un des sites présentant des intensités moyennes d'infection avec ce trématode relativement faibles (« Le Teychan ») alors que pour le second site les niveaux d'infection sont élevés (« La Réousse »). De plus, l'influence du parasitisme ainsi que des niveaux de contamination métallique sur la crevette de vase ont été évaluées à l'échelle moléculaire, i.e. en s'intéressant à l'expression de certains gènes dits « gènes de réponse aux stress », dont les niveaux d'expression peuvent varier selon les conditions environnementales et l'état physiologique des organismes.

Niveaux de contamination

Globalement, les concentrations en éléments métalliques enregistrées chez *U.* cf. *pusilla* sont relativement faibles dans le bassin d'Arcachon et correspondent à celles notées chez d'autres espèces sentinelles au cours de précédentes études (suivis du Réseau d'Observation de la Contamination Chimique effectué sur l'huître *Magallena (Crassostrea) gigas* et les moules *Mytilus* spp. notamment).

Bien que les concentrations y soient faibles, les deux sites échantillonnés diffèrent par les niveaux d'éléments métalliques observés dans leurs sédiments, les concentrations au niveau du site de La Réousse étant plus faibles que celles enregistrées au niveau du site Le Teychan. Nonobstant, les crevettes de vase ne suivent pas exactement ce même schéma puisque les organismes échantillonnés à La Réousse ne présentent pas systématiquement des concentrations en éléments métalliques moins élevées que celles observées chez les individus prélevés au Teychan. Ainsi, le contenu en éléments métalliques des sédiments n'est pas le seul paramètre influant sur la bioaccumulation chez *U.* cf. *pusilla in situ*. Des différences de granulométrie, de contenu en matière organique ou de composition faunistique et microbienne entre les sédiments des deux sites pourraient jouer sur la biodisponibilité des métaux, et *in fine* sur la bioaccumulation de *U.* cf. *pusilla.*

Les faibles niveaux d'éléments métalliques enregistrés chez *U.* cf. *pusilla* dans le bassin d'Arcachon ne permettent pas définir l'un des dix métaux quantifiés comme substance d'étude prioritaire à l'échelle locale. Toutefois, dans d'autres environnements, l'apport de contaminants par les activités humaines pourrait constituer un facteur de stress pour ces organismes. **Dans le cadre de ce**

travail de thèse, nous avons choisi de nous focaliser sur le cadmium comme contaminant métallique modèle. Le choix de cet élément s'explique par le fait que le Cd est considéré comme une substance d'intérêt prioritaire dans le cadre de la Directive Cadre sur l'Eau (Directive 2008/105/EC) du fait de son importante toxicité pour les organismes aquatiques, et particulièrement les crustacés (e.g., Roast *et al.*, 2000; Wallace and Estephan, 2004; Felten *et al.*, 2008; Al Kaddissi *et al.*, 2014; Luo *et al.*, 2015; Zhu *et al.*, 2018). Par ailleurs, à l'échelle régionale, une importante pollution du continuum Lot/Garonne/estuaire de la Gironde a été mise en évidence au cours des années 70, en lien avec les activités minières (extraction de zinc) en amont de l'estuaire. Cette pollution ne s'arrête pas à l'estuaire de la Gironde mais a également été notée au niveau de la baie de Marennes-Oléron (Boutier *et al.*, 2000), aire référencée comme zone d'occurrence de *U.* cf. *pusilla*.

Variations saisonnières des concentrations en éléments métalliques

Il a pu être mis en évidence des variations saisonnières des niveaux d'accumulation pour certains métaux (As, Cu, Mn et Zn), notamment chez les crevettes de vase échantillonnées à La Réousse. Chez ces organismes, les concentrations en métaux sont globalement plus élevées au cours des périodes hivernales qu'estivales. A l'inverse, *U.* cf. *pusilla* est plus active durant l'été qu'en hiver (Pascal, 2017), et nous aurions donc pu nous attendre à ce que les concentrations en métaux suivent cette même saisonnalité. Nos observations suggèrent que d'autres facteurs, environnementaux ou biologiques (autres que les niveaux d'activités des organismes), modulent *in situ* les processus d'accumulation des métaux chez *U.* cf. *pusilla*.

Liens entre parasitisme et accumulation des éléments métalliques

En ce qui concerne le lien entre les parasites *G. branchialis* et *Maritrema* sp. et l'accumulation des éléments métalliques par leur hôte, aucune corrélation entre ces niveaux et la charge parasitaire avec le trématode n'a pu être mise en évidence chez *U.* cf. *pusilla*, et ce quel que soit le site d'origine des organismes échantillonnés. A l'inverse, les organismes parasités par le bopyre *G. branchialis* présentent des concentrations en éléments métalliques relativement plus élevées que les organismes non infestés par ce parasite, et ce pour plusieurs métaux (Ag, As, Cd, Cu, Mn, Se et Zn). Ces différences sont particulièrement marquées chez les organismes échantillonnés à La Réousse. Par ailleurs, il est à noter que le schéma montrant des crevettes de vase parasitées avec *G. branchialis* accumulant globalement plus les éléments métalliques que les individus non-parasités s'inverse en période estivale. Pour l'heure, ces observations restent ininterprétables.

Bien que faibles, les concentrations en éléments métalliques chez *U.* cf. *pusilla* semblent varier en présence du parasite *G. branchialis*. **Si des différences d'accumulation sont d'ores et déjà observées entre organismes sains et parasités dans des environnements relativement peu pollués, il est envisageable que l'influence exercée par le parasite *G. branchialis* soit d'autant plus critique dans un environnement plus pollué.** Ceci renforce l'importance de prendre en compte le parasitisme dans

le cadre d'études évaluant l'influence de paramètres de stress tels la contamination métallique sur les activités du bioturbateur *U.* cf. *pusilla.*

Réponse transcriptomique

Les fluctuations saisonnières d'une dizaine de gènes de réponses aux stress ont été évaluées chez *U.* cf. *pusilla* et mises en relation avec les niveaux d'éléments métalliques ainsi qu'avec la pression parasitaire expérimentés par les organismes. De manière globale, l'expression d'un nombre limité de gènes (12S, *fer*, *rxr* et *vtg*) varie au cours du temps. Ces modifications transcriptomiques ont pu être mises en relation avec les variations saisonnières des niveaux de métaux accumulés (12S et *fer*) ou en lien avec le cycle de vie des crevettes de vase (*vtg*). En ce qui concerne les effets des parasites, nous n'avons pu mettre en évidence que de sporadiques corrélations entre les niveaux d'expression génique et la charge en métacercaires, qui constituent plus probablement des artefacts qu'elles ne traduisent des interactions biologiques. De manière similaire, la présence du bopyre est associée à de faibles modulations de l'expression génique chez *U.* cf. *pusilla.* La seule exception concerne le gène *vtg*, dont l'expression réduite chez les femelles parasitées confirme à l'échelle moléculaire le rôle castrateur de *G. branchialis* sur son hôte. A l'inverse, le bopyre n'est pas associé à des modifications d'expression de ce gène chez les mâles *U.* cf. *pusilla*, confirmant la féminisation imparfaite des mâles infestés par le bopyre.

Cette étude n'a mis en exergue que de potentiels liens entre accumulation des éléments métalliques et réponse génique et entre parasitisme et réponse génique. Il est par ailleurs particulièrement difficile d'appréhender de possibles interactions entre ces facteurs à l'échelle moléculaire. **Des expériences en conditions contrôlées sont ainsi nécessaires de manière à quantifier de façon plus fine ces interactions. Les modifications d'expression géniques peuvent constituer des biomarqueurs précoces de réponse aux stress. Nonobstant, elles ne préludent pas forcément d'altérations physiologiques ou comportementales** (Weis *et al.*, 2001). Dans le cadre de ce travail, le suivi seul des modulations de l'expression génique chez le bioturbateur *U.* cf. *pusilla* en lien avec des facteurs de stress tels les contaminants métalliques et/ou les parasites est insuffisant et doit donc être évalué en parallèle d'autres paramètres, traduisant notamment l'activité générale des organismes. Ainsi, au cours des **Chapitres IV** et **V** nous nous attacherons **à évaluer l'influence des contaminants métalliques, en prenant pour contaminant modèle le Cd, et du parasite *G. branchialis* à l'échelle moléculaire, et également comportementale.**

CHAPITRE IV

Impact de la bioturbation de *Upogebia* cf. *pusilla* dans un environnement pollué et interférence avec le parasitisme : approche *ex-situ*

Influence of the bioturbator *Upogebia* cf. *pusilla* on trace metal remobilization: Does parasitism matter?

DAIRAIN A., de MONTAUDOUIN X., GONZALEZ P., CIUTAT A., BAUDRIMONT M., MAIRE O., GOURVES P.-Y., DAFFE G. and LEGEAY A.

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Abstract

Marine sediments are an important source of contaminants since they are susceptible to be remobilized to the water column. By modifying the physical and biogeochemical characteristics of sediments, bioturbation can influence contaminants remobilization. Within bioturbators, mud shrimp are considered as among the most influential organisms in marine soft-bottom environments. The physiological state of mud shrimp can be impaired by bopyrid parasites. The present study aims to evaluate the influence of bopyrid-uninfested and bopyrid-infested mud shrimp on sediment contaminants resuspension.

Through a 14-days *ex-situ* experiment we showed a moderate effect of mud shrimp on contaminant remobilization compared to molecular diffusion without the bioturbator. Conversely, parasite presence significantly impaired contaminant bioaccumulation in mud shrimp and interfered with genetic expression. The weak effect of mud shrimp on contaminant remobilization may be due to trace metal accumulation and thus we suggested to evaluate the influence of contaminants on activities of bioturbating species.

Key words: mud shrimp, parasitism, cadmium, bioturbation, trace metal bioaccumulation, genetics

I. Introduction

Marine sediments act as a major sink for a wide range of organic and inorganic contaminants. Contaminants are transported from continents to oceans, bound to particulate matter or dissolved in solution. Once in marine environments, they precipitate as solids or are adsorbed to suspended particles on the seafloor bottom. In sediments, contaminants have varied effects on exposed benthic organisms such as alteration of growth capabilities (Chapman and Fink, 1984), impairment of reproduction (Zulkosky *et al.*, 2002), increase of mortality rates (Bat *et al.*, 1998) or change of benthic community structure (Hansen *et al.*, 1996).

Sediments can also act as an important source of contaminants since pollutants are susceptible to be remobilized towards overlying waters (Salomons *et al.*, 1987) and therefore become available to a wide range of aquatic organisms, including pelagic and benthic species (Roberts, 2012; Remaili *et al.*, 2016). Several abiotic factors as well as biologically mediated processes influence the remobilization of contaminants from sediments to the water column (Calmano *et al.*, 1993; Atkinson *et al.*, 2007). Among them, the impact of bioturbation is substantial (Christensen *et al.*, 2002; Ciutat and Boudou, 2003; Thibodeaux and Bierman, 2003; Atkinson *et al.*, 2007). The process of bioturbation is described as any modification of the sediment matrix, including interstitial waters, due to faunal activities such as feeding, burrowing or ventilation of burrow (Kristensen *et al.*, 2012). The burrowing and feeding activities of benthic organisms displace considerable amount of sediment, both vertically and laterally (Kristensen *et al.*, 2012). These sediment reworking activities enhance resuspension of particle-bound contaminants in the water column (Ciutat *et al.*, 2007; Remaili *et al.*, 2016). In addition, burrow ventilation enhances porewater exchanges (Kristensen *et al.*, 2012) and oxygen input in suboxic and anoxic sediments (Glud, 2008). In anoxic sediments, contaminants are primarily bound in sulfide, iron and manganese complexes and to organic matter. The sediment oxidation leads to the re-oxidation of sulfide, iron and manganese complexes allowing for desorption of bound contaminants such as cadmium (Cd) or zinc (Zn) (Salomons *et al.*, 1987; Shine *et al.*, 1998). Thus, by modifying the geochemical and physical characteristics of sediments (Aller, 1982; Volkenborn *et al.*, 2007a; Pascal *et al.*, 2016a), bioturbating species influence the partitioning of metals between the sediment particles and the dissolved phases, i.e. pore- and overlying waters (Green and Chandler, 1994; Peterson *et al.*, 1996), their speciation (Ciutat and Boudou, 2003; Ciutat *et al.*, 2007), and finally their bioavailability to bentho-pelagic species (Atkinson et al. 2007; Remaili et al. 2017).

Within bioturbators, thalassinidean mud shrimp (sensu Felder, 2001) are considered to be among the most influential organisms in marine soft-bottom environments (Cadée, 2001). These organisms live in semi- or permanent, complex and deep burrow, i.e. > 1 m deep (Kinoshita *et al.*, 2010), connected to the sediment-water interface by several distant openings (Dworschak, 1983; Kinoshita *et al.*, 2010). Through their fossorial life style, thalassinidean mud shrimp structure benthic habitats (see Pillay and Branch, 2011 for a review), impact nutrient cycling (D'Andrea and DeWitt, 2009; Pascal *et al.*, 2016a)

and influence benthic communities (Posey *et al.*, 1991; Dittmann, 1996; Papaspyrou *et al.*, 2005). These organisms living in deep and long burrows, they greatly increase the sediment-water interface (Dworschak, 1983) and chemically impact surrounding sediments (Volkenborn *et al.*, 2012a). Consequently, we hypothesize that mud shrimp might impact the distribution of sediment's contaminants, contributing to their remobilization to the water column.

The fate of contaminants in the sediment depends on the mode of bioturbation of species (Petersen *et al.*, 1998; Banta and Andersen, 2003). For instance, studies conducted on two widespread polychaetes species occurring along the European coast, the lugworm *Arenicola marina* and the ragworm *Hediste (Nereis) diversicolor*, highlighted their diverse influence on the fate of organic and inorganic contaminants. The lugworm *A. marina* is a head-down deposit feeder living in J-shaped tubes. It feeds on the bottom of its funnel and defecates on the sediment surface. This upward-conveyor therefore buries the upper layer of the sediment surface. *Via* peristaltic movements, the lugworm also irrigates its burrow. On the other hand, the ragworm *H. diversicolor* is an omnivorous or a filter feeder living in U-shaped burrow highly irrigated. While *A. marina* has been observed to influence pyrene contaminant partitioning between sediment and overlying waters mainly because of its significant bioirrigation behavior (Rasmussen *et al.*, 2000; Christensen *et al.*, 2002), *H. diversicolor* mainly enhances the removal of pyrene from sediment *via* metabolites formation (biotransformation of pyrene) (Christensen *et al.*, 2002). Similarly, these species influence Cd distribution in the sediment. Both can transport Cd from the sediment surface to deep horizons. However, *H. diversicolor* cause shallower transfers of the contaminant compared to *A. marina* (Petersen *et al.*, 1998). The literature evaluating the influence of marine bioturbators on contaminants' fate is growing (Atkinson *et al.*, 2007; Granberg *et al.*, 2008; Josefsson *et al.*, 2010; Remaili *et al.*, 2017). However, these studies mainly focused on organisms living in the first centimeter of the sediment, and underestimate the influence of deep bioturbators such as thalassinidean mud shrimp on the fate of contaminants.

Several factors influencing the behavior and physiological state of bioturbators impair the magnitude of their bioturbation activities. For example, oxygen concentration has been shown to deeply impact bioturbators with organisms reducing (and even ceasing) their bioturbation activities during hypoxia (Diaz and Rosenberg, 1995; Sturdivant *et al.*, 2012). Exposure to contaminated sediments can also reduce bioturbation activities, probably by lessening respiratory activity (Bartsch *et al.*, 1999). In addition, interactions between organisms also affect the activities of bioturbators (Duport *et al.*, 2006; Maire *et al.*, 2010). As an illustration, Duport et *al.* (2006) have demonstrated that the sediment reworking rate of *H. diversicolor* decreases with the increase of the ragworm density. This density-dependent effect is likely due to competition between individuals. Yet, most of the studies taking interest in influence of interactions between organisms on their bioturbation potential focused on predation and competition (Bachteram *et al.*, 2005; Duport *et al.*, 2006; Maire *et al.*, 2010; Premo and Tyler, 2013). In contrast, the influence of parasitism has been overlooked, whereas parasites are widespread in

ecosystems (Dobson *et al.*, 2008), greatly influence their host (Smith *et al.*, 2008; Dumbauld *et al.*, 2011; McGrew and Hultgren, 2011; Pascal *et al.*, 2016b) and, by a cascade effect, ecosystem functioning (Thomas *et al.*, 2005; Mouritsen and Poulin, 2005). Mud shrimp are no exception: they host several types of parasites (Dworschak, 1988; Markham, 2005). Among them, bopyrid parasites are known to alter the physiological state of their host (Smith *et al.*, 2008; Pascal *et al.*, 2016b) and also to reduce their activity levels (Pascal, 2017). Consequently, these parasites could impact the potential for bioturbation of their host.

We performed a 14-days *ex-situ* experiment using the thalassinidean mud shrimp *Upogebia* cf. *pusilla* and its bopyrid parasite *Gyge branchialis* to compare the influence of parasite-uninfested ("uninfested") and parasite-infested ("infested") mud shrimp on the transfer of a metallic contaminant across the sediment-water interface. Because of their significant sediment reworking and bioirrigation activities (Pascal, 2017), we hypothesized that mud shrimp would enhance trace metal transfers from the sediment to the overlying water, especially as particulate forms. A lower remobilization of trace metal would be expected by infested individuals compared to uninfested organisms since the bopyrid parasite deeply reduces the magnitude of the mud shrimp bioturbation activities (Pascal, 2017). Contaminant accumulation was also quantified in the soft bodies of both mud shrimp and bopyrids allowing for the assessment of contaminant bioavailability and its distribution between organs. The mud shrimp *U.* cf. *pusilla* being primarily a suspension feeder (Dworschak, 1987a), bioaccumulation of trace metal in various organs would be likely. In addition, parasitism could interfere with bioaccumulation process by reducing the magnitude of mud shrimp activities. Since the bopyrid parasite feeds on its host internal liquid (Tucker, 1930), it could be trophically contaminated if its host accumulates trace metal. Finally, potential genetic responses to contaminant bioaccumulation was investigated in the mud shrimp since significant regulation (induction or repression) of genes involved in several cellular functions has been noticed in trace metal-contaminated organisms (Paul-Pont *et al.*, 2010a; Al Kaddissi *et al.*, 2014). In the present study, cadmium was used as a model contaminant. This trace metal is highly toxic for marine organisms (Eisler, 1971; Hansen *et al.*, 1996; Manyin and Rowe, 2008) and is consequently considered as a priority substance in European waters (Directive 2008/105/EC).

II. Materials and methods

II.1. Biology of *Upogebia* cf. *pusilla*

The mud shrimp *Upogebia* cf. *pusilla* is a gonochoric crustacean with a life span estimated at > 5 years (Dworschak, 1988). It lives in intertidal and upper sublittoral zones along the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff, 1979; Dworschak, 1983). In Arcachon Bay (France), *U.* cf. *pusilla* preferentially inhabits intertidal seagrass meadows where density can reach 39 ind. m^{-2}. It lives in a deep (up to 49 cm) and complex burrow connected to the sediment-water interface by several distant openings (Pascal 2017). Only one individual inhabits each burrow, except during

breeding period when two adult mud shrimp have been found to occupy the same burrow (personal observation).

The bopyrid isopod *Gyge branchialis* is a parasite of *U.* cf. *pusilla* (Bonnier, 1900; Tucker, 1930; Bourdon, 1968) which lives in one (very rarely both) gill chamber of its host (Pascal *et al.*, 2016b). The bopyrid isopod settles early in the mud shrimp's life, grows with its host and finally occupies most of the space in the gill chamber (Tucker, 1930; Pascal *et al.*, 2016b). The effects of this parasite species on *U.* cf. *pusilla* have recently been documented (Pascal *et al.*, 2016b; Pascal, 2017). This ectoparasite disrupts the mud shrimp's reproduction: females are castrated, males are feminized and their gonad seems to be atrophied (Tucker, 1930; Pascal *et al.*, 2016b). In addition, the bopyrid parasite modifies the behavior of *U.* cf. *pusilla* with infested organisms up to 2.3-fold less active than uninfested individuals. Therefore, infested organisms showed reduce sediment reworking and bioirrigation rates compared to uninfested organisms (Pascal, 2017).

II.2. Sampling of *Upogebia* cf. *pusilla* and sediment

The mud shrimp *U.* cf. *pusilla* was sampled at Le Cla (44°42' N, 1°11' W) in the middle of Arcachon Bay, France, with a bait piston pump. This site was selected because it harbors a relatively dense population of mud shrimp, with a significant proportion infested with the bopyrid isopod *G. branchialis* (Pascal *et al.*, 2016b). Only adult male specimens were selected during this experiment because reproductive state of females may influence their behavior, especially when they are gravid (Sornom *et al.*, 2010). Once collected, each specimen was individually isolated in a plastic pot containing seawater, avoiding fights between mud shrimp, before being brought back to the laboratory.

Naturally Cd-uncontaminated sediment (background concentration = 0.047 ± 0.006 mg Cd kg^{-1} dry weight (DW), Appendix 8) was sampled in the middle of Arcachon bay, France, in April 2017. Sediment consists of muddy-sand sediments (sand = 87.2 %, mud = 12.5 % and gravel = 0.3 %; D_{50} = 141.5 µm).

II.3. Sediment spiking

The sediment was firstly sieved on a 500 µm mesh to remove macrofauna and debris. Then it was spiked with Cd (added as $CdCl_2$; Merck) to achieve a final concentration of 0.5 mg Cd kg^{-1} dry weight (DW). This concentration is ten times the background level of Cd in sediment from Le Cla and is in the range of recorded concentrations in medium contaminated sediments from coastal environments along the Northeast Atlantic coast (Strady *et al.*, 2011). After being spiked, the sediment was stored at 4 °C in the dark for a month to facilitate equilibrium between the Cd, sediment, and porewaters. During this time, it was stirred twice a week by mechanical mixing in order to make it homogenized. The final Cd concentration of sediment after spiking was 0.44 ± 0.01 mg kg^{-1} DW (mean ± SE).

II.4. Experimental design and setup

The experiment was conducted for 14 days in May 2017. It took place in a dark thermo-regulated room maintained at 15 °C. This temperature coincided with field water temperature during mud shrimp

sampling. The basic structure of the experimental unit consisted of a Plexiglas tubing (diameter 9.5 cm, height 40 cm) filled with approximatively 2.9 kg of Cd-spiked sediment in order to obtain a water column of 15 cm height (i.e. volume of water column = 1.06 L). Cadmium-spiked sediment was inserted four days before introducing organisms. This time was necessary to allow for stabilization of the sediment matrix and then avoid collapsing of burrowing structures. Three series of treatments with five replicates were conducted. The three treatments were as follows: (1) no *U.* cf. *pusilla* ("control" treatment, to estimate the diffusive chemical transfer of Cd from the sediment to the overlying water); (2) one uninfested *U.* cf. *pusilla* ("uninfested" treatment, to estimate the role of a fit bioturbator on Cd transfers); (3) one infested *U.* cf. *pusilla* ("infested" treatment, to estimate the impact of a parasite on its host's capabilities to influence Cd transfers). Before introducing *U.* cf. *pusilla* specimens, experimental units were placed under running seawater for 2 h in order to remove overlying Cd-contamination solution. During this time, seawater was added at a rate of ~ 90 mL min^{-1}. This water flow did not induce any significant disturbance of the sediment column. Then, mud shrimp were introduced in experimental units and allowed to burrow for 4 days in a seawater open circuit. Indeed, mud shrimp do not burry as fast as well-studied organisms like lugworm or bivalves. This time was necessary to observe completed burrow construction with their typical two-open ends. The mud shrimp *U.* cf. *pusilla* lives in permanent burrow and a complete burrow building is a one-time event during their life time. Thus, we decided to start the incubation period once this step was completed. Seawater running was stopped after the burrowing time, and the overlying water was then removed with a syringe and carefully replaced by filtered seawater (filtration on 1 µm). A gentle air-bubbling system was finally added in order to keep a sufficient oxygen level in the water column. To avoid water evaporation and mud shrimp escape, a lid was placed on top of each experimental unit.

At the same time, five uninfested and five infested mud shrimp were sacrificed. These individuals were used to determine environmental Cd concentrations in mud shrimp and bopyrids, and to characterize environmental genetic expression levels (see below).

II.5. Sampling procedure

II.5.1. During incubation time

Salinity, temperature and pH were measured daily (Multi 340i; WTW) in the central part of the water column of each experimental unit.

Each day, 20 mL of water were sampled in the central part of each experimental unit to measure turbidity (turbidity meter Turb 430 IR/R; WTW). Turbidity was expressed in nephelometric turbidity units (NTU). Besides, the water column was sampled daily during the first week of the experiment and then at time 9, 11 and 14 d the second week for Cd determination. During each sampling time, 20 mL of water were taken off from each experimental unit of which 9 mL were directly acidified with 1 mL of nitric acid (HNO_3 65 %; Carlo Erba Reagents) for Cd determination on unfiltered water samples (total Cd concentration). From the remaining water, 9 mL were filtered on 0.2 µm (cellulose acetate filters;

Dutscher) and then acidified with 1 mL of nitric acid for Cd quantification in the dissolved phase. Cadmium concentrations in water were then measured by Inductively Coupled Plasma Optical Spectrometry (ICP-OES, 700 Series ICP-OES; Agilent). The detection limit was 0.14 µg Cd L^{-1}. Cadmium concentrations in water samples were expressed in µg L^{-1}. The water column was kept constant during the 14-d experiment by water additions, which compensated losses due to sampling and evaporation.

II.5.2. At the end of the incubation time

After 14 days of incubation, vertical distribution of Cd in the sediment was analyzed for the three experimental conditions. The water column was firstly removed with a syringe and then sediment cores were sliced in 1 cm layers. For the layers with burrow lumen, the sediment around the 5 mm of the burrow lumen (mean thickness of the reworked sediment layer; Pascal 2017) was isolated from the rest of the slice in order to compare Cd concentrations in the burrow lumen vicinity (oxidized area, "burrow") and in the remaining un-oxidized sediment layers ("rest"). During slicing, the water contained in each burrow was sampled using a syringe with a small catheter in order to estimate burrow volumes. Mud shrimp were also sampled at this time and stored on ice until they were dissected for Cd bioaccumulation and genetic analyses (see below).

II.6. Metal determination in sediments

Sediment samples ("burrow" and "rest") were firstly lyophilized. Then, sub-sample of 100–200 mg DW were placed in polypropylene tubes and digested with 2 mL of nitric acid (HNO_3 65 %; Carlo Erba Reagents) at 100 °C for 3 h (HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11 %. Cadmium concentrations in the sediment were determined by atomic absorption spectrophotometry with Zeeman correction (Agilent 240Z AA; Agilent). Method blanks and certified reference materials (MESS-3, marine sediment; NRCC-CNRC) were included in each analytical batch and consequently treated and analyzed in the same way as sediment samples. The detection limit (DL) was 0.12 µg Cd L^{-1}. Cadmium concentrations in sediment samples were expressed in µg g^{-1} DW. The nitric acid digestion does not allow a total extraction of Cd bound to the sediment. The concentrations of Cd in sediments presented here can therefore only be considered for relative differences between the three experimental conditions.

II.7. Dissection

Each *U.* cf. *pusilla* specimen was firstly measured at the lesser mm from the rostrum tip to the telson (Total Length, TL) using a digital caliper. For infested mud shrimp, the bopyrid female was delicately removed from its host under a stereomicroscope (SMZ1500; Nikon). Then, the mud shrimp's hepatopancreas and abdominal muscle were sampled. A small section of both organs was used for genetic analyses and consequently placed in a RNA later solution and kept at - 20 °C pending RNA

extractions. The remaining tissues were used for Cd determination. The bopyrid female was also sampled in order to quantify Cd bioaccumulated by the parasite.

II.8. Metal determination in biological samples

After being dried (at least 48 h at 45 °C), biological samples were placed in polypropylene tubes and digested with nitric acid (1 to 3 mL depending on samples DW, HNO3 65 %; Carlo Erba Reagents) at 100 °C for 3 h (HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11 %. Cadmium concentrations in the hepatopancreas and the abdominal muscle of mud shrimp as well as in the bopyrid female were then measured by ICP-OES (700 Series ICP-OES, Agilent). Method blanks and certified reference materials (DOLT-5, dogfish liver, NRCC-CNRC) were included in each analytical batch and consequently treated and analyzed in the same way as biological samples. The detection limit was 0.19 µg Cd L^{-1}. Cadmium concentrations in biological samples were expressed in µg g^{-1} DW.

II.9. Gene expression

II.9.1. Total RNA extraction and reverse transcription of RNA

Total RNA was extracted from the hepatopancreas and the abdominal muscle of mud shrimp using the "SV Total RNA Isolation System" kit (Promega; Fitchburg, WI, USA) according to the manufacturer-recommended protocol. The quality of RNA extractions as well as RNA concentrations were assessed by spectrophotometry (Spectro Multivolume Epoch; BioTek, Winooski, VT, USA). Immediately following total RNA extractions, reverse transcriptions of RNA (RT) were realized. First-strand cDNA were synthesized using the "GoScript Reverse Transcription System" kit (Promega) according to the manufacturer's instructions. The RT reaction was performed from 1 µg of total RNA in the case of hepatopancreas and from 10 µL of total RNA in the case of abdominal muscle, since the latter contained fewer transcripts compared to the former. The cDNA mixture was then stored at - 20 °C pending real-time PCR reactions.

II.9.2. Search for genes

Since the targeted genes were not available in the GenBank database (NCBI), a search for genes was initially necessary. We firstly looked at sequences of genes in the GenBank database for species taxonomically related to *U.* cf. *pusilla*. Several sequences were then aligned using ClustalW2 program allowing for identification of primer-pairs from conserved regions. Thus, primers were tested on cDNA of *U.* cf. *pusilla* by PCR. The PCR reaction was carried out in a total volume of 50 µL consisting of 10 µL 5X PCR reaction buffer (Promega), 1 µL of dNTPs mix (10 mM), 3 µL of $MgCl_2$ (25 mM), 0.2 µL of Go-*Taq* (5 U μL^{-1}; Promega), 0.5 µL of each primer (100 µM), 1 µL of extracted DNA and 33.8 µL of nuclease-free water. The cycling conditions were as follows: 1 cycle of initial activation at 95 °C for 10 min; followed by 40 cycles at 95 °C for 1 min, primers specific temperature for 30 s and 72 °C for 1 min; then a final step at 72 °C for 10 min. Amplified PCR products were analyzed by electrophoresis in

a 1% p/v agarose gel stained with ethidium bromide. Then, they were purified using the "Wizard SV Gel and PCR Clean-Up System" kit (Promega) according to the manufacturer-recommended protocol. Purified PCR products were sent to GATC Biotech Company for complete double strain sequencing.

For *U.* cf. *pusilla*, genes for elongation factor 1 alpha (*ef1-α*), catalase (*cat*), superoxide dismutase Mn (*sod Mn*), ferritin (*fer*), nitric oxide synthase (*nos*), hemocyanin (*hemo*), subunit 1 of cytochrome C oxidase (*cox-I*), rRNA 12S (12S), retinoid X receptor (*rxr*) and vitellogenin (*vtg*) were obtained (accession numbers are reported in Table IV. 1). For each gene, specific primer pairs were determined using Primer3Plus probe design software (version 2.4.1; Untergasser *et al.*, 2012) (Table IV. 1).

Table IV. 1 Accession numbers and specific primer pairs for the ten genes from *Upogebia* cf. *pusilla* used in quantitative PCR analyses.

Gene	Function	Accession numbers	Primer sequence 5'-3'
Elongation factor 1- alpha (*ef1-a*)	Housekeeping gene	KY555759	CACGTTCTGCTGTGCTTCAC[1] AGCCAGAGATGGGGAGGATT[2]
Catalase (*cat*)	Oxidative stress response	KY555763	GACCCAGACTATGCCATCCG[1] TAGTTCTTGGGGTTGCGGTC[2]
Superoxide dismutase Mn (*sod Mn*)	Oxidative stress response	KY555768	TCAAGTACGCTATGGTGGCC[1] GCCCCCTCCATTGAACTTGA [2]
Ferritin (*fer*)	Oxidative stress response, immune response	KY555762	GGATCACGCCCAGAAGTTCA[1] GGTCGTTGTTCTTGTTGGCC[2]
Nitric oxide synthase (*nos*)	Immune response	KY555765	GTGGTCCATGATGGTCACGT[1] CCTGTGTGACATCCATCGCT[2]
Hemocyanin (*hemo*)	Immune response, respiratory metabolims, mitochondrial metabolism	KY555761	ATGGTGGCTACTTCCCCTCT[1] CGATCAGGTCACCCACAACA[2]
Subunit 1 of cytochrome C oxidase (*cox I*)	Mitochondrial metabolism	KY555767	GGAGCCGTTAATTTTATAACCACA[1] TAGGATCTCCTCCTCCAGCA[2]
rRNA 12S (12S)	Mitochondrial metabolism	KY555758	TTAACTATTAGCTGCACCTT[1] CAAAGAACTTGGCGGTGTT[2]
Retinoid X receptor (*rxr*)	Endocrine processes	KY555764	AGGAGGAACGACAACGAACC[1] GGCCCATTCCACCAGTTGTA[2]
Vitellogenin (*vtg*)	Endocrine processes	KY555760	AGACCAACGGCACCTATGTG[1] GGTCCCTGAGGATTGCTGAC[2]

Abbreviations: [1]Forward primer; [2]Reverse primer.

II.9.3. Real-time PCR

For genetic analyses, ten genes of interest, related to four main cellular functions, were targeted (Table IV. 1). The amplification of cDNA was monitored using the DNA intercaling dye SybrGreen I. Real-time PCR reactions were carried out in a total volume of 20 µL consisting of 10 µL GoTaq® qPCR Master Mix (2X; Promega), 2 µL of a mix of the gene-specific primers pair at a final concentration of 2 µM for each primer and 5 µL of ten-time diluted cDNA mixture and 3 µL of nuclease-free water. Real-

time PCR reactions were performed in a Mx3000P (Stratagene). The cycling conditions were as follow: 1 cycle of initial activation at 95 °C for 10 min; followed by 45 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. Reaction specificity was determined for each reaction from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SybrGreen I fluorescence level during a gradual heating of the PCR product from 65 to 95 °C. Relative quantification of each gene expression was normalized according to the elongation factor 1-alpha (*ef1-α*) gene expression. This reference gene had a constant level of expression for all our conditions of exposure. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to generate relative mRNA expression.

II.10. Statistical analyses

The effect of time of exposure and experimental condition ("control", "uninfested" and "infested") on turbidity and dissolved Cd concentrations in water samples was investigated using a two-way repeated measures ANOVA, followed by a Tukey test (comparison of means) if significant.

The relationship between the concentration of dissolved Cd and the concentration of total Cd in water samples for the three experimental conditions was estimated using a linear regression. Analysis of covariance (ANCOVA) was used to investigate potential differences in the relationship between these two variables with experimental condition ("control", "uninfested" and "infested").

The effect of depth and experimental condition on Cd concentrations in the burrow lumen vicinity and in the non-oxidized zone of the sediment layers was assessed using a two-way ANOVA, followed by a Tukey test if significant. Cadmium concentrations in the burrow lumen vicinity and in the non-oxidized zone of the sediment layers were compared by applying a paired t-test.

Burrow volumes of uninfested and infested mud shrimp were compared using a t-test.

The effect of bopyrid occurrence and exposure to Cd-contaminated sediment on Cd bioaccumulation by mud shrimp was tested using a two-way ANOVA, followed by a Tukey test.

Regarding gene expression, the relative mRNA expressions were compared between treatments by using Student's t-test (parametric) or Mann-Whitney test (non-parametric). If significant, induction factor (IF) of each gene was determined following equation:

$$IF = 2^{-\Delta Ct\ (treatment\ a)} / 2^{-\Delta Ct\ (treatment\ b)}$$

Results are reported as the mean ± SE of *N* replicate measurements. A Levene test was performed to assess homogeneity of variances. If necessary, data were log-transformed. Differences were considered significant for $p < 0.05$.

III. Results

For an undetermined reason one of the replicate for the “infested” condition showed bad quality of RNA extraction with unreliable expression of targeted genes. Consequently, this sample was deleted from all analyses. Therefore, there were five replicates for the “control” and “uninfested” treatments, and four replicates for the “infested” treatment.

III.1. Physicochemical parameters

Over 14 days of experiment, temperatures were maintained at 16.6 ± 0.3, 16.3 ± 0.4 and 16.3± 0.9 °C for “control”, “uninfested” and “infested”, respectively. Average salinity values throughout the experiment were 33.0 ± 0.0, 32.8 ± 0.0 and 32.3 ± 0.0 for “control”, “uninfested” and “infested”, respectively. Average pH values were 8.17 ± 0.00, 8.23 ± 0.00 and 8.18 ± 0.00 for “control”, “uninfested” and “infested”, respectively.

III.2. Turbidity

Water column turbidity has been measured daily during the entire duration of the experiment (Figure IV. 1). Throughout the experiment, water column turbidity was low for the three experimental conditions.

For the “control”, the turbidity was significantly higher during the first two days of the experiment compared to the following days. It is due to the experimental setup: although caution has been taken, the change of the water column at the beginning of the experiment led to resuspension of sediment particles in the water column. After this time, turbidity ranged between 0.6 ± 0.1 (day 4) and 1.6 ± 0.7 NTU (day 6) and did not show any significant variation during the whole experiment (Figure IV. 1; Tukey test $p > 0.05$). On the other hand, there was a significant effect of exposure time on water column turbidity for the “uninfested” and “infested” (Figure IV. 1, Table IV. 2). The turbidity at day 1 were 14.3 ± 3.7 and 4.5 ± 0.7 NTU for the “uninfested” and “infested”, respectively and decreased significantly over the time of experiment (Figure IV. 1, Table IV. 2). There was no difference of water column turbidity between “uninfested” and “infested” throughout the experiment except at day 1 when turbidity for the “uninfested” was significantly higher than for the “infested” and the “control” (Figure IV. 1). Conversely, turbidity of the “control” was significantly lower than the turbidity of the “uninfested” and “infested” from day 2 to day 5. Following day 5, turbidity of the “uninfested” and “infested” slightly came close to the turbidity of the “control” (Figure IV. 1).

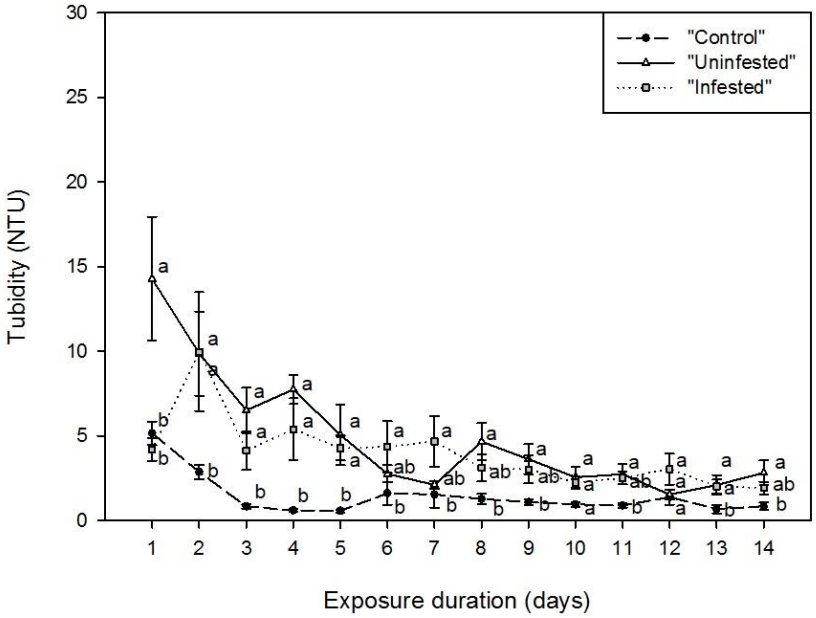

Figure IV. 1 Water column turbidity over the 14-d experiment for the three studied experimental conditions. "Control" = no *Upogebia* cf. *pusilla* in experimental units; "Uninfested" = presence of uninfested *U.* cf. *pusilla*; "Infested" = presence of infested *U.* cf. *pusilla*. Mean values ± standard errors (N = 5 for the "control" and "uninfested" conditions; N = 4 for the "infested" condition). NTU = nephelometric turbidity units. Different letters indicate significant differences of turbidity between the three experimental conditions at each sampling time.

Table IV. 2 Results of two-way repeated ANOVA comparing the effect of time of exposure to Cd-contaminated sediment and experimental condition ("control", "uninfested", "infested") on turbidity and dissolved Cd concentrations. "Control" = no *Upogebia* cf. *pusilla* in experimental units; "Uninfested" = presence of uninfested *U.* cf. *pusilla*; "Infested" = presence of infested *U.* cf. *pusilla*. P-values in bold indicate significant factor effect.

Variable	**Source of variation**	***df***	***F***	***P***
Turbidity	Time of exposure to Cd-contaminated sediment	13	13.17	< **0.001**
	Experimental condition	2	16.27	< **0.001**
	Time of exposure to Cd-contaminated sediment x Experimental condition	26	2.65	< **0.001**
Dissolved-Cd concentrations	Time of exposure to Cd-contaminated sediment	7	45.09	< **0.001**
	Experimental condition	2	1.02	0.392
	Time of exposure to Cd-contaminated sediment x Experimental condition	14	2.39	**0.008**

III.3. Cadmium transfers from the sediment to the overlying water

During the first two days of the experiment, dissolved Cd concentrations were under the detection limit (DL = 0.14 µg L^{-1}) for the three experimental conditions (Figure IV. 2). From day 2 to day 6, in "control", the dissolved Cd concentration steadily increased up to 0.81 ± 0.08 µg L^{-1} and then was stable up to the end of the experiment. The time course was different in "uninfested" and "infested" (Figure IV. 2). Dissolved Cd concentrations increased from day 3 to the end of the experiment up to 1.09 ± 0.08 and 1.15 ± 0.08 µg L^{-1} for the "uninfested" and "infested", respectively. For these two conditions, dissolved Cd did not reach any stable value after 14 days of experiment. However, there was no statistical difference of dissolved Cd concentrations between the three experimental conditions after 14 days of experiment, even though statistical analysis showed a significant interaction between the time of exposure and experimental conditions on dissolved Cd concentrations (Table IV. 2).

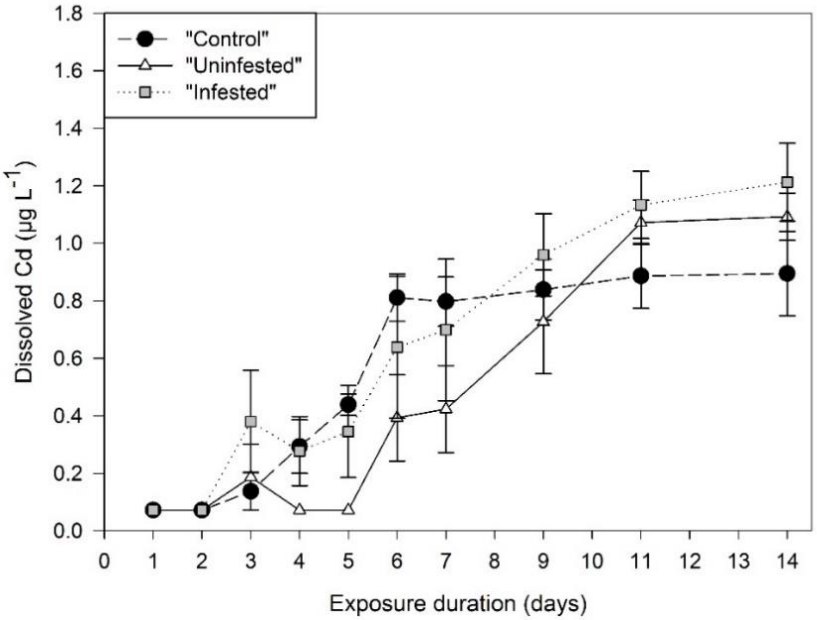

Figure IV. 2 Cadmium concentrations in filtered (< 0.22 µm) water samples as a function of time for the three studied experimental conditions. "Control" = no *Upogebia* cf. *pusilla* in experimental units; "Uninfested" = presence of uninfested *U*. cf. *pusilla*; "Infested" = presence of infested *U*. cf. *pusilla*. Mean values ± standard errors (*N* = 5 for the "control" and "uninfested" conditions; *N* = 4 for the "infested" condition).

Finally, the quantities of Cd in the water column were very low compared to the quantities of Cd contained in the sediments of the different experimental units. At the end of the experiment there was at least 1000-fold more Cd in the sediment than in the water column (1.28 10^3 ± 0.03 10^3 µg Cd in the sediment of each experimental units vs 0.95 ± 0.16, 1.16 ± 0.09 and 1.29 ± 0.14 µg Cd in the water column of the "control", "uninfested" and "infested", respectively) whereas there was 1.8-fold more sediment than water (2.9 kg vs 1.06 kg of sediment and overlying water, respectively).

The time course and value of total Cd concentrations (dissolved + particulate) over the experiment were identical to the ones of dissolved Cd concentrations for the three experimental conditions (data not shown). The linear regression between total Cd and dissolved Cd concentrations showed that Cd was mainly in the dissolved form for the three experimental conditions (R^2 = 0.93, $p < 0.001$, $y = 0.04 + 0.97\,x$) with no significant difference between the three linear regressions (ANCOVA, F= 2.08, p = 0.35). Finally, no significant correlations between turbidities and dissolved Cd concentrations in filtered water samples were noticed (R^2 = 0.00 and p = 0.75, R^2 = 0.00 and p = 0.82, R^2 = 0.05 and p = 0.31 for "control", "uninfested" and "infested", respectively).

III.4. Vertical profiles of cadmium concentrations in the sediment

Profiles of Cd concentrations in the sediment (excluding lumen vicinity) along depth for the three experimental conditions at experiment completion are shown in Figure IV. 3A. There were a significant influence of depth and condition ("control", "uninfested" and "infested") on Cd concentrations in the sediment, with an interaction between both factors (Table IV. 3).

Regarding the "control", there was a 1.4-fold decrease of the Cd concentration from the sediment top layer to 1–2 cm. Then the Cd concentration increased up to 4–5 cm and remained stable up to the core's bottom. The patterns of "uninfested" and "infested" are globally similar to each other and to the "control", except regarding the surface top layer. There was an increase of Cd concentration from the sediment top layer up to 6-7 cm for the "uninfested", and from the sediment top layer up to 4–5 cm for the "infested". Below these depths, Cd concentrations remained constant up to the core's bottom. There was no statistical difference between the vertical profile of Cd in the sediment for the "uninfested" and "infested".

Profiles of Cd concentrations in the vicinity of mud shrimp's burrow along depth at experiment completion are shown in Figure IV. 3B. The vertical profiles of Cd concentrations in the sediment next to the burrow lumen for the "uninfested" and "infested" were similar, with no significant differences of Cd concentrations at each depth between the two treatments except at depth 10-11 cm. There was a significant increase of Cd concentration in the sediment next to the burrow lumen with depth with no influence of experimental condition ("uninfested" and "infested") and an interaction between both factors on Cd concentration (Table IV. 3). Finally, there was no significant difference between the Cd concentrations in the burrow lumen vicinity and in the rest of the sediment layers (paired t-test, t = -0.23, p = 0.82).

III.5. Volume of burrow

The volume of each burrow was estimated after 14 days of experiment by sampling the burrow's water. The average volume of burrow of individuals exposed to Cd-contaminated sediment were 80.8 ± 7.6 and 48.5 ± 2.4 cm^3 for uninfested and infested mud shrimp, respectively (t-test, t = 3.64, p = 0.008).

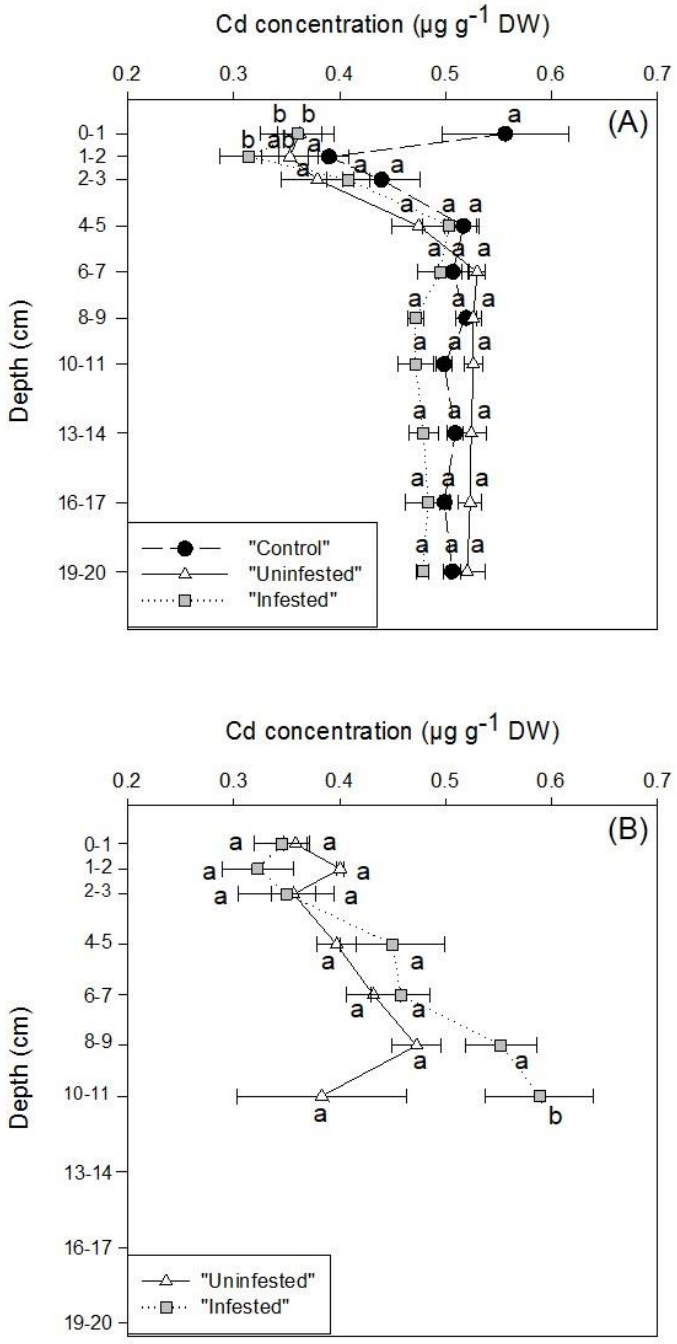

Figure IV. 3 Cadmium concentrations (µg g^{-1} DW) (A) in the non-oxidized sediment zone and (B) in the sediment in the vicinity of the burrow lumen as a function of depth for the three experimental conditions. "Control" = no *Upogebia* cf. *pusilla* in experimental units; "Uninfested" = presence of uninfested *U*. cf. *pusilla*; "Infested" = presence of infested *U*. cf. *pusilla*. Mean values ± standard errors ($N = 5$ for the "control" and "uninfested" conditions; $N = 4$ for the "infested" condition). Different letters indicate significant differences of Cd concentrations between the experimental conditions at each depth.

Table IV. 3 Results of two-way ANOVA comparing the effect of experimental condition ("control", "uninfested", "infested") and depth on sediment Cd concentrations in the vicinity of the burrow lumen and in the non-oxidized zone. "Control" = no *Upogebia* cf. *pusilla* in experimental units; "Uninfested" = presence of uninfested *U.* cf. *pusilla*; "Infested" = presence of bopyrid-infested *U.* cf. *pusilla*. P-values in bold indicate significant factor effect.

Variable	Source of variation	*df*	*F*	*P*
Sediment-Cd concentration in the non-oxidized zone	Experimental condition	2	13.46	< **0.001**
	Depth	9	22.66	< **0.001**
	Experimental condition x depth	18	3.10	< **0.001**
Sediment-Cd concentration in the vicinity of the burrow lumen	Experimental condition	1	2.68	0.108
	Depth	7	6.52	< **0.001**
	Experimental condition x depth	7	3.32	**0.008**

III.6. Cadmium bioaccumulation

Cadmium concentrations in the abdominal muscle and in the hepatopancreas of mud shrimp were determined at the end of the experiment and compared to environmental background levels (Figure IV. 4). Concentrations of Cd in the abdominal muscle were systematically below the detection limit (DL = 0.19 µg L^{-1}), irrespective of bopyrid presence in mud shrimp (uninfested vs. infested organisms) and their contamination status (organisms exposed to Cd-contaminated sediment vs. collected in the environment).

Conversely, there was a significant effect of parasitism and contamination status (exposed to Cd-contaminated sediment vs. collected in the environment) on Cd concentrations in the hepatopancreas of mud shrimp (two-way ANOVA, $F = 7.16$, $p = 0.017$) (Figure IV. 4). The Cd background concentrations in the hepatopancreas of uninfested and infested mud shrimp were similar (Tukey test, $q = 1.27$, $p = 0.38$) and low (Cd concentration = 0.34 ± 0.03 µg g^{-1} DW). The average Cd concentration in the hepatopancreas of mud shrimp exposed to Cd-contaminated sediment was 2.2-fold higher in uninfested compared to infested mud shrimp (Tukey test, $q = 4.00$, $p = 0.013$). Difference in hepatopancreas' Cd-content in mud shrimp exposed to Cd-contaminated sediment was not due to difference of TL between uninfested and infested organisms (TL = 48 ± 1.1 and 44.3 ± 1.3 mm for uninfested and infested mud shrimp, respectively; t-test, $t = 2.21$, $p = 0.063$).

Cadmium contents in female bopyrids were also determined at the end of the experiment and compared to environmental background levels. Concentrations of Cd in female bopyrid were systematically below the detection limit (DL = 0.19 µg L^{-1}), irrespective of the contamination status of the mud shrimp host (i.e. exposed to Cd-contaminated sediment vs. collected in the environment).

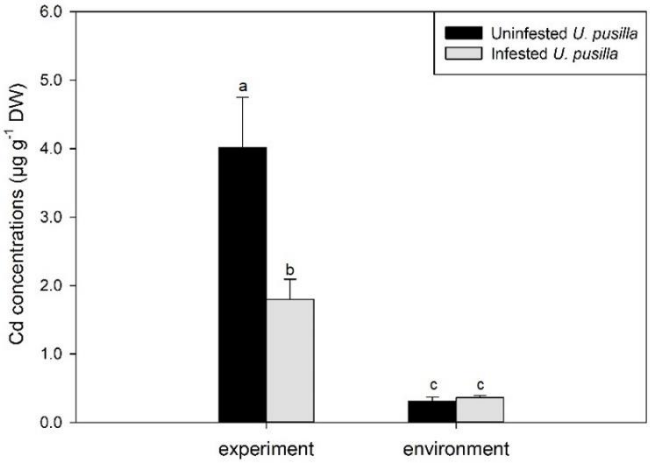

Figure IV. 4 Cadmium concentrations (µg g^{-1} DW) in the hepatopancreas of uninfested and infested *Upogebia* cf. *pusilla* collected in the environment ("environment"; Cd background level) and after 14 days of exposure to Cd-contaminated sediment ("experiment"). (N = 5; N= 4 for infested *U.* cf. *pusilla* from the experiment). Different letters indicate significant differences of Cd concentrations ($p < 0.05$).

III.7. Gene expression

The relative expression of several genes was compared between uninfested and infested mud shrimp collected in the environment (influence of parasitism) and exposed to Cd-contaminated sediment (influence of Cd on uninfested and infested individuals). Results of quantitative PCR are presented in Table IV. 4. Genetic analyses evidenced a tissue-specific expression of genes with larger variations of expression between experimental conditions in the mud shrimp abdominal muscle compared to the hepatopancreas.

In the hepatopancreas, the bopyrid parasite did not trigger any significant differential gene expression in organisms from the environment. Regarding uninfested organisms, there was no influence of exposure to Cd-contaminated sediment on gene expression. On the other hand, exposure to Cd-contaminated sediment triggered significant inductions of the gene *vtg* (x 7.4 to x 7.7; endocrine processes) in infested organisms.

In the abdominal muscle, the bopyrid parasite enhanced the expression of the gene *sod Mn* (x 2.4; antioxidant defenses) in organisms from the environment. Regarding infested organisms, there was no influence of exposure to Cd-contaminated sediment on gene expressions. On the opposite, exposition to Cd-contaminated sediment significantly down-regulated the genes *cat* (x 0.4; antioxidant defenses), *fer* (x 0.3; antioxidant defenses), *nos* (x 0.2; immune response) and 12S (x 0.4; mitochondrial metabolism) and induced the expression of the gene *rxr* (x 2.8; endocrine processes) in uninfested organisms. Finally, exposure to Cd-contaminated sediment and bopyrid parasite induced the expression of the genes *cox I* (x 2.4; mitochondrial respiratory channel), 12S (x 3.7) and *vtg* (x 7.8).

Table IV. 4 Differential gene expression observed in the hepatopancreas and abdominal muscle of uninfested ("Uninfested") and infested ("Infested") mud shrimp *Upogebia* cf. *pusilla* exposed to Cd-contaminated sediment ("exp") or collected on the field ("envt"). Results are given as significant induction (≥ 2) or repression (≤ 0.5) factors. "/" : no significant difference between the two treatments.

	Uninfested exp vs. Uninfested envt	**Infested exp vs. Infested envt**	**Infested exp vs. Uninfested exp**	**Infested envt vs. Uninfested envt**
Hepatopancreas				
vtg	/	7.4	7.7	/
Abdominal muscle				
cat	0.4	/	/	/
sod Mn	/	/	/	2.4
fer	0.3	/	/	/
nos	0.2	/	/	/
cox I	/	/	2.4	/
12S	0.4	/	3.7	/
rxr	2.8	/	/	/
vtg	/	/	7.8	/

IV. Discussion

This study aimed to (1) quantify the influence of the bioturbator *Upogebia* cf. *pusilla* on Cd transfers from contaminated sediment to overlying waters, (2) evaluate the impact of parasite infestation on its host's capabilities to remobilize Cd and (3) measure *U.* cf. *pusilla* contamination in a polluted sediment. Mud shrimp were placed in Cd-spiked sediment for 14 days and concentrations of dissolved and total Cd in overlying waters determined throughout. The bioavailability of dissolved Cd was also determined by quantifying its bioaccumulation in mud shrimp's hepatopancreas and abdominal muscle. Finally, the impact of Cd accumulation on genes expression of mud shrimp was also assessed.

IV.1. Dynamics of cadmium

For the three experimental conditions, there were a significant increase in the total and the dissolved Cd concentrations in overlying waters with time, with no significant differences between the different Cd concentrations at each sampling time. The concentrations of dissolved and total cadmium were almost equal for the three experimental conditions, indicating that the metal was mainly in its dissolved form. This is consistent with turbidity measurements which were low during the whole experiment for the three experimental conditions. Low turbidity values in experimental units with bioturbators may be due to the suspension-feeding activity of mud shrimp (Dworschak, 1987a). However, even if mud shrimp may have maintain a low concentration of particle matter in the water column, elevated salinity in the water column could also have enhanced partial desorption of Cd bound to particulate matter before particles were filtered by mud shrimp (Mantoura *et al.*, 1978; Paalman *et al.*, 1994; Gonzalez and Chiffoleau, 1999). In this scenario, an increase of dissolved Cd concentrations would be expected whereas no high concentration of particulate Cd would be noticed. Since there was no difference of dissolved Cd concentrations between the three experimental conditions, we propose that the molecular diffusion of Cd from sediments to overlying waters was the dominant process for the three experimental

conditions. Thus, over 14 days of experiment, the mud shrimp *U.* cf. *pusilla* had no net impact on Cd release from the sediment to the water column, irrespective of the bopyrid parasite occurrence. However, it seems that there was a difference of time course regarding Cd release between experimental conditions without and with mud shrimp. Even though this difference was not statically significant, it suggests that molecular diffusion is the major process influencing the release of Cd from contaminated sediment only during 14 days. After this time, the influence of *U.* cf. *pusilla* on Cd remobilization still has to be investigated.

The fact that *U.* cf. *pusilla* did not impact Cd remobilization is surprising considering the potential for bioturbation of the mud shrimp. This species lives in deep burrows that organisms maintain over their life time (Dworschak, 1983). The mud shrimp endobenthic life style is associated with important sediment reworking activities, such as expulsion of large amount of sediment particles at the sediment-water interface and their transport inside the burrow (Pascal, 2017). Since Cd in sediments is mainly bound in particulate forms, a significant release of particulate Cd was expected in the overlying water. Once in the water column, desorption of Cd bound on particle matter may occur (Mantoura *et al.*, 1978; Paalman *et al.*, 1994; Gonzalez and Chiffoleau, 1999), which would contribute to increase the pool of dissolved Cd in the water column. On the other hand, mud shrimp living deeper than the oxygen penetration depth, they frequently ventilate their burrow, enhancing exchanges of solutes (as oxygen) between overlying-, burrow- and pore-waters (Pascal *et al.*, 2016a; Pascal, 2017). By doing so, mud shrimp influence pH and redox conditions in sediments. Yet, the stability of trace metals and their mobility in sediments greatly depend of these chemical parameters (Calmano *et al.*, 1993; Atkinson *et al.*, 2007). For instance, the oxidation of acid-volatile sulfide (AVS) releases Cd-bound to aqueous phase (Peterson *et al.*, 1996). In our study, the sediment of the burrow walls displayed a lighter color than the rest of the sediment, attesting that the former was oxidized compared to the latter. Thus, AVS were likely oxidized in the sediment burrow walls while they may have bounded Cd in the remaining sediment. Since we did not noticed any significant difference of Cd concentrations between the sediment in the vicinity of the burrow lumen and the un-oxidized sediment, it strongly suggests that the contribution of AVS to Cd speciation in this study was negligible.

Several studies showed the important role of bioturbators on dynamics of trace metals in aquatic sediments (Rasmussen *et al.*, 1998, 2000; Ciutat and Boudou, 2003; Ciutat *et al.*, 2007; Remaili *et al.*, 2016). For instance, in freshwater environments, tubificid worms allow for a transitorily release of particulate Cd into overlying waters from naturally contaminated sediments (Ciutat *et al.*, 2007). Similarly, the bioturbation activities of the nymph mayfly *Hexagenia rigida* induce a significant release of particulate Cd and Zn into the water column (Ciutat and Boudou, 2003). In marine environments, the literature is scarcer. Nonetheless, it has been demonstrated that the lugworm *Arenicola marina* influences the partitioning of Cd between sediment and the overlying water (Rasmussen et *al.* 2000). Its presence in Cd-contaminated sediments is associated with a release of the metal in the water column,

but this release was more important when the lugworm was not present (Rasmussen *et al.*, 2000). Bioturbation activities of *A. marina* also play a significant role in the distribution of Cd in the sediment. This species is an upward conveyor (Kristensen *et al.*, 2012), i.e. the lugworm is head-down in the sediment, feeds on deep horizons and moves particles to the sediment surface. In contaminated sediment, it transports Cd-bound upwards in the sediment column. Thus the sediment surface layers are enriched in Cd compared to deeper zones (Rasmussen *et al.*, 2000). In this study, we also evaluated the role of *U.* cf. *pusilla* on the distribution of Cd in the sediment column. Conversely to what was observed for *A. marina*, there was presently no enrichment in Cd of the sediment surface layers. On the opposite, surface layers were lightly depleted in Cd compared to deeper horizons. Furthermore, the vertical profiles of Cd concentrations in the sediment were very similar between the three experimental conditions. It is likely due to the fact that *U.* cf. *pusilla* is not an upward conveyor but a regenerator. Regenerators are diggers that maintain a burrow and therefore rework large amount of sediments (Kristensen *et al.*, 2012). Regarding *U.* cf. *pusilla*, the mud shrimp transports sediments outside its burrow but also from the sediment surface to the burrow (Pascal, 2017). Therefore, sediment reworking activities of the mud shrimp are not one-way oriented, which could explain that there was no obvious gradient of Cd concentration in the sediment column. The small Cd depletion in the sediment top layers compared to deeper ones could be explained by two unexclusive hypothesis. First, during the burrow building phase, mud shrimp reworked large amount of sediment, which could result in a transfer of Cd from the sediment to the overlying water. In addition, molecular diffusion of Cd from the top layers to the water column, enhanced by water open circuit before the beginning of incubations (cf materials and methods) could lead to a depletion of Cd in the sediment top layers.

Cadmium concentrations in the sediment in the vicinity of the burrow lumen (i.e. burrow wall) and in the un-oxidized area were also compared. Cadmium concentrations in the two sediment areas were similar to each other all along the sediment column. Conversely, it has been noticed that the sediment of the burrow wall of the ghost shrimp *Glypturus (Callichirus) laurae* was Cd-enriched compared to un-oxidized sediments surrounding the burrow (Abu-Hilal *et al.*, 1988). Klerks *et al.* (2007) also compared Cd concentrations in the burrow walls of the ghost shrimp *Sergio trilobata* and *Lepidophthalmus louisianensis* to the sediment they ejected from their burrow and to surface sediment. They showed that the burrow walls of these organisms were enriched in Cd and Zn compared to surface and ejected sediments. The metal enrichment of the burrow walls of ghost shrimp could be related to higher organic content of the burrow walls compared to ejected and surface sediments (Abu-Hilal *et al.*, 1988; Klerks *et al.*, 2007). This pattern might also be related to shrimp's activities. Especially, ventilation of the burrow might enhance transport of dissolved Cd coming into contact to wall sediment particles. If the burrow ventilation rate of *U.* cf. *pusilla* is lower than the ones of *S. trilobata* and *L. louisianensis*, it might explain there was no Cd enrichment of the burrow walls in this study. Finally, it has to be noted that the burrow walls of ghost shrimp are lined with mucus. It has not been reported for *U.* cf. *pusilla*

burrow walls (Dworschak, 1983). This proteic lining may interfere with trace metal adsorption and/or transport (Petrash *et al.*, 2011).

IV.2. Bioaccumulation of cadmium

Mud shrimp showed important Cd bioaccumulation after 14 days of exposure to Cd-contaminated sediment. This trace metal accumulated in the hepatopancreas, whereas it was not detected in the abdominal muscle of mud shrimp. The partitioning of metals between the different organs varies deeply between metals and greatly depends on the species (Marsden and Rainbow, 2004).

The fact that *U.* cf. *pusilla* exhibited a significant level of Cd bioaccumulation shows that this trace metal was highly bioavailable. The mud shrimp is primarily a suspension-feeder using suspended matter as a diet source (Dworschak, 1987a). In addition, it also filters water for respiration purpose. Therefore, Cd enters animals by the way of gills (water borne Cd) or through the gut after food consumption (dietary Cd). We suggest that the mud shrimp bioaccumulated Cd mostly *via* its diet. Indeed, the mud shrimp drive themselves the resuspension of particles they feed on through their significant activity of burrow ventilation. In addition, mud shrimp transport sediment from the sediment-water interface inside their burrow that they suspend before feeding on. Finally, during the building of the burrow, these organisms can also directly feed on the sediment (Dworschak, 1987a). In light of those, ingestion of Cd-contaminated sediment (particulate Cd) could explain the trace metal accumulation in *U.* cf. *pusilla*. It also implies that accumulation of Cd in the mud shrimp took place during the experimental setting (building phase of the burrow) and the first days of incubation. Indeed, dissolved Cd in the water column were noticed only after three days of experiment. However, the fact that there was no significant difference of dissolved Cd concentrations between experimental units with and without mud shrimp suggests there was no contribution of water borne Cd to mud shrimp contamination during this time. Thus, only particulate Cd would contribute to the pool of Cd accumulated by mud shrimp. Since turbidity in cores containing mud shrimp was low after two days of experiment, mud shrimp would have mainly accumulated Cd before this time. Mud shrimp building their burrow during the experimental set up, they probably ingested a large amount of Cd-contaminated sediment by that time. Moreover, during the first days of incubation, turbidity was still relatively high in the water column of experimental units with mud shrimp compared to the "control". Thus, mud shrimp were still significantly ventilating their burrow, enhancing resuspension of Cd-bound particulate matter they feed on.

IV.3. Influence of parasitism

Over 14 days of experiment, there was no significant influence of the mud shrimp *U.* cf. *pusilla* on dissolved and total Cd resuspension from contaminated sediment to the overlying water column, independently of bopyrid parasite occurrence. Moreover, the mud shrimp did not influence the repartition of Cd in the sediment, independently of parasitic infestation. On the opposite, Cd concentrations in the mud shrimp hepatopancreas differed accordingly to bopyrid burden, with Cd content in infested mud shrimp 2.2-fold lower than in uninfested individuals. It suggests a significant

impact of bopyrid parasite on the Cd bioaccumulation process in mud shrimp. Conversely to mud shrimp, bopyrid parasites did not accumulate any Cd. Thus, the difference of Cd concentrations in the hepatopancreas of uninfested and infested mud shrimp was not due to Cd accumulation by the parasite. Nonetheless, the bopyrid parasite could indirectly interfere with the ability of mud shrimp to accumulate Cd. Previous studies examining impacts of bopyrid parasites on mud shrimp showed that these parasites alter the physiological state of their host (Smith *et al.*, 2008; Dumbauld *et al.*, 2011; Pascal *et al.*, 2016b) and reduce their activity levels (Pascal, 2017). In this study for instance, burrows of uninfested mud shrimp were 1.7-fold larger compared to burrows of infested individuals. It could suggest that, during the building phase, uninfested mud shrimp would have ingested a larger amount of Cd-contaminated sediment than infested organisms. The burrow volume reduction of infested mud shrimp compared to uninfested individuals also contributes to explain the lower bioaccumulation of Cd by mud shrimp. Indeed, reductions in burrow depth and length diminished the surface of exchanges between the water and the sediment column. Lower volume of burrow might limit resuspension of particulate Cd during burrow ventilation events, and therefore Cd accumulation in infested mud shrimp.

IV.4. Gene expression

After 14 days of experiment, mud shrimp showed significant bioaccumulation of Cd in their hepatopancreas, whereas the trace metal was not detected in the abdominal muscle (see above). Regarding Cd, the crustacean hepatopancreas seems to be an important site for metal sequestration (Marsden and Rainbow, 2004; Ahearn *et al.*, 2004). This organ also plays a significant role in detoxification processes, with several pathways involved (Ahearn *et al.*, 2004). One of them implicates mitochondria of hepatopancreatic cells. These organelles remove trace metals from the cytoplasm and sequestrate them as an insoluble complexes, reducing their toxicant effects (Ahearn *et al.*, 2004). This detoxification pathway does not seem to be enhanced in this study. Indeed, there was no induction of both genes involved in mitochondrial metabolism (12S and *cox I*) in the hepatopancreas of mud shrimp exposed to Cd-contaminated sediment compared to specimens sampled in naturally Cd-uncontaminated environment, independently of bopyrid parasite presence.

Although several studies showed a significant regulation (induction or repression) of genes involved in the mitochondrial respiratory chain, in the cellular response to metal and oxidative stresses in the hepatopancreas of various Cd-exposed crustaceans (Soetaert *et al.*, 2007; Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018), there were no significant inductions or repressions of the stress genes targeted in this study, with the exception of the gene *vtg*. The hepatopancreas is a metabolically highly active organ and it may be argue that the basal level of gene expression in this organ would be sufficient to face the stress due to exposure to Cd-contaminated sediment. This theory has already been evoked in studies involving the zebrafish *Danio rerio* and the frog larvae *Xenopus laevis* (Gonzalez *et al.*, 2006; Mouchet *et al.*, 2006). For instance, Gonzalez *et al.* (2006) have demonstrated that the basal gene expression was larger in the digestive gland of *D. rerio* compared to other organs and sufficient to deal with low exposure to Cd.

Finally genetic regulation is a time-dependent process (Soetaert *et al.*, 2007; Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018). In this study, genetic expression was only evaluated after 14 days of exposure to Cd and an earlier genetic response to Cd accumulation should not be excluded.

In the mud shrimp's hepatopancreas, the gene *vtg* was the only one to exhibit significant difference of expression depending on experimental conditions. Bopyrid alone did not induce the *vtg* expression. Similarly, Cd did not trigger induction of *vtg* in uninfested organisms. On the other hand, exposure to Cd induced the *vtg* expression in infested organisms compared to uninfested mud shrimp, suggesting an interaction between both stressors. The *vtg* gene is a biomarker of endocrine disruption in crustaceans (Park *et al.*, 2014). It pointed out the fact that infested mud shrimp are more susceptible to endocrine disruption when exposed to Cd than uninfested organisms. This result is not surprising considering the impact the parasite has on its host. Indeed, this bopyrid parasite is recognized to deeply impair its host reproductive state with females being castrated and males being feminized (Tucker, 1930). Regarding Cd, it is defined as an endocrine-disrupting compound (Rodríguez *et al.*, 2007) which usually down-regulated the expression of lipoprotein genes in arthropods (Soetaert *et al.*, 2007).

While there was no detectable Cd bioaccumulation in the abdominal muscle of mud shrimp, there was a larger genetic response compared to the hepatopancreas. This discrepancy between gene expression and Cd bioaccumulation has already been noticed in *D. rerio* (Gonzalez *et al.*, 2006) and comes to support the hypothesis that the basal genetic expression in the mud shrimp hepatopancreas is sufficient to deal with exposure to Cd. In this study, Cd had no effect on genetic expression in the abdominal muscle of infested mud shrimp. On the opposite, the trace metal triggered a significant down regulation of genes involved in the antioxidant response (*cat*, *fer*) in uninfested mud shrimp, suggesting that this trace metal impairs the ability of these organisms to deal with oxidative stresses. A similar antioxidant response has already been noticed in the hepatopancreas and in the gills of the mud crab *Scylla paramamosain* after 48 h of exposure to Cd (Zhu *et al.*, 2018). At the proteomic level, Cd is also known to inhibit the activity of antioxidant enzymes such as the catalase or the superoxide dismutase (Pan and Zhang, 2006). A significant repression of the gene 12S in the abdominal muscle of uninfested mud shrimp exposed to Cd was also recorded. This gene is taken for a proxy of the mitochondria concentration (or number) (Al Kaddissi *et al.*, 2014; Arini *et al.*, 2015) since it has been evidenced that the number of rRNA copies per mitochondria was constant (Cantatore *et al.*, 1984; Gadaleta *et al.*, 1990). The lower expression of 12S suggests that Cd elicits a reduction of the pool of mitochondria. Impairment of mitochondria due to exposure to Cd has already been noticed in the hepatopancreas of the crab *Eriocheir sinensis*. Cadmium induced apoptosis and necrosis of hepatopancreatic cells with mitochondria exhibiting ruptured membranes (Lin *et al.*, 2017). Even though uninfested mud shrimp exhibited a reduction of their pool of mitochondria due to Cd accumulation, there was no repression of the gene *cox I* (mitochondrial gene) in these mud shrimp compared to the control. It suggests an increase of *cox I* expression in these organisms, which would balance the overall reduction of this gene

expression due to the lower number of mitochondria. Thus, the genetic expression in the abdominal muscle of uninfested mud shrimp suggests these organisms were very sensitive to Cd, which impaired the expression of genes involved in the mitochondrial respiratory chain and in the cellular response to metal and oxidative stresses. In addition, comparison of gene expressions between uninfested and infested mud shrimp exposed to Cd-contaminated sediment evidenced an induction of the genes *cox I* and 12S. These results highlight that uninfested organisms are more sensitive to Cd than infested mud shrimp, with the mitochondrial metabolism of the former reduced compared to the latter. Finally, Cd triggered an induction of *vtg* in the abdominal muscle of infested mud shrimp compared to uninfested organisms. Neither Cd nor bopyrid alone enhanced the *vtg* expression in the mud shrimp abdominal muscle. Thus, there was an interaction between both stress factors, which acted as endocrine disruptors, similarly to what was observed in the hepatopancreas of infested organisms.

At the broader organism level, the discrepancy of gene expression between uninfested and infested mud shrimp exposed to Cd-contaminated sediment may be related to the rate of Cd bioaccumulation and indirectly to the mud shrimp activity. As previously stated, bopyrid parasites deeply impair their host and especially reduce their bioturbation activity. By doing so, bopyrid parasites interfere with the processes of trace metal accumulation by the mud shrimp, uninfested organisms showing greater Cd accumulation rate compared to infested specimens. Consequently, a larger trace metal accumulation may also enhance a larger response at the genetic level in uninfested mud shrimp compared to infested organisms.

V. Conclusion

In this study, we did not observe any influence of the bioturbator *U.* cf. *pusilla* on trace metal transfers from a contaminated sediment to the overlying water, neither on trace metal distribution in the sediment column over 14-days of experiment. The mud shrimp showed an important level of contaminant bioaccumulation and may be very sensitive to this trace metal, especially if uninfested with the bopyrid parasite. Indeed, genetic activity of uninfested mud shrimp seems to be highly impaired because of trace metal, whereas there was no obvious difference between Cd-exposed and unexposed infested mud shrimp. However, potential impairment of the mud shrimp *U.* cf. *pusilla* bioturbation activities due to contaminant toxicity still has to be properly investigated.

The tolerance of a species to contaminants can show great variations according to populations. Populations naturally exposed to contaminants can exhibit local adaptation making them more tolerant and less sensitive to contaminants than organisms unexposed to contaminants (Klerks and Levinton, 1989; Paul-Pont *et al.*, 2010b). Mud shrimp were collected in Arcachon Bay, France, where the concentrations of contaminants in sediments are very low. Thus, we would propose to investigate the influence of trace metals on bioturbation activities of mud shrimp naturally exposed to contaminants.

Conflict of interest

None.

Animal rights

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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BILAN DU CHAPITRE IV ET PERSPECTIVES

Plusieurs études ont évalué le rôle des bioturbateurs marins et d'eau douce sur la remobilisation des contaminants métalliques et organiques contenus dans les sédiments vers la colonne d'eau. Néanmoins, dans leur grande majorité ces études se sont concentrées sur des bioturbateurs de sub-surface, i.e. se trouvant dans les dix premiers centimètres de la colonne sédimentaire. Par ailleurs, les organismes étudiés étaient en grande majorité des biodiffuseurs et des convoyeurs. Ces organismes sont à l'origine de transports de sédiments orientés ou non et ce sur de relatives petites échelles spatiales. A l'inverse, le rôle des régénérateurs comme *U.* cf. *pusilla,* qui créent des terriers profonds, et de ce fait influencent un large volume de la colonne sédimentaire, sur la remobilisation des contaminants n'a pour le moment pas été évalué. Une valeur ajoutée au projet consistait par ailleurs à prendre en compte l'influence du parasitisme sur le potentiel de remobilisation des contaminants métalliques de *U.* cf. *pusilla*. Les parasites sont largement répandus chez les bioturbateurs mais leur influence sur leur hôte n'a que très rarement été évaluée (**Chapitre I-A**).

Au cours de cette étude nous n'avons pas noté d'influence de la crevette de vase *U.* cf. *pusilla* sur la remobilisation du Cd sous forme dissoute ou particulaire depuis le sédiment vers la colonne d'eau, et ce, indépendamment de la présence du parasite bopyre *Gyge branchialis*. Par ailleurs, la crevette de vase n'influence que peu la distribution du Cd dans la colonne sédimentaire, et ce indépendamment de la présence du parasite bopyre. La bioaccumulation significative de Cd par les organismes, les faibles valeurs de turbidité et les modifications d'expression génique en lien avec la contamination métallique permettent d'envisager un impact négatif du Cd sur l'état de santé et, *in fine,* sur les activités de bioturbation d'*U.* cf. *pusilla*. Par ailleurs, nous avons pu mettre en évidence un effet marqué du parasitisme sur les capacités de bioaccumulation de nos organismes modèles ainsi que des modifications d'expression génique atténuées en contexte de multi-stress. Du fait de ces plus faibles niveaux de contamination, il est possible que l'activité de bioturbation des individus parasités par le bopyre soit impactée dans une moindre mesure par rapport à celle des individus non parasités mais accumulant fortement le Cd. L'objectif suivant est ainsi de caractériser et de quantifier l'influence d'une contamination métallique au Cd par l'eau, du parasitisme par *G. branchialis* et de ces deux facteurs combinés sur les activités de ces organismes et fera l'objet du **Chapitre V**.

CHAPITRE V

Influence d'une contamination métallique et d'un stress parasitaire sur la physiologie et l'activité de bioturbation de *Upogebia* cf. *pusilla*: approche *ex-situ*

Do trace metal contamination and parasitism influence the activities of the bioturbating mud shrimp *Upogebia* cf. *pusilla*?

DAIRAIN A., de MONTAUDOUIN X. GONZALEZ P., CIUTAT A., BAUDRIMONT M., MAIRE O. and LEGEAY A.

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Abstract

Mud shrimp are considered as among the most influential ecosystem engineers in marine soft bottom environments because of their significant bioturbation activity and their high density. These organisms play a key role on the physical structure of sediments through intense sediment reworking activity and also deeply influence geochemical properties of sediments *via* frequent bioirrigation events. The influence that mud shrimp have on the environment is related to the magnitude of bioturbation processes and subsequently depends on their physiological condition. In natural environments, several factors act together and influence the well-being of organisms. Among them, the deleterious role of parasites on the physiology and the behavior of their host is well established. Aquatic organisms are also subject to pollutants released by anthropogenic activities. However, the effect of both stressors on the fitness and bioturbation activity of mud shrimp has never been investigated yet.

We conducted a 14-day *ex-situ* experiment to evaluate the influence of trace metal contamination (cadmium Cd) and parasitism infestation on the gene expression (molecular endpoint) and sediment reworking activity (behavioral endpoint) of the mud shrimp *Upogebia* cf. *pusilla*. At completion, mud shrimp exhibited substantial Cd bioaccumulation, with parasitized organisms showing a significantly lower contaminant burden than unparasitized organisms. Cadmium contamination induces modifications of gene expression in both unparasitized and parasitized organisms. We report an antagonistic effect of both stressors on gene expression, which cannot be fully explained by lower Cd bioaccumulation. At the behaviour level, parasitism seems to reduce the sediment reworking activity of mud shrimp, while Cd contamination appears to stimulate this activity. This study highlights that the effects of multiple stressors may be quite different from the effect of each stressor considered individually. It should also motivate for more studies evaluating the influence of multiple stressors on different endpoints encompassing various levels of organization.

Key words: mud shrimp, parasitism, cadmium, gene expression, bioturbation, bioaccumulation

I. Introduction

The shaping and functioning of benthic environments is strongly related to the process of bioturbation (Cadée, 2001; Reise, 2002; Lohrer *et al.*, 2004). Bioturbation is described as any modification of the sediment matrix, including interstitial waters, due to faunal activities (Kristensen *et al.*, 2012). The intense burrowing and feeding activities of bioturbating species lead to important displacement of sediment particles, both vertically and laterally (= sediment reworking) (Kristensen *et al.*, 2012). In addition, some endobenthic bioturbators build semi-permanent or permanent burrows that they have to ventilate. By doing so, these organisms enhance porewater exchanges and displacement of solutes in suboxic and anoxic surrounding sediments (= bioirrigation process) (Glud, 2008; Kristensen *et al.*, 2012). Thus, bioturbators deeply alter the physical and biogeochemical properties of sediments. Because they can occur at high densities in natural environments, bioturbators are recognized as major ecosystem engineers. Thalassinidean mud shrimp (sensu Felder, 2001) are among the most influential organisms in marine soft-bottom environments (Cadée, 2001). They live in complex and deep burrow, i.e. > 1 m deep (Kinoshita *et al.*, 2010), connected to the sediment-water interface by several distant openings (Dworschak, 1983; Kinoshita *et al.*, 2010). Mud shrimp play a significant role in the shaping of benthic habitats (see Pillay and Branch, 2011 for a review), deeply impact nutrient cycling (D'Andrea and DeWitt, 2009; Pascal *et al.*, 2016a) and influence benthic communities (Posey *et al.*, 1991; Dittmann, 1996; Papaspyrou *et al.*, 2005).

The influence that mud shrimp have on their environment is related to the magnitude of bioturbation processes and therefore depends on their physiological condition. Several abiotic factors, as well as biological mediated processes influence the fitness of bioturbators, and thus impair the intensity of their activity (e.g., Bartsch *et al.*, 1999; Duport *et al.*, 2006; Sturdivant *et al.*, 2012; Dedeh *et al.*, 2016). For example, at high density of bioturbators, intraspecific competition decreases individual sediment reworking rates (Duport *et al.*, 2006). Similarly, predation threat modifies preys' behavior and reduced their bioturbating role on solute fluxes at the sediment-water interface (Premo and Tyler, 2013). Conversely, studies evaluating the influence of parasitism on bioturbation activity remain scarce despite the fact that parasites are widespread in ecosystems (Dobson *et al.*, 2008), greatly influence their host fitness and activity (Schulte-Oehlmann *et al.*, 1997; O'Connell-Milne *et al.*, 2016; Pascal *et al.*, 2016b) and, by a cascade effect, affect ecosystem functioning (Thomas *et al.*, 2005; Mouritsen and Poulin, 2005). Mud shrimp are no exception: they host several types of parasites (Dworschak, 1988; Markham, 2005; Dairain *et al.*, 2017). Among them, bopyrid parasites are known to alter the physiological status of their host (Smith et al. 2008; Pascal et al. 2016a) and also to reduce their activity levels (Pascal, 2017). Consequently, these parasites could impact the bioturbation capacities of their host.

In addition to parasitism, bioturbators are naturally exposed to a broad variety of other stressors. Increase in human population densities and associated activities have led to high releases of pollutants in aquatic environments, leading locally to high levels of contamination (Tueros *et al.*, 2009; Pan and

Wang, 2012). Amongst these pollutants, trace metals such as cadmium (Cd), mercury (Hg) or lead (Pb) are of major concern and therefore considered as priority substances by governments (European Water Framework Directive 2008/105/EC; Canadian Environmental Protection Act, 1999) because of the significant toxic effects they have on aquatic organisms. For instance, Cd initiates significant disruption of gene expression (Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018), cellular damages (Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018), physiological alterations (Felten *et al.*, 2008; Luo *et al.*, 2015) and behavior modifications (Bat *et al.*, 1998; Roast *et al.*, 2000; Wallace and Estephan, 2004; Untersteiner *et al.*, 2005) in various aquatic organisms. Regarding bioturbators, studies evaluating the influence of trace metal on their physiology and behavior are still scarce (Ciutat *et al.*, 2005; Lagauzère *et al.*, 2009; Dedeh *et al.*, 2016). They showed contradictory results depending on (1) the species studied and its mode of bioturbation, (2) the contamination way (dietary vs. water borne contamination) and (3) the contaminant tested. As an illustration, Dedeh *et al.* (2016) noticed a significant reduction of the sediment reworking activity of the tubificid oligochaete worm *Tubifex tubifex* exposed to Cd-contaminated sediments whereas Ciutat *et al.* (2005) did not report any influence of water borne Cd on the worms' activity, despite high bioaccumulation of the trace metal.

Since stressors rarely occur independently in natural environments, it is likely that parasites and trace metals act together with additive, synergistic or antagonistic effects on the health of organisms. Parasites might interfere with trace metal sensitivity (Sures, 2008; Marcogliese and Pietrock, 2011) but contaminants might also impair pathogenic organisms themselves and thus reduce their pathogenicity (Lafferty, 1997; Sures, 2008). Studies evaluating the effects of trace metal contamination and parasitism on free-living organisms have received increasing attention over the last two decades. For example, Baudrimont and de Montaudouin (2007) showed that the cockle, *Cerastoderma edule*, infested with trematode parasites bioaccumulated more Cd than uninfested individuals. Moreover, they highlighted that parasites interfered with the trace metal detoxification processes of their host. In a complementary study, Paul-Pont *et al.* (2010a) indicated that both stressors also had an antagonistic effect on mitochondrial gene expression whereas they significantly enhanced the oxidative burst process. Yet, a similar study conducted in the Manila clam *Ruditapes phillippinarum* did not evidence any significant impact of parasitism on Cd bioaccumulation and no synergistic effect of both stressors on the oxidative burst process (Paul-Pont *et al.*, 2010c). The role played by parasites and contaminants depends on the host-pathogen system, but also on the nature of these stressors (de Montaudouin *et al.*, 2010), highlighting the complexity of interactions. Most of these studies evaluated the role of both stressors from a molecular (gene expression) and cellular (count of total haemocytes, phagocytic activity, etc.) perspective, overlooking at potential effects on the behavior and activities of organisms. Regarding bioturbating species, studies assessing the influence of trace metal contamination and/or parasitism on ecosystem engineer traits are still scarce and to our knowledge, none has been conducted taking into account both stressors. In this context, the present study aimed at assessing the effects of trace metal contamination and parasitism, alone and in combination, on the gene expression and the activity of a

bioturbating species. Cadmium was used as a model contaminant. The selected host-parasite system was the mud shrimp *Upogebia* cf. *pusilla* and the bopyrid isopod *Gyge branchialis*. A 14-day *ex-situ* experiment was conducted to investigate (1) the kinetics of trace metal bioaccumulation depending on parasitic infestation, (2) the effect of stressors on a behavioral endpoint by estimating the sediment reworking activity of the mud shrimp and (3) the effect of stressors on a molecular endpoint by quantifying gene expression of target genes involved in key metabolic pathways of the mud shrimp. Cadmium was supplied by water and trace metal bioaccumulation would be expected because of the filtration activities of the mud shrimp. Cadmium induces modifications of gene expression (Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018) and impairs the behavior of crustaceans (Felten *et al.*, 2008; Vellinger *et al.*, 2012), and might thus impair both molecular and behavioral endpoints in mud shrimp as well. Similarly, parasitic infestations have been associated with modifications of gene expression (Paul-Pont *et al.*, 2010a; Morga *et al.*, 2011) and behavior alterations (Mouritsen and Poulin, 2005; Pascal, 2017), and were therefore presently expected. Finally, the bopyrid parasite *G. branchialis* has a detrimental effect on its host condition index (Pascal *et al.*, 2016b). Consequently, bopyrid-infested mud shrimp might bioaccumulate less Cd than uninfested organisms because of a reduced filtration activity in link with a lower energetic balance. Thus, the bopyrid parasite might indirectly interfere with the Cd bioaccumulation process. This lower Cd bioaccumulation might be associated with slighter changes of gene expression and bioturbation activity compared to the ones anticipated in bopyrid-free organisms. Thus, we hypothesize that parasitism would lower the effects of Cd on gene expression and sediment reworking activity of mud shrimp by reducing bioaccumulation rate.

II. Materials and methods

II.1. Sampling of *Upogebia* cf. *pusilla* and sediments

The mud shrimp *Upogebia* cf. *pusilla* was sampled in a mud flat in the middle of Arcachon Bay (44°42' N, 1°11' W), France, with a bait piston pump. This site was selected because it harbours a relatively dense population of mud shrimp (mean density = 4.9 ind. m^{-2}, maximal density = 23 ind. m^{-2}) (Pascal, 2017), with a significant proportion infested with the bopyrid isopod *Gyge branchialis* (Pascal *et al.*, 2016b). Only adult male specimens were selected for the experiment because reproductive status of females may influence their behavior (Sornom *et al.*, 2010). Once collected, each specimen was individually isolated in a plastic pot containing seawater, avoiding fights between mud shrimp, before being brought back to the laboratory. In the laboratory, mud shrimp were kept four days in aquaria containing natural sediments in which they can burrow, before being transferred in experimental units (see below). Theses aquaria were kept in the thermo-regulated room where the experiment was conducted and continuously supplied with seawater. Natural sediment was also collected. The sediment was firstly dried-sieved through a 500 µm mesh to remove macrofauna and debris, kept several days in covered boxes before being introduced in experimental units (see below). The sediment consisted of

muddy sand (sand = 87.2 %, mud = 12.5 % and gravel = 0.3 %; D_{50} = 142 µm). The background Cd concentration in the sediment was 0.047 ± 0.010 µg Cd g^{-1} dry weight (DW).

II.2. Experiment design and setup

The experiment was conducted for 14 days in September 2017, with three sampling times: 3, 7 and 14 days after the first Cd input. This duration was selected based on previous studies evaluating the sediment reworking rate of mud shrimp in "unstressed conditions" (Maire *et al.*, 2016; Pascal, 2017). The sediment reworking rate is maximal four days after fluorescing particles have been introduced in experimental units (see below), decreasing after this time (Pascal, 2017). Nonetheless, crustaceans exposed to Cd exhibit reduced levels of activities (Pestana *et al.*, 2007; Sornom *et al.*, 2010; Dedeh *et al.*, 2016). Thus, we extent the time of experiment to 14 days to be sure that the maximal sediment reworking rate would be recorded.

The experiment took place in a dark thermo-regulated room maintained at 20 °C. This temperature coincides with water temperature in the field at this period. The basic structure of the experimental unit consisted of a Plexiglas tubing (diameter = 9.5 cm, height = 40 cm) filled with approximatively 2.9 kg of sediment in order to obtain an overlying water column of 15 cm height (i.e. volume of the water column = 1.06 L). Sieved sediment was inserted four days before introducing organisms allowing for the stabilization of the sediment matrix. During this time, sediments were placed under a seawater open circuit, allowing for the partial stabilization of geochemical gradients. Mud shrimp were then placed in experimental units. One core contained one organism, which corresponds to a density of 141 ind. m^{-2}. This density is higher than those observed in Arcachon Bay (Pascal, 2017) but is in the range of those documented in other ecosystems (Dworschak, 1987b). Moreover, the density of mud shrimp in our experiment cannot relevantly be compared to field conditions since mud shrimp are alone in their experimental units, without any biotic interactions with conspecifics. Mud shrimp were allowed to burrow for three days in a seawater open circuit. This time was sufficient to observe the building of a two-open-ended burrow (i.e. a complete burrow) in each experimental unit.

Four series of experimental conditions with four replicates per sampling time were conducted: (1) bopyrid-free mud shrimp unexposed to Cd-contaminated seawater ("Control" condition), (2) bopyrid-infested mud shrimp unexposed to Cd-contaminated seawater ("Bop" condition; to estimate the impact of the parasite on its host's gene expression and sediment reworking activity), (3) bopyrid-free mud shrimp exposed to Cd-contaminated seawater ("Cd" condition; to estimate the impact of the trace metal exposure on gene expression and sediment reworking activity of mud shrimp) and (4) bopyrid-infested mud shrimp exposed to Cd-contaminated seawater ("Bop + Cd" condition; to estimate potential interactive effects between both stressors on gene expression and sediment reworking activity of mud shrimp). Thus, the experiment consisted of 48 experimental units: 4 experimental conditions ("Control", "Bop", "Cd" and "Cd + Bop") x 3 sampling times (3, 7 and 14 days) x 4 replicates. Metal contamination was initiated by supplying seawater with a Cd stock solution (added as $CdCl_2$, concentration = 1 g L^{-1};

Merck) at a rate of 180 mL h^{-1} in order to obtain a final concentration of 8.0 µg L^{-1} in each contaminated experimental unit. This concentration is an intermediary between concentrations reported in lightly and heavily contaminated environments (Andrès *et al.*, 1999; Dabrin *et al.*, 2009). Each experimental units was supplied with its one flow of seawater, which allowed for a renewal of the water column every 12 min. The concentration of Cd was measured daily in the water column of contaminated experimental units, and at days 0, 3, 7 and 14 in Cd-uncontaminated experimental units. For this purpose, 9 mL of seawater were sampled in the center of the water column and were directly acidified with 1 mL of nitric acid (HNO_3 65 %; Carlo Erba Reagents). The Cd concentrations in seawater were then determined by Inductively Coupled Plasma Optical Spectrometry (ICP-OES, 700 Series ICP-OES; Agilent). During the experiment, salinity and temperature were also measured daily (Multi 340i; WTW) in the central part of the water column of experimental units.

II.3. Sediment reworking

Sediment reworking rates were determined using inert particles, fluorescing under UV light (luminophores) (Mahaut and Graf, 1987). A mixture of seawater and 10 g DW of luminophores (size range = 100–160 µm, density = 2.5 g cm^{-3}) was homogenously spread on the sediment surface of each experimental unit 2 h before the beginning of the experiment. After 3, 7 and 14 days of experiment, vertical distribution of luminophores in the sediment was analyzed for the four experimental conditions. The water column of each experimental unit was firstly removed with a syringe and then sediment cores were sliced in 1-cm layers from the surface to 10 cm depth and in 2-cm layers from 10 cm depth up to the bottom of the cores. During slicing, mud shrimp were sampled and stored on ice until they were dissected for Cd bioaccumulation and genetic analyses (see below). Each sediment layer was firstly freeze dried and then gently sieved over a 1 mm mesh. Sediments were then homogenized, and subsamples of 1 g were dispersed over a small dish, before being photographed under UV light using a digital camera (Nikon D100) fitted with a yellow filter. Luminophores in each slice were semi-automatically counted using a home-made software, allowing computation of 1-dimensional (1-D) vertical profiles of the percentage of luminophores. From these, the biodiffusion and the bioadvection coefficient (D_b and v_b, respectively) were estimated by fitting vertical profiles with a 1-D biodiffusion-bioadvection model (Maire *et al.*, 2008):

$$\frac{\partial^2 C}{\partial t} = D_b \frac{\partial^2 C}{\partial z^2} - v_b \frac{\partial C}{\partial z}$$

with *C* the luminophore concentration (%), *t* the time (year) and *z* the depth (cm). The accuracy of fitted models was based on low root mean square (RMS) value as well as on visual inspection of similarity between predicted and real vertical profiles of luminophores.

II.4. Dissection

Each *U.* cf. *pusilla* was firstly measured from the rostrum tip to the extremity of the telson (Total Length, TL) using a digital caliper. The measured size was rounded down to the last mm. Then, the mud

shrimp's hepatopancreas and abdominal muscle were sampled. A small part of both organs was used for genetic analyses and consequently placed in a RNA later solution and kept at - 20 °C pending RNA extractions. The remaining tissues were used for Cd determination.

II.5. Metal determination in biological samples

After being dried (at least 48 h at 45 °C), biological samples were placed in polypropylene tubes and digested with nitric acid (1 to 3 mL depending on samples DW, HNO3 65 %; Carlo Erba Reagents) at 100 °C for 3 h (HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11 %. The Cd contents of hepatopancreas and abdominal muscle of mud shrimp were then measured by atomic absorption spectrophotometry with Zeeman correction (Agilent 240Z AA; Agilent). Method blanks and certified reference materials (DOLT-5, dogfish liver, NRCC-CNRC) were included in each analytical batch and consequently treated and analysed in the same way as biological samples. The detection limit (DL) was 0.075 µg g^{-1} DW. Cadmium concentrations in biological samples were expressed in µg g^{-1} DW.

II.6. Gene expression

II.6.1. Total RNA extraction and reverse transcription

Total RNAs were extracted from the hepatopancreas and the abdominal muscle of mud shrimp using the "SV Total RNA Isolation System" kit (Promega; Fitchburg, WI, USA) according to the manufacturer-recommended protocol. The quality of RNA extractions as well as RNA concentrations were assessed by spectrophotometry (Spectro Multivolume Epoch; BioTek). Immediately following total RNA extractions, reverse transcriptions of RNA (RT) were realized. First-strand cDNA were synthesized using the "GoScript Reverse Transcription System" kit (Promega) according to the manufacturer's instructions. The RT reaction was performed from 1 µg of total RNA in the case of hepatopancreas samples and from 10 µL of total RNA in the case of abdominal muscle, since the latter contained fewer transcripts compared to the former. The cDNA mixture was then stored at - 20 °C pending real-time PCR reactions.

II.6.2. Real-time PCR

For genetic analyses, ten genes of interest, related to four main cellular functions which may be impaired by parasitism and/or trace metal contamination, were targeted (Table V-A. 1). Regarding *vtg*, it may be surprising to target this gene as it is closely associated to vitellogenesis in females (Park *et al.*, 2014). However, bopyrid parasites feminize their male host (Tucker, 1930; Pascal *et al.*, 2016b) and one could expected modifications of the expression of genes related to reproductive processes as well. For each gene, specific primer pairs were designed using Primer3Plus probe design software (version 2.4.1; Untergasser *et al.*, 2012) (Table V-A. 1). The amplification of cDNA was monitored using the DNA intercaling dye SybrGreen I. Real-time PCR reactions were carried out in a total volume of 20 µL consisting of 10 µL GoTaq® qPCR Master Mix (2X; Promega), 2 µL of a mix of the gene-specific primer

pairs at a final concentration of 2 µM for each primer, 5 µL of a ten-time diluted cDNA mixture and 3 µL of nuclease-free water. Real-time PCR were performed in a LightCycler® 480 (Roche). The cycling conditions were as follow: 1 cycle of initial activation at 95 °C for 2 min; followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Specificity was determined for each reaction from the dissociation curve of the PCR product. This dissociation curve was obtained by following the SybrGreen I fluorescence level during a gradual heating of the PCR products from 60 to 95 °C. Relative quantification of each gene expression was normalized according to the elongation factor 1-alpha (*ef1-α*) gene expression. This reference gene had a constant level of expression during all our conditions of exposure. The $2^{-\Delta Ct}$ method (Livak and Schmittgen, 2001) was used to generate relative mRNA expression.

Table V-A. 1 Accession numbers and specific primer pairs for the ten genes from *Upogebia* cf. *pusilla* used in quantitative PCR analyses.

Gene	Function	Accession numbers	Primer sequence 5'-3'
Elongation factor 1-alpha (*ef1-a*)	Housekeeping gene	KY555759	CACGTTCTGCTGTGCTTCAC[1] AGCCAGAGATGGGGAGGATT[2]
Catalase (*cat*)	Oxidative stress response	KY555763	GACCCAGACTATGCCATCCG[1] TAGTTCTTGGGGTTGCGGTC[2]
Superoxide dismutase Mn (*sod Mn*)	Oxidative stress response	KY555768	TCAAGTACGCTATGGTGGCC[1] GCCCCCTCCATTGAACTTGA [2]
Ferritin (*fer*)	Oxidative stress response, immune response	KY555762	GGATCACGCCCAGAAGTTCA[1] GGTCGTTGTTCTTGTTGGCC[2]
Nitric oxide synthase (*nos*)	Immune response	KY555765	GTGGTCCATGATGGTCACGT[1] CCTGTGTGACATCCATCGCT[2]
Hemocyanin (*hemo*)	Immune response, respiratory metabolism	KY555761	ATGGTGGCTACTTCCCCTCT[1] CGATCAGGTCACCCACAACA[2]
Subunit 1 of cytochrome C oxidase (*cox I*)	Mitochondrial metabolism	KY555767	GGAGCCGTTAATTTTATAACCACA[1] TAGGATCTCCTCCTCCAGCA[2]
rRNA 12S (12S)	Mitochondrial metabolism	KY555758	TTAACTATTAGCTGCACCTT[1] CAAAGAACTTGGCGGTGTT[2]
Retinoid X receptor (*rxr*)	Endocrine processes	KY555764	AGGAGGAACGACAACGAACC[1] GGCCCATTCCACCAGTTGTA[2]
Vitellogenin (*vtg*)	Reproductive processes	KY555760	AGACCAACGGCACCTATGTG[1] GGTCCCTGAGGATTGCTGAC[2]

II.7. Statistical analysis

The effects of experimental condition ("Control", "Bop", "Cd" and "Bop + Cd") and time of experiment (3, 7 and 14 days) on bioaccumulation of Cd in mud shrimp's hepatopancreas and abdominal muscle were assessed using a two-way ANOVA, followed by a Tukey test (comparison of means) if significant. Data on Cd bioaccumulation were log-transformed to fulfill the assumption of homogeneity of variances. To ensure that potential differences of Cd bioaccumulation were not related to differences of mud shrimp size, TL of organisms were compared between the four experimental conditions using a one-way ANOVA.

The effects of time and experimental condition on the biodiffusion and bioadvection coefficients (D_b and v_b) were assessed using a two-way ANOVA followed by a Tukey test if significant. Data on biodiffusion coefficients were log-transformed to fulfill the assumption of homogeneity of variances.

Regarding gene expression, the relative mRNA expression of each gene was compared with the control (no bopyrid and no Cd) by using Student's t-test (parametric) or Mann-Whitney test (non-parametric). If significant, induction factor (IF) of each gene was determined following equation:

$$IF = 2^{-\Delta Ct\,(treatment)}/2^{-\Delta Ct\,(control)}$$

Results are reported as the mean ± SE of *N* replicate measurements. A Levene test was performed to assess homogeneity of variances. Differences were considered significant for $p < 0.05$.

III. Results

Before the end of the 7 days incubation period, one of the mud shrimp of the "Cd" experimental condition died. Moreover, the burrow of one of the "Bop" mud shrimp collapse before the end of the 14 days incubation period and this shrimp was thus deleted from the analyses. Therefore, there were three replicates for "Cd" at 7 days and for "Bop" at 14 days, while there were four replicates for the others experimental conditions at each of the incubation time (3, 7 and 14 days).

III.1. Physicochemical parameters

Over the 14 days of experiment, the temperature was maintained at 20.6 ± 0.06 °C (mean ± SE) and salinity at 34.3 ± 0.01 in experimental units. The concentration of Cd in the overlying water of contaminated experimental units was 7.4 ± 0.2 µg L^{-1} throughout the experiment and similar between the different experimental units, whereas concentrations of Cd were systematically below the limit of detection in the uncontaminated experimental units (DL = 0.34 µg Cd L^{-1}). There was no difference of mud shrimp TL between the four experimental conditions (TL = 47.8 ± 0.3 mm; ANOVA, $F = 1.06$, $p = 0.38$).

III.2. Bioaccumulation of cadmium

Cadmium concentrations in the abdominal muscle and in the hepatopancreas of mud shrimp were determined after 3, 7 and 14 days of experiment and compared between organisms exposed and unexposed to Cd-contaminated seawater (Figure V-A. 1, Table V-A. 2). In the abdominal muscle, there was a significant effect of experimental conditions and time of exposure on Cd bioaccumulation with no interaction between both factors (Figure V-A. 1A, Table V-A. 2). The Cd concentrations in the abdominal muscle of Cd-unexposed organisms were low and similar between bopyrid-free ("Control") and bopyrid-infested ("Bop") organisms (Cd concentration = 0.09 ± 0.02 µg g^{-1} DW; Figure V-A. 1A) throughout the experiment (Tukey test, $p > 0.05$). When Cd was added to the water column ("Cd" and "Bop + Cd" experimental conditions), the contaminant concentration in the mud shrimp's abdominal muscle increased over time (Figure V-A. 1A, Table V-A. 2) with a significant bioaccumulation observed

from the first sampling time, i.e. 3 days of exposure. However, there was no influence of bopyrid infestation on Cd bioaccumulation in the abdominal muscle over the time of experiment (Tukey test, $p > 0.05$).

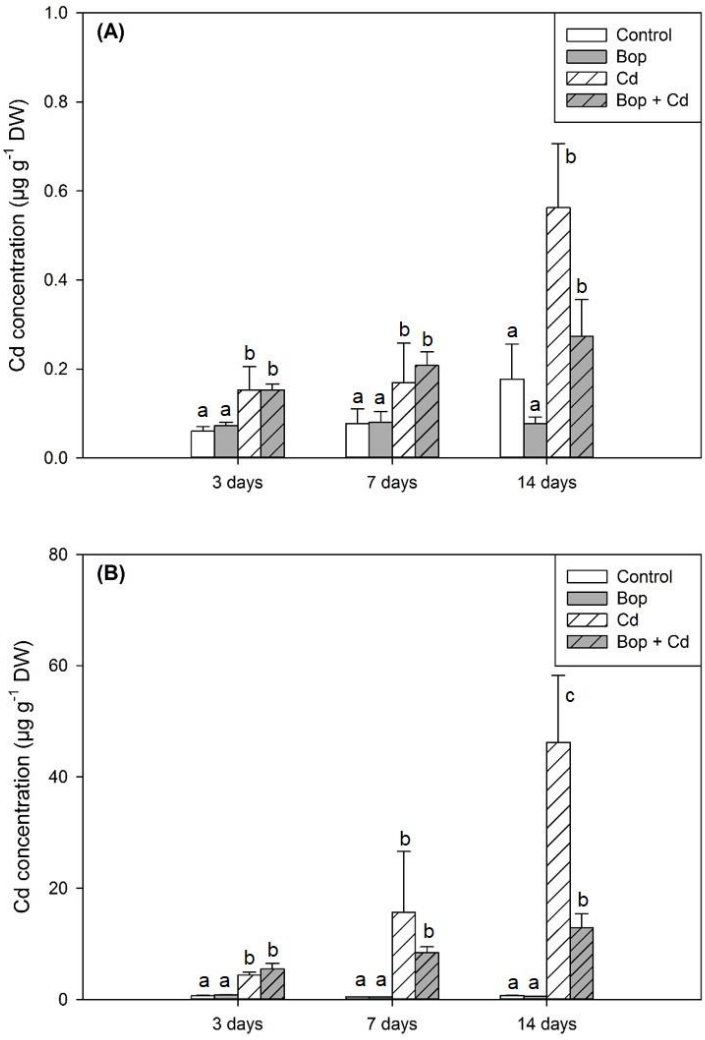

Figure V-A. 1 Cadmium concentrations (µg g^{-1} DW) in the (A) abdominal muscle and (B) hepatopancreas of bopyrid-free and bopyrid-infested *Upogebia* cf. *pusilla* unexposed to Cd-contaminated water ("Control" and "Bop") and exposed to Cd-contaminated water ("Cd" and "Bop + Cd") over a 14-day experiment. Means values + standard errors ($N = 4$ for each experimental condition). Different letters indicate significant differences of Cd concentrations at each sampling time ($p < 0.05$).

Table V-A. 2 Results of two-way ANOVA comparing the effect of experimental condition ("Control", "Bop"; "Cd" and "Bop + Cd") and time of exposure (3, 7 and 14 days) on Cd concentrations in the abdominal muscle and hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla*. P-values in bold indicate significant factor effect.

	Source of variation	***df***	***F***	***p***
Abdominal muscle	Experimental condition	3	10.39	< **0.001**
	Time	2	5.98	**0.006**
	Experimental condition x time	6	1.12	0.37
Hepatopancreas	Experimental condition	3	77.29	< **0.001**
	Time	2	13.11	< **0.001**
	Experimental condition x time	6	3.14	**0.015**

Regarding the mud shrimp hepatopancreas, there was a significant effect of experimental conditions and time of exposure on Cd bioaccumulation with an interaction between both factors due to the dramatic increase of Cd bioaccumulation in mud shrimp form the "Cd" experimental condition at the end of the experiment (day 14) (Figure V-A. 1B, Table V-A. 2). Cadmium concentrations in the hepatopancreas of Cd-unexposed organisms were similar between bopyrid-free ("Control") and bopyrid-infested ("Bop") organisms (Cd concentration = 0.62 ± 0.04 µg g^{-1} DW; Figure V-A. 1B) throughout the experiment (Tukey test, $p > 0.05$). When organisms were exposed to Cd contamination, bioaccumulation was noticed from the first sampling time (3 days of exposure) in bopyrid-free ("Cd") and bopyrid-infested ("Bop + Cd") organisms with no significant difference between mud shrimp at that time (Figure V-A. 1B; Tukey test, $p > 0.05$). The concentration of Cd in the hepatopancreas of bopyrid-free mud shrimp ("Cd") significantly increased over time (Tukey test, $p < 0.05$). In comparison, there was no significant increase of Cd concentration in the hepatopancreas of bopyrid-infested mud shrimp ("Bop + Cd") from day 3 to day 14. Finally, after 14 days of exposition to Cd contamination, bopyrid-free mud shrimp bioaccumulated 3.6-fold more Cd in their hepatopancreas than bopyrid-infested organisms (Figure V-A. 1B; Tukey test, $p < 0.05$).

III.3. Sediment reworking

The vertical profiles of luminophores in the sediment for the four experimental conditions after 3, 7 and 14 days of incubation are presented in Figure V-A. 2. For the "Control", there was an exponential decrease of the proportion of luminophores from the sediment surface to the bottom of the cores, where the tracer's concentrations were very low after 3 and 7 days of experiment. After 14 days, the vertical profiles of luminophores were characterized by a small subsurface peak between 0 and 2 cm of fluorescent particles, suggesting a slight advective transport of sediment particles at depth. Regarding the "Bop" and "Bop + Cd", the vertical profiles of luminophores were similar between both experimental conditions at each of the sampling time. We noticed an exponential decrease of the proportion of luminophores from the sediment surface to the bottom of the cores, where the tracer's concentrations were very low for these two experimental conditions at the three sampling times. The vertical profiles were almost identical between the three sampling times. Concerning the "Cd", the luminophores depth profiles were characterized by a small subsurface peak between 0 and 2 cm of fluorescent particles after 3 days. This small subsurface peak was not noticed after 7 days of experiment. At this time, the vertical profiles of luminophores showed an exponential decrease of the proportion of luminophores from the sediment surface to the bottom of the cores, similar to those reported for "Control", "Bop" and "Bop + Cd". However, it should be pointed out that the sediment top layer of the "Cd" was lightly depleted in fluorescing particles as compared to the top layers of the "Control", "Bop" and "Bop +Cd"; 65 % of the luminophores were located in the 0–1 cm layer for the "Cd" vs. 83, 80, and 82 % for the "Control", "Bop" and "Bop +Cd", respectively. After 14 days of experiment, the luminophores depth profiles for the "Cd" were characterized by a subsurface peak of fluorescent particles between 0 and 5 cm. The different replicates of the "Cd" were dissimilar between each other at that time, whereas there were less inter-individual variations for the "Control", "Bop" and "Bop + Cd".

Biodiffusion coefficients D_b and bioadvection coefficients v_b were estimated from vertical profiles of luminophores and are presented in Figure V-A. 3. D_b ranged from 7.8 ± 2.2 $cm^2\ y^{-1}$ ("Control", day 14) to 47.1 ± 25.8 $cm^2\ y^{-1}$ ("Cd", day 7) (Figure V-A. 3A). There was a significant influence of time of exposure (two-way ANOVA, $F = 10.0$, $p < 0.05$) on D_b, which exhibited a marked decrease over the time of experiment. At day 3, the D_b of the "Bop" experimental condition was similar to the D_b of the "Control" and both seemed to be inferior to the D_b of the "Cd" and "Cd + Bop" experimental conditions. At day 7 and day 14, the D_b of the "Control", "Bop" and "Bop + Cd" were similar to each other. Conversely, the D_b recorded for the "Cd" experimental condition seems to be higher than the D_b for the three other experimental conditions at these two sampling times. Nonetheless, there were no significant influence of experimental conditions ($F = 2.65$, $p = 0.065$) and no interaction between time of exposure and experimental conditions ($F = 0.84$, $p = 0.55$) on D_b over the 14 days of experiment. Bioadvection coefficients v_b were null for "Bop" and "Bop + Cd" after 3 days of experiment and for "Control", "Bop" and "Cd" after 7 days of experiment (Figure V-A. 3B). Low bioadvection was reported for the four experimental conditions after 14 days of experiment: v_b ranged from 3.0 ± 3.0 $cm^2\ y^{-1}$ ("Bop + Cd") to

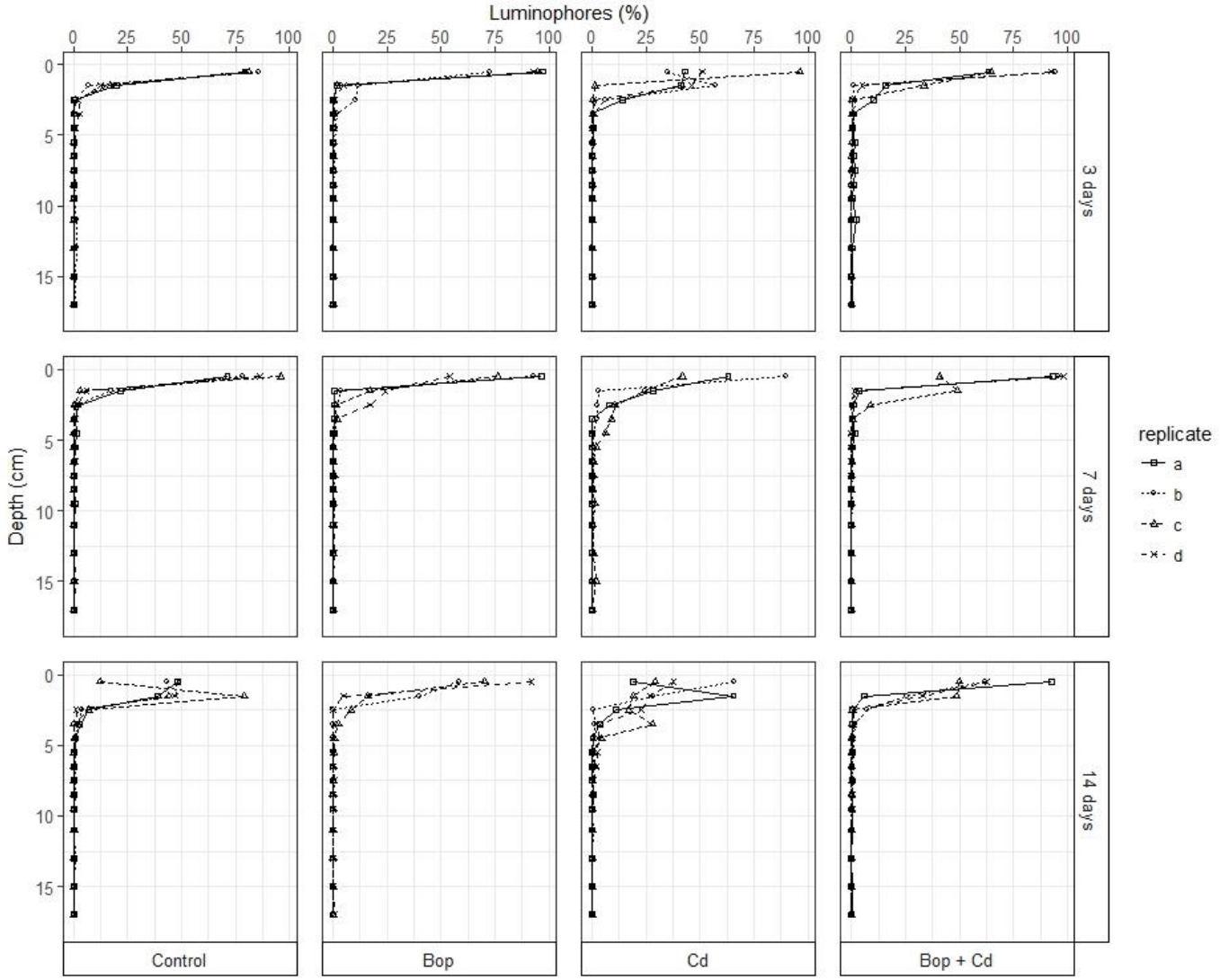

Figure V-A. 2 Vertical profiles of luminophores in the sediment of experimental units with bopyrid-free and bopyrid-infested *Upogebia* cf. *pusilla* unexposed to Cd-contaminated water ("Control" and "Bop") and exposed to Cd-contaminated water ("Cd" and "Bop + Cd") after 3, 7 and 14 days of experiment. The vertical profile of each replicate is given (replicates a, b, c and d) for each condition and sampling time.

29.4 ± 17.6 cm^2 y^{-1} ("Cd") at that time (Figure V-A. 3B). There was a significant influence of experimental condition (two-way ANOVA, $F = 3.71$, $p < 0.05$), no influence of the time of exposure (two-way ANOVA, $F = 2.57$, $p = 0.09$) and no interaction between both factors (two-way ANOVA, $F = 1.4$, $p = 0.23$) on v_b. The bioadvection coefficients of "Cd" were significantly higher than those of "Bop" and "Cd + Bop" (Tukey test, $p < 0.05$) but not different to those of "Control" (Tukey test, $p = 0.63$). Finally, there were no significant relationships between the D_b and v_b coefficients and the Cd bioaccumulated in the abdominal muscle and hepatopancreas of mud shrimp (Appendix 9).

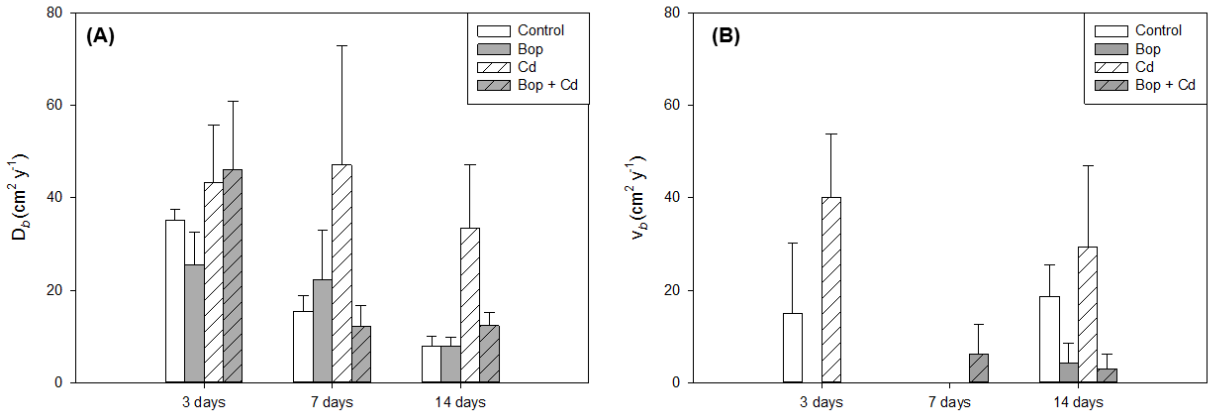

Figure V-A. 3 Vertical (A) biodiffusion D_b and (B) bioadvection v_b coefficients associated with sediment reworking activity of bopyrid-free and bopyrid-infested *Upogebia* cf. *pusilla* unexposed to Cd-contaminated water ("Control" and "Bop") and exposed to Cd-contaminated water ("Cd" and "Bop + Cd") after 3, 7 and 14 days of experiment. Mean value + standard errors.

III.4. Gene expression

The relative gene expression of several genes were compared between (1) bopyrid-free ("Control") and bopyrid-infested ("Bop") mud shrimp unexposed to Cd-contaminated water (influence of parasitism), (2) "Control" and bopyrid-free mud shrimp exposed to Cd-contaminated water ("Cd", influence of Cd contamination) and (3) "Control" and bopyrid-infested mud shrimp exposed to Cd-contaminated water ("Bop + Cd", influence of parasitism and Cd contamination). Results of quantitative PCR are presented in Table V-A. 3. Only genes showing significant variation of their expression levels compared to those of control organisms are reported.

III.4.1. In the abdominal muscle

The bopyrid parasite did not trigger any significant gene induction or repression in the abdominal muscle after 3, 7 and 14 days of experiment, compared to the control. In sharp contrast, Cd contamination was associated with important variations of gene expression levels compared to the control. Additionally, gene expression levels exhibited significant disparity depending on duration of Cd contamination. An early genetic response was observed 3 days following Cd contamination with induction of the genes *sod Mn* (x 3.2; oxidative stress response), *fer* (x 4.3; immune response), 12S (x

Table V-A. 3 Kinetic of relative gene expression (time of exposure: 3, 7 and 14 days) observed in the abdominal muscle and hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla* infested with bopyrid parasite ("Bop"), contaminated with Cd ("Cd") and bopyrid-infested and Cd-contaminated ("Bop + Cd") as compared to bopyrid-free and Cd-uncontaminated ("Control") mud shrimp. Results are given as significant induction (≥ 2) or repression (≤ 0.5) factors compared to "Control" mud shrimp. "/": no significant difference compared to the control. Only genes showing a differential expression are reported. The functions of each gene are given in Table V-A. 1.

	Bopyrid-infested			**Cd-contaminated**			**Bopyrid-infested and Cd-contaminated**		
	3 days	**7 days**	**14 days**	**3 days**	**7 days**	**14 days**	**3 days**	**7 days**	**14 days**
Abdominal muscle									
cat	/	/	/	/	19.7	/	/	17.1	/
sod Mn	/	/	/	3.2	/	/	6.7	3.9	/
fer	/	/	/	4.3	3.0	3.0	2.3	/	/
hemo	/	/	/	/	56.2	78.1	/	30.7	/
cox I	/	/	/	/	3.7	/	/	4.0	/
12S	/	/	/	29.1	16.7	72.0	/	7.7	18.1
rxr	/	/	/	/	/	0.4	/	0.5	/
vtg	/	/	/	23.5	22.4	390.3	/	22.3	/
Hepatopancreas									
fer	/	/	/	2.2	/	/	/	/	/
hemo	/	/	/	/	6.7	/	0.1	/	/
12S	/	/	/	3.0	/	/	/	/	/
rxr	/	/	/	/	/	/	2.0	/	/
vtg	/	/	/	/	/	/	/	/	11.3

29.1; mitochondrial metabolism) and *vtg* (x 23.5; reproductive processes). The genes *fer*, 12S and *vtg* were still induced after 7 and 14 days of exposure to Cd contamination. Conversely, the gene *sod Mn* did not exhibited any significant variation compared to the control after 7 and 14 days of exposure to Cd. A tardive induction of the genes *cat* (x 19.7, oxidative stress response), *hemo* (x 56.2 and x 78.1; immune response and mitochondrial metabolism) and *cox I* (x 3.7; mitochondrial metabolism) was noticed after 7 and 14 days of exposure to waterborne Cd. At experiment completion, the gene *rxr* (endocrine processes) was the only one to show significant down regulation following Cd exposure. Multiple stressors (Cd and parasitism) also enhanced substantial genetic regulation. After 3 days of experiment, we recorded an antagonistic interactive effect of Cd and parasitism in combination on the expression of the genes *fer*, 12S and *vtg* since the induction factors of these genes were lower than in the Cd experimental condition ("Cd") solely. On the other hand, both stressors up-regulated the gene *sod Mn* after 3 days of experiment. The larger differential gene expression in double stressed organisms compared to the control was observed after 7 days of experiment with a massive induction of genes involved in oxidative stress response (*cat* and *sod Mn*, x 17.1 and x 3.9 respectively), mitochondrial metabolism (*hemo*, *cox I* and 12S, x 30.7, x 4.0 and x 7.7, respectively) and in reproductive processes (*vtg*, x 22.3) compared to the control. In contrast, a down-regulation of the gene *rxr* was also observed at that time (x 0.5). After 14 days of experiment, Cd and parasitism only elicited the induction of the gene 12S (x 18.1). Other genes did not show any significant difference of expression compared to the control.

III.4.2. In the hepatopancreas

Similar to the pattern observed in the mud shrimp's abdominal muscle, the bopyrid parasite did not lead to any significant difference of gene expression compared to the control after 3, 7 and 14 days of experiment. Regarding Cd contamination, genetic responses in the abdominal muscle and the hepatopancreas showed marked differences. In the hepatopancreas, Cd contamination was only associated with an early induction of the genes *fer* (x 2.2) and 12S (x 3.0) after 3 days of contamination. None of these two genes were induced after 7 and 14 days of contamination. The gene *hemo* was the only one to show significant variation of expression (x 6.7) after 7 days of contamination. At the end of the experiment, Cd did not trigger any significant differential gene expression compared to the control. Finally, in the mud shrimp's hepatopancreas, Cd and parasitism only triggered a down-regulation of the gene *hemo* (x 0.1) and an induction of the gene *rxr* (x 2.0) after 3 days of experiment. None of the targeted genes displayed any differential expression after 7 days of experiment compared to the control. After 14 days of experiment, there was a significant synergistic effect of parasitism and Cd on the *vtg* expression (x 11.3) since the induction factor of this gene was higher than in the parasite ("Bop") and the Cd ("Cd") experimental conditions.

IV. Discussion

IV.1. Cadmium bioaccumulation and influence of parasitism

Over 14 days of experiment, mud shrimp significantly bioaccumulate Cd in their hepatopancreas and, to a much lower extent in their abdominal muscle. This strong bioaccumulation of Cd in the hepatopancreas is not surprising since this organ is an important site for metal sequestration in crustaceans (Marsden and Rainbow, 2004) and is known to play a significant role in detoxification process (Ahearn *et al.*, 2004). The low bioaccumulation of Cd in the mud shrimp' abdominal muscle is consistent with previous studies showing that this organ is not a main storage compartment for Cd (Marsden and Rainbow, 2004; Gonzalez *et al.*, 2006).

Parasite burden interfered with mud shrimp susceptibility to trace metal in the "Cd" experimental condition. After 14 days of exposure to the waterborne trace metal, bopyrid-free mud shrimp bioaccumulated 3.6-fold more Cd in their hepatopancreas than bopyrid-infested organisms. Even though not statistically significant, the same tendency was noticed in the mud shrimp's abdominal muscle. Parasites associated with reduced trace metal burden in their host is a common pattern and several hypotheses explaining this tendency have been put forward. For instance, Evans *et al.* (2001) suggested that trematode parasites reduce the hepatopancreas' volume of the common periwinkle, *Littorina littorea*, lessening the capacity of trematode-infested individuals to bioaccumulate trace metals. In comparison, Paul-Pont *et al.* (2010c) proposed that trematode parasites could lower the filtration activity of the common cockle *Cerastoderma edule*, restricting its capability to absorb and bioaccumulate contaminants. In our study, the difference of Cd bioaccumulation between bopyrid-free and bopyrid-infested mud shrimp may be due to direct bioaccumulation of Cd by the parasite since the bopyrid parasite feeds on the internal liquid of its host (Tucker, 1930). The mechanisms of parasites acting as a sink for metal has been evidenced in the [*Pomphorhynchus laevis* + *Squalius (Leuciscus) cephalus*] parasite-host system (Sures and Siddall, 1999), with parasitized fishes exhibiting 2-fold less lead (Pb) in their intestine walls compared to unparasitized organisms while parasites bioaccumulated 18.6 to 30.0-fold more Pb than the intestine wall of their host. It is due to the parasite interfering with the fish hepatic-intestinal cycling of Pb, therefore directly diminishing the contaminant uptake through the host's intestinal wall (Sures and Siddall, 1999). The bopyrid could also indirectly interfere with Cd bioaccumulation process by altering the mud shrimp physiological state. The parasite *Gyge branchialis* occurs in one of the branchial chamber of its host where it hooks up to gill lamella (Tucker, 1930). If the bopyrid parasite impairs the respiratory apparatus of its host, the rate of Cd uptake from the water may be lessened, which *in fine* would reduce the bioaccumulation of the trace metal in parasitized mud shrimp compared to bopyrid-free organisms, as observed in our study. Finally, it should be considered that the bopyrid parasite could interfere with the process of bioaccumulation of its host through modifications of the mud shrimp's behavior. Indeed, it has been evidenced that the bopyrid parasite was lowering the time mud shrimp spent to ventilate their burrow as well as their bioirrigation rate (Pascal,

2017). Therefore, it could be suggested that the difference of Cd bioaccumulation between mud shrimp may be related to the bopyrid parasite diminishing the renewal of Cd-contaminated seawater in the mud shrimp's burrow or to a reduction of the filtration activity of the host as well.

IV.2. Influence of Cd contamination and parasitism on sediment reworking activity

Luminophores depth profiles recorded for the "Control" exhibited exponential decrease evidencing that the luminophores spread at the sediment-water interface were progressively buried while deep sediments were transported upward. After 14 days, we noticed a small subsurface peak showing a nonlocal transport of sediment particles This pattern is consistent with previous observations conducted on the mud shrimp (Maire *et al.*, 2016) and leads to define *Upogebia* cf. *pusilla* as a regenerator (Kristensen *et al.*, 2012). Using a 1-D biodiffusion-advection model, we also estimated biodiffusion coefficient D_b and bioadvection coefficient v_b associated to vertical profiles of luminophores. The decrease of D_b from day 3 to day 14 leads to the assumption that the pool of luminophores has been reworked between day 3 and day 7. These results are in accordance with those of Pascal (2017), who showed that the D_b associated to the mud shrimp's sediment reworking activity increased up to day 4 after the luminophores had been spread on the sediment surface and gradually decreased after that time. However, it is worth noting that the D_b determined in our study are ca. 34-fold smaller than the one estimated by Pascal (2017) after 14 days of experiment. A similar remark can be made from the bioadvection coefficient v_b, which was 7.8-fold higher in the study of Pascal (2017) as compared to the value presently documented after 14 days of experiment. These discrepancies could be due to different experimental setup. Indeed, Pascal (2017) used thin aquarium ("ant farm") allowing for mud shrimp to build a 2-D burrow whereas we used tubing allowing for building of 3-D burrows. Moreover, the sediment column was 20-22 cm in our experiment, whereas it was 30 cm in thin aquarium, which allowed for the construction of deeper burrows. It is likely that the architecture of burrow built by mud shrimp were different in both experiments, which may be related to distinct sediment reworking rate. For instance, by using cylindrical experimental units, Maire *et al.* (2016) determined D_b of 10.1 ± 5.2 $cm^2\ y^{-1}$ and v_b of 26.1 ± 15.7 $cm^2\ y^{-1}$ after 16 days of incubation, which is in the range of D_b and v_b estimated in this study.

IV.2.1. Influence of Cd contamination

In this study, mud shrimp largely bioaccumulated Cd in their hepatopancreas. Such high bioaccumulation of Cd may have toxic effects on mud shrimp's physiology and activities. Sediment reworking activity was used as a behavioral endpoint in this study. Although not statistically significant, we noticed that Cd contamination ("Cd") stimulated the sediment reworking activity of mud shrimp. This tendency is surprising considering that Cd contamination is usually associated with lower levels of activity. Indeed, Cd has been evidenced to impair feeding (Pestana *et al.*, 2007), locomotor (Roast *et al.*, 2000; Wallace and Estephan, 2004) and ventilatory (Vellinger *et al.*, 2012) activities and antipredator

behaviors (Sornom *et al.*, 2012) of crustaceans. For instance, Felten *et al.* (2008) evaluated behavior alterations in the amphipod *Gammarus pulex* following a 7-day Cd-contamination period (C = 7.5 µg L^{-1}). They evidenced that Cd-contaminated organisms showed reduced feeding (reduction of 30 %), locomotor (reduction of 38 %) and ventilatory (reduction of 5.7 %) activities compared to uncontaminated individuals. Regarding *U.* cf. *pusilla*, the enhanced sediment reworking activity may result from a potential escape reaction. Such behavior has been reported for the copepod *Eurytemora affinis*, exhibiting increase swimming activity when exposed to Cd contamination (Michalec *et al.*, 2013). Similarly, the amphipod *Corophium volutator* placed in experimental units with Cd-contaminated and Cd-uncontaminated sediments preferentially burrows in uncontaminated areas, evidencing that amphipods were able to detect Cd contamination and tried to avoid it (Bat *et al.*, 1998). Finally, it should be mentioned that there were important inter-individual differences in Cd-contaminated mud shrimp regarding their sediment reworking activity whereas Cd-uncontaminated mud shrimp exhibited less variable sediment reworking activity. However, there were no correlations between the sediment reworking coefficients and the quantity of Cd bioaccumulated by mud shrimp, evidencing that the influence Cd contamination may have on the behavior of mud shrimp was not directly related to the level of contaminant displayed by the organisms.

IV.2.2. Influence of parasitism

While the shape of vertical tracer profiles was similar between bopyrid-free (“Control”) and bopyrid-infested (“Bop”) mud shrimp after 3 and 7 days of experiment, there was a small subsurface peak of luminophores in “Control” which was no present in “Bop”. It suggest that the bopyrid parasite may impair the sediment reworking activity of mud shrimp in the first cm of the sediment column. Nonetheless, we did not record any significant influence of the parasite occurrence on the quantity of sediments reworked at each of the sampling time (similar D_b). These results are in contradiction with those of Pascal (2017), who evidenced that bopyrid-infested mud shrimp were less active than bopyrid-free individuals. As mentioned above, the discrepancy between our result and those of Pascal (2017) may be due to different experimental setups.

IV.2.3. Interaction between Cd and parasitism

Vertical luminophores profiles of mud shrimp subjected to parasitism and Cd contamination in combination (“Cd + Bop”) were similar to the ones of bopyrid-infested mud shrimp (“Bop”) but different to the ones documented for solely Cd contaminated mud shrimp (“Cd”). It may suggest an interactive effect of parasitism and Cd on the sediment mixing mode of the mud shrimp *U.* cf. *pusilla*. While solely bopyrid-infested mud shrimp (“Bop”) and mud shrimp Cd-contaminated and bopyrid-infested (“Cd + Bop”) seemed to induce a biodiffusive-like reworking of the first cm of the sediment column, solely Cd-contaminated mud shrimp (“Cd”) induced a non-local mixing of the sediment. Although not statistical significant, the same tendency was recorded for the biodiffusion coefficient D_b and the bioadvection coefficient v_b: solely Cd-contaminated mud shrimp seemed to rework a larger

amount of sediment as compared to solely bopyrid-infested and Cd-contaminated and bopyrid-infested mud shrimp. Thus, it may be proposed that Cd had a low influence on the sediment reworking activity of bopyrid-infested mud shrimp as compared to its effect on bopyrid-free mud shrimp.

The influence of trace metal contamination and parasitism in combination has never been quantified in bioturbating species and rarely on the behavior of other organisms. Nonetheless, a synergistic effect between parasites occurrence and organic pollutants in the glaucous gulls *Larus hyperboreus* has been observed. Parasites enhanced the detrimental effect of pollutants on the nesting success of *L. hyperboreus* whereas there was no effect of contaminants on uninfested organisms (Bustnes *et al.*, 2006). Contaminants and parasites can impair foraging activity of the amphipod *G. pulex*, with interactive effect (synergistic or antagonistic effect) between both stressors on food consumption (Brown and Pascoe, 1989). On the opposite, the organic contaminant chlorpyrifos has been evidenced to alter anti-predator behavior of the California killifish *Fundulus parvipinnis* through reduced swimming activity, whereas no interactive effect between the contaminant and parasites has been noticed on fishes' behavior (Renick *et al.*, 2016).

IV.3. Influence of contamination and parasitism on gene expression

IV.3.1. Influence of parasitism

Bopyrid parasite did not enhance any significant differential gene expression in the mud shrimp's hepatopancreas and abdominal muscle as compared to the control organisms. This result may seem surprising since parasites are, by definition, deleterious to their host and thus impairment of various functions, as gene regulation, are expected. Indeed, a modification of the expression levels of genes related to mitochondrial metabolism was noticed in cockles *C. edule* stressed with trematode parasites (Paul-Pont *et al.*, 2010a). The oyster *Ostrea edulis* challenged with the protozoan parasite *Bonamia ostreae* also showed an induction of the expression of genes involved in cellular protection mechanisms following parasite infestation (Morga *et al.*, 2011). In these studies, modifications of gene expression were assessed after organisms were experimentally infested with parasites. Thus, modulations of gene expression levels were a response to a recently applied stress. On the opposite, mud shrimp used in our study were not experimentally infested with bopyrid parasites. Indeed, the *G. branchialis*/*U.* cf. *pusilla* association is a long term relationship with the bopyrid parasite settling early in the mud shrimp' life and growing with its host (Tucker, 1930; Pascal *et al.*, 2016b). It could explain that we did not observe any modification of the expression of the genes *nos* and *hemo* encoding proteins implicated in the crustaceans' immunity for instance. Indeed, the modification of gene expression is a time dependent process with genes playing a role in immune response up-regulated early during the infection process while others can show delayed modifications of expression (Clark *et al.*, 2013; Li *et al.*, 2016). For instance, several hemocyanin genes have been showed to be only induced during the earliest time of infection in the American lobster *Homarus americanus* challenged with the protistan parasite *Anophryoides haemophila* (Clark *et al.*, 2013).

IV.3.2. Influence of Cd contamination

Conversely to bopyrid parasites, Cd contamination enhanced an important genetic regulation in the mud shrimp *U.* cf. *pusilla* over the experiment, but there was a large discrepancy between Cd bioaccumulation (mainly in the hepatopancreas) and gene response (mainly in the abdominal muscle). A similar pattern was documented in trophically Cd-contaminated mud shrimp (Dairain *et al.*, 2018a). Indeed, there was any modification of gene expression in the hepatopancreas of mud shrimp exposed to Cd-contaminated sediments for 14 days while a significant bioaccumulation of the trace metal was noticed. Since the hepatopancreas is a metabolically highly active organ, it was suggested that the basal level of gene expression in this organ may be sufficient to face the stress due to Cd bioaccumulation. Despite the fact that mud shrimp exposed to a contaminated sediment bioaccumulated ca. 10-fold less Cd in their hepatopancreas than mud shrimp exposed to waterborne Cd after a 14 days exposure period, we assume that the basal level of gene expression was sufficient to deal with Cd contamination in this study as well.

In the mud shrimp's abdominal muscle, the gene *fer* was consistently up-regulated throughout the experiment. This gene is encoding the ferritin metal binding protein, which is involved in the storage and scavenging of metals, especially iron (Chasteen and Harrison, 1999). This gene has also been evidenced to be up-regulated in the crustacean *Daphnia pulex* exposed to Cd (Poynton *et al.*, 2007). This suggests an adaptive response of the mud shrimp with induction of processes allowing for Cd sequestration in the abdominal muscle of the mud shrimp. Associated to extrusion mechanisms involving cellular transporters of the ABC family for instance (Sturm *et al.*, 2009), this trace metal binding device may also contribute to explain the low Cd bioaccumulation in the abdominal muscle of *U.* cf. *pusilla* (Gonzalez *et al.*, 2006). In addition to the gene *fer*, the genes *sod Mn* and *cat* were also up-regulated. These two genes are encoding the mitochondrial Mn-superoxide dismutase and the catalase protein, respectively. Both enzymes are involved in the detoxification pathway of reactive oxygen species. The induction of *cat* and *sod Mn* was the sign that oxidative stress occurred in muscular cells of mud shrimp. Indeed, among the large range of Cd impacts, the induction of oxidative stress is common (Paul-Pont *et al.*, 2010a; Al Kaddissi *et al.*, 2014; Lin *et al.*, 2017). This trace metal inhibits the functioning of the complexes II and III of the mitochondrial electron transfer chain. By doing so, Cd indirectly stimulates the production of reactive oxygen species, as hydrogen peroxide (H_2O_2) or superoxide anion (O_2^-), in mitochondria (Wang *et al.*, 2004). The up-regulation of the genes *cat* and *sod Mn* allows to postulate that the Cd sequestration process induced over the first week of experiment was not sufficient to face the stress due to the trace metal contamination and that detoxification devices may thus have been induced early in the mud shrimp's abdominal muscle. In addition to detoxification and sequestration devices, induction of mitochondrial metabolism was also enhanced in Cd-contaminated organisms since the gene 12S was largely up-regulated. This gene is taken for a proxy of the number of mitochondria in cells (Al Kaddissi *et al.*, 2014; Arini *et al.*, 2015) since it has been evidenced that the

number of rRNA copies per mitochondria was constant (Cantatore *et al.*, 1984; Gadaleta *et al.*, 1990). The higher expression of 12S in Cd-contaminated mud shrimp compared to control organisms suggests that Cd induces an increase of the pool of mitochondria in the mud shrimp's abdominal muscle. It indicates a higher energetic demand, which was probably associated with the cost of mechanisms involved in the defense against Cd toxicity in the abdominal muscle. Finally, we also noticed a significant and large up-regulation of the gene *vtg* in the mud shrimp's abdominal muscle, which was dose-dependent. The *vtg* gene is a biomarker of endocrine disruptions in crustaceans. For example, this gene was significantly up-regulated in the hepatopancreas and in ovaries of the Asian paddle crabs *Charybdis japonica* exposed to organic pollutants (Park *et al.*, 2014). Yet, the large induction of *vtg* in the abdominal muscle of *U.* cf. *pusilla* was unexpected since this organ is not recognized as an important site of *vtg* expression and vitellogenin production (protein encoded by *vtg*) in crustaceans (Tsang *et al.*, 2003; Park *et al.*, 2014). Regarding the specific influence of Cd on *vtg* expression in crustaceans, studies are contradictory so far. For example, Soetaert *et al.* (2007) showed that this gene was significantly down-regulated in the crustacean *D. magna* after a 96 h exposure to Cd (concentrations = 10 to 100 µg L^{-1}), whereas Hannas *et al.* (2011) evidenced a significant up-regulation of the gene expression after 72 h of exposure to Cd (concentration = 20 µg L^{-1}). Nonetheless, both studies suggest that Cd acts as an endocrine disruptor component and a similar tendency was noticed in the mud shrimp *U.* cf. *pusilla*.

IV.3.3. Interaction between Cd bioaccumulation and parasitism infestation

In the hepatopancreas of mud shrimp, Cd and the bopyrid parasite only induced a low modification of gene expression levels as compared to control organisms. This pattern was similar to the one elicited by Cd in bopyrid-free mud shrimp, highlighting no obvious interactive effect of both stressors on gene expression in the mud shrimp's hepatopancreas. On the opposite, both stressors induced marked modifications of gene expression in the mud shrimp's abdominal muscle. Nonetheless, induction factors were different from those determined in solely Cd-contaminated mud shrimp and the kinetics of genes expression were dissimilar as well. As an illustration, the gene *fer* was only induced after 3 days of experiment in double-stressed organisms whereas it was induced all along the experiment in Cd-contaminated mud shrimp. Moreover, Cd and bopyrid parasites induced a lower up-regulation of this gene compared to the influence of Cd alone on bopyrid-free organisms. Thus, there was an antagonistic effect between Cd and parasitism on the expression of a gene encoding a protein involves in the storage and scavenging of metals (Chasteen and Harrison, 1999). Conversely, both stressors enhanced the expression of the gene *sod Mn* after 3 and 7 days of experiment emphasizing that oxidative stress occurred in multi-stressed organisms over these times. The difference of gene expression between bopyrid-free and bopyrid-infested mud shrimp may suggest the settings up of two strategies allowing to deal with Cd contamination: whereas there was mainly an induction of devices lowering the contamination with Cd in bopyrid-free organisms, there was principally an induction of mechanisms minimizing the debilitating role of Cd contamination in bopyrid-infested mud shrimp. We also noticed

an important antagonistic effect between Cd and parasitism on the expression of the gene 12S. A massive induction of this gene was observed throughout the experiment in Cd-contaminated organisms, whereas it was only induced after 7 and 14 days in Cd-contaminated and parasitized organisms and to a much lower extent. Therefore, the pool of mitochondria seems to be also enhanced in bopyrid-infested mud shrimp exposed to a Cd contamination, but it appears that the parasite was impairing this response. Such antagonistic effect between Cd and parasitism on mitochondrial gene expression has already been noticed in the gills and the visceral mass of the cockle *C. edule* (Paul-Pont et al., 2010a). Finally, the bopyrid parasite also modified the regulation of *vtg* in Cd-contaminated organisms evidencing that the endocrine disrupting role associated to Cd contamination (Soetaert *et al.*, 2007; Hannas *et al.*, 2011) was impaired in bopyrid-infested organisms. This pattern is surprising considering the role played by bopyrid parasites on the sexual state of their host. The bopyrid *G. branchialis* induces the castration of infested females and the feminization of infested males (Tucker, 1930). Thus, it would have been suggested that bopyrid parasites stimulate endocrine disruption as well and that an additive or a synergistic effect of the bopyrid parasite and Cd contamination will be observed in double-stressed mud shrimp.

Together these results evidenced a large antagonistic effect of the bopyrid parasite *G. branchialis* and Cd contamination on the gene expression of the mud shrimp *U.* cf. *pusilla*. Two unexclusive hypotheses could explain this pattern. First of all, the bopyrid parasite may interfere with gene expression in Cd-contaminated mud shrimp by weakening its host. Indeed, bopyrid parasites have been shown to alter the physiological state of their host with bopyrid-infested organisms lighter than bopyrid-free mud shrimp when standardized for TL (Smith *et al.*, 2008; Pascal *et al.*, 2016b). The susceptibility of crustaceans to contaminants depends on their physiological status. For instance, starved green crabs *Carcinus maenas* showed lower antioxidant capabilities when stressed with pyrene than feed organisms (Dissanayake *et al.*, 2008). Thus, it may be proposed that an energy deficit related to the parasitic burden might affect the ability of mud shrimp to deal with trace metal contamination at the molecular level. Reduced genetic response in bopyrid-infested mud shrimp challenged with Cd compared to bopyrid-free organisms could also be related to parasites interfering with the trace metal bioaccumulation process. Indeed, bopyrid-infested mud shrimp were less contaminated with Cd than bopyrid-free organisms. The influence of Cd on gene expression has been showed to be dose-dependent (Soetaert *et al.*, 2007; Al Kaddissi *et al.*, 2014; Zhu *et al.*, 2018). It is likely that a lower trace metal bioaccumulation leads to a lower genetic response. Nonetheless, it should be pointed out that the kinetics of the genetic response to Cd contamination was also impaired in bopyrid-infested mud shrimp compared to bopyrid-free organisms suggesting that the solely lower bioaccumulation of the trace metal cannot explain the difference of genetic response between mud shrimp.

V. Conclusion

In natural environments, organisms are exposed to a broad variety of stressors, which rarely act independently. This study aimed to evaluate the influence of the bopyrid parasite *Gyge branchialis* and Cd contamination on a molecular and a behavioral endpoint in the mud shrimp *Upogebia* cf. *pusilla*. We showed that bopyrid-free mud shrimp bioaccumulated more Cd than bopyrid-infested organisms. Cadmium contamination was associated with a large modulation of gene expression levels involving genes related to the defense against toxic effects of Cd in mud shrimp. However, we noticed that the bopyrid parasite lessened the gene response and modified its temporal dynamics. Regarding the sediment reworking activity of mud shrimp, it seems that parasites decreased the activity of their host whereas Cd stimulated it. These results were unexpected considering the known effects of Cd on the behavior of crustaceans (Roast *et al.*, 2000; Wallace and Estephan, 2004; Felten *et al.*, 2008). Finally, it is suggested that there may be an interactive effect of Cd contamination and parasitism infestation on the sediment reworking activity of mud shrimp.

Molecular endpoints may be used as early biomarkers of adverse effects in ecotoxicological studies. In the present study, modifications of gene expression did not lead to a clear outcome at the behavioral level. Thus, this study highlights the necessity to evaluate the influence of multiple stressors on different levels of organization (Weis *et al.*, 2001). Finally, it is worth noting that the influence of parasitism and Cd contamination was only evaluated on the mud shrimp's sediment reworking activity. However, *U.* cf. *pusilla* living deeper than the oxygen penetration depth in the sediment, it frequently ventilates its burrow enhancing solute exchanges at the sediment-water interface (Pascal *et al.*, 2016a). Since Cd and the bopyrid parasite have been evidenced to interfere with the ventilating behavior of crustaceans (Vellinger *et al.*, 2012; Pascal, 2017), we suggest to evaluate the influence of both stressors on this behavioral endpoint too. It will also allow to determine if the lower bioaccumulation of trace metal in bopyrid-infested organisms compared to bopyrid-free individuals is due to modified ventilating behavior or to other processes.

Compliance with ethical standards

Declarations of interest: none.

Animal rights: All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

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BILAN DU CHAPITRE V-A ET PERSPECTIVES

L'objectif de cette étude était d'évaluer la sensibilité de *Upogebia* cf. *pusilla* à deux facteurs de stress, à savoir le parasite *Gyge branchialis* et une contamination métallique au Cd par l'eau. L'influence de ces stress a été évaluée indépendamment et en combinaison à l'échelle moléculaire (régulation de l'expression génique) et comportementale (activité de remaniement sédimentaire). Bien que les études s'intéressant à l'effet combiné des parasites et des contaminants se soient largement développées au cours des deux dernières décennies, la plupart évalue l'influence de ces stress à l'échelle moléculaire et à l'échelle cellulaire, et très peu d'études se sont finalement intéressées aux modifications comportementales. Par ailleurs, à notre connaissance il s'agit de la première étude évaluant l'influence de ces deux types de facteurs de stress sur les activités d'une espèce bioturbatrice.

Influence de la contamination métallique

La crevette de vase *U.* cf. *pusilla* s'est avérée capable d'accumuler d'importantes quantités de Cd lorsque celui-ci est suppléé par l'eau. La bioaccumulation du Cd s'effectue intensément dans l'hépatopancréas et, dans une moindre mesure, au niveau du muscle abdominal des organismes. En parallèle de cette bioaccumulation, une faible modification de l'expression génique est observée dans l'hépatopancréas alors qu'elle est de relativement grande ampleur au niveau du muscle abdominal. Ceci suggère que les niveaux d'expression génique de base dans l'hépatopancréas sont suffisants pour faire face aux stress cellulaires dus à la contamination par le Cd. A l'inverse, cette contamination métallique stimule l'expression de gènes codant des enzymes impliquées dans la réponse aux stress oxydants et dans le métabolisme mitochondrial dans le muscle abdominal. Bien que nous n'ayons pas suivi ces modifications transcriptomiques au niveau protéomique, cette réponse génique tend à suggérer que *U.* cf. *pusilla* est capable de faire face aux modifications cellulaires provoquées par l'accumulation de Cd. L'influence de la contamination au Cd a également été suivie à l'échelle comportementale. A la différence de la réponse notée à l'échelle moléculaire, la réponse comportementale liée à la contamination métallique est bien moins marquée, du fait notamment d'une importante variabilité inter-individuelle. Les taux de remaniement sédimentaire sont ainsi relativement similaires entre organismes soumis à une contamination au Cd et non exposés à ce métal. Toutefois, l'activité de bioturbation des organismes soumis à la contamination semble plus importante que celle de leurs congénères non exposés au Cd, notamment dans les couches les plus superficielles de la colonne sédimentaire.

Influence du parasitisme et interaction avec la contamination métallique

A l'échelle moléculaire, le parasite n'induit aucune modification de l'expression des gènes de stress ciblés. Cette observation n'est pas surprenante considérant que l'association entre le bopyre et son hôte *U.* cf. *pusilla* est une interaction durable. Le bopyre infeste en effet son hôte relativement tôt dans la vie de ce dernier et se développe avec lui (Tucker, 1930; Pascal, 2017). Or les gènes ciblés au cours de cette

étude, et notamment ceux en lien avec la réponse immunitaire, sont connus pour être induits précocement dans le cadre d'un stress infectieux et relativement peu au cours des stades tardifs d'une infection.

En ce qui concerne l'activité de bioturbation de *U.* cf. *pusilla* et contrairement à ce qui avait été montré au cours de la Thèse de L. Pascal (2017), nous n'avons pas noté d'influence marquée de *G. branchialis* sur le remaniement sédimentaire de la crevette de vase *U.* cf. *pusilla*. Nonobstant, nos résultats suggèrent que le parasite puisse interférer avec le remaniement sédimentaire exercé par les crevettes de vase dans les couches superficielles de la colonne sédimentaire. Les différences entre l'étude de Pascal (2017) et celle présentée ici pourraient être notamment dues à des variations dans les systèmes d'expérimentation (i.e. configuration et volumes des unités d'incubation) et mettent en évidence la nécessité d'être prudent avant d'extrapoler des données obtenues en milieu contrôlé et souvent peu représentatif des conditions environnementales.

Bien que ne semblant pas avoir d'effet marqué seul sur l'expression génique ou sur l'activité de remaniement sédimentaire de *U.* cf. *pusilla*, le parasite *G. branchialis* interfère largement dans la réponse de son hôte à la contamination métallique. Tout d'abord, les individus parasités accumulent une bien moindre quantité de Cd que les individus non parasités. Associée à cette plus faible bioaccumulation, nous avons observé une modification de moins grande ampleur de l'expression génique, ainsi qu'une modification de la cinétique de cette réponse en comparaison à celle observée chez les organismes uniquement soumis à la contamination au Cd et accumulant fortement ce métal. Cela permet de supposer l'existence d'effets antagonistes entre parasitisme et contamination métallique sur la régulation de l'expression génique chez *U.* cf. *pusilla*. Bien que dans une moindre mesure, ces effets antagonistes sont également suggérés à l'échelle comportementale puisque les organismes exposés à la contamination au Cd et parasités présentent une activité de remaniement sédimentaire similaire à celle observée chez les individus seulement parasités mais différente de celle observée chez les crevettes de vase uniquement soumises à la contamination au Cd.

Les effets observés aux échelles moléculaire et cellulaire sont largement utilisés comme des signaux précoces de réponse aux stress et la plupart des études évaluant l'influence de divers facteurs de stress sur les organismes se cantonnent ainsi à l'évaluation de leurs effets à ces échelles d'étude. Néanmoins, les effets observés à ces échelles n'entrainent pas forcément des effets détectables à l'échelle de l'organisme (Weis *et al.*, 2011). Cette étude met ainsi en exergue l'importance d'intégrer des études comportementales dans les études visant à évaluer l'influence de stress multiples sur les organismes, et ce particulièrement dans le cas d'étude portant sur des espèces dont le comportement joue un rôle prépondérant dans le fonctionnement des écosystèmes.

Peut-être l'un des effets le plus marquant du parasite dans cette étude concerne-t-il le processus de bioaccumulation du Cd chez *U.* cf. *pusilla.* Nous avons noté que le parasite limitait significativement l'accumulation de Cd chez son hôte. Ce schéma est plutôt commun puisqu'il a été documenté pour

plusieurs systèmes hôtes-parasites (Cross *et al.*, 2003; Sures and Radszuweit, 2007; Paul-Pont *et al.*, 2010a). Néanmoins, les mécanismes fins qui le sous-tendent restent jusqu'à présent relativement peu étudiés. L'étude des mécanismes par lesquels le parasite *G. branchialis* limite la contamination métallique de son hôte *U.* cf. *pusilla* fera ainsi l'objet du **Chapitre V-B**.

How does the bopyrid isopod *Gyge branchialis* interfere with trace metal bioaccumulation in the mud shrimp *Upogebia* cf. *pusilla*?

DAIRAIN A., LEGEAY A., GERNIGON V. and de MONTAUDOUIN X.

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Abstract

Parasites are widespread in natural environments, and their impacts on the fitness of their host and, at a broader scale, on ecosystem functioning are well recognized. Over the last two decades, there has been an increasing interest in the effects of parasites in conjunction with other stressors, especially pollutants, on the health of organisms. For instance, parasites can interfere with the bioaccumulation process of contaminants in their host leading to parasitized organisms exhibiting lower pollutants burdens than unparasitized individuals for example. However, the mechanisms underlying these patterns are not well understood. This study examined how the bopyrid parasite *Gyge branchialis* could lower the cadmium (Cd) uptake of its mud shrimp host *Upogebia* cf. *pusilla*. When exposed to water-borne Cd, parasites were able to bioaccumulate this trace metal. However, the uptake of Cd by the parasite was low and cannot entirely explained the deficit of Cd contamination of the host. The weight of gills of parasitized organisms was significantly reduced compared with unparasitized organisms. We suggest that by reducing the surface for metal uptake, parasites could lower the contaminant burden of their host.

Key words: Host-parasite interactions; Metal contamination; Physiological alteration; Mud shrimp

I. Introduction

Increasing human population densities and associated activities have led to the release of important contaminants in aquatic environments, leading to high levels of contamination locally (Tueros et al., 2009; Pan and Wang, 2012). These pollutants are of major concern because some have strong deleterious impacts on the health of organisms (e.g., Felten et al., 2008; Al Kaddissi et al., 2014).

Living organisms are naturally exposed to a broad variety of stressors, which can have cumulative effects on their fitness. In particular, the process of contaminant bioaccumulation, as well as the effects of contaminants on organisms, depend on several other environmental parameters (see Holmstrup et al., 2010 for a review). Amongst these factors, the influence of parasites in contaminant bioaccumulation is now well recognized (Sures and Siddall, 1999; Coors and De Meester, 2008; Paul-Pont et al., 2010a). By definition, parasites benefit from their host and are harmful to them. In a contamination context, parasites can further negatively impact their host through enhancing their sensitivity to pollutants (Baudrimont and de Montaudouin, 2007; Coors and De Meester, 2008). In contrast, parasites have also been shown to reduce the contaminant burden of their host (Sures and Siddall, 1999; Evans et al., 2001; Paul-Pont et al., 2010a). The mechanisms underlying this pattern of reduced contaminants burdens in parasitized organisms are not fully understood but parasites could diminish the capacity of their host to absorb and bioaccumulate contaminants by altering their physiological status and/or by reducing their activity levels (e.g., Evans et al., 2001; Paul-Pont et al., 2010a). Moreover, parasites can bioaccumulate contaminants to concentrations which are orders of magnitude higher than their host (Nachev and Sures, 2016). Therefore, they could also directly interfere with contaminant bioaccumulation processes by acting as pollutant sinks. For example, the concentration of lead in the acanthocephalan parasite *Pomphorhynchus laevis* was 3 to 30-fold higher than the concentrations found in the intestine of its fish host, the chub *Squalius (Leuciscus) cephalus*. The parasite *P. laevis* interferes with the fish's hepatic-intestinal cycling of lead, thus directly reducing the lead uptake through the intestinal wall of the host (Sures and Siddall, 1999).

In a previous study, unparasitized mud shrimp, *Upogebia* cf. *pusilla* Petagna 1792, bioaccumulated 3.6-fold and 2.1-fold more Cd in their hepatopancreas and abdominal muscle, respectively, than parasitized organisms after an exposure period of 14 days to water-borne cadmium (Cd) (Dairain *et al.*, 2018b). In this study, we investigate how the bopyrid parasite *Gyge branchialis* Cornalia & Panceri, 1861 may interfere with the process of trace metal bioaccumulation in its mud shrimp host *U.* cf. *pusilla*, using Cd as a model contaminant. The female bopyrid parasite lives in one of the gill chambers of its host and feeds on internal host body fluids (Tucker, 1930). Thus, trophic contamination by trace metal taken up by the host is likely for this parasite. The female bopyrid parasite represents ca. 6.6 % of the total host-parasite biomass for this host-parasite combination. Therefore, we firstly evaluated whether the parasite acts as an important Cd sink in this system. In addition, the mud shrimp *U.* cf. *pusilla* filters water regularly for respiration purposes, and consequently the gills are a major site of metal uptake from

contaminated water. Hence, secondly, due to the parasite position in the gills, we evaluated whether the bopyrid parasite damages the gills of the host, potentially affecting its uptake of Cd.

II. Materials and Methods

II.1. The host-parasite association

The crustacean *Upogebia* cf. *pusilla* is a gebiidean mud shrimp occurring in intertidal and upper sublittoral zones along the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff, 1979; Dworschak, 1983). It lives in a deep (up to 49 cm) and complex burrow connected to the sediment-water interface by several distant openings (Pascal, 2017). The fossorial life style of the mud shrimp is associated with extensive bioturbation activity (Pascal, 2017). The bopyrid isopod *Gyge branchialis* is a parasite of *U*. cf. *pusilla* (Tucker, 1930). A sexually mature female lives in one (very rarely both) gill chamber of its host (Pascal et al., 2016b), with a dwarf male attached to her. This ectoparasite disrupts the mud shrimp's reproduction (Tucker, 1930; Pascal et al., 2016b) and also reduces its activity levels (Pascal, 2017).

II.2. Trace metal bioaccumulation in the bopyrid parasite

The bioaccumulation of Cd in female bopyrids has been investigated by conducting a 14-day contamination experiment on parasitized mud shrimp. Naturally Cd-uncontaminated mud shrimp were used in this experiment. They were sampled in the middle of Arcachon Bay (44°40' N, 1°08' W), France, using a bait piston pump. Only adult male mud shrimp of similar size (Total Length TL = 47.6 ± 0.5 mm, mean ± SE) were selected for the experiment. Experimental units consisted of Plexiglas tubes (diameter 9.5 x 40 cm) filled with sediments up to 20–22 cm depth. The rest was filled with seawater (water column = 1.06 L). Mud shrimp were placed in experimental units (one organism per core) and allowed to burrow for three days. This time was sufficient to observe a complete burrow (two open ends). Two series of treatments with four replicates per sampling time were conducted: (1) parasitized mud shrimp unexposed to Cd-contaminated seawater ("unexposed") and (2) parasitized mud shrimp exposed to Cd-contaminated seawater ("exposed"). Metal contamination was initiated by supplying seawater with a Cd stock solution (added as $CdCl_2$, concentration = 1 g L^{-1}) at a rate of 180 mL h^{-1}. Throughout the experiment, the Cd concentration in the water of contaminated experimental units was 7.4 ± 0.2 µg L^{-1} (mean ± SE), whereas Cd concentrations were systematically below the detection limit (DL) in the water column of uncontaminated experimental units (DL = 0.34 µg Cd L^{-1}).

Mud shrimp were sampled after 3, 7 and 14 days of incubation. Their hepatopancreas and abdominal muscle were sampled for Cd quantifications. The female bopyrid parasite was removed from its host under a stereomicroscope (SMZ1500; Nikon) and measured (Total Length, TL) (NIS-elements 0.4.00.00 software) before Cd analyses were performed.

After being dried (at least 48 h at 45 °C), biological samples were firstly weighed (dry weight, DW), then placed in polypropylene tubes and finally digested with nitric acid (HNO_3 65 %) at 100 °C for 3 h

(HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11 %. Then, Cd concentrations were determined in mud shrimp's abdominal muscle, hepatopancreas, and in bopyrid parasites by atomic absorption spectrophotometry with Zeeman correction (Agilent 240Z AA; Agilent). Method blanks and certified reference materials (DOLT-5, dogfish liver, NRCC-CNRC) were included in each analytical batch, and treated and analysed in the same way as biological samples (DL = 0.075 µg g^{-1} DW).

Prior to any statistical analysis, a Levene test was performed to assess homogeneity of variances. Bopyrid parasites (females only) TL and DW were tested for significant differences between experimental conditions using a Wilcoxon test. There were no significant TL nor DW differences between the bopyrid parasites examined (TL = 10.0 ± 0.3 mm; Wilcoxon test, $W = 0.88$, $p = 0.50$ and DW = 33.8 ± 2.7 mg; Wilcoxon test, $W = 73$, $p = 0.08$). Then, the effects of experimental condition ("unexposed" and "exposed") and time of experiment (3, 7 and 14 days) on Cd bioaccumulation in bopyrid parasites and in the mud shrimp's abdominal muscle and hepatopancreas (log-transformed data) were assessed using a two-way ANOVA. Concentrations of Cd were compared between female bopyrids and the abdominal muscle and the hepatopancreas of the host using a paired t-test and a Wilcoxon test for paired variables, respectively. Quantities of Cd in each organ were deduced from Cd concentrations and DW. Results are reported as the mean ± SE of N replicate measurements. Differences were considered significant for $p < 0.05$.

II.3. Influence of the bopyrid parasite on the mud shrimp's gills

The effect of the bopyrid parasite *G. branchialis* on the gills of the mud shrimp *U.* cf. *pusilla* has been determined by comparing the weight of gills between parasitized and unparasitized organisms. Mud shrimp were sampled on a mud flat in Arcachon Bay (44°40' N, 1°08' W), France, in May–June 2016 using a bait piston pump. Once collected, each specimen was individually isolated in a plastic pot containing seawater, to avoid fighting between the mud shrimp. In the laboratory, all mud shrimp were rinsed of sediment with seawater, and frozen (−20 °C) before being dissected. A total of 51 unparasitized and 39 parasitized mud shrimp were processed.

Each *U.* cf. *pusilla* was measured from the rostrum tip to the telson (Total Length, TL) using a digital calliper. Then, the left and right gill chambers were checked for the presence of the female bopyrid parasite *G. branchialis*. If present, the parasite was delicately removed from the gill chamber of its host under a stereomicroscope (SMZ1500; Nikon), before the left and right gills were carefully sampled. Gills were dried (at least 48 h at 60 °C) before being weighed (dry weight, DW). In a similar way, mud shrimp without their gills were dried and weighed.

Prior to any statistical analysis, a Levene test was performed to assess homogeneity of variances. The effect of the bopyrid parasite on the relationship between the total DW of gills (right and left gills together) and the mud shrimp TL was investigated by applying an analysis of covariance (ANCOVA) on log-transformed data. Regarding parasitized mud shrimp, the DW of the gill infested with the bopyrid

parasite and the DW of the gill unparasitized were compared using a Wilcoxon test for paired variables. Differences were considered significant for $p < 0.05$.

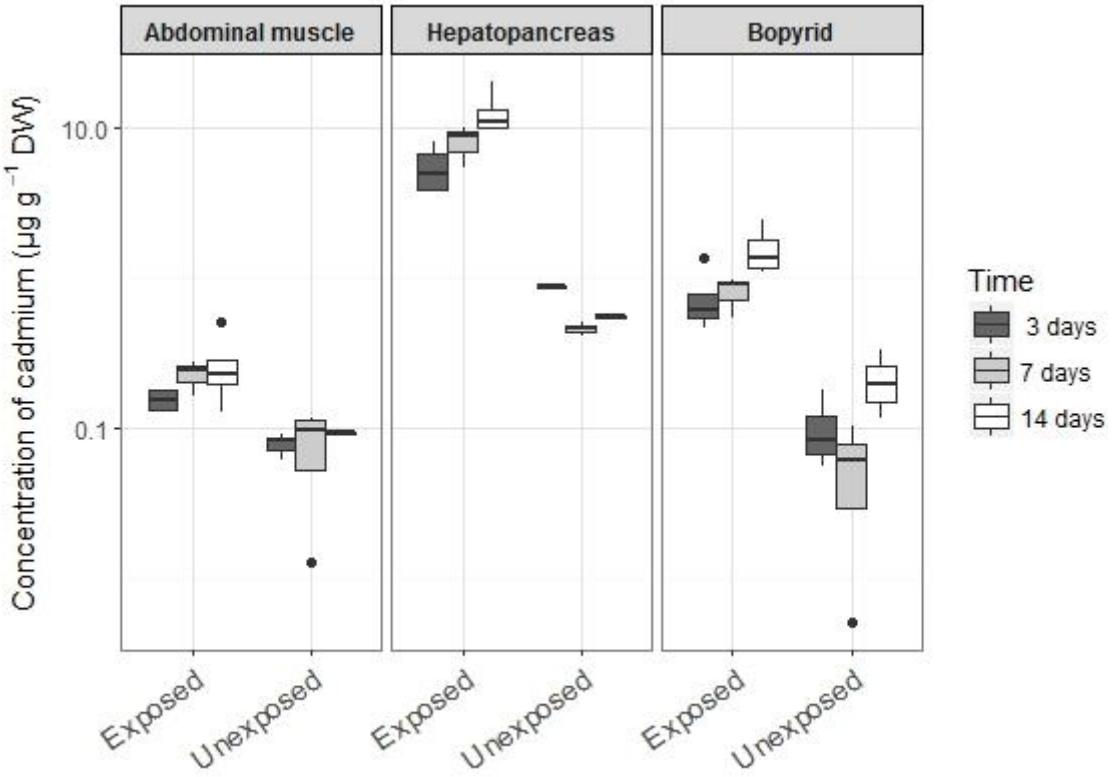

Figure V-B. 1 Concentrations of cadmium (Cd, µg g^{-1} DW; median, first and third quartiles, logarithmic scale) in the abdominal muscle and hepatopancreas of mud shrimp *Upogebia* cf. *pusilla* and in their bopyrid parasites *Gyge branchialis* exposed ("exposed") and unexposed ("unexposed") to Cd contamination over 14 days. $N = 4$ and $N = 3$ for "exposed" organisms at days 7.

Table V-B. 1 Results of two-way ANOVA evaluating the influence of time of exposure (3, 7 and 14 days) and experimental conditions ("exposed" vs. "unexposed") on cadmium concentrations in the abdominal muscle and the hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla* and in their bopyrid parasites *Gyge branchialis*. P-values in bold indicate significant effect.

	Factor	Df	F	*p*
Mud shrimp's abdominal muscle	Time (1)	2	1.20	0.35
	Experimental condition (2)	1	17.02	< **0.05**
	(1) x (2)	2	1.03	0.38
Mud shrimp's hepatopancreas	Time (1)	2	3.30	0.062
	Experimental condition (2)	1	613.96	< **0.05**
	(1) x (2)	2	13.84	< **0.05**
Bopyrid parasite	Time (1)	2	3.51	0.058
	Experimental condition (2)	1	45.38	< **0.05**
	(1) x (2)	2	1.00	0.39

III. Results and discussion

III.1. Trace metal bioaccumulation in the female bopyrid parasite

The Cd concentrations in "unexposed" parasites were low throughout the experiment, whereas "exposed" parasites showed significantly higher levels of Cd (Figure V-B. 1; Table V-B. 1). At experiment completion, Cd concentrations in "exposed" parasites were 7.0 times higher than in "unexposed" bopyrids. There was no effect of time and no interactive effect between experimental condition and time on the bioaccumulation of Cd by bopyrid parasites (Table V-B. 1). The ability of marine parasites to accumulate contaminants is well recognized (Nachev and Sures 2016), even though studies targeting ectoparasites, such as bopyrids, are scarce. The literature suggests that ectoparasites have a reduced ability to bioaccumulate contaminants compared to endoparasites (Bergey *et al.*, 2002), such as acanthocephalans, which often display higher contaminant levels than their host (Sures and Siddall, 1999; Nachev and Sures, 2016). Furthermore, in this study, the Cd concentrations were significantly higher in "exposed" parasites than in the abdominal muscle of their "exposed" host (paired t-test, $t = 4.76$, $p < 0.05$) but lower than in the "exposed" host's hepatopancreas (Wilcoxon test for paired variables, $V = 0$, $p < 0.05$) at each of the sampling times (Figure V-B. 1). A mass balance calculation showed that there were on average 0.24 ± 0.06 µg of Cd in the hepatopancreas, and 0.01 ± 0.00 µg of Cd in the abdominal muscle, of "exposed" parasitized mud shrimp, while bopyrids contained 0.05 ± 0.02 µg of Cd on average at experiment completion. On the other hand, there was on average 0.38 ± 0.19 µg of Cd in the hepatopancreas, and 0.03 ± 0.01 µg of Cd in the abdominal muscle, of unparasitized mud shrimp exposed to Cd contamination over 14 days (Dairain *et al.*, 2018b). Thus, the difference in Cd bioaccumulation between parasitized and unparasitized mud shrimp cannot only be due to a direct effect of the bopyrid parasite acting as a sink in mud shrimp.

III.2. Effect of the bopyrid parasite on the gills of mud shrimp

The bopyrid parasite was negatively associated with the relationship between the total DW of gills and mud shrimp TL (ANCOVA, $F_{1,87} = 19.40$, $p < 0.05$), with the gills of parasitized organisms being lighter than those of unparasitized individuals, when standardized for TL (Figure V-B. 2). These results are in accordance with those of Pascal *et al.* (2016b) who showed that parasitized organisms were almost 10 % lighter than uninfested specimens, when weight was standardized for TL. In addition, we noticed that the gills harboring the bopyrid parasite were significantly lighter than the opposite unparasitized gill (Wilcoxon test for paired variables, $V = 142$, $p < 0.05$). Together these gill weight results suggest that the bopyrid parasite has a particularly detrimental impact on the gills of its host. The mud shrimp *U.* cf. *pusilla* is primarily a suspension feeder (Dworschak, 1987a) but also filters water for respiration purposes. Thus, gills are a major site of direct Cd uptake. By reducing the weight of gills of the mud shrimp, it is likely that the bopyrid parasite reduces the surface area available for Cd uptake by the host. To properly confirm this, an experiment comparing Cd bioaccumulation in the gills of parasitized and unparasitized organisms, as well as between the two gills of parasitized mud shrimp, is required.

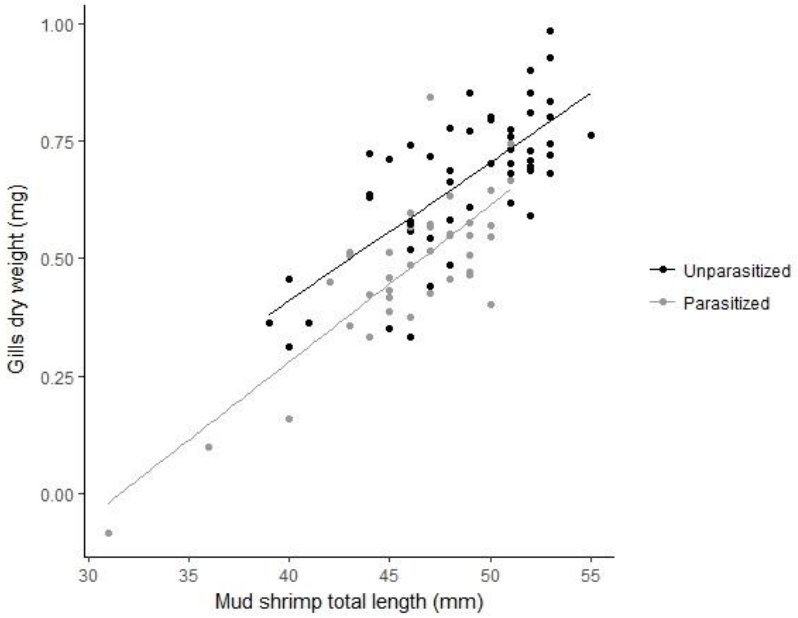

Figure V-B. 2 Influence of the bopyrid parasite *Gyge branchialis* on the biometric relationships between the gills dry weight (log-transformed data; mg) and the mud shrimp *Upogebia* cf. *pusilla* total length (mm). $y = 0.029\ x - 0.76$ for unparasitized mud shrimp; $y = 0.033\ x - 1.05$ for parasitized mud shrimp. $N = 51$ unparasitized mud shrimp and $N = 39$ parasitized mud shrimp.

IV. Conclusion

We found that the bopyrid parasite *Gyge branchialis* bioaccumulates Cd when its host, the mud shrimp *Upogebia* cf. *pusilla*, is exposed to this trace metal. Levels of Cd were higher in the parasite than in the abdominal tissue of the host. However, this was not the case for the hepatopancreas of the host which showed the highest Cd levels recorded. Additionally, the parasite damages the gills of the mud shrimp, which might reduce the surface area of the gills and lessen the Cd uptake by parasitized organisms. Finally, it should be considered that the parasite may also reduce the Cd uptake of its filter feeder host by lessening its filtration and/or ventilation activities (Stier *et al.*, 2015; Pascal, 2017). However, the influence of this bopyrid parasite on the filtration activity of the mud shrimp in regard of the bioaccumulation process of Cd and other contaminants has still to be investigated.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

BILAN DU CHAPITRE V-B ET PERSPECTIVES

Cette courte étude avait pour objectif d'investiguer deux mécanismes pouvant expliquer la différence de contamination notée entre crevettes de vase *Upogebia* cf. *pusilla* parasitées avec le bopyre *Gyge branchialis* et non parasitées au cours d'expériences en laboratoire (**Chapitres IV** et **V-A**). Dans un premier temps nous avons estimé les capacités d'accumulation du Cd par le parasite. En effet, un nombre croissant d'études a permis de mettre en évidence la capacité des parasites à accumuler les contaminants (Nachev and Sures, 2016), amenant à suggérer que les parasites eux-mêmes pourraient être utilisés dans le cadre de programmes de surveillance en tant qu'espèces sentinelles. Par ailleurs, il a été montré que les parasites accumulent les contaminants à des concentrations bien supérieures à celles observées chez leur hôte, venant à proposer que les parasites puissent être des puits de contaminants ce qui pourrait donc être avantageux pour l'hôte dans des environnements pollués. En ce qui concerne *G. branchialis* une accumulation de Cd significative a été notée chez les parasites provenant de crevettes de vase soumises à une contamination au Cd. Bien que les concentrations en Cd des parasites soient supérieures à celles notées dans le muscle abdominal de leur hôte, elles sont inférieures à celles observées dans l'hépatopancreas des crevettes de vase. De plus, la réalisation d'un bilan de masse a montré que la quantité de Cd accumulée par le parasite ne peut expliquer à elle seule la différence de bioaccumulation entre les crevettes de vase parasitées par le bopyre et celles non parasitées.

La seconde hypothèse envisagée pour expliquer la plus faible concentration de Cd notée chez les crevettes de vase infestées par *G. branchialis* était que le parasite vivant dans la cavité branchiale de son hôte, il puisse abimer les branchies de ce dernier. Or les branchies constituent une voie d'entrée majeure des contaminants métalliques dissous. En comparant les poids des branchies entre organismes non parasités et parasités, nous avons en effet pu mettre en évidence que, à taille égale, les individus parasités par *G. branchialis* présentaient une masse branchiale inférieure à celle des organismes non parasités. En réduisant la taille de la surface branchiale de son hôte, le parasite *G. branchialis* pourrait limiter les entrées de Cd à ce niveau et donc limiter la contamination métallique de son hôte. Cependant, il est à noter que pour compenser cette réduction de la masse branchiale, et par conséquent également de la surface de prélèvement de l'oxygène, une augmentation des activités de filtration des *U.* cf. *pusilla* parasitées ne peut être exclue et devrait être évaluée.

CHAPITRE VI

Discussion générale et perspectives de recherche

Le travail de thèse de L. Pascal (2017) a permis de définir le thalassinidé *Upogebia* cf. *pusilla* comme une importante espèce bioturbatrice dans le bassin d'Arcachon. Cet organisme construit des terriers relativement profonds au niveau des zones sablo-vaseuses intertidales où il est à l'origine d'un important remaniement sédimentaire et d'une large bioirrigation du sédiment. Entre autres, ces activités influencent largement les flux biogéochimiques à l'interface eau-sédiment et stimulent les processus de minéralisation de la matière organique par la communauté microbienne dans les sédiments. Chez divers thalassinidés, ces activités ont été associées à un rôle prépondérant de ces organismes dans la structuration des communautés benthiques et notamment des organismes de la micro-, méio- et macrofaune (Pillay and Branch, 2011). De ce fait, les thalassinidés sont considérés comme d'importantes espèces ingénieures des écosystèmes marins sablo-vaseux.

La bonne santé des espèces bioturbatrices détermine l'intensité et le type de bioturbation exercée par ces organismes. Ainsi, il a été montré que les variations de certains facteurs abiotiques (disponibilité en oxygène, salinité) pouvaient modifier le comportement des espèces bioturbatrices (Diaz and Rosenberg, 1995; Przeslawski *et al.*, 2009). Par ailleurs, les interactions entre organismes eux-mêmes (compétition intraspécifique, prédation) peuvent amener à des modifications de ces activités de bioturbation (e.g., Duport *et al.*, 2006; Maire *et al.*, 2010; Premo and Tyler, 2013). Parmi les interactions biotiques, l'influence du parasitisme a jusqu'à présent été relativement peu étudiée chez les espèces bioturbatrices (**Chapitre I-A**). Chez *U.* cf. *pusilla*, le travail de thèse de L. Pascal (2017) a montré que le parasite bopyre *Gyge branchialis* modifiait de façon conséquente le comportement de son hôte, les organismes parasités étant relativement moribonds comparés aux individus non parasités. En lien avec ces modifications du comportement, une diminution conséquente des activités de bioturbation (remaniement sédimentaire et bioirrigation) a été documentée. C'est dans ce contexte général et la continuité de ces précédents travaux de thèse que s'insère le présent travail de thèse, dont le but premier était d'appréhender la pression parasitaire expérimentée par le bioturbateur *U.* cf. *pusilla* et d'en évaluer les conséquences, et ce, à deux niveaux d'intégration : (1) moléculaire, certaines modifications d'expression géniques pouvant être considérées comme des biomarqueurs de réponse aux stress, et (2) comportemental, i.e. en s'intéressant aux modifications de l'intensité des activités de bioturbation de ce crustacé (notamment le remaniement sédimentaire).

Par rapport aux travaux de L. Pascal (2017), nous avons par ailleurs cherché à replacer nos travaux dans le contexte écologique actuel des zones côtières. En effet, plus de 40 % de la population humaine vit à moins de 100 km des côtes, ce qui fait de ces milieux des environnements largement soumis aux pressions anthropiques. Parmi les perturbations anthropiques touchant les environnements côtiers, le rejet de contaminants métalliques ou organiques est particulièrement préoccupant du fait de la diversité de ces composés et de leur gamme d'impacts sur les organismes peuplant ces environnements. Ainsi, en plus de l'influence du parasitisme, il a été choisi d'évaluer l'impact d'une contamination métallique sur le thalassinidé *U.* cf. *pusilla*. Dans cette optique, une approche couplant suivis *in situ* et expérimentations

en laboratoire a été mise en œuvre permettant (1) de caractériser les pressions parasitaires et métalliques à l'échelle locale (i.e. le bassin d'Arcachon) et (2) de quantifier les impacts de ces facteurs de stress, seuls et en interaction, sur la crevette de vase *U.* cf. *pusilla*.

I. Interactions parasitaires chez *Upogebia* cf. *pusilla*

Un des premiers objectifs de ce travail a consisté à caractériser à l'échelle locale, i.e. le bassin d'Arcachon, la pression parasitaire expérimentée par *U.* cf. *pusilla*. La dynamique temporelle de deux parasites, le bopyre *Gyge branchialis* et le trématode *Maritrema* sp., a ainsi été décrite (**Chapitre III-A**). Il a également été possible de caractériser les interactions entre ces deux parasites. Une association négative asymétrique entre le bopyre et le trématode a été mise en évidence, *G. branchialis* interférant de manière directe et indirecte avec les processus d'infection de *Maritrema* sp. (Figure VI. 1). Il est donc proposé que le bopyre diminue les activités de ventilation de son hôte (Pascal, 2017), réduisant ainsi le nombre de cercaires arrivant au contact de ce dernier. Une fois à proximité de l'hôte, la simple présence de *G. branchialis* dans la cavité branchiale pourrait constituer une barrière physique limitant l'accès aux branchies et donc la fixation des cercaires. Par ailleurs, nous avons noté que le parasite réduisait la masse des branchies de son hôte (**Chapitre V-B**) et donc potentiellement aussi leurs surfaces, ce qui *in fine* circonscrirait les possibilités d'infection avec les trématodes, du moins au niveau des branchies. Finalement, en plus de réduire les capacités de *U.* cf. *pusilla* à ventiler son terrier, il est proposé que le bopyre amoindrisse les capacités de filtration de son hôte et donc l'apport de larves parasites cercaires arrivant au contact de ses branchies.

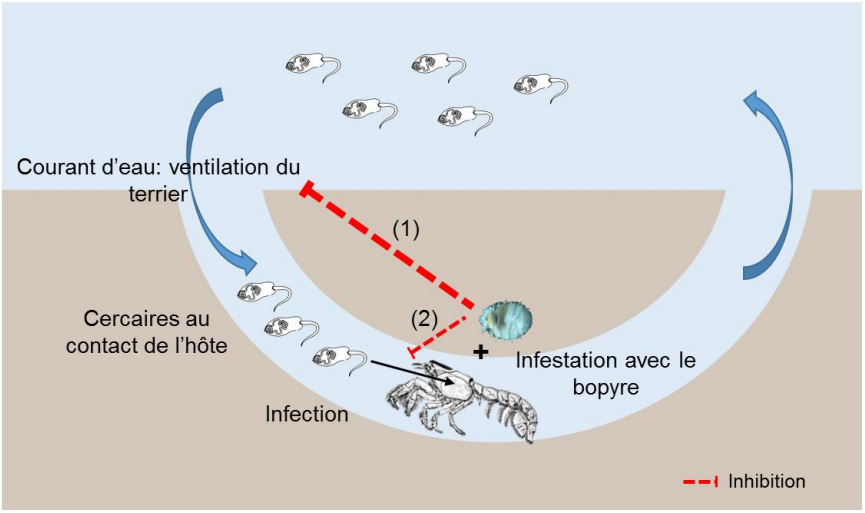


Figure VI. 1 Mécanismes supposés par lesquels le bopyre *Gyge branchialis* limite l'infection avec le trématode *Maritrema* sp chez la crevette de vase *Upogebia* cf. *pusilla* : le bopyre interfère avec les processus d'infection avec *Maritrema* sp. en (1) limitant les processus de ventilation du terrier par la crevette de vase et donc l'arrivée de cercaires au contact du crustacé et (2) réduisant la fixation des cercaires au niveau des branchies par sa simple présence et/ou en réduisant les activités de filtration de *U.* cf. *pusilla*.

II. Impacts du trematode *Maritrema* sp. ?

Le trématode *Maritrema* sp. a été identifié au niveau des branchies, du muscle abdominal et de la cavité viscérale de *U.* cf. *pusilla*. Dans le bassin d'Arcachon, les intensités d'infection sont très variables, i.e. de quelques métacercaires à plus de 1000 individus par hôte. Cette large gamme d'intensité d'infection pourrait être due à des variations de la densité des autres hôtes de ce parasite entre les différents sites, et notamment du premier hôte intermédiaire. Le cycle de vie de *Maritrema* sp. n'étant à ce jour pas élucidé, il est impossible de tester de telles hypothèses. Ce parasite appartient aux groupes des Microphallidea. Chez ces parasites, le premier hôte intermédiaire est un gastéropode et l'hôte final un oiseau (Dawes, 1946; Deblock, 2008) : les recherches devraient donc se concentrer sur les organismes appartenant à ces deux clades.

L'absence de connaissances concernant le cycle de vie de *Maritrema* sp. limite fortement les opportunités d'évaluer l'influence de ce parasite sur *U.* cf. *pusilla*. En effet, il n'est pas possible de déterminer *a priori* la présence/absence de ces parasites ainsi que leurs intensités chez les crevettes de vase, la dissection constituant l'unique moyen de déterminer ces paramètres. Seules des infections contrôlées en laboratoire d'organismes non-infectés par *Maritrema* sp. permettraient de quantifier l'influence de ces parasites sur leur hôtes.

Les trématodes au stade métacercaire sont globalement considérés comme peu délétères pour leur hôte comparativement au stade sporocyste se développant dans l'hôte amont (i.e. dans le premier hôte intermédiaire) (Lauckner, 1980, 1983). En réalité, cette assertion souffre de nombreuses exceptions ; en particulier la présence de métacercaires a été associée à de larges modifications du comportement des hôtes (réductions des niveaux d'activités ou encore comportements aberrants) (e.g., Helluy, 1983; Thomas and Poulin, 1998; Thomas and Renaud, 2001; Mouritsen and Poulin, 2005). Par exemple, les coques de Nouvelle-Zélande *Austrovenus stutchburyi* largement parasitées par des métacercaires d'Echinostomatoidae présentent des capacités de reptation considérablement réduites (Mouritsen and Poulin, 2005). Ces parasites altèrent également les capacités d'enfouissement dans la colonne sédimentaire de leur hôte (Thomas and Poulin, 1998). De telles modifications comportementales chez *U.* cf. *pusilla* pourraient se traduire par une altération de l'intensité de son activité bioturbatrice et, *in fine*, pourrait modifier son impact en tant qu'espèce ingénieure de l'écosystème (Figure I-A. 5). Une première perspective de recherche consisterait ainsi dans un premier temps à **élucider le cycle de vie de *Maritrema* sp.** et notamment à caractériser le premier hôte intermédiaire de ce parasite, pour dans un second temps **réaliser des infections expérimentales permettant de caractériser l'influence de cette espèce de trématode sur le comportement de *U.* cf. *pusilla***. A l'inverse de la méthodologie expérimentale mise en œuvre au cours de ces travaux (**Chapitres IV** et **V**), ces études nécessiteraient de pouvoir observer les organismes dans leurs terriers et donc la mise en place d'unités expérimentales adaptées tels des aquariums plats (« ant farm ») (Pascal, 2017).

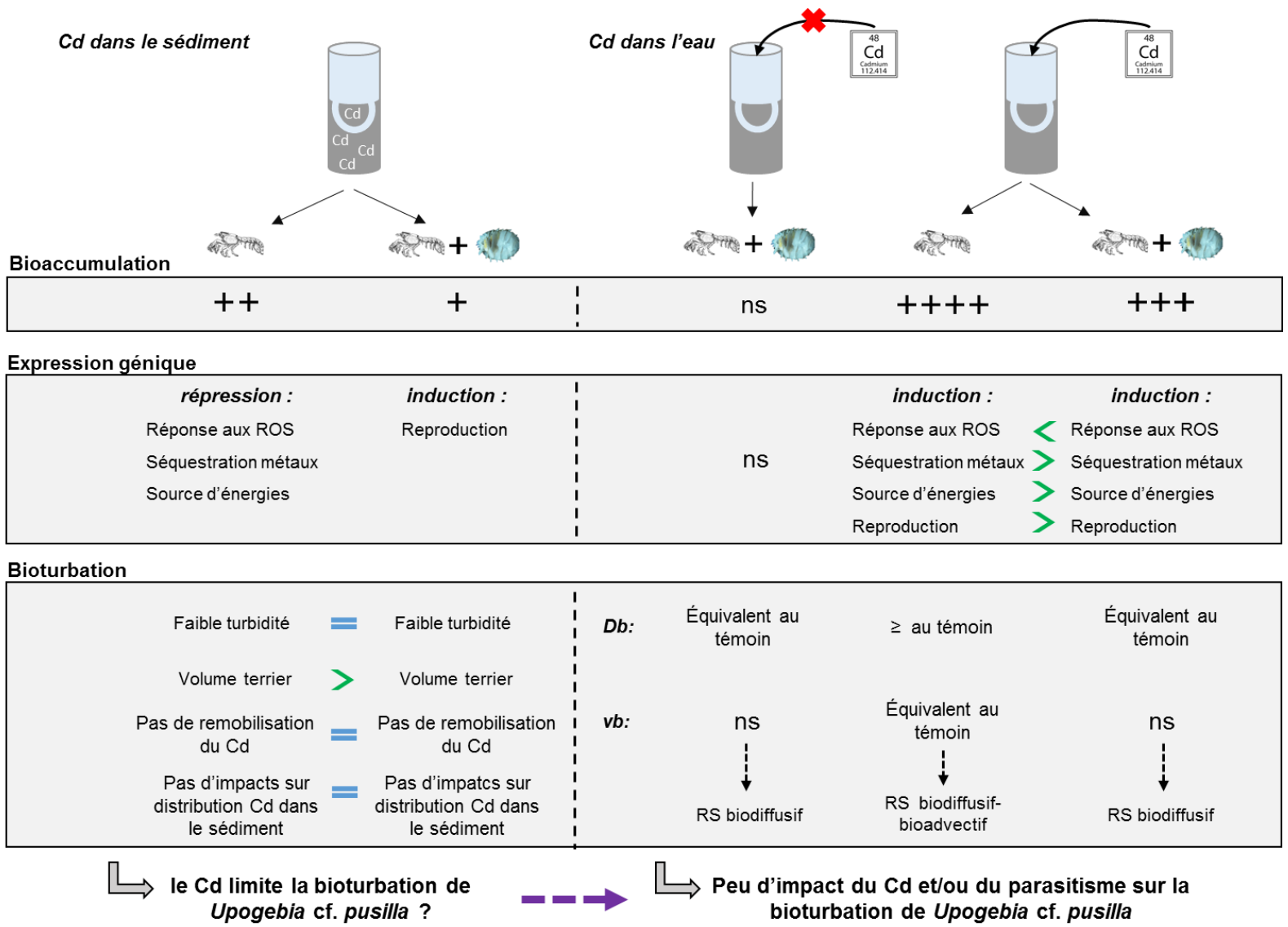


Figure VI. 2 Représentation schématique des principaux résultats obtenus *ex-situ* au cours de ce travail de thèse. ns : non-significatif, *Db* : coefficient de biodiffusion, ROS : espèces réactives de l'oxygène, RS : remaniement sédimentaire, *vb* : coefficient de bioadvection.

III. Impacts du bopyre *Gyge branchialis*

L'influence du bopyre sur son hôte a été évaluée à trois échelles (génétique, physiologique et comportementale) à l'aide d'études *in situ* (**Chapitres III-B** et **V-B**) et d'expérimentations en laboratoire (**Chapitres IV** et **V)**. Un schéma général de synthèse des principaux résultats obtenus expérimentalement est proposé en Figure VI. 2 et sera discuté dans les paragraphes suivants.

III.1. Au niveau génétique

III.1.1. Influence de *Gyge branchialis* seul

Parmi la dizaine de gènes ciblés, seule l'expression du gène *vtg*, en lien avec les processus reproductifs, semble impactée par la présence du parasite bopyre, son expression étant considérablement réduite chez les femelles parasitées au cours des périodes de reproduction (**Chapitre III-B**). Ce résultat confirme ainsi à l'échelle moléculaire l'impact délétère de *G. branchialis* sur les fonctions reproductrices de son hôte femelle. En ce qui concerne l'influence du bopyre sur les capacités de reproduction des mâles, les résultats sont plus ambigus. Tucker (1930) a observé que le fonctionnement des gonades pouvait être altéré chez les mâles parasités par *G. branchialis*, certains d'entre eux présentant même un nombre excessif de gamètes femelles. A l'inverse, à l'échelle moléculaire, nous n'avons pas noté d'induction significative du gène *vtg*. Cependant, il est vrai que chez certains individus mâles parasités ce gène était largement induit, la non-significativité des résultats étant due à d'importantes variations inter-individuelles. De ce fait, il semble que l'influence du bopyre sur les fonctions reproductives des mâles soit modulée par des caractéristiques propres à chaque individu (différence de capacité de résistance à l'infestation et à ses effets par exemple).

En ce qui concerne les autres gènes ciblés et comme précédemment évoqué dans les discussions des **Chapitres III-B** et **V-A**, l'absence de modification de l'expression des gènes ciblés chez les organismes parasités pourrait être due au fait que l'association *U.* cf. *pusilla/G. branchialis* est une association à long terme, le parasite se fixant sur son hôte relativement tôt au cours de la vie de ce dernier (Tucker, 1930; Pascal *et al.*, 2016b). Or, les modifications d'expression génique sont des processus temps-dépendant, certains gènes répondant de façon précoce aux stress et d'autres de façon plus tardive (Figure VI. 3) (Denslow *et al.*, 2007). Il est ainsi possible que les gènes présentement étudiés ne constituent pas de bons biomarqueurs d'effets à un stress chronique parasitaire. De manière à évaluer l'influence de *G. branchialis* sur des adultes *U.* cf. *pusilla* au niveau génique, **un séquençage du transcriptome complet par RNA-seq** pourrait être envisagé et permettrait éventuellement la mise en évidence de modifications de l'expression de gènes dits de « compensation » ou « d'adversité » en lien avec la chronicité du stress exercé par le parasite (Denslow *et al.*, 2007). Cette technique a notamment pour avantage de ne pas nécessiter de connaitre le génome de l'organisme étudié (le génome de *U.* cf. *pusilla* n'étant, à ce jour, pas disponible) et s'effectue sans *a priori.*

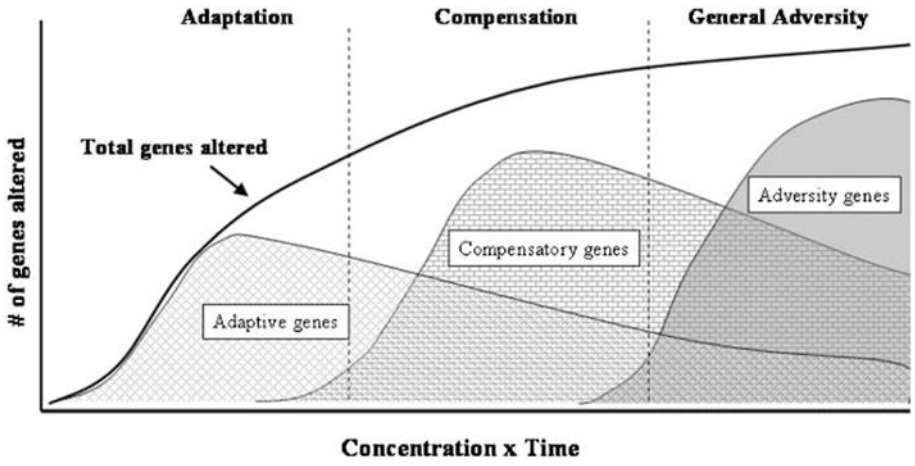


Figure VI. 3 Changements temporels de l'expression génique. Dans un premier temps, une modification de l'expression de gènes dits « adaptatifs » est observée. Ces gènes répondent de manière globale à toute une série de facteurs de stress. A mesure que le temps d'exposition aux facteurs de stress augmente, l'expression de gènes dits « compensatoires » est modifiée. Ces gènes permettent la mise en place de nouvelles voies biochimiques permettant de compenser celles impactées par les facteurs de stress. Ces changements compensatoires sont plus spécifiques que les changements adaptatifs initiés précocement. Si la durée d'exposition s'allonge, l'organisme n'est plus capable de compenser, il est épuisé et certaines fonctions homéostatiques sont interrompues (arrêt de la croissance, de la reproduction, etc.), amenant éventuellement à la mort de l'organisme. D'après Denslow et *al.* (2007).

Finalement, des infestations expérimentales avec *G. branchialis* pourraient être suggérées dans le but d'étudier les effets précoces de ce parasite à l'échelle génique. Néanmoins, et en dehors du fait que ce type d'expérimentation souffre actuellement des mêmes limitations que celles évoquées pour les trématodes, cette perspective de recherche nous semble à exclure puisque ne reflétant aucunement la réalité environnementale (aucun jeune bopyre n'a par exemple été retrouvé chez des crevettes de vase matures). Par ailleurs, ce type d'expérimentation pourrait générer un stress plus important que celui dû au parasite même car nécessitant l'implantation d'un parasite adulte au niveau de la cavité branchiale de *U.* cf. *pusilla* par un expérimentateur.

III.1.2. Contamination métallique au cadmium et parasitisme

A l'inverse du parasite bopyre seul qui est à l'origine de peu de modifications de l'expression des gènes ciblés, la contamination au Cd, couplée ou non à la présence de *G. branchialis*, module largement l'expression de gènes impliqués dans la réponse au stress oxydant, à la séquestration des métaux, à l'apport énergétique ou encore en lien avec les processus reproductifs. A partir de nos résultats il est néanmoins difficile de tirer des conclusions globales concernant l'influence de ces deux facteurs de stress sur l'expression génique puisque les réponses observées se sont notamment montrées dépendantes de la voie de contamination (*via* le sédiment vs. la colonne d'eau), des niveaux d'accumulation ainsi que du temps d'exposition à la contamination. De plus, nous avons noté des divergences de modulation de l'expression génique au moment *t* entre certains gènes impliqués dans une même fonction (e.g., *sod Mn*

et *cat* , tous deux codant des protéines intervenant dans la réponse au stress oxydant). Toutefois, les fortes inductions géniques observées chez les organismes contaminés au Cd et contaminés au Cd et parasités (**Chapitre V-A**) laissent à penser que *U.* cf. *pusilla* est capable de mettre en place des protocoles de lutte contre les effets délétères de la contamination au Cd (notamment le stress oxydant généré par ce métal) ainsi que des processus de détoxication.

Bien qu'une importante réponse génique ait été observée chez les individus exposés au Cd non parasités et parasités par *G. branchialis*, le parasite modifie largement cette réponse que ce soit en termes d'intensité ou de cinétique. De manière générale, les crevettes de vase parasitées accumulent moins de Cd que leur consœurs non parasitées, en lien avec des modulations d'expression génique de moindre importance. Cette plus faible accumulation de Cd par les organismes parasités n'explique néanmoins pas toutes les différences d'expressions géniques notées entre les deux types de crevette de vase et souligne la complexité des interactions entre stress multiples.

L'analyse de l'expression des gènes est une première étape pour comprendre l'influence d'une contamination métallique au Cd et d'une contamination métallique en interaction avec le parasitisme sur les organismes. Toutefois, ces modifications d'expression génique n'aboutiront pas forcément à des modifications des niveaux des protéines permettant, par exemple, de lutter contre le stress oxydant généré par le Cd. Dans ce contexte, il pourrait ainsi être intéressant **d'élargir l'analyse génique à l'échelle protéomique en réalisant notamment des dosages d'activités enzymatiques** de manière à comprendre de façon plus détaillée les mécanismes cellulaires de détoxication des métaux et de lutte contre leurs effets délétères mis en place chez *U.* cf. *pusilla* en réponse à une contamination.

III.2. Au niveau physiologique

L'influence du parasite *G. branchialis* sur la physiologie de son hôte a été évaluée notamment en estimant le rôle joué par ce parasite dans les processus de bioaccumulation des métaux, et plus spécifiquement du Cd. Nous avons ainsi observé qu'au cours de contaminations expérimentales *G. branchialis* limitait de façon conséquente l'accumulation de Cd par son hôte. La façon dont ce parasite interfère avec les processus d'accumulation reste cependant à élucider. Par exemple, les nombreuses dissections réalisées au cours de ce travail nous ont amenés à supposer que le bopyre puisse altérer les branchies de son hôte. Une courte étude visant à tester cette hypothèse a ainsi été menée mettant en évidence, qu'à taille égale, les individus parasités par le bopyre présentaient une masse branchiale inférieure à celles d'organismes non parasités (**Chapitre V-B**). Ainsi, il peut être proposé qu'en limitant la masse et donc potentiellement la taille des branchies, le bopyre limite les échanges de solutés au niveau de cette interface, dont les échanges de Cd. De manière plus globale, l'impact de *G. branchialis* sur la physiologie de son hôte reste relativement peu connu et mériterait des investigations complémentaires de manière à mieux appréhender de potentielles modifications du comportement de *U.* cf. *pusilla* associées à la présence de ce parasite. Par exemple, la réduction des niveaux d'activités de *U.* cf. *pusilla* pourrait être due au parasite interférant avec l'équilibre énergétique de son hôte. Par ailleurs,

le parasite pourrait limiter les capacités de son hôte à faire face à un stress hypoxique au cours des périodes de basse mer et ainsi réduire son niveau d'activité au cours de cette période.

III.3. Au niveau comportemental

Le rôle joué par le parasite *G. branchialis* sur les activités de bioturbation de son hôte a été évalué de manière indirecte, i.e. en s'intéressant à l'influence de *U.* cf. *pusilla* sur le devenir d'un contaminant métallique (remobilisation vers la colonne d'eau et distribution dans la colonne sédimentaire), et directe, i.e. en quantifiant le taux de remaniement sédimentaire à l'aide de traceurs particulaires (Figure VI. 2).

III.3.1. Bioturbation et devenir des contaminants métalliques

a) Rôle de *Upogebia* cf. *pusilla*

Au cours du **Chapitre IV**, nous avons tout d'abord évalué l'influence du bioturbateur *U.* cf. *pusilla* sur le devenir d'un contaminant métallique, le Cd, contenu dans un sédiment artificiellement enrichi. Les crevettes de vase parasitées ou non n'ont influencé ni la remobilisation du Cd depuis le sédiment vers la colonne d'eau ni sa répartition dans la colonne sédimentaire (comparaison des concentrations de Cd entre les zones oxydées et réduites du sédiment). Ces résultats sont surprenants considérant que la bioturbation est reconnue comme un des paramètres clés influant sur le devenir des contaminants dans les sédiments (e.g., Rasmussen *et al.*, 2000; Ciutat, 2003; Atkinson *et al.*, 2007). Néanmoins, il est à noter que, dans un environnement donné, ce devenir dépend en grande partie (1) du type de contaminant et (2) du type de sédiment. Ainsi, il est possible que le comportement du Cd en présence de *U.* cf. *pusilla* ne soit pas totalement représentatif de celui d'autres éléments métalliques. De manière à tester cette hypothèse, une série d'expériences similaires à celle présentée au cours de ce travail, utilisant d'autres éléments métalliques pourrait être envisagée. Néanmoins, outre le fait que de telles expériences seraient particulièrement redondantes, elles ne revêtiraient pas de réalité écologique puisque dans l'environnement les contaminations sont généralement polymétalliques (notion de « cocktail »). Ainsi, une perspective de recherche peut-être plus intéressante serait **d'évaluer l'influence de *U.* cf. *pusilla* sur le devenir d'éléments métalliques en utilisant un sédiment naturellement contaminé**. De plus, *U.* cf. *pusilla* colonisant une large gamme granulométrique de sédiments (Tableau II. 1), de telles expérimentations gagneraient à prendre en compte ce paramètre.

Au cours de ce travail, les mesures de Cd conduites dans la colonne d'eau ont permis de quantifier le Cd dissous et le Cd particulaire. Ces mesures ont été réalisées par spectrométrie d'émission atomique à plasma couplé induit (ICP-OES). Bien que relativement sensible, cette technique l'est bien moins que la spectrométrie de masse à plasma couplé induit (ICP-MS). Seules de faibles concentrations de Cd dans la colonne d'eau des différentes unités expérimentales ont été détectées au cours de cette expérimentation et uniquement à partir de trois jours d'incubation. Par ailleurs, nous avons enregistré une importante variabilité entre les différents réplicas des différentes conditions expérimentales (incluant les contrôles). Ceci pourrait en partie s'expliquer par le fait que les concentrations mesurées étaient relativement proches des limites de détection de l'appareil d'ICP-OES utilisé, notamment dans

les premiers temps des incubations. **Ainsi, dans le cadre d'éléments traces métalliques, de prochaines expérimentations gagneraient à utiliser l'ICP-MS plutôt que l'ICP-OES.**

Finalement, soulignons que les mesures de Cd réalisées dans le sédiment ont été effectuées sur la matrice sédimentaire dans son ensemble, et n'ont permis de comparer que les concentrations en Cd des zones oxydées et réduites. Or, il est envisageable que la bioturbation exercée par *U.* cf. *pusilla* ait influencé la répartition du Cd entre les eaux interstitielles et les particules sédimentaires, modifiant de ce fait la biodisponibilité du Cd dans le sédiment et *in fine* sa toxicité. Il est ainsi suggéré que de prochaines expérimentations évaluant l'influence de la bioturbation de *U.* cf. *pusilla* sur le devenir des contaminants métalliques dans les sédiments **s'intéressent à la fois aux métaux liés aux particules sédimentaires ainsi qu'à ceux contenus dans les eaux interstitielles.** Par ailleurs, le **dosage des AVS (acid volatile sulfides) devrait être envisagé**. En effet, ces composés ont une influence majeure sur la biodisponibilité des métaux et il est ainsi couramment admis que dans le cas d'une concentration en AVS supérieure à celles des métaux, la concentration de ces derniers dans les eaux interstitielles peut être considérée comme négligeable et non toxique pour les organismes benthiques (Ankley *et al.*, 1996). La bioturbation (notamment le remaniement sédimentaire) peut être à l'origine de l'oxydation de ces composés volatiles, *via* leur relocalisation depuis des zones anoxiques vers des zones oxiques de la colonne sédimentaire, et donc influer sur la biodisponibilité des éléments métalliques.

Au cours de cette expérimentation, nous n'avons pas observé d'influence du parasitisme sur les capacités de remobilisation du Cd depuis le sédiment vers la colonne d'eau de *U.* cf. *pusilla* ni sur le rôle joué par cet organisme sur la répartition du Cd dans la colonne sédimentaire. L'hypothèse émise était que le parasite *G. branchialis* diminuant les niveaux d'activité de son hôte, l'influence d'organismes parasités sur le devenir du Cd contenus dans les sédiments serait moindre. Puisque les organismes non-parasités ne jouent eux-mêmes qu'un rôle mineur dans cette dynamique, il n'est pas surprenant de ne pas observer d'effets du parasite *G. branchialis* sur les crevettes de vase. Cependant, il convient tout de même de souligner qu'au cours de cette expérimentation, les individus parasités ont creusé des terriers de moindre envergure que ceux construits par les organismes non parasités, ce qui pourrait traduire une réduction des activités de fouissage (et donc une réduction des taux de remaniement sédimentaire). Néanmoins, dans cette expérience, les crevettes de vase étaient en présence d'un sédiment contaminé et il ne peut être exclu que cela soit le résultat d'une interaction entre la contamination métallique et le parasitisme. En effet, après 14 jours d'exposition à un sédiment contaminé au Cd, les organismes infestés et non-infestés par *G. branchialis* ont montré des contenus en Cd bien supérieurs à ceux observés chez des individus prélevés dans le bassin d'Arcachon, où les concentrations en Cd dans les sédiments sont faibles (**Chapitre III-B**). Ainsi, nous avons émis l'hypothèse que l'activité de bioturbation de *U.* cf. *pusilla* pouvait être réduite du fait de la contamination métallique.

b) Contamination métallique au cadmium et parasitisme

L'expérience menée dans le cadre du **Chapitre V-A** a permis d'évaluer l'influence d'une contamination au Cd seule et associée à la présence du parasitisme sur le remaniement sédimentaire de la crevette de vase *U.* cf. *pusilla* (Figure VI. 2). Les expériences multi-stress sont intéressantes car elles reflètent une réalité environnementale et permettent la mise en évidence de potentiels effets additifs, synergiques ou antagonistes. Par ailleurs, cette expérimentation a été l'occasion de re-tester les effets du parasite *G. branchialis* seul sur l'activité de bioturbation de *U.* cf. *pusilla*.

(i) Influence du parasite seul

Contrairement aux travaux de L. Pascal (2017), nous n'avons observé qu'un effet limité du parasitisme sur la bioturbation exercée par *U.* cf. *pusilla*, les taux de remaniement sédimentaire déterminés étant peu modifiés du fait de la présence du parasite. L'examen des profils verticaux des traceurs particulaires a néanmoins permis de noter que les organismes non parasités étaient à l'origine de transports bioadvectifs dans les premières couches de la colonne sédimentaire à l'inverse des organismes parasités dont le remaniement sédimentaire semble purement biodiffusif.

Comme souligné dans le cadre du **Chapitre V-A,** les importantes différences de résultats concernant l'influence du parasite *G. branchialis* sur les activités de remaniement sédimentaire de son hôte entre les travaux de L. Pascal (2017) et les nôtres pourraient être dus à des systèmes expérimentaux différents. L. Pascal a conduit ses expériences dans des aquariums plats alors que nous avons utilisés des carottes de plexiglas. Les aquariums plats contraignent la construction de terriers en deux dimensions alors que les carottes de plexiglas permettent la construction de terriers en trois dimensions, peut-être plus conformes à ce qui peut être observé dans l'environnement. Par ailleurs, et indépendamment de la présence/absence du parasite *G. branchialis*, il convient de noter que les coefficients de biodiffusion et bioadvection obtenus dans le cadre de ces deux études diffèrent largement, ceux obtenus par L. Pascal étant très supérieurs aux coefficients déterminés présentement. De plus, nos expérimentations ont utilisé des colonnes sédimentaires d'environ 20 cm. Or *U.* cf. *pusilla* peut être à l'origine de terrier pouvant atteindre des profondeurs bien plus importantes (Dworschak, 1983; Pascal, 2017). De ce fait, il est possible que nos unités expérimentales aient contraint l'extension verticale des terriers des crevettes de vase, parasitées par *G. branchialis* ou non, *in fine* limitant le remaniement sédimentaire de ces organismes et, ce, indépendamment de la présence du parasite. Ainsi, il pourrait être judicieux de **réaliser des expérimentations utilisant des systèmes de plus grande envergure** de manière à tester cette hypothèse**.**

(ii) Contamination métallique et interaction avec le parasite

Suite aux résultats obtenus dans le cadre du **Chapitre IV** (Figure VI. 2), l'expérience menée dans le cadre du **Chapitre V-A** visait à tester l'hypothèse selon laquelle une contamination au Cd (apports *via* la colonne d'eau) pouvait réduire l'activité de bioturbation de *U.* cf. *pusilla*. Nos résultats montrent au

contraire que la contamination au Cd semble stimuler le remaniement sédimentaire de cette crevette de vase. Ces résultats sont relativement surprenants car cet élément métallique a souvent été associé à des réductions des niveaux d'activités des organismes contaminés (e.g., Roast *et al.*, 2000; Pestana *et al.*, 2007; Vellinger *et al.*, 2012). Nous suggérons que cette légère augmentation du remaniement sédimentaire de *U.* cf. *pusilla* pourrait traduire une réaction précoce de fuite (Michalec *et al.*, 2013). Ainsi, il pourrait être supposé que la concentration de Cd appliquée, ainsi que les niveaux accumulés, n'étaient pas toxiques au point de limiter l'activité de ce bioturbateur. Une contamination plus importante ou plus longue aurait, à l'inverse, pu conduire à une réduction des niveaux d'activités en lien avec une toxicité accrue de ce contaminant. Les évènements de contamination de l'environnement pouvant être ponctuels ou chroniques, **une exposition de plus longue durée de *U.* cf. *pusilla* aux contaminants** pourrait être envisagée de manière à tester cette hypothèse.

Contrairement aux organismes non parasités exposés au Cd, les individus parasités sont peu sensibles à cette contamination, les activités de remaniement sédimentaire étant similaires entre organismes parasités et organismes parasités soumis à la contamination. Ces résultats suggèrent ainsi que l'influence exercée par le parasite sur son hôte, bien que faible, soit supérieure à celle d'une contamination au Cd. Ces résultats ne sont pas forcément évidents. En effet, le parasite *G. branchialis* étant capable d'accumuler le Cd (**Chapitre V-B**), il aurait pu être envisagé que celui-ci, tout comme son hôte, ait été impacté par l'exposition à ce métal. De manière à mieux comprendre le rôle joué par *G. branchialis* sur son hôte dans le cas d'une contamination métallique, nous gagnerons à caractériser l'influence de contaminations métalliques sur le parasite lui-même. Néanmoins, puisque par définition le parasite est dépendant de son hôte (*U.* cf. *pusilla* est à la fois l'habitat de *G. branchialis* et sa ressource trophique), il n'est pas possible de séparer hôte et parasite. De manière plus réaliste, il pourrait toutefois être proposé **d'évaluer la capacité de *G. branchialis* à infester *U.* cf. *pusilla* dans une eau contaminée** (étude du comportement de nage de la larve cryptoniscus, de sa viabilité, etc.) ; des modifications du succès d'infestation pouvant moduler la prévalence du parasite dans la population, et *in fine* son impact écologique. Evidemment, de telles études nécessiteraient tout d'abord de caractériser le cycle de vie de *G. branchialis* et d'arriver à échantillonner des crevettes de vase suffisamment petites pour que le parasite puisse s'y fixer.

Finalement, il apparait essentiel de souligner que la sensibilité des organismes aux différents facteurs de stress (contamination métallique et parasitisme) dépend du contexte environnemental local et de leur histoire de vie (e.g., Klerks and Levinton, 1989; Bryan-Walker *et al.*, 2007; Paul-Pont *et al.*, 2010b). Ainsi, Paul-Pont *et al.* (2010b) ont montré que la réponse de palourdes japonaises *Ruditapes philippinarum* à une contamination au Cd menée en laboratoire dépendait de leur site d'origine: soumis à une contamination artificielle au Cd, des organismes collectés au niveau d'un site dont le sédiment était légèrement enrichi en cet élément ont montré de plus faibles accumulations de Cd au niveau de leurs branchies que celles observées chez des organismes provenant d'un site non contaminé. Par

ailleurs, il a été mis en évidence que les organismes naïfs n'étaient pas capables de lutter contre la contamination appliquée en laboratoire à l'inverse des individus ayant déjà été confrontés à une légère contamination métallique dans leur environnement. De manière similaire, les capacités de résistance des organismes à l'infestation parasitaire dépendent des niveaux de parasites auxquels les individus sont soumis dans leur milieu ; les organismes provenant de populations naïves accumulant plus de parasites lors d'infestations expérimentales que les individus provenant de populations expérimentant une plus forte pression parasitaire (adaptation locale) (Bryan-Walker *et al.*, 2007). En ce qui concerne *U.* cf. *pusilla*, les expérimentations ayant été menées sur des organismes naturellement infestés par *G. branchialis*, la question du contexte parasitaire local ne semble pas constituer un facteur confondant. A l'inverse, les expérimentations de contamination ont été réalisées sur des organismes collectés dans le bassin d'Arcachon, au sein duquel les niveaux de Cd dans les organismes et l'environnement sont très faibles (**Chapitre III-B**). Ainsi **nous avons testé la réponse d'organismes naïfs à une contamination métallique. Ces résultats pourraient être confrontés à ceux menés sur des individus expérimentant naturellement des concentrations en Cd plus élevées**. Pour ce faire, nous proposons d'utiliser des individus provenant de la région de Marennes-Oléron. En effet, du fait de sa situation géographique, la baie de Marennes-Oléron est sous influence de la pollution historique polymétallique (notamment en Cd et Zn) de l'estuaire de la Gironde (Boutier *et al.*, 2000). Il a ainsi été mis en évidence que les huîtres cultivées en baie de Marennes-Oléron présentent des niveaux de Cd plus élevés que ceux notés pour celles du bassin d'Arcachon (Morin *et al.*, 2016; Oger-Jeanneret *et al.*, 2016), mais sous la concentration de consommation autorisé (C = 1,0 mg kg^{-1} poids frais pour les mollusques) (EC No 1881/2006, amendement No 488/2014). Une courte campagne de prospection menée dans cette zone nous a permis de collecter des spécimens de *U.* cf. *pusilla* et de noter que ces derniers présentaient des concentrations en Cd supérieurs à ceux déterminés chez les organismes prélevés dans le bassin d'Arcachon (Figure VI. 4).

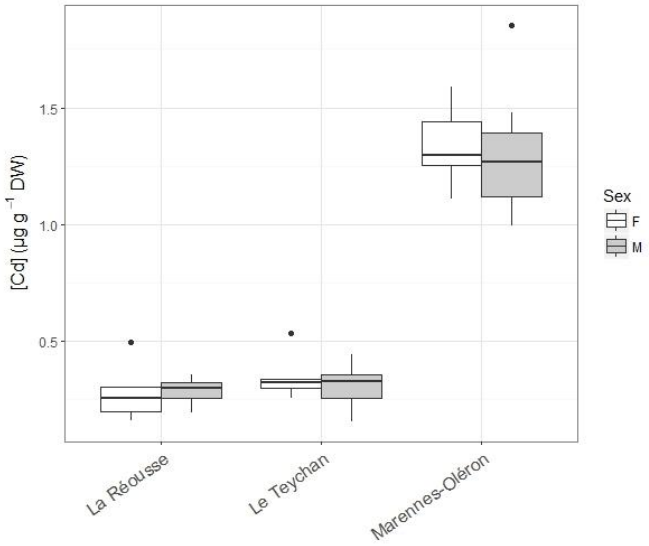


Figure VI. 4 Concentrations en cadmium Cd (µg g^{-1} poids sec (DW)) dans l'hépatopancréas de crevettes de vase *Upogebia* cf. *pusilla* males (« M ») et femelles (« F ») collectées en hiver sur deux sites dans le bassin d'Arcachon (« La Réousse » et « Le Teychan ») et sur un site à Marennes-Oléron. *N* = 5 femelles et 5 males pour les sites du bassin d'Arcachon et *N* = 7 femelles et 10 males pour Marennes-Oléron.

(iii) Et la bioirrigation?

Au cours de ces travaux nous nous sommes concentrés sur l'effet (1) du parasitisme, (2) d'une contamination métallique et (3) du parasitisme et d'une contamination métallique sur le remaniement sédimentaire de *U.* cf. *pusilla*. En revanche, l'effet de ces facteurs de stress n'a été évalué ni sur la bioirrigation ni sur le rôle joué par ce bioturbateur sur les flux biogéochimiques à l'interface eau-sédiment, seconde facette de la bioturbation. En ce qui concerne l'effet du parasitisme seul, ces expérimentations ont déjà été réalisées par L. Pascal (2017) au cours de ses travaux de thèse (dans des unités expérimentales similaires à celles utilisées dans le cadre de nos travaux) et ont montré une réduction des taux de bioirrigation des crevettes de vase parasitées, associée à une diminution du rôle de *U.* cf. *pusilla* dans les processus biogéochimiques. **L'influence d'une contamination au Cd, ainsi que de l'interaction entre contamination au Cd et parasitisme, sur les processus de bioirrigation restent néanmoins à être évaluées**. Par ailleurs, et considérant la large disparité entre les résultats de L. Pascal (2017) et ceux présentés concernant l'influence de *G. branchialis* sur le remaniement sédimentaire de *U.* cf. *pusilla*, il pourrait être intéressant de réitérer l'expérience « bioirrigation » menée par L. Pascal, de manière à **tester la reproductibilité des résultats** initialement obtenus.

c) Parasitisme, bioturbation et fonctionnement des écosystèmes

Bien que les expérimentations menées dans le cadre de ce travail de thèse aient évalué l'influence du parasite *G. branchialis* sur les capacités de bioturbation de *U.* cf. *pusilla*, **l'influence de ce parasite sur le rôle ingénieur de cette espèce, et particulièrement sur sa capacité à structurer les communautés benthiques (notamment de la méio- et de la macrofaune), reste encore à être évaluée.** En effet, la bioturbation des thalassinidés est globalement reconnue comme un facteur structurant majeur des communautés benthiques (Pillay and Branch, 2011), mais l'influence spécifique de *U.* cf. *pusilla* n'a, à notre connaissance, pas encore été évaluée. *A priori*, il semble difficile d'évaluer l'influence de cette crevette de vase sur les communautés benthiques en laboratoire, notamment du fait de la taille des systèmes expérimentaux requis. En revanche, ce type d'expérimentation pourrait être mené sur le terrain *via* la mise en place de **protocoles d'exclusion**, basés sur le modèle décrit par Pillay *et al.* (2007). L'objectif premier serait ainsi d'évaluer de manière globale le rôle d'une population de crevettes de vase (comprenant des organismes parasités et non-parasités par *G. branchialis*) sur la structure de ces communautés. Dans un second temps, il pourrait être envisagé de réaliser des **expériences d'inclusion de crevettes de vases (toutes parasitées par le bopyre vs. non-parasitées) de manière à quantifier l'influence du parasitisme**. Ces types d'expérimentations étant particulièrement lourdes, une première approche pourrait consister à réaliser des inventaires de la meio- et de la macrofaune dans un périmètre délimité autour des terriers de crevettes non parasitées et parasitées, le statut parasitaire des organismes étant déterminé *a posteriori*.

Dans le cadre de telles expérimentations en milieu naturel il n'est évidemment pas concevable de réaliser des contaminations métalliques et il apparait ainsi difficile d'appréhender à la fois l'influence

du parasitisme et de la contamination métallique sur le rôle joué par *U.* cf. *pusilla* en tant qu'ingénieur de l'écosystème. Toutefois, il pourrait être suggéré de réaliser ces expérimentations dans des **environnements présentant des concentrations en éléments métalliques contrastées,** tout en gardant à l'esprit la possible présence d'autres facteurs environnementaux confondants.

Pour conclure, cette étude a été l'occasion de tester l'influence de deux facteurs de stress sur une espèce bioturbatrice majeure du bassin d'Arcachon à l'échelle génétique, physiologique et comportementale. Tout d'abord, nos résultats montrent que des modifications à l'échelle moléculaire ne sont pas forcément extrapolables à l'échelle comportementale et soulignent ainsi l'intérêt des expérimentations prenant en compte différents niveaux de réponses aux facteurs de stress. Par ailleurs, cette étude confirme la complexité des interactions entre des facteurs de stress multiples, la réponse des organismes n'étant pas forcément une simple addition des effets observés en condition « simple stress ». Enfin, ces travaux soulignent l'intérêt de tester la reproductibilité des résultats obtenus *via* l'utilisation de systèmes expérimentaux variés.

ANNEXES

Influence of parasitism on bioturbation: from host to ecosystem functioning

Appendix 1 List of bioturbating species occurring in coastal environments. For each species, one study is quoted as an example of the influence of the organism in bioturbation processes.

Species	Class	Family	Bioturbation parameters estimated	References
ANNELIDA				
***Thalassodrilides* sp.**	Clitellata	Naididae	Influence on sediments geochemistry	(Ito *et al.*, 2016)
Melinna cristata	Polychaeta	Ampharetidae	Influence on sediments geochemistry	(Hannides *et al.*, 2005)
Melinna palmata	Polychaeta	Ampharetidae	Quantification of bioirrigation and sediment reworking	(Massé, 2014)
Abarenicola affinis	Polychaeta	Arenicolidae	Sediment reworking	(Goerlitz *et al.*, 2015)
Abarenicola pacifica	Polychaeta	Arenicolidae	Quantification of bioirrigation	(Waldbusser and Marinelli, 2006)
Arenicola marina	Polychaeta	Arenicolidae	Quantification of bioirrigation	(Delefosse *et al.*, 2015)
Maxmuelleria lankesteri	Polychaeta	Bonelliidae	Quantification of sediment reworking	(Hughes *et al.*, 1996)
Capitella* cf. *teleta	Polychaeta	Capitellidae	Influence on sediments geochemistry	(Ito *et al.*, 2016)
Heteromastus filiformis	Polychaeta	Capitellidae	Quantification of sediment reworking, influence on nutrients cycling	(Quintana *et al.*, 2007)
Heteromastus similis	Polychaeta	Capitellidae	Influence on nutrients cycling	(Figueiredo-Barros *et al.*, 2009)
Notomastus latericeus	Polychaeta	Capitellidae	Quantification of sediment reworking	(Giangrande *et al.*, 2001)
***Notomastus* sp.**	Polychaeta	Capitellidae	Influence on nutrients cycling	(Kikuchi, 1987)
Cirratulus grandis	Polychaeta	Cirratulidae	Observation of sediment transport, quantification of bioturbation rates	(Shull and Yasuda, 2001)
Cirriformia filigera	Polychaeta	Cirratulidae	Influence on nutrients cycling	(Banks *et al.*, 2013)
Timarete (Cirriformia) luxuriosa	Polychaeta	Cirratulidae	Influence on sediments geochemistry	(Zorn *et al.*, 2006)
Glycera alba	Polychaeta	Glyceridae	Influence on nutrients cycling	(Norling *et al.*, 2007)
Lumbrineris latreilli	Polychaeta	Lumbrineridae	Influence on nutrients cycling	(Casado-Coy *et al.*, 2017)
Axiothella rubrocincta	Polychaeta	Maldanidae	Quantification of sediment reworking	(Kudenov, 1982)
***Clymenella* sp.**	Polychaeta	Maldanidae	Influence on sediments geochemistry	(Zorn *et al.*, 2006)
Clymenella torquata	Polychaeta	Maldanidae	Quantification of sediments reworking	(Campbell and Lindsay, 2014)
Maldane sarsi	Polychaeta	Maldanidae	Influence on sediments geochemistry	(Dufour *et al.*, 2008)
Nephtys caeca	Polychaeta	Nephtyidae	Quantification of sediment reworking	(Piot *et al.*, 2008)
Nephtys hombergii	Polychaeta	Nephtyidae	Quantification of bioirrigation and sediment reworking	(Murray *et al.*, 2014)
Nephtys incisa	Polychaeta	Nephtyidae	Influence on sediments structure	(Michaud *et al.*, 2010)

Alitta virens	Polychaeta	Nereididae	Quantification of bioirrigation and sediment reworking	(Murray *et al.*, 2014)
Hediste (Neanthes) japonica	Polychaeta	Nereididae	Influence on nutrients cycling	(Kikuchi, 1987)
Hediste (Nereis) diversicolor	Polychaeta	Nereididae	Quantification of bioirrigation and sediment reworking	(Murray *et al.*, 2014)
Laeonereis culveri (acuta)	Polychaeta	Nereididae	Influence on sediments structure	(Palomo and Iribarne, 2000)
Perinereis aibuhitensis	Polychaeta	Nereididae	Quantification of sediment reworking	(Koo and Seo, 2017)
Perinereis nuntia	Polychaeta	Nereididae	Influence on sediments geochemistry	(Ito *et al.*, 2016)
Diopatra cuprea	Polychaeta	Onuphidae	Influence on sediments geochemistry	(Waldbusser and Marinelli, 2009)
Kinbergonuphis (Onuphis) jenneri	Polychaeta	Onuphidae	Quantification of bioirrigation, influence on nutrients cycling	(Waldbusser and Marinelli, 2009)
Naineris laevigata	Polychaeta	Orbiniidae	Quantification of sediment reworking	(Giangrande *et al.*, 2001)
Scoloplos armiger	Polychaeta	Orbiniidae	Quantification of bioirrigation, influence on sediments geochemistry	(Valdemarsen *et al.*, 2018)
Owenia fusiformis	Polychaeta	Oweniidae	Influence on sediments structure	(Noffke *et al.*, 2009)
Lipobranchius jeffreysii	Polychaeta	Scalibregmatidae	Quantification of sediment reworking	(Lindqvist *et al.*, 2016)
Scalibregma inflatum	Polychaeta	Scalibregmatidae	Quantification of sediment reworking	(Caradec *et al.*, 2004)
Marenzelleria arctia	Polychaeta	Spionidae	Influence on nutrients cycling	(Renz and Forster, 2014)
Marenzelleria neglecta	Polychaeta	Spionidae	Influence on nutrients cycling	(Norling *et al.*, 2007)
Marenzelleria viridis	Polychaeta	Spionidae	Influence on nutrients cycling, quantification of sediment reworking	(Quintana *et al.*, 2007)
Polydora ciliata	Polychaeta	Spionidae	Influence on nutrients cycling	(Bartoli *et al.*, 2009)
Pygospio elegans	Polychaeta	Spionidae	Influence on sediments structure	(Bolam and Fernandes, 2003)
Eupolymnia heterobranchia	Polychaeta	Terebellidae	Influence on nutrients cycling	(Marinelli, 1992)
Eupolymnia nebulosa	Polychaeta	Terebellidae	Observation of sediments transport	(Maire *et al.*, 2007a)
Lanice conchilega	Polychaeta	Terebellidae	Influence on nutrients cycling, quantification of sediment reworking	(Braeckman *et al.*, 2010)
Pectinaria gouldii	Polychaeta	Terebellidae	Influence on sediments resuspension	(Davis, 1993)
Urechis caupo	Polychaeta	Urechidae	Quantification of bioirrigation	(Osovitz and Julian, 2002)
ARTHROPODA				
Alpheus macellarius	Malacostraca	Alpheoidea	Influence on sediments geochemistry and structure	(Vonk *et al.*, 2008)
Calocaris templemani	Malacostraca	Axiidae	Quantification of bioirrigation and sediment reworking	(Gagnon *et al.*, 2013)
Corallianassa longiventris	Malacostraca	Axiidae	Influence on sediments geochemistry	(Abed-Navandi *et al.*, 2005)
Biffarius (Callianassa) filholi	Malacostraca	Callianassidae	Quantification of sediment reworking	(Berkenbusch and Rowden, 1999)

Biffarius arenosus	Malacostraca	Callianassidae	Influence on sediments geochemistry	(Bird *et al.*, 2000)
Callianassa subterranea	Malacostraca	Callianassidae	Quantification of sediment reworking	(Rowden *et al.*, 1998)
Callichirus (Callianassa) kraussi	Malacostraca	Callianassidae	Quantification of sediment reworking	(Branch and Pringle, 1987)
Callichirus islagrande	Malacostraca	Callianassidae	Influence on sediments geochemistry (burrow ventilation)	(Stanzel and Finelli, 2004)
Eucalliax panglaoensis	Malacostraca	Callianassidae	Defined as bioturbators	(Kneer *et al.*, 2013)
Glypturus (Callichirus) armatus	Malacostraca	Callianassidae	Quantification of sediment reworking	(de Vaugelas *et al.*, 1986)
Glypturus (Callichirus) laurae	Malacostraca	Callianassidae	Quantification of sediment reworking	(de Vaugelas and de Saint Laurent, 1984)
Lepidophthalmus bocourti	Malacostraca	Callianassidae	Quantification of sediment reworking, influence on nutrients cycling	(Nates and Felder, 1998)
Lepidophthalmus louisianensis	Malacostraca	Callianassidae	Influence on sediments geochemistry (burrow ventilation)	(Stanzel and Finelli, 2004)
Lepidophthalmus sinuensis	Malacostraca	Callianassidae	Quantification of sediment reworking, influence on nutrients cycling	(Nates and Felder, 1998)
Neotrypaea californiensis	Malacostraca	Callianassidae	Quantification of bioirrigation	(Waldbusser and Marinelli, 2006)
Pestarella (Callianassa) tyrrhena	Malacostraca	Callianassidae	Influence on sediments geochemistry	(Papaspyrou *et al.*, 2005)
Pestarella tyrrhena	Malacostraca	Callianassidae	Influence on nutrients cycling	(Papaspyrou *et al.*, 2004)
Sergio trilobata	Malacostraca	Callianassidae	Influence of bioturbation activity on contaminants' fate, quantification of sediment reworking	(Klerks *et al.*, 2007)
Trypaea australiensis	Malacostraca	Callianassidae	Influence on nutrients cycling, quantification of bioirrigation	(Webb and Eyre, 2004a)
Corophium arenarium	Malacostraca	Corophiidae	Influence on sediments structure	(Jones and Jago, 1993)
Corophium volutator	Malacostraca	Corophiidae	Influence on nutrients cycling, quantification of bioirrigation	(Mermillod-Blondin *et al.*, 2005)
Leptocheirus plumulosus	Malacostraca	Corophiidae	Influence on sediments geochemistry	(Chandler *et al.*, 2014)
Dotilla fenestrata	Malacostraca	Dotillidae	Influence on sediments geochemistry	(Bulcao and Hodgson, 2012)
Victoriopisa australiensis	Malacostraca	Eriopisidae	Influence on nutrients cycling	(Dunn *et al.*, 2009)
Heloecius cordiformis	Malacostraca	Helociidae	Influence on sediments structure	(Katrak and Bird, 2003)
Laomedia astacina	Malacostraca	Laomediidae	Influence on sediments geochemistry	(Koo and Koh, 2013)
Hemiplax hirtipes	Malacostraca	Macrophthalmidae	Quantification of sediment reworking	(Rowden, 2016)
Macrophthalmus japonicus	Malacostraca	Macrophthalmidae	Influence on sediments structure	(Tanaka *et al.*, 2017)
Mictyris longicarpus	Malacostraca	Mictyridae	Influence on nutrients cycling and sediments structure, quantification of bioirrigation	(Webb and Eyre, 2004b)

Sarsamphiascus (Amphiascus) tenuiremis	Malacostraca	Miraciidae	Influence on nutrients cycling	(Chandler *et al.*, 2014)
Austruca (Uca) annulipes	Malacostraca	Ocypodidae	Influence on sediments structure	(Bartolini *et al.*, 2009)
Austruca (Uca) triangularis	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Mokhtari *et al.*, 2016)
Cranuca (Uca) inversa	Malacostraca	Ocypodidae	Influence on sediments structure	(Bartolini *et al.*, 2009)
Gelasimus (Uca) vocans	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Kristensen and Alongi, 2006)
Leptuca (Uca) crenulata	Malacostraca	Ocypodidae	Influence on sediments geochemistry and structure	(Bertics and Ziebis, 2009)
Leptuca (Uca) cumulanta	Malacostraca	Ocypodidae	Quantification of sediment reworking	(Aschenbroich *et al.*, 2016)
Leptuca (Uca) leptodactyla	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Natálio *et al.*, 2017)
Leptuca (Uca) panacea	Malacostraca	Ocypodidae	Quantification of sediment reworking	(Franco *et al.*, 2018)
Leptuca (Uca) uruguayensis	Malacostraca	Ocypodidae	Quantification of sediment reworking, influence on sediments geochemistry	(Botto and Iribarne, 2000)
Minuca (Uca) longisignalis	Malacostraca	Ocypodidae	Quantification of sediment reworking	(Franco *et al.*, 2018)
Minuca (Uca) pugnax	Malacostraca	Ocypodidae	Influence on sediments structure	(Smith *et al.*, 2008)
Tubuca (Uca) forcipata	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Mokhtari *et al.*, 2016)
Tubuca (Uca) paradussumieri	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Mokhtari *et al.*, 2016)
Tubuca (Uca) rosea	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Mokhtari *et al.*, 2016)
Uca maracoani	Malacostraca	Ocypodidae	Influence on sediments geochemistry	(Araújo *et al.*, 2012)
Ucides cordatus	Malacostraca	Ocypodidae	Quantification of sediment reworking	(Aschenbroich *et al.*, 2016)
Hyas araneus	Malacostraca	Oregoniidae	Quantification of sediment reworking	(Solan *et al.*, 2004)
Monoporeia affinis	Malacostraca	Pontoporeiidae	Influence on nutrients cycling	(Karlson, 2007)
Pontoporeia femorata	Malacostraca	Pontoporeiidae	Quantification of sediment reworking	(Lopez and Elmgren, 1989)
Sesarma reticulatum	Malacostraca	Sesarmidae	Influence on sediments geochemistry	(Wilson *et al.*, 2012)
Sphaeroma quoianum	Malacostraca	Sphaeromatidae	Influence on sediments structure	
Neaxius acanthus	Malacostraca	Strahlaxiidae	Influence on sediments geochemistry and structure	(Vonk *et al.*, 2008)
Austinogebia edulis	Malacostraca	Upogebiidae	Influence on sediments geochemistry	(Das *et al.*, 2017)
Upogebia africana	Malacostraca	Upogebiidae	Influence on sediments geochemistry	(Tibbles *et al.*, 1994)
Upogebia deltaura	Malacostraca	Upogebiidae	Influence on sediments geochemistry	(Howe *et al.*, 2004)
Upogebia major	Malacostraca	Upogebiidae	Influence on sediments geochemistry	(Koo and Koh, 2013)
Upogebia pugettensis	Malacostraca	Upogebiidae	Quantification of bioirrigation	(Waldbusser and Marinelli, 2006)
Upogebia pusilla	Malacostraca	Upogebiidae	Influence on nutrients cycling	(Pascal *et al.*, 2016a)
Austrohelice crassa	Malacostraca	Varunidae	Influence on sediments structure	(Needham *et al.*, 2013)

Cyrtograpsus angulatus	Malacostraca	Varunidae	Influence on sediments structure	(Martinetto *et al.*, 2011)
Eriocheir sinensis	Malacostraca	Varunidae	Influence on sediments structure	(Rudnick *et al.*, 2005)
Helice formosensis	Malacostraca	Varunidae	Influence on sediments geochemistry	(Mchenga and Tsuchiya, 2008)
Helice tientsinensis	Malacostraca	Varunidae	Influence of bioturbation activity on contaminants' fate	(Qin *et al.*, 2010)
Hemigrapsus oregonensis	Malacostraca	Varunidae	Influence on sediments geochemistry	(Zorn *et al.*, 2006)
Neohelice (Chasmagnathus) granulata	Malacostraca	Varunidae	Quantification of sediment reworking, influence on sediments geochemistry	(Botto and Iribarne, 2000)
CEPHALOPRHYNCHA				
Halicryptus spinulosus	Priapulida	Priapulidae	Quantification of bioirrigation and sediment reworking	(Powilleit *et al.*, 2012)
ECHINODERMATA				
Brissopsis lyrifera	Echinoidea	Brissidae	Quantification of sediment reworking	(Lindqvist *et al.*, 2016)
Peronella lesueuri	Echinoidea	Laganidae	Influence on nutrients cycling	(Li *et al.*, 2013)
Echinocardium cordatum	Echinoidea	Loveniidae	Quantification of sediment reworking	(Caradec *et al.*, 2004)
Encope emarginata	Echinoidea	Mellitidae	Influence on sediments structure	(Brustolin *et al.*, 2016)
Abatus ingens	Echinoidea	Schizasteridae	Quantification of sediment reworking	(Thompson and Riddle, 2005)
Brisaster latifrons	Echinoidea	Schizasteridae	Sediment reworking trough vertical movement	(Nichols *et al.*, 1989)
Holothuria (Halodeima) atra	Holothuroidea	Holothuriidae	Quantification of sediment reworking	(Uthicke, 1999)
Holothuria (Microthele) whitmaei	Holothuroidea	Holothuriidae	Quantification of sediment reworking	(Shiell and Knott, 2010)
Holothuria princeps	Holothuroidea	Holothuriidae	Quantification of sediment reworking	(Dahlgren *et al.*, 1999)(
Holothuria tubulosa	Holothuroidea	Holothuriidae	Quantification of sediment reworking	(Coulon and Jangoux, 1993)
Molpadia oolitica	Holothuroidea	Molpadiidae	Influence on sediments structure	(Rhoads and Young, 1971)
Apostichopus (Stichopus) japonicus	Holothuroidea	Stichopodidae	Influence on sediments geochemistry	(Michio *et al.*, 2003)
Australostichopus mollis	Holothuroidea	Stichopodidae	Influence on nutrients cycling	(MacTavish *et al.*, 2012)
Stichopus chloronotus	Holothuroidea	Stichopodidae	Quantification of sediment reworking	(Uthicke, 1999)
Stichopus herrmanni	Holothuroidea	Stichopodidae	Influence on sediments structure, quantification of sediment reworking	(Wolfe and Byrne, 2017)
Oestergrenia (Labidoplax) digitata	Holothuroidea	Synaptidae	Quantification of sediment reworking	(Queirós *et al.*, 2015)
Amphiura chiajei	Ophiuroidea	Amphiuridea	Influence on nutrients cycling	(Norling *et al.*, 2007)
Amphiura filiformis	Ophiuroidea	Amphiuridea	Quantification of sediment reworking	(Caradec *et al.*, 2004)
HEMICHORDATA				

Saccoglossus bromophenolosus	Enteropneusta	Harrimaniidae	Influence on sediments geochemistry	(Zorn *et al.*, 2006)
Balanoglossus aurantiaca	Enteropneusta	Ptychoderidae	Influence on sediments geochemistry	(Waldbusser and Marinelli, 2009)
***Schizocardium* sp.**	Enteropneusta	Spengelidae	Influence on sediments geochemistry	(Furukawa *et al.*, 2001)
MOLLUSCA				
Cerastoderma edule	Bivalvia	Cardiidae	Influence on sediments geochemistry and nutrients cycling	(Mermillod-Blondin *et al.*, 2005)
Spisula subtruncata	Bivalvia	Mactridae	Influence on sediments geochemistry and nutrients cycling	(Sospedra *et al.*, 2017)
Mya arenaria	Bivalvia	Myidae	Influence on nutrients cycling	(Michaud *et al.*, 2006)
Nuculana (Nuculana) pernula	Bivalvia	Nuculanidae	Quantification of sediment reworking	(Lindqvist *et al.*, 2016)
Nuculana minuta	Bivalvia	Nuculanidae	Influence on nutrients cycling	(Olsgard *et al.*, 2008)
Nucula hanleyi	Bivalvia	Nuculidae	Influence on nutrients cycling	(Murray *et al.*, 2017)
Nucula nitidosa	Bivalvia	Nuculidae	Influence on nutrients cycling, quantification of bioirrigation and sediment reworking	(Wrede *et al.*, 2017)
Nucula proxima (annulata)	Bivalvia	Nuculidae	Influence on sediments structure	(Richardson and Young, 1980)
Ensis leei (arcuatus var. directus)	Bivalvia	Pharidae	Influence on sediments structure and sediment reworking	(Witbaard *et al.*, 2017)
Nuttallia japonica	Bivalvia	Psammobiidae	Influence on sediments geochemistry	(Kanaya, 2014)
Abra alba	Bivalvia	Semeliae	Influence on nutrients cycling	(Braeckman *et al.*, 2010)
Abra nitida	Bivalvia	Semeliae	Quantification of sediment reworking	(Caradec *et al.*, 2004)
Abra segmentum (ovata)	Bivalvia	Semeliae	Quantification of sediment reworking	(Trannum, 2017)
Scrobicularia plana	Bivalvia	Semeliae	Influence on sediments structure	(Orvain, 2005)
Theora lata	Bivalvia	Semeliae	Influence on nutrients cycling	(Yamada and Kayama, 1987)
Tagelus plebeius	Bivalvia	Solecurtidae	Influence on sediments geochemistry	(Alvarez *et al.*, 2015)
Fabulina fabula	Bivalvia	Tellinidae	Sediment reworking	(Kamp and Witte, 2005)
Limecola (Macoma) balthica	Bivalvia	Tellinidae	Influence on nutrients cycling	(Norling *et al.*, 2007)
Limecola (Macoma) contabulata	Bivalvia	Tellinidae	Influence on sediments geochemistry	(Kanaya, 2014)
Macoma nasuta	Bivalvia	Tellinidae	Quantification of bioirrigation	(Volkenborn *et al.*, 2012b)
Macomangulus (Angulus) tenuis	Bivalvia	Tellinidae	Quantification of bioirrigation and sediment reworking	(Murray *et al.*, 2014)
Macomona (Tellina) deltoidalis	Bivalvia	Tellinidae	Influence of bioturbation activity on contaminants' fate	(Atkinson *et al.*, 2007)
Macomona liliana	Bivalvia	Tellinidae	Quantification of bioirrigation	(Volkenborn *et al.*, 2012b)
Macoploma (Macoma) tenta	Bivalvia	Tellinidae	Influence on sediments resuspension	(Davis, 1993)

Austrovenus stutchburyi	Bivalvia	Veneridae	Influence on sediments structure and nutrients cycling	(Sandwell *et al.*, 2009)
Chamelea gallina	Bivalvia	Veneridae	Influence on nutrients cycling	(Murray *et al.*, 2017)
Cyclina sinensis	Bivalvia	Veneridae	Influence on nutrients cycling	(Nicholaus and Zheng, 2014)
Meretrix meretrix	Bivalvia	Veneridae	Influence on sediments geochemistry	(Shen *et al.*, 2016)
Polititapes aureus (Venerupis aurea)	Bivalvia	Veneridae	Quantification of sediment reworking	(François *et al.*, 1999)
Ruditapes decussatus	Bivalvia	Veneridae	Quantification of sediment reworking	(François *et al.*, 1999)
Ruditapes philippinarum	Bivalvia	Veneridae	Influence on sediments structure	(Sgro *et al.*, 2005)
Yoldia hyperborea	Bivalvia	Yoldiidae	Sediment reworking	(Stead and Thompson, 2006)
Yoldia limatula	Bivalvia	Yoldiidae	Influence on sediments structure	(Bender and Davis, 1984)
Batillaria flectosiphonata	Gastropoda	Batillariidae	Influence on sediments geochemistry	(Kamimura and Tsuchiya, 2006)
Batillaria zonalis	Gastropoda	Batillariidae	Influence on sediments geochemistry	(Kamimura and Tsuchiya, 2004)
Peringia (Hydrobia) ulvae	Gastropoda	Hydrobiidae	Influence on sediments structure	(Andersen *et al.*, 2002)
Tritia (Cyclope) neritea	Gastropoda	Nassariidae	Influence on sediments geochemistry	(Pischedda *et al.*, 2008)
Tritia (Ilyanassa) obsoleta	Gastropoda	Nassariidae	Influence on nutrients cycling	(Premo and Tyler, 2013)
Pirenella (Cerithideopsilla) cingulata	Gastropoda	Potamididae	Influence on sediments geochemistry	(Kamimura and Tsuchiya, 2004)
Terebralia palustris	Gastropoda	Potamididae	Sediment reworking	(Penha-Lopes *et al.*, 2010)
Turritella communis	Gastropoda	Turritellidae	Influence on nutrients cycling	(Murray *et al.*, 2017)
SIPUNCULA				
Sipunculus (Sipunculus) nudus	Sipunculidea	Sipunculidae	Influence on sediments geochemistry	(Li *et al.*, 2015)

Appendix 2 List of macroparasites documented in bioturbating species (identified in Appendix 1). When available, prevalence (percentage of infestation in the population), abundance (mean number of parasite per potential host) and/or intensity of infection (mean number of parasite per infested host) are given. The size of the samples are given as well. Potential impacts on the host are reported.

Bioturbating species	Parasite taxon	Phylum	Class	Form	Prevalence (%)	Abundance (mean or range)	Intensity of infection	N	Impact on host	Location	Reference
ANNELIDA											
Abarenicola affinis	Opecoelid E	Platyhelminthes	Trematoda	Metacercariae	46.8					New Zealand	(Peoples *et al.*, 2012)
Abarenicola pacifica											
Alitta virens	*Zoogonus rubellus*	Platyhelminthes	Trematoda	Metacercariae	50.6				Reduced survival and foraging activity	United States	(McCurdy and Moran, 2004)
Arenicola marina											
Axiothella rubrocincta											
Capitella cf. teleta											
***Capitella* spp.**											
Cirratulus grandis											
Cirriformia filigera											
***Clymenella* sp.**											
Clymenella torquata											
Diopatra cuprea	*Arabella* sp.	Annelida	Polychaeta							United States	(Allen, 1952)
Eupolymnia heterobranchia											
Eupolymnia nebulosa											
Glycera alba											
Hediste (Neanthes) japonica											

	Dichelyne minutus	Nematoda	Chromadorea		5	0.06	1.2	230		Russia	(Pronkina *et al.*, 2017)
Hediste (Nereis) diversicolor	*Dichelyne minutus*	Nematoda	Chromadorea							Denmark	(Køie, 2001)
	Parvatrema minutum (Gymnophallus nereicola)	Platyhelminthes	Trematoda	Metacercariae						France	(Bartoli, 1981)
Heteromastus filiformis	Opecoelid E	Platyhelminthes	Trematoda	Metacercariae	61.2	range = 0 to 15		71	May compromise the structural integrity of the host	New Zealand	(Peoples *et al.*, 2012)
Heteromastus similis											
Kinbergonuphis (Onuphis) jenneri											
Laeonereis culveri (acuta)											
Lanice conchilega											
Lipobranchius jeffreysii											
Lumbrineris latreilli											
Maldane sarsi											
Marenzelleria arctia											
Marenzelleria neglecta											
Marenzelleria viridis	*Himasthla quissetensis*	Platyhelminthes	Trematoda	Metacercariae	Overall: 76.8			33 and 125 (2 sampling times)		United States	(Phelan *et al.*, 2016)
	Lepocreadium setiferoides	Platyhelminthes	Trematoda	Metacercariae							
	Zoogonus lasius	Platyhelminthes	Trematoda	Metacercariae							
Maxmuelleria lankesteri											
Melinna cristata	*Melinnacheres ergasiloides*	Arthropoda	Hexanauplia							Sweden	(Bresciani and Lützen, 1974)
Melinna palmata											

Naineris laevigata										
Nephtys caeca										
Nephtys hombergii										
Nephtys incisa										
Notomastus latericeus										
***Notomastus* sp.**										
Owenia fusiformis										
Pectinaria gouldii										
Perinereis aibuhitensis										
Perinereis nuntia										
Polydora ciliata										
Pygospio elegans	*Lepocreadium setiferoides*	Platyhelminthes	Trematoda	Metacercariae					United States	(McCurdy, 2001)
Scalibregma inflatum										
Scoloplos armiger										
***Thalassodrilides* sp.**										
Timarete (Cirriformia) luxuriosa										
Urechis caupo										
ARTHROPODA										
***Alpheus macellarius*[1]**										
Austinogebia edulis										
***Austrohelice crassa*[1]**	*Microphallus* sp.	Platyhelminthes	Trematoda	Metacercariae	90.0	26 ± 5.8	50		New Zealand	(Koehler and Poulin, 2010)
	Ascarophis sp.	Nematoda	Chromadorea		64.0	3.6 ± 1.0				
	Portunion sp.	Arthropoda	Malacostraca		2.0					
Austruca (Uca) annulipes										

Austruca (Uca) triangularis										
Biffarius arenosus										
Biffarius (Callianassa) filholi										
Callianassa subterranea	*Ione thoracica*	Arthropoda	Malacostraca						Ireland	(Astall *et al.*, 1996)
	Pseudione callianassae	Arthropoda	Malacostraca						United Kingdom	
	Pseudione borealis	Arthropoda	Malacostraca						France	
Callichirus (Callianassa) kraussi										
Callichirus islagrande										
Calocaris templemani										
Corallianassa longiventris										
Corophium arenarium	*Microphallus claviformis*	Platyhelminthes	Trematoda	Metacercariae				Increase of mortality	Denmark	(Jensen *et al.*, 1998)
Corophium volutator	*Microphallus claviformis*	Platyhelminthes	Trematoda	Metacercariae				Increase of mortality		
	Maritrema subdolum	Platyhelminthes	Trematoda	Metacercariae	6.5 to 48		100 per sample and site (16 samples, 2 sites)		Germany	(Meißner and Bick, 1997)
	Levinseniella brachysoma	Platyhelminthes	Trematoda	Metacercariae	0.5 to 28					
	Microphallus claviformis	Platyhelminthes	Trematoda	Metacercariae	75 to 100	1.92 ± 0.38 to 15.8 ± 2.32	76 and 102 (2 sampling)	Could contribute to *C. volutator* emergence at the sediment surface	Denmark	(Damsgaard *et al.*, 2005)
	Maritrema subdolum + Microphallidae sp. no. 15	Platyhelminthes	Trematoda	Metacercariae	41 to 96	0.5 ± 0.11 to 3.19 ± 0.33				
	Levinseniella sp.	Platyhelminthes	Trematoda	Metacercariae	11 to 67	0.19 ± 0.10 to 1.44 ± 0.20				
	Gynaecotyla adunca	Platyhelminthes	Trematoda	Metacercariae					Canada	(Shim *et al.*, 2013)
	Skrjabinoclava morrisoni	Nematoda	Chromadorea		0 to 12.5		24 to 484 (23 sampling times)	Increase the emergence of *C. volutator* at the sediment surface	Canada	(McCurdy, 1999)

Cranuca (Uca) inversa									
Cyrtograpsus angulatus	*Neocancrincola platensis*	Arthropoda	Hexanauplia		8	65		Argentina	(Alda *et al.*, 2011)
	Ascarophis spp.	Nematoda	Chromadorea		8				
	Acuariinae gen. sp.	Nematoda	Chromadorea		6				
	Profilicollis chasmagnathi	Acanthocephala	Palaeacanthocephala		20				
	undefined Cestoda, order Cyclophyllidea	Platyhelminthes	Cestoda		8				
	Maritrema bonaerense	Platyhelminthes	Trematoda	Metacercariae	94				
	Maritrema cf. *orensense*	Platyhelminthes	Trematoda	Metacercariae	70				
	Levinseniella cruzi	Platyhelminthes	Trematoda	Metacercariae	14				
	Odhneria sp.	Arthropoda	Malacostraca		59				
	Microphallus szidati	Platyhelminthes	Trematoda	Metacercariae	87 to 97	21 to 37 (3 sites)		Argentina	(Mendez Casariego *et al.*, 2015)
	Maritrema bonaerense	Platyhelminthes	Trematoda	Metacercariae	32 to 53				
	Profilicollis chasmagnathi	Acanthocephala	Palaeacanthocephala		60 to 68				
Dotilla fenestrata									
***Eriocheir sinensis*[1]**	*Polyascus gregaria*	Arthropoda	Hexanauplia		10.3 to 55.1	89 to 439 (5 sampling times)	Castration, anecdysis and increase of mortality	China	(Li *et al.*, 2011)
Eucalliax panglaoensis									
Gelasimus (Uca) vocans									
Glypturus (Callichirus) armatus									
Glypturus (Callichirus) laurae									
***Helice formosensis*[1]**									
***Helice tientsinensis*[1]**									
Heloecius cordiformis									

	Ascarophis spp.	Nematoda	Chromadorea	1.8 to 75		1 to 8	4 to 150 (monthly samples over one year)		Canada	(Poinar and Kuris, 1975)
Hemigrapsus oregonensis	*Portunion conformis*	Arthropoda	Malacostraca	20.7 to 91.3	range= 0 to more than 5		34 to 97 per sampling site (22 sites)	Female castration	Canada	(Kuris *et al.*, 1980)
	Carcinonemertes epialti	Nemerta	Enopla	0 to 83	0 to 6				United States	(Shields, 1993)
	Profilicollis botulus	Acanthocephala	Palaeacanthocephala	9 to 62	1.3 to 2.4 (intensity)		42 and 692 (2 sampling times)		Canada	(Ching, 1989)
Hemiplax hirtipes										
Hyas araneus										
Laomedia astacina										
Lepidophthalmus bocourti										
Lepidophthalmus louisianensis										
Lepidophthalmus sinuensis										
Leptocheirus plumulosus										
Leptuca (Uca) crenulata										
Leptuca (Uca) cumulanta										
Leptuca (Uca) leptodactyla										
Leptuca (Uca) panacea										
Leptuca (Uca) uruguayensis										
Macrophthalmus japonicus										
Mictyris longicarpus										
Minuca (Uca) longisignalis										

Minuca (Uca) pugnax											
Monoporeia affinis	*Echinorhynchus salmonis*	Acanthocephala	Palaeacantho-cephala		0 to 1.4	1 ± 0		33 to 8084 (17 sites)		Sweden, Finland	(Benesh *et al.*, 2015)
Neaxius acanthus											
***Neohelice (Chasmagnathus) granulata*[1]**	*Microphallus szidati*	Platyhelminthes	Trematoda	Metacercariae	90.1		60.8 ± 13.2	81	Increase of glycogen content in the hepatopancreas, sometimes associated with haemocytic infiltration	Brazil	(Robaldo *et al.*, 1999)
	Neocancrincola platensis	Arthropoda	Hexanauplia		21			36		Argentina	(Alda *et al.*, 2011)
	Ascarophis spp.	Nematoda	Chromadorea		3 to 4						
	Profilicollis chasmagnathi	Acanthocephala	Palaeacantho-cephala		47						
	Maritrema bonaerense	Platyhelminthes	Trematoda		100						
	Maritrema cf. *orensensis*	Platyhelminthes	Trematoda		70						
	Levinseniella cruzi	Platyhelminthes	Trematoda		11						
	Odhneria sp.	Arthropoda	Malacostraca		53						
	Acuariinae gen. sp.	Nematoda	Rhabditida		3						
	Profilicolis chasmagnathi	Acanthocephala	Palaeacanthoce phala		42.4 to 52.8		0.69 to 0.88	142-145 (4 samples)		Argentina	(La Sala *et al.*, 2012)
Neotrypaea californiensis											
Pestarella (Callianassa) tyrrhena											
Pontoporeia femorata											
Sarsamphiascus (Amphiascus) tenuiremis											
Sergio trilobata											
***Sesarma reticulatum*[1]**											
Sphaeroma quoianum											
Trypaea australiensis											

Tubuca (Uca) forcipata										
Tubuca (Uca) paradussumieri										
Tubuca (Uca) rosea										
Uca maracoani										
Ucides cordatus[1]	*Leidya distorta*	Arthropoda	Malacostraca		0.5 to 2.6		40 -196 (3 sites)		Brazil, Venezuela	(Wunderlich *et al.*, 2017)
Upogebia africana	*Progebiophilus kensleyi*	Arthropoda	Malacostraca			1 couple			South Africa	(Markham, 2005)
Upogebia deltaura	*Gyge branchialis*	Arthropoda	Malacostraca			1 couple			United Kingdom	(Astall *et al.*, 1996)
Upogebia major										
Upogebia pugettensis	*Orthione griffenis*	Arthropoda	Malacostraca		27-75	1 couple	100 (5 sites)	Weight loss	United States	[72, 159]
Upogebia pusilla	*Progebiophilus euxinicus*	Arthropoda	Malacostraca			1 couple			France	
	Gyge branchialis	Arthropoda	Malacostraca		6 to 26	1 couple	50 per sampling times and sites (12 sampling times, 10 sites)	Reduced burrowing and ventilation activities associated with lower sediment reworking and bioirrigation rates and lower exchanges of solutes at the sediment-water interface	France	[160, 161]
	Gyge branchialis	Arthropoda	Malacostraca		1.3 to 6.6				Italia	(Dworschak, 1988)
	Unknown Metacercariae	Platyhelminthes	Trematoda	Metacercariae	5 to 100					
	Arhythmorhynchus sp.	Acanthocephla	Palaeacantho-cephala		0 to 28.4					
	Maritrema sp.	Platyhelminthes	Trematoda	Metacercariae	26.7 to 96.6	0 to > 1000	40-121 per sampling time and site (24 sampling times, 10 sites)		France	(Dairain *et al.*, 2017)

Victoriopisa australiensis							
CEPHALORHYNCHA							
Halicryptus spinulosus							
ECHINODERMATA							
Abatus ingens[1]							
Amphiura chiajei							
Amphiura filiformis							
Apostichopus (Stichopus) japonicas[1]							
Australostichopus mollis							
Brisaster latifrons[1]							
Brissopsis lyrifera							
Echinocardium cordatum[1]							
Encope emarginata[1]	*Dissodactylus crinitichelis*	Arthropoda	Malacostraca	87	213	Brazil	(Martinelli Filho *et al.*, 2014)
Holothuria (Halodeima) atra[1]	*Megadenus atrae*	Mollusca	Gastropoda	0 to 10.0	25 to 90 per sampling site (7 sites)	Japan	(Takano *et al.*, 2017)
Holothuria princeps[1]							
Holothuria tubulosa[1]							
Holothuria (Microthele) whitmaei[1]							
Molpadia oolitica[1]							
Oestergrenia (Labidoplax) digitata							
Peronella lesueuri[1]							

***Stichopus chloronotus*[1]**										
***Stichopus herrmanni*[1]**										
HEMICHORDATA										
Balanoglossus aurantiaca										
Saccoglossus bromophenolosus										
***Schizocardium* sp.**										
MOLLUSCA										
Abra alba	*Prosorhynchoides (Bucephaloides) gracilescens*	Platyhelminthes	Trematoda	Sporocyst				Castration, alterations in lysosomal functions	Scotland	(Johnston *et al.*, 1982)
Abra nitida										
Abra segmentum (ovata)	*Paratimonia gobii*	Platyhelminthes	Trematoda	Sporocyst, Metacercariae	0.5				France	(Maillard, 1975)
	Parvatrema (Gymnophallus) rebecqui	Platyhelminthes	Trematoda	Metacercariae					France	(Bartoli, 1983)
	Parvatrema minutum (Gymnophallus nereicola)	Platyhelminthes	Trematoda	Sporocyst, Metacercariae	1 to 40 %				France	(Bartoli, 1981)
***Austrovenus stutchburyi*[1]**	*Curtuteria australis*	Platyhelminthes	Trematoda	Metacercariae	8.6	0.1		70	Reduced shell growth rate, decrease of soft tissu dry weight and body condition	New Zealand	(O'Connell-Milne *et al.*, 2016)
	Echinostome parasites (*Curtuteria* spp. and *Acanthoparyphium* spp.)	Platyhelminthes	Trematoda	Metacercariae	29.2 to 100	min range = 0 to 2; max range = 1 to 2260		10 for each site (17 sites)		New Zealand	(Studer and Poulin, 2013)
	Gymnophallus sp.	Platyhelminthes	Trematoda	Metacercariae	100	46 (range = 23-126)		15		New Zealand	(Leung *et al.*, 2009)
	Pseudomyicola spinosus	Arthropoda	Hexanauplia		4		1	500		New Zealand	(Leung and Poulin, 2007)

	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae				Reduced ability to burry	New Zealand	(Mouritsen and Poulin, 2005)
	Acanthoparyphium sp.	Platyhelminthes	Trematoda	Metacercariae						
	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae				Reduced ability to burry	New Zealand	(Mouritsen, 2004)
	Boccardia acus	Annelida	Polychaeta							
	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae				Reduced the ability of the cockle's foot to contract	New Zealand	(Mouritsen, 2002a)
	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae		73.3 to 402.6 (mean)	72 to 79 (4 sites)		New Zealand	(Poulin *et al.*, 2000)
	Parvatrema (Meiogymnophallus) sp.	Platyhelminthes	Trematoda	Metacercariae		20.1 to 85.3 (mean)				
	Pseudomyicola spinosus	Arthropoda	Hexanauplia		5.1 to 5.6					
	Cercaria pectinata	Platyhelminthes	Trematoda	Sporocyst	0 to 5.4			Castration		
	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae				Organisms unable to burry	New Zealand	(Thomas *et al.*, 1998)
Batillaria flectosiphonata										
Batillaria zonalis										
***Cerastoderma edule*[1]**	*Mytilicola orientalis*	Arthropoda	Hexanauplia		0 to 13	1.2 ± 0.1	1 to 145 (14 sites)		The Netherlands	(Goedknegt *et al.*, 2017)
	Bucephalus minimus	Platyhelminthes	Trematoda	Sporocyst	5 ± 8		15 per site (28 sites)		Portugal	(Freitas *et al.*, 2014)
	Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae	54 ± 40	4 ± 3				
	Himasthla continua	Platyhelminthes	Trematoda	Metacercariae	32 ± 26	1 ± 2				
	Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae	48 ± 43	6 ± 10				
	Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	30 ± 34	3 ± 6				
	Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae	32 ± 26	2 ± 2				
	Monorchis parvus	Platyhelminthes	Trematoda	Sporocyst, Metacercariae	0.2 ± 1					
	Parvatrema fossarum	Platyhelminthes	Trematoda	Metacercariae	4 ± 9	1 ± 1				
	Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	52 ± 34	172 ± 398				

Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	4 ± 7		0.2 ± 1			
Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	3 ± 5		1 ± 1			
Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae	10 to 100		1 ± 0 to 152 ± 70	50 per site (14 sites)		Ireland	(Fermer *et al.*, 2011)
Himasthla continua	Platyhelminthes	Trematoda	Metacercariae	0 to 100		1 ± 1 to 16 ± 11				
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	0 to 35		1 ± 0 to 2 ± 1				
Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	0 to 30		1 ± 0 to 9 ± 11				
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	0 to 50		1 ± 0 to 6 ± 4				
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	76 to 100		8 ± 7 to 1080 ± 511				
Bucephalus minimus (Labratrema minimus)	Platyhelminthes	Trematoda	Sporocyst	0 to 6						
Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst, Metacercariae	0 to 5						
Mytilicola intestinalis	Arthropoda	Hexanauplia		10 to 90		1 ± 0 to 5 ± 2				
Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae	0.2	1.4		10 to 20 monthly over 2 years	Increase of mortality	Morocco	(Gam *et al.*, 2009)
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	0	0.2					
Curtuteria arguinae	Platyhelminthes	Trematoda	Metacercariae	4.1	23.7					
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	91.9	537.1					
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	0	0.1					
Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae	0.2	1.1					
Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae	0.7	4.1		10 to 20 monthly over 2 years	Increase of mortality	France	
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	7.0	41					
Curtuteria arguinae	Platyhelminthes	Trematoda	Metacercariae	0.9	5.1					
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	90.7	530.5					
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	0.5	3.1					

Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae	0.2	1				
Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae		2.7				
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae		41.3 to 88.8		10 per sampling time (2 sampling time)		Spain	(Dang *et al.*, 2009)
Parvatrema fossarum (Meiogymnophallus fossarum)	Platyhelminthes	Trematoda	Metacercariae		21.5 to 139.9				
Curtuteria arguinae	Platyhelminthes	Trematoda	Metacercariae		1.6 to 35				
Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae		0 to 9.3				
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae		0 to 0.5				
Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae		0.1 o 2.3				
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae		163.4 to 2016		30 per site (6 sites)		Morocco	(Gam *et al.*, 2008)
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae		0 to 0.1					
Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst	0 to 7				Castration		
Bucephalus minimus	Platyhelminthes	Trematoda	Sporocyst	0 to 7				Castration		
Monorchis parvus	Platyhelminthes	Trematoda	Sporocyst	0 to 3				Castration		
Paravortex cardii	Platyhelminthes	Rhabditophora			3 to 17					
Pinnotheres pisum	Arthropoda	Malacostraca			10 to 70					
Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae	85.7 ± 21.4		20.5 ± 7.2				
Himasthla continua	Platyhelminthes	Trematoda	Metacercariae	72.3 ± 34.7		15.8 ± 25.1				
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	84.2 ± 38.8		43.7 ± 20.8				
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	77.6 ± 38.4		5.8 ± 2.8	20 to 54 (4 sites)		Germany	(Thieltges *et al.*, 2006)
Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	95.7 ± 6.6		51.8 ± 49.9				
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	22.3 ± 28.6		1.7 ± 0.8				
Gymnophallus gibberosus	Platyhelminthes	Trematoda	Metacercariae	64.4 ± 37.8		5.7 ± 5.2				
Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst	5.2 ± 3.9						
Monorchis parvus	Platyhelminthes	Trematoda	Sporocyst	0.5 ± 1.3						

Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst	7.4 to 71	156 and 240 (2 types of cockles)	Castration, surfacing cockles with higher prevalence of parasite, increased mortality in surfacing cockles	Germany	(Thieltges, 2006)
Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae	87.7	1516		Portugal	(Russell-Pinto *et al.*, 2006)
Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae	78.5				
Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae	26.9				
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	4.2				
Curtuteria sp.	Platyhelminthes	Trematoda	Metacercariae	2.6				
Bucephalus minimus	Platyhelminthes	Trematoda	Sporocyst	2.3				
Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst, Cercariae, Metacercariae	2.2				
Parvatrema minutum	Platyhelminthes	Trematoda	Metacercariae	93.3				
Parvatrema (Meiogymnophallus) fossarum	Platyhelminthes	Trematoda	Metacercariae	83.5				
Monorchis parvus	Platyhelminthes	Trematoda	Sporocyst, Cercariae, Metacercariae	1.0				
Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	8.4				
Bucephalus minimus	Platyhelminthes	Trematoda	Sporocyst			Could contribute to cockle emergence at the sediment surface	France	(Desclaux *et al.*, 2002)
Himasthla quissetensis	Platyhelminthes	Trematoda	Metacercariae			Could contribute to cockle emergence at the sediment surface		
Paravortex cardii	Platyhelminthes	Rhabditophora		0 to 57			Spain	

Ciliates (*Trichodina* sp. + *Rynchodida*-like)				0 to 55		30 per site (34 sites)			(Carballal *et al.*, 2001)
Mytilicola-like	Arthropoda	Hexanauplia		0 to 7			Metaplasia of intestinal epithelial cells		
Gymnophallus choledochus	Platyhelminthes	Trematoda	Sporocyst, Cercariae, Metacercariae	0.2		453		France	(de Montaudouin *et al.*, 2000)
Bucephalus minumus	Platyhelminthes	Trematoda	Sporocyst	2.6					
Monorchis parvus	Platyhelminthes	Trematoda	Sporocyst, Cercariae, Metacercariae	0.2					
Himasthla continua	Platyhelminthes	Trematoda	Metacercariae	47.9	0 to 15				
Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae	0.4					
Himasthla interrupta	Platyhelminthes	Trematoda	Metacercariae	37.5	0 to 30				
Parvatrema (Meiogymnophallus) fossarum	Platyhelminthes	Trematoda	Metacercariae	0					
Parvatrema (Meiogymnophallus) minutum	Platyhelminthes	Trematoda	Metacercariae	59.4					
Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	11.5	0 to 3				
Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	18.8	0 to 6				
Paravortex cardii	Platyhelminthes	Rhabditophora	Metacercariae	30.5	1				
Mytilicola intestinalis	Arthropoda	Hexanauplia		5.1					
unindentified cestode	Platyhelminthes	Cestoda		0.4					
Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae		24 ± 17	220	Reduced ability of cockles to deal with hypoxia	Denmark	(Wegeberg and Jensen, 1999)
Monorchis parvus (Cercaria cerastodermae I)	Platyhelminthes	Trematoda	Sporocyst, Cercariae, Metacercariae	17 to 81 %		147	Castration; reduced muscular tissue in the foot, reduced ability to close the valves	Sweden	(Jonsson and Andé, 1992)

	Paravortex karlingi	Platyhelminthes	Rhabditophora						Scotland	(Pike and Burt, 1981)
	Malacobdella grossa	Nemerta	Enopla		2 to 96.2	0 to 2		77 to 210 (7 sites)	Scotland	(Jones *et al.*, 1979)
	Himasthla elongata	Platyhelminthes	Trematoda	Metacercariae			8.3 ± 0.9	Modifications of genes expression, induction of oxidative burst, increase of the concentration of circulating haemocytes		(Paul-Pont *et al.*, 2010a)
Chamelea gallina										
***Cyclina sinensis*[1]**	*Himasthla alincia*	Platyhelminthes	Trematoda	Metacercariae	100	57.8		20	South Korea	(Han *et al.*, 2009)
***Ensis leei (arcuatus var. directus)* [1]**	Undefined Turbellaria	Platyhelminthes			0 to 7.7			135 to 165 (3 sites)	Spain	(Darriba *et al.*, 2010)
	Undefined trematoda, fam. Bucephallidae	Platyhelminthes	Trematoda	Sporocyst, Cercariae	0 to 3.2					
	Undefined trematoda	Platyhelminthes	Trematoda	Metacercariae	0 to 13.3					
	Cestode-like larvae	Platyhelminthes	Cestoda		0 to 3.7					
***Fabulina fabula*[1]**										
***Limecola (Macoma) balthica*[1]**	*Himasthla elongata*	Platyhelminthes	Trematoda	Metacercariae	7.4 ± 2.1		1.2 ± 0.2	16 to 96 per site (4 sites)	Germany	(Thieltges *et al.*, 2006)
	Himasthla continua	Platyhelminthes	Trematoda	Metacercariae	6.8 ± 8.9		1 ± 0			
	Himsthla interrupta	Platyhelminthes	Trematoda	Metacercariae	2.9 ± 3.3		1 ± 0			
	Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	4.3 ± 5.2		1 ± 0			
	Renicola roscovita	Platyhelminthes	Trematoda	Metacercariae	2.9 ± 3.3		1 ± 0			
	Gymnophallus gibberosus	Platyhelminthes	Trematoda	Metacercariae	43.9 ± 13.6		3.2 ± 1.2			
	Gymnophalloides (Lacunovermis) macomae	Platyhelminthes	Trematoda	Metacercariae	10.1 ± 6.8		1.2 ± 0.2			
	Parvatrema affine	Platyhelminthes	Trematoda	Sporocyst	3.1 ± 6.3					
	Trematoda, fam. Gymnophallidae	Platyhelminthes	Trematoda	Metacercariae	8 to 56 %			50 to 78 per site (6 sites)	Russia	(Gantsevich *et al.*, 2016)

								deformation of the hinge plate		
	Mytilicola orientalis	Arthropoda	Hexanauplia		6 to 7 %	1 ± 0	14 to 116 per site (11 sites)		The Netherlands	(Goedknegt *et al.*, 2017)
***Limecola (Macoma) contabulata*[1]**										
Macomangulus (Angulus) tenuis										
***Macoma nasuta*[1]**										
Macomona (Tellina) deltoidalis	*Curtuteria australis*	Platyhelminthes	Trematoda	Metacercariae	Overall: range = 0 to 288, mean = 20.4		196		New Zealand	(Leung and Poulin, 2008)
	Acanthoparyphium sp.	Platyhelminthes	Trematoda	Metacercariae						
***Macomona liliana*[1]**										
Macoploma (Macoma) tenta										
***Meretrix meretrix*[1]**	*Conchyliurus bombasticus*	Arthropoda	Hexanauplia						Thailand	(Ho and Kim, 1995)
	Ostrincola portonoviensis	Arthropoda	Hexanauplia							
	Lichomolgus similis	Arthropoda	Hexanauplia							
***Mya arenaria*[1]**										
Nucula hanleyi										
Nucula nitidosa										
Nucula proxima (annulata)										
Nuculana (Nuculana) pernula										
Nuculana minuta										
Nuttallia japonica										
Peringia (Hydrobia) ulvae	*Asymphylodora demeli*	Platyhelminthes	Trematoda						Baltic Sea	(Thieltges *et al.*, 2009)
	Cryptogonimidae sp.	Platyhelminthes	Trematoda						North Sea	

Aphalloides coelomicola (timmi)	Platyhelminthes	Trematoda	Baltic Sea
Cercaria sinitzini	Platyhelminthes	Trematoda	Baltic Sea, South European Atlantic Shelf
Bunocotyle progenetica	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Bunocotyle cingulata	Platyhelminthes	Trematoda	Baltic Sea
Haploporidae sp. no. 3	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Haploporidae sp. no. 4	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Haploporidae sp. no. 6	Platyhelminthes	Trematoda	South European Atlantic Shelf
Haploporus benedeni	Platyhelminthes	Trematoda	South European Atlantic Shelf
Haploporidae sp. no. 7	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Deropristis inflata	Platyhelminthes	Trematoda	North Sea
Himasthla continua	Platyhelminthes	Trematoda	Baltic Sea, North Sea, Celtic Sea

Himasthla interrupta	Platyhelminthes	Trematoda	Baltic Sea,North Sea, Celtic Sea and South European Atlantic Shelf
Echinostomatidae sp. no. 8	Platyhelminthes	Trematoda	North Sea
Echinostomatidae sp. no. 9	Platyhelminthes	Trematoda	South European Atlantic Shelf
Psilochasmus aglyptorchis	Platyhelminthes	Trematoda	North Sea
Psilochasmus oxyurus	Platyhelminthes	Trematoda	Baltic Sea
Psilostomum brevicolle	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Cryptocotyle jejuna	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Cryptocotyle lingua	Platyhelminthes	Trematoda	Baltic Sea
Cryptocotyle concava	Platyhelminthes	Trematoda	Baltic Sea,North Sea, Celtic Sea and South European Atlantic Shelf
Pygidiopsis ardae	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf

Paramonostomum alveatum	Platyhelminthes	Trematoda	Baltic Sea
Notocotylid sp. no. 10	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Notocotylid sp. no. 11	Platyhelminthes	Trematoda	North Sea, Celtic Sea
Notocotylid sp. no. 12	Platyhelminthes	Trematoda	North Sea, Celtic Sea
Cercaria microphallidarum	Platyhelminthes	Trematoda	Baltic Sea
Microphalllus claviformis	Platyhelminthes	Trematoda	Baltic Sea,North Sea, Celtic Sea and South European Atlantic Shelf
Maritrema subdolum	Platyhelminthes	Trematoda	Baltic Sea,North Sea, Celtic Sea and South European Atlantic Shelf
Microphallid sp. no. 15	Platyhelminthes	Trematoda	North Sea, Celtic Sea and South European Atlantic Shelf
Microphallid sp. no. 16	Platyhelminthes	Trematoda	South European Atlantic Shelf
Maritrema oocysta	Platyhelminthes	Trematoda	Noth Sea, Celtic Sea
Microphallus pirum	Platyhelminthes	Trematoda	North Sea
Microphallus abortivus	Platyhelminthes	Trematoda	North Sea, Celtic Sea

				and South European Atlantic Shelf	
Levinseniella sp. no. 17	Platyhelminthes	Trematoda		North Sea, Celtic Sea and South European Atlantic Shelf	
Microphallus primas	Platyhelminthes	Trematoda		Noth Sea, Celtic Sea	
Microphallus scolectroma	Platyhelminthes	Trematoda		North Sea	
Microphallus papillorobustus	Platyhelminthes	Trematoda		Baltic Sea	
Levinseniella brachysoma	Platyhelminthes	Trematoda		North Sea, Celtic Sea	
Cercaria camarguensis	Platyhelminthes	Trematoda		North Sea and South European Atlantic Shelf	
Levinseniella sp.	Platyhelminthes	Trematoda		Baltic Sea	
Timoniella praeterita	Platyhelminthes	Trematoda		North Sea, Celtic Sea and South European Atlantic Shelf	
Timoniella imbutiforme (Acanthostomum balthium)	Platyhelminthes	Trematoda		Baltic Sea	
Cryptocotyle concava	Platyhelminthes	Trematoda	Indirect impact on diatoms community: parasites reduce snail's bioturbation activity and, by a cascade effect primary production. Parasites also influence	Denmark	(Mouritsen and Haun, 2008)
Himasthla spp.	Platyhelminthes	Trematoda			
Maritrema spp.	Platyhelminthes	Trematoda			
Microphallus claviformis	Platyhelminthes	Trematoda			

						diatoms' communities (favorization of epipelic diatoms)		
Microphallus claviformis	Platyhelminthes	Trematoda	Sporocyst	1.5 ± 1.3	363-470 (4 sites)		Germany	(Thieltges *et al.*, 2006)
Maritrema subdolum	Platyhelminthes	Trematoda	Sporocyst	3.5 ± 1.5				
Maritrema gratiosum	Platyhelminthes	Trematoda	Sporocyst	0.2 ± 0.4				
Levinseniella brachysoma	Platyhelminthes	Trematoda	Sporocyst	0.3 ± 0.6				
Himasthla continua	Platyhelminthes	Trematoda	Sporocyst	1.9 ± 1.2				
Himasthla interrupta	Platyhelminthes	Trematoda	Sporocyst	0.3 ± 0.2				
Cryptocotyle jejuna	Platyhelminthes	Trematoda	Sporocyst	0.1 ± 0.2				
Cryptocotyle concavum	Platyhelminthes	Trematoda	Sporocyst	0.5 ± 0.3				
Himasthla sp.	Platyhelminthes	Trematoda	Sporocyst	0.5 ± 0.7				
Notocotylus (Cercaria) ephemera	Platyhelminthes	Trematoda	Sporocyst	0.7 ± 0.7				
Psilochasmus aglyptorchis	Platyhelminthes	Trematoda	Sporocyst	0.2 ± 0.3				
Deropristis inflata	Platyhelminthes	Trematoda	Sporocyst	0.1 ± 0.1				
Haploporidae	Platyhelminthes	Trematoda		0 to 16 (community of parasites)	100 (3 sites, monthly samples over one year)		France	(de Montaudouin *et al.*, 2003)
Himasthla spp.	Platyhelminthes	Trematoda		0 to 16 (community of parasites)				
Unindentified sporocysts	Platyhelminthes	Trematoda	Sporocyst	0 to 16 (community of parasites)				
Cryptocotyle concavum	Platyhelminthes	Trematoda		0 to 16 (community of parasites)		Increase of mortality		
Microphallidae	Platyhelminthes	Trematoda		0 to 16 (community of parasites)				
Notocotyle sp.	Platyhelminthes	Trematoda		0 to 16 (community of parasites)				

Cercaria sinitzini	Platyhelminthes	Trematoda		0 to 16 (community of parasites)				
Himasthla continua	Platyhelminthes	Trematoda	Cercariae	0 to 6.7	161-401 (3 sites)	Potentially associated with gigantism, may be associated to reduced ability to burry and to slower moving rates	United Kingdom	(Huxham *et al.*, 1995)
Microphallidae sp. no. 15	Platyhelminthes	Trematoda	Cercariae	0.5 to 3.8				
Cryptocotyle jejuna	Platyhelminthes	Trematoda	Cercariae	0 to 0.5				
Notocotylidae sp. no. 10	Platyhelminthes	Trematoda	Cercariae	0 to 2.9				
Maritrema oocysta	Platyhelminthes	Trematoda	Metacercariae, cercariae	0 to 2.9				
Levinseniella sp. no. 17	Platyhelminthes	Trematoda	Metacercariae, cercariae	0 to 0.6				
Maritrema subdolum	Platyhelminthes	Trematoda	Cercariae			Reduced penis size, reduced oviposition rate, increased growth rate, females with higher egestion rates, reduced locomotory activity	Denmark	(Mouritsen and Jensen, 1994)
Microphallus claviformis	Platyhelminthes	Trematoda	Cercariae					
Levinseniella sp.	Platyhelminthes	Trematoda	Cercariae					
Cryptocotyle concava	Platyhelminthes	Trematoda	Cercariae					
Himasthla continua	Platyhelminthes	Trematoda	Cercariae					
Atriophallus minutus (Levinseniella minuta)	Platyhelminthes	Trematoda	Metacercariae				France	(Deblock, 1978)
Cercaria sinitzini	Platyhelminthes	Trematoda						
Bunocotyle progenetica	Platyhelminthes	Trematoda	Cercariae					
Deropristis inflata	Platyhelminthes	Trematoda	Cercariae					
Himasthla continua	Platyhelminthes	Trematoda	Cercariae					
Himasthla interrupta	Platyhelminthes	Trematoda	Cercariae					
Himasthla militaris?	Platyhelminthes	Trematoda	Cercariae					
Himasthla leptosoma?	Platyhelminthes	Trematoda	Cercariae					
Haploporidae sp. no. 3	Platyhelminthes	Trematoda	Cercariae					

Haploporidae sp. no. 4	Platyhelminthes	Trematoda	Cercariae
Haploporidae sp. no. 5	Platyhelminthes	Trematoda	Cercariae
Haploporidae sp. no. 6	Platyhelminthes	Trematoda	Cercariae
Haploporus benedeni	Platyhelminthes	Trematoda	Cercariae
Haplosporus sp. no. 7	Platyhelminthes	Trematoda	Cercariae
Echinostomatidae sp. no. 8	Platyhelminthes	Trematoda	Cercariae
Echinostomatidae sp. no. 9	Platyhelminthes	Trematoda	Cercariae
Psilostomum brevicolle	Platyhelminthes	Trematoda	Cercariae
Notocotylidae sp. no. 10	Platyhelminthes	Trematoda	Cercariae
Notocotylidae sp. no. 12	Platyhelminthes	Trematoda	Cercariae
Notocotylidae sp. no. 13	Platyhelminthes	Trematoda	Cercariae
Notocotylidae sp. no. 14	Platyhelminthes	Trematoda	Cercariae
Microphallus claviformis	Platyhelminthes	Trematoda	Cercariae
Maritrema subdolum	Platyhelminthes	Trematoda	Cercariae
Microphallidae sp. no. 15	Platyhelminthes	Trematoda	Cercariae
Microphallidae sp. no. 16	Platyhelminthes	Trematoda	Cercariae
Maritrema oocysta	Platyhelminthes	Trematoda	Cercariae
Microphallus pirum	Platyhelminthes	Trematoda	Cercariae
Levinseniella sp. no. 17 ?	Platyhelminthes	Trematoda	Cercariae
Cercaria camarguensis	Platyhelminthes	Trematoda	Cercariae
Cercaire ocellée sp. no. 20	Platyhelminthes	Trematoda	Cercariae
Cercaria tetralophocerca	Platyhelminthes	Trematoda	Cercariae
Cryptocotyle jejuna	Platyhelminthes	Trematoda	Cercariae

	Timoniella praeteritum	Platyhelminthes	Trematoda	Cercariae						
	Gymnophallus glandosa	Platyhelminthes	Trematoda	Metacercariae						
Pirenella (Cerithideopsilla) cingulata	Unindentified cercaria	Platyhelminthes	Trematoda	Cercaria	4.8 to 38.5			42-73 (3 sites)		Thailand	(Sri-aroon *et al.*, 2005)
	Cercaria caribbea	Platyhelminthes	Trematoda	Cercariae and sporocysts						South Korea	(Han *et al.*, 2012)
***Polititapes aureus (Venerupis aurea)*[1]**	*Curtuteria arguinae*	Platyhelminthes	Trematoda	Metacercariae		1.1		10		France	(Dang *et al.*, 2009)
	Himasthla sp.	Platyhelminthes	Trematoda	Metacercariae		21.1					
	Diphterostomum brusinae	Platyhelminthes	Trematoda	Metacercariae		0.7					
	Unknown Metacercariae	Platyhelminthes	Trematoda	Metacercariae		19.2					
***Ruditapes decussatus*[1]**	*Curtuteria arguinae*	Platyhelminthes	Trematoda	Metacercariae		1.2					
	Himasthla sp.	Platyhelminthes	Trematoda	Metacercariae		2					
	Parvatrema (Meiogymnophallus) fossarum	Platyhelminthes	Trematoda	Metacercariae		83.5 to 139.9		10		Spain	
	Bucephalus labracis	Platyhelminthes	Trematoda	Sporocyst, cercaria	0 to 0.11			84-176 per sampling time (monthly samples over one year)		Tunisia	(Dhrif *et al.*, 2015)
	Cercaria lata	Platyhelminthes	Trematoda	Sporocyst, cercaria	10.2 to 23.8						
	Caecincola parvulus	Platyhelminthes	Trematoda	Metacercariae	0 to 0.1		1				
	Acanthoparyphium sp.	Platyhelminthes	Trematoda	Metacercariae	1.1 to 22.7		4.2 to 16.8				
	Curtuteria australis	Platyhelminthes	Trematoda	Metacercariae	13.9 to 61.1		6.9 to 12.2				
	Parvatrema (Gymnophallus) fossarum	Platyhelminthes	Trematoda	Metacercariae	23.2 to 98.4		7.1 to 213				
	Parvatrema (Gymnophallus) rebecqui	Platyhelminthes	Trematoda	Metacercariae	0 to 3.5		3.2 to 7.6				
	Lepocreadium pegorchis	Platyhelminthes	Trematoda	Metacercariae	0 to 0.1		1				
	Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae	0 to 1.2		0.4 to 1.2				
	Robphildollfusium fractum	Platyhelminthes	Trematoda	Metacercariae	0 to 0.1		1				
	Parazoogonus sp.	Platyhelminthes	Trematoda	Metacercariae	0.01		1				

	Mytilicola-like	Arthropoda	Hexanauplia		0 to 4			25 per site (2 sites)		Canada	(Marshall *et al.*, 2003)
	Cercaria tapidis	Platyhelminthes	Trematoda	Sporocyst	0 to 12			48-50 per sampling time (14 samples)	Castration, alteration of connective tissues	South Korea	(Ngo and Choi, 2004)
	Curtuteria arguinae	Platyhelminthes	Trematoda	Metacercariae		0.1 to 0.7		10 per sampling time (monthly samples over 1 year)		France	(Dang *et al.*, 2009)
	Himasthla sp.	Platyhelminthes	Trematoda	Metacercariae		0.1 to 0.2					
	Psilostomum brevicolle	Platyhelminthes	Trematoda	Metacercariae		0.3					
	Himasthla alincia	Platyhelminthes	Trematoda	Metacercariae	25	8.8 (intensity)		10		South Korea	(Han *et al.*, 2009)
***Ruditapes philippinarum*[1]**	*Parvatrema duboisi*	Platyhelminthes	Trematoda	Sporocyst, cercaria, metacercaria	97.5		61.3 ± 93.5	40		Japan	(Yanagida *et al.*, 2009)
	unindentified sporocysts	Platyhelminthes	Trematoda	Sporocyst	7.5 to 10			40 per site (2 sites)		South Korea	(Yang *et al.*, 2010)
	unindentified Metacercariae	Platyhelminthes	Trematoda	Metacercariae	15 to 20		0.33 ± 1.05 to 0.45 ± 1.38				
	unindentified cestodes	Platyhelminthes	Cestoda		15 to 22.5		0.2 ± 0.56 to 0.43 ± 1.08 (intensity)				
	Himashtla alincia	Platyhelminthes	Trematoda	Metacercariae	30 to 80		1.6 to 5.8	10 and 15 (2 sites)		South Korea	(Sohn *et al.*, 2017)
	Parvatrema sp.	Platyhelminthes	Trematoda	Metacercariae	6.7 to 100		126 to 238				
	Nymphonella tapeti	*Arthropoda*	Pycnogonida		27 to 86		1.9 to 3.2	50-59 (3 sampling types)	Reduced burial ability, lower somatic index, reduced adductor strength	Japan	(Tomiyama *et al.*, 2016)
***Scrobicularia plana*[1]**	*Parvatrema minutum (Meiogymnophallus minutus)*	Platyhelminthes	Trematoda	Sporocyst, Cercariae	10.5 to 22			50-56 per sampling time (monthly samples over one year)		Ireland	(Fermer *et al.*, 2010)

	Proctoeces (subtenuis) maculatus	Platyhelminthes	Trematoda	Adult	0 to 98	0.016 ± 0.016 to 4.4 ± 0.4	10-55 per sampling time (8 sites with 3 samplings)		United Kingdom	(White, 1972)
	Afropinnotheres monodi	Arthropoda	Malacostraca		4.1	1	9441		Spain	(Drake *et al.*, 2014)
	Oxydromus (Parasyllidea) humesi	Annelida	Polychaeta		0.2 to 4.7	1	269-1065 (5 sampling times)	Reduced biomass	Spain	(Martin *et al.*, 2012)
	Tetrastemma fozensis	Nemerta	Enopla		0 to 100	1 to 2	45-72 (6 sampling sites)		Spain	(Thiel and Zubillaga, 1997)
	Parvatrema (Gymnophallus) fossarum	Platyhelminthes	Trematoda	Sporocyst, Cercariae					France	(Bartoli, 1972)
***Spisula subtruncata*[1]**										
Tagelus plebeius	Gymnophallidae	Platyhelminthes	Trematoda	Metacercariae				Increased susceptibility to predation	Argentina	(Addino *et al.*, 2010)
	Parvatrema sp.	Platyhelminthes	Trematoda	Metacercariae	0 to 100		17-33 (3 sampling times)		Argentina	(da Silva *et al.*, 2009)
	Echinostomatidae	Platyhelminthes	Trematoda	Metacercariae	0 to 26.7					
	Fellodistomidae	Platyhelminthes	Trematoda	Sporocyst	0 to 0.96		14-104 (3 sites)		Argentina	(Vazquez *et al.*, 2006)
	Gymnophallidae	Platyhelminthes	Trematoda	Sporocyst, Metacercariae	0.96 to 100					
	Spirurida	Nematoda	Chromodorea		21.4 to 35.6					
Terebralia palustris										
Theora lata										
Tritia (Cyclope) neritea	*Macvicaria obovata*	Platyhelminthes	Trematoda	Metacercariae	96.7 to 100		60 and 69 (2 sampling times)		Spain	(Born-Torrijos *et al.*, 2012)
	Microphallidae	Platyhelminthes	Trematoda		0 to 1		29-33 (3 sites)		France	(Bachelet *et al.*, 2004)
Tritia (Ilyanassa) obsoleta	*Pleurogonius malaclemys*	Platyhelminthes	Trematoda	Redia	1.2 to 2.6		3922		United States	(Chodkowski *et al.*, 2016)
	Pleurogonius malaclemys	Platyhelminthes	Trematoda	Metacercariae	57.9	2.6 ± 2.4				
	Himasthla quissetensis	Platyhelminthes	Trematoda	Sporocysts	2.9					
	Zoogonus lasius	Platyhelminthes	Trematoda	Sporocysts	2.3			Castration		

Austrobilharzia variglandis	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	1.7				
Stephanostomum tenue	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	0.2				
Lepocreadium setiferoides	Platyhelminthes	Trematoda	Redia	0.1				
Gynaecotyla adunca	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	0.03				
Diplostomum nassa	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	0.03				
Gynaecotyla adunca	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	16 to 21			Canada	(Shim *et al.*, 2013)
Lepocredium setiferoides	Platyhelminthes	Trematoda	Larval stages (sporocysts or rediae)	0 to 16 %				
Zoogonus rubellus	Platyhelminthes	Trematoda		7.8 to 13	1955-2660 (3 sites)		United States	(Blakeslee *et al.*, 2012)
Lepocreadium setiferoides	Platyhelminthes	Trematoda		5.4 to 5.8				
Stephanostomum tenue	Platyhelminthes	Trematoda		0.5 to 7.1				
Stephanostomum dentatum	Platyhelminthes	Trematoda		0.8 to 4.1				
Himasthla quissetensis	Platyhelminthes	Trematoda		0.2 to 4.7				
Austrobilharzia variglandis	Platyhelminthes	Trematoda		0.05 to 0.6				
Gynaecotyla adunca	Platyhelminthes	Trematoda		0.15 to 1				
Diplostomum nassa	Platyhelminthes	Trematoda		0 to 0.26				
Pleurogonius malaclemys	Platyhelminthes	Trematoda		0 to 0.7				
Zoogonus rubellus	Platyhelminthes	Trematoda		0 to 1.4				
Lepocreadium setiferoides	Platyhelminthes	Trematoda		0 to 1.0				
Stephanostomum tenue	Platyhelminthes	Trematoda		0 to 0.4				
Himasthla quissetensis	Platyhelminthes	Trematoda		0.7 to 4.7				
Austrobilharzia variglandis	Platyhelminthes	Trematoda		0.6 to 3.1				

Turritella communis	*Cercaria pythionoke*	Platyhelminthes	Trematoda	Cercariae	2	541		United Kingdom	(Rothschild, 1935)
	Cercaria herpsyllis	Platyhelminthes	Trematoda	Cercariae	0.5				
	Cercaria doricha	Platyhelminthes	Trematoda	Cercariae	5				
	Cercaria nicarete	Platyhelminthes	Trematoda	Cercariae	1				
	Cercaria ampelis	Platyhelminthes	Trematoda	Cercariae	4				
	Cercaria ranzii	Platyhelminthes	Trematoda	Cercariae	1				
Yoldia hyperborea									
Yoldia limatula									
SIPUNCULA									
Sipunculus (Sipunculus) nudus[1]	*Myzomolgus sipunculensis*	Arthropoda	Hexanauplia					Brazil	(Kihara *et al.*, 2007)
	Myzomolgus stupendus	Arthropoda	Hexanauplia					France	
	Myzomolgus tenuis	Arthropoda	Hexanauplia					South Korea	

[1] Bioturbating species of economic interest (wild-caught or farmed).

Appendix 3 List of microparasites reported for bioturbating species (identified in Appendix 1). When available, prevalence (percentage of infestation in the population) is given. The size of the samples are given as well. Potential impacts on the host are reported.

Bioturbating species	Microparasite species	Phylum	Class	Prevalence (%)	N	Impact on the host	Location	Reference
ANNELIDA								
Abarenicola affinis								
Abarenicola pacifica								
Alitta virens								
Arenicola marina								
Axiothella rubrocincta	*Haplozoon axiothellae*	Miozoa	Dinophyceae				United States	(Siebert, 1973)
Capitella cf. teleta								
***Capitella* spp.**	*Ancora sagittata*	Myzozoa	Conoidasida				Denmark, Sweden	(Ronny Larsson and Køie, 2006)
	Sphaerospora dicentrarchi	Cnidaria	Myxozoa	0 to 0.1	121 and 916 (2 sites)		Portugal	(Rangel *et al.*, 2016)
Cirratulus grandis								
Cirriformia filigera								
***Clymenella* sp.**	*Pterospora clymenellae*	Myzozoa	Conoidasida	28.6	35		United States	(Landers, 1991)
Clymenella torquata								
Diopatra cuprea								
Eupolymnia heterobranchia								
Eupolymnia nebulosa								
Glycera alba								
Hediste (Neanthes) japonica								
Hediste (Nereis) diversicolor	Undefined Myxosporea (gen. *Tetractinomyxon*)	Cnidaria	Myxozoa	2	50		Denmark	(Køie, 2000)
	Ellipsomyxa (Zschokkella) mugilis	Cnidaria	Myxozoa	0 to 3.2	126-528 (seasonal samples over one year)		Portugal	(Rangel *et al.*, 2009)
	Eucoccidium (Coelotropha) duchorni	Myzozoa	Conoidasida				France	(Porchet-Hennere, 1967)

	Iridovirus					France	(Devauchelle and Durchon, 1973)
	Lecudina tuzetae	Myzozoa	Conoidasida			France	(Kuriyama *et al.*, 2005)
Heteromastus filiformis							
Heteromastus similis							
Kinbergonuphis (Onuphis) jenneri							
Laeonereis culveri (acuta)							
Lanice conchilega							
Lipobranchius jeffreysii							
Lumbrineris latreilli							
Maldane sarsi							
Marenzelleria arctia							
Marenzelleria neglecta							
Marenzelleria viridis							
Maxmuelleria lankesteri							
Melinna cristata							
Melinna palmata							
Naineris laevigata	*Kudoa septempunctata*	Cnidaria	Myxozoa	0 to 40	62 to 126 (7 sampling times)	Korea	(Paari *et al.*, 2017)
Nephtys caeca							
Nephtys hombergii							
Nephtys incisa							
Notomastus latericeus							
***Notomastus* sp.**							
Owenia fusiformis							
Pectinaria gouldii							
Perinereis aibuhitensis							
Perinereis nuntia							
Polydora ciliata							
Pygospio elegans	*Lecudina* spp.	Myzozoa	Conoidasida			United States	(Douglass and Jones, 1991)

	Coccidium spp.	Myzozoa	Conoidasida	80			United States	(Douglass and Jones, 1991)
Scalibregma inflatum								
Scoloplos armiger	*Eleutheroschizon duboscqi*	Myzozoa	Conoidasida				Russia	(Valigurová *et al.*, 2015)
***Thalassodrilides* sp.**	*Endocapsa rosulata*	Cnidaria	Myxozoa				Australia	(Hallett *et al.*, 1999)
	Sphaeractinomyxon ersei	Cnidaria	Myxozoa				Australia	(Hallett *et al.*, 1999)
Timarete (Cirriformia) luxuriosa								
Urechis caupo								
ARTHROPODA								
***Alpheus macellarius*[1]**								
Austinogebia edulis								
***Austrohelice crassa*[1]**								
Austruca (Uca) annulipes								
Austruca (Uca) triangularis								
Biffarius (Callianassa) filholi								
Biffarius arenosus								
Callianassa subterranea								
Callichirus (Callianassa) kraussi								
Callichirus islagrande								
Calocaris templemani								
Corallianassa longiventris								
Corophium arenarium								
Corophium volutator	Unknown microsporidian	Microsporidia		females =27, males = 1.5	306 females, 65 males	Increase of offsprings production	Canada	(Mautner *et al.*, 2007)
Cranuca (Uca) inversa								
Cyrtograpsus angulatus	*Epistylis* sp.	Ciliophora	Oligohemenophorea		65		Argentina	(Alda *et al.*, 2011)
	White spot syndrome virus		Nimaviridae	0 to 66.7	6 to 16 (2 sampling times)		Argentina	(Martorelli *et al.*, 2010, 2017)

Dotilla fenestrata								
***Eriocheir sinensis*[1]**	*Hepatospora (Endoreticulatus) eriocheir*	Microsporidia	Microsporea	0 to 18.8	23 and 64 (2 sampling sites)		China	(Wang and Chen, 2007)
	Hepatospora eriocheir (Endoreticulatus eriocheir)	Microsporidia	Microsporea	up to 70	60 per month over one year		United Kingdom	(Stentiford *et al.*, 2011)
	Aphanomyces astaci	Oomycota	Saprolegniales	100	6		Sweden	(Svoboda *et al.*, 2014)
	Hepatospora eriocheir	Microsporidia	Microsporea			Hypertrophy of hepatopancreatic cells, metabolic stress	China	(Ding *et al.*, 2018)
	Spiroplasma eriocheiris	Tenericutes	Mollicutes			Associated with tremor disease. Could be related to mass mortality	China	(Wang and Gu, 2002; Wang *et al.*, 2011)
	White spot syndrome virus					Increase of mortality rate	China	(Ding *et al.*, 2015)
	Eriocheir sinensis ronivirus (*Es*RNV)					Increase of mortality rate	China	(Zhang and Bonami, 2007)
	Vibrio parahaemolyticus	Proteobacteria	Gammaproteobacteria	100			United Kingdom	(Wagley *et al.*, 2009)
	Vibrio parahaemolyticus	Proteobacteria	Gammaproteobacteria	89.5	19		China	(Yano *et al.*, 2006)
Eucalliax panglaoensis								
Gelasimus (Uca) vocans								
Glypturus (Callichirus) armatus								
Glypturus (Callichirus) laurae								
***Helice formosensis*[1]**								
***Helice tientsinensis*[1]**								
Heloecius cordiformis								
Hemigrapsus oregonensis	*Taeniella grandis*	Choanozoa	Ichthyosporea	50			United States	(McDermott, 2011)
	Taeniella carcini	Choanozoa	Ichthyosporea					
Hemiplax hirtipes								
Hyas araneus	*Hematodinium* sp.	Myzozoa	Dynophycae	45	40		Greenland	(Eigemann *et al.*, 2010)
Laomedia astacina								
Lepidophthalmus bocourti								
Lepidophthalmus louisianensis								

Lepidophthalmus sinuensis							
Leptocheirus plumulosus							
Leptuca (Uca) crenulata							
Leptuca (Uca) cumulanta							
Leptuca (Uca) leptodactyla							
Leptuca (Uca) panacea							
Leptuca (Uca) uruguayensis							
Macrophthalmus japonicus							
Mictyris longicarpus							
Minuca (Uca) longisignalis							
Minuca (Uca) pugnax							
Monoporeia affinis	Unknown microsporidian	Microsporidia	Microsporea	12		Sweden	(Jacobson *et al.*, 2010)
Neaxius acanthus							
Neohelice (Chasmagnathus) granulata[1]	White spot syndrome virus		Nimaviridae	23.3	150	Brazil	(Marques *et al.*, 2011)
	Clostridium perfringens	Firmicutes	Clostridia			Argentina	(La Sala *et al.*, 2015)
	Undefined filamentous bacteria			11	36	Argentina	(Alda *et al.*, 2011)
	Epistylis sp.	Ochropyta		8			
	Infectious hypodermal and hematopoietic necrosis virus			0 to 88	30 and 50 (2 sites)	Brazil	(Cavalli *et al.*, 2013)
Neotrypaea californiensis							
Pestarella (Callianassa) tyrrhena							
Pontoporeia femorata							
Sarsamphiascus (Amphiascus) tenuiremis							
Sergio trilobata							
Sesarma reticulatum[1]							
Sphaeroma quoianum							
Trypaea australiensis							
Tubuca (Uca) forcipata							

Tubuca (Uca) paradussumieri							
Tubuca (Uca) rosea							
Uca maracoani							
***Ucides cordatus*[1]**	*Exophiala cancerae*	Ascomycota	Eurotiomycetes		Causing lethargic crab disease (LCD), increase of mortality, moribund crab, necrosis of tissue, destruction of gills lamellae tissue, haemocytic infiltration		(Vicente *et al.*, 2012)
	Fonsecaea brasiliensis	Ascomycota	Eurotiomycetes				
Upogebia africana							
Upogebia deltaura							
Upogebia major							
Upogebia pugettensis							
Upogebia pusilla							
Victoriopisa australiensis							
CEPHALORHYNCHA							
Halicryptus spinulosus							
ECHINODERMATA							
***Abatus ingens*[1]**							
Amphiura chiajei							
Amphiura filiformis							
***Apostichopus (Stichopus) japonicus*[1]**	*Boveria labialis*	Ciliophora	Oligohemenophorea		Associated with massive mortality	China	(Zhan *et al.*, 2018)
	Boveria subcylindrica	Ciliophora	Oligohemenophorea				
	Vibrio cyclitrophicus	Proteobacteria	Gammaproteobacteria	20 per year (3 years)	Pathogens of skin ulceration disease, associated with high mortality rate	China	(Deng *et al.*, 2009)
	Vibrio tasmaniensis	Proteobacteria	Gammaproteobacteria				
	Vibrio harveyi	Proteobacteria	Gammaproteobacteria				
	Vibrio splendidus	Proteobacteria	Gammaproteobacteria				
	Photobacterium sp.	Proteobacteria	Gammaproteobacteria				
	Spherical viruses				Pathogen of viscera ejection syndrome, associated with high mortality rate		
	Pseudoalteromonas tetraodonis	Proteobacteria	Gammaproteobacteria		Pathogens of skin ulceration and peristome tumescence, associated with decrease of tentacle activity	China	(Liu *et al.*, 2010)
	Pseudoalteromonas sp.	Proteobacteria	Gammaproteobacteria				
	Skin ulceration and peristome tumescence syndrome virus (SUPTSV)						

	Arenibacter latericius	Bacteroidetes	Flavobacteria		Russia	(Ivanova *et al.*, 2001)
	Salegentibacter holothuriorum	Bacteroidetes	Flavobacteria		Russia	(Nedashkovskaya, 2004)
	Vibrio alginolyticus	Proteobacteria	Gammaproteobacteria		China	(Zhang *et al.*, 2015)
	Shewanella marisflavi	Proteobacteria	Gammaproteobacteria	Viscera ejection, swollen mouth, skin ulceration, increase of mortality rate	China	(Li *et al.*, 2010)
	Aeromonas salmonicida	Proteobacteria	Gammaproteobacteria	Pathogen of bacterial ulceration disease, increase of mortality rates at low temperatures	China	(Yang *et al.*, 2008)
Australostichopus mollis						
Brisaster latifrons[1]						
Brissopsis lyrifera						
Balanoglossus aurantiaca						
Echinocardium cordatum[1]	*Lithocystis schneideri*	Myzozoa	Conoidasida	Initiation of a coelomocytic reaction	France	(De Ridder and Jangoux, 1984)
Encope emarginata[1]						
Holothuria (Halodeima) atra[1]						
Holothuria princeps[1]						
Holothuria tubulosa[1]						
Holothuria (Microthele) whitmaei[1]						
Molpadia oolitica[1]						
Oestergrenia (Labidoplax) digitata						
Peronella lesueuri[1]						
Stichopus chloronotus[1]						
Stichopus herrmanni[1]						
HEMICHORDATA						
Saccoglossus bromophenolosus						
***Schizocardium* sp.**						
MOLLUSCA						
Abra alba						

Abra nitida								
Abra segmentum (ovata)								
***Austrovenus stutchburyi*[1]**								
Batillaria flectosiphonata								
Batillaria zonalis								
***Cerastoderma edule*[1]**	*Minchinia mercenariae*-like parasite	Cercozoa	Ascetosporea	4.6 to 10	22 to 32 (5 sampling times)		Spain	(Ramilo *et al.*, 2018)
	Disseminated neoplasia (unknown etiological agent)			0 to 50		Haemocytic infiltration	Spain	(Villalba *et al.*, 2014)
	Marteilia cochillia	Cercozoa	Ascetosporea	0 to 100		Causing marteiliosis, heavy haemocytic infiltration in the connective tissue, destruction of digestive tubules, increase of mortality rates		
	Perkinsus chesapeaki	Myzozoa	Perkinsea	23.3 to 84.4	30 per sampling time (7 sampling time)		Spain	(Carrasco *et al.*, 2014)
	Disseminated neoplasia (unknown etiological agent)			15.0 to 38.3	60 to 95 (6 sampling times)	Haemocytic infiltration, may enhance surfacing of cockles	Ireland	(Morgan *et al.*, 2012)
	Steinhausia sp.	Microsporidia	Microsporea	11.8 to 20.0	30 to 60 (3 sites)		France	(Comtet *et al.*, 2003)
	Haplosporidium edule	Cercozoa	Ascetosporea				Spain	(Azevedo *et al.*, 2003)
	Disseminated neoplasia (unknown etiological agent)			0 to 32	30 per site (34 sites)	Haemocytic infiltration	Spain	(Carballal *et al.*, 2001)
	Nematopsis sp.	Myzozoa	Conoidasida	30 to 100		Haemocytic reaction		
	Rickettsia-like	Proteobacteria	Alphaproteobacteria	0 to 43				
	Pseudoklossia sp.	Myzozoa	Conoidasida	0 to 39		Hypertrophy of renal cells		
	Bacterial extracellular cysts			0 to 93		Alteration of gill architecture in heavily infected organisms		
	Perkinsus sp.	Myzozoa	Perkinsea	3 to 67	30 per site (5 sites)		France	(Lassalle *et al.*, 2007)
	Vibrio tapetis	Proteobacteria	Gammaproteobacteria	17 to 43		Sometimes associated with brown ring disease signs		
	Vibrio tapetis	Proteobacteria	Gammaproteobacteria			Increased mortality rates, modifications of genes expression, induction of oxidative burst, reduced haemocytes adhesion		(Paul-Pont *et al.*, 2010a)

Chamelea gallina	*Perkinsus mediterraneus*	Myzozoa	Perkinsea	25	24		Spain	(Valencia *et al.*, 2014)
	Marteilia refringens	Cercozoa	Ascetosporea	4.4	69	Associated to important mortality event	Spain	(López-Flores *et al.*, 2008)
	Vibrio parahaemolyticus	Proteobacteria	Gammaproteobacteria				Italy	(Rahman *et al.*, 2017)
	Nematopsis sp.	Myzozoa	Conoidasida	100	1172			(Berrilli *et al.*, 2000)
	Porospora sp.	Myzozoa	Conoidasida	0.6				
	Ancistrum sp.	Ciliophora	Oligohemenophorea	5.5				
	Boveria sp.	Ciliophora	Oligohemenophorea	13.8				
***Cyclina sinensis*[1]**								
***Ensis leei (arcuatus var. directus)*[1]**	Prokaryote-like colonies			0 to 1.8	135 to 165 (3 sites)		Spain	(Darriba *et al.*, 2010)
	Renal coccidia	Myzozoa	Conoidasida	16.5 to 19.6				
	Nematopsis sp.	Myzozoa	Conoidasida	49.6 to 89.1				
***Fabulina fabula*[1]**								
***Limecola (Macoma) balthica*[1]**	*Perkinsus chesapeaki*	Myzozoa	Perkinsea				United States	(Burreson *et al.*, 2005)
	Disseminated neoplasia (unknown etiological agent)			0 to 26.1	5 to 199 per sampling time (29 times)		Finland	(Pekkarinen, 1993)
***Limecola (Macoma) contabulata*[1]**								
Macomangulus (Angulus) tenuis								
Macoma nasuta								
Macomona (Tellina) deltoidalis								
***Macomona liliana*[1]**								
Macoploma (Macoma) tenta								
***Meretrix meretrix*[1]**	*Trichodina meretricis*	Ciliophora	Oligohemenophorea				China	(Xu *et al.*, 1999)
	Nematopsis spp.	Myzozoa	Conoidasida	44	50		Saudi Arabia	(Abdel-Baki *et al.*, 2012)
	Perkinsus sp.	Myzozoa	Perkinsea	48				
	Marteilia spp.	Cercozoa	Ascetosporea	0.19	1026		China	(Xie and Xie, 2018)
	Vibrio vulnificus	Proteobacteria	Gammaproteobacteria	28.5	26		India	(Sangeetha *et al.*, 2017)

	Vibrio parahaemolyticus	Proteobacteria	Gammaproteobacteria	20 to 29.4 %	5 and 17 (2 sampling times)		Thailand	(Vuddhakul *et al.*, 2006)
	Vibrio sp. strain MM5	Proteobacteria	Gammaproteobacteria			Increase of mortality	China	(Yue *et al.*, 2011)
***Mya arenaria*[1]**	*Perkinsus* spp. (probably *marinus* and *chesapeki*)	Myzozoa	Perkinsea	11.7	240	Lesions in the gills connective tissues (loss of tissue structure), lesions in the digestive gland and gonadic gland, haemocytic infiltration in muscle tissues. May affect fertitliy and growth	United States	(McLaughlin and Faisal, 1998)
	Disseminated neoplasia (unknown etiological agent)			19 to 43	30 to 189 (18 sampling times)	Increase of mortality	United States	(Cooper *et al.*, 1982)
	Campylobacter sp.	Proteobacteria	Epsilonproteobacteria	47.2	72		Canada	(Lévesque *et al.*, 2006)
	Cryptosporidium parvum	Myzozoa	Conoidasida	70.7				
	Giardia sp.			63.4				
	Salmonella sp.	Proteobacteria	Gammaproteobacteria	1.4				
Nucula hanleyi								
Nucula nitidosa								
Nucula proxima (annulata)								
Nuculana (Nuculana) pernula								
Nuculana minuta								
Nuttallia japonica								
Peringia (Hydrobia) ulvae								
Pirenella (Cerithideopsilla) cingulata								
***Polititapes aureus (Venerupis aurea)*[1]**	*Perkinsus olsenii*	Myzozoa	Perkinsea				Spain	(Ramilo *et al.*, 2015)
***Ruditapes decussatus*[1]**	*Vibrio alginolyticus*	Proteobacteria	Gammaproteobacteria			Increase of mortality of larvae	Tunisia	(Mechri *et al.*, 2015)
	Vibrio tubiashii subsp. *europaeus*	Proteobacteria	Gammaproteobacteria			Increase of mortality of larvae	Spain	(Dubert *et al.*, 2017)
	Vibrio barjaei	Proteobacteria	Gammaproteobacteria				Spain	(Dubert *et al.*, 2016b)
	Vibrio spp. (*V. alginolyticus, V. cholerae, V. parahaemolyticus*)	Proteobacteria	Gammaproteobacteria	51.8	83		Tunisia	(Gdoura *et al.*, 2016)
	Marinomonas atlantica	Proteobacteria	Gammaproteobacteria				Spain	(Lasa *et al.*, 2016)
	Marinomonas gallaica	Proteobacteria	Gammaproteobacteria					

Vibrio bivalvicida	Proteobacteria	Gammaproteobacteria				Spain	(Dubert *et al.*, 2016a)
Vibrio spp.	Proteobacteria	Gammaproteobacteria			Associated with Brown Ring Disease	Tunisia	(El Bour *et al.*, 2011)
Vibrio artabrorum	Proteobacteria	Gammaproteobacteria				Spain	(Dieguez *et al.*, 2011)
Vibrio atlanticus	Proteobacteria	Gammaproteobacteria					
Vibrio breoganii	Proteobacteria	Gammaproteobacteria				Spain	(Beaz Hidalgo *et al.*, 2009)
Vibrio alginolyticus	Proteobacteria	Gammaproteobacteria			Increase of mortality of larvae	Spain	(Gomez-Leon *et al.*, 2005)
Vibrio splendidus	Proteobacteria	Gammaproteobacteria			Increase of mortality of larvae		
Vibrio tapetis	Proteobacteria	Gammaproteobacteria	20 to 40			France, Spain	(Paillard, 2004)
Cryptosporidium sp.	Myzozoa	Conoidasida				Portugal	(Azevedo, 1989a)
Perkinsus olsenii (atlanticus)	Myzozoa	Perkinsea	100% in clams with gaping valves			Portugal	(Azevedo, 1989b)
Rickettsia-like organisms	Proteobacteria	Alphaproteobacteria	3 to 20	28-60 (3sites)		Spain	(Navas *et al.*, 1992)
Perkinsus olsenii (atlanticus)	Myzozoa	Perkinsea	66.6 to 92.9				
Nematopsis sp.	Myzozoa	Conoidasida	0 to 3.4				
Coccidians	Myzozoa	Conoidasida	0 to 5.1				
Microsporidia-like organisms	Microsporidia		0 to 6.8				
Trichodina sp.	Ciliophora	Oligohemenophorea	3.3 to 15.4				
Minchinia tapetis	Cercozoa	Ascetosporea	32.1 to 96.6				
Brown ring disease			0.9 to 93.3	30 per site (4 sites)		Spain	(Figueras *et al.*, 1996)
Brown ring disease			11.1 to 37			Portugal	
Chlamydia-like organisms			10 to 86.2			Spain	
Chlamydia-like organisms			30 to 71.4			Portugal	
Haplosporidia-like organisms (HLOs)	Cercozoa	Ascetosporea	3.3 to 100			Spain	
Haplosporidia-like organisms (HLOs)	Cercozoa	Ascetosporea	0 to 20			Portugal	
Perkinsus-like organisms	Myzozoa	Perkinsea	0 to 60			Spain	
Urosporidium sp.	Cercozoa	Ascetosporea	36 to 71.4	overall = 184		Spain	(Novoa *et al.*, 2004)
Perkinsus olsenii	Myzozoa	Perkinsea	20 to 100	40 per sampling time (7 samplings)		Spain	(Elandaloussi *et al.*, 2009)

	Perkinsus olsenii	Myzozoa	Perkinsea			Reduced bioturbation activity (lower ability to remobilize iron)		(Simão *et al.*, 2010)
	Perkinsus olsenii	Myzozoa	Perkinsea			Inhibition of gonadic development	Spain	(Casas and Villalba, 2012)
	Perkinsus olsenii	Myzozoa	Perkinsea				France	(Arzul *et al.*, 2012)
	Perkinsus chesapeaki	Myzozoa	Perkinsea					
	Marteilia refringens	Cercozoa	Ascetosporea	0 to 68.9	30 per sampling time (monthly sample over one year)		France	(Boyer *et al.*, 2013)
	Perkinsus olsenii	Myzozoa	Perkinsea	46 to 100	25 per sampling time (monthly sample over 2 years)	Increase of mortality	Spain	(Villalba *et al.*, 2005)
	Perkinsus olsenii	Myzozoa	Perkinsea	0 to 80	35 per sampling time (3 sites sampled twice a year for 4 years)		Tunisia	(El Bour *et al.*, 2012)
	Brown ring disease			0 to 20	100 per sampling time (3 sites sampled twice a year for 4 years)			
	Lacinutrix venerupis	Bacteroidetes	Flavobacteria		30 per sampling time (sample every 2 months over 2 years)		Spain	(Lasa *et al.*, 2015)
	Perkinsus olsenii	Myzozoa	Perkinsea	68 to 100		Reduced growth rate	Spain	(Dang *et al.*, 2013)
***Ruditapes philippinarum*[1]**	Ostreid herpes virus OsHV-1 in larvae						France	(Arzul *et al.*, 2001)
	Herpes-like virus					Increase of mortality, cell lesions in connective tissues	France	(Renault *et al.*, 2001)
	Brown muscle disease associated with virus-like particles			0 and 48	100 per sampling time (monthly samples over 1 year)	Associated with high mortality rates, lesions of the posterior adductor muscle (necrotic and atrophied muscular cells), conchiolin deposits	France	(Dang *et al.*, 2008)
	Virus-like particles			26 to 93	29-150 per site (5 sites)	Associated with high mortality rates, hypertrophy of cells in connective tissues and striated	United Kingdom	(Bateman *et al.*, 2012)

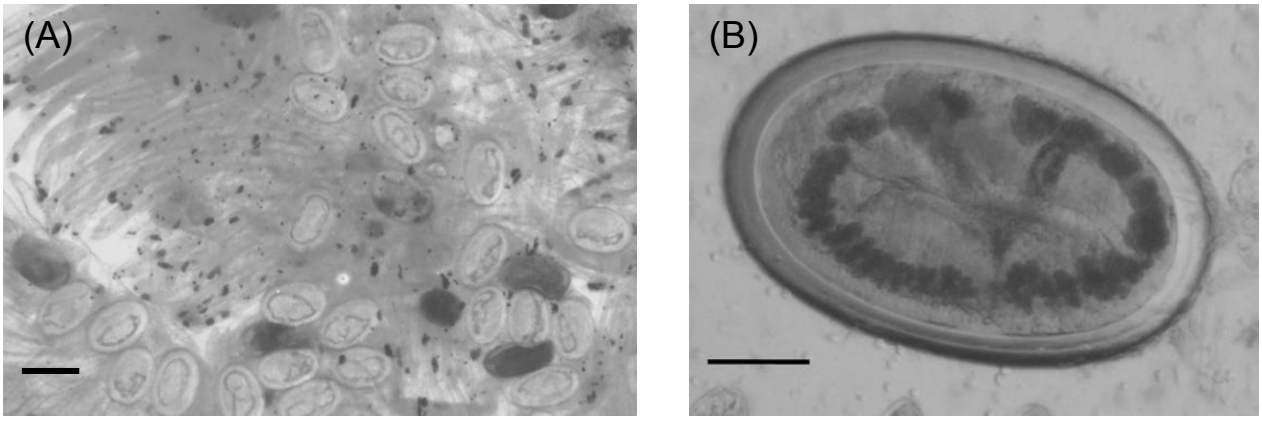
					muscle fibers, haemocytic infiltration		
Rickettsia-like organisms	Proteobacteria	Alphaproteobacteria	0 to 17.9	12-30 per site (4 sites)	Haemocytic response with destruction of the tissue structure	Spain	(Navas *et al.*, 1992)
Perkinsus olsenii (atlanticus)	Myzozoa	Perkinsea	20 to 83.3				
Trichodina sp.	Ciliophora	Oligohemenophorea	0 to 13.5				
Brown ring disease			6.6 to 84.2	19-106 per site (4 sites)		Spain	(Figueras *et al.*, 1996)
Brown ring disease			3.3 to 4.9			Portugal	
Chlamydia-like organisms	Chlamydiae	Chlamidiia	11.5 to 41.6			Spain	
Chlamydia-like organisms	Chlamydiae	Chlamidiia	10 to 16.6			Portugal	
Haplosporidian-like organisms (HLOs)	Myzozoa	Perkinsea	0 to 19.2			Spain	
Perkinsus-like organisms	Myzozoa	Perkinsea	0 to 8.3			Spain	
Perkinsus sp.	Myzozoa	Perkinsea			Damages of the connective tissue, possible disturbance of the reproduction ability	Japan	(Hamaguchi *et al.*, 1998)
Trichodina ruditapicis	Ciliophora	Oligohemenophorea				China	(Xu *et al.*, 1999)
Perkinsus sp.	Myzozoa	Perkinsea	0 to 100	18-75 per site (22 sites)	May disturb reproduction and metabolism	South Korea	(Park and Choi, 2001)
Rickettsia or Chlamydia			8 to 32	25 per site (2 sites)		Canada	(Marshall *et al.*, 2003)
Trichodina sp.	Ciliophora	Oligohemenophorea	20 to 56	25 per site (2 sites)			
Perkinsus sp.	Myzozoa	Perkinsea	6 to 86	48-50 per sampling time (14 samples)		South Korea	(Ngo and Choi, 2004)
Haplosporidium sp.	Cercozoa	Ascetosporea	2.5	40	Alterations of connective tissues	Japan	(Itoh *et al.*, 2005)
Marteilia sp.	Cercozoa	Ascetosporea	2.5				
Marteilioides sp.	Cercozoa	Ascetosporea	2.5				
Perkinsus sp.	Myzozoa	Perkinsea	0 to 80	30 per site (5 sites)		France	(Lassalle *et al.*, 2007)
Vibrio tapetis	Proteobacteria	Gammaproteobacteria	20 to 40	30 per site (5 sites)	Sometimes associated with brown ring disease		
Perkinsus olsenii	Myzozoa	Perkinsea	Overall = 97	50	Alterations of connective tissues	Japan	(Dungan and Reece, 2006)
Perkinsus honshuensis	Myzozoa	Perkinsea					
Perkinsus olsenii	Myzozoa	Perkinsea				France	(Arzul *et al.*, 2012)
Perkinsus chesapeaki	Myzozoa	Perkinsea					
Perkinsus olsenii	Myzozoa	Perkinsea	43.3 to 76.7	30 per site (2 sites)		South Korea	(Yang *et al.*, 2010)

Brown ring disease			1.4 to 5.7	70 per site (2 sites)			
Marteilioides sp.	Cercozoa	Ascetosporea	0.4	1840 over 23 sites		South Korea	(Yanin *et al.*, 2013)
Marteilia granula	Cercozoa	Ascetosporea	0 to 28.6	6-14 (15 samples)		Japan	(Itoh *et al.*, 2014)
Perkinsus olsenii	Myzozoa	Perkinsea	100	100-200 per sampling times (6 samples)	Increase of mortality	Italia	(Pretto *et al.*, 2014)
Mikrocytos-like parasite			3 to 93.3	30 per site (4 sites)	Haemocytic infiltration	Spain	(Ramilo *et al.*, 2014)
Perkinsus olsenii	Myzozoa	Perkinsea	0 to 46.7	30 per site (4 sites)		Spain	(Ramilo *et al.*, 2016)
Vibrio tapetis	Proteobacteria	Gammaproteobacteria			Causing brown ring disease. Increase of mortality, conchiolin deposits		(Allam *et al.*, 2002)
Vibrio tapetis	Proteobacteria	Gammaproteobacteria			Causing brown ring disease, weight loss, decrease of respiration and clearance rates in heavy infected clams	France	(Flye-Sainte-Marie *et al.*, 2007)
Vibrio tapetis-like bacteria	Proteobacteria	Gammaproteobacteria	35.3 to 71	7 and 17 (2 sampling times)	Associated with signs of brown ring disease	Norway	(Paillard *et al.*, 2008)
Vibrio breoganii	Proteobacteria	Gammaproteobacteria				Spain	(Beaz Hidalgo *et al.*, 2009)
Vibrio gallaecicus	Proteobacteria	Gammaproteobacteria				Spain	(Beaz-Hidalgo *et al.*, 2009)
Vibrio artabrorum	Proteobacteria	Gammaproteobacteria				Spain	(Dieguez *et al.*, 2011)
Vibrio atlanticus	Proteobacteria	Gammaproteobacteria					
Vibrio celticus	Proteobacteria	Gammaproteobacteria				Spain	(Beaz-Hidalgo *et al.*, 2010b)
Aliivibrio finisterrensis	Proteobacteria	Gammaproteobacteria				Spain	(Beaz-Hidalgo *et al.*, 2010a)
Vibrio toranzoniae	Proteobacteria	Gammaproteobacteria				Spain	(Lasa *et al.*, 2013)
Marinivirga aestuarii	Bacteroidetes	Flavobacteria				South Korea	(Park *et al.*, 2013)
Vibrio tubiashii subsp. *europaeus*	Proteobacteria	Gammaproteobacteria			Increase of mortality in the initial stages of development	Spain	(Prado *et al.*, 2015)
Perkinsus olsenii	Myzozoa	Perkinsea	0 to 95.8	23-120 (7 sites)		China	(Wu *et al.*, 2011)
Perkinsus spp.	Myzozoa	Perkinsea	0 to 100	30 per sampling (monthly	Reduced growth rates	France	(Dang *et al.*, 2013)

					samples > 1 year)			
	Perkinsus olsenii	Myzozoa	Perkinsea			Clams emerging at the surface	South Korea	(Nam *et al.*, 2018)
	Vibrio tapetis	Proteobacteria	Gammaproteobacteria			Reduced concentration of granulocytes, reduced haemocytes viability		(Paul-Pont *et al.*, 2010c)
***Scrobicularia plana*[1]**								
***Spisula subtruncata*[1]**								
Tagelus plebeius	*Perkinsus chesapeaki*	Myzozoa	Perkinsea	0 to 91	22-30 (11 sites)		United States	(Reece *et al.*, 2008)
Terebralia palustris								
Theora lata								
Tritia (Cyclope) neritea								
Tritia (Ilyanassa) obsoleta								
Turritella communis								
Yoldia hyperborea								
Yoldia limatula								
SUPUNCULA								
***Sipunculus (Sipunculus) nudus*[1]**								

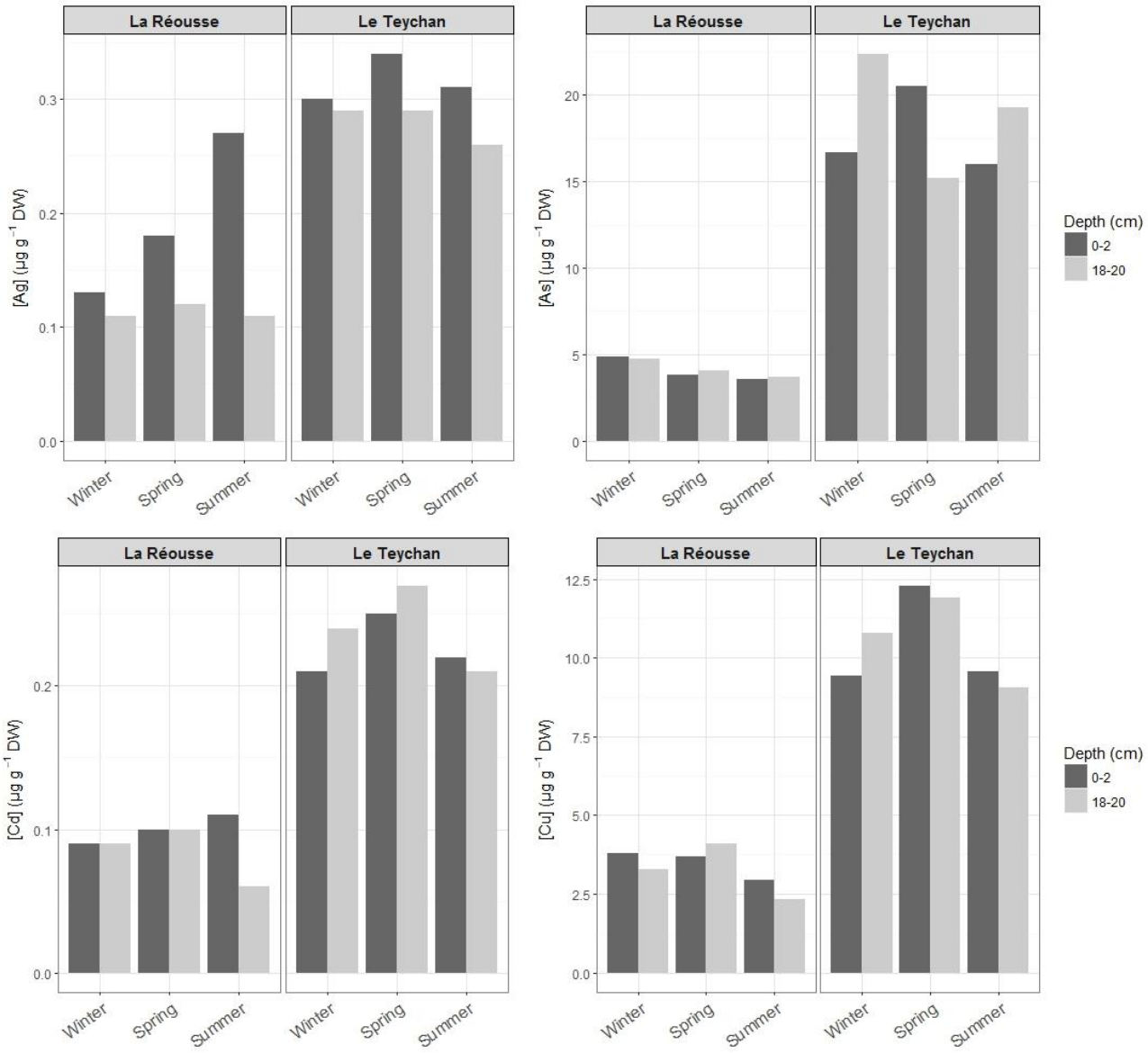
[1] Bioturbating species of economic interest (wild-caught or farmed).

Parasite interactions in the bioturbator *Upogebia pusilla* (Decapoda: Gebiidae): a case of amensalism?

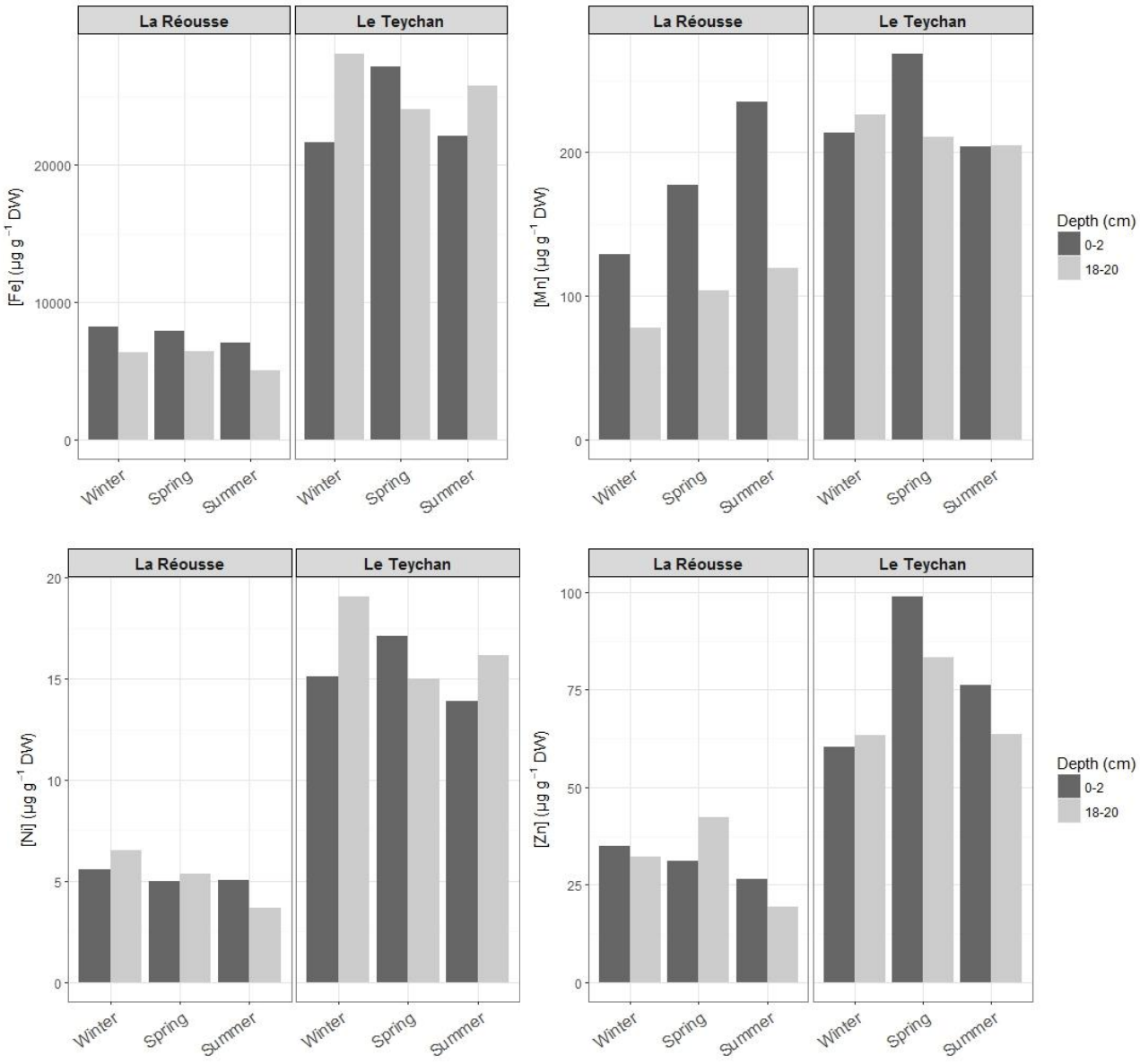


Appendix 4 (A) *Upogebia* cf. *pusilla* gill tissue infected with numerus metacercariae. Scale bar = 250 µm; (B) isolated encysted metacercaria. Scale bar = 100 µm.

Relationships between metal content and parasitic pressure in the bioturbating mud shrimp *Upogebia* cf. *pusilla*: a field survey



Appendix 5 Concentrations of metals (µg g^{-1} DW) over time (seasonal sampling) in the sediments of the two sampling sites (La Réousse and Le Teychan) of the seasonal survey conducted in Arcachon Bay, France. Concentrations were determined in sediments at the sediment-water interface (depth = 0–2 cm) and in deeper horizons of the sediment column (depth = 18–20 cm).



Appendix 5 Suite.

Appendix 6 Results of Kendall rank correlations evaluating the relationship between trematode abundances in bopyrid-uninfested mud shrimp *Upogebia* cf. *pusilla* and their metal burden. P-values in bold indicate statistically significant effect. Mud shrimp were categorized according to the site they have been sampled on (La Réousse and Le Teychan) and their sex.

	La Réousse				**Le Teychan**			
	Females		**Males**		**Females**		**Males**	
	τ	p	τ	p	τ	p	τ	p
FALL								
Ag	-0.40	0.48	0.40	0.48	-0.32	0.45	0.32	0.45
As	0.20	0.82	0.60	0.23	-0.11	0.80	-0.32	0.45
Cd	0.20	0.82	0.60	0.23	0.32	0.45	0.53	0.21
Cu	0.00	1	0.80	0.083	0.32	0.45	0.11	0.800
Fe	0.00	1	0.60	0.23	0.11	0.80	0.31	0.45
Mn	0.80	0.083	0.60	0.23	0.53	0.21	0.53	0.21
Ni	-0.60	0.23	0.80	0.083	0.32	0.45	0.11	0.80
Zn	0.00	1.00	0.60	0.23	0.32	0.45	-0.11	0.80
WINTER								
Ag	0.60	0.23	-0.40	0.48	-0.53	0.21	0.36	0.41
As	0.60	0.23	0.60	0.23	-0.32	0.45	0.12	0.78
Cd	0.80	0.083	0.00	1	0.32	0.45	0.12	0.78
Cu	0.80	0.083	0.20	0.82	-0.53	0.21	0.36	0.41
Fe	-0.20	0.82	0.40	0.48	-0.53	0.21	0.12	0.78
Mn	0.00	1	0.40	0.48	-0.53	0.21	0.60	0.17
Ni	0.20	0.82	0.40	0.48	-0.53	0.21	-0.12	78.00
Zn	0.40	0.48	-0.20	0.82	0.11	0.80	0.12	0.78
SPRING								
Ag	0.20	0.82	0.00	1	0.11	0.80	-0.36	0.41
As	0.20	0.82	0.80	0.08	0.53	0.21	0.84	0.052
Cd	-0.40	0.48	0.00	1.00	-0.32	0.45	0.84	0.052
Cu	-0.20	0.82	0.40	0.48	-0.11	0.80	0.60	0.17
Fe	0.20	0.82	-0.20	0.82	0.32	0.45	-0.12	0.78
Mn	0.60	0.23	0.40	0.48	0.74	0.08	-0.60	0.17
Ni	0.20	0.82	0.20	0.82	0.11	0.80	-0.12	0.78
Zn	0.40	0.48	0.20	0.82	0.53	0.21	0.12	0.78
SUMMER								
Ag	-0.20	0.82	0.40	0.48	-0.53	0.21	-0.32	0.45
As	-0.20	0.82	0.20	0.82	0.32	0.45	0.53	0.21
Cd	0.60	0.23	0.20	0.82	-0.11	0.80	-0.11	0.80
Cu	0.20	0.82	0.20	0.82	0.11	0.80	0.11	0.80
Fe	0.40	0.48	0.00	1	-0.53	0.21	0.32	0.45
Mn	0.40	0.48	0.00	1	0.11	0.80	0.32	0.45
Ni	0.40	0.48	0.20	0.82	-0.11	0.80	-0.11	0.80
Zn	-0.20	0.82	0.20	0.82	0.11	0.80	0.32	0.45

Appendix 7 Results of Kendall rank correlations evaluating the relationship between trematode abundances in bopyrid-uninfested mud shrimp *Upogebia* cf. *pusilla* and gene expression. P-values in bold indicate statistically significant effect. Mud shrimp were categorized according to the site they have been sampled on (La Réousse and Le Teychan) and their sex.

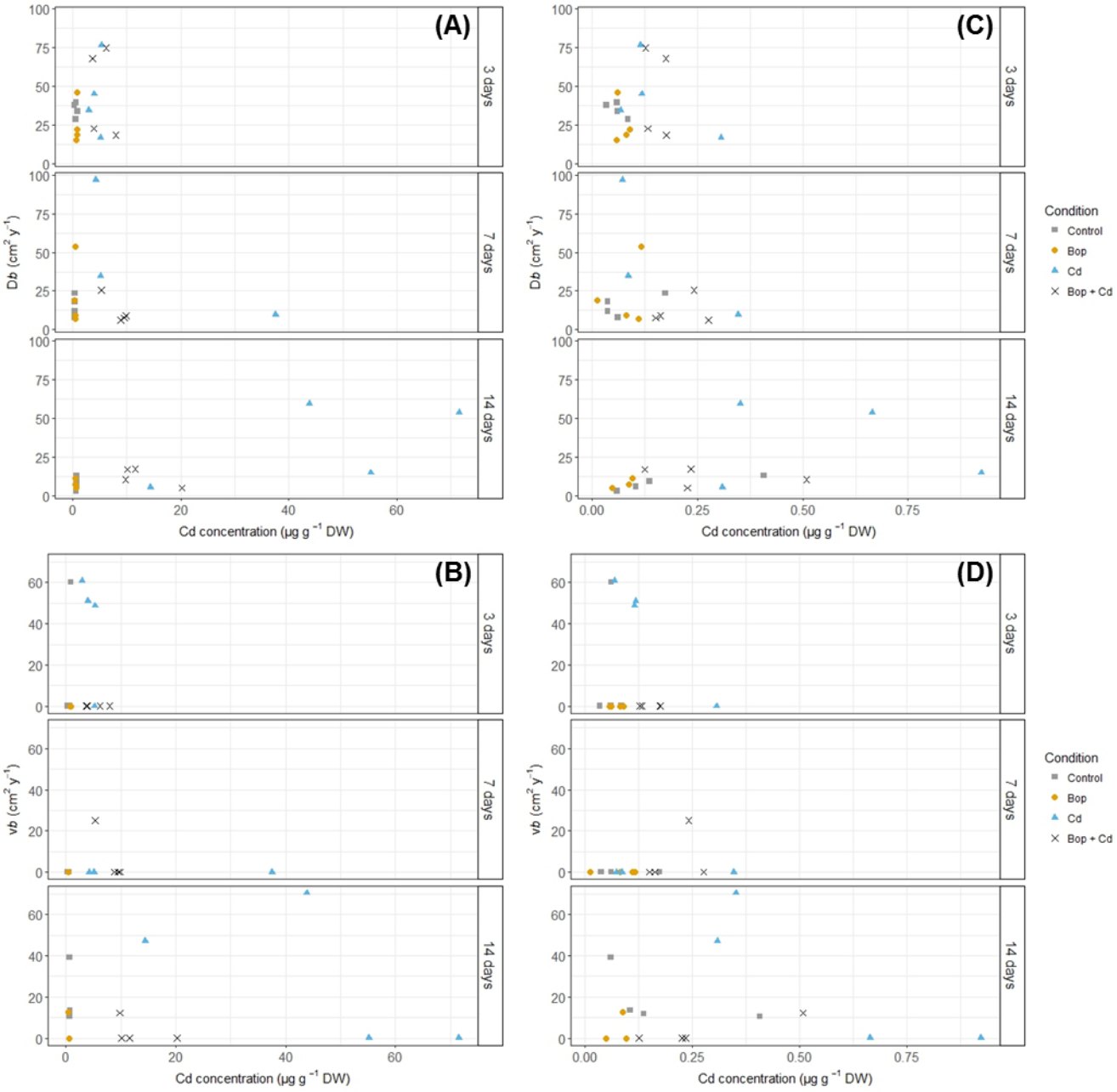
	La Réousse				**Le Teychan**			
	Females		**Males**		**Females**		**Males**	
FALL	τ	p	τ	p	τ	p	τ	p
12S	-0.60	0.23	-0.40	0.48	-0.11	0.80	0.110	0.80
cat	-0.20	0.82	0.40	0.48	0.11	0.80	0.32	0.45
cox I	-0.20	0.82	0.0	1	-0.53	0.21	0.00	1
fer	-0.40	0.48	-0.20	0.82	-0.11	0.80	0.53	0.21
hemo	-0.67	0.33	0.40	0.48	-0.11	0.80	0.32	0.45
nos	0.80	0.08	0.11	0.80	0.11	0.80	-0.11	0.80
rxr	0.60	0.23	-0.80	0.08	0.53	0.21	-11.00	0.80
sod Mn	0.60	0.23	-0.20	0.82	0.53	0.21	-0.33	0.75
vtg	-0.20	0.82	0.60	0.23	0.32	0.45	0.32	0.45
WINTER								
12S	-0.40	0.48	-0.53	0.21	0.32	0.45	-0.36	0.41
cat	-0.11	0.80	0.32	0.45	-0.11	0.80	-0.12	0.78
cox I	0.20	0.82	-0.20	0.82	-0.11	0.80	-0.36	0.71
fer	-0.20	0.82	-0.20	0.82	-0.53	0.21	-0.12	0.78
hemo	0.00	1	-0.20	0.82	-0.53	0.21	-0.36	0.41
nos	-0.20	0.82	-0.20	0.82	0.55	0.28	0.04	0.41
rxr	-0.20	0.82	-0.40	0.48	-0.74	0.077	0.36	0.41
sod Mn	-0.95	**< 0.05**	-0.80	0.083	-0.53	0.21	0.12	0.78
vtg	0.32	0.45	0.40	0.48	0.11	0.80	-0.84	0.052
SPRING								
12S	-0.20	0.82	1.00	**< 0.05**	-0.33	0.75	0.12	0.78
cat	-0.20	0.82	0.2	0.82	0.32	0.45	-0.12	0.78
cox I	-0.40	0.48	-0.80	0.083	0.53	0.21	0.36	0.41
fer	-0.40	0.48	0.20	0.82	0.32	0.45	-0.12	78.00
hemo	-0.40	0.48	-0.60	0.23	0.11	0.80	0.12	0.78
nos	-0.11	0.8	1.00	**< 0.05**	-0.82	0.22	-0.91	0.071
rxr	-0.20	0.82	-0.20	0.82	0.11	0.80	0.36	0.41
sod Mn	-0.60	0.23	0.20	0.82	-0.11	0.80	-0.36	0.41
vtg	0.00	1	-0.20	0.82	0.32	0.45	-0.91	0.071
SUMMER								
12S	-0.60	0.23	0.20	0.82	0.11	0.80	0.22	0.60
cat	0.00	1	0.20	0.82	-0.32	0.45	0.11	0.80
cox I	-8.00	0.083	0.40	0.48	-0.32	0.45	0.11	0.80
fer	0.00	1	1.00	**< 0.05**	0.11	0.80	-0.32	0.45
hemo	-0.67	0.33	0.20	0.82	0.33	1.00	0.32	0.45
nos	0.60	0.23	0.00	1	0.32	0.45	0.33	0.75
rxr	0.00	1	0.20	0.82	0.32	0.45	0.32	0.45
sod Mn	0.00	1	0.40	0.48	0.11	0.80	0.32	0.45
vtg	0.20	0.82	0.00	1	-0.32	0.45	0.11	0.80

Influence of the bioturbator *Upogebia* cf. *pusilla* on trace metal remobilization: Does parasitism matter?

Appendix 8 Trace metal concentrations (µg g^{-1} dry weight) in Cd-spiked sediment. Trace metals were extracted with hydrofluoric acid.

Samples	As	Ag	Cd	Co	Cu	Ni	Pb	V	Zn
S1	4.62	0.150	0.430	2.57	3.93	7.23	12.2	31.1	46.4
S2	5.28	0.180	0.440	2.78	4.12	7.52	13.0	33.7	36.1
S3	5.46	0.190	0.450	2.97	4.59	6.82	13.1	34.6	47.8

Do trace metal contamination and parasitism influence the activities of the bioturbating mud shrimp *Upogebia* cf. *pusilla*?



Appendix 9 Relationships between biodiffusion (D_b; (A) and (C)) or bioadvection (v_b; (B) and (D)) coefficients and the Cd content in the mud shrimp hepatopancreas ((A) and (C)) or abdominal muscle ((B) and (D)) after 3, 7 and 14 days of experiment and depending on experimental conditions ("Control", "Bop"; "Cd" and "Bop + Cd"). "Control": bopyrid-free and Cd-uncontaminated *Upogebia* cf. *pusilla*; "Bop": bopyrid-infested and Cd-uncontaminated *U.* cf. *pusilla*; "Cd": bopyrid-free and Cd-contaminated *U.* cf. *pusilla* and "Bop + Cd": bopyrid-infested and Cd-contaminated *U.* cf. *pusilla*.

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