Évaluation de la contamination chimique et changement du régime alimentaire des marsouins communs (Phocoena Phocoena) échoués au sud de la mer du Nord

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Thèse de doctorat de l'Université du Littoral

École Doctorale 104 *"Sciences de la matière, du rayonnement et de l'environnement"*
Spécialité: Géosciences, Ecologie, Paléontologie, Océanographie

An assessment of the chemical contamination and the diet changes of the harbour porpoise (*Phocoena phocoena*) stranded along the southern North Sea

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Soutenue le 26 Novembre 2014

pour obtenir le grade de Docteur de l'Université du Littoral Côte d'Opale

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Acknowledgements

This thesis was co-directed and funded by the CNRS Lebanon and ULCO. I would like to thank both organizations for their financial support during the three years of this PhD.

I would like to thank the Jury members: Professors Florence Caurant, Zitouni Boutiba, Baghdade Oudane and Tarik Meziane for accepting to judge this work.

I would like to express my deepest gratitude to my two PhD supervisors Professors Gaby Khalaf and Rachid Amara for their expert supervision and mentorship. Thank you Gaby for your enthusiastic, energetic and generous support. Thank you Rachid for your availability, your instructive comments and continuous support and for sharing your knowledge with me. I could not have asked for better supervisors!

I am very grateful to my PhD co-supervisor Dr. Françoise Henry for her generous commitment for helping me in this thesis and for carefully driving me to its final completion. Thank you Françoise for your careful and instructive comments and for your friendly and continuous support.

I would like to thank OCEAMM and CRMM for providing the samples for this study. Thanks to Dr. Thierry Jauniaux for executing post-mortem examinations and providing samples and information on the animals from the southern North Sea. Thanks to Willy Dabin for providing samples from the Bay of Biscay.

Parts of the analyses in this thesis were done in foreign labs. I would like to thank Professor Tarik Meziane for his involvement in the thesis. Thank you for forming and initiating me to the fatty acids world! Thank you for letting me perform the fatty acids analyses in the lab BOREA, MNHN and thanks to Mrs. Najet Thiney for her help in the analyses during my stay. I would also like to thank Professor Florence Caurant for welcoming me in the LIENSs lab, ULR to perform the stable isotope analyses and Ms. Paula Méndez-Fernandez for her instructions on how to prepare the samples.

I wish to thank all my colleagues at the National Centre for Marine Sciences (Lebanon) and at the MREN (France) which have provided a pleasant and inspiring atmosphere during the thesis.

I couldn’t imagine my life in France without you Louis, Mira, Joelle and Sahar! From the first month you made my life easier. You helped me in every little detail. In spite of the distance, Nadine your encouragement and support have remained constant. You all know that you are more than friends to me! Thank you.

My big happy family! I would like to thank you all. Each one of you helped me and encouraged me in a way. Thank you Eddy, Nancy, Sandy, Jad, Chady, Joanne, Fida, Soha, Wassim and Joe. My Nephews and nieces, I love u loads Joey, Rayo, Kouda, Alexou, Bicho and Touti.

Zahi, you were present from the beginning of the thesis and you always found the perfect words to support me. You made these three years easier! There are no words to convey how much I love you. You have been a true and great supporter and have unconditionally loved me during my good and bad times. My best friend, soul-mate and future husband, here’s to many more years to come.

Mom and Dad! I am here today thanks to your unconditional love and care support. I would not have made it this far without you. For your love and everything, I dedicate this thesis to you.

"If you really think that the environment is less important than the economy

Try holding your breath while you count your money"

Dr. Guy McPherson

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PREFACE

This thesis is in collaboration with Université du Littoral (ULCO) and the Lebanese National council for scientific research (CNRS).

As part of this agreement, the student Celine Mahfouz has received a research grant financed by the Lebanese CNRS and ULCO.

The work of this thesis was performed in Laboratoire d'Océanologie et de Géosciences (LOG) at Maison de la Recherche en Environnement Naturel (MREN) in Wimereux under the supervision of Rachid AMARA and the co-supervision of Françoise HENRY.

RESUME DETAILLE DE LA THESE

La thèse étant rédigée en anglais, ce résumé présente en français, de façon synthétique, le contexte, les objectifs et les principaux résultats et conclusions de ce travail.

Les mammifères marins sont largement distribués dans les mers et les océans du monde entier. Différentes études ont montré que leur distribution est influencée par différents facteurs physiques et biologiques, notamment la bathymétrie, la topographie, la température, la salinité, les courants, la répartition des proies, les stratégies de reproduction, la prédation et la compétition interspécifique (Baumgartner et al., 2000; Davis et al., 2002; Kiszka et al., 2007). Les mammifères marins en tant que prédateurs supérieurs jouent un rôle d'intégrateur des perturbations du réseau trophique et par suite contribuent à établir un équilibre au niveau de l'écosystème marin. En retour, la diminution de l'abondance des mammifères marins peut affecter d'autres composants de l'écosystème (Mangel and Hofman, 1999). De plus, les mammifères marins sont de plus en plus affectés par les activités humaines telles que les prises accidentelles dans les engins de pêche, les collisions avec les navires, les changements de la ressource trophique en qualité et en quantité, le changement climatique, les pollutions de toute natures (chimiques, sonores, etc.), les maladies, la dégradation de l'habitat, etc. En raison de leur statut particulier d'espèces protégées dans la plupart des pays, les mammifères marins sont difficiles à étudier et ne peuvent faire l'objet d'échantillonnages et de prélèvements comme cela peut être fait avec d'autres organismes marins. Les informations disponibles sont limitées aux observations visuelles ou acoustiques (localisation, comptages, suivi), les échouages et les prises accidentelles dans les filets de pêche (Haelters and Camphuysen, 2009). Des investigations post-mortem peuvent être menées sur les mammifères marins échoués afin d'évaluer entre autres leur stade de reproduction. De même les prélèvements d'organes et de tissus permettent d'effectuer des analyses biologiques et chimiques (Jauniaux et al., 2002a). Par conséquent, les mammifères marins échoués peuvent fournir des informations sur leur écologie et renseigner sur les impacts des activités anthropiques. Ces prédateurs supérieurs peuvent aussi être utilisés comme modèle biologique pour l'étude des polluants dans les écosystèmes marins (Mangel and Hofman, 1999).

Notre étude s'est focalisée sur le marsouin commun (*Phocoena phocoena*) qui est le mammifère marin le plus commun et le plus abondant dans la mer du Nord et les mers adjacentes (Haelters and Camphuysen, 2009; Hammond et al., 2013). Cette espèce est généralement rencontrée au niveau du plateau continental des eaux tempérées de

l'hémisphère Nord (Reid et al., 2003). Depuis les années 2000, le nombre de marsouins communs échoué au sud de la mer du Nord n'a cessé d'augmenter surtout le long des côtes françaises et belges (Haelters and Camphuysen, 2009). Sur le littoral Français en 2013, le marsouin commun est pour la première fois depuis que les relevés existent, l'espèce la plus représentée dans les échouages avec 43.7% des échouages, juste devant le dauphin commun (42.4%) (Van Canneyt et al., 2014). De plus, parmi les 6 espèces de mammifères marins échouées dans la Manche et la mer du Nord (sur la côte française) en 2013, le marsouin commun est l'espèce prédominante dans la composition des échouages avec plus de 90% du total des animaux échoués. Une campagne d'observation à grande échelle soutenue par la Commission Européenne et plusieurs organisations internationales a été mise en place en été 1994 pour déterminer l'abondance des petits cétacés dans la mer du Nord et les mers adjacentes (SCANS). Cette campagne a déterminé l'abondance du marsouin commun à près de 340 000 animaux (Hammond et al., 2002). Près de dix ans plus tard, une deuxième campagne réalisée durant l'été 2005 (SCANS II) a montré que l'abondance du marsouin n'a pas évoluée (Hammond et al., 2013). Par contre ces 2 campagnes mettent en évidence un changement majeur dans la répartition spatiale du marsouin qui s'est déplacé du Nord vers le Sud de la mer du Nord.

Dans ce contexte, du fait des pressions anthropiques importantes au sud de la mer du Nord, cette étude vise à définir la ou les causes du déplacement de la population vers le sud ainsi que l'augmentation des échouages observés ces dernières années. Nous avons focalisé notre étude sur la pollution chimique et le régime alimentaire en lien avec la disponibilité des proies. Le premier objectif de cette étude a été d'évaluer le niveau de la contamination chimique de cette espèce sur des animaux en bon état de conservation échoués sur les côtes françaises et belges du sud de la mer du Nord. Un deuxième objectif a été de déterminer si les changements dans la répartition des marsouins dans le sud de la mer du Nord peuvent être le résultat du changement de la disponibilité de leurs proies. L'étude du régime alimentaire a été appréhendée par trois méthodes : l'analyse des contenus stomacaux, l'analyse des isotopes stables (carbone et azote) et l'analyse des acides gras. L'intérêt de l'utilisation conjointe de ces trois méthodes est aussi discuté.

Evaluation du niveau de la contamination métallique du marsouin commun échoué au sud de la mer du Nord

Cette première partie propose un suivi de l'état de la contamination métallique sur des marsouins échoués sur la période de 2006 à 2013 au sud de la mer du Nord (côtes française et belge). Les teneurs en éléments traces (As, Cd, Cr, Cu, Hg, Mn, Se, V et Zn) ont été déterminées dans les foies et reins de 105 marsouins échoués de 2006 à 2013 et 27 marsouins échoués dans le golfe de Gascogne à titre de comparaison géographique (côte française de 2009 à 2012). Ces éléments ont été analysés par ICP-MS (spectrométrie de masse par plasma à couplage inductif) ou ICP-AES (spectrométrie d'émission atomique par plasma à couplage inductif) et le mercure a été dosé par SAA (spectrométrie d'absorption atomique). Les niveaux de concentration ont été discutés en rapport avec l'âge, le genre et l'état de santé des individus.

Les marsouins échoués au sud de la mer du Nord et ceux échoués dans le golfe de Gascogne présentent des niveaux comparables de contaminants dans leurs tissus. Les concentrations en métaux sont du même ordre de grandeur que les valeurs rapportées pour des marsouins échoués dans le sud de la mer du Nord (Nord de la France et Belgique) entre 1994 et 2001 (Das et al., 2004b) et des marsouins échoués sur les côtes Françaises entre 1997 et 2003 (Lahaye et al., 2007). Ces niveaux de concentration déterminés dans la présente étude restent inférieurs à ceux obtenus dans les marsouins échoués sur les côtes Anglaises (Jepson, 2003).

Les marsouins qui sont morts suite à des maladies infectieuses montrent des concentrations hépatiques en Cd, Hg, Se, Zn et V significativement plus élevées que ceux échoués suite à des chocs accidentels. De plus, les marsouins adultes montrent des concentrations significativement plus élevées en Cd, Cr, Hg, Se and V dans leurs foies comparés aux juvéniles. Le foie et le rein sont connus comme étant les organes de rétention du Hg et du Cd, respectivement. Le Hg est particulièrement préoccupant en raison de ses propriétés de bioamplification dans les niveaux supérieurs de chaîne trophique, sa mobilité et sa persistance dans le milieu marin (Palmisano et al., 1995; Das et al., 2003a). Sa bioaccumulation avec l'âge a été décrite chez différents mammifères marins (Wagemann and Muir, 1984; Wagemann et al., 1996). Le Hg hépatique a été associé à la sévérité de la maladie chez les marsouins dans les eaux allemandes (Siebert et al., 1999). Dans notre étude, seulement trois animaux morts suite à des maladies infectieuses ont des concentrations en Hg supérieures au seuil estimé par AMAP (1998) (200 $\mu g.g^{-1}$ dw) mais reste inférieures au seuil estimé par Wagemann et Muir (1984) (350 $\mu g.g^{-1}$ dw) à partir desquels des dommages hépatiques pourront avoir lieu chez les mammifères marins.

Pour le Cd, les concentrations sont significativement plus élevées chez les adultes comparés aux juvéniles. La principale source de contamination des mammifères marins se fait principalement par voie trophique (Wang and Rainbow, 2010). Des concentrations élevées en Cd dans les foies et les reins des mammifères marins ont été attribuées dans des études précédentes aux céphalopodes qui font partie de leur régime alimentaire et qui sont considérés comme une source importante de Cd (Bustamante et al., 1998; Das et al., 2000). Des différences dans le régime alimentaire entre juvéniles et adultes ont été reportées dans des études précédentes en suggérant que les juvéniles ne peuvent pas plonger aux mêmes profondeurs à cause de leurs petites tailles (Santos and Pierce, 2003). Les résultats de notre étude suggèrent que les différences d'enrichissement en métaux entre juvéniles et adultes sont dues au processus de bioaccumulation avec l'âge, en relation avec un comportement alimentaire différent plus ou moins marqué pour certains éléments.

Les marsouins échoués au sud de la mer du Nord, pour la période 2006-2013 (cette étude) et 1994-2001 (Das et al., 2004b) ne montrent pas d'évolution temporelle significative des concentrations en métaux. Le niveau de contamination métallique des marsouins communs dans la région est a priori stable depuis 1994. Ces résultats suggèrent que la contamination chimique au sud de la mer du Nord ne peut pas être la principale cause de l'augmentation du nombre d'échouage dans cette zone.

Les organochlorés dans les tissus des marsouins communs échoués au sud de la mer du Nord entre 2010 et 2013

7 polychlorobiphényles (PCBs), 6 dichlorodiphényltrichloroéthane (DDXs) et 8 polybromodiphényléther (PBDEs) ont été déterminés dans le lard de 20 marsouins communs échoués au sud de la mer du Nord entre 2010 et 2013. En raison de leur caractère lipophile, les polluants organiques persistants (POPs) ont une forte affinité pour les tissus adipeux comme le lard.

Les marsouins morts suite à des maladies infectieuses montrent des niveaux supérieurs en PCBs comparés aux animaux sains qui sont morts suite à des captures accidentelles. Les mêmes tendances ont été reportées dans d'autres études sur des marsouins échoués sur les côtes anglaises (Jepson et al., 1999 ; 2005). Ces auteurs ont suggéré que les maladies infectieuses préexistantes peuvent provoquer une dégradation métabolique des réserves lipidiques et ainsi révéler des niveaux élevés en PCBs dans le lard des marsouins.

L'accumulation des POPs chez les mammifères marins adultes a été démontrée dans la littérature (Kleivane et al., 1995; Weijs et al., 2009b). Cette tendance ne peut pas être vérifiée dans notre étude puisqu'un seul marsouin adulte a été analysé. Par contre, la somme des 7 CBs (CB 28, 52, 101, 118, 153, 138 and 180) et des 6 DDXs (*o,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT and *p,p'*-DDE) sont plus élevées chez les juvéniles par rapport aux adultes femelles. A l'exception de 3 individus, les concentrations en PBDEs sont inférieures à la limite de quantification dans le lard des animaux analysés. Il a été démontré que les faibles niveaux de POPs chez les femelles adultes sont expliqués par le transfert des contaminants organochlorés à leurs descendants durant la gestation et la lactation (Aguilar and Borrell, 1994; Tanabe et al., 1994; Westgate et al., 1997; Jepson et al., 1999). Ces résultats peuvent expliquer les variations du niveau des POPs entre les adultes femelles et les marsouins juvéniles échoués au sud de la mer du Nord.

Les concentrations en PCBs et DDXs obtenues dans le lard des marsouins analysés dans notre étude sont du même ordre de grandeur et même inférieures aux niveaux déterminés chez les marsouins échoués le long du Nord Est de l'Atlantique et de la mer Noire entre 1987 et 2013. Du fait de l'interdiction de l'utilisation de ces composés en 1970s et 1980s, la présence des organochlorés devrait être en diminution dans l'environnement marin. Différents auteurs ont montré cette tendance à la diminution des niveaux des POPs chez les mammifères marins (Westgate et al., 1997; Jepson et al., 2005; Law, 2014). Les études actuelles sur cette thématique suggèrent que même si les niveaux d'organochlorés diminuent dans le milieu marin, ils demeurent préoccupant dans les tissus et donc capable de causer des effets néfastes immunologiques. Dans notre étude, 60% des animaux analysés présentent des concentrations en PCBs supérieures au seuil déterminé (17 $\mu g.g^{-1}$ lipides) à partir duquel des effets néfastes immunologiques peuvent avoir lieu chez les mammifères marins (Kannan et al., 2000). Par conséquent, il est important de continuer à surveiller les niveaux des organochlorés dans les tissus du marsouin commun en tant qu'intégrateurs des différents maillons trophiques de l'écosystème marin.

Le régime alimentaire du marsouin commun dans la mer du Nord en relation avec la disponibilité des proies

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Le changement de l'abondance du marsouin commun ainsi que son déplacement vers le sud de la mer du Nord a été évalué et interprété en relation avec l'abondance des proies

potentielles. Dans ce contexte, le régime alimentaire des marsouins communs échoués au sud de la mer du Nord entre 2010 et 2013 a été déterminé par le biais de trois méthodes qui renseignent sur les proies consommées à différentes échelles de temps: l'analyse des contenus stomacaux, l'analyse des isotopes stables (carbone et azotes) dans le muscle et l'analyse des acides gras dans le lard. Le profil des acides gras et les valeurs des isotopes stables des marsouins ont été comparés à ceux des proies potentielles du marsouin commun. A cet effet, 59 marsouins communs et 14 proies potentielles ont été analysés.

L'analyse des contenus stomacaux consiste à identifier les restes de proies ingérées qui sont présents sous forme d'otolithes, d'os de poissons et de becs de céphalopodes jusqu'au plus petit taxon possible. Elle renseigne sur les proies consommées récemment (quelques jours). La méthode des isotopes stables se base sur le fait que la signature isotopique se conserve ou est prévisible avec un enrichissement attendu d'un maillon trophique à l'autre (DeNiro and Epstein, 1978). Enfin, la méthode des acides gras est basée sur le fait que ces derniers sont déposés dans les tissus adipeux avec peu de modification ou d'une manière prévisible, pouvant ainsi fournir des renseignements sur le régime alimentaire à l'échelle de quelques mois (Budge et al., 2006).

La combinaison des trois techniques a mis en évidence la présence des proies potentielles suivantes dans le régime alimentaire du marsouin commun: gobies, merlan, lançon, sprat, trisopterus sp., hareng et sardine. A l'exception de la sardine, ces espèces figurent parmi les espèces de poisson les plus abondantes en mer du Nord (Daan et al., 1990; ICES, 2013b). Depuis les années 2000, l'abondance relative du lançon a fortement diminuée en mer du Nord (SIH-Ifremer, 2014). Dans plusieurs écosystèmes, le lançon est une espèce très importante pour les prédateurs supérieurs, aussi bien pour les mammifères marins que pour l'avifaune (Hain et al., 1995; Frederiksen et al., 2007). Une étude antérieure a montré qu'une sous alimentation constaté chez les marsouins dans la mer du Nord aux printemps 2002 et 2003 au niveau de l'Écosse était le résultat d'une raréfaction du lançon et d'une diminution de sa consommation (MacLeod et al., 2007). Par ailleurs, la sardine n'a jamais été présente dans le régime alimentaire du marsouin dans les études antérieures selon les contenus stomacaux. Depuis les années 90, le retour de la sardine au sud de la mer du Nord a été attribué au changement climatique (Beare et al., 2004 a; b). Par conséquent, peu d'informations sont disponibles sur cette espèce qui est considérée comme occupant occasionnel de la mer du Nord.

Dans notre étude, le déplacement des marsouins du nord jusqu'au sud de la mer du Nord peut être attribué à la baisse de l'abondance du lançon dans le nord, ainsi qu'à la ré-invasion du sud de la mer du nord par la sardine. Aussi, le changement dans la répartition des marsouins est probablement lié au changement dans la disponibilité des proies. Néanmoins, d'autres facteurs tels que les captures accidentelles (augmentation des activités de pêche), les pollutions acoustiques suite aux aménagements de certaines zones côtières en mer du Nord et le réchauffement climatique peuvent aussi conduire à des changements dans la répartition des marsouins et l'augmentation des échouages.

Le régime alimentaire des marsouins communs dans le sud de la mer du Nord et dans le golfe de Gascogne déduit d'une approche multi-analyses

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L'étude de l'écologie alimentaire des mammifères marins permet entre autres d'étudier leur stratégie alimentaire, les relations prédateurs-proies, leurs interactions avec la pêche et leurs réponses aux changements climatiques. Cette partie compare le régime alimentaire des marsouins communs échoués dans le sud de la mer du Nord entre 2010 et 2013 et ceux échoués dans le golfe de Gascogne entre 2009 et 2012. Ces deux zones diffèrent par la nature et la diversité des proies, le type de fond et les conditions océanographiques.

D'après l'analyse des contenus stomacaux, les marsouins du sud de la mer du Nord se nourrissent surtout d'espèces démersales, benthiques et côtière, alors que les marsouins du golfe de Gascogne se nourrissent plutôt d'espèces benthiques, démersales et pélagiques. Les animaux échoués dans le sud de la mer du Nord ont montré des signatures isotopiques significativement plus élevées en $\delta^{15}N$ par rapport à ceux échoués dans le golfe de Gascogne. Cette différence a été attribuée à la signature du rapport $\delta^{15}N$ à la base de la chaîne alimentaire qui est différente (Jennings and Warr, 2003) plutôt qu'à des différences dans le réseau trophique des marsouins habitant chaque zone. De même, les comparaisons des signatures en $\delta^{13}C$ des deux consommateurs, ne permet pas d'établir si les différences reflètent des changements dans les stratégies alimentaires ou bien si c'est lié à une signature de base différente du réseau trophique (Barnes et al., 2009). Les acides gras qui proviennent de l'alimentation tels que le 20:1ω9 et 22:1ω11/9 présents dans le lard des marsouins du sud de la mer du Nord suggèrent que ces animaux se nourrissent de proies planctonivores telles que le hareng. En outre, l'acide gras 20:4ω6, marqueur des algues benthiques littorales (Dalsgaard et al., 2003) est présent en grandes quantités dans le muscle des poissons

benthiques telles que les gobies, le merlan et le tacaud. Ces marqueurs n'ont pas été trouvés dans le lard du marsouin, bien que selon l'analyse des contenus stomacaux ces trois espèces sont présentes dans le régime alimentaire du marsouin. L'analyse des contenus stomacaux peut ne refléter que l'alimentation à court terme en faveur des espèces côtières, alors que l'analyse des isotopes stables et des acides gras reflètent les proies consommées à moyen terme. La comparaison des résultats de cette étude avec des études précédentes confirme que le régime alimentaire des marsouins varie selon les zones et probablement selon les années, les saisons et l'état de maturité (Bjørge et al., 2003; Santos et al., 2014). De plus, des différences dans le régime alimentaire du marsouin ont été attribuées à la disponibilité des proies dans le milieu ainsi qu'aux caractéristiques de l'habitat telle que la topographie (la pente et la profondeur).

Cette étude confirme la nécessité d'utiliser une approche multi-analyses qui intègre des informations complémentaires à différentes échelles de temps pour étudier le régime alimentaire des prédateurs supérieurs en lien avec ses proies dans une région donnée. En outre, il faut prendre des précautions sur les extrapolations des données sur le régime alimentaire du marsouin d'une zone à l'autre.

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CHAPTER 1

INTRODUCTION

Chapter 1 - Introduction

Marine mammals are widely distributed throughout the world's seas and oceans. A number of studies have identified a range of physical and biological factors that affect the distribution of marine mammals including bathymetry, topography, sea surface temperature, salinity, tidal currents, prey distribution, reproductive strategies, predation and inter-specific competition (Baumgartner et al., 2000; Davis et al., 2002; Kiszka et al., 2007). Due to their position as top predators in the ocean, marine mammals play an important role in the food web and help insure balance in the ocean's ecosystem; hence the decrease in the abundance of marine mammals affects other ecosystem components (Mangel and Hofman, 1999). Marine mammals are becoming increasingly affected by human activities and their worldwide distribution requires conservation and management on a global scale. Indeed, growing human population and an increase in the diversity of human uses and activities in the marine environment, especially concentrated along the coast, put marine resources under increased pressure. Actually 40% of the world's oceans are heavily impacted by human activities, particularly around European coasts (Halpern et al., 2008) (Figure 1.1). In this context, marine mammals are exposed to several potential threats such as bycatch, collision with vessels, depletion of prey resources, climate change, pollution, disease, habitat degradation, etc. Due to their conservation status, marine mammals are difficult to study. However, the little information available and limited data are obtained through sightings, strandings and incidental catches in commercial fisheries (Haelters and Camphuysen, 2009). Peaks in the number of stranded animals might reflect peaks in the number of animals at the sea. One should however take into account that bias can exist due to meteorological conditions, to an increased seasonal mortality due to bycatch or to a high mortality rate of juvenile individuals (Haelters and Camphuysen, 2009). Although, post-mortem investigations might be carried out on stranded or bycatch marine mammals to assess their health status, reproduction and other aspects of life history and to preserve samples for chemical and genetic analyses (Jauniaux et al., 2002a; OSPAR, 2013). Hence, stranded marine mammals may provide valuable information on their ecology and on human impacts and may be used as a useful indicator of certain pollutants in coastal marine ecosystems (Mangel and Hofman, 1999).

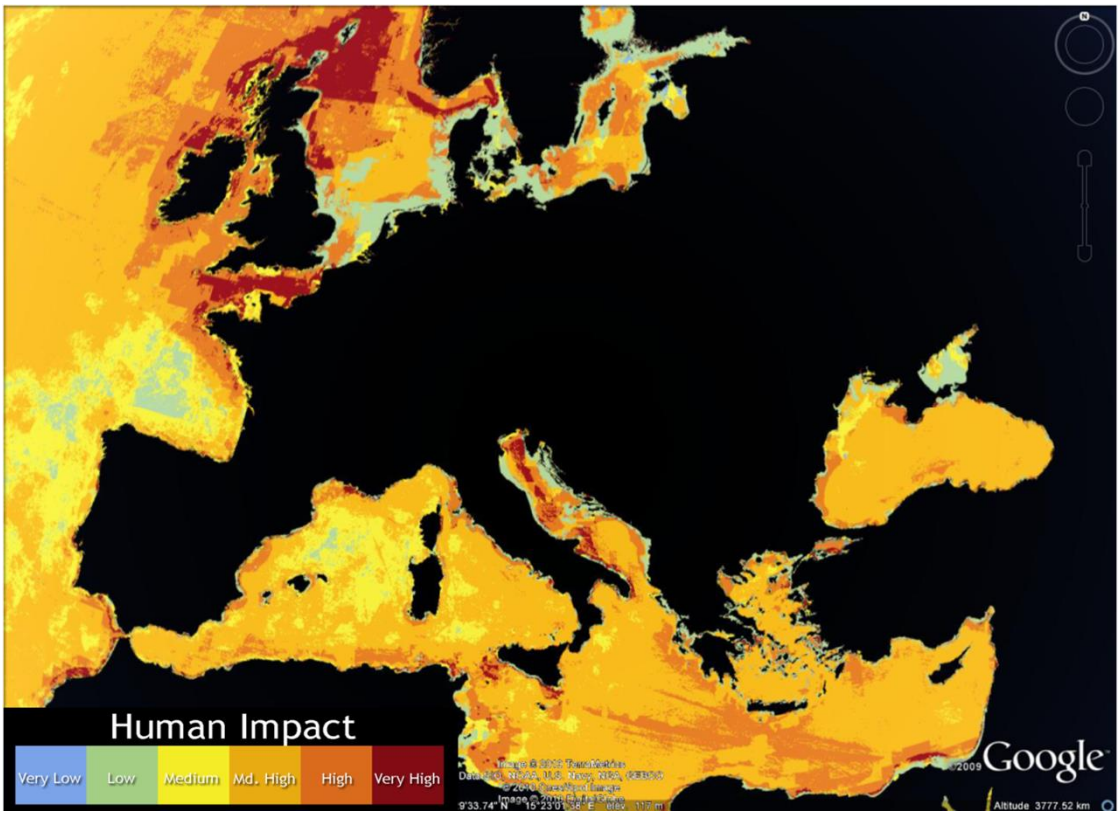


Figure 1.1 Global map of cumulative global impact in European marine Ecosystems (From Halpern et al., 2008).

The harbour porpoise (*Phocoena phocoena*)

Distribution and description

Harbour porpoise (*Phocoena phocoena*) (Linnaeus, 1767) is known to inhabit the continental shelf of temperate waters in the northern Hemisphere (Reid et al., 2003) as shown in figure 1.2. Within the eastern North Atlantic, harbour porpoise is common and widely distributed and abundant in Icelandic and Norwegian waters, the North Sea, the Kattegat, the Skagerrak, the British Isles, the eastern Channel and the Atlantic coast of France and Spain. The distribution of porpoises extends southward along the African coast to Mauritania (Boisseau et al., 2007; Haelters and Camphuysen, 2009; Hammond et al., 2013). However, two genetically distinct groups of porpoises along the Atlantic coasts of France were identified. One closely related to the Iberian and African harbour porpoises and the second related to the individuals from the more northern waters of Europe (Alfonsi et al., 2012).

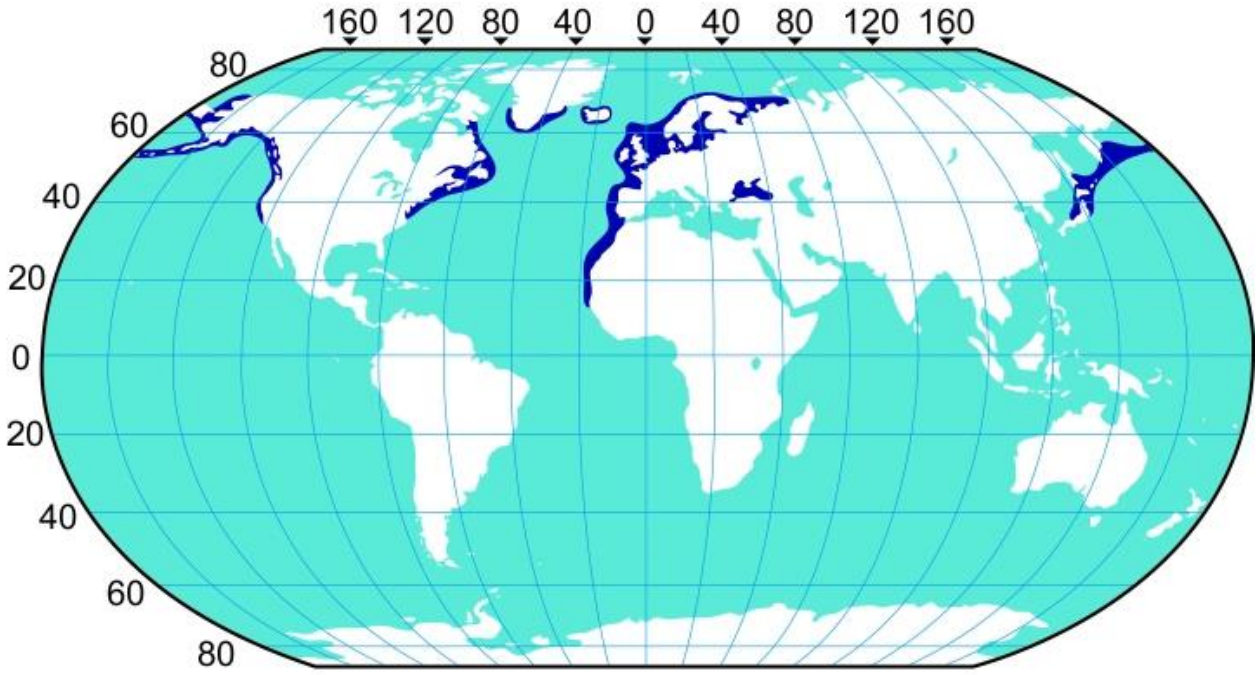


Figure 1.2 Harbour porpoise's spatial distribution map shown by the areas in dark blue. (Map from the American Cetacean Society, www.acsonline.org).

This odontocete is characterized by small flippers, rounded at the tips and oval in shape (Figure 1.3). The dorsal fin is broad based, low and triangular and is located slightly after the center of the body. Harbour porpoises are small cetaceans with extremely thick blubber layer which primarily role is insulation and energy storage (Koopman et al., 2002). They have an average life span of 20 years and they have a maximum length of only 1.8 m. At birth they weight about 5 kg with a range of 3-9 kg corresponding to 60-80 cm. They reach sexual maturity at the age of 3 to 4 years which corresponds to a body length of around 1.35 m in males, whereas the females reach sexual maturity at an age of 4 to 5 which corresponds to a body length of around 1.40 m. The gestation period is about 10 to 11 months with a lactation period that lasts about 8 months. Females give birth once a year (Gaskin, 1984; Lockyer, 1995).

Figure 1.3 Photo of a stranded harbour porpoise on the coast of northern France (OCEAMM).

Distribution on a local scale

The harbour porpoise is the most common and abundant marine mammal inhabiting the North Sea and adjacent waters (Haelters and Camphuysen, 2009; Hammond et al., 2013). This species is generally limited to the continental shelf. The shallow southern part of the North Sea and especially its coastal waters can be very turbid. However, porpoises in the North Sea can frequently be observed feeding close inshore, sometimes even in the shallow waters of the surf zone (Haelters and Camphuysen, 2009). In addition, in offshore waters of the southern North Sea, coordinated drive hunts for pelagic fish are frequently seen and typically conducted by small numbers of harbour porpoises sighted in groups of 1 to 5 animals (IFAW, 2011).

Throughout the southern North Sea, harbour porpoises were considered abundant in coastal waters from 1900 to the early 1950's. It appears that the number started to decline in this area and by the 1970's sightings were so rare. Therefore, during the 1970s and 1980s, stranding data of harbour porpoises in the southern North Sea was almost absent. However, since the 1990s increased number of stranded porpoises was recorded (Haelters and Camphuysen, 2009). Alongside, a large-scale survey supported by the European commission and a number of international organizations (SCANS: Small Cetacean Abundance in the North Sea) conducted along the North Sea in summer 1994 estimated the abundance of harbour porpoises to be 340 000 animals. During this survey, that covered the North Sea and adjacent waters, 9 ships (from 27 June to 26 July 1994) and 2 aircraft (between 26 June and 3

August) were used (Hammond et al., 2002). Almost 10 years later and during the second survey in summer 2005 (SCANS II), the abundance was estimated to be 375 000 animals (Hammond et al., 2013). SCANS II was surveyed by 7 ships (from 29 June to 28 July 2005) and 3 aircraft (from 27 June to 4 August 2005). Moreover, in SCANS II the Atlantic shelf from the Bay of Biscay to Portugal were added to the study area. A comparison between the results of the two major abundance and distribution surveys in the North Sea clearly highlights a major shift in the distribution from the northern parts of the North Sea to its eastern parts rather than a population increase. Abundance estimates did not show any significant change in the overall population sizes. In the SCANS 1994 survey, high density distribution of harbour porpoises were observed off the coastline of south-east Scotland and North East England and off the north and west coasts of Denmark, whereas in SCANS II 2005 survey, the main concentration in distribution has shifted further south to the southern North Sea (Figure 1.4).

Protection status of harbour porpoises in the European waters

In the European waters, the status of harbour porpoises has been of concern for many years. Conservation measures in form of several international, European and national (French) conventions and regulations were implemented in order to protect the harbour porpoise. At the international level, the Bern convention (1979) aims to conserve wild flora and fauna and their natural habitats and also aims to promote European cooperation in order to protect migratory species. In addition, the harbour porpoise figures on the IUCN (International Union for Conservation of Nature) red list of threatened species. Moreover, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) cited cetaceans as protected species against over-exploitation through international trade. At the European level, the ASCOBANS (Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas) conservation plan obliges signatories to apply the conservation, research and management measures prescribed in the annex. Those involve bycatch reduction, pollution control, research and monitoring. The Habitat Directive (92/43/EEC) list harbour porpoise in annex II and IV, which means that favorable conservation status of this species has to be achieved and Special Areas of Conservation (SACs) are to be designated for this species where needed. The European regulation (812/2004) determines the measures to take concerning incidental catches of cetaceans and aims to reduce by-catch in some fisheries. Finally at the national level, this species figures in

the French Decree of 27 July 1995 and the Decree of 9 July 1999 as protected endangered species. Moreover, the Oceans Round Table (Grenelle de la Mer 2009) has advised in the article 14.f to strengthen measures to protect/restore threatened marine species and/or marine sanctuaries and mammals by helping to create new sanctuaries and has also advised in the article 16.b to take the necessary measures to limit noise pollution, collisions with ships and incidental catches in fishing gear.

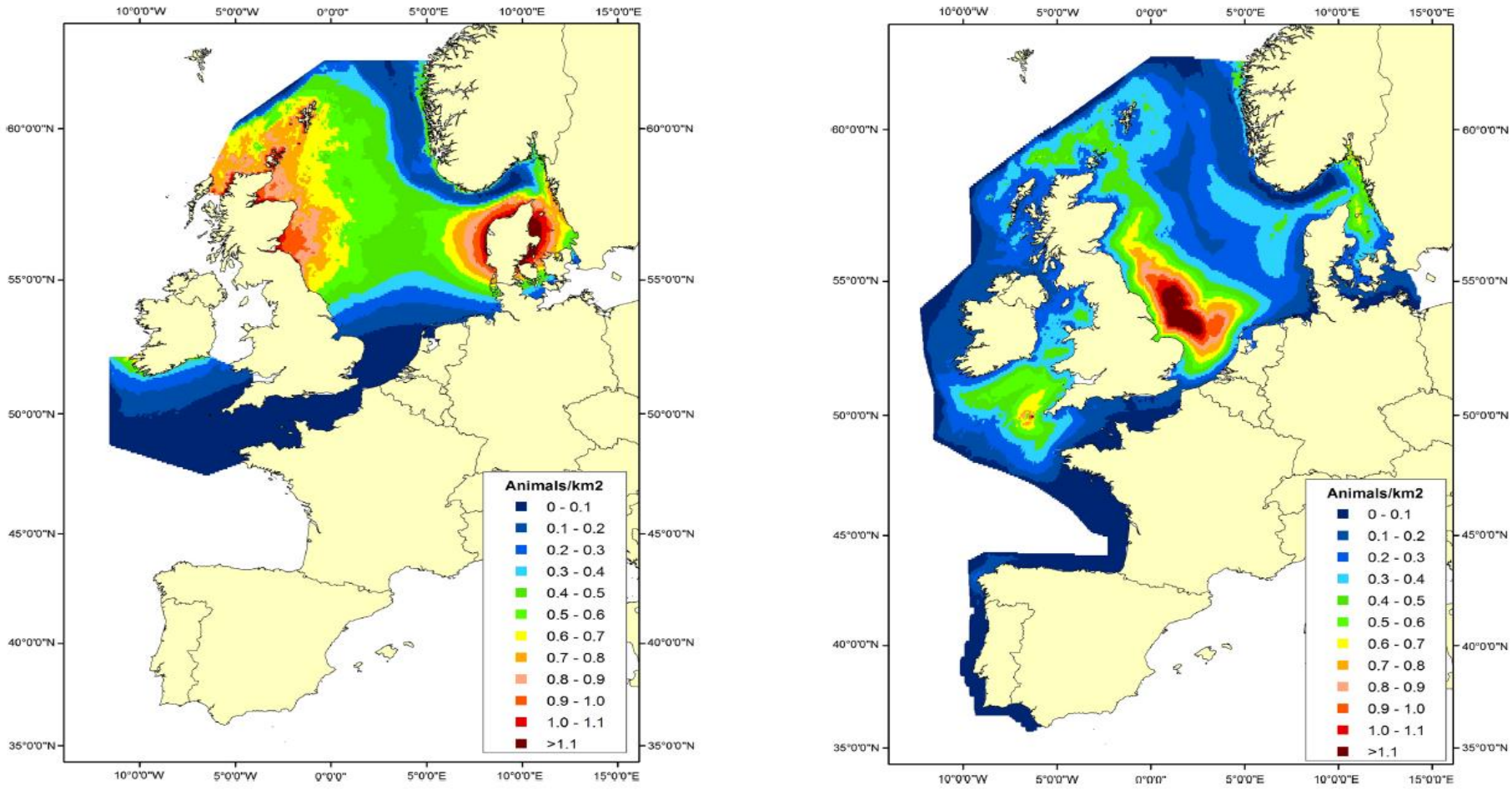


Figure 1.4 Predicted density surface for harbour porpoises in 1994 (SCANS survey, figure on the left) and in 2005 (SCANS II survey, figure on the right); the color bar indicates porpoises density (number of animals per km^2) (Hammond et al., 2013).

Diet of harbour porpoises

The diet arising from stomach content analysis for about 100 harbour porpoises stranded between 1989 and 1994 along the UK waters consisted mainly on gadoids, sandeels and gobies (Martin, 1996). In Scottish waters, about 188 stomachs investigated for porpoises stranded between 1992 and 2003 mainly included whiting, sandeels and gadoids such as Trisopterus sp. (Santos et al., 2004). In 2006, stomach contents of 64 porpoises stranded along the Dutch waters mainly included gobies, sandeels, sprat, herring, whiting and twait shad (Leopold and Camphuysen, 2006). A more recent study on 64 porpoises stranded between 1997 and 2011 along the Belgian waters showed that porpoise's stomachs mainly included gobies, sandeels and gadoids such as whiting and Trisopterus sp. (Haelters et al., 2012). More studies on the diet of harbour porpoises are presented in table 1.1.

Table 1.1 Some studies on harbour porpoise's diet inferred from stomach content analysis in the North Sea and adjacent areas. n: number of stomachs analyzed.

Area (year of stranding)	n	Main prey	Reference
Belgian coast (1997-2011)	64	Gobies, sandeels, whiting, trisopterus sp.	Haelters et al., 2012
Dutch coast (2006)	64	Gobies, sandeels, sprat, herring, whiting, twait shad	Leopold and Camphuysen, 2006
English Channel (1998-2003)	7	Pouting, gobies	De Pierrepont et al., 2005
Scotland (1992-2003)	188	Whiting, sandeels, gadids, trisopterus sp.	Santos et al., 2004
United Kingdom (1989-1994)	100	Gadids, sandeels, gobies	Martin, 1996
Germany	34	Sandeels, sole	Benke and Siebert, 1996
Denmark, Sweden, Norway	197	Herring, gadids	Aarefjord et al., 1995
Germany	36	Sole, cod	Lick, 1991
France	8	Blue whiting, scad, hake	Desportes, 1985
Scotland (1959-1971)	93	herring, sprat, whiting	Rae, 1965; 1973
Bay of Biscay (1988-2003)	26	Blue whiting, sardine, scads, whiting	Spitz et al., 2006

Threats

The North Sea is a large, semi-enclosed, epi-continental sea with a relatively shallow mean depth of 90 m with deeper areas in the north of the Norwegian Trench (700 m) (Ducrotoy et al., 2000) and surrounded by large and highly developed societies (Ducrotoy and Elliott, 2008). This area is subject to several anthropogenic activities such as fishing pressures and

coastal industrial activities (artisanal and industrial use of the coast). It supports large-scale commercial fishing and is an example of a region where fishing has substantially impacted forage fish populations (Dickey-Collas et al., 2014). The North Sea is regarded as a moderately polluted sea area where the input and transport of contaminants have been discussed in previous studies (Anderson et al., 1996; OSPAR, 2010). It is a productive and biologically rich area, and therefore it sustains numerous marine mammals. Most marine mammals depend on an abundant supply of local food; for that reason fishing may negatively affect their survival by reducing the availability of prey or by inducing its dispersal (Lassalle et al., 2012). The depletion of fish stocks is a result of serial demand on fish protein explained by overfishing (Read et al., 2006). Major changes in the North Sea food web are taking place which might introduce changes in marine mammals feeding habits. The decrease of sandeel and the increase of sprat along with the lower energy value of both species in the diet were linked to breeding failure of seabirds in the North Sea in 2004 (Wanless et al., 2005). A lack of sandeel consumption was linked to an increased starvation in harbour porpoises in the Scottish North Sea (MacLeod et al., 2007). These authors suggested that the decrease of the sandeel availability in response to climate change may have negative effects on habrour porpoises population in the North Sea by increasing the starvation in spring.

Overall on the French coasts in the Channel and the southern North Sea, the Atlantic and the Mediterranean, 11 species of cetaceans have been recorded in the stranding in the year 2013. Among these species, the harbour porpoise is for the first year (for more than 40 years of monitoring in France) the most represented species in the strandings with 43.7 % just before the common dolphin (*Delphinus delphis*) with 42.4 %. Moreover, among the 6 species found in the English Channel and the southern North Sea (along the French coast) in 2013, the harbour porpoise was predominant in the composition of stranding with more than 90% of the total stranded animals (Figure 1.5) (Van Canneyt et al., 2014).

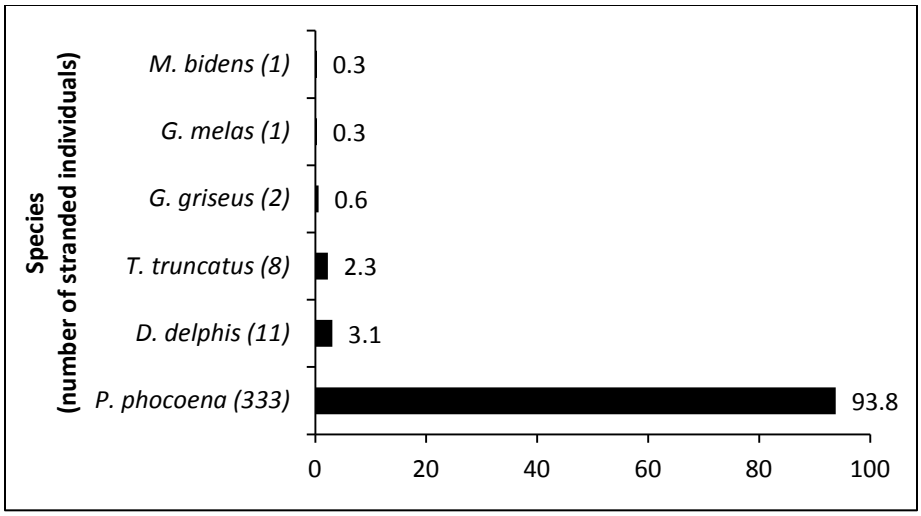


Figure 1.5 Species composition (%) of stranded cetaceans along the English Channel and the North Sea (French coast) in 2013. Sowerby's beaked whale *Mesoplodon bidens*, long-finned pilot whale *Globicephala melas*, Risso's dolphin *Grampus griseus*, Common bottlenose dolphin *Tursiops truncatus*, Short-beaked common dolphin *Delphinus delphis* and Harbour porpoise *Phocoena phocoena*; n = 368 (Van Canneyt et al., 2014).

More specifically, over the past decade harbour porpoises stranding has increased in the southern North Sea particularly in the French, Belgian and Dutch coastal waters (Camphuysen et al., 2008; Haelters and Camphuysen, 2009; Van Canneyt et al., 2014). According to the stranding data in the Channel and the North Sea (French coast), an increase in the number of stranded animals from the late 90s onwards was found (Figure 1.6a). Only a few animals were stranded between 1990 and 1998 (maximum 9 individuals per year with a total of 29 animals stranded in 9 years). This number increased to more than 58 stranded individuals per year in the period 2004-2011 with a maximum of 94 individuals stranded in 2007. In the year 2012, the number of stranded porpoises (186 animals) was almost the double of the previous year (90 stranded animals in 2011). The same trend was observed in 2013 with almost the double of porpoises stranded (333 animals) compared to the previous year 2012. Alongside, in the southern North Sea on the Belgian coast similar trends of stranding occurred in the past few decades. As shown in the figure 1.6b, an increase in the number of stranded animals from the late 90s onwards was found. Only a few animals were stranded between 1990 and 1997 (maximum 6 individuals per year). This number increased to more than 85 stranded individuals per year in the period 2005-2007. In 2008 and 2009 the increase was interrupted, with respectively 62 and 66 stranded individuals per year. In 2011, the stranding of porpoises increased again.

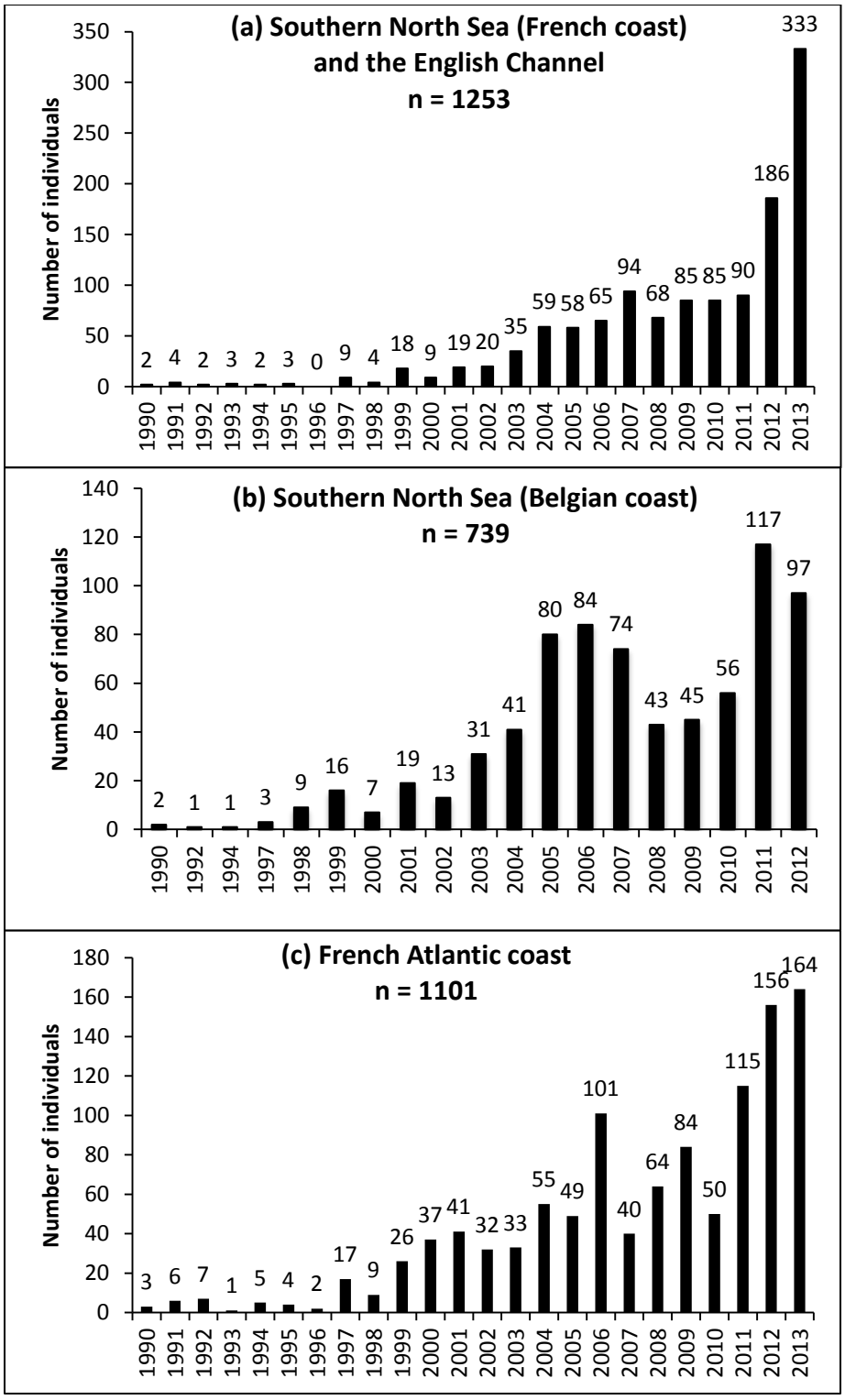


Figure 1.6 Annual distribution of stranded harbour porpoises in (a) the English Channel and the North Sea (French coast) between 1990 and 2013 (Van Canneyt et al., 2014), (b) the southern North Sea on the Belgian coast between 1990 and 2012 (T. Jauniaux personal communication) and (c) the French Atlantic coast between 1990 and 2013 (Van Canneyt et al., 2014).

The increase in stranding harbour porpoises has also been observed in Dutch waters between 1998 and 2007. A minimum of 59 stranded individuals in 1998 and a maximum of 539 individuals in 2006 were recorded (Camphuysen et al., 2008). Similarly on the French Atlantic coast (Bay of Biscay) since the late 1990s, the increase of stranded porpoises has been observed reaching a maximum of 164 stranded individuals in 2013 (Figure 1.6c) (Van Canneyt et al., 2014). Stranding data seems to indicate that the stranded individuals are mainly composed of juveniles with significantly more males than females. The increase in number of stranded porpoises in Belgian and Dutch coastal waters consisted mainly of juveniles (Haelters and Camphuysen, 2009). Distinct peaks of stranding were remarkable between March and May for the French coast in the southern North Sea and between December and January for the Atlantic French coast. This increase in numbers of stranded porpoises was believed to be probably an anomaly of abundance and/or particular mortalities in the French waters (Van Canneyt et al., 2014). As for the Belgian and Dutch coasts, distinct peaks were remarkable between March and May followed by another peak in August. This increase in numbers of stranded porpoises in the southern North Sea is probably food related and is believed to be due to an influx of animals from more northern waters (Haelters and Camphuysen, 2009; Hammond et al., 2013).

Harbour porpoises in the North Sea are very sensitive to anthropogenic disturbances and the main threats on their distribution/abundance may be represented by the interaction with different human activities. Despite all the conventions and regulations, this species is still exposed to several potential threats due to human impacts. The major threats are the incidental catches in fishing gears (bycatch), the contaminant exposure and the depletion of favorite, nutritive rich prey species through overfishing (Reijnders, 1992; Bennett et al., 2001; Herr et al., 2009; Bjørge et al., 2013). However, other anthropogenic disturbances such as noise pollution caused by sonar and climate change might also threaten porpoise's status.

- The **bycatch in fisheries** is the main direct threat to small cetaceans in the European Atlantic waters (ICES, 2013a). Monitoring programs of Danish set-net fisheries in the North Sea revealed an average of 5 591 porpoises taken annually in the period 1987-2001 in the center and southern North Sea (Vinther and Larsen, 2004). In addition, by-catch in coastal gill net fisheries in Norway was estimated at more than 20 500 porpoises during

2006-2008 (Bjørge et al., 2013). According to the management objectives defined by ASCOBANS this annual by-catch is not sustainable. Moreover, from 43 stranded porpoises investigated along the Belgian coast, 11 specimens were suspected to have been caught incidentally in fishing gear (ICES, 2013a). A threshold of 1.7% of the best estimate of population abundance should not be exceeded for annual by-catch. Unfortunately, no specific monitoring programs for marine mammal's by-catch took place in most countries of the North Sea (ICES, 2013a).

- The **contaminant exposure** is a potential threat for the harbour porpoises in the North Sea. This species is known to inhabit coastal waters close to pollution sources. This long-lived species feeds at a high trophic level, thus it can accumulate relatively high levels of contaminants. Several studies have been interested in the study of chemical contaminants in harbour porpoises from the North Sea (Jepson et al., 1999; Siebert et al., 1999; Bennett et al., 2001; Das et al., 2004b).
- The **overfishing** can lead to a decline of favored, nutritive rich prey species for porpoises. This can have an impact on their diet composition and their abundance and/or distribution. Harbour porpoises are small cetaceans with limited body fat and energy storage capacity; therefore, they must feed at a high daily rate without prolonged periods of fasting to maintain energy requirements (Koopman et al., 2002). Hence, this species is highly sensitive to changes in food availability for instance caused by overfishing and/or other changes in environmental conditions (Read and Hohn, 1995). Many fish species consumed by harbour porpoises have commercial value and are highly exploited. A shift in the diet of porpoises has been witnessed after the collapse of the herring stock in the southern North Sea (Santos and Pierce, 2003).
- The **noise pollution** such as construction of offshore wind farms (Haelters, 2009), shipping, military activities, etc may have direct effects on individuals. Harbour porpoises use sound for navigation, finding food and communication and are therefore sensitive to acoustic pollution. The harbour porpoise mass stranding in Danish waters in 2005 was partly related to the military activity in the region (Wright et al., 2013).
- **Climate change** may act on harbour porpoise population in different ways. Indirect effects of climate change include changes in prey availability affecting distribution, abundance and migration patterns, community structure, susceptibility to disease and

contaminants (Learmonth et al., 2006; Hammond et al., 2013). For instance, the sandeel availability in response to climate change may have negative effects on habrour porpoises population in the North Sea by increasing the starvation in spring. In fact, an increased starvation in harbour porpoises in the Scottish North Sea was linked to a lack of sandeel consumption (MacLeod et al., 2007).

Assessment of chemical contamination

A potential threat for marine mammals in response to human activities is represented by the contaminant exposure (Bjørge, 2003). The contaminant load in the tissues and organs of dead or washed ashore marine mammals might provide a useful indicator of certain pollutants in coastal marine ecosystems. However, a healthy or unhealthy ecosystem might not be directly related to the robust or declining marine mammals stock (Mangel and Hofman, 1999). Marine mammals are top predators and consequently, they tend to accumulate relatively high levels of contaminants (Duinker et al., 1989; Tanabe et al., 1994). Hence, the toxic effects of contaminants on vulnerable animals such as cetaceans should be considered from an ecotoxicological perspective (Tanabe et al., 1994; Kleivane et al., 1995).

Metals are encountered in the environment as a result of both human activities and natural processes. Some of these metals such as Cu, Cr, Mn, Se and Zn are essential for effective immune functioning, while others such as As, Cd, Hg, Pb and V are non essential elements and therefore leading to autoimmune diseases (Lynes et al., 2006). Uptake from water and food (trophic transfer) are several sources contributing to metal accumulation in marine animals (Wang and Rainbow, 2010). Essential trace elements are slightly variable from animal to animal, whereas the potential toxic elements are much greater variable with concentration ranges often covering several orders of magnitude (Mackey et al., 1995). High levels of metal contaminants have been documented in various studies on marine mammals. These studies suggested that metal contaminants may have immunological effects on marine mammal's health. For instance, the exposition of seals to high Zn concentrations in Caspian Sea resulted in the disturbance of homeostatic control and nutritional status of essential elements (Anan et al., 2002). Moreover, correlations between severities of disease and liver Hg levels were detected in harbour porpoises from the German waters (Siebert et al., 1999). Metals have been shown to produce alteration in

the immune function of harbour seals from the Antarctic, which may decrease resistance to infectious diseases encountered by marine mammals (Frouin et al., 2010). High Zn concentrations in harbour porpoises form UK that died of infectious disease may represent a response to infection and other stressors (like cold) causing Zn redistribution (Bennett et al., 2001). Moreover, porpoises from the North Sea displaying lesions of the respiratory tract had higher hepatic Zn burden than porpoises without lung lesions (Das et al., 2004b). In addition, Lahaye et al., (2007) suggested that different biological and ecological factors might be related to trace element levels in harbour porpoises, which can explain the significant geographical differences in hepatic Zn concentrations. Moreover, the elevated Cd levels obtained in Scottish porpoises could be related to their feeding preferences. A study on five toothed whales from the Northwest Iberian Peninsula revealed that differences in metallic concentrations are probably related to biological factors such as age and sex and/or to ecological factors such as feeding habits or bioavailability of the various elements. Pilot whales and striped dolphins showed the highest concentrations of Cd and Hg compared to harbour porpoise, common dolphin and bottlenose dolphin (Méndez-Fernandez et al., 2014b). Along the North Sea, harbour porpoises form England and Whales, northern France, Belgian and German coasts tend to accumulate some metals according to their health status (Siebert et al., 1999; Bennett et al., 2001; Das et al., 2004b). Table 1.2 represents an overview of some metal levels in organs of harbour porpoises stranded between 1990 and 2004 in different areas of the North Sea and adjacent waters.

Table 1.2 Some trace element concentrations (µg.g^{-1} dw) in tissues of harbour porpoises stranded in different areas of the North Sea and adjacent waters. Year of stranding; Mean ± SD; range of concentration (min-max) and n: number of samples analyzed. [a] Converted value from wet weight (ww) to dry weight (dw) using the factor 0.29 for livers; [b] Harbour porpoises that died from infectious disease; [c] harbour porpoises that died from physical trauma.

Area and year of stranding		Cd	Cu	Hg	Se	Zn
North Sea and Baltic Sea	Liver			38.8 ± 76.5		
German waters (1991-1993)				(0.6 - 449)		
(Siebert et al., 1999)				n=56		
	Kidney			9.6 ± 21.2		
				(0.5 - 160)		
				n=57		
Coasts of England and Whales	Liver [a,b]	0.8 ± 0.2	41 ± 8	69 ± 19	34 ± 12	267 ± 24
(1990-1994)		n=37	n=37	n=37	n=29	n=37
(Bennett et al., 2001)	Liver [a,c]	0.66 ± 0.1	50 ± 7	42 ± 12	26 ± 8	148 ± 9
		n=49	n=49	n=49	n=40	n=49
Southern North Sea	Liver	0.5 ± 0.6	39 ± 38	23 ± 66	14 ± 21	234 ± 172
(1994-2001)		(<0.05-2.5)	(9-257)	(0.6 - 344)	(0.6 - 99)	(40-684)
(Das et al., 2004b)		n=49	n=49	n=27	n=37	n=49
	Kidney	3.1 ± 3.1	17 ± 11	8 ± 13	6 ± 4	107 ± 27
		(<0.05-12)	(7-73)	(0.9 - 42)	(1 - 21)	(68-201)
		n=48	n=48	n=18	n=34	n=48
North Sea (1994-2001)	Liver	0.7 ± 1.3	58 ± 49	14 ± 18	11 ± 11	219 ± 181
German waters		(<0.05 - 5)	(20 - 169)	(1 - 56)	(1 - 39)	(71-727)
(Das et al., 2004b)		n=14	n=14	n=14	n=13	n=14
	Kidney	4 ± 9	15 ± 2	nd	nd	103 ± 26
		(<0.05 - 33)	(12 - 19)			(69-157)
		n=12	n=12			n=12
Baltic Sea (1994-2001)	Liver	0.2 ± 0.2	62 ± 77	4.5 ± 3.6	6 ± 3	135 ± 56
German waters		(<0.05 – 0.5)	(18 - 260)	(0.9 - 12)	(2 - 10)	(78-242)
(Das et al., 2004b)		n=9	n=9	n=9	n=8	n=9
	Kidney	1.1 ± 1.5	16 ± 3	nd	nd	90 ± 20
		(<0.05 - 5)	(13 - 21)			(68-136)
		n=9	n=9			n=9
Baltic Sea	Liver	0.24 ± 0.07	19.8 ± 4.74	21.9 ± 56.4		91.9 ± 17.5
Polish waters (1996-2003)		(0.15-0.34)	(14.3-29.4)	(1.53-217)	(<1.7-6.09)	(66-134)
(Ciesielski et al., 2006)		n=14	n=14	n=14	n=5	n=14

Other chemical contaminants that harbour porpoises are exposed to in the North Sea are the persistent organic pollutants (POPs). POPs have a strong affinity to lipid-rich tissues and organs because of their lipophilicity and hence they are retained mainly in the blubber of cetaceans (Aguilar and Borrell, 1994). They can bioaccumulate and magnify in the food chain. Small cetaceans are known to have lower biotransformation capacity of PCBs and DDXs compared to seals, birds and terrestrial mammals (Duinker et al., 1989; Tanabe et al., 1994; Boon et al., 1997; Law et al., 1998). The POP chemicals include important classes and families of chlorinated aromatics such as polychlorinated biphenyls (PCBs), different organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT) and polybrominated diphenyl ethers (PBDE). PCBs and PBDEs have been synthesized for industrial uses and DDXs as agrochemicals (Jones and de Voogt, 1999). The production of these compounds was banned in Europe since the end of 1970s generating the EU directive (79/117/EEC) for DDXs and the council directive (96/59/EC) for disposal of PCBs. As for the PBDEs, banning the use of these compounds was represented in the Restriction of Hazardous Substances Directive (ROHS) 2002/95/EC, adopted in February 2003 by the European Union. However, even after the ban on use of these compounds a continuous long-term contamination by toxic organochlorines over many generations is expected (Tanabe et al., 1994).

Exposure to persistent organochlorines such as PCBs and DDT and their related compounds has caused abnormalities in higher trophic feeding animals from North Sea, UK and various seas (Duinker et al., 1989; Tanabe et al., 1994; Aguilar and Borrell, 1995; Jepson et al., 1999). Accumulation patterns of POPs might be different from a species to another. For instance harbour seals (pinnipeds) and harbour porpoises (cetaceans) have different ability for metabolic breakdown reflected by different PCB or PBDE profiles (Weijs et al., 2009 a; b). Moreover, factors such as age and gender are important for the bioaccumulation of POPs. For example, adult males had the highest concentrations of PCBs indicating bioaccumulation with age (Weijs et al., 2009 a; b). A study on five toothed whales from the Northwest Iberian Peninsula revealed that differences in PCB and PBDE concentrations among the species are highly dependent on age, sex and ecological factors such as trophic level, prey type and habitat. For instance, bottlenose dolphin and harbour porpoise showed the greatest concentrations of PCBs compared to common dolphin, long-finned pilot whale and striped dolphin (Méndez-Fernandez et al., 2014a). Moreover, organic pollutants may have possible adverse effects on marine mammal

populations. For instance harbour porpoises that died from infectious disease or parasitic infection had higher concentrations of POPs than animals that died from other causes (Pierce et al., 2008). It has been demonstrated that thymic atrophy and spleenic depletion in harbour porpoises from German North and Baltic Seas were significantly correlated to increased PCB and PBDE levels (Beineke et al., 2005). Moreover, PCBs and DDT increasing led to decreasing lymphocyte responses in bottlenose dolphins from Florida, Atlantic Coast (Lahvis et al., 1995) and high levels of organochlorines led to high prevalence of cancer in white whales from St. Lawrence estuary (De Guise et al., 1994). It is important to keep monitoring the levels of chemical compounds in the tissues and organs of the top predator harbour porpoise in the North Sea. Table 1.3 represents an overview of some organochlorine levels in blubber of harbour porpoises stranded between 1978 and 2008 in different regions of the North East Atlantic Ocean and the Black Sea.

Table 1.3 Mean concentrations of the sum of PCBs, and DDXs (µg.g^{-1} lipids) in blubber of harbour porpoises from different regions of the North East Atlantic Ocean and the Black Sea. Years in brackets refer to the date of stranding. A: Adults; J: Juveniles; AM: Adult males; AF: Adult females; JM: Juvenile males; JF: Juvenile females; n: number of samples. * median; ** Σ7CBs.

		∑PCBs			∑DDXs			∑PBDEs			
Area	Age/Gender	Mean ± SD	(min - max)	n	Mean ± SD	(min - max)	n	Mean ± SD	(min - max)	n	References
Dansih and Norwegian waters (1987-1991)	M	23.3	(3.7-65)	34	16.39	(3.2 - 45.1)	34				Kleivane et al., 1995
Baltic sea (1985 - 1993)	JM	16 ± 8	(2.9 - 32)	13	15 ± 18	(1.5 - 59)	11				Berggren et al., 1999
Baltic sea (1988 - 1989)	AM	46 ± 29	(14 - 78)	4	116 ± 134	(20 - 308)	4				
Kattegat-Skagerrak Seas (1989-1990)	JM	11 ± 5.0	(2.2 - 20)	10	20 ± 13	(5.7 - 36)	8				
Kattegat-Skagerrak Seas (1988-1990)	AM	13 ± 5.2	(6.7 - 22)	7	25 ± 20	(2.8 - 61)	7				
Kattegat-Skagerrak Seas (1978-1981)	AM	40 ± 22	(17 - 67)	5	98 ± 43	(35 - 154)	5				
West coast of Norway (1988-1990)	AM	15 ± 11	(7.2 - 33)	8	9.1 ± 7.4	(3.1 - 22)	6				
Southern North Sea (2001-2003)	F	15 ± 8.6		19				1.06 ± 0.8		19	Pierce et al., 2008
Scotland (2001-2003)	F	10.5 ± 13.2		31				1.4 ± 1.4		31	
Ireland (2001-2003)	F	53.5 ± 48		12				0.7 ± 0.5		12	
France (2001-2003)	F	13.8 ± 11		2				1.4 ± 0.9		2	
Galicia (2001-2003)	F	53 ± 42		3				0.3 ± 0.04		3	
Southern North Sea (1999-2004)	JF	12.9 ± 11.9	(1.3 - 39.3)	9				0.70 ± 0.41	(0.22 - 1.48)	9	Weijs et al., 2009a
	JM	15.4 ± 10.7	(5.3 - 39.8)	12				1.73 ± 1.77	(0.50 - 5.93)	12	
	AF	7.3 ± 2.0	(4.4 - 8.9)	5				0.85 ± 0.60	(0.32 - 1.56)	5	
	AM	82.9 ± 31.8	(38.7 - 125.5)	8				1.54 ± 0.96	(0.28 - 3.10)	8	
East England (1991-2005)	M	11.6 ± 9.7		23							Law et al., 2010b
Southern North Sea (1991-2005)	M	46.4 ± 30.7		21							
Around the UK (1992-1998)	AM							1.34	(0.03 - 7.32)	95	Law et al., 2010a
	AF							1.03	(0.09 - 8.19)	93	
	J							1.62	(0.04 - 15.73)	227	
Black Sea (1998)	A	13.2*	(8.8 - 24.9)	11	77.3*	(55 - 157)	11	0.07*	(0.04 - 0.09)	11	Weijs et al., 2010a
	J	7.0*	(4.9 - 13.7)	9	40.9*	(27.4 - 82)	9	0.06*	(0.05 - 0.07)	9	
North Sea (1990-1999)	A	81.5		1	22.9		1	1.9*		1	Weijs et al., 2010b

North Sea (2000-2008)	A	24.9	(15.3-34.5)	2	3.4	(1.2-1.4)	2	1.2*	(0.6 - 1.8)	2	
North Sea (1990-1999)	J	19.1		1	4.5		1	4.8*		1	
North Sea (2000-2008)	J	9.9	(1.1-68.2)	5	1.7	(0.4-6.4)	5	0.5*	(0.3 - 1.5)	5	
North West Iberian Peninsula	JF	10.8 ± 2.8		5							Méndez-Fernandez et al., 2014a
(2004-2008)	JM	9.4 ± 3		3				0.57		1	
	AF	37.5 ± 30.8		3							
	AM	50.8		1							

Studying the feeding ecology of harbour porpoises

Changes in abundance and occurrence of porpoises in the North Sea are illustrated as consequences of changes in environmental factors (Reijnders, 1992). Due to their limited body size, harbour porpoises need to feed regularly without prolonged periods of fasting (Koopman et al., 1996). Thus, prey availability might be responsible for the changes in the distribution of porpoises (Santos and Pierce, 2003; Santos et al., 2004). Accordingly, studying the feeding ecology of harbour porpoises serves among others to investigate their feeding strategy, the predator-prey relationships, and their responses to changes in food webs dynamics, climate changes or fishery interaction. Methods to investigate the feeding ecology of marine mammals have been developed from traditional methods such as the stomach content analysis (Santos et al., 2004; Leopold and Camphuysen, 2006; Haelters et al., 2012) to more sophisticated methods based on biochemical techniques such as the stable isotope analyses (Das et al., 2003b; Jansen et al., 2012; Mèndez-Fernandez et al., 2012) and the fatty acids analyses (Jansen, 2013). Only few studies have combined the 3 techniques to assess the foraging ecology of marine mammals (Hooker et al., 2001; Jansen, 2013) and birds (Karnovsky et al., 2008). Each method has its strength and weakness and gives different aspects of the dietary information, timeframe perspective and prey detail (Figure 1.7). Even if all methods are subject to bias, it is expected that by combining the three techniques together the limitations of each method could be overcome.

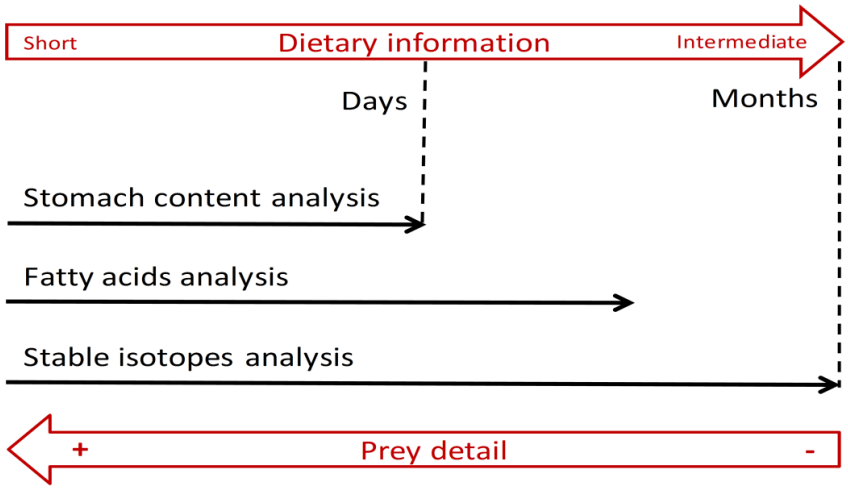


Figure 1.7 Representation of the different techniques used in the study of harbour porpoises diet.

The **stomach content analysis** is a traditional method for dietary study based on the identification of the remains of ingested preys in the stomach contents (otoliths, fish bones, cephalopod beaks, etc) to the lowest taxonomic level. The central assumption of this method is that the relative frequency and size of prey, estimated from hard remains, reflect the frequency and size of prey consumed. However, the otoliths of different species erode at different rates. Subsequently, counts and measures of otoliths are likely to underestimate the number and sizes of fish ingested (Tollit et al., 1997). Correction factors to reduce bias are not usually available (Bowen and Iverson, 2012). Another weakness of this method is that stranded cetaceans might be expected to have stomach contents biased towards inshore prey species (Pierce and Boyle, 1991). Moreover, this technique represents short-term dietary information; only the last ingested meal(s) may be studied. The identification of prey DNA in stomach contents (Dunn et al., 2006) and feces shows promise as a method to estimate the species composition of diet. The major constraint of this method being the high unit cost, further development and testing is needed to validate its use (Bowen and Iverson, 2012).

The **Stable isotope analysis** is currently among the most effective tools for the study of trophic relationships and feeding habits (Hobson and Welch, 1992; Caut et al., 2009). Using the muscle as reference tissue for the stable isotope analyses (Hobson and Welch, 1992) allows to compare between individuals and taxa and to overcome inter-tissue differences explained by protein turnover rate and metabolic routing (Cherel et al., 2009). The ratios of naturally occurring isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) in specific tissues of the predator may be directly related to those of their prey with an expected enrichment called Trophic Enrichment Factor (TEF). This TEF varies between 0 to 1‰ for $\delta^{13}C$ and between 2 to 4‰ for $\delta^{15}N$ depending on the analyzed tissue (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001). The $\delta^{13}C$ value may be used as a tracer of the origin of the food chain and the feeding zone of organisms (inshore vs. offshore) (Hobson et al., 1994; France, 1995), whereas the $\delta^{15}N$ value may be used as an indicator of the trophic position of organisms relatively to the primary consumers (Hobson, 1999). The Stable Isotope Analysis in R computing language (SIAR) is a mixing model used to estimate the contribution of prey species to the predators' diet. SIAR modeling can deal with more sources than isotopes and it

can incorporate trophic enrichment and elemental concentrations allowing quantitative estimation of prey composition. When using SIAR, results might be biased if a species that is consumed is not included among the potential prey species or if two prey species have isotopic composition that cannot be reliably distinguished (Parnell et al., 2010).

The **fatty acid analysis** in the blubber of marine mammals has witnessed a potential development in the last few decades. It has been evolved from a tool for revealing food webs to a more sophisticated technique for qualitative (Falk-Petersen et al., 2004) and quantitative (Iverson et al., 2004) analysis of diet by comparing the fatty acids found in the predator blubber with those found in their potential prey. The inner blubber layer is more active metabolically than the outer blubber layer in terms of lipid deposition and mobilization (Koopman et al., 1996). This technique is based on the fact that fatty acids (FAs) are deposited into adipose tissue with little change or in a predictable manner, thus reflecting the diet of the predator over a period of up to several months (Budge et al., 2006). The qualitative method reside on the comparison of fatty acids patterns in the blubber of marine mammals to the FAs patterns in the muscle of prey species. Recently, a method to use FAs to quantitatively estimate the species composition of the diet has been developed. The quantitative fatty acid signature analysis (QFASA) is a mixing model that requires information on the FA composition of prey species of predator fat store, a subset of total FAs that reflect dietary sources, calibration coefficients (CC) to account for predator metabolism of ingested FAs prior to their deposition in blubber and a statistical model to minimize the statistical distance between the predator and the weighted mixture of prey species representing the diet (Iverson et al., 2004: Bowen and Iverson, 2012). However, calibration coefficients from grey seals are available and given the differences between adipose tissues the application of these coefficients to cetaceans might create bias in QFASA estimates (Bowen and Iverson, 2012).

Furthermore, the **compound-specific stable isotope analysis** (CSIA) is a new research tool used to avoid many limits encountered when using bulk tissue stable isotopes (measurement of $\delta^{13}C$ and $\delta^{15}N$ values in a sample) or FAs analyses (Gladyshev et al., 2012). This approach tracks the stable carbon isotope of specific FA structure that can only arise from diet (Budge et al., 2011). CSIA is expected to provide a greater specificity to biomarkers. Its strength relies when studied organisms cannot be physically isolated from each others, when tracing

quantitatively minor but qualitatively important component and when different food sources have similar bulk carbon isotopes and fatty acids signatures.

Objectives of the study

A major shift in the distribution of harbour porpoises from the northern parts of the North Sea to its eastern parts was highlighted. Alongside, over the past few decades harbour porpoises stranding has increased in the southern North Sea particularly in the French, Belgian and Dutch coastal waters. In this context, the present study aimed to delineate the cause of the increased number of stranded porpoises in the southern North Sea and to define the cause of the southward shift of the population. Since the stranded porpoises provide valuable information on their ecology and on human impacts and since one of many threats that porpoises are subject to in the North Sea is the contaminant exposure, it was crucial to assess the population status. Therefore, a **first objective** of this study was to determine levels of metals and persistent organic pollutants in the organs and tissues of harbour porpoises from the southern North Sea. A second threat for porpoises in the North Sea is the depletion of favored, nutritive rich prey species through overfishing. Hence, a **second objective** of this study was to investigate whether the changes in the distribution of porpoises in the southern North Sea may be a result of the changes in prey availability. Finally, a **third objective** of this study was to combine three techniques to determine the diet of harbour porpoises.

Outline of the study

The thesis consists of seven chapters of which four chapters (Chapters 3, 4, 5 and 6) are presented as scientific publications. Chapters 3 and 4 deal with the chemical contamination, whereas chapters 5 and 6 deal with the feeding ecology of harbour porpoises. Chapter 3 is published in the journal "Environmental Research", chapter 4 is published in the journal "Environmental Science: Processes and Impacts", chapter 5 is in preparation to be submitted to "Journal of applied ecology" and finally chapter 6 is in preparation to be submitted to "Journal of Experimental Marine Biology and Ecology".

Chapter 1 introduces the status of harbour porpoises in the North Sea, the different threats to which this species is exposed to with the stranding data in the southern part of the region. Two aspects were developed as an introduction for the present study, (1) The interest to assess the chemical contamination with the focus on the metallic and organic contaminants and (2) the various methods to study the feeding ecology of marine mammals. Moreover, the objectives of this study were presented.

Chapter 2 presents the strategies, protocols and methodologies used during this study. It evokes the methodology applied from the carcasses collection and necropsies on the field to the laboratory applications.

Chapter 3 deals with the chemical contamination in tissues of harbour porpoises stranded in the southern North Sea along northern France and Belgian coasts between 2006 and 2013. Moreover, the population status was assessed by comparing levels of metal contaminants in organs of porpoises stranded in the southern North Sea to porpoises stranded in the Bay of Biscay (French Atlantic coast) between 2009 and 2012. The results indicate that some metals appeared to be higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma. Hence, the chemical contamination may represent one of many threats encountered by harbour porpoises, but it cannot explain alone the increase in the number of stranded individuals.

Chapter 4 deals with the organic contamination in harbour porpoises stranded along the southern North Sea between 2010 and 2013. Potential associations between organic contaminants (PCBs and pesticides) and the cause of death (traumatic or infectious) of porpoises were investigated. Moreover, the current contamination status of harbour porpoises in the study area was assessed through a comparison with other porpoises stranded along European waters (North East Atlantic Ocean and the Black Sea). The results indicate that levels of PCBs were significantly higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma. And the sum of PCBs and DDXs were higher in juvenile porpoises compared to adult females. Levels of PCBs and DDXs obtained in the blubber of porpoises from this study were in the same order of magnitude or even lower than porpoises stranded along the North East Atlantic Ocean and the Black Sea.

Chapter 5 presents the diet of harbour porpoises in the southern North Sea in order to investigate whether the changes in the distribution of porpoises may be a result from changes in prey availability. For this purpose, three different techniques were combined: the stomach content, the stable isotopes and the fatty acids analyses. The combined techniques resulted in greater insight into the feeding ecology of harbour porpoises. Data were interpreted in the light of fish prey species abundances evolution. The results suggest that the feeding changes and the southward shift of porpoise's population encountered the past few years in the North Sea may be related to the sandeel abundance decline in the northern parts of the North Sea along with the re-invasion of the southern North Sea by the sardine species, perhaps in response to climate change

Chapter 6 compares the feeding habits of harbour porpoises from the southern North Sea and the Bay of Biscay by combining 3 different methods: the stomach content, the stable isotopes and the fatty acids analyses. Different habitat characteristics of each area that might result in differences in the feeding habits of porpoises inhabiting each zone were investigated. Harbour porpoises feed on a variety of demersal, benthic and coastal prey in the southern North Sea and a variety of demersal, benthic and pelagic prey in the Bay of Biscay. The results suggest that the differences in the diet between both regions might be related to the different prey species availability along with the different topography such as the depth and the slope in the region.

Chapter 7 summarizes all the results obtained from the study. The value of using a multi approach dietary analysis was also discussed.

CHAPTER 2

STRATEGIES, PROTOCOLS AND METHODOLOGIES

Chapter 2 - Strategies, protocols and methodologies

1. Sample collection and necropsies

1.1. Carcasses collection

The national stranding network in France (RNE; Réseau National d'échouage) established in 1972 consists of local correspondents available to intervene when a stranding of marine mammal occurs along the French coast. The network is coordinated by the "Centre de Recherche sur les Mammiferes Marins" (CRMM) under the supervision of the Ministry of the Environment. Once a stranding of marine mammal is reported, the CRMM prevent the corresponding of the area to intervene and examine the animal. In our study area, the "Observatoire pour la Conservation et l'Etude des Animaux et Milieux Marins" (OCEAMM) and the "Ligue Protectrice des Animaux du Nord" (LPA) Calais were the correspondents in case of a stranding along northern France coast. For the strandings along the Bay of Biscay coast, different correspondents may intervene (such as CRMM, Ligue pour la Protection des Oiseaux (LPO) marais Breton, LPO marais de Mullembourg, etc). Whereas for the marine mammals stranded along the Belgian coast, the Belgian Marine Mammals Network intervenes in case of stranding. On the coast where the stranding occurred, basic information such as the species in question, sex, size, status, date and place were systematically recorded. Other parameters as well as tissues and organs collection were obtained after dissection.

1.2. Protocol of dissection, collection and preservation of samples

Freshly dead or slightly decomposed carcasses of porpoises stranded along the southern North Sea (Figure 2.1) were transported to the Veterinary Medicine, University of Liege as soon as possible to be preserved in freezing rooms for further necropsies and investigations (Figure 2.2).

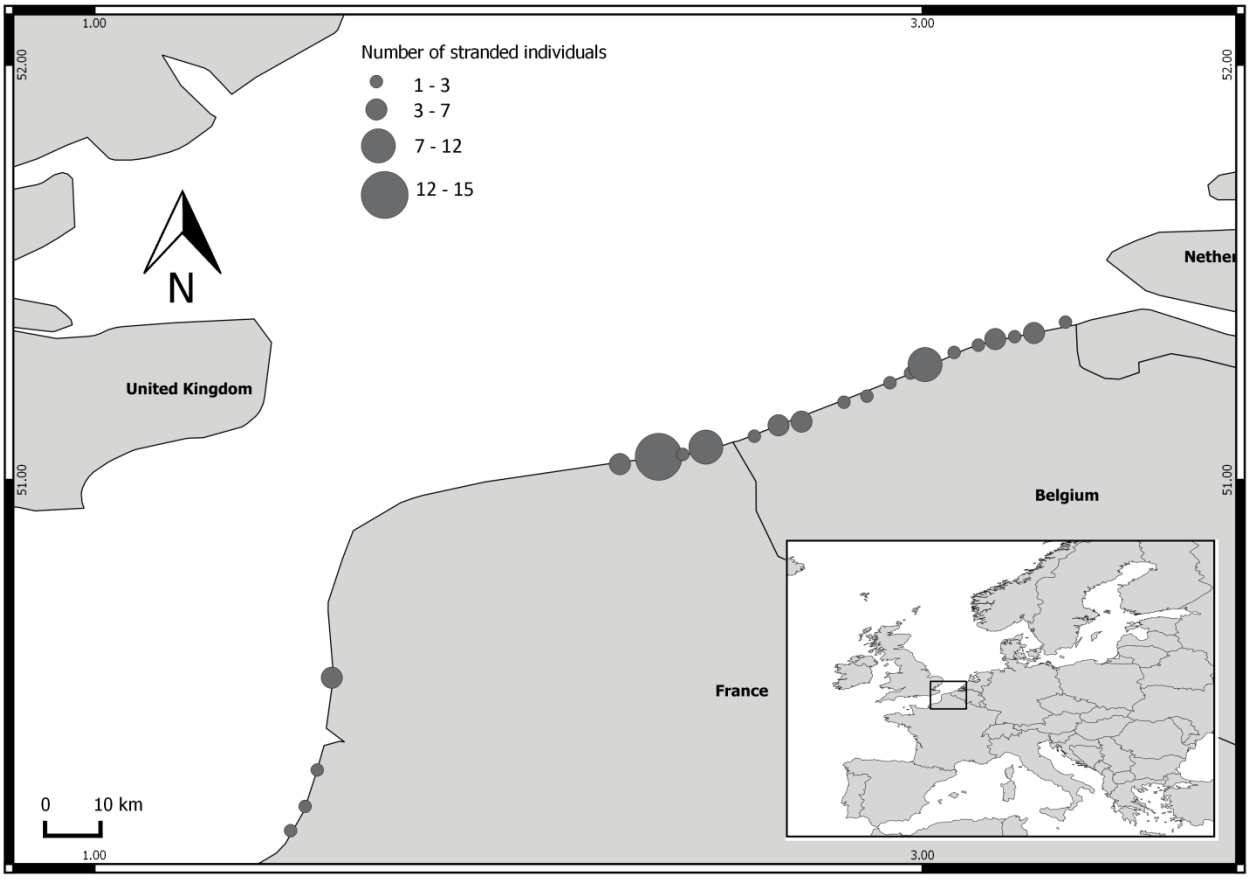


Figure 2.1 Harbour porpoises stranding locations and numbers along the southern North Sea (northern France and Belgian coast) analyzed in this study (2006-2013).

Figure 2.2 Harbour porpoise (*Phocoena phocoena*) stranded along northern France prior to necropsy.

All individuals provided for necropsies were labeled. Animals were measured, weighed and the sex and the overall length were determined. For the harbour porpoises stranded along the Bay of Biscay, necropsies were held at the CRMM, La Rochelle - France. Stranding locations are presented in figure 2.3.

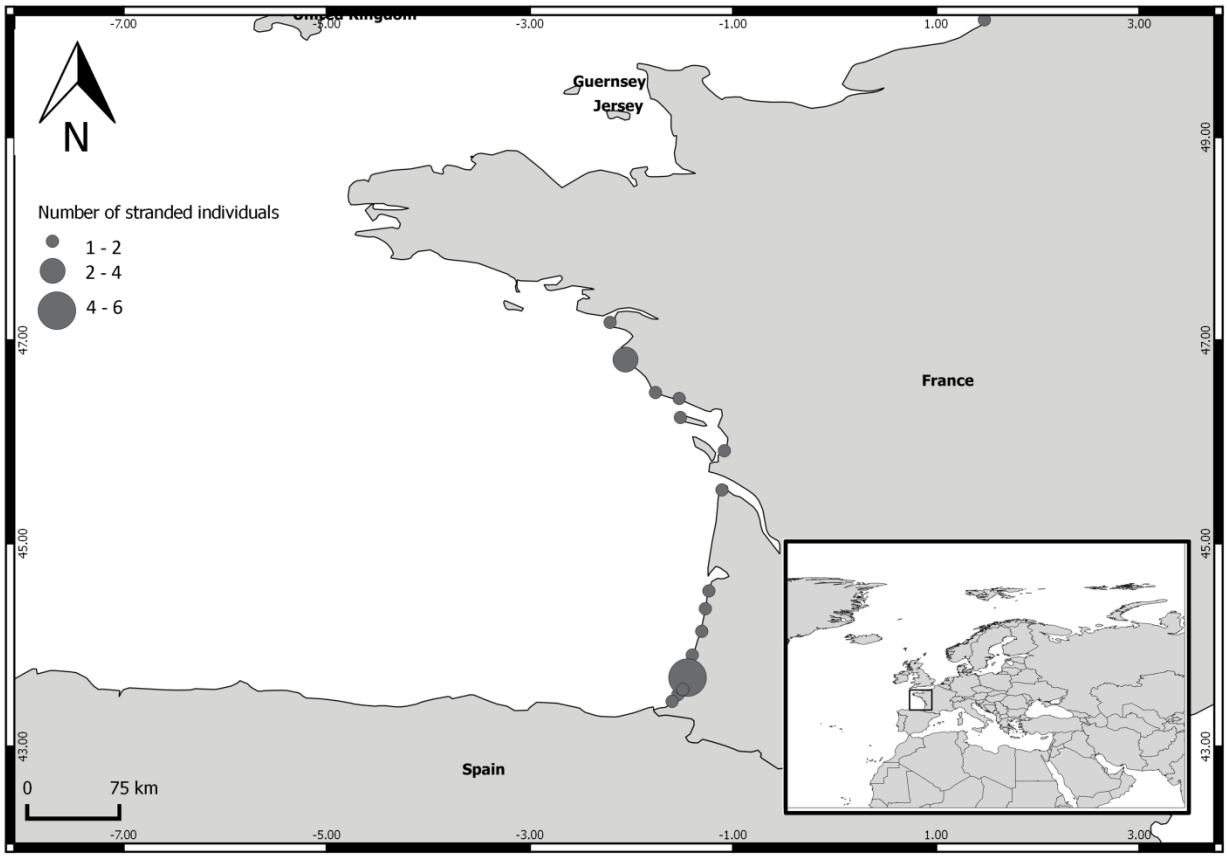


Figure 2.3 Harbour porpoises stranding locations and numbers along the Bay of Biscay (french coast) analyzed in this study (2009-2012).

Based on published protocols (Kuiken and Hartmann, 1993; Jauniaux et al., 2002), the Decomposition Condition Code (DCC), codification from 1 to 5 describes the conservation status of the individual (Table 2.1), it allows a better estimation of the freshness of the carcass in the first few days after the death of the animal.

Table 2.1 Decomposition Condition Code of the washed ashore carcasses based on the observations during necropsies (Modified from Kuiken and Hartmann, 1993; Jauniaux et al., 2002a).

DCC 1	**Extremely fresh animal**, within 48 hours after death Uninflated carcass May show signs of rigor mortis Blood still separates serum Non glassy eye
DCC 2	**Fresh animal** Glassy eye Blood does not separate the serum Intact viscera, not distended by the putrefaction gases No protrusion of the tongue and penis
DCC 3	**Moderate decomposition** Inflated carcasses Separation of the upper part of the skin Moderate alteration of organs Protrusion of the tongue and penis Viscera distended by the putrefaction gases
DCC 4	**Advanced decomposition** Liquid flow through orifices Separation of skin flaps over large body surfaces Severe impairment of organs Some unidentifiable viscera
DCC 5	**Very old putrefied carcass** Organs are beyond clear recognition or absent

Body condition was estimated on the basis of the Condition Code. The skin was carefully examined for wounds, scars, infections and other damage. All orifices: mouth, eyes, external auditory canals, genital and anal fissures were inspected and the presence of any damage or parasite secretion was described and sampled. All individuals used in this study were freshly dead or slightly decomposed (DCC 1 to DCC 3). Samples collection depends on the Condition Code of the carcasses. Some analyses require specific storage conditions. According to the DCC, samples were chosen for further analyses:

- DCC 2 – 5 : Demography
- DCC 2 – 3: Histopathology
- DCC 2 – 3: Virology
- DCC 2 – 3: Bacteriology

- DCC 2 – 4: Parasitology
- DCC 2 – 3: Toxicology

The blubber thickness was measured at the cranial insertion of the dorsal fin. The body was opened in order to sample the organs. The liver was inspected on all sides and samples were taken out for toxicology, histopathology, virology and parasitology. The kidneys were examined in the same manner and sampled. An overview of all types of analyses and samples collected is given below in table 2.2.

Table 2.2 Samples collection and storage.

Teeth (age determination)	At least 5 teeth were removed from each mandible and stored at -20°C
Food remains	the entire gastrointestinal tract was removed and stomachs were stored at -20°C for further investigation
Genetics	2 pieces of skin with blubber were fixed in 70% ethanol
Maturity	Gonads (testes or ovaries) were removed, weighed and fixed in 10% neutral - buffered formalin
Histopathology	Damaged tissues were collected and fixed in 10% neutral - buffered formalin
Virology	In case of viral infection suspicion, a tissue sample was collected and stored at -80°C
Bacteriology	Liver and spleen samples were collected and stored at -20°C for later laboratory research on *Brucella sp.*
Parasitology	All organs were inspected for parasites presence; parasites were collected and preserved in 70% ethanol with 5% glycerine
Toxicology	2 large pieces of skin with blubber collected along the dorsal fin of the animal were sampled for organochlorines and fatty acid analyses and stored in aluminum foils at -20°C Large pieces of muscle, liver and kidney were collected for organochlorines (in aluminum foils) and heavy metals (in plastic bags) and stored at -20°C

In our study, samples of livers and kidneys were collected from washed ashore porpoises along the southern North Sea and the Bay of Biscay for metallic analysis. Blubber was collected

from porpoises stranded along the southern North Sea for persistent organic pollutants (POPs) analyses and from both areas for Fatty Acids analyses (FAs). Finally stomachs were collected from both areas for dietary investigations. Data on porpoises stranded along the southern North Sea are reported in the annex 1a: the date when the carcass was found, the location, the country, the maturity status, the gender, the length, the weight, the blubber thickness , the cause of death and finally the type of analysis that have been carried out on each sample. Moreover, data on porpoises stranded along the Bay of Biscay are reported in the annex 1b: the date when the carcass was found, the location, the maturity status, the gender, the length, the weight and finally the type of analysis that have been carried out on each sample.

2. Chemical analyses

2.1. Metallic analyses

Livers and kidneys were collected for metal analysis and stored at -20°C in plastic bags until analysis were carried out. In order to avoid contamination for trace metal analyses, two acidified bath wash (molar mixture HCl: HNO_3 for analysis, Merck) were used to decontaminate all the used equipments (vials, beakers, syringes, etc). After decontamination, all materials were rinsed with Milli-Q ultrapure water (18.2 MΩ.cm, Millipore Corporation). The drying of equipments as well as all the handling (dissection, grinding, preparation of solutions, etc) were carried out under a laminar flow hood "Federal Standard" (n°209a, class 100). Tissues of livers and kidneys were freeze-dried for 48 hours then homogenized with an agate mortar and pestle. In Teflon vials (Savillex PFA), an aliquot of ~150 mg from each material was digested in a concentrated solution of nitric acid (65 %, Suprapur, Merck) at room temperature for 24 hours and then at 100°C for 4 hours. After digestion, all samples were diluted to ~15% then filtered out using polyethylene syringes equipped with cellulose acetate filter (0.2µm). Finally, before spectroscopic analysis, all samples were diluted to ~2%. This dilution helps to reach a concentration below $2g.L^{-1}$ in total dissolved salt for best instrument performance and stability. For each analysis series, reagent blanks were treated and analyzed in the same conditions as samples. The signal of all blanks remains below 1% compared to the signal of all samples and for all elements reported in this study. Therefore, no corrections have been made.

Besides total mercury analysis which were conducted on an Advanced Mercury Analyzer Spectrophotometer (AMA-254, Altec), other metal concentrations were determined by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Thermo Scientific iCAP 6500) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Varian 820-MS) depending on concentration levels of each metal. Metal concentrations are reported in $\mu g.g^{-1}$ dry weight (dw). ICP-AES and ICP-MS are analytical techniques used for multi-elemental measurements available at Centre Commun de Mesure (CCM, Université du Littoral Côte d'Opale, Dunkerque). The inductively coupled plasma principle is common for the two spectroscopic techniques but the detection mode is different (figure 2.4).

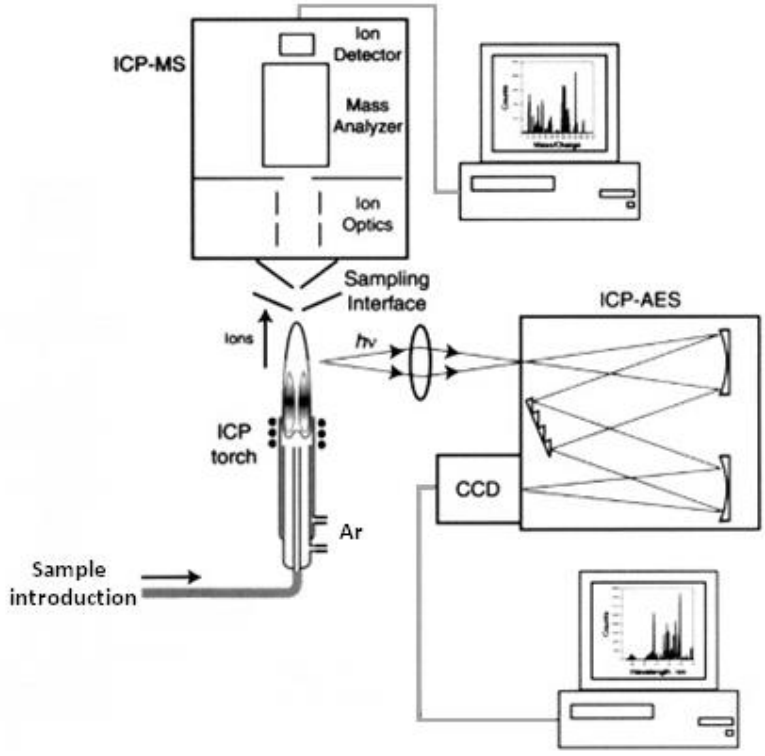


Figure 2.4 Spectroscopic techniques ICP-MS and ICP-AES with the different detection modes specific for every technique (Modified from Russo et al., 2002).

After digestion and dilution, the sample is injected in the ICP into a nebulizer by aspiration which converts the liquid sample into an aerosol. Only droplets with a diameter less than 10 microns are converted into gaseous atoms and carried into the argon plasma with a stream of argon gas. The electrons of the element in the sample are therefore thermally excited due to the high plasma temperature ~7000°C and conducted towards the end of the plasma.

2.1.1. Analysis on ICP-AES

After excitation by high temperature argon plasma, the relaxation of the excited electrons is accompanied by the emission of energy at a given wavelength as they return to the ground state. The energy emitted is characteristic of the element present in the sample and the intensity of that energy at a given wavelength is proportional to the concentration of the element in the sample analyzed. The ICP-AES used is available with radial and axial view configurations (Figure 2.5).

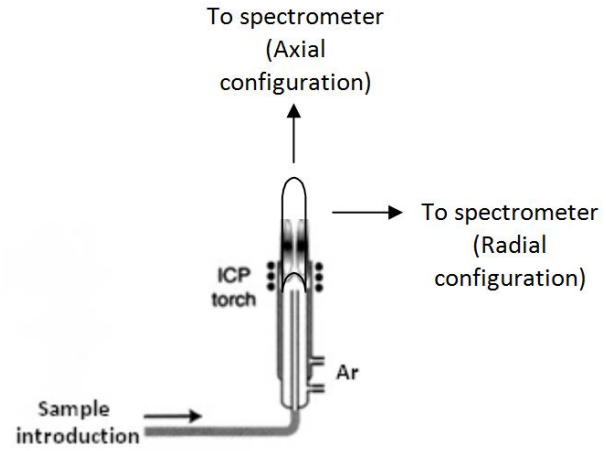


Figure 2.5 Radial and axial view configurations available for ICP-AES, Thermo Scientific iCAP 6500.

We used the axial view since it improves on the sensitivity and quantification limits of the radial view by about a factor of 10 or more depending on the element. These improvements are not without inconveniences. Axially viewed plasma is much more susceptible to interferences for some elements. ICP-AES is a fast, multi-elemental technique and has the advantage of minimizing the matrices effects compared to other spectroscopic methods. Elements such as Cu, Mn, Se and Zn were analyzed using ICP-AES in both livers and kidneys of

harbour porpoises. Whereas As and Cd which were relatively concentrated in some samples of kidneys, these elements were analyzed using both ICP-AES and ICP-MS depending on their concentrations in samples. Limits of quantification are presented in table 2.3.

2.1.2. Analysis on ICP-MS

Via the interface cone, the elements in the sample converted into ions are bought into the mass spectrometer. An intermediate vacuum region created by two intermediate cones: the sampler and the skimmer (~1mm) transmit the ions coming from the ICP torch with an atmospheric pressure into a low pressure region of the mass spectrometer. The mass spectrometer used is a quadrupole mass filter. It consists on the fact that electrostatic lenses with a positive charge serve to collimate the ion beam (also positively charged) and focus it into the entrance slit of the mass spectrometer. The ions are therefore separated by their mass-to-charge ratio (m/e). This mass filter only transmits the ions with an m/e ratio according to the frequency previously applied to the quadrupole. For a given isotope, the signal is the number of pulses which is converted into concentration after calibration. ICP-MS is a fast, multi-elemental technique and has much lower detection capabilities compared to ICP-AES. Elements such as As, Cr, Cd, Pb and V were determined in livers and kidneys (depending on concentration levels in the sample) using ICP-MS. Limits of quantification are presented in table 2.3.

Table 2.3 Limits of quantification (LOQ) expressed in µg.g^{-1} dw for metallic elements determined by ICP-AES, ICP-MS and SAA; *: LOQ for relatively concentrated elements determined in kidneys of harbour porpoises.

	As	Cd	Cr	Cu	Hg	Mn	Pb	Se	V	Zn
ICP-AES	1.5*	1.5*	-	2	-	2	-	2	-	2.5
ICP-MS	0.35	0.05	0.35	-	-	-	0.05	-	0.2	-
SAA	-	-	-	-	0.005	-	-	-	-	-

The main inconvenient of ICP-MS is the interferences which can bias the results over some masses. In order to overcome the matrix effects and the attenuation of the signal over time, a standard addition technique was applied before analyzing the samples on ICP-MS. It was used for the elements with low concentration in the samples such as As, Cd, Cr and V. Briefly, the standard addition technique consists in making replicates of the sample and adding a solution of

different increasing concentrations to known volumes of the sample. Depending on the element, standard additions between 0.5 and 10 $\mu g.L^{-1}$ were realized.

2.1.3. Analysis on AMA-254

The liver is the main storage organ for the mercury element (Wagemann and Muir, 1984); therefore we only determined Hg concentrations in livers of harbour porpoises. Samples of mineralized livers (liquid form 100µl) were injected directly in the Advanced Mercury Analyzer Spectrophotometer (AMA-254, Altec) available at Maison de la Recherhe en Environnement naturel (MREN, Wimereux). In fact, this analyzer allows direct measurements of solid samples, but considering that Hg is relatively concentrated in the livers of marine mammals and in order to reduce the effects of memory of the apparatus, we chose to inject samples in liquid form. Once introduced in the mercury analyzer, the liquid sample reaches the first furnace where a rise in temperature (~800°C) under an oxygen stream provides the drying of the matrix, its decomposition and calcination. The decomposed sample is drawn into a second furnace with a continuous heating (~900°C) and the Hg vapors are subsequently amalgamated on a gold net. Hg is then released by evaporation and measured by Atomic Absorption Spectrophotometry (AAS) at a characteristic wavelength (253.65 nm). The absorbance is quantified by prior calibration and is proportional to the concentration of Hg in the sample. Amounts of Hg ranging between 0.5 and 2 ng were obtained. The limit of quantification is presented in table 2.3.

2.1.4. Quality control procedures

For each analysis series, reagent blanks were analyzed. Signals of all blanks were below 1% compared to the signal of all samples. In addition, replicated analysis and analysis of standard reference material were adapted for quality control procedures. The standard reference materials, DOLT-4 and DORM-3 (dogfish liver and fish protein, respectively) from National Research Council (Canada) were treated and analyzed in the same conditions as samples in order to monitor the method sensitivities. Results are reported in table 2.4. According to the results, selected rays for ICP-AES and isotopes for ICP-MS were chosen for the different elements. For ICP-AES, the elements analyzed and wavelengths selected were: As 189.0 nm, Cd 214.4 nm, Cu 324.7 nm, Se 196.0 nm and Zn 213.8 nm. Whereas for ICP-MS, elements analyzed and isotopes

selected were: As 75, Cd 111, Cr 52, Pb 208 and V 51. Finally for the Hg element, the detection by AAS was held at the wavelength 253.65 nm. Yields from 87 % to 115 % proved that the results were in good agreement with the certified values.

Table 2.4 Comparison of concentrations (Mean ± SD) in µg.g^{-1} dry weight between certified reference materials DOLT-4 and DORM-3and measured values. * Values are not certified but given as an indication. Depending on the method used (ICP-MS or ICP-AES), mass of the isotope or wavelength in italic (nm) of the element were presented in the table.

Element	DOLT-4 (n=8)		DORM-3 (n=6)	
(Mass / *Wavelength*)	Certified	Measured	Certified	Measured
As 75	9.66 ± 0.62	8.37 ± 1.1	6.88 ± 0.30	7.25 ± 1.64
As 189.0		8.37 ± 0.3		7.12 ± 0.51
Cd 111	24.3 ± 0.8	22.93 ± 3.4	0.29 ± 0.02	0.27 ± 0.04
Cd 214.4		24.1 ± 0.4		
Cu 324.7	31.2 ± 1.1	30.5 ± 2	15.5 ± 0.63	15.6 ± 2.5
Cr 52	1.4*	1.35 ± 0.2	1.89 ± 0.17	1.85 ± 0.24
Hg	2.58 ± 0.22	2.40 ± 0.04	0.38 ± 0.06	0.35 ± 0.05
Pb 208	0.16 ± 0.04	0.14 ± 0.05	0.39 ± 0.05	0.38 ± 0.05
Se 196.0	8.3 ± 1.3	7.67 ± 0.8		
V 51	0.6*	0.69 ± 0.1		
Zn 213.8	116 ± 6	116 ± 8	51.3 ± 3.1	51.5 ± 10.2

2.2. Persistent Organic Pollutants analyses

POPs were determined in 20 samples of blubber from harbour porpoises stranded along the southern North Sea between 2010 and 2013. 10 to 20 g of blubber was freeze-dried and water content was determined by difference of weight before and after lyophilization. Samples were extracted for 8 hours by soxhlet apparatus with a mixture of nonpolar solvents cyclohexane/toluene (1/1; v/v). 10 to 40 % of lipids were dissolved in 5 mL of hexane. 1 mL of sulfuric acid (96 %) was added in order to precipitate lipids. From the extract, separate aliquots were taken for PCB, DDX and PBDE analyses.

Seven PCB congeners (whose IUPAC numbers are: CB 28, 52, 101, 118, 153, 138 and 180) recommended by the International Council for the exploration of the Sea (ICES) were considered. PCBs were measured with an Agilent 6890N gas chromatograph coupled to a 5973 Network MSD (GC-MS). The injector temperature was initially 80°C and after 1 min the

temperature was elevated by 20°C.min^{-1} up to 130°C, thereafter the temperature was elevated by 7°C.min^{-1} up to 270°C and kept for 6 min. The $^{13}C_{12}$-labeled PCB congeners 28, 52, 101, 138, 153, and 180 were used as internal standards. Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The limit of quantification (LOQ, according to the "Norme Française" EN 1528) was 0.01 µg.g^{-1} lipids.

Six DDXs (*o,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT and *p,p'*-DDE) were measured with an Agilent 6890A gas chromatograph coupled with a 5973 Network MSD (GC-MS). The GC was equipped to an Rxi XLB 30 m x 0.25 mm x 0.25 µm silica column. The injector temperature was initially 100°C and after 3 min the temperature was elevated by 12°C.min^{-1} up to 180°C, thereafter the temperature was elevated by 5°C.min^{-1} up to 300°C and kept for 6 min. The internal standard used was $^{13}C_{12}$-labeled *p,p'*-DDE Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The LOQ (according to the "Norme Française" EN 1528) was 0.01 µg.g^{-1} lipids.

Polybrominated diphenyl ethers (PBDE) congeners (BDE 28, 47, 99, 100, 153, 154, 183 and 209) were measured with an Agilent 7890A gas chromatograph coupled with a mass spectrometer system (GS/MS/MS Quattro Micro Waters). The GC was equipped to an Rtx 1614, 15 m x 0.25 mm x 0.1 µm silica column. The injector temperature was initially 250°C and after 2 min the temperature was elevated by 20°C.min^{-1} up to 310°C at which it was maintained for 4 min. The carrier gas was helium with a constant flow (3ml.min^{-1}). The internal standards added were: $^{13}C_{12}$-labeled BDE congeners 28, 47 and 99 (50 ng.ml^{-1}), $^{13}C_{12}$-labeled BDE congeners 153, 154 and 189 (100 ng.ml^{-1}) and $^{13}C_{12}$-labeled BDE 209 (250 ng.ml^{-1}). Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 70%. The LOQ was 0.05 µg.g^{-1} lipids.

2.3.Data treatment

The software "XLSTAT – Pro" 2013 (Addinsoft) was used in order to process statistical data. Half the limit of quantification was assigned for statistical analyses when values were below the limit of quantification. The level of significance was set at $\alpha = 0.05$. Parametric tests

such as student's *t*-test and Analysis of Variances (ANOVA) were used when the necessary assumptions of normality and homogeneity of variances for parametric statistics were satisfied. Non-parametric tests, such as Mann-Whitney *U* test and Kruskal-Wallis followed by Dunn test for multiple comparisons to check for pairwise differences, were used when the necessary assumptions of normality and homogeneity of variances for parametric statistics were not satisfied.

3. Feeding ecology

3.1. Stomach content analysis

Stomachs were collected from animals freshly dead or slightly decomposed along the southern North Sea and the Bay of Biscay and were stored at -20ºC until analysis. From all the 29 stomachs investigated (Figure 2.6), only 1 stomach related to a porpoise stranded in the southern North Sea was empty.

Figure 2.6 Stomach of a male harbour porpoise stranded along the Bay of Biscay in 2011. The photo shows the content of a whole prey species.

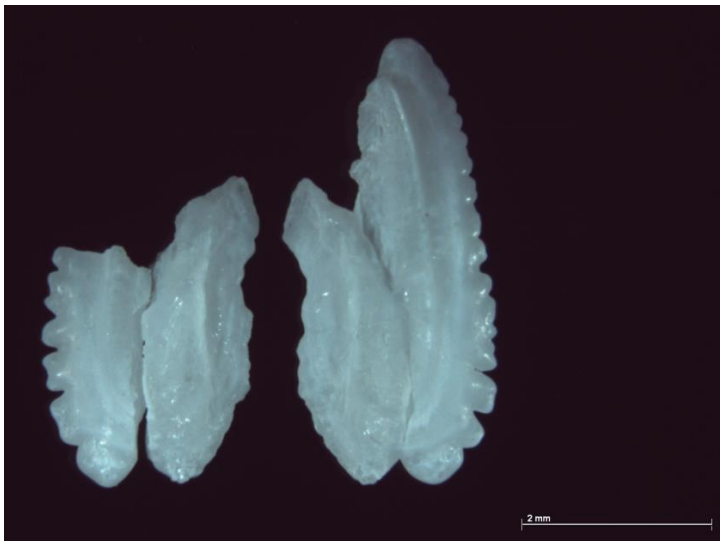
The total weight of stomach was recorded as well as the empty stomach weight. Therefore, the weight of the stomach content was obtained by difference. Stomachs were rinsed through running water and the content separated through a sieve with a mesh size of 0.2 mm. The remains in the sieve such as fish bones and otoliths were stored dry, whereas whole or partly digested prey items, cephalopod beaks as well as any remains with flesh attached were stored in 70% ethanol until identification. All remains were identified to the lowest possible taxon using our reference collection of specimens caught in the North Sea and English Channel (Laboratoire

d'Océanologie et de Géosciencs, Wimereux) and published data from Leopold et al., (2001) then counted and measured. Paired structures (such as otoliths) and impaired structures (such as carapaces for crustaceans) were used to estimate the total number of food items.

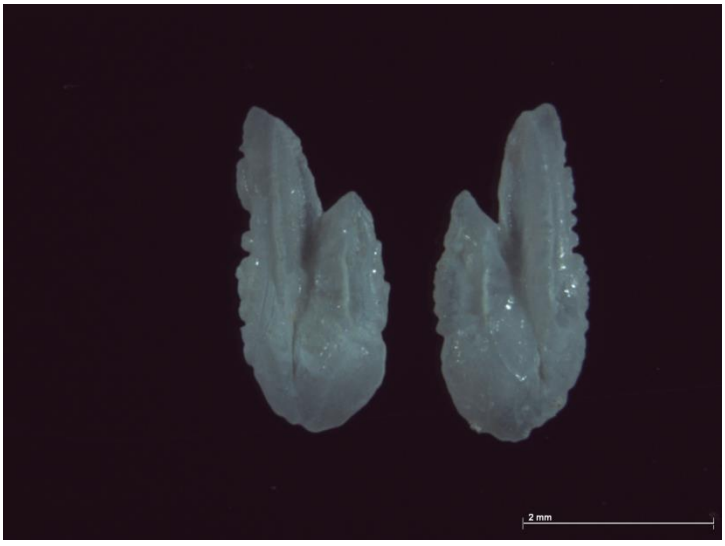
3.1.1. Otoliths

Teleost fishes consumed by the predators may be rapidly digested making them unrecognizable from external morphological features. However, hard parts such as bones and otoliths are much more resilient to digestion and have regularly been used to aid in the identification of partially digested remains. The form and structure of fish otoliths are species specific, allowing them to be used as an aid in identification of prey species consumed by predators. Thus the shape and size of preserved or undigested otoliths may be used to reconstruct the species and size composition of the diet of fish predators (Campana and Casselman, 1993). Various terms are used to describe the otolith's outline (Hour-glass, lobate, pyriform, tall, cuneiform, irregular, elliptic, etc) (Tuset et al., 2003). For some species it was only possible to identify the prey at the genera level. This was the case of the otoliths of gobiidaes which are very similar in shape and are hard to identify to species level.

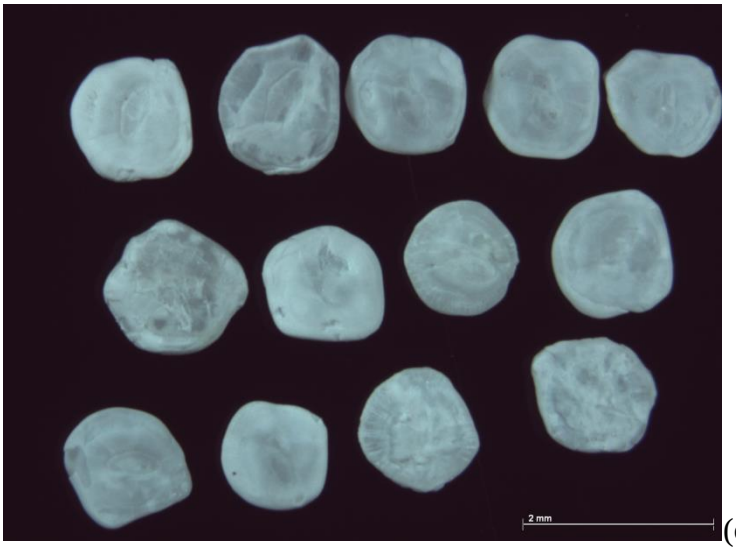
Acquisitions of otoliths were performed with the software TNPC 5.0 (Traitement Numérique des Pièces Calcifiées), digital processing of calcified parts developed by LASAA (Laboratoire de Sclérochronologie des Animaux Aquatiques, IFREMER) and in collaboration with NOESIS, images software developers. This software allows taking digital photos, storing them as databases and making accurate measurements on the otoliths. Digital photographs were taken for each otolith found in the stomach of harbour porpoises (Figure 2.7; Annex 2). The otolith was placed on a small black plate to provide a black background in order to obtain a good representation of the sagitta contour. The otoliths were represented with the dorsal margin to the top of the image and anterior (rostral) region to the right. Pictures of the otoliths were taken with a digital camera (BAUMER TX D13C) under a binocular microscope (Leica M10) connected to a computer. To get a clear image with the highest possible contrast, a lighting reflection from microscopic optical fiber (Intralux 5000-1) has set the direction and intensity of the light.



(a)



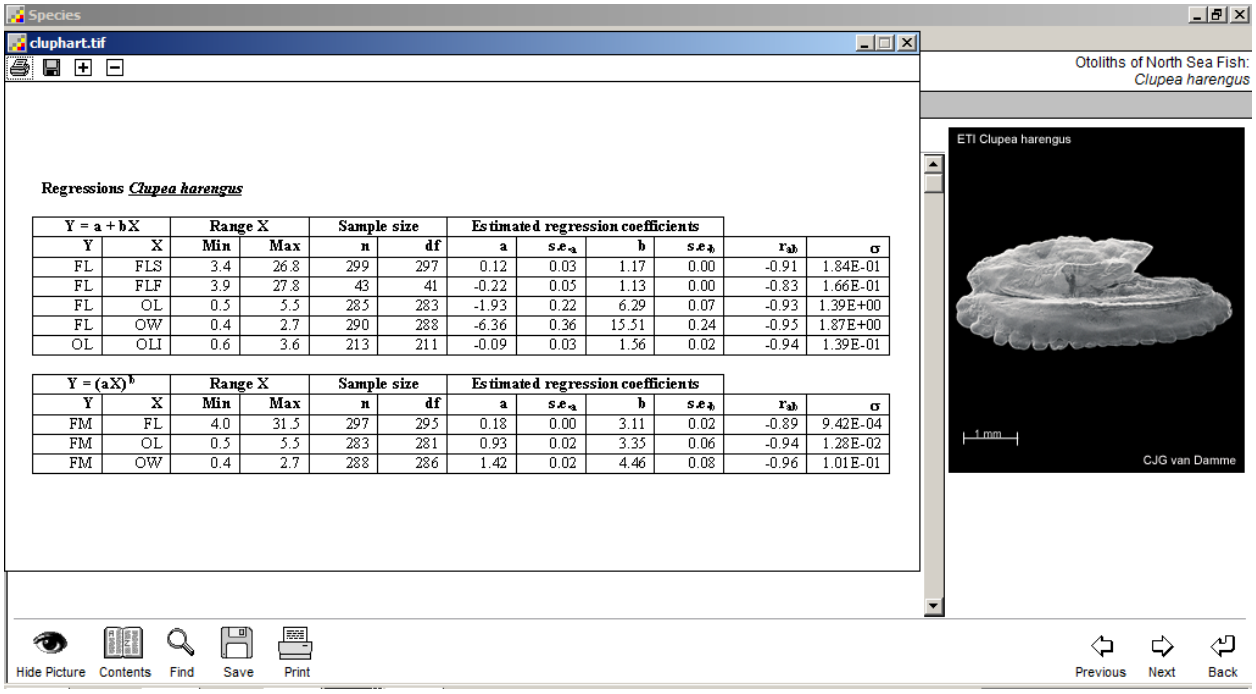
(b)



(c)

Figure 2.7 Different photos of otoliths found in the stomachs of harbour porpoises stranded along the southern North Sea and the Bay of Biscay; (a) *Clupea harengus*; (b) *Sardina pilchardus* and (c) *Gobidae sp.*

Therefore, measurements of otoliths length (OL, mm) and otoliths width (OW, mm) were used to reconstruct the total body length and individual body mass by using allometric relationships from Leopold et al., (2001) (Figure 2.8). When otoliths tips were broken, we used the otoliths width instead of the length to calculate the fish length and weight. That was the case of almost all *Merlangius merlangus* (Whiting) and *Merluccius merluccius* (European hake) otoliths which happened to have relatively the biggest otoliths.



Regressions *Clupea harengus*

Y = a + bX		Range X		Sample size		Estimated regression coefficients					
Y	X	Min	Max	n	df	a	$s.e._a$	b	$s.e._b$	r_{ab}	σ
FL	FLS	3.4	26.8	299	297	0.12	0.03	1.17	0.00	-0.91	1.84E-01
FL	FLF	3.9	27.8	43	41	-0.22	0.05	1.13	0.00	-0.83	1.66E-01
FL	OL	0.5	5.5	285	283	-1.93	0.22	6.29	0.07	-0.93	1.39E+00
FL	OW	0.4	2.7	290	288	-6.36	0.36	15.51	0.24	-0.95	1.87E+00
OL	OLI	0.6	3.6	213	211	-0.09	0.03	1.56	0.02	-0.94	1.39E-01

$Y = (aX)^b$		Range X		Sample size		Estimated regression coefficients					
Y	X	Min	Max	n	df	a	$s.e._a$	b	$s.e._b$	r_{ab}	σ
FM	FL	4.0	31.5	297	295	0.18	0.00	3.11	0.02	-0.89	9.42E-04
FM	OL	0.5	5.5	283	281	0.93	0.02	3.35	0.06	-0.94	1.28E-02
FM	OW	0.4	2.7	288	286	1.42	0.02	4.46	0.08	-0.96	1.01E-01

Figure 2.8 Print screen for the regressions of the herring *Clupea harengus* from the CD-ROM "Otoliths of North Sea Fish", Fish identification key by means of otoliths and other hard parts (Leopold et al., 2001). FL: Fish Length; FLS: Standard Length; FLF: Fork Length; FM: Fish Mass; OL: Otolith Length; OW: Otolith Width; OLI: Indented Otolith Length.

3.1.2. Diet composition

Four indices were used to quantify the diet composition of harbour pospoises:

- Percentage frequency of occurrence (*%O*):

$$\%O = \frac{e_i}{\mathrm{E}} \times 100$$

where e_i is the number of stomachs where the particular prey species was found and *E* is the total number of non empty stomachs,

- Percentage by number (*%N*):

$$\%N = \frac{\mathrm{n_i}}{\mathrm{N}} \times 100$$

where n_i is the number of prey of each taxonomic group and *N* is the total number of prey items,

- Percentage by mass ($\%W$):

$$\%W = \frac{w_i}{\mathrm{W}} \times 100$$

where w_i is the total weight of the particular prey species and W is the total weight of all prey species, prey mass provides the best proxy for the energetic importance of each prey type to porpoises

3.1.3. Feeding strategy

The feeding strategy was analyzed according to the graphical method proposed by Amundsen et al., (1996) which incorporated the prey-specific abundance (P_i) into Costello (1990) analysis. P_i is defined as the percentage that a prey taxon comprises of all items in only those predators in which the actual prey occurs, as follows:

$$P_i = \frac{\Sigma\, S_i}{\Sigma\, S_{ti}} \times 100$$

where P_i is the prey-specific abundance of prey i, S_i the stomach content comprised of prey i and S_{ti} the total stomach content in only those predators with prey i in their stomachs. This method allows prey item importance and type of feeding specialization (Amundsen et al., 1996).

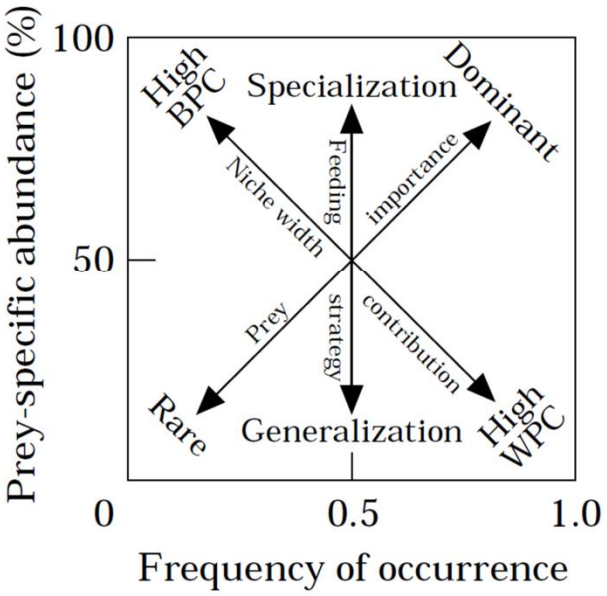


Figure 2.9 Explanatory diagram for interpretation of feeding strategy, niche width contribution and prey importance from the method Amundsen et al., (1996). BPC: between-phenotype component; WPC: within-phenotype component.

The examination of the distribution of data points along the diagonal and axes of the diagram can be used to interpret information about prey importance and feeding strategy of the predator (Figure 2.9). The measure of prey importance is provided by the percent abundance increasing along the diagonal from the lower left to the upper right corner, with the dominant prey at the upper and rare or unimportant prey at the lower end. The feeding strategy of the predator in terms of specialization or generalization is represented by the vertical axis. The upper part of the graph indicates that the predator have specialized on the prey type, whereas the lower part indicates that the prey have been eaten more occasionally (generalized). Prey points located to the upper right of the diagram must necessarily be restricted to a single or a few points, reflecting a predator population with a narrow niche width. If all prey points are located along or below the diagonal from the upper left to the lower right, with no prey points to upper right, the predator population will have a broad niche width. Prey with high Pi and low occurrence (upper left) will have been consumed by a few individuals displaying specialization, whereas prey with low Pi and high occurrence (lower right) will have been consumed occasionally by most individuals. Differences in feeding strategy are related to the between- and within-phenotype contributions (BPC and WPC, respectively) to the niche width. Different individuals specialize

on different resource types in a population with a high BPC, whereas in a population with a high WPC, most of the individuals utilize many resources types simultaneously (Amundsen et al., 1996).

3.2. Stable isotopes analysis

Muscle from porpoises stranded along the southern North Sea and the Bay of Biscay were stored at -20°C until further analysis. Based on previous studies describing harbour porpoises diet (Santos et al., 2004; Haelters et al., 2012), 14 potential prey species (*Limanda limanda, Platichthyus flesus, Pleuronectes platessa, Merlangius merlangus, Trisopterus luscus, Solea solea, Echiichthys vipera, Clupea harengus, Sprattus sprattus, Sardina pilchardus, Hyperoplus lanceolatus, Gobidae sp.*, *Crangon crangon* and *Sepia officinalis*) were collected along the southern North Sea. In order to take into consideration the seasonality variations, three campaigns were considered to collect prey species; the cruise SEPIA (INSU-CNRS) during June 2012 and October 2012 and the RV "Thalassa" (Ifremer) during November 2012. Prey samples were selected in order to cover the size-classes found in the stomach contents of harbour porpoises. Selected preys were also stored at -20º C until further analysis.

Stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were determined in the muscle of harbour porpoises as well as the muscle of prey species except for the gobies (*Gobidae sp.*) and the crustacean (*Crangon crangon*) where the whole body was ground. Samples were freeze-dried for 48 hours then ground into fine powder. An aliquot of approximately 100 mg from each sample was mixed with 4 ml of cyclohexane for lipid extraction, since lipids are highly depleted in ^{13}C compared to other tissue components (Tieszen et al., 1983). Samples were agitated for 1 h at 800 rpm then centrifuged for 5 min at 4000 rpm. The upper solution containing the lipids was removed and the samples were dried in an oven at 50º C for 48 hours. Subsamples of dried and lipid free muscle powder (0.35 ± 0.05 mg) were weighed into tin cups for stable isotopes analysis. Stable isotope measurements were performed with an elemental analyzer coupled to an isotope ratio mass spectrometer (DELTA V ADVANTAGE Isotope Ratio MS – Thermo Scientific) available at the laboratory "LIttoral ENvironnement et Societés" (LIENSs), University of La Rochelle, France.

Stable isotope abundances are expressed in "delta notation, δ" in parts per thousands (‰) following the equation:

$$\delta X = [\frac{R_{Sample}}{R_{Standard}} - 1] \times 1000$$

where *X* represents ^{13}C or ^{15}N and R_{Sample} represents the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic ratio of the sample. Ratios are expressed relative to the international standards Vienna PeeDee Belemnite (V-PDB) and atmospheric Nitrogen (N_2) for ^{13}C and ^{15}N measurements, respectively. Replicate measurements of internal laboratory standards (acetanilide) during each series of measurement indicated errors less than 0.15 ‰ and 0.20 ‰ for carbon and nitrogen, respectively.

3.3. Fatty acids analysis

Blubbers of harbour porpoises previously stored at -20°C were freshly used for extractions whereas muscles of potential prey species were freeze-dried before extractions. In addition to the prey species used for stable isotopes analysis, 2 prey species were added for the fatty acids analysis: the sand smelt (*Atherina presbyter*) and the thinlip grey mullet (*Liza ramada*).

A slightly modified version of Bligh and Dyer, (1959) as in Meziane et al., (2007) was used for the fatty acids extractions. Approximately 50 mg of fresh blubber from harbour porpoise or freeze-dried muscle from prey species were placed in 5 ml Reacti-Therm Vials to which a known quantity (typically around 20µg) of tricosanoic acid (23:0) was added as an internal standard. Samples were subject to ultrasonication for 20 min with distilled water: $CHCl_3$: MeOH (1:1:2, v: v: v). Afterwards, the addition of a distilled water: $CHCl_3$ mixture (1:1, v: v) and the centrifugation (5 min; 3000 rpm) of the mixture formed a two layer system. The lower $CHCl_3$ layer containing the lipids was carefully pipetted with a glass Pasteur Pipettes with rubber bulbs and retained in new vials. The upper layer was reprocessed with $CHCl_3$ (2 ml) in order to ensure that all the lipids in the sample were extracted. After the second extraction, the new lower $CHCl_3$ layer was added to the first one already retained in the vials and the whole mixture containing the lipids was concentrated under a N_2 flow.

In order to obtain the total lipids as methyl esters, saponification and methylation were conducted according to Meziane and Tsuchiya, (2002).

- Saponification

A mixture of 2 mol NaOH: MeOH (1: 2, v: v) was added to the residue and after mixing, the vials were placed in the heating block for 90 min at 100°C. Afterwards, hydrochloric acid (35%) was added to stop the reaction. 1.5 ml $CHCl_3$ was added to the mixture, then mixed, centrifugated (5 min; 3000 rpm) and the lower phase containing lipids was retained in new vials. This step was repeated one more time to ensure that all lipids were extracted. After the 2 consecutive extractions, the mixture was concentrated under a N_2 flow.

- Methylation

Boron trifluoride - methanol (BF_3, 1 ml) was added to the residue of the saponification as an esterification reagent. After mixing, the vials were kept in the heating block for 10 min at 100°C. When cooled down, a mixture of distilled water: $CHCl_3$ (1:1, v: v) was added, mixed and centrifugated (5 min; 3000 rpm). The upper layer containing the distilled water and the MeOH was withdrawn with a glass Pasteur Pipettes and discarded. This step was repeated one more time to ensure that all lipids were extracted. Finally the $CHCl_3$ lower layer containing the fatty acids methyl esters (FAMEs) was pipetted off and transferred into a 1 ml pre-weighed vial. The mixture was concentrated under a N_2 flow, and then weighed. The total lipids were obtained by weight differences. The FAMEs were dissolved in an adequate amount of $CHCl_3$ (generally about 200 µl per 1 mg of FAMEs) and all vials were kept at -20°C for later chromatographic analysis.

- Chromatographic analysis

For the identification, FAMEs were dissolved in an adequate amount of hexane (generally about 150 µl per 0.5 mg of FAMEs). Individual FAMEs were separated and quantified by gas chromatography equipped with a flame ionization detector (GC; Varian CP – 3800; available at the Museum National d'Histoire Naturel – MNHN, BOREA, Paris). The GC was fitted with a Supelco OMEGAWAX 320 column (30 m, 0.32 mm ID, 0.25 µm film thickness). Helium was the carrier gas. The sample (1µl) was injected at 60°C and the temperature was raised to 150°C

at 40°C.min^{-1}, then ramped up to 240°C at 3°C.min^{-1} and kept at there for 14 minutes. A typical chromatogram produced under these conditions is shown in Figure 2.10.

Peaks were identified by comparing their retention times with those of authentic standards (Supelco^{TM} 37, PUFA Mix – No 1 Marine Source and Bacterial mix; Supelco Inc., Bellefonte, PA, USA). Moreover, peaks of FAs were confirmed with GC-mass spectrometry (GC-MS; ThermoFinnigan TRACE DSQ; available at the Museum National d'Histoire Naturel – MNHN, BOREA, Paris) for some samples. Standard nomenclature is used for the identified FAs:

$$X{:}Y\ \omega\ Z$$

where X is the number of carbon atom, Y is the number of double bonds and Z is the position of the ultimate double bond from the terminal methyl. For example, the 20:5ω3 fatty acid has 20 carbon atoms, 5 double bonds with double bond closest to the methyl-end situated between the carbon atoms at positions 17 and 18.

According to Schomburg, (1987), the concentration of each FA (C_{FA}, mg) was calculated:

$$C_{FA} = \frac{A_S}{A_{IS}} \times \frac{C_{IS}}{W_S}$$

where A_S is the peak area of the FA, A_{IS} is the peak area of the internal standard, C_{IS} is the concentration of the internal standard (mg) and W_S is the dry weight of the sample (g) for prey species and fresh weight (g) for the blubber of porpoises.

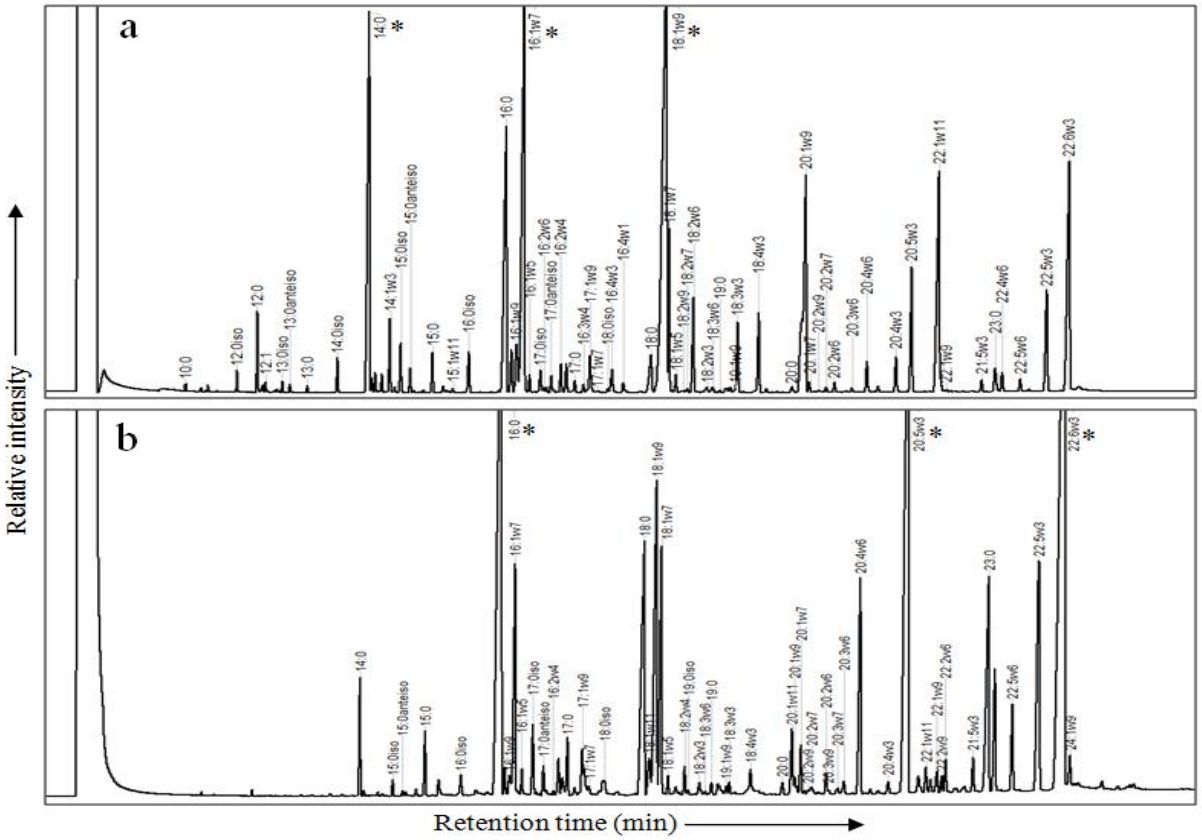


Figure 2.10 Gas chromatogram (FID) of fatty acid methyl esters derived (a) from harbour porpoise stranded along the southern North Sea in 2011 and (b) from the prey species *Gobidae sp.* collected from the cruise SEPIA (INSU-CNRS) during October 2012. *: Peak was cut off at the top.

3.4.Compound-Specific Stable Isotope Analysis (CSIA)

The Compound-Specific ^{13}C isotope analysis of the fatty acid methyl esters was determined in the blubber of 10 harbour porpoises and some prey species such as *Clupea harengus, Sardina pilchardus, Gobidae sp.*and *Liza ramada*. All CSIA analyses were held at the Stable Isotope Facility, University of California, Davis. Therefore, the protocol will be briefly discussed. After extraction of the FAMEs as discussed above, stable carbon isotope ratios of the FAMEs were measured with a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to Thermo Finnegan Delta Plus Advantage isotope ration mass spectrometer (GC-IRMS). The GC was equipped with a splitless injector and an BP x 70 (60 m; 0.25 mm O.D; 0.25 mm film thickness) column with a constant flow of 1.5 $mL.min^{-1}$. A known quantity (typically around 20µg) of dodecanoic acid (12:0) was added as an internal standard to the FAMEs dissolved in hexane before injection into the GC. The sample (3µl) was injected at

100°C (held for 1 min) and the temperature was raised to 210°C at 3°C.min^{-1}(held for 5 min), then ramped up to 245°C at 3°C.min^{-1} and kept at there for 5 minutes.

The stable carbon isotope composition of the individual FAs are expressed as δ13C values, which are defined as parts per thousands (‰) differences from an international standard:

$$\delta^{13}C = [(^{13}C/^{12}C_{sample} / {}^{13}C/^{12}C_{standard}) - 1] * 1000$$

the international standard used as a reference is the Peedee Belemnite (PBD) standard. C12, C15, C17, C18, C19, C20 and C22 *n*-Alkanes were co-injected with the FAMEs and used as internal isotopic references. Yields between 73 and 99% proved the accuracy and precision of the GC-IRMS. *n*-Alkane standards were also used as an additional check for any drift between runs.

3.5. Data treatment

3.5.1. Mixing model: Stable isotope analysis in R (SIAR)

Through mixing models, the proportional contribution of each source (prey species) to the isotopic signature (accumulated diet) of the predator may be estimated. For this purpose, several isotopic mixing models have been developed to link isotopic signatures of predators to isotopic signatures of potential prey species, taking into account the isotopic fractionation between prey and predator (Phillips and Gregg, 2001; Phillips and Gregg, 2003). The stable isotope mixing model SIAR (stable isotope analysis in R) was used to evaluate the relative contribution of each prey species in the diet of harbour porpoises stranded in the southern North Sea. This Bayesian stable isotope mixing model is able to deal with more sources than variables (Phillips and Gregg, 2001; Phillips and Gregg, 2003) and can also include uncertainties such as natural variations and analytical errors, producing results as probability distributions with residual errors (Parnell et al., 2010). Our model was based on the trophic enrichment factors (TEFs) for captive cetaceans as published by Hobson et al., (1996) (1.3 ± 0.1‰ and 2.4 ± 0.3‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively) and Caut et al., (2011) (1.26 ± 0.2‰ and 1.23 ± 0.15‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively). Individual isotope ratios of porpoises (n=52) were entered in the model, whereas for prey species (n=49), means and standard deviations were used. The mixing model was run using default parameters (iterations: 500 000; burn in: 50 000 and thinning by: 15).

3.5.2. Fatty acids data treatment

To investigate for variations in FA composition among samples, Bray-Curtis similarity matrices were calculated on the FA dataset with no transformation applied. Analysis of similarity (ANOSIM) was performed using PRIMER 5 and the statistic test was computed after 5000 permutations. Significant differences in FAs profiles were identified using the global *R*-values. Similarity percentages routine (SIMPER) was used to determine FAs contributing to the differences. Factor used for the analysis were gender and maturity status, cause of death and blubber thickness.

3.6. Environmental data

To link the abundance of potential prey species in the North Sea to the spatial distribution and feeding ecology of harbour porpoises, we used the data on the evolution of fish abundance in the North Sea available from the database SIH-Ifremer (http://sih.ifremer.fr/). The population and community indicators for the survey data have been collected since the end of the 1970s. After scientific surveys carried out by Ifremer along the French coasts, data such as species distribution map by area, population indices by species, by area and community indices by area were available.

CHAPTER 3

HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) STRANDED ALONG THE SOUTHERN NORTH SEA: AN ASSESSEMENT THROUGH METALLIC CONTAMINATION

Chapter 3 - Harbour porpoises (*Phocoena phocoena*) stranded along the southern North Sea: An assessment through metallic contamination

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Published in "Environmental Research", 133 (2014) 266-273.

Abstract

Throughout the last few years, the southern North Sea has witnessed an increase in the number of stranded marine mammals, particularly the harbour porpoise (*Phocoena phocoena*). This species is subject to several potential threats such as exposure to contaminants, changes in food supply, marine traffic and fishery by-catch. The aims of this study were to investigate potential associations between contaminants and health status and to analyze spatial and temporal trends of metal concentrations in harbour porpoises. Selected trace elements (As, Cd, Cr, Cu, Hg, Mn, Se, V and Zn) were measured in kidneys and livers of 105 harbour porpoises stranded along the southern North Sea (French and Belgian coasts from 2006 to 2013) and 27 stranded along the Bay of Biscay (French coast from 2009 to 2012). Porpoises that died from infectious disease displayed significant higher hepatic concentrations of Cd, Hg, Se and Zn compared to healthy porpoises that died from physical trauma. Adult porpoises displayed significant higher concentrations of Cd, Cr, Hg, Se and V in livers compared to juveniles. No spatial or temporal trends in metal concentrations were detected in our study. The results of the present study suggested that chemical contamination may represent one of many threats encountered by harbour porpoises, but it cannot explain alone the increase in the number of stranded individuals.

Keywords: harbour porpoise; stranding; metals; southern North Sea

Introduction

The harbour porpoise (*Phocoena phocoena*) is the most common and abundant marine mammal inhabiting the North Sea and adjacent waters. The abundance of porpoises has increased significantly in the last decade along the southern North Sea (Hammond et al., 2013). This species is very sensitive to anthropogenic disturbances, mostly fishery activities such as by-catch and food depletion (Haelters and Camphuysen, 2009; Herr et al., 2009), organic pollutants and metal contaminants (Bennett et al., 2001; Das et al., 2004b) and recently the exponential growth of industrial activity at sea through the construction of offshore wind farms (Haelters, 2009). The North Sea is a productive and biologically rich area, and therefore it provides abundant food for marine mammals. It is regarded as a moderately polluted sea area (OSPAR, 2010). The North Sea is a semi-enclosed sea surrounded by large and highly developed societies. Therefore this area supports intense fishing activities and coastal industrial activities. It is a major navigation route for some of the world's most developed and highly populated economies, thus shipping in the area is the most intense in the world (Ducrotoy and Elliott, 2008).

Due to their role as top predators within the marine food web, marine mammals such as porpoises have been used as indicators for ecosystem changes. Over the last decade, porpoises stranding has increased in French, Belgian and Dutch coastal waters (Haelters and Camphuysen, 2009; Jauniaux et al., 2008). Many studies tried to identify the causes of death and monitored the health status of marine mammals. In the southern North Sea, infectious diseases and bycatch in fishing nets were the two prevailing causes of death (Jepson et al., 1999, Siebert et al., 1999, Jauniaux et al., 2002b; Haelters and Camphuysen, 2009). However, high levels of metal contaminants have been documented in various studies on marine mammals. For instance, harbour porpoises from England and Wales, northern France, Belgian and German coasts tend to accumulate metal contaminants according to their health status (Bennett et al., 2001; Das et al., 2004a). Correlations between severities of disease and liver Hg levels were detected in harbour porpoises from the German waters (Siebert et al., 1999). These studies suggested that metal contaminants may have adverse effects on harbour porpoise's health or that metals are redistributed from other tissues to liver during disease or cachexia.

Since chemical contaminants may affect the health of harbour porpoises, contamination by metals may be associated with the increased stranding of harbour porpoises in the southern

North Sea along northern France and Belgian coasts. Therefore, the aims of the present work were (1) to establish a temporal trend of metal levels in organs of porpoises from the southern North Sea stranded between 2006 and 2013 and to investigate potential associations between metal concentrations and the cause of death (traumatic or infectious) of porpoises; (2) to assess the population status by comparing levels of metal contaminants in organs of porpoises stranded in the southern North Sea to porpoises stranded in the Bay of Biscay (French Atlantic coast) between 2009 and 2012.

1. Materials and methods

1.1. Sampling and data collection

From 120 harbour porpoises stranded in the southern North Sea along the northern France and Belgian coasts between 2006 and 2013, livers and kidneys were collected for analyses. Similarly in the Bay of Biscay, livers and kidneys were collected from 35 harbour porpoises stranded between 2009 and 2012. According to the protocol from Kuiken and Hartmann, (1993) and Jauniaux et al., (2002b), post-mortem investigations were performed on all freshly dead or slightly decomposed carcasses washed ashore along the southern North Sea. Age groups were determined from the length of individuals. Porpoises with lengths ranging from 91 to 130 cm were considered as juveniles and animals greater than 130 cm were considered as adults (Jauniaux et al., 2002b). The nutritional status of animals was evaluated according to the blubber thickness measured at the cranial insertion of the dorsal fin. Four categories were identified according to the cause of death of the animal. The first category represented harbour porpoises that died from infectious diseases including parasitic, bacterial, mycotic and viral infections and those that died from lung edema, pneumonia and emaciations. The second category represented porpoises that died from physical trauma associated to suffocation, traumatic injuries and entanglement in fishing nets. Porpoises who died of other causes (tumor, starvation, etc) or whose cause of death could not be determined mostly due to the advanced decomposition of carcasses represented the third category. Finally porpoises that died from seal predations represented the fourth category. Livers and kidneys were stored at -20°C in plastic bags until analyses were carried out.

1.2. Metal analysis

Livers and kidneys were freeze-dried and homogenized with an agate mortar and pestle. An aliquot of 150 mg from each material was digested in a concentrated solution of nitric acid (65 %, Suprapur, Merck) at room temperature for 24 h and then at 100°C for 4 h. Metal concentrations (As, Cd, Hg, Pb and V as non essential elements and Cu, Cr, Mn, Se and Zn as essential elements) were determined by Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES, Thermo Scientific iCAP 6500) and Inductively Coupled Plasma Mass Spectrometer (ICP-MS, Varian 820-MS) depending on concentration levels of each metal. In order to resolve the matrix effects, a standard addition technique was applied for the determination of As, Cd, Cr, and V on ICP-MS. Metal concentrations are reported in $\mu g.g^{-1}$ dry weight (dw). For conversion into wet weight (ww) in order to compare with other studies, the following factors according to the weight before and after lyophilisation are applicable: 0.29 for liver and 0.24 for kidney. Limits of quantification were 0.05 $\mu g.g^{-1}$ dw for Cd and Pb, 0.20 $\mu g.g^{-1}$ dw for V, 0.35 $\mu g.g^{-1}$ dw for As and Cr, 2 $\mu g.g^{-1}$ dw for Cu, Se and Mn, and 2.5 $\mu g.g^{-1}$ dw for Zn. Lead concentrations were consistently below the quantification limit and therefore will not be mentioned in the table of results.

Total mercury analyses were conducted on an Advanced Mercury Analyzer Spectrophotometer (AMA-254, Altec). Concentrations were only determined in the livers of harbour porpoises since it is the main storage organ for Hg (Paludan-Müller et al., 1993). This analyzer allows direct measurements of solid samples, but considering that Hg is relatively concentrated in the livers of marine mammals, we injected samples in liquid form (100 µL) after mineralization. Amounts of Hg ranging between 0.5 and 2 ng were obtained. This method reduces the effects of memory of the apparatus and allows having a representative weight of the considered sample. The limit of quantification was 0.005 $\mu g.g^{-1}$ dw.

Procedural blanks, replicated analysis and analysis of standard reference material were adapted for quality control procedures. As for standard reference materials, DOLT-4 and DORM-3 (dogfish liver and fish protein, respectively, National Research Council, Canada) were treated and analyzed under the same conditions as samples in order to monitor the above mentioned method sensitivities. Yields from 87 % to 115 % proved that the results were in good agreement with the certified values.

1.3. Data treatment

Data analysis was performed using "XLSTAT – Pro" 2013 (Addinsoft). When values were below the limit of quantification, half the limit of quantification was assigned for statistical analyses. Concentrations in livers and kidneys were tested using a Mann-Whitney *U* test or a student's *t*-test when the necessary assumptions of normality and homogeneity of variances for parametric statistics were satisfied. These statistical tests were used to compare metal accumulation in juvenile and adult porpoises from the southern North Sea, as well as to compare metal levels between the southern North Sea and the Bay of Biscay. Moreover, metal concentrations were tested using Kruskal-Wallis test followed by the Dunn test for multiple comparisons to check for pairwise differences. These statistical tests were used to delineate the evolution of metal concentrations in livers and kidneys of porpoises from the southern North Sea between 2006 and 2013. Finally, correlations between metals in livers and kidneys were tested using the Spearman coefficient. The level of significance was set at $\alpha = 0.05$.

2. Results

Mean tissue concentrations of metals determined in livers and kidneys of harbour porpoises from the southern North Sea and the Bay of Biscay are presented in table 3.1. The results show that Mn, Cu, Se and Zn presented higher levels in livers compared to kidneys, whilst V, As and Cr had almost same levels in both organs. The Cd element had higher values in kidneys compared to livers. Zn had the highest mean levels in livers of porpoises stranded in southern North Sea followed by Cu, Hg, Se, Mn, As, Cr, V and Cd. Similar order occurred for metal concentrations in the livers of porpoises from the Bay of Biscay except for Hg which had higher mean levels than Cu. In kidneys of porpoises from the southern North Sea, mean levels in descending order were: Zn, Cu, Se, Mn, As, Cd, Cr and V. Porpoises stranded in the Bay of Biscay, displayed similar order except for Cd which had higher mean levels than As.

For the geographical comparison, hepatic Zn concentrations displayed significantly higher levels in porpoises of the southern North Sea compared to porpoises stranded in the Bay of Biscay ($p < 0.05$), whereas V and As displayed higher levels of hepatic concentrations in porpoises stranded in the Bay of Biscay compared to the southern North Sea area ($p < 0.05$ and $p < 0.001$, respectively). Other metallic contaminants such as Cd, Cr, Cu, Hg, Mn and Se showed

no significant variations between both studied areas. For renal analysis, results showed that Cr, Cu, Mn, V and Zn displayed higher concentrations in the kidneys of porpoises from the southern North Sea compared to animals from the Bay of Biscay ($p < 0.05$), while As, Cd and Se showed no significant interregional variations.

Table 3.1 Trace element ($\mu g.g^{-1}$ dw) concentrations in tissues of harbour porpoises stranded between 2006 and 2013 along the Southern North Sea (French and Belgian coasts) and porpoises stranded between 2009 and 2012 in the Bay of Biscay. Mean ± SD; range of concentrations (minimum – maximum); number of samples.

	Metal concentrations								
	Mn	**Cu**	**Se**	**Zn**	**V**	**As**	**Cr**	**Cd**	**Hg**
Livers									
Southern North Sea	18 ± 5	38 ± 36	24 ± 46	193 ± 106	0.6 ± 0.4	2.5 ± 0.9	2 ± 1	0.4 ± 0.5	30 ± 51
n = 105	(2.4 - 38)	(6 - 320)	(1.9 - 311)	(67 - 635)	(0.2 – 2.4)	(<0.35 - 6.6)	(0.6 - 7)	(<0.05 – 3.3)	(1.8 - 292)
Bay of Biscay	17 ± 6	33 ± 23	24 ± 31	152 ± 62	0.7 ± 0.4	3.0 ± 0.9	1.5 ± 0.2	0.5 ± 0.5	45 ± 67
n = 25	(9 - 28)	(12 - 136)	(2 - 106)	(77 - 303)	(0.4 – 1.76)	(1.4 – 5.2)	(1.4 – 2.4)	(<0.05 – 1.5)	(2.1 - 225)
Kidneys									
Southern North Sea	3 ± 1.6	16 ± 4.5	11 ± 3.5	94 ± 17	0.6 ± 0.3	2.8 ± 1	2 ± 1	2.3 ± 3.1	-
n = 100	(<2 - 18)	(5 - 44)	(3.6 - 22)	(40 - 150)	(0.2 – 1.4)	(<0.35 – 5.5)	(0.6 – 4.5)	(<0.05 - 18)	-
Bay of Biscay	3 ± 0.7	15 ± 2.5	12 ± 6	87 ± 12	0.4 ± 0.1	2.4 ± 1.3	1.1 ± 0.1	2.8 ± 2.6	-
n = 27	2 - 4	11 - 22	3 - 23	68 - 120	0.2 – 0.7	0.9 - 7	0.6 – 1.3	<0.05 - 8	-

For harbour porpoises stranded in the southern North Sea between 2006 and 2013, correlations in livers (n = 105) and kidneys (n = 100) between metals are shown in table 3.2. In the livers of porpoises, there was a strong correlation between Hg and Se concentrations (Spearman correlation value $Rs = 0.868$, $p < 0.0001$). Hg was also correlated with Cd ($p < 0.0001$), V ($p < 0.005$) and negatively correlated with Mn ($p < 0.05$). In kidneys, Cd showed significant correlations with Cu, Se and Zn. A significant correlation was also detected between Cu and Zn in livers ($p < 0.0001$). A high correlation was observed between V and Cr in livers ($Rs = 0.871$) and kidneys ($Rs = 0.955$) of porpoises ($p < 0.0001$). Similarly to the porpoises stranded in the southern North Sea, almost same correlations between metals were found in livers (n = 25) and kidneys (n = 27) of porpoises stranded in the Bay of Biscay (Data not shown). Hg and Se were highly correlated in livers ($Rs = 0.923$, $p < 0.0001$). Hg was also correlated to Cd and V ($p < 0.0001$). In kidneys, Cd showed significant correlations between Se, Zn ($p < 0.05$)

and V ($p < 0.0001$). For V and Cr, this correlation is not as pronounced in the case of porpoises from the Bay of Biscay.

Table 3.2 Spearman rank correlation matrix between trace elements in livers (n=105) and kidneys (n=100) of harbour porpoises stranded in the Southern North Sea between 2006 and 2013.

	Mn	Cu	Se	Zn	V	As	Cr	Cd	Hg
livers									
Mn	**1**								
Cu	**0.360****	**1**							
Se	-0.147	0.157	**1**						
Zn	**0.281****	0.134	0.071	**1**					
V	-0.016	-0.082	**0.337****	-0.067	**1**				
As	-0.068	-0.051	-0.015	**-0.297****	0.034	**1**			
Cr	-0.010	-0.033	**0.262***	-0.003	**0.871*****	-0.111	**1**		
Cd	-0.152	0.027	**0.411*****	0.170	0.109	-0.016	0.088	**1**	
Hg	**-0.203***	0.043	**0.868*****	0.129	**0.286****	-0.033	0.173	**0.458*****	**1**
Kidneys									
Mn	**1**								
Cu	**0.434*****	**1**							
Se	-0.012	-0.185	**1**						
Zn	**0.321****	**0.476*****	**-0.247***	**1**					
V	-0.004	**-0.251***	-0.008	-0.026	**1**				
As	0.056	-0.143	**0.249***	**-0.409*****	-0.093	**1**			
Cr	0.043	**-0.229***	-0.027	-0.017	**0.955*****	-0.084	**1**		
Cd	0.127	**0.253***	**0.294****	**0.296****	0.094	-0.168	0.103	**1**	

*$P < 0.05$
**$P < 0.005$
***$P < 0.0001$

2.1. Metal contaminants and maturity status

Metal contaminants were determined in livers of 85 juveniles (33 females and 52 males) and 18 adults (13 females and 5 males). Results (Figure 3.1a) showed that adult porpoises displayed significantly higher Cd, Cr, Hg, Se and V hepatic concentrations compared to juveniles ($p < 0.05$). No such differences between juveniles and adults were detected for As, Cu and Zn in livers. Hepatic concentrations of Cd, Hg and Se in adult porpoises were 4, 9 and 6 fold higher than in juvenile porpoises, respectively.

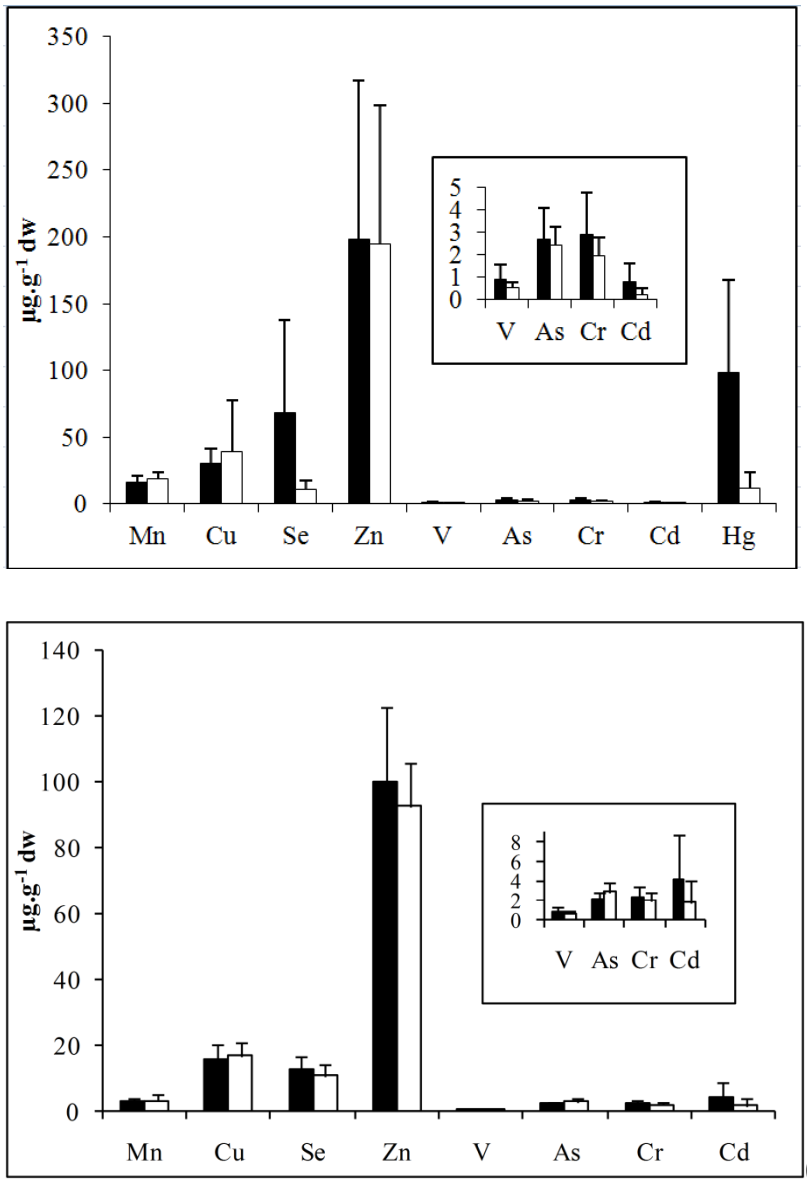


Figure 3.1 Mean metal concentrations (µg.g^{-1} dw) in livers (a) and kidneys (b) of porpoises stranded along the French and Belgian coasts between 2006 and 2013 according to the maturity status. Black bars represent adult porpoises (n=18, livers and n=19, kidneys) and white bars juvenile porpoises (n=85, livers and n=79, kidneys).

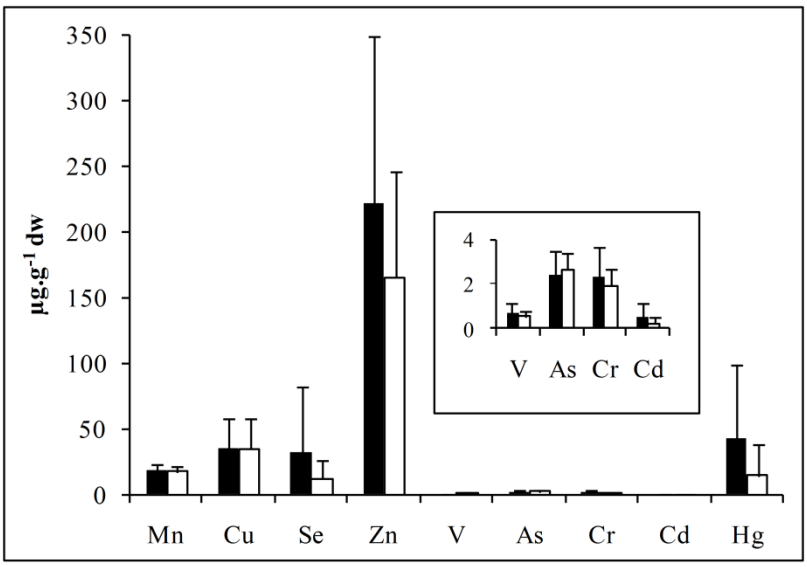
For renal analysis, 79 juveniles (31 females and 48 males) and 19 adults (13 females and 6 males) were analyzed (Figure 3.1b). Juveniles exhibited significantly higher As concentrations compared to adults ($p < 0.05$). For the Cd, mean renal concentrations were significantly higher in adult porpoises compared to juveniles ($p < 0.05$). No such differences were detected for Cr, Cu, Mn, Se and V in kidneys between juveniles and adults.

2.2. Metal contaminants and causes of death

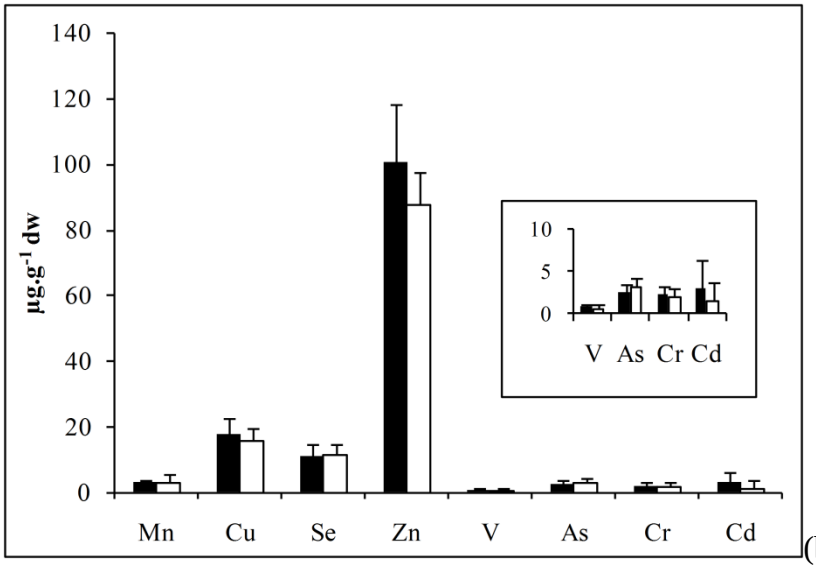
For porpoises stranded in the southern North Sea between 2006 and 2013, we compared the mean blubber thickness of porpoises that died from physical trauma (20 ± 6 mm) and those that died from infectious disease (14 ± 6 mm). Statistical analysis showed that porpoises that died from physical trauma displayed significantly mean thicker blubber than porpoises that died from infectious disease (Mann-Whitney, $p < 0.0001$).

Harbour porpoises that died from infectious diseases ($n = 47$) showed significantly higher mean liver concentrations of Cd, Hg, Se, V and Zn compared to healthy porpoises ($n = 44$) that died from physical trauma ($p < 0.05$). However porpoises that died from traumas showed significantly higher concentrations of As in the liver compared to those that died from diseases ($p < 0.05$) (Figure 3.2a).

In kidneys (Figure 3.2b), significant differences for Cd, Cr, Cu and Zn concentrations were detected in porpoises that died from infectious disease ($n = 44$) compared to those that died from traumas ($n = 44$) ($p < 0.05$). Similarly to the liver, significantly higher concentrations of As were detected in the kidneys of porpoises that died from traumas compared to those that died from diseases ($p < 0.05$).



(a)



(b)

Figure 3.2 Mean metal concentrations (µg.g^{-1} dw) in livers (a) and kidneys (b) of porpoises stranded along the French and Belgian coasts between 2006 and 2013 according to the cause of death. Black bars represent porpoises that died from infectious disease (n=47, livers and n=44, kidneys) and white bars porpoises that died from physical trauma (n=44, livers and n=44, kidneys).

3. Discussion

Exposure to metal contaminants may impair the immune system of marine mammals. High levels of metal contaminants have been documented in various studies on harbour porpoises (Jepson et al., 1999; Siebert et al., 1999; Bennett et al., 2001; Das et al., 2004b). In the present study, Metal levels were compared in organs of harbour porpoises stranded in a semi-

enclosed sea (North Sea) and in a very large opened bay (Bay of Biscay) (table 3.1). Results showed that only Zn element had significant higher concentrations in livers and kidneys of stranded porpoises from the southern North Sea. Porpoises from this area, with high Zn and poor body condition are already facing stressful conditions such as elevated levels of organochlorines and other toxic compounds (Jepson et al., 1999; Das et al., 2004b). Metal concentrations determined in livers and kidneys of harbour porpoises stranded in the southern North Sea and the Bay of Biscay were within the range of values reported for other porpoises stranded in the southern North Sea (northern France and Belgium) between 1994 and 2001 (Das et al., 2004b) and porpoises stranded on the French coasts between 1997 and 2003 (Lahaye et al., 2007), whereas hepatic and renal concentrations in porpoises from the present study were lower than levels determined in porpoises stranded on the UK coasts between 1989 and 2001 (Jepson, 2003). From a geographical comparison, harbour porpoises from the southern North Sea and the Bay of Biscay may have comparable levels of metallic contaminants in their tissues. However in our study, differences of metal concentrations are much more pronounced taking into account the cause of death rather than the stranding location. Indeed, we have constantly found that porpoises that died from infectious diseases displayed significantly higher Hg, Se, Zn, Cd and V levels in their livers compared to those that died from physical trauma. These findings are in agreement with the study of Bennett et al. (2001) who found same trends for Hg, Se and Zn levels but not for Cd levels, whereas another study showed that Cu, Cd and Hg concentrations in livers and kidneys did not vary significantly with deteriorated health condition (Das et al., 2004b).

3.1. Non essential elements

Several studies showed that, among the non essential elements, Hg is the most concentrated metal in porpoise livers (present study, Bennett et al., 2001; Das et al., 2004b; Ciesielski et al., 2006; Lahaye et al., 2007). Mercury is of particular concern due to its properties of biomagnifications in the upper levels of the food web, high mobility and persistence in the marine ecosystem (Palmisano et al., 1995; Das et al., 2003b). Liver Hg and MeHg concentrations were associated with the severity of disease in porpoises from the German waters (Siebert et al., 1999). Additionally, Hg can accelerate and exacerbate disease manifestations in inbred strains of mice prone to autoimmune disease (Reviewed by Theron et al., 2012). Total mercury levels in livers of porpoises stranded in the southern North Sea varied widely within individuals; adult

porpoises displayed significantly higher hepatic Hg concentrations compared to juveniles ($p < 0.0001$). For instance, the highest value of mercury (292 $\mu g.g^{-1}$ dw) was obtained in the liver of a porpoise which age class and sex were undetermined. While the second highest value (276 $\mu g.g^{-1}$ dw) was determined in the liver of an adult female who died from infectious disease. This latter concentration was more than 150 fold higher than the lowest value (1.8 $\mu g.g^{-1}$ d.w.) determined in the liver of a juvenile male who died after capture. Bioaccumulation of mercury with age has been described in various marine mammal species (Wagemann and Muir, 1984; Wagemann et al., 1996). Consequently, the difference in mercury levels between juveniles and adults is presumably linked to a lower exposure and / or lower prey intake for the juvenile porpoises. It has been proved that in marine mammals mercury is not stored solely as methylmercury (MeHg). Mercury is ingested mainly in its highly toxic form methylmercury. Through a relatively slow biochemical process, the MeHg is biotransformed to the inorganic form and biochemically inert mercury selenide (HgSe). Indeed there was a strong correlation between selenium and mercury concentrations in the livers of porpoises in the present study (table 3.2). It is well known that mercury and selenium occurs in a 1:1 molar ratio in marine mammals (Paludan-Müller et al., 1993; Nigro and Leonzio, 1996; Wagemann et al., 1998) only after a certain threshold for the total mercury concentration (Palmisano et al., 1995). Harbour porpoises from the southern North Sea exhibited a Hg:Se molar ratio lower than 1. Same findings were observed on stranded porpoises along the French coasts between 1997 and 2003 (Lahaye et al., 2007). In fact, porpoises that died from infectious disease displayed significant higher mean Hg:Se molar ratio compared to those that died from physical trauma (Mann-Whitney, $p < 0.05$). These results are in agreement with the study of Bennett et al., (2001), who suggested that high Hg concentrations in livers of porpoises from coastal waters of England and Wales could not be detoxified by available Se since porpoises were already facing infectious diseases. It should be noted that healthy group who died from physical trauma included 43 juveniles and only 2 adults, whereas the infectious disease group included 32 juveniles and 15 adults. In addition, in our study adult porpoises exhibited significantly higher mean molar ratio (0.6) compared to juvenile porpoises (0.4) (Mann-Whitney, $p < 0.001$). Therefore, the difference in mean Hg:Se molar ratio in our study may be associated to the maturity status along with the fact that in the infectious disease group with high Hg concentrations the detoxification process with Se may be limited (Bennett et al., 2001). The Hg limit for hepatic damage in liver tissue of marine mammals is estimated to

100 – 400 $\mu g.g^{-1}$ ww (350 – 1400 $\mu g.g^{-1}$ dw approximately) (Wagemann and Muir, 1984). Furthermore, a threshold level of 60 $\mu g.g^{-1}$ ww (200 $\mu g.g^{-1}$ dw approximately) was estimated for liver damage in mammals (AMAP, 1998). Bennett et al., (2001) did not reject the hypothesis that Hg exposure may influence the health of harbour porpoises and contribute to their mortality. Only three adult animals from the southern North Sea and two adult animals from the Bay of Biscay exhibited higher hepatic Hg concentrations than the second threshold level estimated by AMAP (1998) (200 $\mu g.g^{-1}$ dw) in mammal livers but lower than the first threshold (350 $\mu g.g^{-1}$ dw) estimated by Wagemann and Muir (1984). These animals, exhibiting Hg levels in their livers higher than the above-mentioned estimated threshold, died due to infectious diseases. The variability found in Hg concentrations may be related to differences in methylmercury elimination capacity and/or age related storage of detoxified forms among individuals (Nigro and Leonzio, 1996). In this study, most of the Hg levels were below the estimated threshold and the mean Hg:Se molar ratio was below the unit. These findings suggest that harbour porpoises from the southern North Sea may not be exposed to high levels of Hg and therefore Hg levels in livers were not sufficient to induce tiemannite granules as result of coprecipitation of Hg with Se. We urge caution in such interpretation since stranded animals in this study were predominant by younger age of porpoises.

Unlike mercury which is known for its accumulation in livers, renal tissues were typically higher in Cd levels than hepatic tissues (Wagemann and Muir, 1984; Paludan-Müller et al., 1993). Indeed, our study also revealed that Cd concentrations in kidneys were significantly higher than liver concentrations (Mann-Whitney, $p < 0.0001$). In both organs, Cd concentrations were significantly higher in adult porpoises compared to juveniles (Figure 3.1). Uptake from water and food (trophic transfer) are several sources contributing to metal accumulation in marine animals (Wang and Rainbow, 2010). High Cd concentrations have been reported previously in livers and kidneys of marine mammals suggesting that oceanic cephalopods constituted a significant part of their diet (Bustamante et al., 1998; Das et al., 2000) or that marine mammals were ingesting prey from polluted areas (Wagemann and Muir, 1984). Indeed, in marine trophic food chains the oceanic cephalopods are considered as an important source of Cd for top predators (Bustamante et al., 1998). Several authors reported different feeding habits related to sex and/or age in harbour porpoises. For instance, adult female porpoises fed at a higher trophic level than adult males, while no differences as a function of sex were detected for

juvenile porpoises (Das et al., 2004a). Evidence of sex or age related variation in diet may be consistent with opportunistic predation. Differences in diet between adult and juvenile porpoises have been found in several studies. Some prey species are more important in the diet of young porpoises, such as gobies (Gobiidae) and shrimps, compared to adult ones (Santos et al., 2004). Santos and Pierce, (2003) suggested that juveniles cannot dive as deep as adults and could be limited by their small size from catching and eating big prey. Furthermore, the bioaccumulation of Cd element with age in marine mammal species has been well described (Mackey et al., 1996; Anan et al., 2002; Ikemoto et al., 2004; Harper et al., 2007). Our data suggest that the different Cd levels between juveniles and adults may be related to the maturity status, the bioaccumulation with age along with the differences in the feeding habits between juveniles and adults. We found a significant correlation between Cd and Se in livers and kidneys of stranded porpoises (Table 3.2). This correlation is probably not a result of a biochemical interaction. It is more likely due to the fact that both elements are age-dependent; the interactions of Hg and Se probably lead to age-dependent accumulation of Se in livers (Paludan-Müller et al., 1993; Anan et al., 2002).

For the other two non essential elements considered in this study, differences in concentrations according to the maturity status were less pronounced than Hg and Cd elements. Arsenic, showed no differences in the liver of porpoises in both juveniles and adults (Figure 1a). In kidneys, the As element showed significantly higher concentrations in juveniles (Figure 1b). Moreover, porpoises that died from physical trauma showed significantly higher concentrations of As than porpoises that died from infectious disease in both livers and kidneys (Figure 3.2). It should be mentioned that juvenile porpoises represent more than 89% of porpoises that died from physical trauma. Thus, the significantly higher As concentrations found in porpoises that died of physical trauma might be influenced by the higher proportion of younger animals in this group rather than the cause of death of porpoises. Frouin et al., (2010) showed that high As concentrations were able to induce genotoxic effects in a harbour seal cell line, and therefore produce alterations in the immune function, which may decrease resistance to infectious diseases encountered in marine mammals. In our study, As concentrations were within the range of values reported for various stranded marine mammals. For instance, California sea lions from southern California (Harper et al., 2007) and bottlenose dolphins from south Carolina and Florida waters (Stavros et al., 2011). The bottlenose dolphins from this latter study also exhibited same range of V levels in their livers compared to porpoises stranded in our study. Moreover, the present

hepatic V levels were in the same order of magnitude compared to those reported in other marine mammals such as, Beluga whales (Mackey et al., 1996), various pinnipeds (Saeki et al., 1999) and Caspian seals (Anan et al., 2002). An increase in the concentrations of V with age has been reported in several species of marine mammals and in addition V levels were correlated with Hg levels in the liver (Mackey et al., 1996; Saeki et al., 1999; Anan et al., 2002; Ikemoto et al., 2004; Stavros et al., 2011). Similarly, adult porpoises showed significant higher levels of V in livers compared to juveniles and a significant correlation was found between hepatic V and Hg accumulation (table 3.3). It has been suggested that the correlation of Hg and V in livers with aging was caused by the increase in the nuclei and mitochondria fraction since these hepatic elements existed mainly in these cells. Hence V may be accumulated in the tissues as a contaminant similar to Hg (Saeki et al., 1999).

3.2. Essential elements

Apart from Se, Zn and Cu concentrations were also significantly correlated with Cd concentrations in kidneys of porpoises stranded in the southern North Sea (Table 3.2). Another study suggested that Zn and Cd correlation is due to the fact that Cd bonds to the zinc-metallothionein, which in turn induces the synthesis of new zinc-metallothionein (Paludan-Müller et al., 1993). The primary function of metallothioneins is the homeostatic regulation of essential metals as Cu and Zn. Moreover metallothioneins are able to bind non-essential metals such as Cd and Hg (Reviewed by Das et al., 2003a). Therefore, the functions of metallothioneins are considered to be metal storage / detoxification of non-essential metals (Cd and Hg) or homeostatic regulation of essential metals (Zn and Cu) (Reviewed by (Wang and Rainbow, 2010). This correlation between toxic Cd and essential Cu and Zn would suggest induction of metallothioneins and possible competition or increase in metal binding sites (Wagemann et al., 1988). Whereas other studies who found no correlation between cited metals suggested that the low Cd concentrations to which porpoises may be exposed in European waters are probably not sufficient to induce Cu and / or Zn ion displacement from metallothioneins and consequently leading to co-accumulation with Cd (Lahaye et al., 2007). Das et al., (2004b) suggested that porpoises from the southern North Sea, which are already facing stressful conditions such as elevated levels of organochlorines and other toxic compound, could be exposed to an additional source of stress due to a combination of high Zn and Hg levels and poor body condition. Zn

concentrations in our study were not significantly different between adults and juveniles. These results were in agreement with the study of Bennet et al., (2001) who showed no accumulation of Zn with age. Zn concentrations have been rather related to disease status. For instance, high Zn concentrations in porpoises that died of infectious disease may represent a response to infection and other stressors (like cold) causing Zn redistribution (Bennett et al., 2001). Furthermore, porpoises displaying lesions of the respiratory tract had higher hepatic Zn burden than porpoises without lung lesions (Das et al., 2004b). The exposition of seals to high Zn concentrations in Caspian Sea resulted in the disturbance of homeostatic control and nutritional status of essential elements (Anan et al., 2002). Similarly, in our study porpoises from the southern North Sea that died from infectious disease showed significantly higher levels of Zn compared to those that died from physical trauma (Figure 2). Hence the blubber thickness of infected porpoises was 12.9 ± 6.4 mm, which was significantly lower than healthy porpoises that died from physical trauma (19.8 ± 5.9 mm) (Kruskal-Wallis; $p<0.0001$). Accordingly, high Zn concentrations may be linked to a deteriorated body condition and nutritional status.

High correlation between Cr and V was observed in livers and kidneys of porpoises (Table 3.2). Similarly to V, Cr was also correlated with age in livers. Adult porpoises displayed significantly higher levels of hepatic Cr compared to juveniles (Figure 3.1a). Therefore, this observed correlation between V and Cr may be related to an age-dependant bioaccumulation. Mn and Cr are essential trace elements (Theron et al., 2012) hence slightly variable from animal to animal. Mn is required as a cofactor in at least two classes of enzymes, phosphotransferases and arginases (Mackey et al., 1995). When compared with harbour porpoises from other regions, Mn and Cr levels appear to be in the same order of magnitude than those reported in previous works (Bennett et al., 2001; Szefer et al., 2002; Ciesielski et al., 2006; Aubail et al., 2013).

3.3. Temporal trends

From 2006 to 2013, we compared the mean metal concentrations in livers and kidneys of porpoises stranded in the southern North Sea. The number of stranded porpoises ranged between 9 stranded animals for the year 2006 and 16 stranded animals for 2007. Statistical analysis showed that in both livers and kidneys, no significant differences were found in metal concentrations along the years 2006 to 2013. In order to broaden the period for comparison, we

may compare our results with previous work (Das et al., 2004b). Elements such as Zn, Cd, Cu, Fe, Se and Hg were determined in liver, kidney and muscle of harbour porpoises from northern France and Belgium (almost same area as the present study) stranded between 1994 and 2001. Metal levels were almost similar in both studies. Hg superior limits were higher in porpoises stranded between 1994 and 2001, whereas Se limits were lower (344 vs. 292 $\mu g.g^{-1}$ dw and 99 vs. 311 $\mu g.g^{-1}$ dw for Hg and Se in periods 1994 – 2001 and 2006-2013, respectively). Porpoises from the southern North Sea (1994 – 2001) displayed high Zn and Hg concentrations compared to porpoises from Norwegian waters and Baltic Sea. This variation was linked to both differences in pollutant exposure and nutritional status between individuals (Das et al., 2004b). The results of our study are in agreement with these findings. As already mentioned, Zn and Hg concentrations were significantly higher in porpoises that died from infectious diseases. However Das et al., (2004b) showed that the increase in Hg and Se with deteriorating body condition in porpoises was not significant, whereas in our study Hg and Se levels were higher in infected porpoises that died between 2006 and 2013. Considering the metallic concentration levels in organs, we may assume that the population status has been stable from 1994 to 2013. Human activities and changes in emission of pollutants have a noticeable effect on the trace metal and organic pollutant levels in rivers and coastal zones. The recent study of (Gao et al., 2013) showed that there is a general decrease of chemical pollution in the southern North Sea (Belgian coastal zone and Scheldt River and Estuary). These authors related the chemical pollution decrease to a decrease in atmospheric and aquatic emissions related to industrial, transportation and domestic activities. Therefore, the stable metal concentrations in porpoises organs stranded between 1994 and 2013 may be related to the decrease of chemical pollution in the environment.

Conclusion

In the present study a passive monitoring of stranded animals was presented, which can provide insight into environmental impacts on marine mammals. Levels of Hg, Se, Zn, Cd and V appeared to be higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma, although synergetic effects of metallic contaminants on health status was not elucidated. Our findings indicate that we cannot reject the hypothesis that metallic contaminants may influence the health of harbour porpoises and contribute to the increased stranding encountered the last decade for the population in the southern North Sea.

This leads us to the fact that the contamination status in the southern North Sea may represent one of many threats encountered by the harbour porpoises population in this area. Furthermore, the SCANS surveys (1994) showed that porpoises were encountered in the North and Celtic seas except for the channel and southern part of the North Sea (Hammond et al., 2002). These highest densities that were encountered in northern areas in 1994 showed a south shifting in 2005 (Hammond et al., 2013). The authors suggested that the cause of this shifting may be likely due to a change in the distribution and / or availability of harbour porpoises preys. Therefore, several threats such as habitat degradation and climate change may affect distribution and availability of preys (Harwood, 2001). Further work is required to evaluate whether southward shifting population may be related to prey availability which may contribute to the strikingly increase stranding.

Acknowledgements

The samples used in this study were collected by the French Stranding Network. The "Grand Port Maritime de Dunkerque" (France) is acknowledged for the financial support of this study. Mahfouz Celine is financially supported by a PhD fellowship from the National Council for Scientific Research (Lebanon) and Université du Littoral Côte d'Opale (France). We would like to thank two anonymous reviewers for their helpful comments on an earlier version of this manuscript.

CHAPTER 4

ORGANOCHLORINES IN HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) STRANDED ALONG THE SOUTHERN NORTH SEA BETWEEN 2010-2013

Chapter 4 - Organochlorines in harbour porpoises (*Phocoena phocoena*) stranded along the southern North Sea between 2010-2013

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Published in "Environmental Science: Processes and Impacts", 16 (2014) 2774-2781.

Abstract

7 polychlorinated biphenyls (PCBs), 6 dichlorodiphenyltrichloroethane (DDXs) and 8 Polybrominated diphenyl ethers (PBDE) were measured in the blubber of 20 harbour porpoises stranded on the coasts of the southern North Sea between 2010 and 2013. Results showed that porpoises that died from infectious disease displayed significant higher levels of PCBs in their blubber compared to healthy porpoises that died from physical trauma. ∑7CBs and ∑DDXs were higher in juvenile porpoises compared to adult females. Except for three individuals, PBDE concentrations were below the limit of quantification in the blubber samples treated. In general, levels of PCBs and DDXs obtained in the blubber of porpoises from this study were in the same order of magnitude or even lower than porpoises stranded along the North East Atlantic Ocean and the Black Sea over the period 1987 and 2013. The results of the present study suggest that even if the status of marine pollution has been improved, a continuous long-term contamination by toxic organochlorines over many generations may be observed.

Keywords: Harbour porpoise; stranding; PCBs; DDXs; southern North Sea

Introduction

Harbour porpoise (*Phocoena phocoena*) is a representative top predator species for the North Sea ecosystem. This long-lived species feeds at a high trophic level, thus it can accumulate relatively high levels of contaminants and is vulnerable to the effects of environmental changes (Duinker et al., 1989; Tanabe et al., 1994; Aguilar and Borrell, 1995). In the past few years, an increased number of stranded porpoises in the southern part of the North Sea (Jauniaux et al., 2008; Haelters et al., 2011) has generate a special interest toward this species. Concern has also been expressed about other potential threats such as food depletion (MacLeod et al., 2007) and pollutants (Bennett et al., 2001; Das et al., 2004b; Mahfouz et al., 2014).

Exposure to persistent organochlorines such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT) and their related compounds has caused abnormalities in higher trophic feeding animals from North Sea, UK and various seas (Duinker et al., 1989; Tanabe et al., 1994; Aguilar and Borrell, 1995; Jepson et al., 1999). PCBs have been synthesized for industrial uses and DDXs as agrochemicals (Jones and de Voogt, 1999). The production of these organochlorines was banned in Europe since the end of 1970s generating the EU directive (79/117/EEC) for DDXs and the council directive (96/59/EC) for disposal of PCBs. However a continuous long-term contamination by toxic organochlorines over many generations is expected (Tanabe et al., 1994). POPs are lipophilic compounds and can bioaccumulate and magnify in the food chain, therefore their impact on top predator species is of particular concern (Jones and de Voogt, 1999). Organic pollutants may have possible adverse effects on marine mammal populations. It has been demonstrated that thymic atrophy and spleenic depletion in harbour porpoises from German North and Baltic Seas were significantly correlated to increased PCB and polybrominated diphenyl ethers (PBDE) levels (Beineke et al., 2005).

Compared to seals, birds and terrestrial mammals, harbour porpoises are suggested to have lower capacity to metabolize organochlorine compounds (Duinker et al., 1989; Tanabe et al., 1994; Boon et al., 1997; Law et al., 1998). Since organochlorines are known to exert immunotoxicity in harbour porpoises individuals, a special interest may be given to the contaminant levels in their organs and tissues. A previous study on the metallic contaminants in livers and kidneys of harbour porpoises stranded along the southern North Sea did not reject the hypothesis that chemical contaminants may influence the health of harbour porpoises and

contribute to the increased level of stranding seen during the last decade for the population in this area (Mahfouz et al., 2014). Therefore, the aims of the present study were (1) to relate organochlorine concentrations and profiles to the maturity status and the gender of stranded porpoises, (2) to investigate potential associations between organic contaminants (PCBs and pesticides) and the cause of death (traumatic or infectious) of porpoises and (3) to compare the contaminant levels in porpoises from this study to other porpoises stranded along European waters (North East Atlantic Ocean and the Black Sea) in order to assess the current contamination status of harbour porpoises in the study area.

1. Materials and methods

1.1. Sampling and data collection

Harbour porpoises stranded in the southern North Sea along the northern France and Belgian coasts between 2010 and 2013 were collected for POP analyses (Figure 4.1). Due to their lipophilic nature, POPs are known to accumulate in the fatty tissues, hence in the blubber of cetaceans (Tanabe et al., 1994; Aguilar and Borrell, 1994). Therefore blubber was sampled from the cranial insertion of the dorsal fin and stored wrapped in aluminum foil at -20°C. All washed ashore carcasses were freshly dead or slightly decomposed. Post-mortem investigations were performed according to the protocol from Kuiken and Hartmann, (1993) and Jauniaux et al., (2002a). The length of individuals was used to determine age groups. Porpoises with lengths ranging from 91 to 130 cm were considered as juveniles and animals greater than 130 cm were considered as adults (Jauniaux et al., 2002b). According to the blubber thickness measured at the cranial insertion of the dorsal fin, the nutritional status of animals was evaluated. All animals were divided into 4 groups according to the cause of death. Harbour porpoises that died from infectious diseases including parasitic, bacterial, mycotic and viral infections and those that died from lung edema, pneumonia and emaciations represented the first group. Porpoises that died from physical trauma associated to suffocation, traumatic injuries and entanglement in fishing nets represented the second group. The third group represented porpoises that died of other causes (tumor, starvation, etc) or whose cause of death could not be determined. Finally porpoises that died from seal predations represented the fourth group.

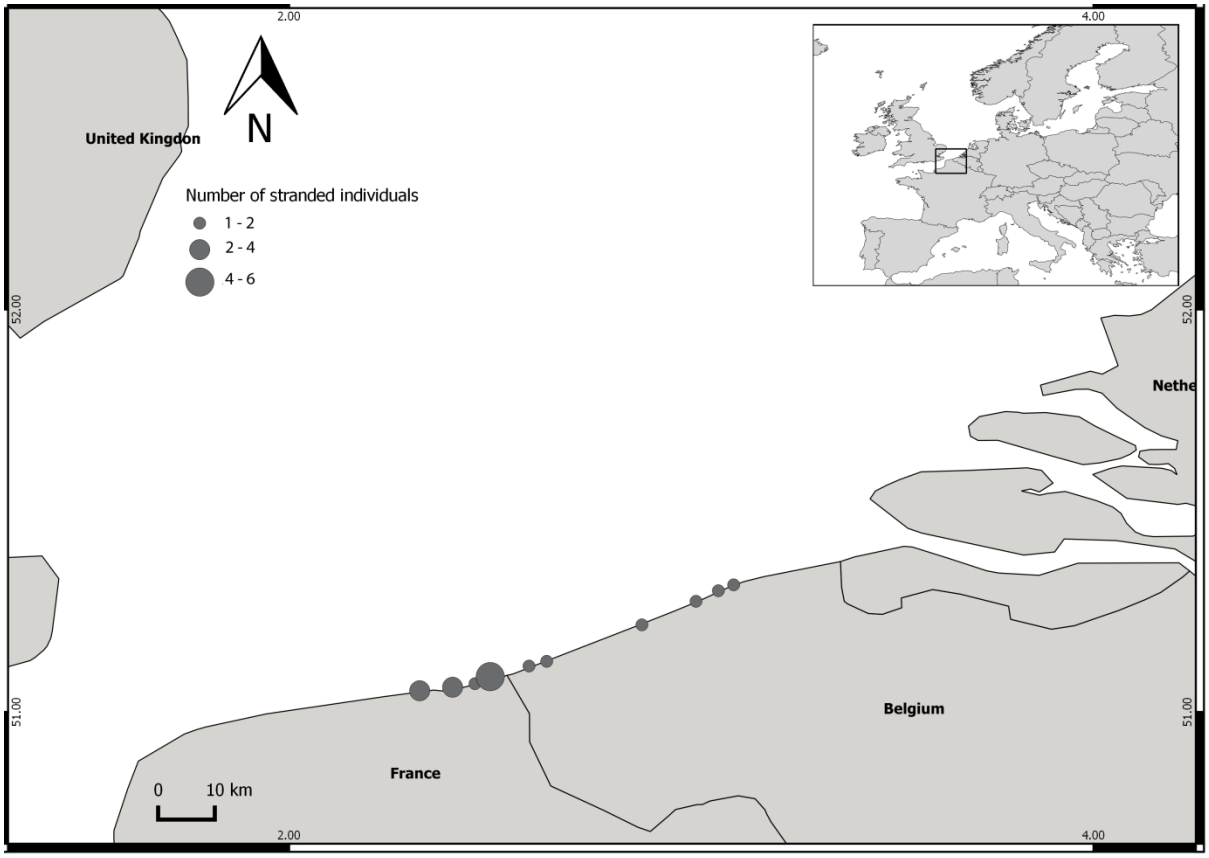

Figure 4.1 Harbour porpoises stranding locations and numbers along the southern North Sea analyzed in this study (2010-2013).

1.2. POP analysis

POPs were determined in 20 samples of blubber from harbour porpoises stranded along the southern North Sea between 2010 and 2013. 10 to 20 g of blubber was freeze-dried and water content was determined by difference of weight before and after lyophilization. Samples were extracted for 8 hours by soxhlet apparatus with a mixture of nonpolar solvents cyclohexane/toluene (1/1; v/v). 10 to 40 % of lipids were dissolved in 5 mL of hexane. 1 mL of sulfuric acid (96 %) was added in order to precipitate lipids. From the extract, separate aliquots were taken for PCB, DDX and PBDE analyses.

Seven PCB congeners (whose IUPAC numbers are: CB 28, 52, 101, 118, 153, 138 and 180) recommended by the International Council for the exploration of the Sea (ICES) were considered. PCBs were measured with an Agilent 6890N gas chromatograph coupled to a 5973 Network MSD (GC-MS). The injector temperature was initially 80°C and after 1 min the

temperature was elevated by 20°C.min^{-1} up to 130°C, thereafter the temperature was elevated by 7°C.min^{-1} up to 270°C and kept for 6 min. The $^{13}C_{12}$-labeled PCB congeners 28, 52, 101, 138, 153, and 180 were used as internal standards. Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The limit of quantification (LOQ, according to the "Norme Française" EN 1528) was 0.01 µg.g^{-1} lipids.

Six DDXs (*o,p'*-DDD, *o,p'*-DDT, *o,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT and *p,p'*-DDE) were measured with an Agilent 6890A gas chromatograph coupled with a 5973 Network MSD (GC-MS). The GC was equipped to an Rxi XLB 30 m x 0.25 mm x 0.25 µm silica column. The injector temperature was initially 100°C and after 3 min the temperature was elevated by 12°C.min^{-1} up to 180°C, thereafter the temperature was elevated by 5°C.min^{-1} up to 300°C and kept for 6 min. The internal standard used was $^{13}C_{12}$-labeled *p,p'*-DDE Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 80%. The LOQ (according to the "Norme Française" EN 1528) was 0.01 µg.g^{-1} lipids.

Polybrominated diphenyl ethers (PBDE) congeners (BDE 28, 47, 99, 100, 153, 154, 183 and 209) were measured with an Agilent 7890A gas chromatograph coupled with a mass spectrometer system (GS/MS/MS Quattro Micro Waters). The GC was equipped to an Rtx 1614, 15 m x 0.25 mm x 0.1 µm silica column. The injector temperature was initially 250°C and after 2 min the temperature was elevated by 20°C.min^{-1} up to 310°C at which it was maintained for 4 min. The carrier gas was helium with a constant flow (3ml.min^{-1}). The internal standards added were: $^{13}C_{12}$-labeled BDE congeners 28, 47 and 99 (50 ng.ml^{-1}), $^{13}C_{12}$-labeled BDE congeners 153, 154 and 189 (100 ng.ml^{-1}) and $^{13}C_{12}$-labeled BDE 209 (250 ng.ml^{-1}). Replicate analyses and procedural blanks were adopted with no significant amount of analytes observed. Recoveries of internal standards were more than 70%. The LOQ was 0.05 µg.g^{-1} lipids.

1.3. Data treatment

Data analysis was performed using "XLSTAT – Pro" 2013 (Addinsoft). The level of significance was set at $\alpha = 0.05$. When values were below the limit of quantification, half the limit of quantification was assigned for statistical analyses. To assess the differences in POP

concentrations between porpoises that died from infectious diseases and those that died from physical trauma, Mann-Whitney *U* test or a student's *t*-test were used when the necessary assumptions of normality and homogeneity of variances for parametric statistics were satisfied. Moreover, to compare POP concentrations between juveniles and adults (males and females) in the blubber of porpoises from the southern North Sea, Kruskal-Wallis test followed by the Dunn test for multiple comparisons were used to check for pairwise differences. Finally, correlations between POPs in blubber were tested using the Spearman coefficient.

2. POP results

Results for POPs analysis in the blubber of 20 harbour porpoises stranded in the southern North Sea between 2010 and 2013 are presented in table 4.1. PCB concentrations (sum of 7 congeners) varied widely between individuals reporting an average of 18 ± 25 $\mu g.g^{-1}$ lipids and ranging between 0.6 and 110 $\mu g.g^{-1}$ lipids. The CB profiles in the blubber of all harbour porpoises analyzed were dominant by the recalcitrant congener CB 153 with proportions more than 40% of total CB. In descending order, levels were: CB 153, CB 138, CB 180, CB 101, CB 118, CB 52 and CB 28. Results for CB 153 are also shown in table 1 in order to compare with other studies. Similarly, DDX concentrations varied largely between individuals with an average of 15 ± 20 $\mu g.g^{-1}$ lipids. Smallest and largest values ranged between 0.7 and 96 $\mu g.g^{-1}$ lipids. The DDX profiles were dominant by *p,p'*-DDE and *p,p'*-DDD contributing to more than 80% to the sum of DDXs. In descending order, levels were: *p,p'*-DDE, *p,p'*-DDD, *o,p'*-DDE, *o,p'*-DDD, *o,p'*-DDT and *p,p'*-DDT.

All PBDE concentrations were below the limit of quantification (0.05 $\mu g.g^{-1}$ lipids) in the blubber samples treated except for three juvenile harbour porpoises. The congener BDE 47 was the most concentrated with values of 1.06 $\mu g.g^{-1}$ lipids (juvenile that died from infectious disease) and 0.19 and 0.15 $\mu g.g^{-1}$ lipids (juveniles that died from physical trauma). Moreover, two other congeners (BDE 99 and BDE 153) were also detected in the juvenile that died from infectious disease with concentrations 0.64 and 0.18 $\mu g.g^{-1}$ lipids, respectively. The harbour porpoises that displayed the maximum level of PBDE (sum of the 3 BDEs: 1.89 $\mu g.g^{-1}$ lipids) also exhibited the maximum level of Σ7CBs (110 $\mu g.g^{-1}$ lipids).

A Spearman rank correlation matrix was established in order to track the correlation between POPs in the blubber. A significant positive correlation was observed between ∑7CBs and ∑6DDXs ($p < 0.05$).

2.1. POPs and maturity status

Since only one adult male was analyzed for POPs, values were excluded for the rest of the statistical tests. Juveniles exhibited higher ∑7CB and ∑DDXs levels than adult females (Table 4.1). More specifically, juvenile males were the most contaminated individuals compared to juvenile females and adult females. Due to the low number of animals analyzed, statistical analysis did not show differences between maturity status for the CB compounds ($p > 0.05$), whereas for DDX levels, juveniles displayed significantly higher concentrations compared to adult females ($p < 0.05$). Consequently, juveniles will be considered as one group for further comparisons.

2.2. POPs and causes of death

From 20 porpoises analyzed for POPs, post-mortem investigations showed that 10 porpoises died from infectious diseases, 7 died from physical trauma, 2 whose cause of death could not be determined and one died from seal predation. The blubber of porpoises that died from infectious diseases displayed significantly lower thickness than porpoises that died from physical trauma ($p < 0.05$). Figure 4.2 showed that the mean ∑7CBs level in the diseased group displayed higher concentrations than the trauma group, but these differences were not statistically significant. Both groups displayed nearly the same levels of ∑DDX (Figure 4.2), hence no significant differences were found between both groups for DDXs levels.

Table 4.1 Mean concentrations of the sum of PCBs, CB 153 and DDXs (µg.g^{-1} lipids) in blubber of harbour porpoises from different regions of the North East Atlantic Ocean and the Black Sea. Years in brackets refer to the date of stranding. A: Adults; J: Juveniles; AM: Adult males; AF: Adult females; JM: Juvenile males; JF: Juvenile females; n: number of samples. * median; ** Σ7CBs.

		∑PCBs			CB 153			∑DDXs			
Area	Age/Gender	Mean ± SD	(min - max)	n	Mean ± SD	(min - max)	n	Mean ± SD	(min - max)	n	References
Dansih and Norwegian waters (1987-1991)	M	23.3	(3.7-65)	34				16.39	(3.2 - 45.1)	34	Kleivane et al., 1995
Baltic sea (1985 - 1993)	JM	16 ± 8	(2.9 - 32)	13	6.6 ± 3.6	(1.1 - 13)	13	15 ± 18	(1.5 - 59)	11	Berggren et al., 1999
Baltic sea (1988 - 1989)	AM	46 ± 29	(14 - 78)	4	20 ± 13	(5.9 - 33)	4	116 ± 134	(20 - 308)	4	
Kattegat-Skagerrak Seas (1989-1990)	JM	11 ± 5.0	(2.2 - 20)	10	4.8 ± 2.5	(1.0 - 10)	10	20 ± 13	(5.7 - 36)	8	
Kattegat-Skagerrak Seas (1988-1990)	AM	13 ± 5.2	(6.7 - 22)	7	5.7 ± 2.3	(3.0 - 9.5)	7	25 ± 20	(2.8 - 61)	7	
Kattegat-Skagerrak Seas (1978-1981)	AM	40 ± 22	(17 - 67)	5	19 ± 12	(6.0 - 33)	5	98 ± 43	(35 - 154)	5	
West coast of Norway (1988-1990)	AM	15 ± 11	(7.2 - 33)	8	5.6 ± 4.6	(2.5 - 14)	8	9.1 ± 7.4	(3.1 - 22)	6	
Southern North Sea (2001-2003)	F	15 ± 8.6		19							Pierce et al., 2008
Scotland (2001-2003)	F	10.5 ± 13.2		31							
Ireland (2001-2003)	F	53.5 ± 48		12							
France (2001-2003)	F	13.8 ± 11		2							
Galicia (2001-2003)	F	53 ± 42		3							
Southern North Sea (1999-2004)	JF	12.9 ± 11.9	(1.3 - 39.3)	9	3.7 ± 4.1	(0.2 - 13.4)	9				Weijs et al., 2009a
	JM	15.4 ± 10.7	(5.3 - 39.8)	12	3.9 ± 3.0	(1.2 - 11.5)	12				
	AF	7.3 ± 2.0	(4.4 - 8.9)	5	1.7 ± 0.6	(1.0 - 2.3)	5				
	AM	82.9 ± 31.8	(38.7 - 125.5)	8	28.7 ± 12.0	(11.6 - 46.0)	8				
East England (1991-2005)	M	11.6 ± 9.7		23							Law et al., 2010b
Southern North Sea (1991-2005)	M	46.4 ± 30.7		21							
Black Sea (1998)	A	13.2*	(8.8 – 24.9)	11				77.3*	(55 – 157)	11	Weijs et al., 2010a
	J	7.0*	(4.9 – 13.7)	9				40.9*	(27.4 – 82)	9	

North Sea (1990-1999)	A	81.5		1				22.9		1	Weijs et al., 2010b
North Sea (2000-2008)	A	24.9	(15.3-34.5)	2				3.4	(1.2-1.4)	2	
North Sea (1990-1999)	J	19.1		1				4.5		1	
North Sea (2000-2008)	J	9.9	(1.1-68.2)	5				1.7	(0.4-6.4)	5	
North West Iberian Peninsula	JF	10.8 ± 2.8		5	2.9 ± 0.8		5				Méndez-Fernandez et al., 2014a
(2004-2008)	JM	9.4 ± 3		3	2.8 ± 1		3				
	AF	37.5 ± 30.8		3	12.0 ± 9.7		3				
	AM	50.8		1	16.6		1				
Southern North Sea (2010-2013)	JF	32 ± 21**	(7.4 - 48)	3	14 ± 10	(3 - 22)	3	16 ± 10	(8 - 27)	3	Present study
	JM	20 ± 31**	(0.6 - 110)	12	9 ± 15	(0.3 - 54)	12	19 ± 25	(2.4 - 96)	12	
	AF	4 ± 1,8**	(2.5 - 7)	4	1.8 ± 0.9	(1 - 3)	4	1.9 ± 1.3	(0.7 – 3.5)	4	
	AM	22**	-	1	10	-	1	13	-	1	

3. Discussion

3.1. PCB levels

Unlike some essential trace elements, persistent organic pollutants are non essential for survival. They have a strong affinity to lipid-rich tissues and organs because of their lipophilicity and hence they are retained mainly in the blubber of cetaceans (Tanabe et al., 1994; Aguilar and Borrell, 1994). The biotransformation capacity of PCBs and DDXs is known to be lower in small cetaceans compared to seals, birds and terrestrial mammals (Duinker et al., 1989; Tanabe et al., 1994; Boon et al., 1997; Law et al., 1998).

The wide range between the minimum and the maximum for PCB concentrations (Table 1) may underline the involvement of numerous biological factors for instance age, gender, diet, body condition and metabolic capacity to degrade toxic contaminants regarding PCB lipid accumulation (Duinker et al., 1989; Tanabe et al., 1994; Weijs et al., 2009a; Weijs et al., 2009b). It has been documented that CB 153 levels are higher in the majority of the samples from aquatic mammals (Kleivane et al., 1995; Boon et al., 1997). The levels of CB 153 in juvenile harbour porpoises from this study (Table 4.1) ranged between 0.3 - 54 $\mu g.g^{-1}$ lipids. These levels were generally higher than those reported in immature male porpoises (1985 – 1990) from the Baltic Sea (1.1 - 13 $\mu g.g^{-1}$ lipids) and the Kattegat-Skagerrak Seas (1.0 - 10 $\mu g.g^{-1}$ lipids) (Berggren et al., 1999). Similarly, juvenile porpoises stranded in our study exhibited higher CB 153 contents compared to juvenile porpoises from the southern North Sea stranded between 1994 and 2004 (0.2 – 13.4 $\mu g.g^{-1}$ lipids) (Weijs et al., 2009a) and those from the North West Iberian Peninsula stranded between 2004 and 2008 (2.9 ± 0.8 $\mu g.g^{-1}$ lipids) (Méndez-Fernandez et al., 2014a) (Table 1). However, cautions should be taken in interpreting the findings of the present study since the total sample size of 20 individuals may be relatively small for temporal comparisons. A significant accumulation of PCBs with age is apparent in male porpoises from the Scandinavian Waters (Kleivane et al., 1995) and from the southern North Sea (Weijs et al., 2009a) as well as in male fin whales from the coasts of Spain (Aguilar and Borrell, 1994). Unfortunately such accumulation with age could not be verified for our study due to the fact that we were not able to analyze more than one adult male harbour porpoise (Table 4.1). Juveniles had higher PCB levels than adult females with similar trends for the congener CB 153. It has been reported that adult

females have decreasing levels of organic contaminants explained by the transfer of organochlorines to their offspring during gestation and lactation (Aguilar and Borrell, 1994; Tanabe et al., 1994; Westgate et al., 1997; Jepson et al., 1999). Such findings may explain variations in PCB levels between adult females and juvenile porpoises stranded in the southern North Sea.

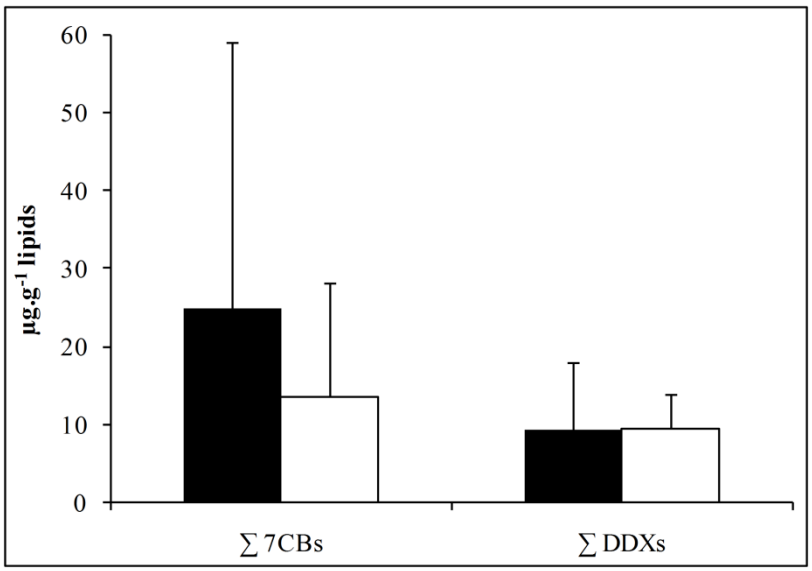

Figure 4.2 Mean blubber concentrations (µg.g^{-1} lipids) of the ∑7PCBs and ∑DDXs of harbour porpoises stranded in the Southern North Sea between 2010 and 2013 for animals that died from infectious disease (n=10; black bars) and physical trauma (n=7; white bars).

Furthermore, porpoises that died from infectious disease exhibited higher PCB levels (25 ± 34 µg.g^{-1} lipids) compared to those that died from physical trauma (14 ± 15 µg.g^{-1} lipids) (Figure 4.2). Same trends were found in other studies with a more representative sampling for porpoises stranded in the United Kingdom (Jepson et al., 1999; Jepson et al., 2005) and western European seas (Pierce et al., 2008). Jepson et al., (1999; 2005) suggested that pre-existing disease processes may cause mobilization and metabolic breakdown of blubber lipid stores which lead to highlighting levels of PCBs in harbour porpoise's blubber and support a causal relationship between PCB exposure and infectious disease mortality. Moreover, porpoises that died from physical trauma (n=7) displayed thicker blubber compared to porpoises that died from infectious diseases (n=10) ($p<0.05$). It has been suggested that pollutants may be diluted in a thicker blubber layer (Kleivane et al., 1995). This leads us to the fact that porpoises could be exposed to the same levels of organochlorines in the environment, but concentrations of pollutants may be more pronounced in diseased porpoises due to emaciation processes.

A toxic threshold concentration in liver (17 µg.g^{-1} lipids) for total PCBs determined for adverse health effects in marine mammals is proposed (Kannan et al., 2000). In order to compare the levels of PCBs in the blubber of porpoises from the present study with the proposed limit, PCBs concentrations had to be converted given that the threshold is based on the commercial PCB mixture Aroclor 1254. The conversion factor, from the seven ICES congeners (CB 28, 52, 101, 118, 153 and 180) to total PCBs, may be obtained by multiplying the sum of the seven congeners by three. According to the equation: Total PCB concentration (as Aroclor 1254) = 3.0 * Sum of the seven ICES congeners (lipid weight) (Jepson et al., 2005). In the present study, 60% of the animals analyzed exceeded this threshold. In the UK, the porpoises with total PCB levels that exceeded the threshold (17 µg.g^{-1} lipids), total PCB levels were significantly higher in porpoises that died due to infectious disease compared to healthy porpoises that died due to physical trauma (Jepson et al., 2005). Moreover, 74% of harbour porpoises from the southern North Sea (Jepson et al., 2005) and 75% of the harbour porpoises from the North West Iberian Peninsula exceeded this threshold (Méndez-Fernandez et al., 2014a). However, cautions should be taken when applying this threshold, since this value was derived from the liver of laboratory aquatic mammals (seals, European otters and minks) that were fed with field food items. The extrapolation of this threshold level to the blubber of stranded harbour porpoises that were feeding on a variety of prey species may be questionable.

3.2. DDX levels

Because of the different chemical nature of organochlorines, the distribution patterns of DDXs in different tissues and organs of animals are generally more variable than those of the CBs (Duinker et al., 1989). In the present study, *p,p'*-DDE and *p,p'*-DDD had the largest contribution to the sum of DDX (more than 80%) which is in agreement with previous studies (Duinker et al., 1989; Tanabe et al., 1997; Berggren et al., 1999; Weijs et al., 2010b). Marine mammals have induced levels of cytochrome P450-1A and 2B that are capable of metabolizing *p,p'*-DDT (Boon et al., 1997). Thus, relatively high concentrations of DDT metabolites (*p,p'*-DDE and DDD) in marine mammal tissues were related to the higher metabolism of DDT in marine mammals along with the bioaccumulation of DDT metabolites through their life span (Hoekstra et al., 2003). In addition, ratios of *p,p'*-DDE/∑DDXs may indicate whether a new

source of DDT is entering the environment. Therefore, a ratio greater than 0.6 implies a stable system indicating that there is no new or recent input of DDXs in the environment (Hall et al., 1992; Tanabe et al., 1997). Mean ratios in the blubber of porpoises from the southern North Sea were 0.6 which may indicate that there is no new input of DDXs in this region. Levels of the sum of DDX in porpoises from this study were 15 ± 20 (0.7 – 96) $\mu g.g^{-1}$ lipids (Table 1), higher than those reported in livers of porpoises stranded between 1997 and 2000 (3.4 ± 2.3; 0.3 – 44.3 $\mu g.g^{-1}$ lipids) (Covaci et al., 2002) and in blubber of juvenile porpoises stranded between 2000 and 2008 (1.7 ; 0.6 – 6.4 $\mu g.g^{-1}$ lipids) (Weijs et al., 2010b) along the southern North Sea. Male porpoises (16.4; 3 – 45 $\mu g.g^{-1}$ lipids) from Scandinavian Waters stranded between 1987 and 1991 (Kleivane et al., 1995) and juvenile porpoises from the Baltic Sea (15 ± 18; 1.5 – 59 $\mu g.g^{-1}$ lipids) and Kattegat-Skagerrak Seas (20 ± 13; 5.7 – 36 $\mu g.g^{-1}$ lipids) stranded between 1985 and 1993 (Berggren et al., 1999) showed almost the same concentrations of DDXs compared to porpoises from our study, whereas porpoises from the Black Sea stranded in 1993 and 1998 (Tanabe et al., 1997; Weijs et al., 2010a) had higher concentrations (Table 4.1). Juveniles displayed significantly higher DDXs levels compared to adult females ($p < 0.005$). As mentioned earlier, this could be explained by the transfer of organochlorines from adult females to their offsprings. Furthermore, the great differences in organochlorine concentrations between the adult male analyzed and females were related to the lactational period. This sexual difference is more pronounced in harbour porpoises compared to other cetaceans due to their longer lactation period (Tanabe et al., 1997). An age-dependent accumulation was also found for all DDT residues in porpoises from various regions (Aguilar and Borrell, 1994; Kleivane et al., 1995; Tanabe et al., 1997; Weijs et al., 2010b).

Unlike PCB trends, porpoises that died from infectious disease (9.3 ± 9 $\mu g.g^{-1}$ lipids) showed almost same levels of DDXs compared to healthy porpoises that died from physical trauma (9.5 ± 4 $\mu g.g^{-1}$ lipids) (Figure 2). A significant correlation was observed between DDXs and PCBs for the 20 animals analyzed ($p < 0.001$). This finding was in agreement with the study of Jepson (2003) showing significant correlation between ∑25CBs and DDTs ($p < 0.001$) for a more representative sampling (n = 169) of porpoises stranded on UK coasts between 1989 and 2001.

In the present study, levels of BDE congeners were detected in only three juvenile porpoises (>LOQ = 0.05 $\mu g.g^{-1}$ lipids). The concentrations obtained seems to be in the same order of magnitude than harbour porpoises stranded along the southern North Sea between 1999 – 2004 (range 0.22 – 5.93 $\mu g.g^{-1}$ lipids) (Weijs et al., 2009a), between 2001-2003 (1.06 – 0.8 $\mu g.g^{-1}$ lipids) (Pierce et al., 2008) and more recently between 2000 – 2008 (range 0.28 – 1.83 $\mu g.g^{-1}$ lipids) (Weijs et al., 2010b). Similarly as PCBs, Law et al., (2010a) observed also a decline for Σ9BDE concentrations in the blubber of harbour porpoises stranded or bycaught from the UK during the period 1992 – 2008. However, ΣBDE concentrations in stranded porpoises dying due to infectious disease were higher than levels in bycaught animals (Law et al., 2010a).

It has been suggested that after the ban in 1970s and 1980s, organochlorines in biota were in continuous decline (Aguilar et al., 2002). For instance, Berggren et al., (1999) found a temporal decline in ∑DDT and ∑PCB levels between porpoises collected in 1978-81 compared to those from 1988-90 in the Kattegat-Skagerrak Seas. A decline in organochlorines has been documented for harbour porpoises in Danish Waters (Granby and Kinze, 1991) and in the Bay of Fundy, Canada (Westgate et al., 1997). A temporal variation was also observed in ∑25CB levels between 1989 and 2001 for porpoises from United Kingdom demonstrating a gradual decline from early 1990s to 2001 (Jepson et al., 2005). A recent study for Law et al., (2010b) showed a quite slow decline for CBs concentrations in UK porpoises stranded from 1991 to 1998 then reached a plateau thereafter until 2009. Tanabe et al., (1994) suggested that the high transmission rate of organochlorines is pronounced in cetaceans. Thus, even if the status of marine pollution has been improved, a continuous long-term contamination by toxic organochlorines over many generations may be observed.

Conclusion

The present study provides an assessment of some persistent organic pollutant levels in the blubber of harbour porpoises stranded along the southern North Sea between 2010 and 2013. Levels of PCBs were significantly higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma. Furthermore, the sum of PCBs and DDXs were higher in juvenile porpoises compared to adult females, which is in agreement with previous studies. According to the ratio *p,p'*-DDE/∑DDXs, our results suggest that there is

no new input of DDXs in this region. In addition, levels of PCBs and DDXs obtained in the blubber of porpoises from this study were in the same order of magnitude or even lower than porpoises stranded along the North East Atlantic Ocean and the Black Sea over the period 1987 and 2013. We believe that even though the levels of organochlorines are slowly declining in the marine environment, they are still high enough in harbour porpoises and still capable of causing negative effects. Moreover, a threshold level is proposed only for total PCBs (17 $\mu g.g^{-1}$ lipids) above which there are health effects in mammals. Along with the fact that this threshold is questionable, no such threshold is proposed in the literature for other organochlorines such as DDXs and PBDEs. Therefore, it is important to keep monitoring the levels of these compounds in the top predator harbour porpoise in the North Sea.

Acknowledgements

We would like to thank the French Stranding Network for their efforts in collecting the samples, especially "Centre de Recherche sur les Mammiferes Marins" (CRMM) and "Observatoire pour la Conservation et l'Etude des Animaux et Milieux Marins" (OCEAMM). The "Grand Port Maritime de Dunkerque" (France) is acknowledged for its financial support of this study. Mahfouz Celine is financially supported by a PhD fellowship from the National Council for Scientific Research (Lebanon) and Université du Littoral Côte d'Opale (France). We would like to thank two anonymous reviewers for their helpful comments on an earlier version of this manuscript.

CHAPTER 5

THE DIET OF HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) IN THE SOUTHERN NORTH SEA: A RELATIONSHIP WITH PREY AVAILABILITY

Chapter 5 – The diet of harbour porpoises (*Phocoena phocoena*) in the southern North Sea: A relationship with prey availability

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In prep., manuscript will be submitted to "Journal of Applied Ecology".

Abstract

Over the past few years, harbour porpoises population has witnessed a southward shift along the North Sea. This shift has led to an increase in the number of stranded porpoises in this area. The aims of the present work were to investigate whether the changes in the distribution of porpoises in the North Sea may be a result from changes in prey availability. Therefore, the diet of harbour porpoises stranded along the southern North Sea (northern France and Belgian coast) over the past 3 years was assessed through 3 complementary methods: the stomach content analysis, the stable isotopes (carbon and nitrogen) analysis determined from muscle samples and the fatty acids analysis determined from blubber samples. Fatty acid patterns and stable isotope values from porpoises were compared to potential preys collected from the southern North Sea. Overall, 59 harbour porpoises and 14 potential prey species were investigated. The results of the present study suggested that the feeding changes and the southward shift of porpoise's population encountered the past few years in the North Sea may be related to the sandeel abundance decline in the northern parts of the North Sea along with the re-invasion of the southern North Sea by the sardine species, perhaps in response to climate change.

Keywords: harbour porpoises; North Sea; shift; stomach contents; stable isotopes; fatty acids

Introduction

Harbour porpoises (*Phocoena phocoena*) are the most common cetacean species in the North Sea (Hammond et al., 2013). A large-scale survey (SCANS) held along the North Sea estimated the abundance of harbour porpoises to be 340 000 animals (Hammond et al., 2002). Almost 10 years later and during the second survey (SCANS II), the abundance was estimated to be 375 000 animals (Hammond et al., 2013). The abundance of porpoises was almost the same, but SCANS II highlighted a marked shift in the distribution from the northern parts of the North Sea to its eastern parts. Alongside, a clear increase in the number of stranded animals was observed along the southern North Sea including the Netherlands, Belgium and Northern France since the beginning of the 21st century (Jauniaux et al., 2008; Haelters and Camphuysen, 2009).

Harbour porpoises are small cetaceans with limited body fat and energy storage capacity; therefore, they must feed at a high daily rate without prolonged periods of fasting to maintain energy requirements. This species cannot afford to be too specialized and is generally believed to be an opportunistic feeder (Koopman et al., 2002; Santos et al., 2004). Most marine mammals depend on an abundant supply of local food; for that reason fishing may negatively affect their survival by reducing the availability of prey or by inducing its dispersal (Lassalle et al., 2012). Different authors linked the changes over the last few decades in the distribution and relative abundance of porpoises in the southern North Sea to the changes in prey availability (Camphuysen, 2004; MacLeod et al., 2007; Hammond et al., 2013). The distribution of harbour porpoises is expected to follow the distribution of their main prey species (Santos et al., 2004). This species is known to consume a wide selection of prey species; for instance in the North Sea, harbour porpoises are expected to feed on gobies (*Gobiidae*), sandeels (*Ammodytidae*) and different species of gadidae (*Merlangius merlangus* and *Gadus morhua*) (Santos et al., 2004; Haelters et al., 2012) and to a smaller extent some clupeids (*Clupea harengus* and *Sprattus sparttus*) (Santos et al., 2004). Accordingly, studying the feeding ecology of harbour porpoises serves to investigate their feeding strategy, the predator-prey relationships, and their responses to changes in food webs dynamics, climate changes or fishery interactions (Haelters and Camphuysen, 2009; Herr et al., 2009). Different methods and organs may be used when studying the feeding ecology of marine mammals. Each method provides different details and temporal indications on the diet consumed.

Stomach content analysis is a traditional method for dietary study based on the identification of the remains of ingested preys in the stomach contents (otoliths, fish bones, cephalopod beaks, etc) to the lowest taxonomic level. However, this technique represents short-term dietary information; only the last ingested meal(s) may be studied. Recently, new biochemical techniques have been applied to investigate the diet or trophic positions of organisms.

Stable isotope analysis is currently among the most effective tools for the study of trophic relationships and feeding habits (Hobson and Welch, 1992; Caut et al., 2009). The ratios of naturally occurring isotopes of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$, respectively) in specific tissues of the predator may be directly related to those of their prey with an expected enrichment called Trophic Enrichment Factor (TEF). This TEF varies between 0 to 1‰ for $\delta^{13}C$ and between 2 to 4‰ for $\delta^{15}N$ depending on the analyzed tissue (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 2001). The $\delta^{13}C$ value may be used as a tracer of the origin of the food chain and the feeding zone of organisms (inshore vs. offshore) (France, 1995), whereas the $\delta^{15}N$ value may be used as an indicator of the trophic position of organisms relatively to the primary consumers (Hobson, 1999). The Stable Isotope Analysis in R computing language (SIAR) is a mixing model used to estimate the contribution of prey species to the predators' diet. SIAR modeling can deal with more sources than isotopes and it can incorporate trophic enrichment and elemental concentrations allowing quantitative estimation of prey composition. It is most useful when dealing with few prey species having distinct isotopic composition (Parnell et al., 2010).

Fatty acid analysis in the blubber of marine mammals has witnessed a potential development in the last few decades. It has been evolved from a tool for revealing food webs to a more sophisticated technique for qualitative (Falk-Petersen et al., 2004) and quantitative (Iverson et al., 2004) analysis of diet by comparing the fatty acids found in the predator blubber with those found in their potential prey. This technique is based on the fact that fatty acids (FAs) are deposited into adipose tissue with little change or in a predictable manner, thus reflecting the diet of the predator over a period of up to several months (Budge et al., 2006). Furthermore, the compound-specific stable isotope analysis (CSIA) is a new research tool used to avoid many limits encountered when using bulk tissue stable isotopes (measurement of $\delta^{13}C$ and $\delta^{15}N$ values

in a sample) or FAs analyses (Gladyshev et al., 2012). This approach tracks the stable carbon isotope of specific FA structure that can only arise from diet (Budge et al., 2011). CSIA is expected to provide a greater specificity to biomarkers.

The above-mentioned techniques are powerful complementary methods to the stomach content analyses. The objectives of this paper are (1) to determine the feeding ecology of harbour porpoises along the southern North Sea using different and complementary methods, and (2) to investigate whether the changes in the distribution of porpoises in the southern North Sea may be a result of the changes in prey availability.

1. Materials and methods

1.1. Sampling and data collection

This study was conducted on 59 harbour porpoises stranded in the Southern North Sea along northern France and Belgian coasts between 2010 and 2013 for fatty acids and stable isotopes analyses in blubber and muscle, respectively. From the above-mentioned animals, 15 stomachs were collected for stomach content analysis. Muscles, blubbers and stomachs were stored at -20º C until further analysis. All animals were freshly dead or slightly decomposed. Post-mortem investigations according to the protocol from Kuiken and Hartmann, (1993) and Jauniaux et al., (2002a) were performed. Morphometric data such as sex and length of the animals were collected and according to the length of the animal, age groups were determined. Porpoises with lengths ranging from 91 to 130 cm were considered as juveniles and animals with measures greater than 130 cm were considered as adults. The causes of death of the animals were: infectious diseases, lung edema and physical trauma. Moreover there were porpoises which cause of death could not be determined.

We collected potential prey species (as identified in previous studies) along the Southern North Sea covering two different seasons. In June 2012 and October 2012, preys were collected in coastal areas with the RV SEPIA (INSU-CNRS), and in November 2012, preys were collected with the RV “Thalassa” (Ifremer). Prey samples were stored at -20º C until further analysis. Prey samples were selected in order to cover the size-classes found in the stomach contents of harbour porpoises.

1.2. Stomach content analyses

Stomachs were weighed then rinsed through running water and contents were emptied in a sieve with mesh size of 0.2 mm. Empty stomachs were weighed again to obtain the mass of the stomach contents by difference. Remains such as otoliths and fish vertebrates were stored dry, whereas whole or partly digested prey items and cephalopod beaks were stored in 70 % ethanol. All remains were counted, measured and identified to the lowest taxonomic level using our reference collection of specimens (LOG) and published data from Leopold et al., (2001). Otoliths were identified and measured (otolith width and length) using a video system fitted to a compound microscope and an Image Analysis System (TNPC 5.0). Fish weight and length estimates were calculated from the measured length and width of otoliths using regressions from Leopold et al., (2001). No corrections were made for loss or reduction of size of prey remains due to digestion.

Four indices were used to estimate the prey importance in the diet of harbour porpoises. The percentage frequency of occurrence (% O) relies on the number of stomachs where the particular prey species was found divided by the total number of non empty stomachs * 100. The percentage by number (% N) is calculated upon the number of the particular prey species found divided by the total number of preys found * 100. The percentage by mass (% W) relies on the total weight of the particular prey species divided by the total weight of all prey species * 100.

1.3. Stable isotopes analyses

Stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were determined in the muscle of harbour porpoises and fishes except for the gobies (*Gobiidae sp.*) where the whole body was ground. Samples were freeze-dried for 48 hours then ground into fine powder. An aliquot of approximately 100 mg from each sample was mixed with 4 ml of cyclohexane for lipid extraction, since lipids are highly depleted in ^{13}C compared to other tissue components (Tieszen et al., 1983). Samples were agitated for 1 h at 800 rpm then centrifuged for 5 min at 4000 rpm. The upper solution containing the lipids was removed and the samples were dried in an oven at 50º C for 48 hours. Subsamples of dried and lipid free muscle powder (0.35 ± 0.05 mg) were weighed into tin cups. Stable isotope measurements were performed with an elemental analyzer coupled to an isotope ratio mass spectrometer (DELTA V ADVANTAGE Isotope Ratio MS –

Thermo Scientific). The stable carbon isotope composition of an aliquot of the freeze-dried and powdered sample is referred to as the “bulk” stable carbon isotope composition in this article.

Stable isotope abundances are expressed in "delta notation, δ" in parts per thousands (‰) following the equation:

$$\delta X = [\frac{R_{Sample}}{R_{Standard}} - 1] \times 1000$$

Where X represents ^{13}C or ^{15}N and R_{sample} is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ isotopic ratio of the sample. Ratios are expressed relative to the international standards Vienna PeeDee Belemnite (V-PDB) and atmospheric Nitrogen (N_2) for ^{13}C and ^{15}N measurements, respectively. Replicate measurements of internal laboratory standards (acetanilide) during each series of measurement indicated errors less than 0.15 ‰ and 0.20 ‰ for carbon and nitrogen, respectively.

1.4. Fatty acids composition

Fatty acid (FA) compositions were determined in the blubber of harbour porpoises and the muscle of prey species. In addition to the preys used for the stable isotope analysis, the sand smelt (*Atherina presbyter*) and the thinlip grey mullet (*Liza ramada*) were added for the FAs analysis. Before FAs extraction, an internal standard (23:0; 20 µg) was added to approximately 50 mg of fresh blubber from harbour porpoise or freeze-dried muscle from prey. A slightly modified version of Bligh and Dyer, (1959) as in Meziane et al., (2007) was adopted for extraction. Samples were subject to ultrasonication for 20 min with distilled water:$CHCl_3$:MeOH (1:1:2, v:v:v). Afterwards, the addition of a distilled water:$CHCl_3$ mixture (1:1, v:v) and the centrifugation (5 min; 3000 rpm) of the mixture formed a two layer system. The lower $CHCl_3$ layer containing the lipids was retained. This step was repeated one more time with $CHCl_3$ (2 ml). The residue obtained from the consecutive extractions was concentrated under a N_2 flow, then saponified in a mixture of 2 mol NaOH:MeOH (1:2, v:v) and heated for 90 min at 100°C In order to obtain the total lipids as methyl esters, saponification and methylation were conducted according to Meziane and Tsuchiya, (2002). For the identification, FAMEs were separated and quantified by gas chromatography equipped with a flame ionization detector (GC; Varian CP – 3800). The GC was fitted with a Supelco OMEGAWAX 320 column (30 m, 0.32 mm ID, 0.25

μm film thickness). Helium was the carrier gas. The sample (1μl) was injected at 60°C and the temperature was raised to 150°C at 40°C.min^{-1}, then ramped up to 240°C at 3°C.min^{-1} and kept at there for 14 min. Peaks were identified by comparing their retention times with those of authentic standards (Supelco^{TM} 37, PUFA Mix – No 1 Marine Source and Bacterial mix; Supelco Inc., Bellefonte, PA, USA). For some samples, peaks of FAs were confirmed with GC-mass spectrometry (GC-MS; ThermoFinnigan TRACE DSQ).

Standard nomenclature is used for the identified FAs (X:YωZ), where X is the number of carbon atom, Y is the number of double bonds and Z is the position of the ultimate double bond from the terminal methyl.

According to Schomburg, (1987), the concentration of each FA (C_{FA}, mg) was calculated:

$$C_{FA} = \frac{A_S}{A_{IS}} \times \frac{C_{IS}}{W_S}$$

Where A_S is the peak area of the FA, A_{IS} is the peak area of the internal standard, C_{IS} is the concentration of the internal standard (mg) and W_S is the dry weight of the sample (g) for prey species and fresh weight (g) for the blubber of porpoises.

1.5. Compound-Specific Stable Isotope Analysis (CSIA)

The Compound-Specific ^{13}C isotope analysis of the fatty acid methyl esters was determined in the blubber of harbour porpoises and some prey species such as *C. harengus, Sardina pilchardus, Gobiidae sp.* and *L. ramada*. CSIA analyses were held at the Stable Isotope Facility, University of California, Davis. Therefore, the protocol will be briefly discussed. After extraction of the FAMEs as discussed above, stable carbon isotope ratios of the FAMEs were measured with a Trace GC Ultra gas chromatograph (Thermo Electron Corp., Milan, Italy) coupled to Thermo Finnegan Delta Plus Advantage isotope ration mass spectrometer (GC-IRMS). The GC was equipped with a splitless injector and an BP x 70 (60 m; 0.25 mm O.D; 0.25 mm film thickness) column with a constant flow of 1.5 mL.min^{-1}. A known quantity (typically around 20μg) of dodecanoic acid (12:0) was added as an internal standard to the FAMEs dissolved in hexane before injection into the GC. The sample (3μl) was injected at 100°C (held for 1 min) and the temperature was raised to 210°C at 3°C.min^{-1}(held for 5 min), then ramped up

to 245°C at 3°C.min^{-1} and kept at there for 5 minutes. C12, C15, C17, C18, C19, C20 and C22 *n*-Alkanes were co-injected with the FAMEs and used as internal isotopic references. Yields between 73 and 99 % proved the accuracy and precision of the GC-IRMS. *n*-Alkane standards were also used as an additional check for any drift between runs.

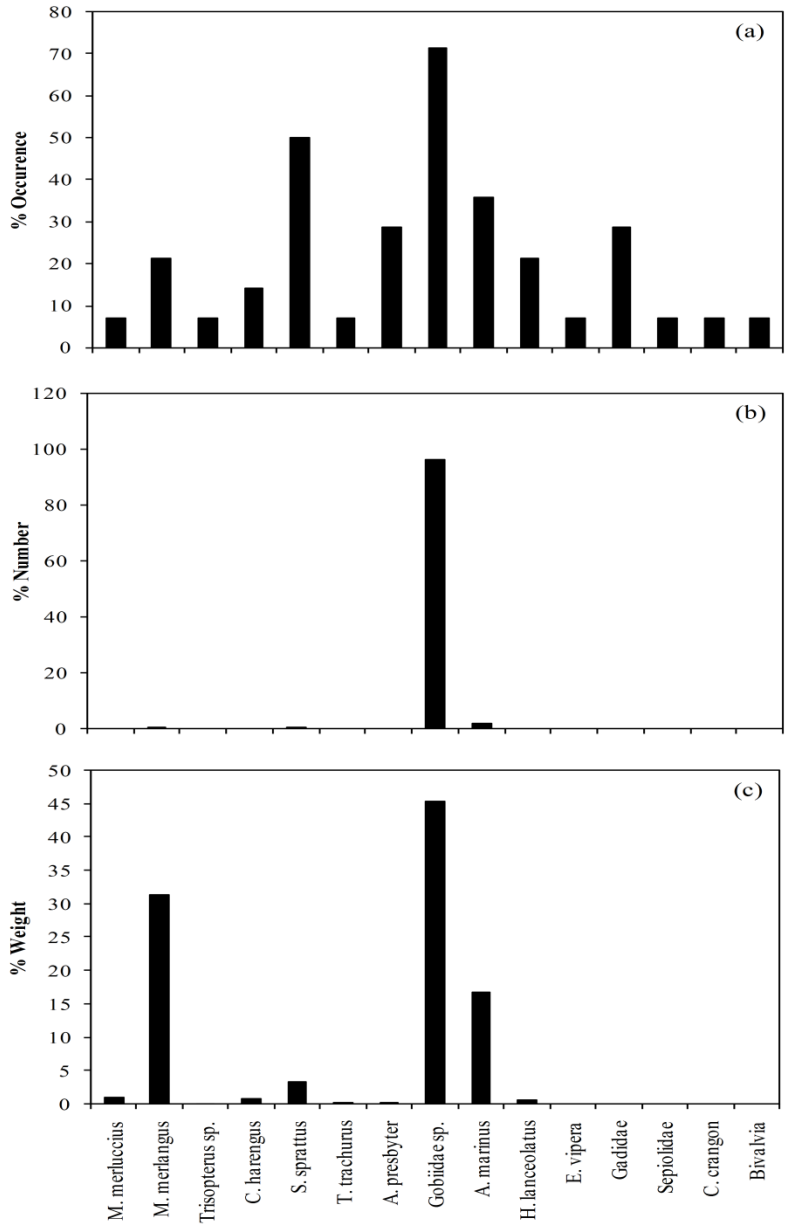


Figure 5.1 (a) Frequency of occurrence (% O); (b) Percentage by number (% N) and (c) percentage by mass of prey species in stomachs of harbour porpoises (*Phocoena phocoena*).

1.5. Data treatment

Data analysis of stable isotopes was performed using "XLSTAT – Pro" 2013 (Addinsoft). The level of significance was set at $\alpha = 0.05$. $\delta^{15}N$ and $\delta^{13}C$ values in muscle were tested using ANOVA followed by post-hoc multiple comparison tests to compare the data between age classes (juveniles and adults) and years of stranding.

The stable isotope mixing model SIAR (Stable Isotope Analysis in R Development Core Team, 2010) was used to evaluate the relative contribution of each prey species in the diet of harbour porpoises stranded in the southern North Sea. The main prey species contributing to the total diet of porpoises as determined by weight from stomach content analyses were considered as sources: gobies (*Gobiidae*), sandeels (*Ammodytidae*), different species of gadidae (*M. merlangus* and *Trisopterus sp.*) and some clupeids (*C. harengus* and *S. sparttus* and *S. pilchardus*) (Santos et al., 2004; Haelters et al., 2012). Our model was based on the trophic enrichment factors (TEFs) for captive cetaceans as published by Hobson et al., (1996) (1.3 ± 0.1‰ and 2.4 ± 0.3‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively) and Caut et al., (2011) (1.26 ± 0.2‰ and 1.23 ± 0.15‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively). Individual isotope ratios of porpoises (n=52) were entered in the model, whereas for prey species (n=49), means and standard deviations were used. The mixing model was run using default parameters (iterations: 500 000; burn in: 50 000 and thinning by: 15).

To investigate for variations in FA composition among samples, Bray-Curtis similarity matrices were calculated on the FA dataset with no transformation applied. Analysis of similarity (ANOSIM) was performed using PRIMER 5 and the statistic test was computed after 5000 permutations. Significant differences in FAs profiles were identified using the global *R*-values. Similarity percentages routine (SIMPER) was used to determine FAs contributing to the differences. Factor used for the analysis were gender and maturity status, cause of death and blubber thickness.

2. Results

2.1. Stomach contents

Fourteen stomachs of harbour porpoises stranded along the southern North Sea between 2012 and 2013 were investigated. The sampling included 10 juvenile males, 2 adult females, 1 adult male and 1 undetermined porpoise. More than 8000 individual prey remains (mainly otoliths) representing 13 species from 9 families were identified in the 15 stomachs analyzed of harbour porpoises. The longest fish found in the stomach contents examined was a whiting *M. merlangus* with an estimated length of 32 cm which otoliths were found in the stomach of a 161 cm long adult female weighing 50 kg.

In terms of occurrence (% O), *Gobiidae sp.*, sprat *S. sprattus*, lesser sandeel *Ammodytes marinus*, sand smelt *A. presbyter* and undetermined Gadidae dominated as preys with 71, 50, 36, 29 and 29 %, respectively (Figure 5.1a). In terms of percentage by number (% N), *Gobiidae sp.* were by far the most important preys with 96 % followed by *A. marinus* with 2.0 % (Figure 5.1b). In terms of percentage of composition by weight (% W), the main prey species was *Gobiidae sp.* with a contribution of 45 %, followed by *M. merlangus* with a contribution of 31 % and *A. marinus* with 17 % (Figure 5.1c).

2.2. Stable isotopes and SIAR

Stable isotope values measured in the muscle of 52 harbour porpoises ranged between -18.5 ‰ to -16.3 ‰ for $\delta^{13}C$ and between 13.5 ‰ to 18.4 ‰ for $\delta^{15}N$ (Table 5.1). Harbour porpoises were distributed as follow: 15 juvenile females, 27 juvenile males, 7 adult females and 3 adult males. No significant differences were found in $\delta^{13}C$ values for these 4 groups (ANOVA, $p > 0.05$). $\delta^{15}N$ values of juvenile females and adult females were significantly different (ANOVA, Post-hoc, $p < 0.05$), whereas comparing the other groups, $\delta^{15}N$ values showed no significant differences ($p > 0.05$). The sampling covered 4 years of porpoises stranding of which 14, 9, 15 and 14 porpoises were stranded in 2010, 2011, 2012 and 2013, respectively. No significant differences were found in $\delta^{13}C$ and $\delta^{15}N$ values in the muscle of porpoises stranded among these years (ANOVA, $p > 0.05\%$). For the prey species, selected for SIAR model, $\delta^{13}C$ values ranged between -20.3 ‰ and -16.2 ‰ for *Gobiidae sp.* and *S. sprattus*, respectively. As

for the $\delta^{15}N$, values ranged between 9.9 ‰ and 17.9 ‰ for *C. harengus* and *Gobiidae sp.*, respectively (Table 5.1).

Table 5.1 Sizes (cm), $\delta^{13}C$ and $\delta^{15}N$ values in the muscles of Harbour porpoises (*Phocoena phocoena*) and fish species selected from the southern North Sea. n: number of individuals; Data are presented as mean ± standard deviation; (minimum; maximum).

		Size (cm)		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
Family / Species	n	Mean ± SD	(min ; max)	Mean ± SD	(min ; max)	Mean ± SD	(min ; max)
Harbour porpoise (Southern North Sea)	52	120 ± 18	(92; 161)	-17 ± 0.5	(-18.5; -16.3)	15.8 ± 1	(13.5; 18.4)
Juvenile females	15	115 ± 10	(98; 133)	-17.4 ± 0.5	(-18.2; -16.8)	16.1 ± 1.0	(14.1; 18.4)
Juvenile males	27	110 ± 7	(92; 120)	-17.3 ± 0.5	(-18.3; -16.3)	15.9 ± 1.1	(13.8; 18.0)
Adult females	7	156 ± 6	(145; 161)	-17.5 ± 0.5	(-18.5; -17.0)	14.7 ± 1.1	(13.5; 16.3)
Adult males	3	146 ± 4	(143; 150)	-17.3 ± 0.5	(-17.6; -16.9)	15.4 ± 0.6	(14.7; 15.9)
FISH							
Gadidae							
Merlangius merlangus	10	23 ± 0.7	(22; 24.2)	-16.9 ± 0.3	(-17.3;-16.3)	16.3 ± 0.4	(15.6;17.0)
Trisopterus luscus	9	12 ± 6.7	(4.7; 19.3)	-17.8 ± 0.6	(-18.7;-16.7)	14.5 ± 1.1	(13.7;17.0)
Clupeidae							
Clupea harengus	5	29 ± 1	(27.8; 30)	-18.5 ± 0.5	(-19.0;-17.6)	10.8 ± 0.7	(9.9;11.4)
Sprattus sprattus	10	7.5 ± 1.3	(6.2; 9.5)	-17.7 ± 0.7	(-18.3;-16.2)	13.5 ± 0.7	(12.4;14.5)
Sardina pilchardus	5	22.8 ± 0.9	(22; 24)	- 18.5 ± 0.4	(-19.0;-18.0)	12.4 ± 0.7	(11.4;13.2)
Ammodytidae							
Hyperoplus lanceolatus	5	24.3 ± 5.5	(20.3; 32.6)	-17.7 ± 0.6	(-18.4;-16.9)	13.5 ± 0.7	(13.1;14.6)
Gobiidae							
Gobies	5	6.2 ± 0.6	(5.6; 7.3)	-18.2 ± 1.4	(-20.3;-16.9)	15.5 ± 1.6	(14.1;17.9)

Percentages of the estimated contribution of prey species in the diet of porpoises according to SIAR modeling are represented in figure 2. For the model 1 (Figure 5.2a) based on the TEFs from Hobson et al., (1996), gobies, sardine and herring were the preys that contributed the most to the diet of juvenile porpoises with a contribution of more than 60% of the total diet for juvenile females and more than 70% for juvenile males. The other species constituted 3 to 12 % of the diet of juvenile porpoises. For the adult females, *C. harengus* constituted 26 % of the diet followed by *S. pilchardus* with a contribution of 22 %. Finally, all prey species had almost the same contribution to the diet of adult male porpoises with estimated contribution varying from 9 % for *M. merlangus* to 20 % for *C. harengus*. According to the model 2 (Figure 5.2b) based on Caut et al., (2011) TEFs, results suggested a high contribution of *Gobiidae sp.* (39 %-

46 %), followed by *S. pilchardus* (13 %-17 %) and *Trisopterus sp.* (15 %-12 %) to the diet of juvenile female and juvenile male porpoises, respectively. These three species constituted more than 67% of the total diet of juveniles. The other species contributed from 5 to 9 % to the diet of juvenile porpoises. For the adult females, *C. harengus* constituted 19 % of the diet followed by *S. pilchardus* (18%). Finally, according to SIAR modeling for adult male porpoises, all prey species had almost the same contribution to the diet with estimated contribution varying from 12 % to 17 %, although confidence intervals were wide. The difference between the 2 models was remarkable in the diet of juveniles where *C. harengus* was relevant in the diet according to the model 1, whereas *Trisopterus sp.* contributed most to the diet in the model 2. Besides that, the two models revealed different percentages of prey contribution to the diet of porpoises.

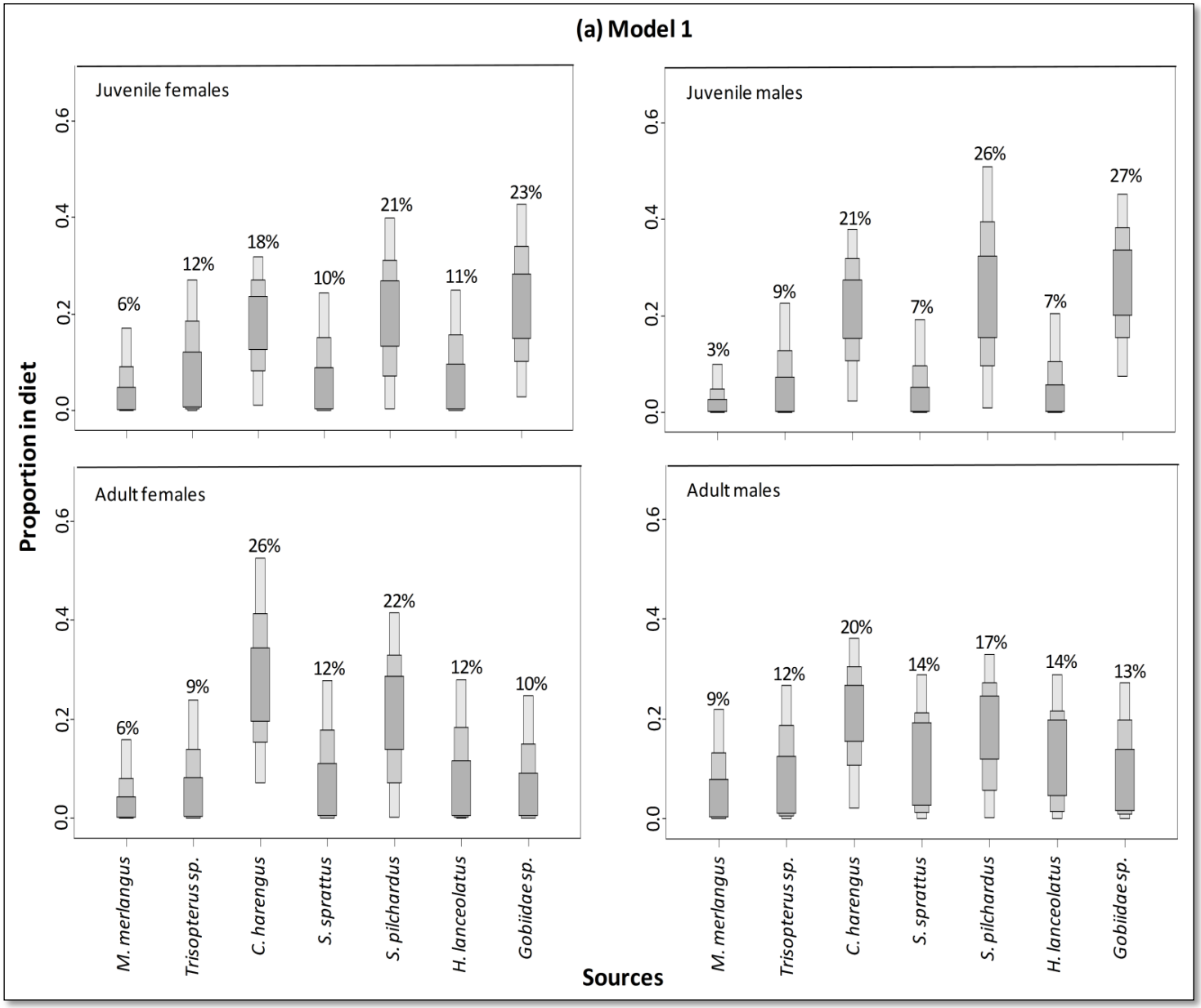
2.3. Fatty acids composition and CSIA

A common spectrum of marine FAs was obtained for the harbour porpoises with about 40 compounds present in relative amounts > 0.1 %. Large standard deviations (SDs) indicated large individual differences between porpoises. There were 15 juvenile females, 31 juvenile males, 10 adult females, 2 adult males and 1 porpoises whose gender could not be identified. FA profiles of juvenile females, juvenile males, adult females and adult males were not significantly different (ANOSIM, $p > 0.05$). Similarly, no differences were found in the FA profiles of porpoises according to the cause of death (ANOSIM, $p > 0.05$), of which 28 died from infectious diseases, 24 from accidental captures and 7 of them whose cause of death could not be identified. Additionally, since the blubber thickness is an index of the nutritional status or body condition (Koopman et al., 2002), porpoises were divided into 3 groups according to the blubber thickness (A: porpoises whose blubber thickness was < 10 mm; B: between 11 and 20 mm, C porpoises with blubber thickness > 21 mm and only 1 porpoise whose blubber thickness was not determined). The FA profiles of porpoises with blubber thickness < 10 mm (n=10) were significantly different compared to those with blubber thickness between 11 and 20 mm (n=28) (ANOSIM, $R = 0.38$, $p < 0.05$), with 16:1ω7, 22:6ω3, 14:1ω3 and 14:0 primarily accounting for the differences (SIMPER, with percent dissimilarities for the FAs of 20.3, 14.0, 5.7 and 5.7%, respectively). Similarly, FA profiles of porpoises with blubber thickness < 10 mm (n=10) were significantly different compared to those with blubber thickness > 21 mm (n=20) (ANOSIM, R =

0.94, $p < 0.001$), with 16:1ω7, 22:6ω3, 14:0 and 20:5ω3 primarily accounting for the differences (SIMPER, with percent dissimilarities for the FAs of 19.6, 15.3, 6.2 and 5.8 %, respectively).

Similarly, in the muscle of potential prey species about 40 FAs were present in relative amounts > 0.1 %. Sandeel exhibited the highest amounts of polyunsaturated fatty acids (PUFAs) with 55.32 ± 5.07 %, while herring exhibited the lowest amounts 24.18 ± 12.55 %. For the monounsaturated fatty acids (MUFAs), herring exhibited the highest amounts (51.16 ± 12.80 %), whereas sandeel exhibited the lowest amounts of MUFAs (11.78 ± 2.88 %).

Non-metric MDS of FA proportions from total FAs (Figure 5.3) in the blubber of all harbour porpoises showed that according to some specific FAs, porpoises may be divided into 3 groups. Porpoises where the proportion of the FA 16:1ω7 is the highest, thereafter referred to group a (> 19 %, figure 5.3a), porpoises where the FAs 22:1ω11/9 and 20:1ω9 were relatively dominant, thereafter referred to group b (> 3 %, figue 3b) and those who exhibited the largest proportion of 20:5ω3, thereafter referred to group c (> 5 %, figure 5.3c). 3 to 4 samples were chosen from each group in order to analyze the Compound-Specific stable C isotope of the above-mentioned FAs as well as from some potential prey species such as *Gobiidae sp.*, *C. harengus*, *S. pilchardus* and *L. ramada*. These species were chosen following the preliminary results obtained for the FAs analyses. In addition, we suspected the presence of a potential herbivivorous prey, therefore thinlip grey mullet *L. ramada* was chosen. The $\delta^{13}C$ values of FAs that arise directly from the diet and could not be synthesized *de novo* by the consumer are shown in table 5.2. The FA $\delta^{13}C$ values varied widely in porpoises blubber, from -34.6 ± 12.2 ‰ for the FA 22:1ω11/9 to -15.4 ‰ for the FA 20:1ω9. For the prey species, FA $\delta^{13}C$ values ranged between -29.2 ± 1.5 ‰ for the FA 16:1ω7 in *C. harengus* to -21.3 ± 0.9 ‰ for the FA 20:1ω9 in *Gobiidae sp.*.


(a) Model 1
Juvenile females
Juvenile males
Adult females
Adult males
Proportion in diet
0.0
0.2
0.4
0.6
6%
12%
18%
10%
21%
11%
23%
3%
9%
21%
7%
26%
7%
27%
6%
9%
26%
12%
22%
12%
10%
9%
12%
20%
14%
17%
14%
13%
M. merlangus
Trisopterus sp.
C. harengus
S. sprattus
S. pilchardus
H. lanceolatus
Gobiidae sp.
Sources

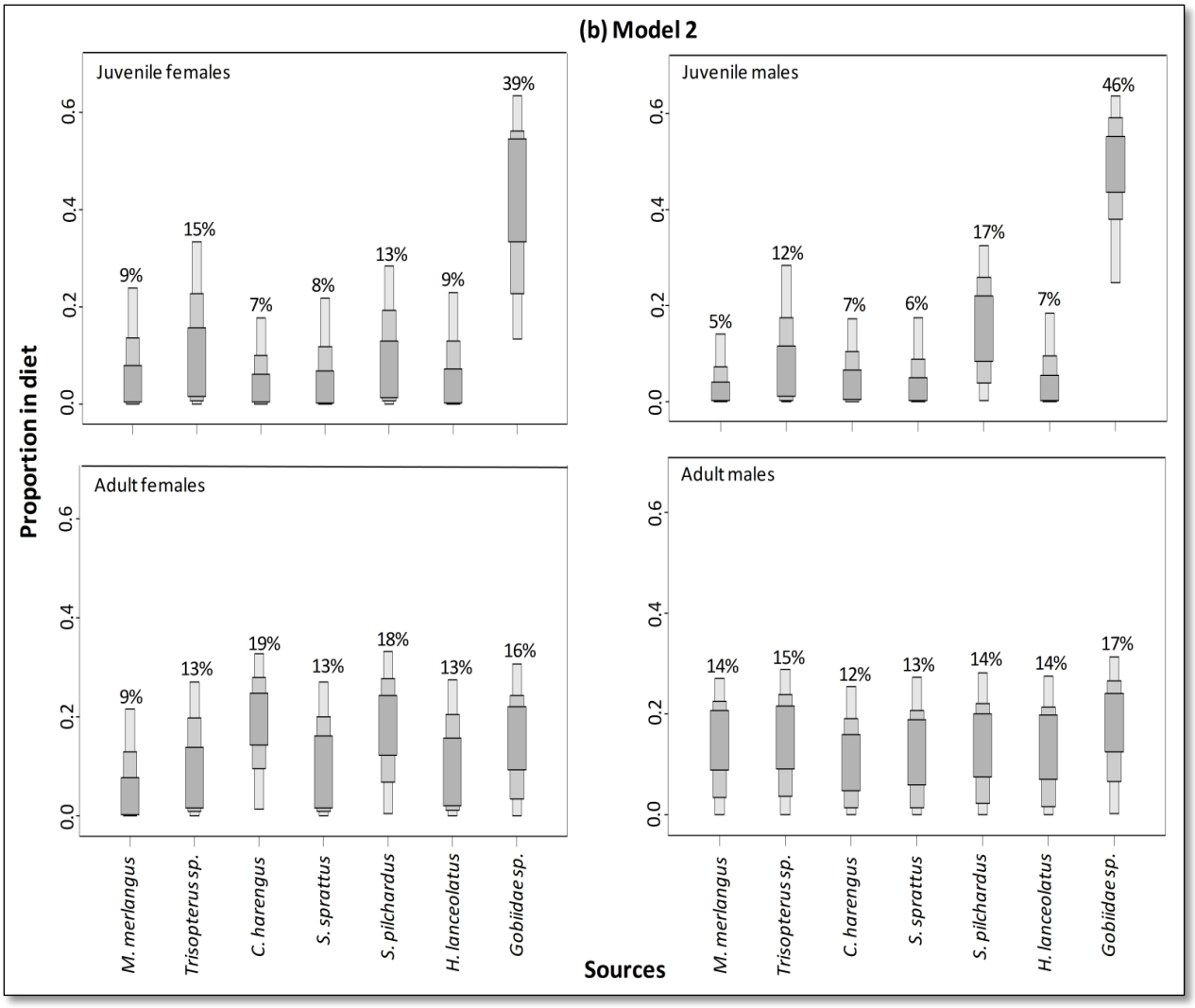


Figure 5.2 SIAR modeling: boxplots of the estimated prey contributions in the diet of harbour porpoises, (a) Model 1: using TEFs of 1.3 ± 0.1‰ and 2.4 ± 0.3‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively by Hobson et al., (1996) and (b) Model 2 using TEFs of 1.26 ± 0.2‰ and 1.23 ± 0.15‰ for $\Delta^{13}C$ and $\Delta^{15}N$, respectively by Caut et al., (2011). Confidence intervals (CI): CI_{95}: light grey; CI_{75}: medium grey and CI_{50}: dark grey.

3. Discussion

3.1. Diet of harbour porpoises stranded along the southern North Sea

The need to combine several techniques to analyze the diet of marine mammals is required in order to overcome the inconveniences of each method. To our knowledge, only few studies have combined the 3 methods to assess the foraging ecology of marine mammals and birds (Hooker et al., 2001; Karnovsky et al., 2008; Jansen, 2013). Stomach content analysis may

be subject to possible bias due to the degradation of preys at different rates; even hard parts such as otoliths are expected to be degraded by the stomach gastric acids (Pierce and Boyle, 1991). The results from stomach content analyses revealed in terms of occurrence that the most important prey items were gobies , followed by sprat, sandeel and to a lesser extent some unidentified gadoids (*Gadidae sp.*) and whiting. The numerically most important prey items were gobies. Reconstructing the original weight of prey items revealed that gobies constituted the most important prey by weight, followed by the whiting and the sandeel. Based on the stomach content analyses, it has been demonstrated that differences in the feeding habits might exist according to the maturity status and the gender of porpoises (Santos and Pierce, 2003). Therefore, segregation of porpoises appears to be essential. Thus, previous studies showed that juvenile porpoises were mainly feeding on gobies (Santos and Pierce, 2003; Santos et al., 2004; Haelters et al., 2012). Similarly in this study, gobies were the most relevant species in the diet of juveniles (n=10) followed by sprats, while gobies and sandeels were co-existent in the diet of adult porpoises (n=3) and to a lesser extent whiting. Overall, the fact that stranded juveniles represented almost 80% of the sampling in this study reflected gobies as the numerically most important prey items. Teleosts of the family Gobiidae are the most abundant and widespread species in shallow coastal waters of the North Sea and play an important role within this ecosystem. It has been suggested that juvenile porpoises fed more on gobies because they cannot dive as deep as adults along with the fact that their small size prevent them from taking big preys (Santos and Pierce, 2003; Haelters et al., 2012). In addition, the importance of gobies in the stomach content may also be explained by the fact that before the stranding, porpoises are usually near the coast and feeding on benthic species such as gobies.

The stable isotope approach provides insight in feeding ecology over a longer period of time (Tieszen et al., 1983) and allows a trophic segregation (France, 1995). The significant differences in $\delta^{15}N$ values between juveniles and adult porpoises may explain differences in feeding habits related to the maturity status; juveniles exhibiting higher $\delta^{15}N$ values. A previous study suggested that the low $\delta^{15}N$ signatures exhibited by adult males might be related to a more offshore prey feeding with low $\delta^{15}N$ signatures such as herring (Das et al., 2003b). However, SIAR modeling used to estimate the proportional contribution of preys to the assimilated diet of porpoises (Parnell et al., 2010) showed that gobies had the highest contribution to the diet of juvenile porpoises (Figure 5.2). Therefore higher number of gobies, identified as the main

contributor by weight to the total diet of juveniles from stomach content analysis (present study, Santos et al., 2004; Haelters et al., 2012), induced higher isotopic values (Table 5.1). As modeled by SIAR, adult females fed mainly on herrings and sardines. Indeed, both species exhibited the lowest $\delta^{15}N$ values (10.8 ± 0.7 ‰ and 12.4 ± 0.7 ‰, respectively). Therefore, the importance of both preys as part of the diet of adult porpoises may have induced lower $\delta^{15}N$ signatures in the muscle of adult females compared to juveniles (Table 5.1).

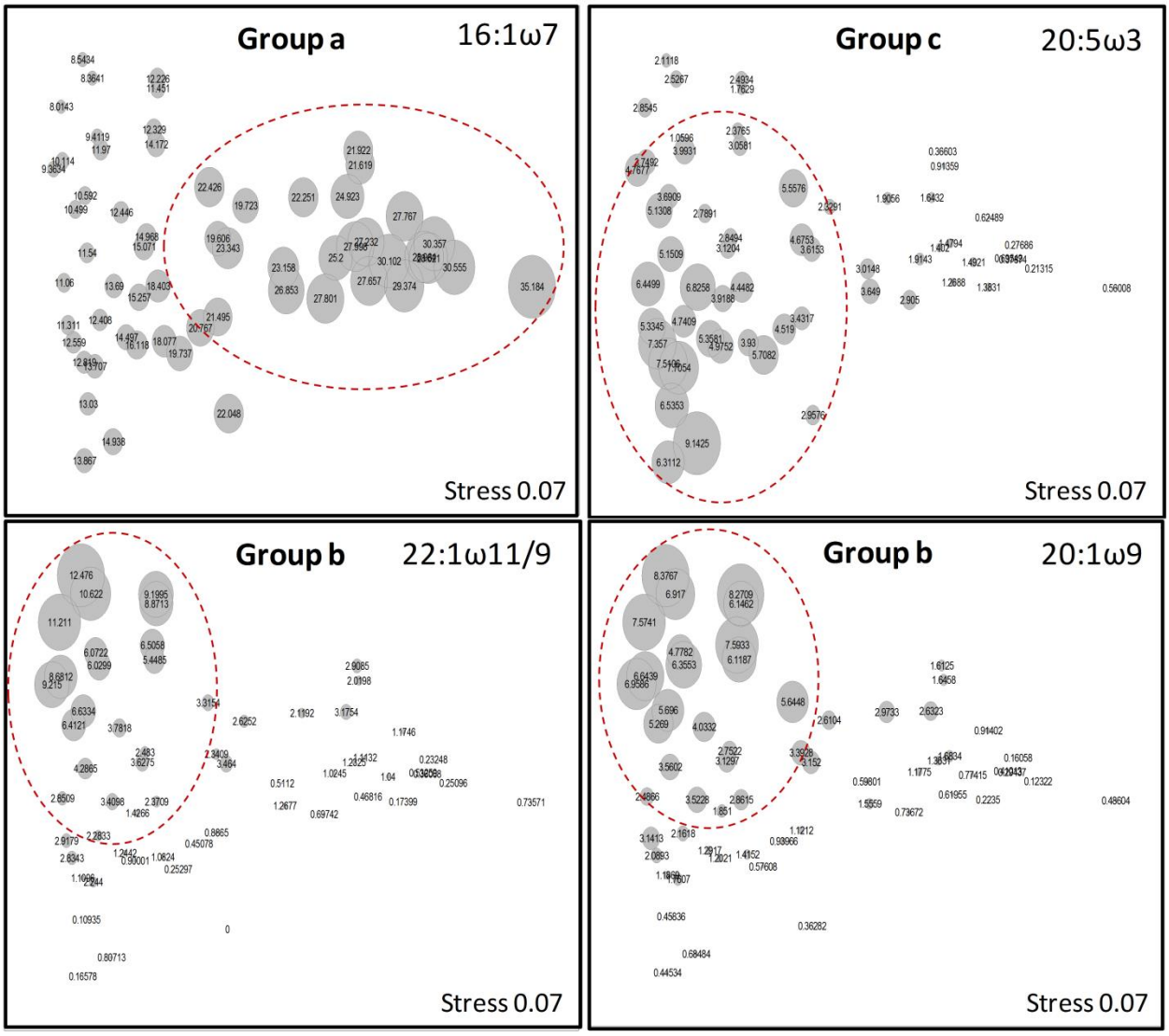


Figure 5.3 Non-metric MDS of FA proportions from total FAs (%) in harbour porpoise's blubber. Group a where the proportion of the FA 16:1ω7 is the highest (> 19%), group b where the FAs 22:1ω11/9 and 20:1ω9 were relatively dominant (> 3%) and group c where the proportion of the FA 20:5ω3 is the highest (> 5%).

The FAs analyses in the blubber also reflect the assimilated diet of porpoises through a period of up to several months (Budge et al., 2006). A decline in the body condition of porpoises induce changes in blubber (Koopman et al., 2002), therefore changes in FAs composition. 16:1ω7 and 22:6ω3 were the main FAs that primarily accounted for the differences between porpoises according to the body condition. The FA 16:1ω7 is originally synthesized by diatoms in the open sea and will preserve $\delta^{13}C$ transmitted by algae (Dalsgaard et al., 2003). According to the $\delta^{13}C$ of the FA 16:1ω7 (Table 5.2), the group *a* exhibiting the highest proportions of 16:1ω7 (Figure 5.3) may be feeding on gobies and sardine. These 2 species having the $\delta^{13}C$ of 16:1ω7 values (-26.4 ± 1.1 ‰ and -25.4 ± 1.6 ‰ for gobies and sardine, respectively) the closest to the group *a* (-25.7 ± 0.4 ‰). The $\delta^{13}C$ of 18:2ω6 values showed that the group *c* (-27.8 ± 2.5 ‰) might be feeding on herring (-27.7 ± 2.2 ‰). In addition, elevated levels of 20:1ω9 and 22:1ω11/9 in muscles of herring reflected the consistent diet of this species (amounts >13 % and 22 % for 20:1ω9 and 22:1ω11/9, respectively in herring muscles). Indeed, these particular FAs are known to be biomarkers and produced by zooplankton (such as calanoid copepods) whose role is to transfer FAs to higher trophic levels such as herring in the northern hemisphere (Tocher, 2003). These 2 FAs exhibiting high proportions in the group *b* (Figure 3) indicated that herring may be potential prey species in the diet of these porpoises. For the FA trophic marker 20:5ω3 which is also originally synthesized by diatoms, its $\delta^{13}C$ values in the group *c* (-28.1 ± 1.4 ‰) lead to the herring (-28.6 ± 1.8 ‰) as a potential prey species. However, the porpoises of the group *c* and the group *b* were feeding differently.

Overall, each technique has its advantages and disadvantages. However, in order to overcome the inconveniences of each method the association of several techniques was required to analyze the diet of porpoises. Gobies were the only species that figured in all techniques. Besides that, sprat, sandeel and whiting were only found to contribute to the diet of porpoises according to the stomach contents. The stable isotopes and the fatty acids approaches were found to converge in a way, where gobies, sardine and herring were simultaneously encountered in the diet of porpoises. Trisopterus sp. was found to contribute to the diet of porpoises only according to SIAR (Model 2). Therefore, stable isotopes and FAs analyses provided complementary information for the stomach content analysis. Combining techniques that integrate diet over days and weeks allowed gaining more complete understanding (Karnovsky et al., 2008) of harbour porpoise's diet relative to stomach contents.

Table 5.2 $\delta^{13}C$ values of selected FAs (mean ± SD) in the blubber of harbour porpoises and the muscle of some prey species; "a" refers to harbour porpoises where the proportion of the FA 16:1ω7 is the highest; "b" refers to porpoises where the FAs 22:1ω11/9 and 20:1ω9 were relatively dominant; "c" refers to porpoises exhibiting the largest proportion of 20:5ω3; "-": not determined.

Species	Harbour porpoises			Prey species			
FA $\delta^{13}C$ (‰)	a (n=3)	b (n=4)	c (n=3)	*Gobiidae sp. (n=3)*	*Clupea harengus (n=3)*	*Sardina pilchardus (n=3)*	*Liza ramada (n=3)*
16:1ω7	-25.7 ± 0.4	-26.9 ± 1.4	-24.2 ± 0.3	-26.4 ± 1.1	-29.2 ± 1.5	-25.4 ± 1.6	-23.4 ± 1.5
18:2ω6	-31.5 ± 1.6	-30.0 ± 1.9	-27.8 ± 2.5	-	-27.7 ± 2.2	-	-24.2 ± 0.3
20:1ω9	-15.4	-23.9 ± 2.6	-22.6 ± 1.0	-21.3 ± 0.9	-24.6 ± 1.0	-24.1 ± 1.5	-23.5 ± 1.8
20:5ω3	-30.9 ± 0.5	-29.7 ± 1.0	-28.1 ± 1.4	-25.8 ± 0.5	-28.6 ± 1.8	-26.2 ± 0.6	-24.8
22:1ω11/9	-33.9 ± 3.1	-24.6 ± 2.0	-34.6 ± 12.2	-22.4 ± 0.8	-23.1 ± 1.4	-24.3 ± 1.4	-23.4 ± 0.0
22:6ω3	-30.0	-27.4 ± 1.4	-24.4 ± 0.5	-26.4 ± 0.5	-26.1 ± 1.7	-25.4 ± 1.7	-26.4 ± 0.9

3.2. General diet composition

The diet arising from stomach content analysis for about 100 harbour porpoises stranded between 1989 and 1994 along the UK waters consisted mainly on gadoids, sandeels and gobies (Martin, 1996). In Scottish waters, about 188 stomachs investigated for porpoises stranded between 1992 and 2003 mainly included whiting, sandeels and gadoids such as Trisopterus sp. (Santos et al., 2004). In 2006, stomach contents of 64 porpoises stranded along the Dutch waters mainly included gobies, sandeels, sprat, herring, whiting and twait shad (Leopold and Camphuysen, 2006). A more recent study on 64 porpoises stranded between 1997 and 2011 along the Belgian waters showed that porpoise's stomachs mainly included gobies, sandeels and gadoids such as whiting and Trisopterus sp. (Haelters et al., 2012). Therefore, the diet of porpoises along the North Sea since earlier 90s till nowadays primarily comprises 7 prey species: gobies, whiting, sandeel, sprat, trisopterus sp., herring and sardine. Beside the sardine, these species are among the most numerous fish and widely distributed along the North Sea (Daan et al., 1990; ICES, 2013b). Hence, the opportunistic predator character of porpoises as described in previous studies (Santos and Pierce, 2003; Fontaine et al., 2007) tends to be justified considering that porpoises are relying for their main dietary intake on abundant existent preys that are easily available in high numbers. Thus, the diet of porpoises may be expected to reflect the availability of food items in the area to which the animal is exposed (Christensen and Richardson, 2008). Although, a previous study suggested that the narrow range of prey species on which porpoises appear to feed on suggest a specialized feeding character (Santos et al., 2004).

Demonstrating effects of prey distributions on harbour porpoise's diet is difficult as it is not subject to experimentation. Therefore, we have tried to link the total abundance of potential prey species in the North Sea to the distribution and consequently to the shift of porpoises from the northern areas of the North Sea (ICES Div. IVb) to its southern areas (ICES Div. IVc). Herring, sprat, sandeel and whiting are common species in the North Sea with an economic interest (ICES, 2013b). The spatial trend for total landings in almost all areas of the North Sea was similar (SIH - Ifremer, 2014). Relative abundance of the most common species, data from the International Bottom Trawl Survey (IBTS), in the North Sea from 1983 to 2010 are presented in figure 4 (SIH - Ifremer, 2014). Between both surveys (SCANS in summer 1994 and SCANS II in summer 2005), relative abundance of herring and sprat showed no clear temporal variations, whereas the whiting and sandeel species witnessed a striking decline in total abundance since 2001. In the case of sandeel, the decline was more pronounced from about 1 400 000 000 (relative value) in 2002 to 44 000 000 (relative value) in 2003 (Figure 5.4). This decline may be particularly attributed to the sandeel total landings decline in the northern parts of the North Sea since the late 90s (MacLeod et al., 2007; ICES, 2007). In addition, in the period between 1994 and 2009, 84 % of the total sandeel landings came from the southern areas of the North Sea (ICES, 2014). The North Sea is an example of a region where fishing has substantially impacted forage fish populations (Dickey-Collas et al., 2014). It has been suggested that the starvation of harbour porpoise populations in spring 2002 and 2003 in the Scottish North Sea was due to the negative effects of climate change on sandeels availability (MacLeod et al., 2007). We suggest that after the decline of the relative abundance of sandeel since 2002 in the northern parts of the North Sea, harbour porpoises have started to move southern along the North Sea to follow their potential prey.

Gobies and trisopterus sp. are also important prey species in harbour porpoise's diet but contrary to the previous species exploited by targeted fisheries (ICES), few data are available on their distribution and abundance. However, data from the International Bottom Trawl Survey (IBTS) in the North Sea showed that the relative abundance of trisopterus sp. decreased since 2000, whereas gobies showed no clear temporal variations. Gobies are the most abundant fish species of the shallow coastal areas and are abundant all year round. Gobies and Trisopterus sp. figured in almost all previous studies, therefore they may be considered as traditional common preys in the diet of harbour porpoises in the North Sea.

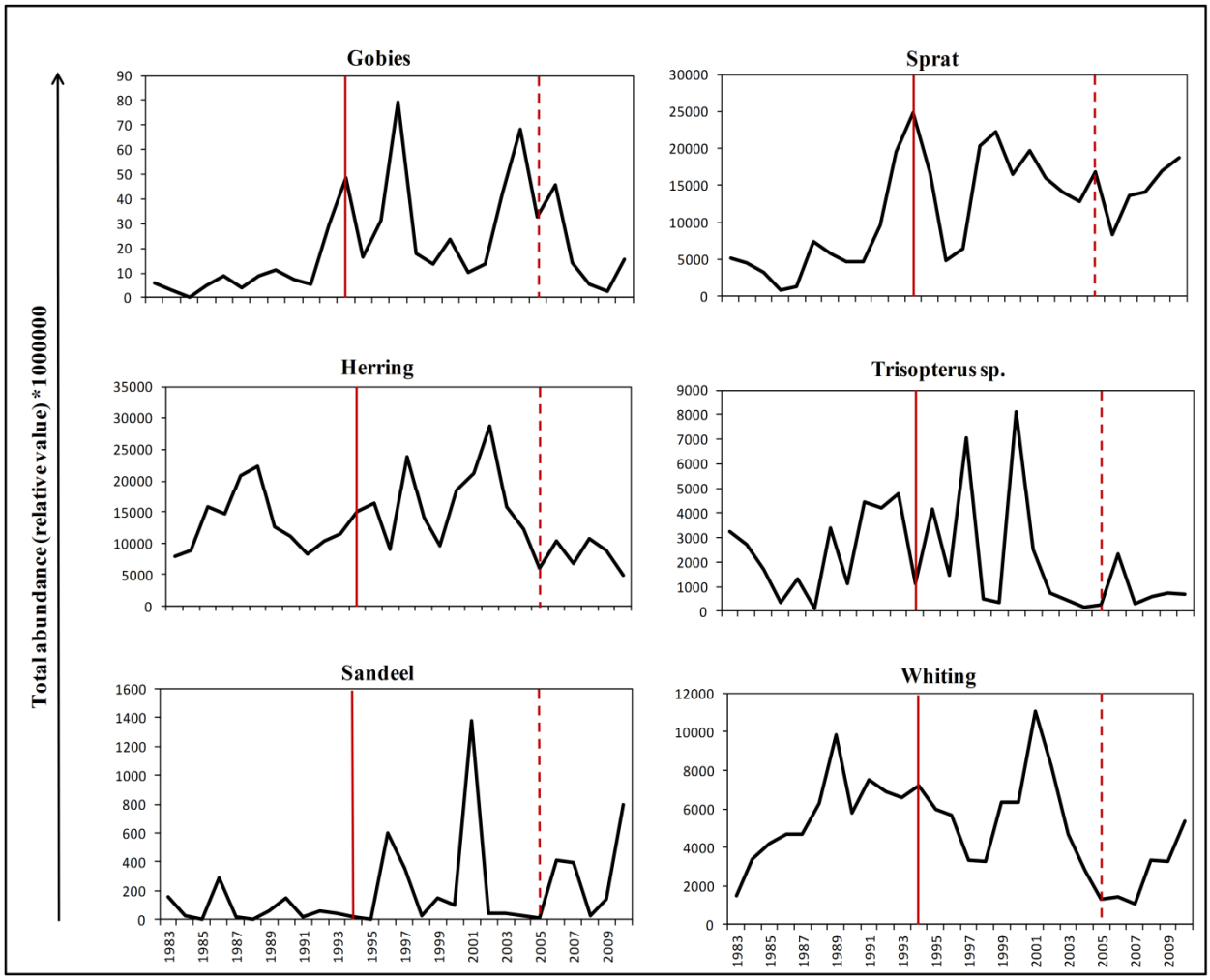


Figure 5.4 Total abundance (relative value) (*1000 000) of potential prey species in the North Sea from 1983 to 2010. Data are available from the International Bottom Trawl Survey (IBTS) for the areas IVb and IVc (SIH – Ifremer, 2014). Red line represents SCANS and red dashed line represents SCANS II surveys held in summer 1994 and 2005, respectively.

In the present study sardine was found to contribute to the diet of porpoises according to SIAR and to the FAs analyses. However, sardine did not figure as potential prey in harbour porpoise's diet in previous studies relying on stomach content analyses (Leopold and Camphuysen, 2006; Haelters et al., 2012). This small pelagic fish with more southern distribution is important commercial species in southern Europe. However, sardine distribution has witnessed an increase in the North Sea over time. Since the mid 1990s, the re-invasion by sardines into the North Sea has been highlighted and attributed as a response to climate change (Beare et al., 2004 a; b). Therefore, little information is known about the abundance of sardine and it is considered occasional occupant of the North Sea and rarely occurs at a biomass large

enough to attract fisheries exploitation. Hence, sardines may be considered as a "new" potential prey species in the diet of porpoises or it may be a "backup" prey replacing the sandeel decline. The results also suggest that porpoises may prey in offshore water on pelagic shoaling species such as sardine probably to compensate for the decrease in abundance of demersal coastal species.

Conclusion

The combined techniques resulted in greater insight into the feeding ecology of harbour porpoises. Thus, any one approach alone could not provide similar outcomes. The present study highlighted the decrease of sandeel as potential prey in the diet of porpoises along the northern parts of the North Sea and suggested that the shift of the distribution to the southern parts may be likely due to the decline of sandeel in northern North Sea. In addition, the feeding changes may also be due to the occurrence since the mid 90s of sardines in the North Sea making this species a new potential prey contributing to the diet of porpoises. Finally, harbour porpoises shift toward the southern parts of the North Sea have induced an increase of the abundance of porpoises in this area especially along northern France and Belgian coasts. However, prey availability may only be considered as one of several potential components influencing the shift of porpoises along the North Sea. We cannot reject the presence and influence of several connected factors that can also affect the abundance and distribution of porpoises such as climate changes, fishery interactions, contamination, and marine traffic.

Acknowledgements

The French Stranding Network is acknowledged for the collection of harbour porpoises samples used in this study. Special thanks to the "Centre de Recherche sur les Mammifères Marins" (CRMM) and "Observatoire pour la Conservation et l'Etude des Animaux et Milieux Marins" (OCEAMM). The authors would also like to thank Thierry Jauniaux for executing post-mortem examinations and providing samples and information on the animals from the southern North Sea. Florence Caurant and Sebastien Lefebvre are acknowledged for their help in the stable isotopes and SIAR. We thank Najet Thiney from the MNHN for her help in the FAs

analysis. M. C. is financially supported by a PhD fellowship from the National Council for Scientific Research (Lebanon) and Université du Littoral Côte d'Opale (France).

CHAPTER 6

FEEDING HABITS OF HARBOUR PORPOISES (*PHOCOENA PHOCOENA*) FROM THE SOUTHERN NORTH SEA AND THE BAY OF BISCAY INFERRED FROM A MULTI APPROACH DIETARY ANALYSES

Chapter 6 – Feeding habits of harbour porpoises (*Phocoena phocoena*) from the southern North Sea and the Bay of Biscay inferred from a multi approach dietary analyses

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In prep., manuscript will be submitted to "Journal of Experimental Marine Biology and Ecology".

Abstract

The study of the feeding ecology of harbour porpoises serves among others to investigate their feeding strategy, the predator-prey relationships, and their responses to changes in food webs dynamics, climate changes or fishery interactions. This study compares the feeding ecology of harbour porpoises stranded along the southern North Sea between 2010-2013 (Northern France and Belgian coast) and along the Bay of Biscay between 2009-2012. The diet was assessed through a combination of stomach content analysis, the stable isotope analysis (carbon and nitrogen) determined from muscle samples and the fatty acids analysis determined from blubber samples. The combined approach has proven to be particularly complementary and helpful toward a better and general insight into the feeding ecology of the harbour porpoise. Our study suggests that the opportunistic or specialized character of harbour porpoises is related to the prey availability in the environment. In addition, the different habitat characteristics of each area resulted in differences in the feeding habits of porpoises inhabiting each zone.

Keywords: harbour porpoises; North Sea; Bay of Biscay; stomach contents; stable isotopes; fatty acids

Introduction

The harbour porpoise (*Phocoena phocoena*) is a relatively small cetacean widespread in cooler coastal waters of the North Atlantic (Reid et al., 2003). Beside the several potential threats such as exposure to contaminants, marine traffic and fishery by-catch, the harbour porpoise may be subject to extra environmental stress such as depletion of favored, nutritive rich prey species through overfishing (Bjørge, 2003). Depending on the region where it operates, it is subject to different environment and human impacts. Studying the feeding ecology of harbour porpoises serves to investigate their feeding strategy, the predator-prey relationships, and their responses to changes in food webs dynamics, climate changes or fishery interactions (Haelters and Camphuysen, 2009; Herr et al., 2009). Harbour porpoises are known to consume a wide variety of prey species including numerous commercial fish species such as sandeels (*Ammodytidae*), scads (*Trachurus trachurus*), different species of gadidae (*Merlangius merlangus* and *Gadus morhua*) and to a smaller extent some clupeids (*Clupea harengus*, *Sprattus sparttus* and *Sardina pilchardus*) (Santos et al., 2004; Haelters et al., 2012; Spitz et al., 2006).

The North Sea and the Bay of Biscay are characterized by different environmental and anthropogenic pressures. The North Sea is a large, semi-enclosed, epi-continental sea with a relatively shallow mean depth of 90 m with deeper areas in the north of the Norwegian Trench (700 m) (Ducrotoy et al., 2000) and surrounded by large and highly developed societies (Ducrotoy and Elliott, 2008). It supports large-scale commercial fishing and is an example of a region where fishing has substantially impacted forage fish populations (Dickey-Collas et al., 2014) which in turn can introduce changes in marine mammals feeding habits. The North Sea sustains numerous marine mammals (Jauniaux et al., 2002b). The harbour porpoise is the most common and abundant marine mammal inhabiting the North Sea and adjacent waters. The abundance and stranding of this species has increased along the southern North Sea over the last few decades (Jauniaux et al., 2008; Haelters and Camphuysen, 2009; Hammond et al., 2013). Changes in abundance and occurrence of porpoises in the North Sea are illustrated as consequences of changes in environmental factors (Reijnders, 1992). On the other hand, the Bay of Biscay is a very large open oceanic bay in the North East Atlantic with a mean width continental shelf varying between 50 and 180 km on the French coast and a narrow continental shelf on the Spanish coast with a mean width of 30 to 40 Km (Koutsikopoulos and Le Cann,

1996). Numerous anthropogenic activities are exerted in the Bay of Biscay such as important fisheries (Lorance et al., 2009) and the increase in fishing pressure has induced changes in fishing composition and fishing grounds (Guénette and Gascuel, 2012). It has been suggested that porpoises inhabiting the Bay of Biscay are more impacted by their incidental captures in fishing gears than resource depletion (Lassalle et al., 2012).

The feeding habits of harbour porpoises have been derived through several methods. The analysis of stomach contents (Santos and Pierce, 2003; Santos et al., 2004; Spitz et al., 2006) is based on the identification of the remains of ingested preys in the stomach contents (otoliths, fish bones, cephalopod beaks, etc) to the lowest taxonomic level. The analysis of muscle stable isotopes (^{13}C and ^{15}N) (Das et al., 2003b; Jansen et al., 2012; Mèndez-Fernandez et al., 2012) is based on the fact that $\delta^{13}C$ and $\delta^{15}N$ in tissues of predators may be directly related to those of their prey with an expected enrichment called Trophic Enrichment Factor (TEF) (Vander Zanden and Rasmussen, 2001). Finally, the analysis of blubber fatty acids composition (Jansen, 2013) is based on the fact that fatty acids (FAs) are deposited into adipose tissue with little change or in a predictable manner, thus reflecting the diet of the predator over a period of up to several months (Budge et al., 2006). However, rare are the studies that combined the 3 methods together in order to assess the feeding ecology of organisms in general (Hooker et al., 2001; Karnovsky et al., 2008; Jansen, 2013).

The aims of the present study are (1) to compare the diet of harbour porpoises from the southern North Sea and the Bay of Biscay by combining 3 different methods, (2) to investigate if the different habitat characteristics of each area might result in differences in the feeding habits of porpoises inhabiting each zone and (3) to assess for a long-term trend or changes in diet composition by relating changes to prey availability and comparing our results to previous studies.

1. Materials and methods

1.1. Sampling and data collection

Harbour porpoises freshly dead or slightly decomposed, stranded along the southern North Sea between 2010 and 2013 and the Bay of Biscay between 2009 and 2012, were collected

for necropsies. According to the protocol from Kuiken and Hartmann, (1993) and Jauniaux et al., (2002a), post-mortem investigations were performed. Animals were grouped into age-classes according to the total body length: porpoises whose length ranged from 91 to 130 cm were considered as juveniles and porpoises whose length was greater than 130 cm were considered as adults. Stomachs were collected for the stomach content analysis, muscles for the stable isotope analysis and finally blubber was collected for the fatty acids analysis. All samples were stored at -20° C until analyses were carried out.

Overall 16 potential prey (n=117) were collected along the southern North Sea. Prey samples were selected in order to cover the size-classes found in the stomach contents of harbour porpoises. RVs covered two different seasons. The RV SEPIA (INSU-CNRS) provided preys from coastal areas in June 2012 and October 2012, whereas the RV "Thalassa" (Ifremer) provided preys in November 2012. Prey samples were stored at -20º C until further analysis.

1.2. Stomach content analysis

Fourteen stomach contents of stranded porpoises from the southern North Sea between 2010 and 2013 were previously analyzed in Chapter 5. In addition, thirteen stomachs from porpoises stranded along the Bay of Biscay between 2009 and 2012 were analyzed for this study. Stomachs were cut open and the contents were washed under running tap-water and collected in a sieve with mesh size of 0.2 mm. Remains such as otoliths and fish vertebrates were stored dry, whereas whole or partly digested prey items and cephalopod beaks were stored in 70 % ethanol. Hard prey remains were identified to the lowest taxonomic level possible following our reference collection of specimens (LOG) and published data from Leopold et al., (2001). Otoliths were analyzed using an image analysis system consisting on a high resolution Sony video camera connected to an optical microscope. Measurements of otoliths width and length, obtained with image analysis software (TNPC 5.0 NOESIS), were used to reconstruct the original fish size and mass using regressions from Leopold et al., (2001). No corrections were made for loss or reduction of size of prey remains due to digestion.

Prey composition was expressed as the percentage of frequency of occurrence (%O, the percentage of stomachs in which a certain prey item occurred, excluding empty stomachs) and percentage composition by number (%N, the number of a particular prey item as a proportion of

the total number of all prey items in the stomach) and percentage composition by weight (%W, the mean of the weight of a given prey species as a percentage of the total prey weight in each stomach). In addition, the predator feeding strategy was analyzed according to the graphical method proposed by Amundsen et al., (1996), which incorporated the prey-specific abundance (P_i) into Costello, (1990) analysis. P_i is defined as the percentage a prey taxon comprises of all prey items in only those predators in which the actual prey occurs, as follows:

$$P_i = \frac{\Sigma\, S_i}{\Sigma\, S_{ti}} \times 100$$

where P_i is the prey-specific abundance of prey i, S_i the stomach content comprised of prey i and S_{ti} the total stomach content in only those predators with prey i in their stomach. The examination of the distribution of data points along the diagonal and axes of the diagram can be used to interpret information about prey importance and feeding strategy (specialization) of the predator. For a detailed description see Amundsen et al., (1996).

1.3. Stable isotopes analysis

Muscle samples of harbour porpoises stranded along the southern North Sea (n=53) were previously analyzed for $\delta^{13}C$ and $\delta^{15}N$ (Chapter 5). As for porpoises stranded along the Bay of Biscay (n=34), lipids were extracted from muscle samples (see details in Chapter 5) as they are highly depleted in ^{13}C relative to other tissue component (Tieszen et al., 1983). Stable isotope ratios ($^{15}N/^{14}N$ and $^{13}C/^{12}C$) were also determined in the muscle of prey species except for the gobies (*Gobiidae sp.*) and the crustacean (*Crangon crangon*) where the whole body was ground. Subsamples of lipid-free powder (0.35 ± 0.05 mg) were weighed into tin cups for stable isotopes analyses. Stable isotope measurements were performed with an elemental analyzer coupled to an isotope ratio mass spectrometer (DELTA V ADVANTAGE Isotope Ratio MS – Thermo Scientific). The results are expressed in the δ notation in parts per thousands (‰) relative to the international standards Vienna PeeDee Belemnite (V-PDB) and atmospheric Nitrogen (N_2) for ^{13}C and ^{15}N measurements, respectively. The experimental precision based on replicate measurements of internal laboratory standards (acetanilide) during each series of measurement was ± 0.15 and ± 0.20‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

1.4. Fatty acids analysis

Fatty acids were previously determined in the blubber of harbour porpoises stranded along the southern North Sea (n=59) (Chapter 5). As for the porpoises stranded along the Bay of Biscay (n=32) and the prey species, extractions were done as described chapter 5. Briefly, samples were processed using a slightly modified version of Bligh and Dyer, (1959) as in Meziane et al., (2007). Saponification and methylation were conducted according to Meziane and Tsuchiya, (2002) in order to obtain the total lipids as methyl esters. For the identification, FAMEs were separated and quantified by gas chromatography equipped with a flame ionization detector (GC; Varian CP – 3800). The GC was fitted with a Supelco OMEGAWAX 320 column (30 m, 0.32 mm ID, 0.25 µm film thickness). Helium was the carrier gas. Finally, peaks were identified by comparing their retention times with those of authentic standards (Supelco^{TM} 37, PUFA Mix – No 1 Marine Source and Bacterial mix; Supelco Inc., Bellefonte, PA, USA). For some samples, peaks of FAs were confirmed with GC-mass spectrometry (GC-MS; ThermoFinnigan TRACE DSQ). The identified FAs are designated as X:YωZ, where X is the number of carbon atom, Y is the number of double bonds and Z is the position of the ultimate double bond from the terminal methyl. The concentration of each FA (C_{FA}, mg) was calculated according to Schomburg, (1987):

$$C_{FA} = \frac{A_S}{A_{IS}} \times \frac{C_{IS}}{W_S}$$

Where A_S is the peak area of the FA, A_{IS} is the peak area of the internal standard, C_{IS} is the concentration of the internal standard (mg) and W_S is the dry weight of the sample (g) for prey species and fresh weight (g) for the blubber of porpoises.

Table 6.1 Prey found in the stomach contents of harbour porpoises stranded along the southern North Sea (2010 – 2013) and the Bay of Biscay (2009 – 2012). Data are expressed as mean ± SD (standard deviation); (minimum – maximum); %O: frequency of occurrence; %N: composition by number and %W: composition by weight.

Species	Southern North Sea							Bay of Biscay						
	Fish length (cm)		Fish weight (g)					Fish length (cm)		Fish weight (g)				
	mean ± SD	(min - max)	mean ± SD	(min - max)	% O	%N	% W	mean ± SD	(min - max)	mean ± SD	(min - max)	%O	%N	%W
Demersal and benthic fish														
Boops boops	-	-	-	-	-	-	-	-	-	-	-	7.7	1.8	-
E. vipera	-	-	-	-	7.1	0.01	-	-	-	-	-	-	-	-
Gadidae	-	-	-	-	28.6	0.2	-	-	-	-	-	7.7	0.5	-
Gobiidae sp.	4.5 ± 1.3	(2.7 - 6.6)	1 ± 0.8	(0.2 - 2.7)	71.4	96.5	45.4	6.8 ± 1.1	(5.2 - 8.5)	2.9 ± 1.3	(1.3 - 5.2)	23.1	54.3	4.6
M. merlangus	22.5 ± 3.3	(17 - 32)	95.9 ± 46.5	(38 - 253)	21.4	0.4	31.3	-	-	-	-	-	-	-
M. merluccius	18.3 ± 2.8	(17 - 22)	38.7 ± 19.5	(27 - 61)	7.1	0.04	1.1	24.9 ± 8.9	(4.2 - 44)	140 ± 138	(0.4 - 590)	69.2	13.4	64.7
Trisopterus sp.	6.1 ± 2.5	(4.3 - 8)	2.9 ± 3.1	(0.7 - 5.1)	7.1	0.02	0.1	17.4 ± 5.3	(8.0 - 23)	83 ± 60.7	(5.4 - 170)	30.8	1.1	3.0
Pelagic fish														
C. harengus	17.1 ± 8.8	(11 - 23)	47.2 ± 55.3	(8.1 - 86)	14.3	0.04	0.9	-	-	-	-	-	-	-
E. encrasicolus	-	-	-	-	-	-	-	15.3 ± 2.7	(8.7 - 20)	22.7 ± 9.7	(3.9 - 44)	7.7	3.3	2.6
H. lanceolatus	19.9 ± 3.5	(17 - 23)	21.4 ± 11.4	(13 - 34)	21.4	0.04	0.6	-	-	-	-	-	-	-
Lampanyctus sp.	-	-	-	-	-	-	-	-	-	-	-	7.7	1.2	-
M. poutassou	-	-	-	-	-	-	-	17.8 ± 3.3	(16 - 22)	33.7 ± 18.6	(23 - 55)	15.4	0.5	0.5
S.pilchardus	-	-	-	-	-	-	-	20.3 ± 4.6	(5.8 - 24)	101 ± 46.6	(2.1 - 152)	30.8	3.2	10.9
S. sprattus	10.2 ± 1.8	(6.3 - 14)	9.5 ± 5.6	(1.6 - 23)	50.0	0.5	3.4	-	-	-	-	-	-	-
T. trachurus	10.8 ± 0.8	(10 - 11)	11.4 ± 2.6	(10 - 13)	7.1	0.02	0.2	16.9 ± 5.2	(7.7 - 27)	54.9 ± 47.2	(4.1 - 169)	38.5	6.5	12.2
M. muelleri	-	-	-	-	-	-	-	-	-	-	-	7.7	1.7	-
Coastal fish														
A. marinus	15 ± 2.7	(12 - 22)	10.8 ± 7.5	(4.4 - 34)	35.7	2.0	16.8	12.3 ± 2	(11 - 14)	5.1 ± 2.6	(3.3 - 7)	15.4	0.3	0.1
A. presbyter	8.2 ± 1.4	(7 - 10)	3.8 ± 2	(1.6 - 7.3)	28.6	0.1	0.3	8.2 ± 1.9	(5.4 - 14)	4.2 ± 3.3	(0.9 - 17)	15.4	11.0	1.6

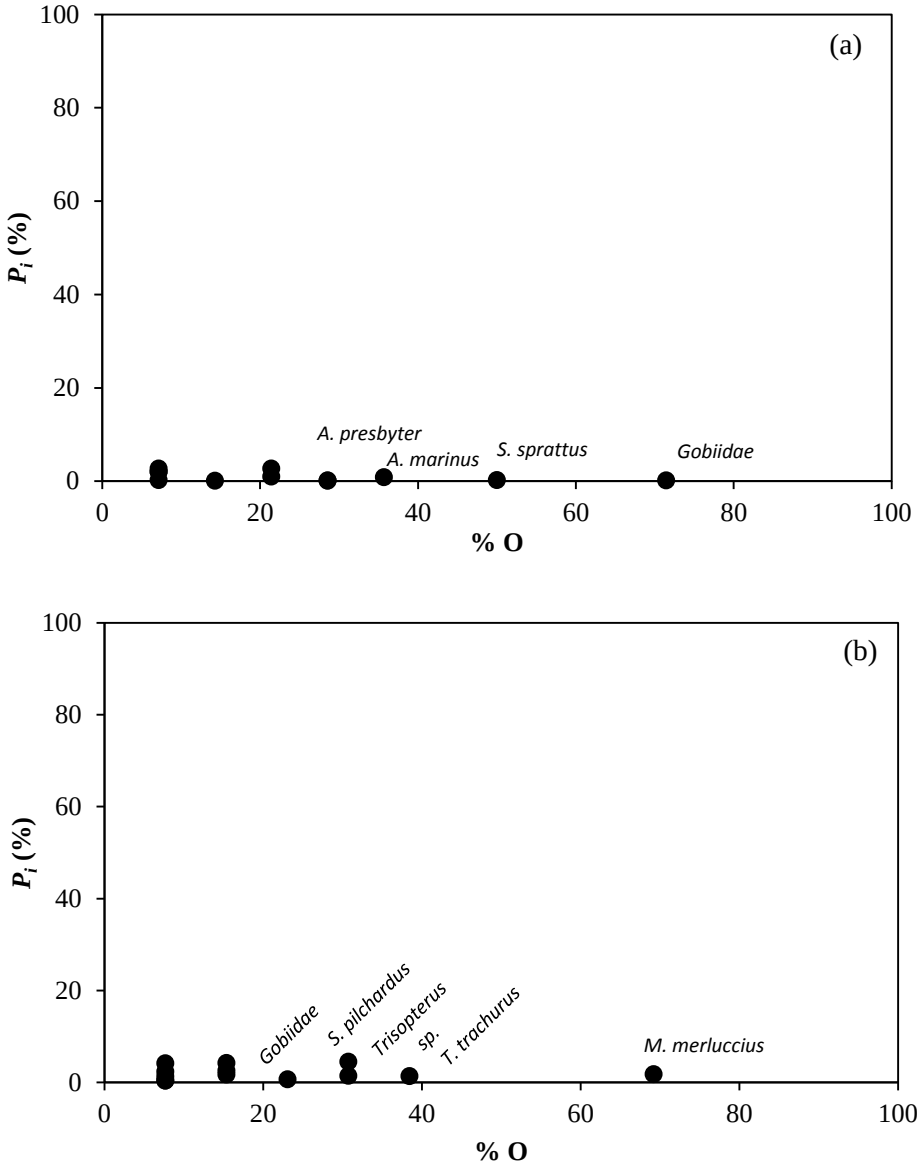

Figure 6.1 Feeding strategy of harbour porpoises stranded along (a) the southern North Sea and (b) the Bay of Biscay analyzed by the graphic method of Amundsen et al. (1996). Specific abundance of prey (P_i %) plotted against the frequency of occurrence of prey (%O).

1.5. Data treatment

Data analysis of stable isotopes was performed using "XLSTAT – Pro" 2013 (Addinsoft). The level of significance was set at $\alpha = 0.05$. $\delta^{15}N$ and $\delta^{13}C$ values in muscle were tested using a Mann-Whitney *U* test or a student's *t*-test when the necessary assumptions of normality and homogeneity of variances for parametric statistics were satisfied. These statistical tests were used

to compare stable isotope values in the muscle of porpoises from the southern North Sea and the Bay of Biscay. Moreover, ANOVA followed by post-hoc multiple comparison tests were used to compare the data between age classes (juveniles and adults).

To investigate for variations in FA composition among samples, Bray-Curtis similarity matrices were calculated on the FA dataset with no transformation applied. Analysis of similarity (ANOSIM) was performed using PRIMER 5 and the statistic test was computed after 5000 permutations. Significant differences in FAs profiles were identified using the global *R*-values. Factors used for the analysis were the sampling regions (southern North Sea and the Bay of Biscay), gender and maturity status.

2. Results

2.1. Stomach contents

The diet of harbour porpoises investigated from both regions was mostly composed of fish species (Table 6.1, only fish species were presented). For porpoises stranded along the southern North Sea (10 juvenile males, 2 adult females, 1 adult male and 1 undetermined porpoise) the overall prey-size distribution ranged from 2.7 to 32 cm, whereas the fish weight ranged from 0.2 to 253 g. Three fish species made up 93.6 % of the biomass (%W). Along with a high relative abundance (96.5%), gobies was the most important prey in term of ingested biomass with 45.4%, followed by whiting 31.3% and lesser sandeel (*A. marinus*) 16.8%, the other species accounted for less than 2% of the diet of porpoises either in weight or in number. For porpoises stranded along the Bay of Biscay (1 juvenile male, 4 adult females, 6 adult males and 2 undetermined porpoises), the overall prey-size distribution ranged from 4.2 to 44 cm, whereas the fish weight ranged from 0.4 to 590 g. Three fish species made up 87.7 % of the biomass (%W). Despite a relatively low relative abundance (13.4%), the European hake (*M. merluccius*) was the most important prey in term of ingested biomass with 64.7%, followed by scads (*T. trachurus*) 12.2% and sardine (*S. pilchardus*) 10.9%, the other species accounted for less than 5% of the diet of porpoises in weight. Some fish species such as gobies, and sand smelt were important in term of relative abundance (54.3 and 11%, respectively) but relatively low in term of biomass (4.6 and 1.6%, respectively).

Analysis of feeding strategy based on the Amundsen's method showed that harbour porpoises from the southern North Sea and the Bay of Biscay have comparable generalized feeding strategy (Figure 6.1). Most individuals of porpoises stranded along the southern North Sea (Figure 6.1a) are feeding occasionally on gobies. In addition, gobies had been eaten by more than half of harbour porpoises but their average contribution to the stomach contents of porpoises was low, indicating a generalized feeding strategy. Likewise, most individuals of porpoises stranded along the Bay of Biscay (Figure 6.1b) are feeding occasionally on European hake. Therefore, this species has been eaten by more than half of harbour porpoises but their average contribution to the stomach contents of porpoises was low, indicating a generalized feeding strategy. In addition for both regions prey points indicate a broad niche width.

2.2. Stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$

Overall, $\delta^{15}N$ and $\delta^{13}C$ values (Table 6.2) in muscles of harbour porpoises stranded along the southern North Sea showed significant differences compared to porpoises stranded along the Bay of Biscay (Mann-Whitney, $p < 0.0001$ and Student-*t* test, $p < 0.05$ for $\delta^{15}N$ and $\delta^{13}C$, respectively).

The mean value of $\delta^{15}N$ in the muscle of harbour porpoises stranded along the southern North Sea was 15.8 ± 1‰ and the mean value of $\delta^{13}C$ was -17.3 ± 0.5‰. Adult females had the lowest mean value of $\delta^{15}N$, whereas juvenile females exhibited the highest mean value (14.7 ± 1.1‰ and 16.1 ± 1.0‰, respectively). Between age-classes, $\delta^{15}N$ values of juvenile females and adult females were significantly different (ANOVA, Post-hoc, $p < 0.05$). No significant differences were found in $\delta^{13}C$ values between age-classes (ANOVA, $p > 0.05$). For the prey species collected along the North Sea, $\delta^{13}C$ values ranged between -20.3 ‰ and -14.3 ‰ for *Gobiidae sp.* and *C. Crangon*, respectively. As for the $\delta^{15}N$, values ranged between 9.9 ‰ and 17.9 ‰ for *C. harengus* and *Gobiidae sp.*, respectively (Table 6.2). Except for *C.crangon*, all pelagic prey species (*C. harengus, S. pilchardus* and *S. sprattus*) showed lower values of $\delta^{15}N$ and $\delta^{13}C$ compared to harbour porpoise's values (Figure 6.2a). As for the demersal and benthic preys, some species showed almost same $\delta^{13}C$ values but lower $\delta^{15}N$ values (such as *Solea solea* and *Limanda limanda*), while other species showed lower values for both $\delta^{15}N$ and $\delta^{13}C$ (*Trisopterus luscus*, *Platychtys flesus* and *Sepia officinalis*).

Table 6.2 Sizes (cm), $\delta^{13}C$ and $\delta^{15}N$ values in the muscles of harbour porpoises (*Phocoena phocoena*) stranded along the southern North Sea and the Bay of Biscay and prey species collected along the North Sea. n: number of individuals; Data are presented as mean ± standard deviation; (minimum; maximum); na: not applicable.

		Size (cm)		$\delta^{13}C$ (‰)		$\delta^{15}N$ (‰)	
Species	n	Mean ± SD	(min ; max)	Mean ± SD	(min ; max)	Mean ± SD	(min ; max)
Harbour porpoise (Southern North Sea)	52	120 ± 18	(92; 161)	-17.3 ± 0.5	(-18.5; -16.3)	15.8 ± 1	(13.5; 18.4)
Juvenile females	15	115 ± 10	(98; 133)	-17.4 ± 0.5	(-18.2; -16.8)	16.1 ± 1.0	(14.1; 18.4)
Juvenile males	27	110 ± 7	(92; 120)	-17.3 ± 0.5	(-18.3; -16.3)	15.9 ± 1.1	(13.8; 18.0)
Adult females	7	156 ± 6	(145; 161)	-17.5 ± 0.5	(-18.5; -17.0)	14.7 ± 1.1	(13.5; 16.3)
Adult males	3	146 ± 4	(143; 150)	-17.3 ± 0.5	(-17.6; -16.9)	15.4 ± 0.6	(14.7; 15.9)
Harbour porpoise (Bay of Biscay)	34	140 ± 22	(105; 179)	-17.1 ± 0.6	(-18.1; -15.7)	13.1 ± 0.9	(11.7; 15.2)
Juvenile females	9	119 ± 8	(105; 128)	-17.2 ± 0.8	(-18.1; -15.7)	13.3 ± 1.2	(12.2; 14.7)
Juvenile males	5	118 ± 13	(95; 129)	-17.4 ± 0.3	(-17.7; -17.0)	12.9 ± 0.5	(12.2; 13.5)
Adult females	6	165 ± 12	(150; 179)	-16.7 ± 0.3	(-17.1; -16.3)	13.1 ± 0.6	(12.5; 14.1)
Adult males	10	158 ± 10	(143; 174)	-17.1 ± 0.5	(-17.9; -16.2)	13.1 ± 1.0	(12.1; 14.9)
PREY							
Demersal and benthic							
Merlangius merlangus	10	23 ± 0.7	(22; 24.2)	-16.9 ± 0.3	(-17.3;-16.3)	16.3 ± 0.4	(15.6;17.0)
Trisopterus luscus	9	12 ± 6.7	(4.7; 19.3)	-17.8 ± 0.6	(-18.7;-16.7)	14.5 ± 1.1	(13.7;17.0)
Gobiidae sp.	5	6.2 ± 0.6	(5.6; 7.3)	-18.2 ± 1.4	(-20.3;-16.9)	15.5 ± 1.6	(14.1;17.9)
Echiichtys vipera	10	13.7 ± 0.8	(12.5; 15.3)	-16.6 ± 0.4	(-17.2;-16.0)	14.8 ± 0.2	(14.5;15.1)
Limanda limanda	10	18.5 ± 4.4	(13.4; 23.5)	-16.8 ± 0.5	(-18;-16.3)	13.1 ± 0.8	(12.3;14.4)
Platichthys flesus	10	20 ± 6.6	(13.2; 27.5)	-17.5 ± 2	(-21.5;-14.7)	13.6 ± 0.9	(12.4;14.9)
Pleuronectes platessa	9	19.5 ± 6.6	(13; 33.5)	-16.2 ± 0.6	(-17.2;-15.3)	13.0 ± 0.7	(11.9;14.1)
Solea solea	10	20.9 ± 1	(19.3; 22.2)	-16.9 ± 0.9	(-18.2;-15.7)	13.8 ± 0.7	(12.3;14.6)
Sepia officinalis	5	na	na	-17.3 ± 0.8	(-18.4;-16.4)	14.3 ± 0.7	(13.1;15.0)
Pelagic							
Hyperoplus lanceolatus	5	24.3 ± 5.5	(20.3; 32.6)	-17.7 ± 0.6	(-18.4;-16.9)	13.5 ± 0.7	(13.1;14.6)
Clupea harengus	5	29 ± 1	(27.8; 30)	-18.5 ± 0.5	(-19.0;-17.6)	10.8 ± 0.7	(9.9;11.4)
Sprattus sprattus	10	7.5 ± 1.3	(6.2; 9.5)	-17.7 ± 0.7	(-18.3;-16.2)	13.5 ± 0.7	(12.4;14.5)
Sardina pilchardus	5	22.8 ± 0.9	(22; 24)	- 18.5 ± 0.4	(-19.0;-18.0)	12.4 ± 0.7	(11.4;13.2)
Crangon crangon	10	na	na	-15.2 ± 0.9	(-17.2;-14.3)	13.4 ± 0.8	(11.8;14.7)

The mean value of $\delta^{15}N$ in the muscle of harbour porpoises stranded along the Bay of Biscay was 13.1 ± 0.9‰ and the mean value of $\delta^{13}C$ was -17.1 ± 0.6‰. Between age-classes, $\delta^{15}N$ and $\delta^{13}C$ values in muscles showed no significant differences (ANOVA, $p > 0.05$). Prey species from the Bay of Biscay included in the biplot (Figure 6.2b) were extracted from a previous study where more than 142 species were sampled from the continental shelf to the shelf-edge along the French part of the Bay of Biscay in the autumns of 2001-2010 (Chouvelon et al., 2012). Unlike prey species of the North Sea, all pelagic prey from the Bay of Biscay did not show lower values of $\delta^{15}N$ and $\delta^{13}C$ compared to harbour porpoises values (figure 6.2b). The same trend was observed for the demersal, benthic and coastal prey.

2.3. Lipid composition

About 40 fatty acids were present in relative amounts > 0.1% in the blubber of harbour porpoises. Large individual differences between porpoises were remarkable due to the large standard deviations (SDs). The FA profiles of porpoises stranded along the southern North Sea and those stranded along the Bay of Biscay did not show significant differences (ANOSIM, $p > 0.05$). Harbour porpoises stranded along the southern North Sea analyzed for fatty acids included 46 juveniles, 10 adult females, 2 adult males and 1 individual whose gender could not be identified. The body masses ranged between 14 and 50 Kg (mean 26 ± 11 Kg) and body length ranged between 92 and 172 cm (mean 121 ± 20 cm). The differences were not significant comparing FA profiles of the groups with different gender and maturity status (ANOSIM, $p > 0.05$). Harbour porpoises stranded along the Bay of Biscay analyzed for fatty acids included 14 juveniles, 5 adult females, 10 adult males and 3 individuals whose gender could not be identified. Unfortunately body masses data were not available and body length ranged between 110 and 179 cm (mean 139 ± 22 cm). FA profiles of the groups with different gender and maturity status were not significantly different (ANOSIM, $p > 0.05$). Muscles of prey species collected along the North Sea had also about 40 FAs present in relative amounts > 0.1 %. The great sandeel (*Hyperoplus lanceolatus*) exhibited the highest amounts of polyunsaturated fatty acids (PUFAs) with 55.32 ± 5.07 %, while the herring exhibited the lowest amounts 24.18 ± 12.55 %. For the monounsaturated fatty acids (MUFAs), the herring exhibited the highest amounts (51.16 ± 12.80 %), whereas the great sandeel exhibited the lowest amounts of MUFAs (11.78 ± 2.88 %).

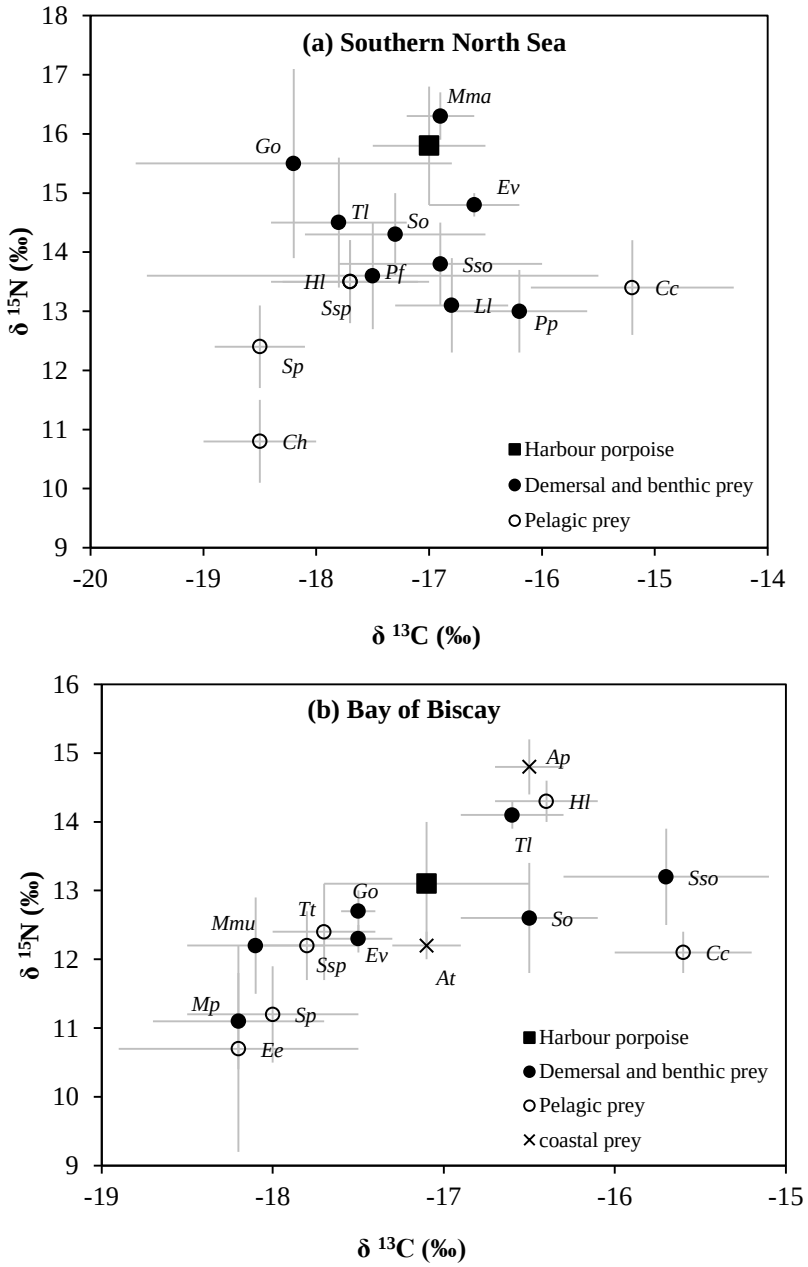

Figure 6.2 Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in muscles of harbour porpoises and potential prey species in (a) the southern North Sea and (b) the Bay of Biscay. Values for prey from the Bay of Biscay were extracted from Chouvelon et al. (2012). *Ammodytes tobianus* (At), *Atherina presbyter* (Ap), *Clupea harengus* (Ch), *Crangon crangon* (Cc), *Echiichthys vipera* (Ev), *Engraulis encrasicolus* (Ee), *Gobiidae sp.* (Go), *Hyperoplus lanceolatus* (Hl), *Limanda limanda* (Ll), *Merlangius merlangus* (Mma), *Merluccius merluccius* (Mmu), *Micromesistius poutassou* (Mp), *Platychtys flesus* (Pf), *Pleuronectes platessa* (Pp), *Sardina pilchardus* (Sp), *Sepia officinalis* (So), *Solea solea* (Sso), *Sprattus Sprattus* (Ssp), *Trachurus trachurus* (Tt) *and Trisopterus luscus* (Tl).

Some FAs that arise directly from the diet and could not be synthesized *de novo* by the consumer along with ΣSFA (saturated FAs), Σ Branched, ΣMUFA (Monounsaturated FAs) and ΣPUFA (Polyunsaturated FAs) are presented in table 3. To obtain combined information from all FAs, data were presented as non-metric MDS for the specific FAs (Figures not shown). Each FA was presented in the blubber of porpoises from the southern North Sea and the Bay of Biscay along with the FA proportions in the muscle of prey species. Elevated levels of 20:1ω9 and 22:1ω11/9 in muscles of herring reflected the consistent diet of this species (amounts >13 % and 22 % for 20:1ω9 and 22:1ω11/9, respectively in herring muscles). Therefore, *C. harengus* was excluded from the non-metric MDS in order to better represent prey species. In addition, non-metric MDS of 20:1ω9 and 22:1ω11/9 in the blubber of some porpoises from the southern North Sea indicated a diet relying on zooplanktonivorous fish such as herring. However, FAs such as 16:1ω7, 18:1ω9, 20:5ω3 and 22:6ω3 were also present in high levels in the muscle of some prey species and blubber of harbour porpoises (Table 6.3).

3. Discussion

The application of several techniques to analyze the diet of harbour porpoises has allowed a better and general insight into the feeding ecology of this species. This method has permitted a more complete understanding of the diet by integrating different techniques with different dietary information (days to months). In the present study, the stomach content analysis was the only quantitative method. Therefore, stable isotopes and FAs analyses provided complementary information for the stomach content analysis. Combining techniques that integrate diet over days and weeks allowed gaining more complete understanding (Karnovsky et al., 2008) of harbour porpoise's diet relative to stomach contents.

Table 6.3 Relative amounts (% of total) of some FAs that arise directly from diet in the blubber of harbour porpoises and muscle of prey species. n: number of individuals analyzed; "-" amount < 1%; SFA: Saturated FAs; MUFA: Monounsaturated FAs; PUFA: Polyunsaturated FAs.

	Harbour porpoises (southern North Sea)	Harbour porpoises (Bay of Biscay)	European flounder *Platichtys flesus*	Gobies *Gobiidae sp.*	Atlantic herring *Clupea harengus*	Common dab *Limanda limanda*	Sandeel *Hyperoplus lanceolatus*	Whiting *Merlangius merlangus*	European plaice *Pleuronectes platessa*
Fatty acids	n=59	n=32	n=10	n=4	n=5	n=10	n=5	n=10	n=10
16:1w7	18.5 ± 7.3	21.18±7.47	3.1 ± 1.6	3.5 ± 0.2	4.2 ± 1.3	4.3 ± 1.7	2.8 ± 0.3	2.4 ± 0.7	3.2 ± 0.5
18:1w9	20.03 ± 2.51	22.39±2.48	6.81 ± 3.36	7.46 ± 0.74	6.45 ± 0.92	6.83 ± 2.44	5.26 ± 1.62	6.87 ± 0.46	6.05 ± 0.65
18:2w6	1.64 ± 0.46	1.64±0.3	0.37 ± 0.26	-	1.34 ± 0.11	0.23 ± 0.09	1.28 ± 0.29	0.34 ± 0.05	0.39 ± 0.10
20:1w9	2.79 ± 2.40	1.66±0.78	0.69 ± 0.28	0.23 ± 0.04	13.14 ± 3.34	0.62 ± 0.34	0.13 ± 0.07	0.41 ± 0.08	0.80 ± 0.25
20:4w6	0.61 ± 0.36	0.61±0.31	4.53 ± 1.13	3.41 ± 0.53	0.53 ± 0.28	4.61 ± 1.27	2.29 ± 0.53	3.73 ± 0.64	5.15 ± 1.98
20:5w3	3.44 ± 2.15	2.88±1.77	21.19 ± 5.11	14.66 ± 1.62	7.24 ± 2.83	17.53 ± 2.46	14.72 ± 1.12	15.47 ± 1.51	22.77 ± 3.46
22:1w11/9	3.08 ± 3.15	1.16±0.61	0.21 ± 0.36	0.26 ± 0.11	22.70 ± 5.81	-	-	-	0.12 ± 0.07
22:6w3	8.32 ± 5.40	7.71±5.44	16.88 ± 4.47	25.65 ± 1.40	11.32 ± 7.87	17.66 ± 4.44	32.02 ± 1.65	29.47 ± 6.56	17.10 ± 3.10
∑ SFA	20.44 ± 4.93	20.4±4.9	28.17 ± 2.74	27.17 ± 1.39	24.02 ± 4.62	29.81 ± 4.13	32.27 ± 3.23	26.04 ± 1.66	28.13 ± 2.91
∑ Branched	4.14 ± 2.87	4.1±2.9	1.93 ± 1.20	1.94 ± 0.74	0.65 ± 0.17	1.71 ± 0.95	0.63 ± 0.18	2.14 ± 0.88	1.40 ± 0.46
∑ MUFA	53.78 ± 21.43	53.8±24.1	17.81 ± 8.71	19.23 ± 2.45	51.16 ± 12.80	18.33 ± 7.70	11.78 ± 2.88	16.83 ± 3.06	17.42 ± 3.22
∑ PUFA	21.64 ± 12.73	21.6±12.7	52.09 ± 14.87	51.66 ± 4.88	24.18 ± 12.55	50.14 ± 12.30	55.32 ± 5.07	54.99 ± 10.83	53.05 ± 11.06

	European pilchard *Sardina pilchardus*	Common sole *Solea solea*	Sprat *Sprattus sprattus*	Pouting *Trisopterus luscus*	Lesser weever *Echiichthys vipera*	Sand smelt *Atherina presbyter*	Thinlip grey mullet *Liza ramada*	Common shrimp *Crangon crangon*	Common cuttlefish *Sepia officinalis*
Fatty acids	n=5	n=10	n=9	n=5	n=10	n=5	n=5	n=9	n=5
16:1w7	3.2 ± 1.4	5.7 ± 1.6	3.7 ± 1.2	2.8 ± 0.4	4.9 ± 1.5	4.2 ± 1.3	12.1 ± 2.4	5.1 ± 1.2	1.1 ± 0.2
18:1w9	14.49 ± 5.54	8.79 ± 0.84	5.90 ± 1.74	6.71 ± 1.25	9.86 ± 2.27	3.76 ± 0.25	4.50 ± 0.72	9.99 ± 0.93	3.39 ± 0.56
18:2w6	0.57 ± 0.15	0.37 ± 0.09	0.32 ± 0.25	-	0.42 ± 0.05	-	0.37 ± 0.28	0.86 ± 0.26	0.92 ± 0.23
20:1w9	2.51 ± 1.10	0.32 ± 0.07	0.23 ± 0.12	0.30 ± 0.05	0.39 ± 0.05	0.46 ± 0.12	0.48 ± 0.10	0.41 ± 0.23	4.11 ± 0.67
20:4w6	1.11 ± 0.31	3.69 ± 1.14	1.85 ± 0.33	4.03 ± 0.59	3.21 ± 1.05	3.24 ± 0.46	1.20 ± 0.39	3.01 ± 0.95	1.34 ± 0.20
20:5w3	8.23 ± 2.25	7.24 ± 0.96	13.03 ± 1.09	18.32 ± 1.10	9.51 ± 0.98	13.67 ± 2.71	19.14 ± 1.80	22.58 ± 1.52	18.77 ± 0.64
22:1w11/9	0.40 ± 0.20	0.19 ± 0.09	0.11 ± 0.10	-	0.10 ± 0.07	-	-	-	-
22:6w3	20.57 ± 8.57	21.26 ± 2.88	32.77 ± 3.67	26.53 ± 3.86	21.58 ± 12.08	20.61 ± 4.78	8.39 ± 2.48	14.38 ± 1.96	27.18 ± 1.41
∑ SFA	35.51 ± 9.36	29.64 ± 2.56	29.99 ± 4.99	26.93 ± 2.00	27.46 ± 3.48	32.87 ± 1.86	33.53 ± 3.30	26.02 ± 2.37	35.37 ± 2.67
∑ Branched	4.05 ± 7.83	2.48 ± 0.86	1.58 ± 0.61	1.71 ± 0.51	2.89 ± 2.04	0.32 ± 0.10	0.24 ± 0.09	2.30 ± 0.94	0.85 ± 0.12
∑ MUFA	25.27 ± 10.49	22.98 ± 5.13	16.56 ± 4.86	16.87 ± 3.52	27.00 ± 11.17	20.47 ± 4.10	25.20 ± 5.33	26.02 ± 4.66	13.03 ± 2.08
∑ PUFA	35.17 ± 13.11	44.90 ± 7.92	51.88 ± 6.90	54.49 ± 6.67	42.64 ± 17.79	46.34 ± 9.74	41.02 ± 7.93	45.67 ± 6.18	50.74 ± 2.90

3.1. Diet composition

Harbour porpoises stranded along the southern North Sea showed a generalized feeding strategy with the prevalence of demersal, benthic and coastal fish in their diet. The ingested biomass (%W) was primarily dominated by 4 prey species: gobies, whiting, lesser sandeel (*A. marinus*) and sprat. These species are probably among the most abundant fish species in the North Sea (Daan et al., 1990). Relatively high mean $\delta^{15}N$ signatures of juveniles compared to adult porpoises suggest that adults are preying on more offshore species with low $\delta^{15}N$ signatures such as herring (Das et al., 2003b). Indeed the biplot revealed that herring had the lowest signature of $\delta^{15}N$ and $\delta^{13}C$ followed by sprat. It has been suggested that juveniles could be prevented by their small size and cannot dive as deep as adults; moreover adults are able to eat a bigger variety of preys with bigger sizes (reviewed in Santos and Pierce, 2003). Porpoises from the Bay of Biscay showed similar generalized feeding strategy with the prevalence of demersal, benthic and pelagic fish in their diet. The ingested biomass was primarily dominated by European hake, scads and sardine. Juveniles and adults from the Bay of Biscay showed no significant differences in $\delta^{15}N$ and $\delta^{13}C$ signatures.

Relatively large standard deviations in FA signatures indicate differences between individual porpoises. Variations may likely be due to differences in the diet of animals regardless the gender and the maturity status. Elevated levels of 20:1ω9 and 22:1ω11/9 in the blubber of harbour porpoises from the southern North Sea reflected that some individuals may be feeding on zooplanktonivorous fish such as herring (Tocher, 2003). Other FAs such as 16:1ω7 present in high levels in the blubber of porpoises from both regions indicate that porpoises are feeding on herbivorous prey species. The FA 20:4ω6 marker of benthic littoral algae (Dalsgaard et al., 2003), is present in elevated amounts in muscle of prey benthic species such as gobies, whiting and pouting. However, these amounts could not be found in porpoises blubber, although according to stomach content analysis these 3 species contributed to the diet of porpoises. Gobies, whiting and pouting does not seem to contribute to the diet according to the FAs analysis. Therefore both methods appear to converge in a way. Gobies, whiting and pouting are benthic species, their presence in stomachs of porpoises may be explained by the last meal consumed before stranding (inshore) whereas the FAs signatures reflect the diet of porpoises over a period of up to several months (Budge et al., 2006).

In terms of habitats and diet composition, harbour porpoises appear to have large variations over relatively small spatial scales (Bjørge, 2003). Mean $\delta^{15}N$ signatures obtained in the muscle of porpoises from the present study are comparable to signatures in porpoises from almost same areas (Das et al., 2003b; Jansen et al., 2012; Chouvelon et al., 2012). The present study highlighted some differences in feeding ecology of harbour porpoises from the southern North Sea compared to the porpoises stranded along the Bay of Biscay. Indeed, harbour porpoises from the southern North Sea had significantly higher $\delta^{15}N$ signatures in muscle compared to porpoises from the Bay of Biscay. Moreover, prey species collected in the North Sea had also higher $\delta^{15}N$ signatures compared to prey species from the Bay of Biscay (data from Chouvelon et al., 2012). A previous study showed that spatial comparisons of trophic position are easily biased if fine-scale information on base $\delta^{15}N$ is not available especially in coastal areas (Jennings and Warr, 2003). Therefore, the difference in $\delta^{15}N$ signatures could be attributed to differences in $\delta^{15}N$ at the base of food chain (Jennings and Warr, 2003) rather than apparent differences in trophic position. Moreover, when comparing differences in consumer $\delta^{13}C$ from different regions, it is difficult to know if the differences reflect source changes or baseline variation (Barnes et al., 2009).

3.2. Comparison with previous studies

The diet of porpoises stranded in the same study areas (North Sea and the Bay of Biscay) or adjacent areas (Dutch coast, United Kingdom, Germany and English Channel) inferred from stomach content analysis are presented in table 6.4 for comparison purpose. Overall, the diet arising from stomach content analysis for porpoises in the North East Atlantic mainly comprises gobies, whiting, sandeel, sprat, trisopterus sp. and herring since earlier 90s (Martin, 1996, Santos et al., 2004; De Pierrepont et al., 2005; Leopold and Camphuysen, 2006; Haelters et al., 2012; present study). Bjørge, (2003) suggested a shift in the diet of porpoises from pelagic prey species in the deeper northern waters (Barents Sea) to more benthic prey species in the relatively shallow North Sea and Skagerrak waters (Based on data from Aarefjord et al., 1995). Hence, in the relatively shallow southern North Sea, porpoises are expected to feed on benthic prey species. Indeed in the present study, porpoises fed on demersal and benthic species such as whiting and gobies along with coastal fish such as the lesser sandeel. Pelagic fish species were less encountered in the diet of porpoises from the southern North Sea according to the stomach content analysis. All these studies confirm

that the diet of harbour porpoises varies between areas and probably between years and seasons and maturity status (Bjørge, 2003; Santos et al., 2004).

Table 6.4 Some studies on harbour porpoise's diet inferred from stomach content analysis in same and adjacent areas. n: number of stomachs analyzed.

Area (year of stranding)	n	Main prey	Reference
Southern North Sea (2010-2013)	14	Gobies, whiting, sandeel	Present study
Belgian coast (1997-2011)	64	Gobies, sandeels, whiting, trisopterus sp.	Haelters et al. 2012
Dutch coast (2006)	64	Gobies, sandeels, sprat, herring, whiting, twait shad	Leopold and Camphuysen 2006
English Channel (1998-2003)	7	Pouting, gobies	De pierrepont et al. 2005
Scotland (1992-2003)	188	Whiting, sandeels, gadids, trisopterus sp.	Santos et al., 2004
United Kingdom (1989-1994)	100	Gadids, sandeels, gobies	Martin, 1996
Germany	34	Sandeels, sole	Benke and Siebert 1996
Denmark, Sweden, Norway	197	Herring, gadids	Aerefjord et al. 1995
Germany	36	Sole, cod	Lick 1991
France	8	Blue whiting, scad, hake	Desportes 1985
Scotland (1959-1971)	93	herring, sprat, whiting	Rae 1965, 1973
Bay of Biscay (2009-2012)	13	European hake, scads, sardine	Present study
Bay of Biscay (1988-2003)	26	Blue whiting, sardine, scads, whiting	Spitz et al. 2006

In the Bay of Biscay, only one quantitative study of the diet of porpoises was available (Spitz et al., 2006). Harbour porpoises stranded between 1988 and 2003 along the Bay of Biscay fed mostly on blue whiting, sardine, scads and whiting (Spitz et al., 2006). The blue whiting was the most frequent prey, whereas in the present study it was almost absent in the diet of porpoises (2009-2012) with only 4 otoliths found in the stomachs of 2 individuals analyzed. According to the Evohe (EValuation des resources Halieutiques de l'Ouest Européen) database, the relative abundance of the Blue Whiting in the Bay of Biscay (ICES Div.VIII) between 1988 and 2003 was almost stable, whereas between 2009 and 2011 this species has witnessed a sticking decline (SIH-Ifremer, 2014). Accordingly, the decrease of the blue whiting in the diet of porpoises from the Bay of Biscay may be a response to the decline of the relative abundance of this species the past few years. The European hake which was absent in the diet of porpoises (Spitz et al., 2006) and had lower relative abundance before 2011 (SIH-Ifremer, 2014) was found to contribute to the diet of porpoises in the present study. Hence, the abundance of prey species in the environment is reflected in the diet of harbour porpoises.

Differences in the diet of porpoises according to the geographical variations were reported (reviewed by Santos and Pierce, 2003). They may be related, among others, to the

prey availability and to the habitat characteristic such as the topography of the environment inhabited by porpoises. Indeed some prey species such as scads, blue whiting and sardine are almost absent or found in lower numbers in the southern North Sea compared to the Bay of Biscay (Trenkel et al., 2009; Ifremer, 2014). Moreover, differences in the topography between both regions have induced differences in the feeding habits of porpoises. Several authors suggested that the distribution and habitat of cetaceans may be defined by the topography such as the depth and the slope in the region (Hooker et al., 2001; Macleod et al., 2004). In the southern North Sea with a relatively shallow continental shelf, harbour porpoises fed mainly on demersal, benthic and coastal fish, whereas in the Bay of Biscay with deeper continental shelf porpoises fed mainly on demersal, benthic and pelagic fish. This finding is in agreement with a previous study who suggested that in the deeper waters porpoises are expected to feed on pelagic fish species (Bjørge, 2003).

Conclusion

The combined stomach contents, stable isotopes and fatty acids analyses have proven to be particularly helpful toward a better and general insight into the feeding ecology of the harbour porpoise. This species feeds on a variety of demersal, benthic and coastal prey in the southern North Sea and a variety of demersal, benthic and pelagic prey in the Bay of Biscay. The differences in the diet between two areas that differ in prey species diversity, bottom type and physical oceanography might be related to the different prey species availability along with the different topography such as the depth and the slope in the region. Even if harbour porpoise is known to be mainly coastal in its distribution, information on diet and habitat use may not be extrapolated from one area to another. In addition, changes in diet compositions of the harbour porpoise might be in response to ecological changes that are taking place due to the temperature rise and the climate change.

Acknowledgements

Harbour porpoise samples used in this study were collected by the French Stranding Network. Special thanks to the "Centre de Recherche sur les Mammifères Marins" (CRMM) and "Observatoire pour la Conservation et l'Etude des Animaux et Milieux Marins" (OCEAMM). Thierry Jauniaux is acknowledged for executing post-mortem examinations and providing samples and information on the animals from the southern North Sea. Thanks to

Willy Dabin for providing porpoises samples from the Bay of Biscay and to Najet Thiney from the MNHN for the help in FAs analyses. Mahfouz Celine is financially supported by a PhD fellowship from the National Council for Scientific Research (Lebanon) and Université du Littoral Côte d'Opale (France).

CHAPTER 7

GENERAL DISCUSSION

Chapter 7 – General discussion

Contamination status of harbour porpoises in the southern North Sea

Harbour porpoises inhabit coastal waters in areas that are known to be point sources of contaminants and can frequently be observed feeding close inshore, sometimes even in the shallow waters of the surf zone (Bjørge, 2003; Haelters and Camphuysen, 2009). Moreover this species feeds at high trophic level and has small body size. Overall, these three factors combine synergistically to place harbour porpoises in an ecological situation where it is highly exposed to environmental contaminants (Aguilar and Borrell, 1995). Measurable quantities of contaminants have been detected in the tissues of harbour porpoises since the late 1960s. Contaminants enter the body of marine mammals mostly through food and some of them are accumulative and increase progressively with the animal age.

Non essential metals such as Hg, Cd and Pb are potentially toxic even at low concentrations. Numerous physiological and ecological factors might affect heavy metal contamination such as geographic location, diet, age, sex, the tissues considered and metabolic rates (Das et al., 2003a). Indeed, concentration levels were different in livers and kidneys tissues. Some metals such as Hg tend to accumulate in livers (Bennett et al., 2001), whereas others such as Cd has affinity to kidneys (Paludan-Müller et al., 1993) (chapter 3). Moreover, according to the maturity status some elements such as Cd, Cr, Hg, Se and V showed higher concentrations in livers of adult harbour porpoises compared to juveniles. Same trends were found in different marine mammals such as beluga whales (*Delphinapterus leucas*), ringed seals (*Phoca hispada*), Caspian seals (*Phoca caspica*) and California sea lions (*Zalophus californianus*) (Mackey et al., 1996; Anan et al., 2002; Harper et al., 2007). In addition, the health status appeared to influence concentration levels in marine mammals. In the present study, levels of Hg, Se, Zn, Cd and V appeared to be higher in porpoises that died from infectious diseases compared to healthy porpoises that died from physical trauma (chapter 3). Same trends were found in the harbour porpoises from England and Whales (Bennett et al., 2001). In the present study, only three adult animals from the southern North Sea exhibited higher hepatic Hg concentrations than the threshold level estimated by (AMAP, 1998) (200 $\mu g.g^{-1}$ dw) above which liver damage in mammals is expected. Between 2006 and 2013, contaminant levels in harbour porpoises tissues stranded along the southern North Sea did not show significant variations (Figure 7.1). Almost same elements were determined in

tissues of porpoises stranded in the same area between 1994 and 2001 (Das et al., 2004b). The comparison of metallic levels showed that concentrations are in the same order of magnitude; therefore the population status of harbour porpoises in terms of metallic concentration has been stable from 1994 to 2013.

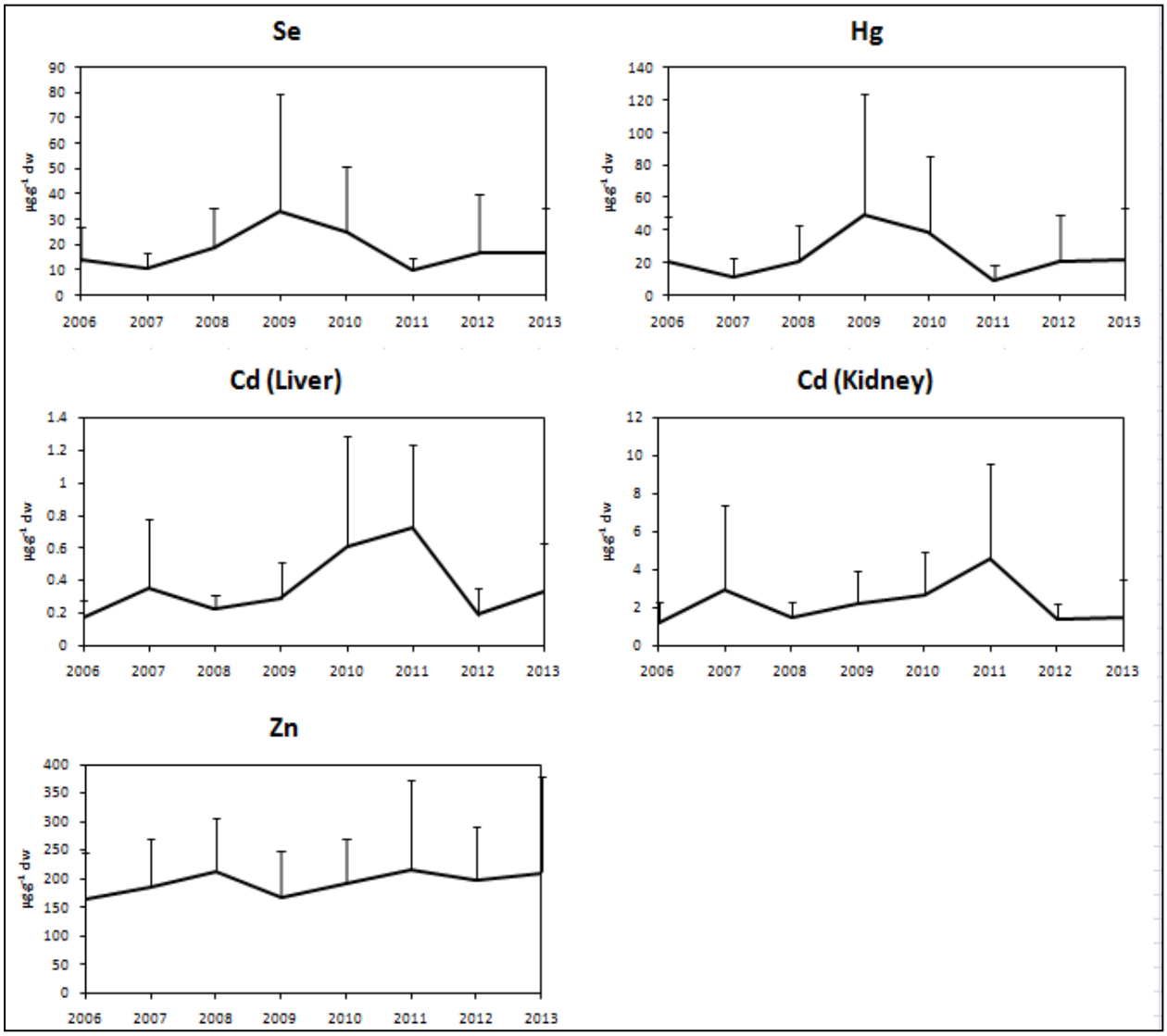

Figure 7.1 Temporal trend of some metal concentrations (Mean and SD µg.g^{-1} dw) in tissues of harbour porpoises stranded along the southern North Sea between 2006 and 2013.

Alongside, the monitoring network of chemical contamination (ROCCH) conducted by Ifremer in the North of France (Pas-de-Calais and Somme), showed that the three regulated metals Cd, Hg and Pb determined in mussels exhibited values far below regulatory limits over the period 2009-2013. The trend for the Cd concentrations suggests a slow and steady decline, whereas for the Hg and Pb concentrations show some variability but stay far below regulatory limits (Blondel et al., 2014). Moreover, the recent study of (Gao et al., 2013) showed that there is a general decrease of chemical pollution in the southern North Sea

(Belgian coastal zone and Scheldt River and Estuary). These authors related the chemical pollution decrease to a decrease in atmospheric and aquatic emissions related to industrial, transportation and domestic activities.

Several biological factors such as sex, age, trophic level, health status and metabolic capacity to degrade/metabolize toxic contaminants may influence the retention of organic pollutants in cetaceans (Aguilar et al., 1999; Weijs et al., 2009 a; b). The concentration of lipophilic pollutants usually increases with the age in males since inputs exceed the ability of the organism to excrete pollutants (Aguilar and Borrell, 1994; Kleivane et al., 1995; Weijs et al., 2009b; 2010b). Unfortunately such accumulation with age could not be verified for our study due to the fact that we were not able to analyze more than one adult male harbour porpoise. In addition, adult females have decreasing levels of organic contaminants explained by the transfer of organochlorines to their offspring during gestation and lactation (Westgate et al., 1997; Jepson et al., 1999). Indeed, the results of the present work showed that juveniles had higher PCB and DDX levels compared to adult females (chapter 4). Moreover, the health status may be responsible for the variety of pollutant concentrations. In the present work, porpoises that died from infectious disease exhibited higher PCB levels compared to those that died from physical trauma (chapter 4) with same trends found in previous studies (Jepson et al., 1999; 2005; Pierce et al., 2008). In addition, 60% of the animals analyzed exceeded the threshold (17 $\mu g.g^{-1}$ lipids) concentration for total PCBs determined in livers for adverse health effects in marine mammals (Kannan et al., 2000). In contrast, DDX levels were comparable in porpoises that died from infectious disease and those that died from physical trauma. No such threshold has been proposed for DDX levels in marine mammals. A recent study has reviewed recent trends for organic contaminant concentrations in marine mammal tissues worldwide reported in the literature from 2008 to date (Law, 2014). This study suggested that temporal trends are downwards for many compound groups such as some perfluorinated compounds, organochlorine pesticides and butylins. In contrast, PCB concentrations have witnessed earlier reductions mostly due to regulation of use (since 1980s) but this trend seems to reach a plateau in many locations (Law, 2014). For instance, in UK porpoises stranded between 1991 and 1998 downward trends of CB concentrations were reported but reached a plateau thereafter until 2009 (Law et al., 2012). It has been suggested that further reductions are likely to take decades. Thus, even if the status of marine pollution has been improved, a continuous long-term contamination by toxic organochlorines over many generations may be observed (Tanabe et al., 1994). For classic contaminants such as

PCBs, DDX and PBDEs the study of time trends is important because it allows assessing the effectiveness of earlier legislation, whereas for emerging contaminants such as novel brominated flame retardants (BFRs) and phosphorous flame retardants (PFRs), it allows assessing their significance and priorities for future studies to be defined (Law, 2014). The DDTs present in North Atlantic marine ecosystems comprise mostly old DDT, which is confirmed by the high percentage of metabolized forms such as DDE (Aguilar and Borrell, 1995). Indeed, in the present study *p,p'*-DDE and *p,p'*-DDD had the largest contribution to the sum of DDX (more than 80%) which is in agreement with previous studies (Duinker et al., 1989; Tanabe et al., 1997; Berggren et al., 1999; Weijs et al., 2010b). Marine mammals have induced levels of cytochrome P450-1A and 2B that are capable of metabolizing *p,p'*-DDT (Boon et al., 1997). Thus, relatively high concentrations of DDT metabolites (*p,p'*-DDE and DDD) in marine mammal tissues were related to the higher metabolism of DDT in marine mammals along with the bioaccumulation of DDT metabolites through their life span (Hoekstra et al., 2003).

The present study contributes towards a continuous assessment of the pollutant load in harbour porpoises. As marine top predators, marine mammals act as sentinel species for both oceans and human health. Many species share the coastal environment with human and consume the same food and therefore they provide one approach to evaluating aquatic ecosystem health (Bossart, 2011). When comparing levels of pollutants, substantial variations in tissue levels among individuals of different sex, age, reproductive status or nutritive condition might exist. Nevertheless, comparing results from several surveys may cause undesired heterogeneity due to differences in sampling and analytical techniques used and differences in the biological characteristics of the individuals sampled (Aguilar et al., 2002). The exposure of organism to pollutants might be monitored through the concentration of the targeted pollutant in selected tissues of the organism (Aguilar et al., 1999). Marine mammals are protected species therefore the information about their contaminant loads are almost only available from tissues such as livers, kidney, muscle, blubber of post-mortem examinated animals. However in order to evaluate the current exposure situation on a wild marine population, samples collected from living animals are required (Griesel et al., 2008). To this end, concentrations of metallic elements were evaluated in whole blood samples of free ranging harbour seals (*Phoca vitulina*). The results showed that concentrations of some metals such as As, Cr, Se and V were higher than human levels (Griesel et al., 2008). Hence, seal blood appeared to be useful to monitor the influence of different conditions or

contaminations in a specific area on marine mammals. In order to evaluate the use of biopsy samples as non-destructive tool for assessing trace element concentrations in marine mammals, more recent studies have attempted to predict liver concentrations in free ranging animals by examining concentrations of trace elements in skin, blubber, liver and kidney of stranded animals (Stavros et al., 2011; Aubail et al., 2013). The results of both studies showed that this method can only be used to investigate Hg bioaccumulation in internal tissues of cetaceans. Overall, it should be recognized that it is important to keep monitoring levels of contaminants in abundant yet still vulnerable marine mammal species. Hence, the overall threats to marine mammals worldwide form chemical and organic pollutants which were introduced into the marine environment by human activities should be assessed.

The results of the present study showed that most of the animals analyzed exhibited relatively low levels of contaminants in their organs and that temporal trend are constant or downwards depending on the contaminant analyzed. Moreover, the samples analyzed in this study mostly included juveniles; hence we comprise a recent picture of the level of contamination and its evolution. However, when analyzing adult animals the picture is not clear whether animals are exposed to a recent or old input of contaminant in the environment mainly due to the bioaccumulation process of some contaminants with age.

Changes in the distribution of harbour porpoises in the North Sea

A comparison between the results of the two major abundance and distribution surveys (SCANS and SCANS II) in the North Sea clearly highlights a major shift in the distribution of harbour porpoises from the northern parts of the North Sea to its eastern parts rather than a population increase (c.f. chapter 1, figure 1.4). In SCANS (1994), the abundance was estimated to be 340 000 animals (Hammond et al., 2002) and in SCANS II the abundance was estimated to be 375 000 animals (Hammond et al., 2013). Therefore, the abundance estimates did not show any significant temporal changes in the overall population sizes. In order to explain this movement, several authors suggested that this shift may be related to the distribution and abundance of prey species (Haelters and Camphuysen, 2009; Hammond et al., 2013). Several studies showed a relation between the diet changes of marine mammals and the changes in prey abundance (Bowen and Harrison, 1996; Santos and Pierce, 2003; Macleod et al., 2004 ;2007). For instance, the variation in the composition of harbour seal (*Phoca vitulina*) diet in two areas, which differ in prey species diversity, bottom type and

physical oceanography, was associated with broad-scale changes in prey abundance (Bowen and Harrison, 1996). Shifts in prey distribution and abundance are the most likely factor governing the distribution and abundance of the mink whale inhabiting the isle of Mull off the west coast of Scotland (Macleod et al., 2004). A shift in the diet of porpoises has been witnessed after the collapse of the herring stock in the North Sea in the mid of the 1960s and therefore, the porpoises have switched their diet to the predation on sandeel and gadoid fish (Santos and Pierce, 2003).

Harbour porpoises have a tight energy budget due to their small size, their limited body fat and energy storage capacity. Hence, they must feed at a high daily rate without prolonged periods of fasting to maintain energy requirements (Koopman et al., 2002). It has been suggested that migration related to movements of large quantities of fish exists (Reijnders, 1992). Demonstrating effects of prey distributions on harbour porpoise's diet is difficult as it is not subject to experimentation. To assess whether prey changes and distributions are related to changes in harbour porpoise's distribution, we have tried to link the total abundance of potential prey species in the North Sea to the distribution and consequently to the shift of porpoises from the northern areas of the North Sea (ICES Div. IVb) to its southern areas (ICES Div. IVc). We have demonstrated that the diet of porpoises along the North Sea since earlier 90s till nowadays primarily comprises 7 prey species: gobies, whiting, sandeel, sprat, trisopterus sp., herring and sardine (chapters 5 and 6). The majority of these prey species are among the most numerous fish and widely distributed along the North Sea (Daan et al., 1990; ICES, 2013b). These species are also important forage fish species (e.g. preyed on by large predators for food and caught in large quantities for trasformation to fishmeal or fish oil for use in the agriculture or aquaculture). The North Sea is an example of a region where fishing has substancially impacted on forage fish population (Dickey-Collas et al., 2014). In the North Sea, sprat, herring, sandeel, Norway pout, sardine and anchovy are the main forage fish species. The first four of these species are exploited by targeted fisheries, whereas the two latter species are considered occasional occupants of the North Sea and rarely occur at a biomass large enough to attract fisheries exploitation (Engelhard et al., 2013). It is well known that forage fish species display fluctuations in their distribution and abundance (Reid et al., 2001; Rijnsdorp et al., 2009). From the International Bottom Trawl Survey (IBTS), the relative abundance of the sandeel in the North Sea from 1983 to 2010 have witnessed a pronounced decrease in the total abundance since the year 2001 (SIH-Ifremer, 2014). Figure 7.2 shows the spatial distribution

of sandeel landings in the North Sea from 1995 to 2008. After the year 2001, a decrease in the total landings of sandeel along the north and the centre of the North Sea is noticeable. In addition, in the period between 1994 and 2009, 84 % of the total sandeel landings came from the southern areas of the North Sea (ICES, 2014). In many ecosystems, sandeel is a key prey fish linking trophic levels (Hain et al., 1995; Frederiksen et al., 2007). Sandeels are an important prey item for a range of seabirds and form an important part of the habrour porpoise's diet. Forage fish can exert buttom-up control on top predators; these effects can be expected to be strongest in cases where the predator is a specialist and lying mainly on the availability of this particular forage fish for its diet (Engelhard et al., 2013). Climate change and fishing are affecting these forage fish and so probably impact on predators such as seabirds and marine mammals (Wanless et al., 2005; MacLeod et al., 2007; Anderson et al., 2014). The decrease of sandeel and the increase of sprat along with the lower energy value of both species in the diet were linked to breeding failure of seabirds in the North Sea in 2004 (Wanless et al., 2005). Moreover, the decline in the importance of sandeels in the diet of Common Guillemot *Uria aalge* chicks was shown in the long-term data for the North Sea (Figure 7.3), with proportions decreasing form 1980s to 2011 (Anderson et al., 2014).

As a consequence, variability in the abundance of sandeels in response to fisheries pressure and climate change is likely to have broad effects on the entire ecosystem and have negative effects on harbour porpoise's population in the North Sea by increasing the starvation. In fact, an increased starvation in harbour porpoises in the Scottish North Sea was linked to a lack of sandeel consumption in spring 2002 and 2003 (MacLeod et al., 2007).

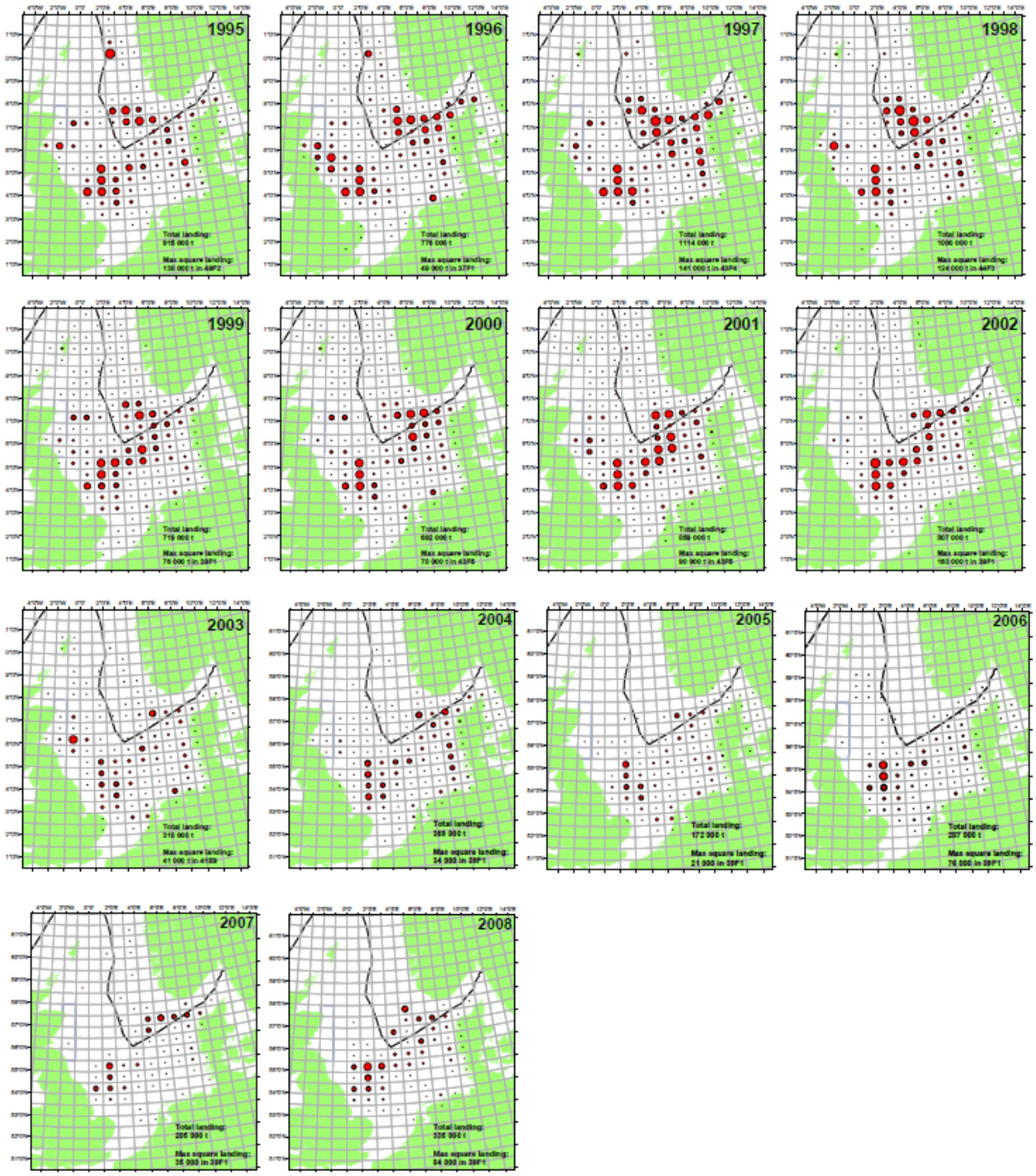

Figure 7.2 Map of spatial distribution of sandeel landings (tones) by year and ICES's rectangles for the period 1995-2008 (from ICES WGNSSK 2008).

On the other hand, in the present study the sardine appeared to be a potential prey in the diet of harbour porpoises from the southern North Sea. This species did not figure in previous studies on the stomachs of porpoises from the North Sea (Leopold and Camphuysen, 2006; Haelters et al., 2012). This small pelagic fish with more southern distribution is important commercial species in southern Europe. However, sardine distribution has

witnessed an increase in the North Sea over time. Since the mid 1990s, the re-invasion by sardines into the North Sea has been highlighted and attributed as a response to climate change (Beare et al., 2004 a; b).

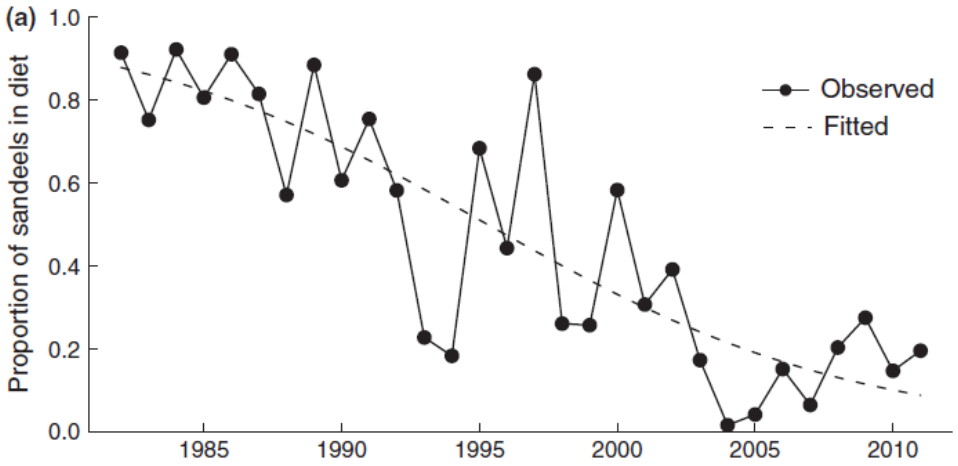

Figure 7.3 Changes in the proportion of sandeels in the diet of Common Guillemot chicks at the isle of May (North Sea) 1982 – 2011 (dashed line indicates fitted values from a logistic regression against year) (Extracted from Anderson et al., 2014).

The shift of harbour porpoises out of the coastal areas in the southern North Sea in the late 1980s has been tentatively related to the scarcity of main prey species (herring and mackerel) along with a no readily available alternative prey. It has been suggested that the lower survival or emigration for harbour porpoises in the central and southern North Sea in the late 1980s was either the result of food depletion or food lower in caloric content (Reijnders, 1992). It is necessary to find out whether the decline in one prey species could be counterbalanced by other species in the same area. Predators should prefer prey that yield more energy than the cost of foraging (Sih and Christensen, 2001). Among the main forage species of the continental shelf in terms of energy density, the families clupeidae (herring, sardine and sprat), scombridae (Atlantic mackerel) and carangidae (scads) are considered as high quality fishes, whereas the families gadidae (trisopterus sp., whiting and blue whiting) and ammodytidae (lesser sandeel) have moderate quality and the merluccidae (European hake) have lower quality (Spitz and Jouma'a, 2013). The quantitative relationship between the diet and the prey availability drives the animal to choose between switching prey which is in decline or leaving the area (Reijnders, 1992). We suggest that after the decline of the relative abundance of sandeel since 2002 in the northern parts of the North Sea, harbour porpoises have started to move southern along the North Sea to follow their potential prey. Such spatial movements have been previously observed in harbour porpoises at seasonal

scales, presumably due to variations in prey availability (Gaskin et al., 1975; Read and Westgate, 1997). Moreover, sardines may be considered as a "new" potential prey species in the diet of porpoises or it may be a "backup" prey replacing the sandeel decline. The results also suggest that porpoises may prey in offshore water on pelagic shoaling species such as sardine probably to compensate for the decrease in abundance of demersal coastal species such as the lesser sandeel. Changes in distribution of harbour porpoises is most likely related to changes in prey distribution, though not necessarily the only reason behind these changes. Other factors such as fishery bycatch, climate change and noise pollution might also combine synergistically and therefore lead to changes in harbour porpoise's distribution.

Value of a multi-approach dietary analysis

Several techniques have been used to study the feeding ecology of marine mammals. Among others, the feces analysis, the stomach content analysis, the stable isotopes analysis and the fatty acids analysis have been widely used and extensively reviewed (Pierce and Boyle, 1991; Hobson et al., 1994; Santos and Pierce, 2003; Iverson et al., 2004; Budge et al., 2006). All diet estimation methods make assumptions and have requirements that should be met in order to make the best estimation possible, to take advantages form its strength and to overcome its limitations. The table 7.1 represents the features of the three techniques used in the present study to investigate the diet of harbour porpoises.

One of the oldest techniques is the **stomach content analysis**. Compared to other methods, the strength of this analysis lies in the determination of the prey size and number. The requirements such as the otoliths and bones identification following the reference collection of prey species otoliths and bones, the measurements of otoliths size and the regressions of otoliths-prey size must be accomplished in order to determine the prey size. For this purpose, several references that comprise photos of otoliths and regressions for otoliths-prey size are available (e.g. for northeast Atlantic fish: Harkonen, 1986; Leopold et al., 2001). However, correction factors to reduce bias due to otoliths erosion in the stomachs of marine mammals which are strongly acidic environment are not usually available. The otoliths of some prey species are completely digested leading to false negatives or a biased view of their importance in diet. Indeed, stomach content analysis may cause an over-representation with large, robust hard parts. For instance, the otoliths of whiting are large, robust and very distinct. This makes them easy to identify even if they are already affected by

digestion. In contrast, otoliths of herring and sprat are more fragile and less recognizable due to digestion and decomposition. This bias may lead to an over-representation of whiting and under-representation of species such as herring and sprat (Grellier and Hammond, 2006). Several *in vitro* studies and experiments on marine mammals show that otoliths and other calcified prey structures have been completely digested or partly eroded during their passage in the stomach (Pierce and Boyle, 1991; reviewed in Bowen and Iverson, 2012). A number of factors can influence the digestion of prey hard parts for instance species differences, intraspecific individual variation, meal characteristics such as frequency, size, composition and energy density (Bowen and Iverson, 2012). Moreover, the diet of species with long foraging trips might be biased towards the last meal ingested and towards inshore prey species (Pierce and Boyle, 1991). Another limitation of the stomach content analysis is that only stranded animals are examined and that often almost half of the individuals analyzed present empty stomachs. For instance, from 250 harbour porpoises analyzed in the UK, 150 individuals presented empty stomachs (Martin, 1996). In the present study, we only disposed stomachs with content; hence we could not track the emptiness of stomachs. Moreover, in the prey remains, it is hard to distinguish between primary and secondary prey unless a prey remain is found in the stomach of another prey. In fact, primary preys are those eaten directly by the harbour porpoise, whereas secondary preys are small prey species eaten larger prey species such as whiting and cod (De Pierrepont et al., 2005). An over-representation of the number of the small secondary prey species are often to occur since they might be taken into account as primary prey. Finally, feeding habits of the animal could not be obtained from the stomach content analysis. Actually, diet data are either available from stranded animals which could be biased towards diseased and unhealthy animals (Craddock et al., 2009), or from bycaught animals which tend to be biased towards prey that are being caught by the fishery. All these aspects limit the robustness of the stomach content method and feeding study must be completed by other approaches.

The first advantage of the **stable isotope analysis** compared to the stomach content analysis is that this technique reveals the dietary history over a period of up to several months. Typically, the stable isotopic analyses of nitrogen have been used to indicate an animal's trophic level and the stable isotope analyses of carbon can indicate the source of the diet (benthic *vs.* pelagic; marine *vs.* terrestrial) (DeNiro and Epstein, 1978; Minagawa and Wada, 1984; Hobson et al., 1994; France, 1995; Vander Zanden and Rasmussen, 2001). However with the development of the mixing model the Stable Isotope Analysis in R (SIAR),

quantitative estimates of the species composition in the diet is possible. In general mixing models have witnessed a potential development since their beginning in the 1990s to further improve the performance of models and to overcome their limitations. Parnell et al., (2010) recently developed the use of Bayesian statistics to calculate the possible contributions of many sources in the diet of a consumer, hence developing a package for ecologists in R (SIAR). One should however keep in mind that strong assumptions are made when stable isotopes are used in the trophic ecology as well as in mixing models to estimate the sources contribution. These assumptions may be summarized as follows: (1) the variability associated with sources and the uncertainty associated with the trophic enrichment factors (TEFs) is normally distributed, (2) prey stable isotopes signatures are distinguishable enough and (3) the TEF between the consumer and the sources is relatively stable over time. Some of these uncertainties in assumptions might be overcome since SIAR can include (Mean ± SD). Along with the limitation that the size of prey remains unknown, false positives or negatives may be encountered when dealing with SIAR. Harbour porpoise might be foraging on a prey that is not included in the model (false negative) or we might include a prey that harbour porpoise did not eat (false positive). Therefore, given the number of prey species typically consumed by predators, mixing models might have several limitations in estimating the diet composition of marine mammals.

The **fatty acids analysis** also reveals the dietary history of the consumer over a period of up to several months. The fatty acids (FAs) are deposited into adipose tissue with little change or in a predictable manner, thus reflecting the diet of the predator. The changes in the FA distributions or patterns of the predator alone can reveal changes about spatial or temporal variations in diet, both among and within individuals or populations (Budge et al., 2006). Also a qualitative use of FAs resides in the use of biomarquers and tracers. It is based on the fact that a unique FA found in a predator can be traced to a single origin or prey species. A limitation of this use lies in the fact that FAs present in marine and terrestrial environments are ubiquitous. However to overcome this limitation, ratios among FAs can be attributed to some prey types therefore indicating their importance in the diet (Reviewed in Dalsgaard et al., 2003). Although, some metabolisms of FAs occurs within the predator and therefore the composition of predator tissue will not exactly match that of their prey (Budge et al., 2006). Recently a quantitative estimation of the proportional contribution of prey to the fatty acid signature of the predator has been developed. The quantitative fatty acid signature analysis (QFASA) is a mixing model that requires information on the FA composition of prey species

and of predator fat stores, the FAs of dietary sources, calibration coefficients (CC) to account for predator metabolism of ingested FAs prior to their depositions in blubber and a statistical model to minimize the statistical distance between the predator and the weighted mixture of prey species representing the diet (Iverson et al., 2004). However similar to SIAR, false positives or negatives may be encountered when dealing with the QFASA. This procedure requires knowledge of the FA compositions of all important potential prey species, as well as sufficient within-species sampling to assess variability or overlap in signatures with ecological and demographic factors (Budge et al., 2006). Furthermore, the **compound-specific stable isotope analysis** (CSIA) is a new research tool used to avoid many limitations encountered when using bulk tissue stable isotopes (measurement of $\delta^{13}C$ and $\delta^{15}N$ values in a sample) or FAs analyses. These limitations are summarized as follows: (1) some studied organisms cannot be physically isolated from each other, (2) tracing quantitatively minor by qualitatively important component and (3) different food sources may have similar bulk carbon isotope and FA signatures, (4) isotopic routing and fractionations from diet to tissue can complicate interpretation where isotopes are incorporated differentially into different tissues (Gannes et al., 1997; Gladyshev et al., 2012). This approach tracks the stable carbon isotope of specific FA structure that can only arise from diet (Budge et al., 2011). CSIA is expected to provide a greater specificity to biomarkers (Evershed et al., 2007). Several studies have reported the CSIA in members of various food webs (Budge et al., 2008; Budge et al., 2011; Bec et al., 2011; Gladyshev et al., 2012). However, most of these studies followed the assumption that $\delta^{13}C$ values of individual FA is transmitted from the diet to the consumer and few of them addressed the issue of molecular fractionation or change in $\delta^{13}C$ values during metabolism of dietary FA (Budge et al., 2011; Bec et al., 2011).

Each method has its requirements, strengths and limitations. When combining several techniques, it is expected to overcome or reduce these limitations. Therefore, in the present study the stomach content analysis, the stable isotopes analysis using SIAR and the fatty acids analysis using CSIA were combined in order to investigate the diet of harbour porpoise from the southern North Sea (Chapter 5). Moreover to compare the feeding habits of harbour porpoises from the southern North Sea and the Bay of Biscay, two areas that differ in prey species diversity, bottom type and physical oceanography, methods were used typically as stomach contents, stable isotopes (bulk) and fatty acids (patterns) (Chapter 6). The results obtained from the Chapter 5 are presented in table 7.2 in order to compare the methods. Gobies were the only species that figured in all techniques. Moreover sprat, lesser sandeel

and whiting were only found to contribute to the diet of porpoises according to the stomach content analysis. Previous studies on harbour porpoise's diet in the North Sea found also that these four species contributed to the diet (Leopold and Camphuysen, 2006; Haelters et al., 2012). Beside sprat, all these species are benthic and coastal species which can explain their presence in the stomach content analysis and leads to the limitation of the method since stranded individuals have stomach contents biased towards inshore prey species. Sandeels have a pelagic and benthic life stage: they buried in the sand during part of the day and turn to a pelagic life stage to feed. It is not known whether harbour porpoises can take sandeels in the water column or while these fish are buried. Herring and sardine were identified with both the stable isotopes and the fatty acids approaches. Both species are pelagic fish which can justify their presence in the intermediate term diet. In the past, clupeids constituted an important part of the diet of harbour porpoises. This apparently changed after the collapse of the herring stock (Santos and Pierce, 2003). Since recent years, herring and also sardine are becoming more common again and their return could explain why clupeids (relatively fat fish, and therefore form an energetic prey in term of energy density) formed an important part of the diet of habrour porpoises in our study.

Table 7.1 Requirements, strengths and limitations of methods that were used in the present study to estimate the diet of harbour porpoises (Modified from Bowen and Iverson, 2012).

Method	Stomach content analysis	Stable isotopes analysis (SIAR)	Fatty acids analysis (CSIA)
Tissue	Stomachs	Muscle	Blubber
Dietary history	Last few meals	Days to months	Days to months
Species composition	yes	yes	no
Prey size and number	yes	no	no
Requirements	Reference collection of prey species otoliths and bones	Fractionation factors for tissues	Prey fat content
	Otolith size measurements	Reference isotope levels from lower trophic levels	Predator adipose tissue
	Otoliths-prey size regressions	Distinguishable prey stable isotope signature	
Strengths	Moderate-large sample sizes possible	Integrates diet over time	Integrates diet over time
	demographic traits of individuals known	demographic traits of individuals known	demographic traits of individuals known
		Used as independent check of trophic level	Sampling location less likely to bias composition
Limitations	Only stranded animals	Size and number of prey not known	Size and number of prey not known
	Prey must have species-specific hard parts and these must be ingested	False positives or negatives possible	False positives or negatives possible
	Correction factors to reduce bias, but they are not usually available		Because of long integrated time, location of foraging less well identified
	Hard parts must resist digestion		
	False positives or negatives possible		
	May not be representative of species with long foraging trips		
	Often many empty stomachs		
	Differential digestion may further bias results		

A similar study in the Southern North Sea along Dutch coastal waters has combined the three techniques to assess the diet of stranded harbour porpoises (Jansen, 2013). The results showed that gobies, sprat, herring, cod and lesser sandeel were all found as the most important prey species in the short-term (stomach content) and the intermediate term (stable isotopes and fatty acids) diet. In our study, gobies, sprat and lesser sandeel were also found to contribute to the diet in the stomach content analysis. Whiting was only found to be an important prey species according to the stomach content analysis, which is in agreement with our results. Moreover, proportions of each prey in the contribution to the diet of habrour porpoises according to the stomach content analysis are close in both studies. For instance gobies accounted for 37 % in the previous study (Jansen, 2013) and 45 % in the present study. Whiting contributed to 25 % and 31 % to the diet of porpoises in the previous and present study, respectively. Sandeel was found to contribute with 13 % and 17 % and sprat contributed with 4 % and 3.4 % to the diet of harbour porpoises in the previous study and the present stud, respectively. According to SIAR modeling, only gobies and poor cod were found in both studies. Proportions of the cod were almost similar in both studies with percentages varying between 13-18 % and 9-15 % in the previous study and the present study, respectively. In contrast, for the gobies species, proportions in the present study (10-46%) were much higher compared to the previous study (13-18%). As for the FAs analysis, gobies and herring were both found to contribute to the diet of porpoises (Table 7.2). Despite all the limitations of the techniques used, SIAR and CSIA analyses provided complementary information for the stomach content analysis. Therefore, combining techniques that integrate diet over days and weeks allowed gaining more complete understanding of harbour porpoise's diet relative to stomach contents.

Table 7.2 The diet of harbour porpoise from the North Sea inferred from a combination of three techniques; n=number of samples analyzed; SIAR: Stable isotopes analysis in R; CSIA: Compound-Specific Stable Isotope Analysis; QFASA: Quantitative Fatty Acid Signature Analysis. Prey species are classified from the most important prey to the less important.

	Stomach content analysis	Stable isotopes analysis	Fatty acids analysis	
Technique	Hard parts	SIAR	CSIA	Present study
Tissue	Stomachs	Muscle	Blubber	(Northern France and
n	14	52	59	Belgian coasts)
Dietary history	Short term Days	Intermediate term Several Months	Intermediate term Several months	
Species	Gobies	Gobies	Gobies	
	Sprat	Herring	Herring	
	Lesser sandeel	Sardine	Sardine	
	Whiting	Trisopterus sp.		
Technique	Hard parts	SIAR	QFASA	Jansen, 2013
Tissue	Stomachs	Muscle	Blubber	(Dutch coastal waters)
n	76	90	73	
Dietary history	Short term Days	Intermediate term Several Months	Intermediate term Several months	
Species	Gobies	Poor cod	Gobies	
	Whiting	Mackerel	Mackerel	
	Lesser sandeel	Greater sandeel	Smelt	
	Herring	Lesser sandeel	Herring	
	Cod	Sprat	Dragonet	
	Sprat	Gobies	Cod	

When methods were used typically, the stable isotopes as bulk and the fatty acids as patterns (Chapter 6), only qualitative evaluation was possible in the comparison of the diet between porpoises from the southern North Sea and those from the Bay of Biscay. The stomach content was the only quantitative technique and provided information about the species composition. The stable isotopes gave insight into the feeding habits of porpoises. Animals from the southern North Sea had significantly higher $\delta^{15}N$ signatures compared to those from the Bay of Biscay. This difference was attributed to the differences in $\delta^{15}N$ at the base of food chain (Jennings and Warr, 2003) rather than apparent differences in trophic position. Hence, the reference isotope level from the lower trophic levels is required (Table 7.1). As for the FAs analysis, only comparison of patterns between the FAs signatures in the blubber of harbour porpoises and those in the muscle of prey species was possible. For instance, FAs that arise from diet such as elevated levels of 20:1ω9 and 22:1ω11/9 in the blubber of harbour porpoises from the southern North Sea reflected that some individuals are feeding on zooplanktonivorous fish such as herring. In addition, The FA 20:4ω6 marker of

benthic littoral algae (Dalsgaard et al., 2003), is present in elevated amounts in muscle of benthic prey species such as gobies, whiting and pouting. However, these amounts could not be found in porpoises blubber, although according to stomach content analysis these 3 species contributed to the diet of porpoises. Here again, stomach contents are biased towards inshore species and reflected short term diet, whereas the FAs patterns reflected medium term diet where signature of more pelagic prey species are present in elevated amounts.

Conclusions and perspectives

The present study evaluated two major threats that harbour porpoises are exposed to along the North Sea. The contamination status was evaluated through an assessment of chemical contaminants. To this end, a passive monitoring of stranded animals was presented. In fact, due to the protective status of this species, only stranded animals could be included which gave insight into the contamination status of harbour porpoises in the southern North Sea over a past period. In order to evaluate the current exposure situation on a wild marine population, several studies have attempted to correlate chemical contaminant levels in blubber and blood biopsies from free ranging animals to organs such as livers and kidneys. These studies appeared to be useful indicators for current exposure situation but revealed that only Hg can be used in order to investigate the bioaccumulation in internal tissues of cetaceans. Therefore, more studies on different species of marine mammals along with new experiments may help to understand the current exposure situation of sentinel species. On the other hand, in order to detect a long-term exposure to contaminants, hard tissues might be used such as bones and teeth. These tissues are easily collected and preserved; therefore they may allow depicting whether animals were exposed to a long-term contamination in their environment. Moreover, uptake from food (trophic transfer) is the main pathway of contaminants in marine mammals. In the present study 7 prey species were found to contribute the most to the diet of harbour porpoises. Therefore, for a future study it might be useful to determine the contaminant loads in the tissues of these prey species in order to relate the levels found in organs of porpoises.

The shift in the abundance of harbour porpoises was evaluated and interpreted in the light of prey species availability and abundance changes. The diet of porpoises was investigated via three techniques each presenting different requirements, strength and limitations. However, the interpretation of the diet of harbour porpoise's results is limited by

a lack of species-specific parameters such as digestion rates, turnover times, isotopic fractionation and lipid metabolism which are necessary for each technique. Controlled feeding experiments with animals in captivity made available these parameters for only a limited number of marine mammal species such as harbour seals, ringed seals, harp seals, killer whales, bottlenose dolphins, etc. Unfortunately, no controlled feeding experiments have been previously done on the harbour porpoise species. These experiments are difficult to conduct because of the protective status of most marine mammals along with the elevated financial cost that feeding experiments might generate. Once an animal is rescued and transported to human care, it might be a good opportunity to conduct feeding experiments and therefore financial cost and protective status might be overcome (Lockyer et al., 2003). More studies on different species of marine mammals along with more experiments on the digestion rates, turnover times and isotopic fractionation might be useful towards a better understanding and interpretation of the dietary results of marine mammals.

The present study highlighted the existence of seven prey species to contribute to the diet of harbour porpoises. Although the number of samples investigated is still small, the limited number of prey species encountered so far in the stomachs of harbour porpoises was surprising. There is more than 200 fish species living in the North Sea, and species such as the flatfish (dab, plaice, sole) are very abundant in coastal areas. Apparently, harbour porpoises are not opportunistic feeders as it was suggested to be in previous studies (Santos and Pierce, 2003; Fontaine et al. 2007). Given the narrow range of prey species on which porpoises appear to feed on compared to the high variety of prey species existing in its environment, this study suggests that porpoises comprise a specialized feeding character. The distribution of harbour porpoises were found to be affected by the main prey species distribution and prey diversity as well as other environmental variables such as bathymetry, topography, etc. It comes out that putting together and comparing the results of the diet analysis from different countries bordering the North Sea would be interesting to further reveal the reasons behind the seasonal movements of harbour porpoises in the area. Moreover, the management of fish stocks could be included in the management and conservation of harbour porpoises. On the other hand, harbour porpoises movements could potentially be a useful indicator of changes in distribution and abundance of forage fish. Given the speed of magnitude of changes in fisheries and climate, there is a need for an up-to-date assessment of the diet of marine mammals in general to establish a baseline against which to measure subsequent changes.

The comparison of the two SCANS surveys (1994 and 2005) only allows a geographically limited trend analysis for the summer distribution and abundance of harbour porpoises which is subject to several assumptions. The observed southward shift could equally be explained by a change in habitat use and/or movement patterns. In some locations, porpoises appear to have seasonal inshore/offshore movements, for instance into the southern German bight as well as Dutch and Belgian coastal waters especially in late winter and early spring (Haelters et al. 2011; OSPAR, 2013). Therefore, we need a solid knowledge of the drivers that influence the movements and consequently the distributions of harbour porpoises in order to understand the temporal and spatial fluctuations in porpoise's distribution. Since almost all individuals analyzed in the present study were stranded in the period between January and May, the seasonal variation in the diet of harbour porpoises was not elucidated. Hence, in order to gain a more complete understanding on the diet, it is recommended to compare the diet of the animal in different seasons and therefore to confirm the driver of its movements. This might also help to understand the reason behind the low diversity of the prey species in the diet of porpoises. Moreover, the correlation between porpoises distribution and biological and environmental variables must be studied in different countries bordering the North Sea in order to have a more complete understanding on the movement and distribution of this species. Finally, the harbour porpoise is one of the smallest cetacean and its habitat and life history impose very high energy demands. Understanding its distribution in relation to its environment, especially its prey, is vital for the conservation status of this species as required in the framework of the European Habitats Directive (92/43/EEC).

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SCIENTIFIC CONTRIBUTIONS

Scientific contributions

Part of this work was presented at national and international conferences in form of oral presentations and posters. A scientific report after the request of the Grand port Autonome de Dunkerque also emerged from this thesis:

1. *Report:*

- Henry F, **Mahfouz C**, Pezeril S, Bouveroux T, Jauniaux T, Amara R (2013) Contribution scientifique à l'étude générale de la surveillance littorale des mammifères marins. Convention de Recherche, Université du Littoral – Grand Port Autonome de Dunkerque, 64p.

2. *Oral communications :*

- **Mahfouz C**, Meziane T, Henry F, Khalaf G, Amara R (2014) Utilisation de trois méthodes complémentaires pour l'étude du régime alimentaire des cétacés : contenus stomacaux, isotopes stables et acides gras. Troisième Conférence Biennale sur la Conservation des Cétacés dans les Pays du sud de la Méditerranée, 21 – 23 Octobre 2014, Jounieh, Liban.

- Mahfouz C, **Henry F**, Khalaf G, Amara R (2014) Méthodologie employée pour l'analyse des contaminants chimiques pour de futures applications dans l'aire ACCOBAMS : Cas du marsouin commun (*Phocoena phocoena*). Troisième Conférence Biennale sur la Conservation des Cétacés dans les Pays du sud de la Méditerranée, 21 – 23 Octobre 2014, Jounieh, Liban.

- **Mahfouz C**, Henry F, Jauniaux T, Khalaf G, Amara R (2013) La contamination métallique pourrait-elle être une des causes de l'augmentation du nombre de marsouins communs (*Phocoena phocoena*) échoués au sud de la mer du Nord ? Secondes Journées Franco-Libanaises, 22 – 25 Octobre 2013, Dunkerque.

- **Mahfouz C**, Henry F, Jauniaux T, Khalaf G, Amara R (2013) Application des isotopes stables (C et N) comme traceurs du régime alimentaire du marsouin commun (*Phocoena phocoena*) échoués sur le littoral Français. 6^{e} journées des jeunes

chercheurs de la Société Française des Isotopes Stables, 28 – 30 Octobre 2013, Dunkerque.

- Mahfouz C, **Henry F**, Meziane T, Jauniaux T, Pezeril S, Bouveroux T, Dabin W, Khalaf G, Amara R (2014) Causes d'un nombre croissant d'échouage de marsouins communs (*Phocoena phocoena*) au sud de la mer du Nord : Etudes de la contamination chimique et du régime alimentaire. XVI^{e} séminaire du RNE, 27 – 28 Septembre 2014, Dunkerque.

3. Poster presentations :

- **Mahfouz C**, Henry F, Jauniaux T, Khalaf G, Amara R (2013) Could the chemical contamination be the cause of the increase in the number of stranded harbour porpoises (*Phocoena phocoena*) along the southern North Sea and the Bay of Biscay? 20th Biennial Conference on Marine Mammals, 9 – 13 December 2013, Dunedin, New Zealand. (**Annex 3**).

- **Mahfouz C**, Henry F, Meziane T, Caurant F, Pezeril S, Bouveroux T, Jauniaux T, Khalaf G, Amara R (2013) Does prey availability influence harbour porpoises (*Phocoena phocoena*) diet, abundance and distribution? 28th Annual Conference of the European Cetacean Society, 5 – 9 April 2014, Liege, Belgium. (**Annex 4**).

ANNEX

Annex 1: Samples analyzed and analyses that has been carried out on each sample; J: Juvenile; A: Adult; F: Female; M: Male; nd: not determined.

a) Samples from porpoises stranded along the southern North Sea

Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Analyses (organ/tissue)					
										Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A13/1932	4/9/2013	Oye Plage	France	J	F	106	18.5	18	lung edema	X	X	X	X	X	
A13/1939	5/18/2013	Bray Dunes	France	A	F	154	50	15	infection	X	X	X	X	X	X
A13/1937	3/31/2013	Bray Dunes	France	J	M	94	16	23	lung edema	X	X		X	X	
A13/1933	3/23/2013	Bray Dunes	France	J	M	112	20	20	capture	X	X	X	X	X	X
A13/1432	3/18/2013	La Panne	Belgium	J	F	111	22.5	27	capture	X	X	X	X	X	
A13/1935	4/20/2013	Dunkerque	France	J	M	114	24	21	infection	X	X	X	X	X	X
A13/1934	4/8/2013	Bray Dunes	France	J	M	105	22.5	22	capture	X	X	X	X	X	X
A13/1938	3/9/2013	Bray Dunes	France	J	M	108	21	28	capture	X	X	X	X	X	X
A13/1757	5/8/2013	Lombsijde	Belgium	A	F	161	50	10	infection	X	X	X	X	X	X
A13/1931	3/24/2013	Malo les Bains	France	J	M	102	16.5	18	capture	X	X	X	X	X	X
A13/1936	3/17/2013	Bray Dunes	France	J	M	112	21.5	22	lung edema	X	X	X	X	X	
A13/1930	4/10/2013	Leffrinckoucke	France	J	M	110	17.5	6	lung edema	X	X	X	X	X	X
A13/1282	2/24/2013	Oye Plage	France	J	M	119	21	13	Infection	X		X	X	X	
A13/1089	5/2/2013		Belgium	J	M	111	25	26	Capture	X			X	X	
A13/1019	10/29/2012	Oye Plage	France	J	M	103	14	20	Infection		X	X	X	X	
A13/330				J	F	123	33.5	30	Capture	X	X			X	
A13/1285				A	F	165	45	12	Infection	X	X			X	X
A13/1020	9/11/2012	Bray-Dunes	France	J	F	124	20	6	lung edema	X	X	X	X	X	
A13/1284				J	M	113	23	25	Capture	X	X			X	X
A13/331	4/28/2012	Equihen	France	J	M	103	20.5	16	Capture	X	X		X	X	

Annex 1a. Continued

Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Analyses (organ/tissue)					
										Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A13/443	5/11/2012	Hardelot	France	J	M	115	21.8	10	nd	X	X		X	X	
A13/444	3/24/2012	Dunkerque, digue du Braek	France	A	M	150	47	28	Capture		X	X	X	X	X
A13/1262				J	M	114	21	13	Infection						
A13/1384			Belgium	J	F	108	20	21	Infection	X				X	
A12/2048	3/9/2012	Wimereux	France	J	M	115	26.5	19	Capture	X			X	X	
A12/1284	1/13/2012	Malo les Bains	France	J	M	120	25	20	nd	X					
A12/1327	1/3/2012	Wimereux	France	A	M	127	28	19	nd					X	
A12/1381	1/5/2012	Wimereux	France	J	F	112	21.5	18	Infection	X	X		X	X	
A12/1326	1/21/2012	Hardelot	France	J	F	110	24.5	21	seal predation	X	X		X	X	
A12/1329	2/29/2012	Camiers	France	A	F	161	49	19	lung edema	X	X		X	X	
A12/2041	3/10/2012	Oye Plage	France	J	M	113	22.5	23	Capture	X	X		X	X	
A12/2053	3/18/2012	Bray-Dunes	France	J	F	105	21	20	Capture	X	X		X	X	
A12/2052	3/27/2012	Dunkerque,Malo les Bains	France	J	F	115	24.8	25	Capture	X	X		X	X	
A12/2061	3/25/2012	Coxyde (Koksijde)	Belgium	J	M	107	18.2	16	Infection	X	X		X	X	
A12/2039	3/21/2012	Bray-Dunes	France	J	M	114	18	8	Infection	X	X		X	X	
A12/2046	1/12/2012	Wimereux	France	J	F	120	21	20	Infection	X	X		X	X	
A12/333	7/26/2011	Oostende	Belgium	J	M	114	15	6	Infection	X	X		X	X	
A11/1168									nd	X	X		X	X	
A11/1584	3/26/2011	Nieuwpoort	Belgium	J	F	98	18.5	16	Capture	X	X		X	X	
A11/1772	3/9/2011	Coxyde	Belgium	J	M	115	26	24	Capture	X	X	X	X	X	
A11/1768	2/28/2011	Oostende	Belgium	J	M	92	11.6	5	nd	X	X	X	X	X	
A11/1763	4/7/2011	Blankenberge	Belgium	J	M	110	19.6	13	Infection	X	X		X	X	
A11/1761	3/24/2011	Blankenberge	Belgium	J	M	107	18	13	Capture	X	X			X	

Annex 1a. Continued

Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Analyses (organ/tissue)					
										Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A11/1770	3/11/2011	Mariakerke	Belgium	A	F	155	41.5	16	Infection	X	X	X	X	X	
A11/1767	3/26/2011	De Haan	Belgium	J	F	128	26.2	23	Capture	X	X		X	X	
A11/1771	4/17/2011	Bredene	Belgium	J	M	118	17.6	18	nd	X	X	X	X	X	
A12/484	9/18/2011	Camiers	France	A	F	158	54	8	Lung edema	X			X	X	
A11/1099			France	J	F	122	26.5	14	Capture	X	X		X	X	
A11/1098			Belgium	J	F	133	28.8	22	Infection	X	X		X	X	
A11/1057			Belgium	A	F	160	41	18	Capture	X	X		X	X	
A10/1965			France	A	F	172	46	22	Lung edema+emaciation				X	X	
A10/1435	3/16/2010	Nieuwpoort	Belgium	J	M	119	28	18	pneumonie					X	
A10/1963			France	J	M	116	24.5	22	Capture	X	X			X	
A10/1415			Belgium	J	M	114	24	16	Capture	X	X		X	X	
A10/1672	May-10	Bredene	Belgium	A	M	143	29	16	Lung edema+emaciation	X	X		X		
A10/1009	Jan-10	Dunkerque	France	A	F	145	39.5	18	Infection	X	X		X	X	
A10/1968			Belgium	J	M	106	15.5	8	nd	X			X	X	
A10/1962			France	J	F	119	23	30	Capture	X	X		X	X	
A10/1964			Belgium	J	F	104	24	23	nd	X	X		X		
A10/1961			Belgium	J	M	114	18	8	emaciation	X	X		X	X	
A10/1277			Belgium	A	M	144	52	20	Infection	X	X		X		
A10/1040			France	J	F	115	17	2	Infection	X	X		X		
A10/1306			France	J	M	120	21.5	12	Lung edema	X	X		X	X	
A10/553	9/28/2009	Bredene	Belgium	A	F	157	38	14	Lung edema	X	X				
A10/959	12/11/2009	Wenduine	Belgium	A	M	149	40	26	Infection	X	X				
A09/1885	3/24/2009	Dunkerque	France	J	M	123	28.5	20	Infection	X	X				
A09/1943	Jun-09	Dunkerque	France	A	F	148	41	13	Infection	X	X				

Annex 1a. Continued

Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Analyses (organ/tissue)					
										Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A09/2041	Aug-09		France	J	M	109	22	30	nd	X	X				
A09/1884	1/2/2009	Coxyde	Belgium	J	F	97	13	8	Lung edema+emaciation	X	X				
A09/1494			France	J	M	105	18.5	10	nd	X	X				
A09/1889	1/21/2009	Oostende	Belgium	J	F	116	17.7	8	emaciation	X	X				
A09/1888	1/30/2009	De Haan	Belgium	J	M	86	13.2	14	Capture	X	X				
A09/2000	8/10/2009	en mer	Belgium	J	F	120	26	12	nd	X	X				
A09/1374	9/8/2008	Dunkerque	France	J	M	88	9.5	8	Capture	X	X				
A09/1140	9/29/2008	Coxyde	Belgium	J	F	114	17	5	Infection	X	X				
A09/766	Sep-08	Dunkerque	France	A	F	140	33.5	20	Capture	X	X				
A08/1433	2/2/2008	De Haan	Belgium	J	M	104	17.5	19	Capture	X	X				
A08/1510	4/9/2008	Blankenberge	Belgium	J	M	113	19.5	11	Capture	X					
A08/1442	1/27/2008	Oostende	Belgium	J	M	120	23	20	Infection	X	X				
A08/1841	4/5/2008	Westende	Belgium	J	F	105	14	8	Infection	X	X				
A08/1843	Apr-08	Bray-Dunes	France	J	M	101	21	25	Capture	X	X				
A08/1721	4/15/2008	Oostende	Belgium	J	F	96	13.8	9	emaciation	X	X				
A08/1842	Feb-08	Middelkerke	Belgium	J	F	94	11.6	10	Infection	X					
A08/1840	4/25/2008	Wenduine	Belgium	J	F	106	16	17	nd	X	X				
A12/1329	2/28/2008	Camiers	France	A	F	161	49	19	Infection						
A12/1286	1/14/2008	Oye Plage	France	A	F	162	50	23	Infection	X	X	X		X	
A12/1283	1/5/2008	Coxyde	Belgium	J	M	105	17.5	18	Capture	X	X			X	X
A12/1326	1/20/2008	Hardelot	France	J	F	110	24.5	21	Seal predation						
A12/1381	1/4/2008	Wimereux	France	J	F	122	21.5	18	Infection						
A08/1847	10/20/2007	Dunkerque	France	J	M	118	25	20	Capture	X	X				
A08/1443	10/28/2007	Oostduinkerke	Belgium	J	F	95	10	4	nd	X					

Annex 1a. Continued

Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Analyses (organ/tissue)					
										Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A08/1853	9/27/2007	Oostduinkerke	Belgium	J	F	110	13.5	3	Infection	X	X				
A08/001	9/15/2007	Middelkerke	Belgium	A	F	142	37	12	Infection	X	X				
A07/1685	Feb-07	Bray-Dunes	France	J	M	112	21.5	22	Capture	X	X				
A07/1681	3/16/2007	Oostende	Belgium	J	M	102	18.5	17	Capture	X	X				
A07/1690	Mar-07	Dunkerque	France	J	F	120	19	8	Lung edema	X	X				
A07/1688	2/22/2007	Oostende	Belgium	J	M	100	14	6	Lung edema	X	X				
A07/1682	Apr-07	Bray-Dunes	France	J	M	128	22.7	24	Capture	X					
A07/1692	3/30/2007	Oostende	Belgium	J	M	106	20.8	27	Capture	X					
A07/1686	2/9/2007	Oostende	Belgium	J	M	100	11.7	5	Lung edema	X	X				
A07/1608	4/9/2007	Oostduinkerke	Belgium	J	M	109	19	13	Capture	X	X				
A07/1691	3/25/2007	Oostende	Belgium	J	F	96	12.5	5	Lung edema	X	X				
A07/1698	2/19/2007	Coxyde	Belgium	J	F	122	22	21	Capture	X	X				
A07/1615	3/31/2007	Coxyde	Belgium	J	M	98	14	9	Capture	X	X				
A07/1684	3/1/2007	Leffrinckoucke	France	J	M	116	22.4	15	Capture		X				
A07/1695	2/26/2007	Blankenberge	Belgium	A	M	140	40	22	Infection		X				
A07/1693	3/27/2007	Loon	France	J	F	116	26	30	Capture		X				
A12/333	7/25/2007	Oostende	Belgium	J	M	114	15	6	Infection						
A12/430	9/23/2007	Knokke	Belgium	J	M	114	18.3	7	Seal predation						
A12/429	9/2/2007	Raversijde	Belgium	J	M	127	28	15	Seal predation	X					
A07/1687	12/30/2006	Blankenberge	Belgium	J	M	90	16	22	Capture	X	X				
A07/1683	12/25/2006	Oostduinkerke	Belgium	J	M	117	18	9	Capture	X	X				
A07/1060	4/27/2006	De Haan	Belgium	J	M	108	17	22	Capture	X	X				
A07/937	12/10/2006	Dunkerque	France	J	M	125	22	8	Infection	X	X				
A07/1689	12/29/2006	Oostduinkerke	Belgium	A	M	121	26	25	Capture	X	X				

Annex 1a. Continued

										Analyses (organ/tissue)					
Reference number	Date found	Location	Country	Maturity status	Gender	length (cm)	Weight (Kg)	Blubber thickness (mm)	Cause of death	Metals (livers)	Metals (kidneys)	POPs (blubber)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
A07/1064	12/12/2006	Dunkerque	France	A	F	164	46	11	Capture		X				
A06/1269	3/26/2006	De Panne	Belgium	J	F	103	21	18	Capture	X	X				
A06/1267	3/30/2006	Middelkerke	Belgium	J	F	113	18	8	Capture	X	X				
A06/1268	3/30/2006	Blankenberge	Belgium	J	F	130	32.7	24	Capture	X	X				
A06/1326	1/26/2006	Knokke	Belgium	A	M	146	32	9	emaciation	X					

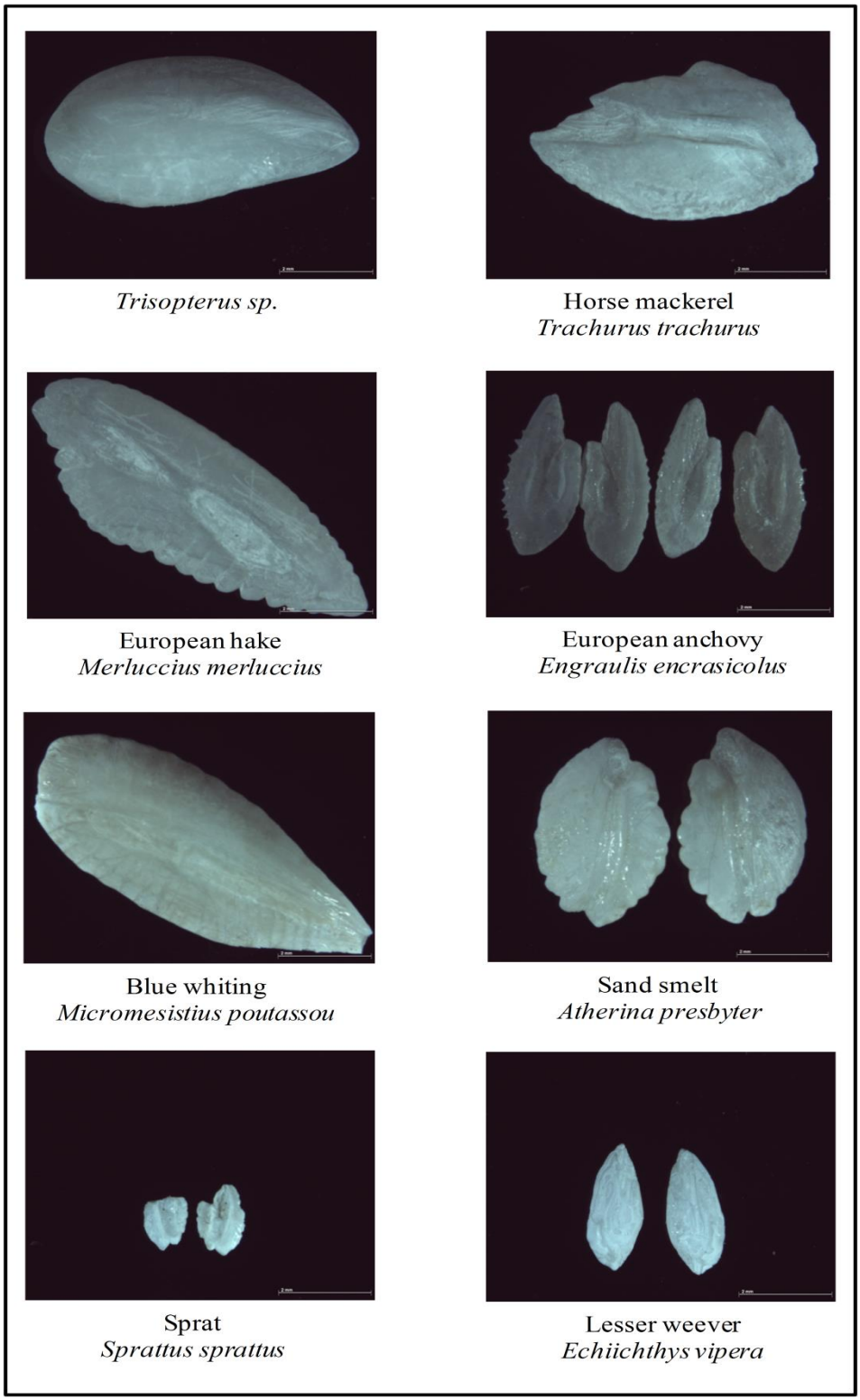
b) Samples from porpoises stranded along the Bay of Biscay

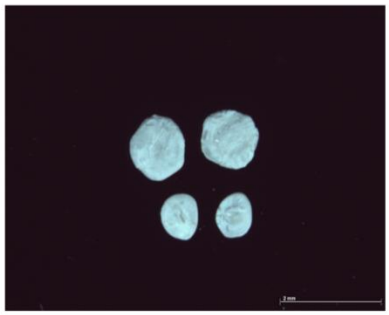
								Analyses (organ/tissue)				
Reference number	Date found	Location		Maturity status	Gender	length (cm)	Weight (Kg)	Metals (livers)	Metals (kidneys)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
11112095	12/2/2011		Mimizan	J	F	119	30	X	X	X	X	
11112092	12/13/2011		Fouras	J	F	110	15	X	X	X	X	
11112126	3/3/2011	Plage des Boucholeurs (sud)	Barbatre	J	F	120		X	X	X	X	
11203037	3/6/2012	Saigresse casernes	Seignosse	J	M	129	20	X	X	X	X	
11204050	4/19/2012	Le corps de garde	La tranche sur mer	J	M	117	26	X	X	X	X	
11205052	5/12/2012	Les corches	Les rivieres (porte en Ré)	J	M	126.5	25	X	X	X	X	
11112125	1/17/2011	Plage de la Coquette/Pointe de la Loire	La Gueriniere	J	M	128		X	X	X	X	
11111088	x/10/2011		Seignosse	A	F	150	45	X	X	X		
11206068	6/24/2012	Nord près du poste MNS	Messanges	A	F	179		X	X	X	X	X
11112089	12/8/2011	Plage	St Girons	A	F	174	50	X	X	X	X	
11201006	1/12/2012		Capbreton	A	M	129.5	34	X	X	X	X	X
11207077	1/11/2012	Les Bourdaines	Seignosse	A	M	143				X	X	

Annex 1b. Continued

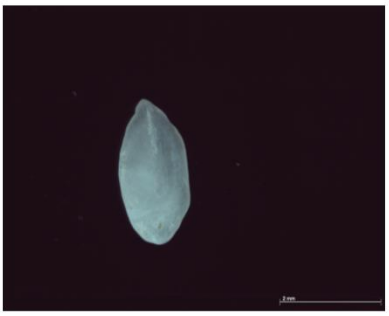
								Analyses (organ/tissue)				
Reference number	Date found	Location		Maturity status	Gender	length (cm)	Weight (Kg)	Metals (livers)	Metals (kidneys)	stable isotopes (muscle)	Fatty acids (blubber)	Stomachs
11203045								X	X	X		
11103054	2/28/2011	Plage de la paraca	les sables d'olonne		f			X	X	X	X	X
11208086	3/6/2012	Metro	Tarnos	A	M	162		X	X	X	X	X
11001003	14-Feb-09	Arcachon		A	M	165	70	X	X	X	X	X
11012052	10-Dec-10	BISCAROSSE PLAGE	BISCARROSSE	A	F	152		X	X	X	X	X
11210092					M				X	X	X	X
11012054	14-Dec-10		SOULAC-SUR-MER	A	M	162			X	X	X	X
11012053	19-Aug-10	POINTE DU DEVIN	L'EPINE	A	M	151				X	X	
11208085	3/6/2012	Metro	Tarnos	A	F	167		X	X	X	X	X
10912147	9-Mar-09		ONDRES	A	F	167		X	X	X	X	X
11012046	28-Nov-10	CELM 3,4KM LIMITE SUD ST EULALIE	BISCARROSSE	J	F	127		X	X	X	X	
10901055	20-Jan-09	LA DUCHESSE ANNE	SAINT-BREVIN-LES-PINS	A	M	157		X	X	X	X	X
10912325	3-Apr-09	LES SABLEAUX	NOIRMOUTIER-EN-L'ILE	J	F	105				X	X	
11208087	6/18/2012	plage de la Davière	SAINT-JEAN-DE-MONTS	J	M	95		X	X			
11208083	1/24/2012	Les sable d'Or	Cap ferret	A	M	174		X	X	X	X	X
10912327	30-Mar-09	ECOLE DE VOILE GOLF	SAINT-JEAN-DE-MONTS	J	F	125		X	X	X	X	
11208084	3/3/2012		SEIGNOSSE	A	M	149				X	X	
11002018	12-Feb-10	FACE AU BANC DU BUCHERON	LES PORTES-EN-RE	J	M	112	19	X	X	X	X	X
10912326	17-Apr-09		SAINT-JEAN-DE-MONTS	J	F	128				X	X	
11001006	22-Jan-10	LA JAOUGUE	VIELLE-SAINT-GIRONS	A	M	129		X	X	X	X	
11112127	28-Dec-11	LES BOURDAINES	SEIGNOSSE		nd					X	X	
11207081	3/3/2012	Soustons plage	Vieux Boucau	J	F	124				X	X	
11208082	1/11/2012	LES BOURDAINES	SEIGNOSSE	J	F	111				X	X	

Annex 2: Digital photographs of different otoliths found in the stomachs of harbour porpoises stranded along the southern North Sea and the Bay of Biscay.

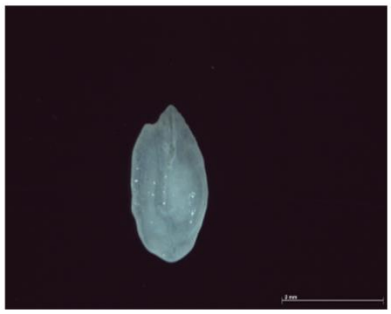




Gobidae sp.

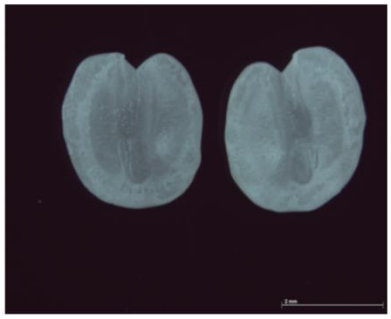


Sandeel
Ammodytes marinus

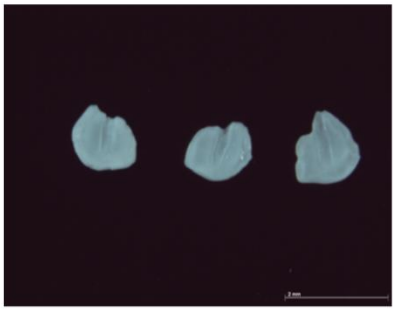


Great sandeel
Hyperoplus lanceolatus

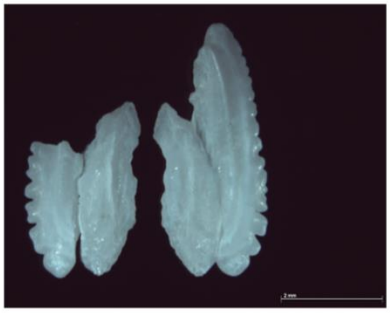
Whiting
Merlangius merlangus



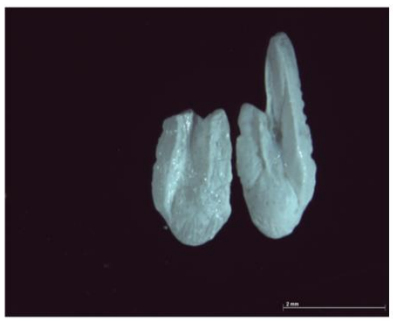
Lampanyctus sp.



Mueller's Pearlside
Maurolicus muelleri



Herring
Clupea harengus



European pilchard
Sardina pilchardus

Annex 3

Could the chemical contamination be the cause of the increase in the number of stranded harbour porpoises (*Phocoena phocoena*) along the Southern North Sea and the Bay of Biscay (France)?

(Poster)

Mahfouz C., Henry F., Jauniaux T., Khalaf G., Amara R.

20th Biennial Conference on Marine Mammals, Dunedin, New Zealand, 9-13 December 2013

20th Biennial Conference on Marine Mammals, Dunedin, New Zealand, 9-13 December 2013

Could the chemical contamination be the cause of the increase in the number of stranded harbour porpoises (*Phocoena phocoena*) along the Southern North Sea and the Bay of Biscay (France)?

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Laboratoire d'Océanologie et de Geosciences – UMR LOG 8187

Introduction

➢ Harbour porpoise (*Phocoena phocoena*) is subject to several potential threats such as exposure to contaminants, changes in food supply, marine traffic and fishery by-catch.
➢ Throughout the last years the Southern North Sea and the English channel (*Fig. 1 – Region 1*) has witnessed an increase in the number of stranded harbour porpoises.
➢ We investigated whether the chemical contamination may explain the rise of porpoises stranding?

Fig. 1. Stranded harbour porpoises; Region 1 : Southern North Sea - English Channel ; Region 2: Bay of Biscay

Fig. 2. Number of Stranded harbour porpoises in the Southern North Sea and the English Channel from 1990 to 2012 (OCEAMM)

Material and Methods

➢ As, Cd, Cr, Cu, Hg, Mn, Se, V and Zn were measured in kidneys and livers of 100 harbour porpoises stranded in region 1 (2006-2013) and 25 porpoises stranded in region 2 (2009-2012) (*Fig. 1*):
- Determination by ICP-MS and ICP-AES
- Hg determination by AAS
➢ POPs: 7 PCBs congeners (IUPAC numbers: CB 28, 52, 101, 118, 153, 138 and 180) and 6 DDXs (o,p'-DDD, o,p'-DDT, o,p'-DDE, p,p'-DDD, p,p'-DDT and p,p'-DDE) were measured in blubber of 20 porpoises stranded in region 1 and were determined by GC-MS

Fig. 3. Stranded Harbour porpoise before autopsy

Results and discussion

Metals

Association between contaminants and health status:
➢ Region 1, Porpoises that died of infectious disease displayed significant higher hepatic concentrations of Cd, Se, Hg and Zn compared to healthy porpoises that died of physical trauma (*Fig. 4*).
(Mann-Whitney, p<0.05).

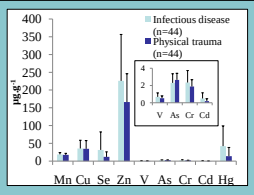

Fig. 4. Mean metal liver concentration (µg.g^{-1}d.w.) in 88 porpoises that died in region 1 from 2006 to 2013

Association between contaminants and maturity status:
➢ Region 1, juveniles displayed significant higher hepatic concentrations of Cd, Hg and Se compared to adult porpoises. (*Fig. 5*)
(Mann-Whitney, p<0.05).

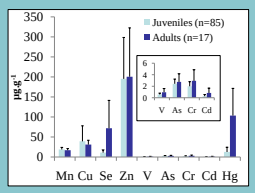

Fig. 5. Mean metal liver concentration (µg.g^{-1}d.w.) in 85 porpoises juveniles and 17 adults that died in area 1 from 2006 to 2013

Temporal trends and comparison with other study in region 1:
➢ Hepatic concentrations in porpoises stranded (2006 to 2013) → No significant differences . (Mann-Whitney, p<0.05).
➢ Comparison with other study → metal concentration in hepatic and renal samples do not differ substantially from that reported for the same area (1994-2001) (*Table. 1*)

Table. 1. Metal concentrations (µg.g^{-1}d.w.) in liver of harbour porpoises from Southern North Sea. Mean ± SD; (Minimum – Maximum); n: number of samples.

	Hg	Cd	Cu	Zn	Se	
Mean ± SD (min-max) n	23 ± 66 (0.6-344) 27	0.5 ± 0.6 (<0.05-2.5) 49	39 ± 38 (9-257) 49	234 ± 172 (40-684) 49	14 ± 21 (0.6-99) 37	*Das et al., 2004* (1994-2001)
Mean ± SD (min-max) n	29 ± 51 (1.8-292) 104	0.3 ±0.5 (<0.5-3.3) 104	38 ± 36 (2.4-37.8) 105	193 ± 106 (67-635) 105	24 ± 46 (1.9-311) 105	*This study* (2006-2013)

Comparison between porpoises in 2 different regions:
➢ Hepatic (*Fig. 6*) and renal (*Fig. 7*) metals determined in the Southern North Sea (2009-2013) and the Bay of Biscay (2009-2012) → No interregional variability.

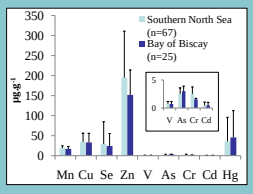

Fig. 6. Mean metal liver concentration (µg.g^{-1}d.w.)

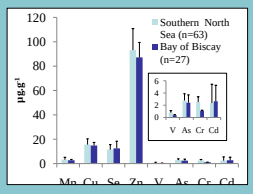

Fig. 7. Mean metal kidney concentration (µg.g^{-1}d.w.)

POPs

Association between contaminants and health status:
➢ Region 1, Porpoises that died of infectious disease displayed higher concentrations of PCBs compared to healthy porpoises that died of physical trauma (*Fig. 8*).
➢ DDXs → same level

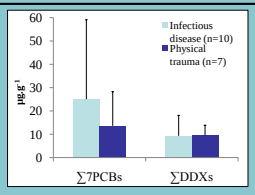

Fig. 8. Mean POPs and DDXs concentrations (µg.g^{-1} lipid) in blubber of porpoises from Southern North Sea

Association between contaminants and maturity status:
➢Region 1, juveniles showed higher concentrations of POPs compared to adults (*Fig. 9*)
(Kruskal-Wallis followed by DUNN; p<0.05 for DDXs)
➢ Previous studies → adults showed higher concentrations
(Weijs et al., 2009 and 2010)

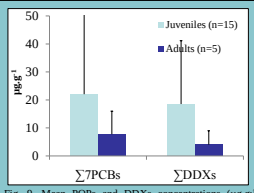

Fig. 9. Mean POPs and DDXs concentrations (µg.g^{-1} lipid) in blubber of porpoises from Southern North Sea

Multivariate analysis:
➢ Confirmation of a positive correlation between Hg, Se, Cd and adult status
➢ POPs → may be correlated to juvenile status
➢ infectious disease → higher levels of metal contaminants
(*Fig. 10*)

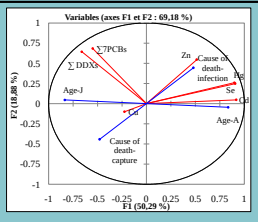

Fig. 10. PCA based on metals (Cadmium (Cd), Copper (Cu), Mercury (Hg), Selenium (Se) and Zinc (Zn) , POPs (∑7PCBs and ∑DDXs), maturity status (Adults (Age-A) and juveniles (Age-J) and cause of death (infectious disease (Infection) and physical trauma (capture)

Conclusion

➢ Exposure to chemical contaminants may influence harbour porpoise health status
➢ Chemical contamination may not be the principal cause of the rise of harbour porpoise stranding
➢ Feeding ecology studies are needed to explain the southward shift of the population (Hammond et al., 2013) → Which may explain the significant rise of porpoises stranding

References

➢ Das, K., et al., Mar .Ecol. Prog. Ser. 281 (2004) 283-295.
➢ Weijs, L., et al., Environ.Int.35 (2009) 893-899.
➢ Weijs, L., et al., Sci. Total Environ. 409 (2010) 228-237.
➢ Hammond, P.S., et al., Biol. Cons. 164 (2013) 107-122.

We are thankful to the "Grand Port Autonome de Dunkerque" for his contribution to this study

Annex 4

Does prey availability influence harbour porpoises (*Phocoena phocoena*) diet, abundance and distribution?

(Poster)

Mahfouz C., Henry F., Meziane T., Caurant F., Pezeril S., Bouveroux T., Jauniaux T., Khalaf G., Amara R.

28th Annual Conference, European Cetacean Society, Liège, Belgium, 5-9 April 2014

Does prey availability influence Harbour porpoises (*Phocoena phocoena*) diet, abundance and distribution?

Céline Mahfouz [1,2,3,4*], Françoise Henry [1,2,3], Tarik Meziane [5], Florence Caurant [6], Sylvain Pezeril [7], Thibaut Bouveroux [7], Thierry Jauniaux [8], Gaby Khalaf [4], Rachid Amara [1,2,3]

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[3] CNRS, UMR 8187, F-62930 Wimereux, France ; [4] CNRS, National Centre for Marine Sciences, Batroun, Lebanon
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[6] Littoral Environnement et Sociétés, UMR 7266 CNRS – Université La Rochelle, 2 Rue Olympe de Gouges, F-17042, La Rochelle Cedex 01, France
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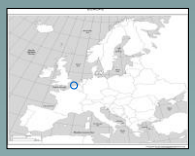
Laboratoire d'Océanologie et de Géosciences – UMR LOG 8187

Introduction

➢ The European large scale surveys SCANS (1994) and SCANS II (2005) have highlighted a marked shift in the distribution of harbour porpoises from the northern part of the North Sea toward its eastern part (Hammond et al. 2013).
➢ Alongside, the last few years the Southern North Sea and the English channel have witnessed an increase in the number of stranded harbour porpoises.
➢ We investigated whether the prey availability may explain the shift of porpoises distribution?



Study area: porpoises stranded in the southern North Sea along French and Belgian coasts

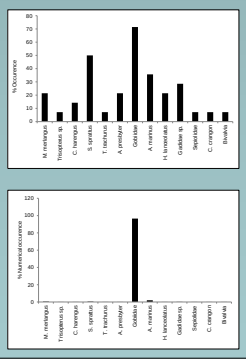
Material and Methods

Short → Intermediate dietary information

Schematic representation of methods used for diet analysis and corresponding dietary information

Results and discussion

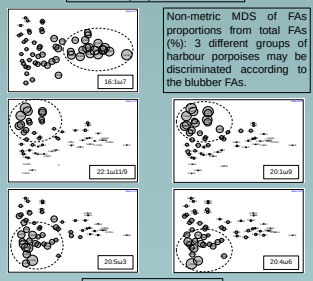
Stomach content analysis | Fatty acids analysis (blubber) | Stable isotopes analysis (muscle)



Frequency of occurrence (%O) and numerical occurrence (%N) of fish species in 14 stomachs of harbour porpoises stranded between 2012 and 2013.

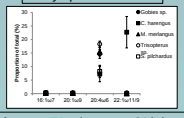
Based on the stomach content analysis, the most frequently occurring species (%O) in the diet of harbour porpoises are gobies followed by European sprat, lesser sand eel, gadidae and whiting.
Gobies are the most abundant in term of fish number (%N).

Harbour porpoises blubber



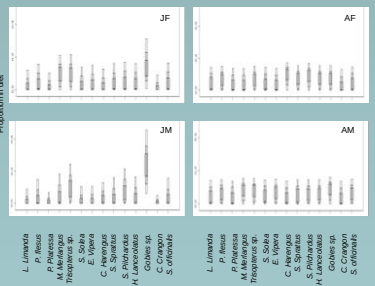
Non-metric MDS of FAs proportions from total FAs (%): 3 different groups of harbour porpoises may be discriminated according to the blubber FAs.

Prey species muscle



Proportions of some FAs (mean ± SD) in prey species muscle.

Based on the FAs analysis, harbour porpoises (group B) are feeding on herring. This species is known to feed on calanus copepod whose FA trophic markers are 22:1ω11 and 20:1ω9.



Boxplots of the contribution of prey sources to the diet of harbour porpoises as modeled by SIAR. Credible intervals (CI): dark grey CI_{50}; medium grey CI_{75}; light grey CI_{95}. TEFs by Caut et al. (2011).

Based on the stable isotope analysis, gobies are the principal feeding source of juvenile harbour porpoises followed by pouting and whiting.
For adult porpoises, all sources have almost same proportions.

➢ The feeding ecology of harbour porpoises was studied combining 3 complementary methods. Each method gave different dietary informations.
➢ Gobies, herring and whiting were the most 3 relevant prey species in the diet of porpoises. There are about 1900 species of gobies in the southern North Sea with very little information available on their distribution and abundance. Herring have witnessed a collapse in the late 1970s followed by a stock recovery by the mid 80s (Simmonds, 2007). Finally whiting is one of the most numerous and widespread species in the North Sea.
➢ According to the feeding ecology of harbour porpoises, we suggest that the shift of their distribution might not be related to the availability of prey species. The prey species that were found to contribute to the diet of porpoises were always numerous and widespread in the North Sea the last few years. Therefore, further investigations are needed to better understand that shift.

References

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The samples used in this study were collected by the French Stranding Network. We are thankful to the "Grand Port Autonome de Dunkerque" for his contribution to this study

Evaluation de la contamination chimique et changement du régime alimentaire des marsouins communs (*Phocoena phocoena*) échoués au sud de la mer du Nord

De par l'importance et la diversité des pressions anthropiques en mer du Nord, les mammifères marins en tant que prédateurs supérieurs se trouvent de plus en plus exposés à ces activités (trafic maritime, activités industrielles et portuaires, surpêche, pollution chimique, etc.). Les campagnes d'observation SCANS et SCANS II mises en place en 1994 et 2005 pour estimer l'abondance des petits cétacés, ont mis en évidence un changement majeur dans la distribution du marsouin commun (*Phocoena phocoena*) en mer du Nord avec un déplacement du Nord vers le Sud. Ce changement peut être lié à une migration de ses proies préférentielles en mer du Nord et/ou à une adaptation de son régime alimentaire par rapport à la disponibilité des proies. Parallèlement à ces modifications de distribution spatiale, un nombre croissant d'échouages de marsouin commun en Manche Orientale et sur les côtes belges a été observé depuis une dizaine d'années avec une augmentation conséquente ces deux dernières années. Pour étudier les causes responsables de ces échouages, un des objectifs de cette étude a été d'évaluer l'état de la contamination chimique des animaux échoués en relation avec l'état sanitaire des individus. L'analyse de deux familles de composés chimiques (éléments traces métalliques et polluants organiques persistants) sur des animaux échoués sur la période 2006-2013 révèlent des concentrations significativement plus élevées dans les organes des animaux présentant des pathologies que celles obtenues chez les animaux sains. Cette tendance a déjà été observée en Atlantique Nord pour le marsouin. Les comparaisons des niveaux de concentration mesurées avec ceux d'études antérieures effectuées sur des marsouins échoués dans la même zone ou dans le Golfe de Gascogne suggèrent que l'augmentation du nombre de marsouin échoué n'est pas liée à une dégradation du milieu en termes de pollution chimique.

Pour déterminer le régime alimentaire du marsouin commun, trois techniques complémentaires ont été utilisées : l'analyse des contenus stomacaux, des isotopes stables (carbone et azote) et des acides gras dans différents tissus. Pour ces deux dernières méthodes, les signatures obtenues pour le marsouin ont été comparées à celle de leurs proies potentielles. Les résultats ont mis en évidence la présence des gobies, merlan, lançon, sprat, trisopterus sp., hareng et sardine comme proies potentielles. Le déplacement des marsouins du nord jusqu'au sud de la mer du Nord a été attribué à la baisse de l'abondance du lançon dans le nord, ainsi qu'à la ré-invasion du sud de la mer du Nord par la sardine, probablement suite au changement climatique. Enfin cette étude confirme la nécessité d'utiliser une approche multi-analyses qui intègre des informations complémentaires à différentes échelles de temps pour étudier le régime alimentaire de ces prédateurs supérieurs.

Mots-clés: marsouin commun, sud mer du Nord, contamination chimique, régime alimentaire, contenus stomacaux, isotopes stable, acides gras

An assessment of the chemical contamination and the diet changes of the harbour porpoise (*Phocoena phocoena*) stranded along the southern North Sea

The North Sea is heavily impacted by human activities such as overfishing and pollution. Due to their position as top predators in the ocean, marine mammals are becoming increasingly affected by anthropogenic activities. The large-scale surveys SCANS in 1994 and SCANS II in 2005 that were held in the North Sea to estimate the abundance of small cetaceans highlighted a major shift in the distribution of harbour porpoises (*Phocoena phocoena*) from the northern parts of the North Sea to its eastern parts. Alongside, over the past few decades harbour porpoises stranding has increased in the southern North Sea particularly along the French and Belgian coastal waters. Since the contaminant exposure presents, among others, a potential threat to harbour porpoises inhabiting the North Sea, the first objective of the present study was to assess the contamination status of this species in the southern North Sea. On the other hand, the distribution and abundance of marine mammals is expected to follow the distribution of their main prey species. Hence, the second objective of this study was to investigate whether the changes in the distribution of porpoises in the southern North Sea may be a result of the changes in prey availability. Moreover, the third objective was to evaluate the interest of combining three methods to investigate the diet of harbour porpoises: stomach contents, stable isotopes (carbon and nitrogen) and fatty acids analyses.

First, the contamination status was evaluated through the determination of two components of chemical contaminants (metals and persistent organic pollutants) in tissues of harbour porpoises stranded along the southern North Sea between 2006 and 2013. Several chemical contaminants presented higher concentrations in diseased animals compared to healthy animals. In addition, some metallic contaminants showed bioaccumulation with age. Comparison with previous study suggests that the population status of harbour porpoises in term of chemical concentration has been stable from 1994 to 2013. This work suggested that the increase in the number of stranded individuals is not related to the decline in the quality of the environment.

Secondly, the shift in the abundance of harbour porpoises was evaluated and interpreted in the light of prey species abundance. Three techniques were used in order to determine the diet of porpoises. Results highlighted the presence of gobies, whiting, sandeel, sprat, trisopterus sp., herring and sardine as potential preys. The shift of the abundance of porpoises form the northern parts of the North Sea to its southern parts was attributed to the sandeel abundance decline in the northern parts of the North Sea along with the re-invasion of the southern North Sea by the sardine species, probably in response to climate change.

Finally, the value of a multi-approach dietary analysis was evaluated. Besides overcoming the limitations of each method, combining different techniques that integrate diet over days and weeks allowed gaining more complete understanding of harbour porpoise's diet.

Keywords: harbour porpoise, southern North Sea, chemical contamination, diet, stomach contents, stable isotopes, fatty acids