Individual and environmental drivers of the foraging behaviour in a long-lived coastal seabird

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Université de Strasbourg

Faculté des Sciences de la Vie

Individual and environmental drivers of the foraging behaviour in a long-lived coastal seabird

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Dr. Yan Ropert-Coudert	Chargé de recherche, CNRS, Strasbourg	Directeur de thèse
Dr. André Chiaradia	Chercheur, PINP, Australie	Co-directeur de thèse

I was raised up believing I was somehow unique
Like a snowflake distinct among snowflakes, unique in each way you can see
And now after some thinking, I'd say I'd rather be
A functioning cog in some great machinery serving something beyond me

But I don't, I don't know what that will be
I'll get back to you someday soon you will see

What's my name, what's my station, oh, just tell me what I should do
I don't need to be kind to the armies of night that would do such injustice to you
Or bow down and be grateful and say "sure, take all that you see"
To the men who move only in dimly-lit halls and determine my future for me

And I don't, I don't know who to believe
I'll get back to you someday soon you will see

If I know only one thing, it's that everything that I see
Of the world outside is so inconceivable often I barely can speak
Yeah I'm tongue-tied and dizzy and I can't keep it to myself
What good is it to sing helplessness blues, why should I wait for anyone else?

And I know, I know you will keep me on the shelf
I'll come back to you someday soon myself

If I had an orchard, I'd work till I'm raw
If I had an orchard, I'd work till I'm sore
And you would wait tables and soon run the store

Gold hair in the sunlight, my light in the dawn
If I had an orchard, I'd work till I'm sore
If I had an orchard, I'd work till I'm sore
Someday I'll be like the man on the screen

Helplessness Blues– Fleet Foxes

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Publications

Articles included in the thesis

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Article B : Pelletier L, Kato A, Ropert-Coudert Y, Chiaradia A. Long-term linkage of the thermal structure of the water column with the foraging behaviour of a coastal seabird. (in preparation).

Article C: Pelletier L, Chiaradia A, Kato A, Ropert-Coudert Y. Fine-scale spatial segregation in the limited foraging area of an inshore seabird species; the effect of age and sex. (to submit to *Ecology*).

Article D: Pelletier L, Ropert-Coudert Y, Kato A, Stier A, Reichert S, Chiaradia A. The cost of having chicks: foraging and oxidative stress in a freely living seabird increase as chicks grow. (to submit to *Functional Ecology*)

Article in annexe

Article E: MacIntosh AJJ, **Pelletier L**, Chiaradia A, Kato A, Ropert-Coudert Y. **2013**. Temporal fractals in seabird foraging behaviour: diving through the scales of time. Scientific Report. 3, 1884; DOI:10.1038/srep01884

Communications

Scientific communication: poster & oral presentations to international congress

Oral presentation

To come: Pelletier L., Ropert-Coudert, Y, Kato K., Stier A, Reichert S, Chiaradia A. Foraging strategies and oxidative stress – Consequences of growing chick on foraging strategies of the little penguin (*Eudyptula minor*). 8th Internationl Penguin Conference. September 2013. Bristol, United-Kingdom (*Oral Communication)*

MacIntosh AJJ, **Pelletier L**, Cottin M, Kato A, Ropert-Coudert Y. Bio-Logging for fractal patterns in penguin behaviour: diving into the world of bio-complexity. PRI Student seminar. **December 2011**. Kyoto, Japan. (*Oral communication*).

Pelletier L, Kato A, Chiaradia A, Ropert-Coudert Y. Can thermoclines be a cue to distribution of prey for little penguins? 4th International Science Symposium on Bio-logging. **March 2011**. Hobart, Australia. (*Oral Communication*).

Poster presentation

Pelletier L, Ropert-Coudert Y, Kato A, Chiaradia A. Environmental conditions drive foraging behaviour. 9th Ecology and Behaviour Annual Meeting (SERL). **April 2013**. Strasbourg, France (*Poster presentation)*.

Pelletier L, Kato A, Chiaradia A, Ropert-Coudert Y. Age-specific spatial segregation in the foraging zone of little penguins. 14th Congress of the International Society for Behavioural Ecology. **August 2012**. Lund, Sweden. (*Poster presentation*).

MacIntosh AJJ, Cottin M, **Pelletier L**, Kato A, Ropert-Coudert Y. Primates, penguins, and periodicity: maintaining complexity in the face of ecological challenge. 14th Congress of the International Primatological Society. **August 2012**. Cancun, Mexico. (*Poster presentation*).

Scientific animations and other activities

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- Participation to "Fête de la Science" (annual science fair in France) 2012: general topic '*L'énergie*', Palais Universitaire, Strasbourg (12-14 October 2012)

Press Releases:

- '*Le manchot pygmée utilise les variations de température de l'eau pour mieux pêcher*' Press release from the CNRS, 23 April 2012 (http://www.cnrs.fr/inee/communication/breves/laure_pelletier.htm)

- '*Le manchot pygmée pêche entre deux eaux*' article in "Science et Vie", July 2012.

TABLE OF CONTENTS

CHAPTER 4 : INDIVIDUAL CHARACTERISTICS AND FORAGING BEHAVIOUR 89

LIST OF FIGURES

General Introduction

General Materials and Methods

Article A

thermocline is framed in bold. The seabed is situated between 60 m (dotted, horizontal grey line) to 80 m (solid, solid, horizontal grey line). We only represented the dive/temperature profiles of those birds that dived deeper than 25 m (see Materials and Methods for details).

Figure 2: Changes of the hunting efficiency during the five weeks. Mean hunting efficiency 6SE (see Materials and Methods) of penguins for each week is represented. Letters a, b and c indicate significant differences (0.05) following GLMM-binomial and Tukey's post hoc tests. n = number of birds.

Article B

Figure 1: The 4 categories of water column defined from the Figure 3 and used for the statistical analysis.

Figure 2: Observation of the evolution of the sea surface temperature (SST) and the difference of temperature between the surface and 50 m (ΔT) over 5 breeding seasons. Boxes defined the sampling interval for each year.

Figure 3: Temperature/depth profiles calculated with the accelerometer temporally attached to the lower back of little penguin over the 5 breeding seasons. Data were presented in function of the timing in a season to see evolution of the structure of a water column.

Figure 4: The number of dives performed by the birds in the different water column conditions. The different letters indicates significative differences (0.05)

Figure 5: The median of dives depth performed by the birds in the different water column conditions. The different letters indicates significative differences (0.05)

Figure 6: proportion of time spent at the bottom of dives in the different water column conditions. The different letters indicates significative differences (0.05)

Figure 7: Hunting efficiency in the different water column conditions. The different letters indicates significative differences (0.05)

Figure 8: The total duration of prey pursuit in a one-day foraging trip (in min) in the different water column conditions. The different letters indicates significative differences (0.05)

Figure 9: The average duration of a prey pursuit during a dive (in sec) in the different water column conditions. The different letters indicates significative differences (0.05)

Article C

Figure 1: Distribution of little penguins in their foraging zone according to age and sex. Kernel density estimates zone of high prey encounter, (a) 25% and (b) 50% of the foraging

Article D

General Discussion & Conclusion

LIST OF TABLES

Article A

Article B

Article C

Article D

General Discussion & Conclusion

Abstract of the Thesis in French

Individual and environmental drivers of the foraging behaviour in a long-lived coastal seabird

Les bouleversements climatiques et les activités humaines ont profondément touché les différents écosystèmes. Ces modifications jouent sur la dynamique des populations d'un grand nombre d'espèces, jusqu'à, dans certain cas, en accélérer la disparition. Bien que l'écosystème marin semble répondre plus rapidement aux changements climatiques que les autres écosystèmes, il est paradoxalement celui qui est le moins étudié dans le cadre de cette thématique. Pour étudier l'impact des bouleversements climatiques sur cet écosystème, il est nécessaire d'utiliser des espèces indicatrices de ces changements (i.e. des indicateurs écologiques). Les espèces placées au sommet des chaînes trophiques intègrent ainsi les modifications qui ont lieu sur chaque maillon de cette chaine. Les prédateurs, tels que les oiseaux ou mammifères marins, qui reviennent à terre de manière périodique pour la saison de reproduction, peuvent donc servir d'outil adéquat pour diagnostiquer l'état de cet écosystème. Afin de comprendre comment les espèces animales réagissent aux changements de l'environnement, il est important de séparer et de comprendre l'influence des caractéristiques propres à l'individu sur les différentes activités individuelles (tels que les comportements de reproduction et de recherche et prise de nourriture). Cela est devenu envisageable grâce aux développements de nouvelles technologies qui permettent de suivre les activités des animaux en mer (*Bio-logging*). Il est possible d'obtenir des informations concernant les efforts produits par les individus lors de la recherche alimentaire. Pour exemple, les données récoltées par le manchot pygmée (*Eudyptula minor*) ont permis de fournir aux chercheurs une estimation de l'effort produit par cet oiseau lors de la recherche alimentaire, mais également une idée approximative de la disponibilité des proies. Cette espèce est l’indicateur écologique le plus important du détroit de Bass qui est situé dans le sud de l’Australie. Dans ce contexte, le but de ma thèse est d’examiner l’influence des paramètres physiques de l’écosystème sur l’activité de recherche alimentaire en mer des manchots pygmée. Il s’agira également de voir si le statut individuel de ces oiseaux joue sur la mise en place de stratégies alimentaire et reproductrice en fonction des conditions changeantes du milieu.

On peut s’attendre à ce que (1) des oiseaux d’âge, de sexe, et/ou d’expérience différentes adoptent des comportements différents et que (2) l’efficacité des stratégies mises en place soit d’autant plus importante que l’individu acquerra de l’expérience avec l’accumulation des saisons de reproduction. (3) En outre ces stratégies vont s’adapter aux conditions océanographiques du moment, comme la disponibilité ou l’absence de certaines proies.

Le projet a été mené au Phillip Island Nature Park en Australie, en collaboration avec le Dr. A. Chiaradia, avec qui le Dr. Y. Ropert-Coudert de l'Institut Pluridisciplinaire Hubert Curien collabore depuis 2004. Grâce à un système d’identification automatique mis en place depuis 1999 nous avons eu accès à des informations sur les saisons de reproduction passées des individus ainsi qu’à l’âge, au sexe et au statut reproducteur de chaque sujet. J’ai récolté des données sur les activités de recherche alimentaire ont été récoltées entre septembre et décembre 2010 et 2011. Des prises de sang ont également été effectuées pour mesurer les variations du ratio des isotopes stables du carbone et de l’azote dans les échantillons de sang, cela afin d’identifier le type de proies ingérées par les manchots. Toujours à partir des échantillons de sang, les mesures du taux de radicaux libres et des barrières anti-oxydantes ont mis en évidence l’état du statut oxydatif de chaque individu. Celui-ci est un indicateur de l’impact de la reproduction sur la condition physiologique de l’organisme. Le statut oxydatif, avec en parallèle une mesure de la longueur des télomères, serviront ici de proxys à la qualité individuelle. Pour déterminer l’activité en mer, des accéléromètres miniatures ont été attachés temporairement sur le dos de certains manchots. J’ai ainsi pu obtenir des informations sur l’effort de nage de ces individus ainsi qu’un indice de la fréquence de rencontre avec leurs proies. J’ai ensuite croisé ces informations avec les conditions océanographiques à fine échelle grâce à l’enregistrement de la température des masses d’eaux où se trouvaient les oiseaux. Des GPS ont également été employés pour localiser les individus en mer et déterminer leur rayon d’action.

La première étude (article A) étudie plus en détails l'évolution de la structure thermique de la colonne d'eau et du comportement de recherche alimentaire au cours d'une seule saison de reproduction. Sur une période de 5 semaines, la colonne d'eau change de manière importante. Lors des trois premières semaines une thermocline est observée, variant à des profondeurs différentes. Au cours de la deuxième semaine, elle est notamment très peu profonde dans la colonne d'eau. Lors des deux dernières semaines elle a disparu des profils de température, il s'en suit alors un changement dans le comportement de recherche alimentaire, mais également une diminution du succès de pêche. L'article suivant (article B) cherchait donc à mettre en évidence l'influence des caractéristiques thermiques de la colonne d'eau sur le comportement de recherche alimentaire des manchots. Pour cela, des données récoltées au cours de 5 saisons de reproduction successives ont été analysées. Les données de température de l'eau de surface, la différence de température entre 0 et 50m de profondeur et la présence d'une thermocline dans la colonne d'eau sont des facteurs qui varient au cours de ces 5 saisons. L'effort et le succès de recherche alimentaire ont été caractérisés pour tous les oiseaux (n= 61). Il a pu être montré que la différence de température n'affecte pas le comportement de plongée. À l'inverse, la présence d'une thermocline est finalement le paramètre qui influence le plus la recherche de proies. En effet, le nombre de plongées effectuées lors du voyage alimentaire est bien plus important quand la thermocline est présente. Cette thermocline affecte la localisation des proies dans la colonne d'eau ce qui permet aux individus de faire des plongées moins profondes, de passer plus de temps dans la phase de fond des plongées et ainsi de réduire l'effort de recherche.

Les articles C et D sont consacrés aux aspects individuels qui peuvent également affecter le comportement de recherche alimentaire. On peut s'attendre à ce que des adultes reproducteurs plus jeunes (dans leurs deux premières années de reproduction) adoptent un comportement qui les rendent moins efficaces que leurs ainés plus expérimentés. La première étude (article C) montre que les oiseaux d'âges médians (entre 5 et 11 ans) n'utilisent pas les mêmes zones de recherche alimentaire que les oiseaux plus âgés (au-delà de 11 ans), ce qui se traduit par une ségrégation spatiale entre les deux groupes. Cependant le comportement de plongée, ainsi que le succès de pêche, ne sont pas différents entre les trois classes d'âges. Sachant que les manchots quittent la colonie en petits groupes pour diminuer la pression de prédation et que l’âge est un facteur important dans la formation de ces associations, on pourrait penser que ces groupes perdurent en mer. Ainsi les oiseaux décideraient de fourrager dans des zones où se trouvent des individus de même classe d’âge et qui possèdent les mêmes besoins. Le dernier article (Article D) porte sur l’importance de la condition physiologique des individus, ainsi que l’influence de la croissance du poussin sur les décisions de recherche alimentaire. Ainsi avec l’âge des poussins, et donc l’augmentation de la demande en énergie de ces derniers, les parents vont adapter leur comportement de recherche alimentaire en fonction de leurs besoins, sans pour autant que l’on puisse observer de différence entre les mâles et femelles ou de leur âge. En effet, on peut observer qu‘au cours de la croissance du poussin les individus vont augmenter leur effort de recherche alimentaire, ayant pour conséquence une augmentation du stress oxydant. Les individus négligent l’investissement dans leurs maintenances propres lors des périodes à forte demande énergétique. Cela laisse à penser que l’état oxydatif des individus pourrait avoir un impact sur la décision d’un individu d’arrêter la période intense d’élevage des poussins et de commencer à restaurer ses propres réserves dans le but de ne pas impacter ses chances de survie, mais également les reproductions futures.

Les deux articles C et D ne montrent pas d’effet direct des caractéristiques individuelles, tel que l’âge et le sexe, sur le comportement et le succès de recherche alimentaire. Cependant, ces deux articles soulignent un facteur important à prendre en compte: l’âge du poussin. En effet, sur une petite période du cycle reproducteur, la croissance du poussin va fortement affecter le comportement de recherche alimentaire ainsi que l’état physiologique des parents.

En conclusion, nous avons vu que les individus adaptaient leurs comportements de recherche alimentaire face aux conditions changeantes de l’environnement, dans la limite de leurs possibilités physiques et physiologiques. La présence d’une thermocline dans la colonne d’eau permet aux individus de diminuer leurs efforts de recherche alimentaire, et donc d’avoir un meilleur succès. Cette adaptation du comportement de recherche alimentaire avec les conditions du milieu suit la distribution des proies affectées par les changements de la structure thermique. Le comportement de recherche alimentaire et le succès associé des oiseaux sont les reflets de l’état de la chaîne trophique en amont, faisant du manchot pygmée un indicateur écologique pertinent de l’écosystème qu’il exploite. Cela souligne également la nécessité qu’ont les individus de bien coordonner leurs efforts

reproducteurs avec les conditions du milieu, c'est à dire la disponibilité des proies. Cependant au sein d'une saison, il a pu également être montré des variabilités dans le comportement qui n'étaient pas forcément liées aux conditions du milieu. Ces variabilités ne sont pas nécessairement liées à l'âge ou au sexe des individus, mais elles peuvent être dues à la position de l'individu dans son cycle reproducteur. Finalement, les poussins semblent être le paramètre qui va influencer le plus le comportement des individus lors des voyages alimentaires. Afin de s'affranchir de l'effet du poussin et ainsi d'examiner l'influence des caractéristiques individuelles, il serait aussi intéressant d'étudier le comportement de pêche des oiseaux lors de la période d'incubation. Il serait notamment intéressant de voir comment ces stratégies de recherche alimentaire évoluent chez un individu au cours de l'ensemble de la saison de reproduction, en fonction notamment de paramètres physiologiques tel que le statut oxydatif, pour une meilleure compréhension des mécanismes impliqués dans les compromis entre survie et reproduction chez les oiseaux marins longévifs.

CHAPTER 1 : GENERAL INTRODUCTION

General Introduction

I. Environmental changes

1. Global Change

The report from the Experts from Intergovernmental Panel on Climate Change (IPCC) of 2007 is unequivocal: the climate is undergoing complex changes, with a notable increase in the global atmospheric temperature (IPCC 2007). All ecosystems, cryosphere, hydrology and water resources, marine and freshwater biological systems, terrestrial biological systems, agriculture and forestry, are affected (IPCC 2007). The cumulative, negative effects of climatic variations and anthropogenic actions are less predictable, particularly at a local scale, i.e. for species with low range of mobility (Beniston 2009). As such, species composition of different communities has changed in many parts of the world (Walther et al. 2002). These modifications are partly due to invasion, i.e. introduction of non-native species that over-compete the native ones, and/or extinction or immigration/emigration.

Emerging evidence suggests that aquatic systems may be extremely vulnerable to climate change and that response of marine organisms to climate change may be faster than that of on-land species, despite slower ocean warming rates (Richardson & Poloczanska 2008). However the Fourth Assessment Report of the IPCC signals a dichotomy between research on marine and terrestrial biological impacts (Figure 1; Richardson & Poloczanska 2008). The amount of knowledge accumulated on terrestrial-based changes does not help understanding changes occurring in marine environments. Understanding impact of ocean changes will require exclusive research focused on marine investigation to improve marine coverage in the IPCC process (Richardson & Poloczanska 2008).

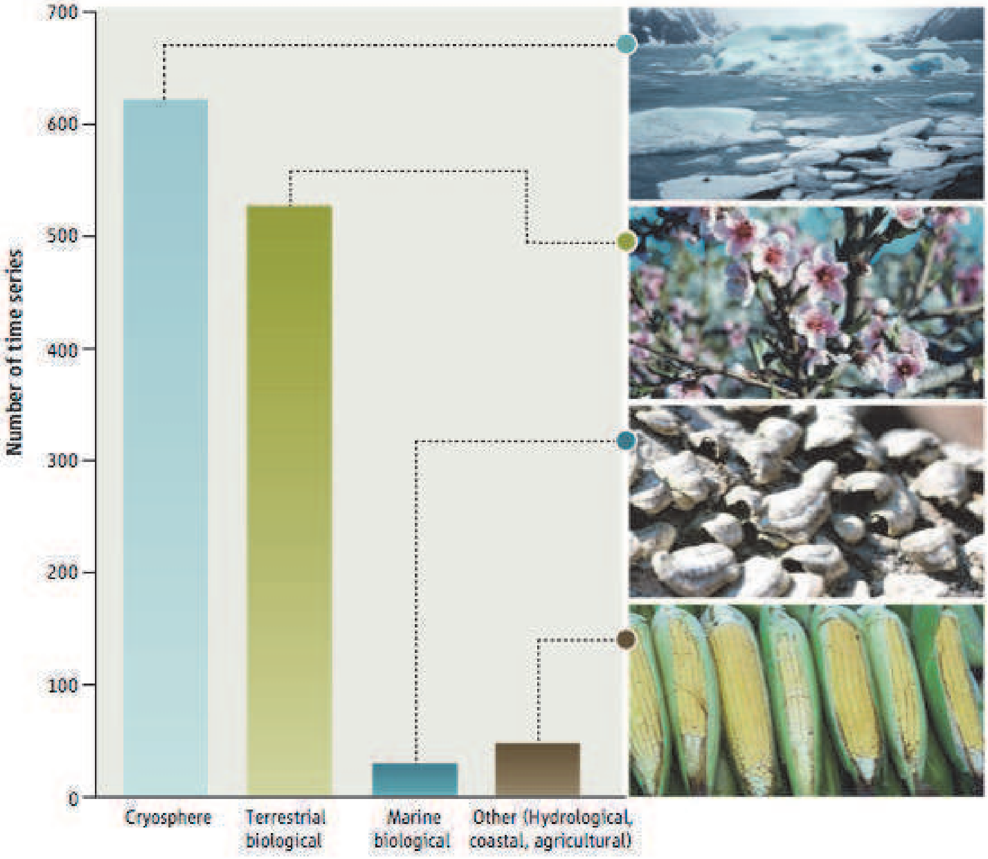


Figure 1: The number of time series from different environments included in the Fourth Assessment Report of the IPCC (2007) (figure from Richardson & Poloczanska 2008)

2. Changes affecting the marine and coastal environments

The marine pelagic ecosystem occupies 70% of the planetary surface and plays a fundamental role in modulating the global climate, as well as regulating biogeochemical cycles (Legendre & Rivkin 2002). Oceans are undergoing drastic changes at both large and local scales.

a. Global, climate-driven changes

The increasing trend observed in air temperature worldwide is mirrored in the increase in sea surface temperature (SST). Ocean warming has already exceeded the range of its natural variability (Field et al. 2006). From 1901 to 2011, SST rose at an average rate of 0.1°C per decade (Figure 2; Gordon et al. 2000; Lima & Wethey 2012). This SST increase affects the circulation patterns of the currents that transport warm and cold waters around the globe (Gordon et al. 2000). The increase in SST would also increase the amount of atmospheric water vapour that supplies weather systems and has for consequences to change the precipitation regime and to shift in storm tracks (Trenberth 2005). Anomalies in the SSTs and the atmospheric circulation are known to be associated with the El Niño Southern Oscillation phenomenon (ENSO; Klein et al. 1999; Meyers et al. 2007).

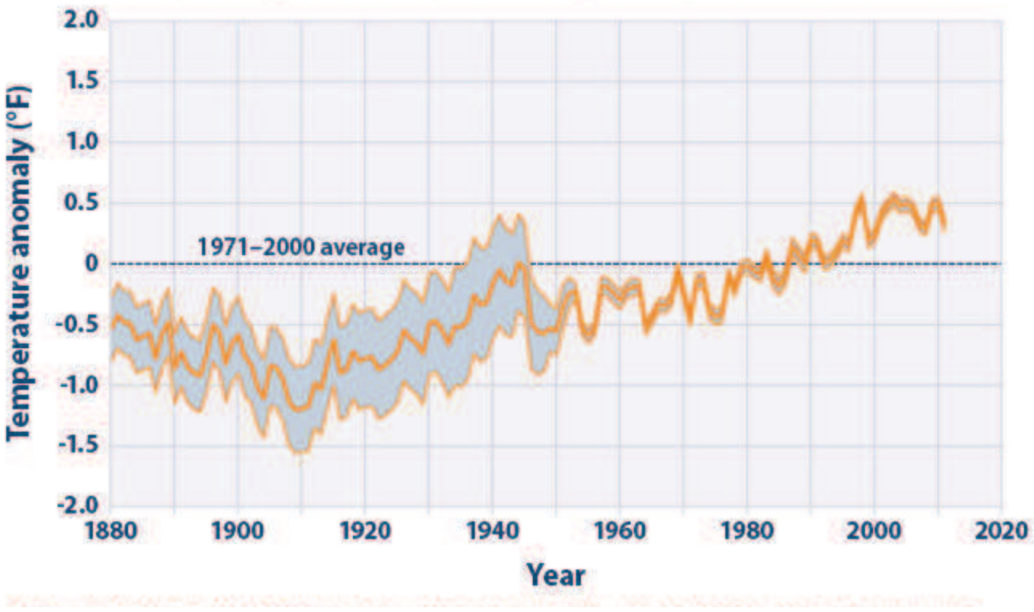


Figure 2: Average global Sea Surface Temperature anomaly 1880-2011. This graph shows how the average surface temperature of the world's oceans has changed since 1880. The shaded band shows the range of uncertainty in the data, based on the number of measurements collected and the precision of the methods used. Data Source: NOAA (National Oceanic and Atmospheric Administration). April 2012. www.ncdc.noaa.gov/ersst/

The Southern Oscillation Index (SOI), which is calculated using the pressure difference between Tahiti and Darwin in Australia (Klein et al. 1999), gives an indication of the development and intensity of El Niño or la Niña events in the Pacific Ocean (Figure 3). The El Niño episodes are distinguished by continuous negative values of SOI below -8 (Klein et al. 1999). El Niño is causing heavy rainfall in the Southern United State and severe droughts in Australia, Indonesia and Southern Asia (Klein et al. 1999; Meyers et al. 2007). La Niña episodes are distinguished by sustained positive values of SOI above +8 (Klein et al. 1999). They are associated with stronger Pacific trade winds and warmer sea temperature to the north Australia (Klein et al. 1999; Meyers et al. 2007). Moreover, during La Niña episodes waters in the central and eastern tropical Pacific Ocean become cooler during this time. Together these give an increased probability that eastern and northern Australia will be wetter than normal (Klein et al. 1999; Meyers et al. 2007).

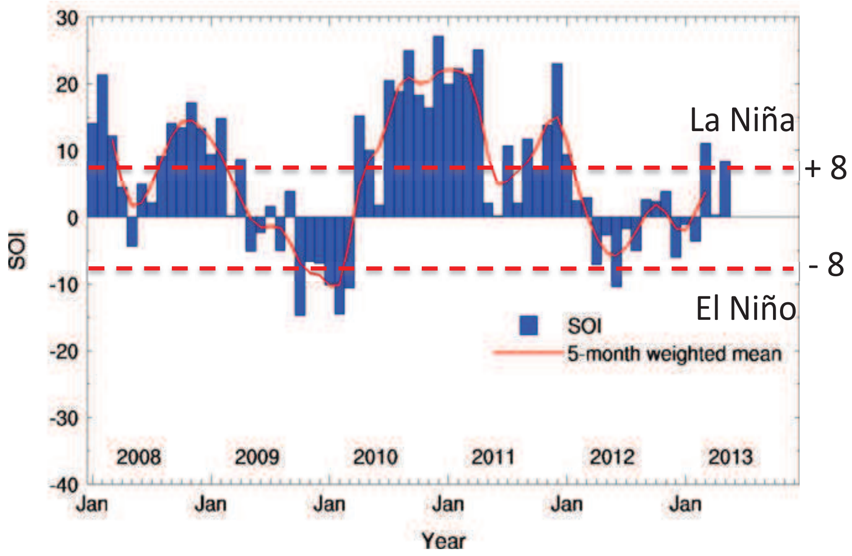


Figure 3: Southern Oscillation Index (SOI) from 2008 to 2013. Continuous positive values above +8 distinguish La Niña episodes and continuous negative values below -8 El Niño episodes. Data source: Australian Government-Bureau of Meteorology: http://www.bom.gov.au/climate/glossary/soi.shtml

An increase in the SST will thus drastically modify the biogeographical distribution, the phenology, physiology and overall abundance of all ectothermic species (Edwards & Richardson 2004). For some fish species migratory paths, time of reproduction, level of fecundity and larval survival rate will be modified by the increase or decrease of water temperature (Loeng et al. 2005).

Variation in temperature of the surface would be also accompanied by changes in the vertical temperature gradients. Indeed, the solar heating and long period of reduced wind stirring warmed up the water column, which becomes thermally stratified with the formation of a thermocline (Fiedler 2010). Thermoclines are defined as an abrupt change in water temperature with depth forming a boundary between the warm well-mixed surface layer and the much colder well-mixed deep layer (Hansen et al. 2001; Fiedler 2010). Thermoclines are, therefore, considered as an important interface for trophic dynamics, that will limit the nutrient passage, represent a suitable zone for fish spawning, as well as for egg and larvae development (Hansen et al. 2001). Moreover these highly productive zones would attract predators (Hansen et al. 2001; Biuw et al. 2007; Kokubun et al. 2010).

b. Anthropogenic, local changes

Over a third (41%) of the world's oceans is medium high to very highly impacted, both directly and indirectly, by human activities (Figure 4, Halpern et al. 2008).

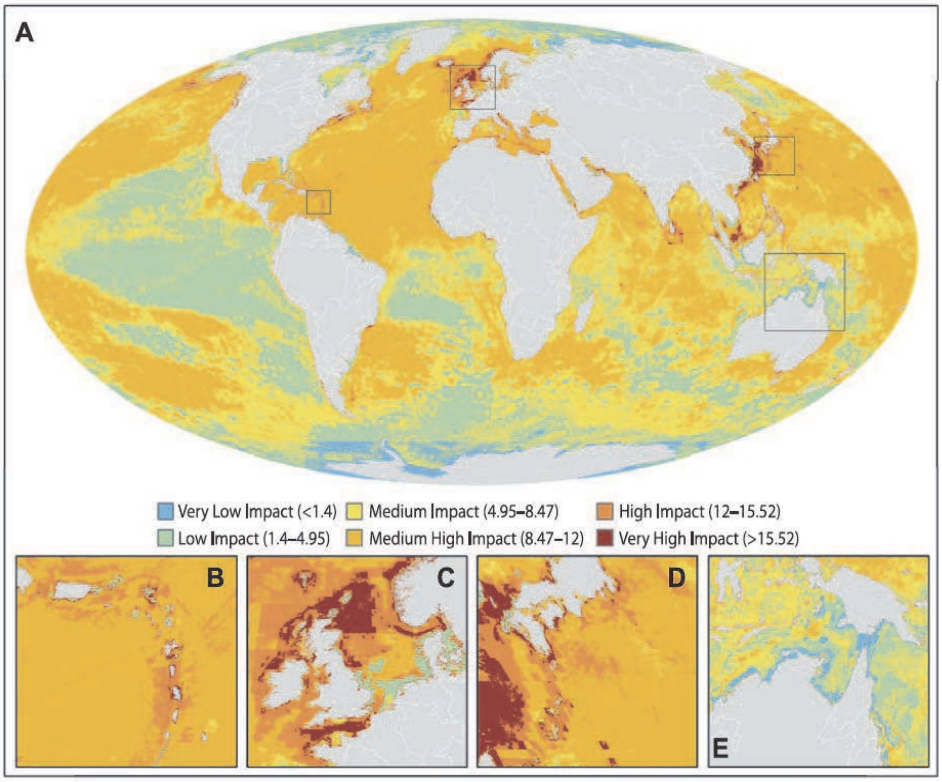


Figure 4: Global map (A) of cumulative human impact. (Insets) Highly impacted regions in Eastern Caribbean (B), the North Sea (C), and Japanese Waters (D) and one of the least impacted regions, in northern Australia and the Torres Strait (E) (figure from Halpern et al. 2008).

Anthropogenic factors impacting marine biological systems are noticed like over-fishing and pollution from terrestrial activities (e.g. deforestation, agriculture and urban development) and atmospheric deposition. Continental shelves and slopes are predicted to be the most impacted of the oceanographic systems, as they are subjected to both land- and ocean-based anthropogenic drivers (Figure 5, Harley et al. 2006; Halpern et al. 2008). IPCC scenarios predict that global changes in atmospheric circulation will also change the frequency of severe weather events like storms at regional scales, as has already been observed in some regions (Bromirski et al. 2003). These changes in the weather at a regional scale will have substantial implications for the inter-tidal and shallow sub-tidal communities that are vulnerable to hydrodynamic disturbance.

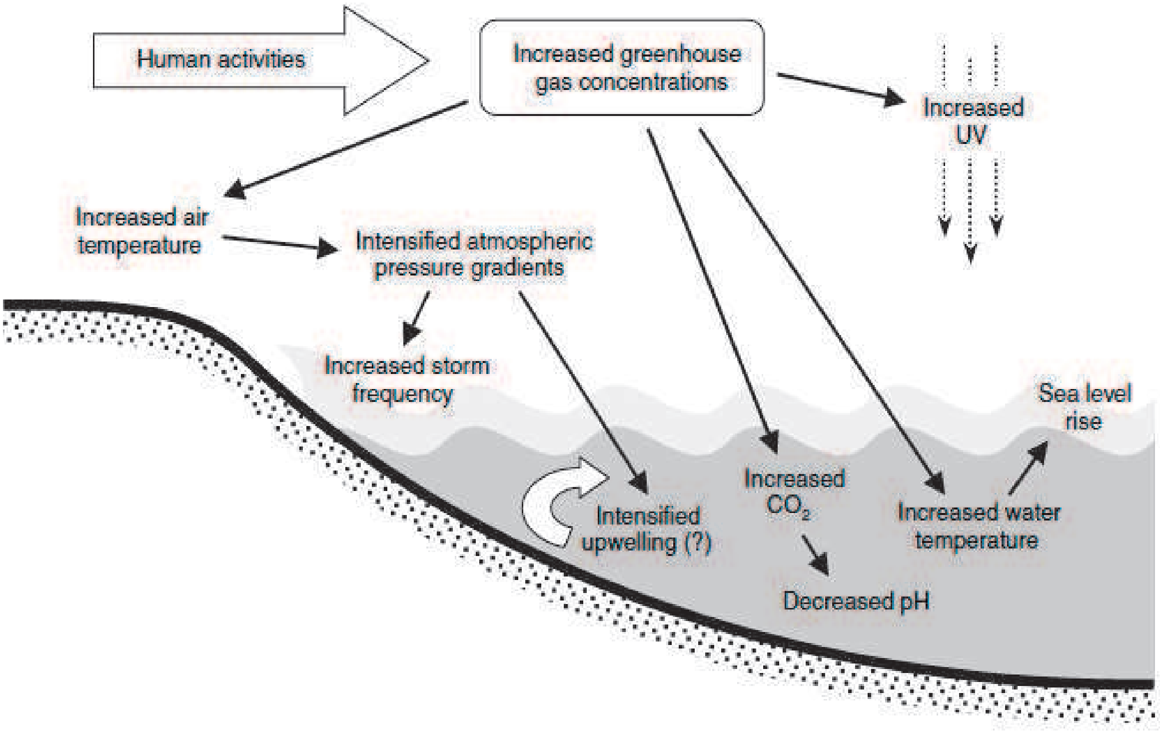


Figure 5: Important physical and chemical changes associated with climate change in coastal oceans (figure from Harley et al. 2006)

c. Effect on ecosystem

Satellite data and *in situ* records of chlorophyll *a* (i.e. an index of phytoplankton production) indicate that annual primary production has declined by more than 6% since the early 1980s in the world's oceans (Gregg et al. 2003). Variations of physical and chemical factors such as nutrient availability, light penetration, salinity and, to a lesser extent, temperature, govern primary production, i.e. phytoplankton, over the time (Belgrano 2005). Phytoplankton will serve as food to grazing species (zooplankton, fish), which will, in turn, be preyed upon by other predators (fish, seabird, marine mammal). Stability of lower trophic levels is thus affecting the predators positioned at the pinnacle of the food webs (Moloney et al. 2011; Figure 6). In the Southern Ocean, the decline in krill has been associated with the gradual warming of the ocean (Atkinson et al. 2004), as for example the sea-ice habitat of the krill breaks up earlier in the season than before. This decline in krill will have repercussions along the food webs and has often been correlated to a decrease in the size of the population of many top predators that depend on this resource, e.g seabirds and marine mammals (Figure 6; Forcada et al. 2005, 2006; Stirling & Parkinson 2006; Weimerskirch et al. 2003). The ability of these marine top consumers to adjust their strategies to fluctuations in food resources is paramount to their survival at both the individual and population levels.

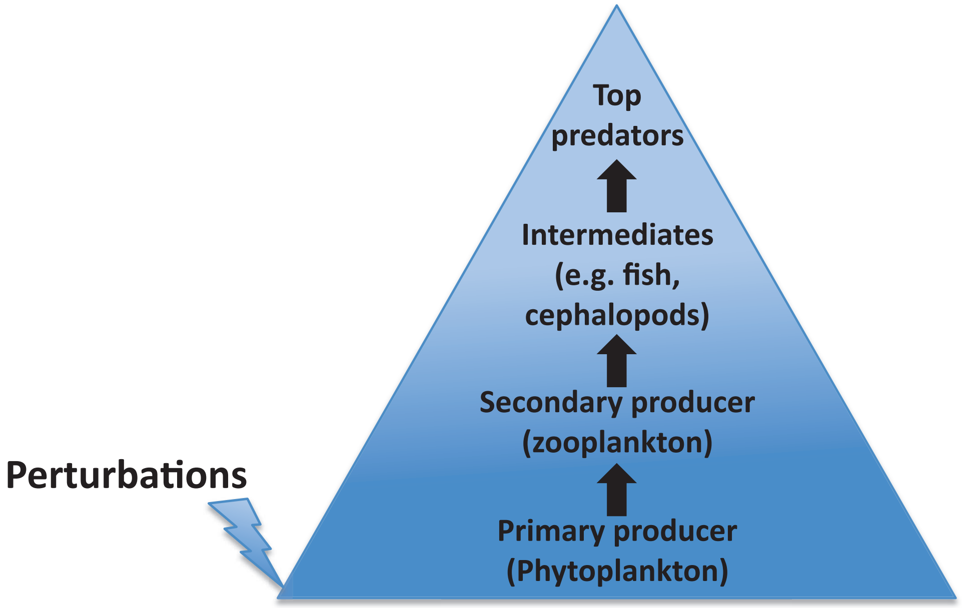


Figure 6: Integration of perturbations from the base of the marine food chain to the top.

Top-predator would have to rely on zone of high productivity and this relationship varies with the spatial scale. At large (>1000km) to meso scales (100-1000 km) the marine currents create zones of convergence where water masses with contrasted temperature and salinity conditions collapse. The upwelling movements resulting from the encounter of these water masses make these oceanographic structures a source of nutrients for primary producers. These structures, termed fronts and eddies, are relatively stable in time and space. Eddies can have a life span of several months in average (Pond & Pickard 1983) and represent therefore predictable zone of high food availability for consumers up to the highest level of the food chains (Hunt et al. 1999).

In order to cope up with environmental heterogeneity, marine predators should favour these predictable structures as eddies, gyres, where they can find their prey more efficiently (Fauchald et al. 2000). King penguins (*Aptenodytes patagonicus*) travel hundreds of kilometres towards oceanic fronts or meso-scale eddies using currents and temperature gradients to locate these features (Charrassin & Bost 2001; Cotté et al. 2007; Trathan et al. 2008). At a small scale <1-100 km, we can find zones of strong productivity but variations in the density or location of prey aggregates are larger and less predictable (Hunt & Schneider 1987). Compare to king penguin, African penguins (*Spheniscus demersus*) during breeding foraged at a much finer scale and they have to locate prey within 10-50 km (Wilson 1985), where processes are more ephemeral than at meso-scale features (Hunt & Schneider 1987).

The continental shelfs and the coastal upwelling are zones where the primary production is important (Weimerskirch 2007). This productivity is ensured by the presence of the seabed (photic zone of the continental shelf between 0 and 200 m) for the establishment of sessile or weakly motile organisms, and the constant supply of nutrients of terrestrial origin. Moreover, this wealth is sometimes amplified by the hydrodynamic properties of coastal environments that promote the import of nutrients from the sea, as in the case of upwelling, tidal or coastal winds (Allanson et al. 1985; Atkinson & Peck 1990; Hunt et al. 1999).

d. Ecological indicators

To evaluate and understand the current condition – or predict the future conditions – of ecosystems, indicators are needed to monitor, assess and manage the ecological integrity of the ecosystem. In that context, a biomarker refers to any biological response related to exposure or toxic effect of a particular chemical condition of an environment, leading to the organism to deviate from its initial form (Van der Oost et al. 2003). A bio-indicator corresponds to an organism that can provide information on the environmental conditions of its habitat (Van der Oost et al. 2003). The bio-indicator has particular requirements regarding the physical or chemical variables of its environment. Changes in the presence/absence, numbers, morphology, physiology or behaviour of the bio-indicator indicate that the given physical or chemical parameters of the environment are outside their preferred limits. Most studies that used biomarkers or bio-indicators were concerned with the estimation of pollution level, environmental quality or were related to human health issues (Burger 2006). Ecological indicators, in contrast, can be used to gauge the environmental condition (Cairns et al. 1993). They can provide an early warning signal that a change is taking place in the environment, and they can be used to diagnose the cause of the environmental problem (Dale & Beyeler

2001). The information collected by ecological indicators can also be used to predict future changes in the environment and to help in establishing remedial actions (Niemi & McDonald 2004).

Ecological indicators should remain reasonably predictable in unperturbed ecosystems and be enough sensitive to react in a noticeable way as soon as its habitat is disturbed (Frost et al. 1992). Practically, the choice of indicators should, of course, depend on the questions being asked. The success of a monitoring program depends on the selection of valuable and relevant indicators. In general, ecological indicators need to be accessible enough to be easily and routinely monitored (Dale & Beyeler 2001). As such, species that can be monitored at low costs would be favoured. An eco-indicating species consists in, ideally, a single species, which may be representative of several other species that share similar ecological requirements (Niemi & McDonald 2004). Indicator species are used in three distinct ways: (a) to reflect the biotic or abiotic state of the environment; (b) to disclose evidence of the impacts of environmental change; or (c) to give indication on the diversity of other species within an area (Lawton & Gaston 2001). Each ecological indicator responds to different spatial and temporal scales (Niemi & McDonald 2004). Thus, for each ecological indicator, these scales must be explicitly stated.

Ecological indicators are used in order to evaluate the changes in the physical conditions of their environment and, more importantly, a change in the resource availability, including their abundance, distribution, seasonality, and predictability (Niemi & McDonald 2004). Seabirds have been identified as useful indicators of the health and status of marine ecosystems (Furness & Camphuysen 1997; Piatt et al. 2007). The concept of seabirds as indicators of fish stocks was well established by the early 1980s (Cairns 1987; Montevecchi 1993). However, the response can also be modulated by biotic factors that are either extrinsic (competition for resources with congeners or other species, predation, parasitism, etc.) or intrinsic. Differences in physiology, morphology or behavioural parameters could be found among individuals from a same population, as each individual is unique. To use ecological indicator, we need to understand individual characteristics that contribute most to the expression of a given performance (breeding or foraging) and identify those that may limit the performance.

II. The contribution of individual characteristics to foraging performance

Theoretically, an organism ideally adapted to the constraints of its environment would have its 'fitness' close to the maximum. The fitness of an individual is defined as the capacity of this individual to transmit their genes in the population by producing viable descendants (Hamilton 1964; Williams 1966). However, this "perfect" being does not exist! According to the principle of allocation of resources, animals must allocate their available energy between different activities that cannot be expressed simultaneously. One of the best-known trade-off of energetic investment for an individual concerns the differential benefit that individuals must make between growth, maintenance (survival) and reproduction (Stearns 1989). The limitation of these trade-offs is enhanced by high rate of energy acquisition.

The acquisition of resources requires a set of processes that implies experience, memorisation and decision-making processes regarding the effort to be invested in foraging, the prey choice and the quantity of prey to ingest (Charnov 1976; Stephens & Krebs 1986). It is assumed that animals have sensorial, perceptual and cognitive mechanisms that allow them to take the option that has the greatest positive effect on its immediate performance. The 'optimal foraging theory' (Stephens & Krebs 1986) predicts that animals adjust their behaviour in order to maximize the net energy gain by minimizing the time/energy spent foraging, while increasing the energy ingested (MacArthur & Pianka 1966; Charnov 1976). Animals have indeed a definite amount of energy and time that they can allocate to prey search, capture and digestion (Cuthill & Houston 1997). Moreover, individuals from a same population are different from each other. This heterogeneity of individuals' characteristics is responsible of the variability in foraging and breeding performance observed in one population (McNamara & Houston 1996). Constraints can be found for each individual that have to find solution to maximize their foraging performance.

1. Life history traits

Organisms are firstly limited by the phylogenetic constraints that define their genetic background, their physiological and biomechanical status. Ecologically, individuals from a given species are defined by a series of life-history traits, e.g. birth size, age at first reproduction, longevity or the number and size of offspring. The life history theory can help us understand how natural selection shapes an individual's characteristics (Stearns 1992; Roff 1992). Even if traits are determined primarily by the genotype of an individual, each individual has the ability to express multiple phenotypes from a given genotype to best adapt to changes occurring in its environment. This ability is called phenotypic plasticity (Dewitt & Scheiner 2004), which can be seen as a proximal response to environmental changes by opposition to a longer-term, population-level response (Pianka 1972). Phenotypic plasticity promotes the 'fitness' of an individual, i.e. the capacity of this individual to transmit their genes in the population by producing viable descendants (Hamilton 1964; Williams 1966).

Even if behaviours are limited by physiological and morphological parameters, behavioural plasticity enables animal to respond extremely rapidly to changes in its environment (Komers 1997), especially in terms of foraging behaviour, where animals should cope up with sudden changes in resources availability (Brown 2000).

2. Body mass, size and sex

Depending on their body size and mass each individual will perform differently. Differences in body size/mass can affect, for instance, an individual's locomotors capacity, i.e. the speed it can attain, the distance it can travel in a day, and/or the maximum depth or altitude reached. For example, the difference in male body size in sea lion (*Zalophus californianus*) is reflected on their diving efficiency (Weise et al. 2010). Bigger red fox (*Vulpes vulpes*) would have access to a larger territory and also to a more varied diet, even in the absence of large size dimorphism (Lossa et al. 2008). Similarly, differences in bill shape lead to a differentiation in prey selection. The African estrildid finch (*Pyrenestes ostrinus*) exhibits a polymorphism in bill size, not related to sex, that influences the choice of food resource and lead to a decrease in the overlap of food preference among individuals (Smith 1990). Body size may also influence other aspects of foraging behaviour, such as foraging mode and choice of microhabitat (Huey & Pianka 1981; Price 1983). Smaller individuals are more vulnerable to predators than larger ones and, hence, allocate more time to predator avoidance and alert behaviour (Berger & Cunningham 1988). Most differences in body mass/size are related to sexual dimorphism and induced sexual differences in foraging behaviour. For example, in albatrosses, *Thalassarche spp.,* variation in body mass and wing morphology has a functional role in flight performance and is an important determinant of at-sea distribution between males and females (Shaffer et al. 2001; Phillips et al. 2004). However, differences in foraging behaviour can also be observed in monomorphic species (e.g. the northern gannet, *Morus bassanus*, Lewis et al. 2002). Foraging can also be different between sexes as the energetic cost of reproduction could differ (Perrigo & Bronson 1985), males and females can be therefore specialised in different types of prey (Newton 1998). Males and females can differ in their susceptibility to predation or their ability to reduce the risk of predation, then differences may be observed in the foraging strategies of males and females (Power 1980; Shine 1989).

3. Age and experience

The increase in age-dependent reproductive performance could be due to difference in foraging skills (Curio 1983). Juveniles foraging behaviour may differ from adults in several ways, with in general young individuals being less efficient than older individuals (Wunderle 1991). Juvenile southern elephant seals (*Mirounga leonina*) remained closer to the island in shallower waters and performed a greater number of foraging trips than adults because of their physiological and morphological constraints (Field et al. 2005). The different metabolic requirement corresponding to an age-specific need and will result in a specialization in food resources (Wunderle 1991). The difference in foraging efficiency can also be the result of the inability of juveniles to identify the most profitable patch of food resources (Wunderle 1991). Juvenile mantled howler monkeys (*Alouatta palliata*), for example, often attempt to eat inedible objects, such as galls on trees that have a similar appearance to edible fruits (Whitehead 1986). Lack of skills in prey handling, capturing and/or processing compared with adults forces juveniles to spend more time foraging than adults. Foraging skills can be enhanced through trial and error learning or by watching congeners (Wunderle 1991). Wild meerkats (*Suricata suricatta*) teach pups prey handling skills by providing them with opportunities to interact with live prey (Thornton & McAuliffe 2006). Young may suffer from poorer access to resources due to competition and exclusion from older individuals (Curio 1983) and use sub-optimal feeding sites because of pressure of adults (Wunderle 1991; Sol et al. 2000). As age and experiences accumulate, individuals would improve their capacity to find food, to detect areas for storing food or favourable areas previously used and/or prey capture.

4. Physiological parameters susceptible to influence performances

Ecologists have demonstrated great relevance in the use of physiological markers as indicators of the health of animal population but also individual performances (e.g. Beaulieu et al. 2013). So far, studies focused on endocrine and immunological parameters responding to environmental perturbation (Stevenson et al. 2005). Animal behaviours are partly controlled and regulated by neuroendocrine processes thanks to chemical messengers, i.e. hormones (reviewed by Lovejoy 2005). For example, Adélie penguins (*Pygoscelis adeliae*) implanted with corticosterone modified their breeding and foraging performances (Spée et al. 2011, Cottin et al. 2011) indicating that hormonal responses are involved in the allocation of resources between self-maintenance and reproduction (Cichon et al. 2003). In parallel, the immune system can also influence foraging performance as it is considered as one of the self-maintenance mechanisms that prevent individuals from suffering harmful damages of pathogens and counteract the decline in physiological functions linked with senescence (Cichon et al. 2003). In this context, oxidative balance is a recent ecological framework compared to endocrine and immunological ecology (Beaulieu et al. 2013) that can bring relevant information about the physiological status of an individual. The measurement of markers of oxidative balance, such as antioxidant defences and oxidative damage, may be a valuable tool to understand differences in individual performances. Indeed, antioxidant defences or low oxidative damage have been positively correlated to fertility and survival of individuals (Bize et al. 2008; Losdat et al. 2011; Beaulieu et al. 2013). Moreover, environmental constraints act on oxidative balance of an individual (Beaulieu et al. 2010).

Oxidative stress is defined as a state of imbalance between 'Reactive Oxygen Species' (ROS) production and antioxidant defences of the organism (Figure 7; Finkel & Holbrook 2000; Monaghan et al. 2009). Oxidative stress occurs when the antioxidants cannot fully neutralize the ROS that are produced (Monaghan et al. 2009). The basic homeostatic situation can change either as a consequence of increased ROS production, or by reduced defences

(Figure 7; Monaghan et al. 2009). The vulnerability of tissues to ROS attack may increase with age as a consequence of changes in the composition of these tissues (Hulbert et al. 2007).

Oxidative stress has been defined as mediator in the trade-off between reproduction, self-maintenance and growth (Monaghan et al. 2009). However, the results of studies focusing on oxidative stress as an indicator of the investment in reproduction are contrasting (Metcalfe & Monaghan 2013). The levels of oxidative stress sustained by organisms are not constant. They vary with developmental stage, environmental conditions and levels of activity (Monaghan et al. 2009). The ROS production could increase the rate at which unrepaired cellular damage accumulates, leading to accelerated senescence, unless there is an increase in the antioxidant system investment (Monaghan et al. 2009). These ROS will cause damage to other molecules, e.g. lipids, proteins or telomeres (Finkel & Holbrook 2000).

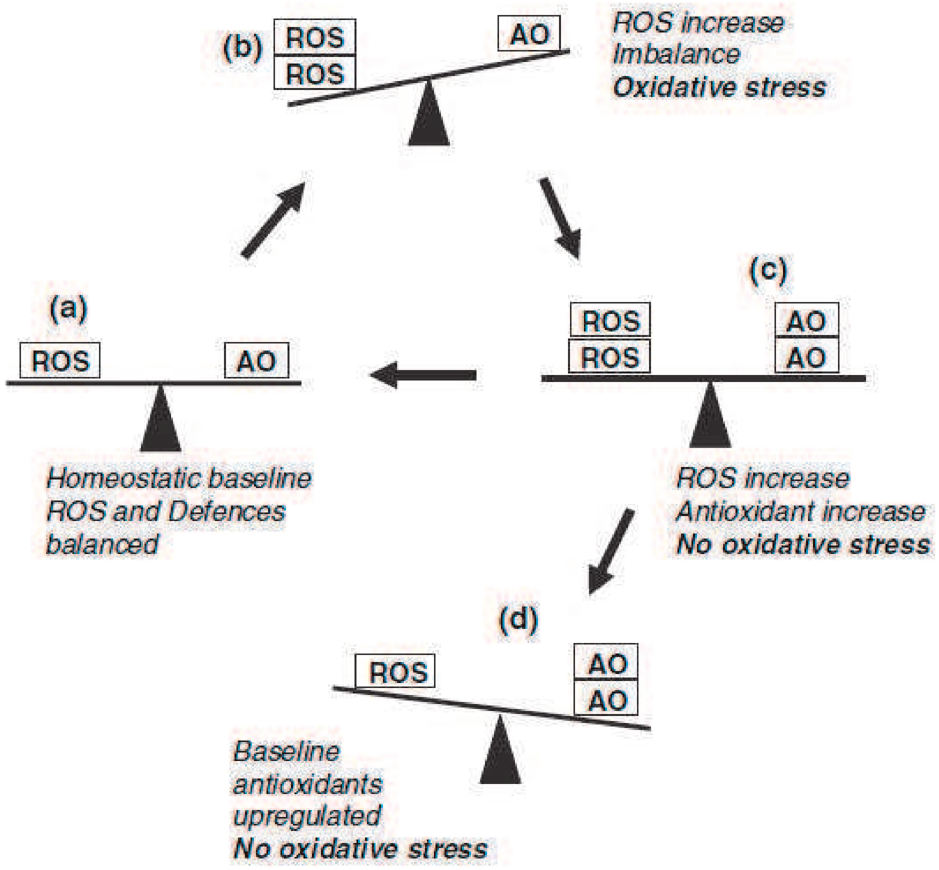


Figure 7: Illustration of the relationships between reactive oxygen species (ROS) and antioxidant defence (AO).

(a) In homeostatic baseline condition there is no oxidative stress as both ROS and antioxidant levels are low, with sufficient defences to neutralise ROS production. **(b)** An increase in ROS production may exceed the capacity of the antioxidant system, leading to an episode of oxidative stress. **(c)** If the increase in ROS is minor, it can be neutralized by an increase of antioxidants, avoiding advance oxidative stress. If the elevation of ROS is only temporary, there will then be a return to (a). **(d)** Exposure to more prolonged elevation of ROS can induce the organism to permanently increase its baseline antioxidant levels, to better cope with future oxidative events. Oxidative stress cannot be decided simply by measuring only ROS or only antioxidants, e.g. a given level of antioxidant defences does not indicate whether oxidative stress is (b) occurring or not (a). Moreover, an elevation of antioxidant defences does not necessarily avoid the appearance of oxidative damage; it will depend on the efficiency of the antioxidant defence. (From Monaghan et al. 2009)

Telomeres are repetitive, non-coding sequences of DNA that occur at the ends of the linear chromosomes of eukaryotes (Blackburn 1991; Monaghan & Haussmann 2006; Monaghan 2010). Telomeres protect chromosome and play a fundamental role in the maintenance of genome integrity (Monaghan & Haussmann 2006; Monaghan 2010). During the process of DNA replication, the very end part of the chromosome is not completely replicated (Monaghan & Haussmann 2006; Monaghan 2010). The risk to lose important coding sequences during cell division is prevented by functional telomeres (Monaghan & Haussmann 2006; Monaghan 2010). Dysfunctional telomeres make the genome of the cell unstable. This genome instability results in the cell no longer being able to divide, a state termed 'replicative senescence', which may be followed by cell death (Monaghan 2010). Consequently, without telomere restoration, there is a fixed number of times that cell can divide (Monaghan 2010). Telomere loss and restoration is expected to be important in determining lifespan, as there is good evidence that telomeres get shorter with age (Monaghan & Haussmann 2006; Monaghan 2010). Telomere length has been thought to reflect phenotypic quality and to highlight individual heterogeneity (Bauch et al. 2013). Telomeres give an indication on biological (rather than chronological) age at the organism level, suggesting the current position of the individual in its journey through life (Monaghan & Haussmann 2006). For some individuals the reduction of telomere length can run faster than for others (Monaghan & Haussmann 2006). Evidence is growing that individual life histories can be associated to telomere dynamics (Monaghan & Haussmann 2006).

III. Seabird as model for studies of foraging plasticity

1. Generalities on seabirds

Seabirds are important components of marine ecosystems and occupy the upper levels of marine food webs. Seabirds represent 300 species divided up to 4 large groups: Sphenisciformes, Procellariiformes, Pelecaniformes and Charadriiformes (Gaston 2004). Seabirds are well distributed all over the marine environment and have been able to colonize all marine areas, from the equator to the poles. Seabird species are very diverse, whether in terms of morphology, behavior or diet. The basic strategies at sea adopted by seabirds are: surface feeding, plunge diving and pursuit diving. Some species dive to great depths of several tens or hundreds of meters. For example the emperor penguins (*Aptenodytes forsteri*) is well known to perform extreme deep dives (564 m; Wienecke et al. 2007). Seabirds feed at variety of trophic levels and in all zones from littoral to pelagic (Furness & Monaghan 1987). For example, in the south of the North Atlantic, Bulwer's petrel (*Bulweria bulweria*) and Madeiran storm petrel (*Oceanodroma castro*) feed extensively in mesopelagic food chains (Monteiro 1996). Whereas other species occurring in the same breeding sites feed in epipelagic food chains, such as Cory's shearwater (*Calonectris diomedea*) and common tern (*Sterna hirundo*). Generalist feeding yellow-legged gull (*Larus cachinnans*), which feeds on the coast, inshore and offshore are also found in the same breeding sites (Monteiro 1996). Many species are also famous for undertaking long annual migrations, crossing the equator or circumnavigating the Earth in some cases. For example, the Cory's shearwaters (*Calonectris diomedea*) travel a minimum distance of 15000 to 35000 km between two consecutive breeding season, when effectuating trans-equatorial migration (González-Solís et al. 2007). A common characteristic of seabird species is that they are long-lived species. A large number of studies have shown that climate changes affect the seabirds' survival and/or reproduction (Croxall et al. 2002; Barbraud & Weimerskirch 2003; Gaston et al. 2005; Le Bohec et al. 2008). Seabirds may thus be good indicators of environmental changes (Frederiksen et al. 2007; Piatt et al. 2007), especially regarding fluctuations in prey abundance and distribution (Frederiksen et al. 2007).

Seabirds spent the majority of their life at sea and depend year-round on marine resources. However, during the reproduction, they return regularly to their nest on land to tend their chicks making them easily accessible to researchers at that time. Throughout this period, birds commute between the colony and the foraging site and are thus called 'central place foragers' (Orians & Pearson 1979). The need to commute between the nest and the food patch represents a constraint in which intensity depends on the distance between breeding and foraging sites and the abundance and distribution of resources in space and time. In addition, the length of time that an individual can leave its partner incubating before it deserts the nest or before the chicks starve, must be taken into account. All these factors limit individuals' movement at sea and their time spent in a food patch. This separation between the breeding and foraging sites has thus a cost that is growing with increasing colony size due to intra-specific competition (Lewis et al. 2001; Ainley et al. 2003).

2. Little penguin (*Eudyptula minor*)

The little penguin (Figure 8) is a long-lived species with an average life expectancy of breeding adult birds of approximately 6.5 years (Reilly & Cullen 1979; Dann et al. 1995).

Figure 8: A couple of little penguins (*Eudyptula minor*) in their artificial nest box.

However, some individuals in south-eastern Australian have lived far in excess of the average life expectancy, e.g. a bird was recorded still breeding at the age of 21 years old (Dann et al. 2005). Breeding performance is age-related in little penguin (Nisbet & Dann 2009; Figure 9). During the first breeding seasons, the inexperienced birds will improve their breeding output until they attain a stable and high reproductive success. The conflict between maintenance and reproduction will then become higher when individuals reach the ‘senescence’ stage, corresponding to the degradation of the physiological and behavioural performances of an organism (Monaghan et al. 2008).

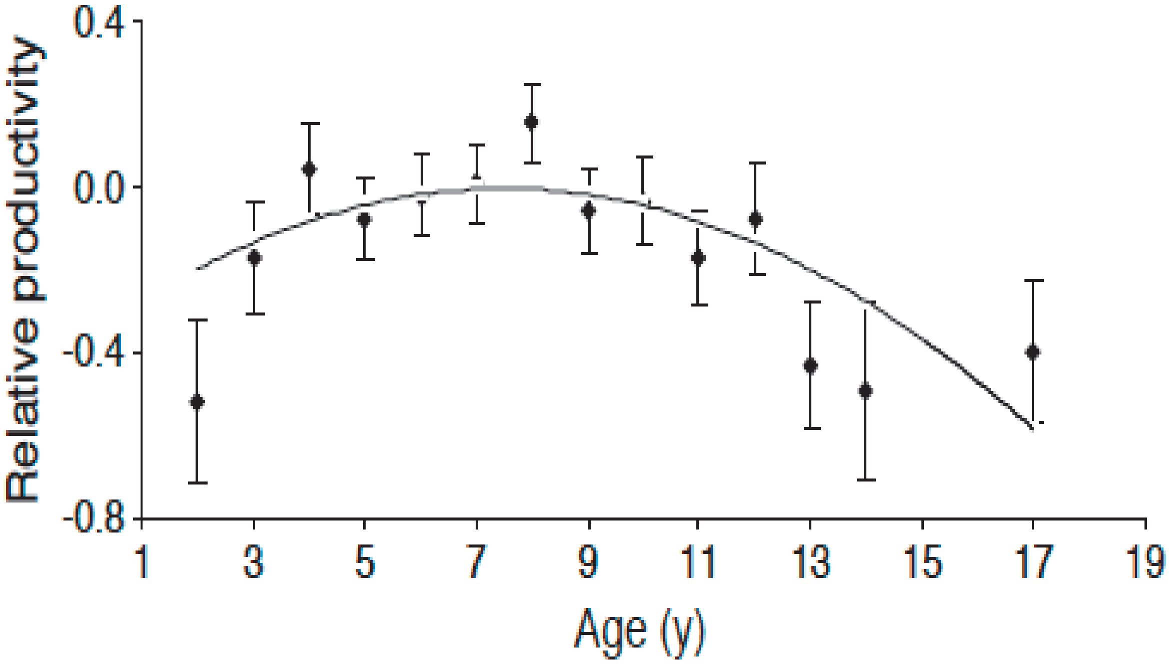


Figure 9: Relationship between reproductive performance (here expressed by an index of productivity that includes information about mass of chicks, duration of the rearing period, and reproductive success) and age of the little penguin (*Eudyptula minor*) (from Nisbet & Dann 2009).

This decrease in breeding performance may be also related to a difference in foraging skills as a decrease in foraging performance was found in very young little penguins (<5 years old), as well as in very old birds (>11 years old) that were probably in non optimal physical and physiological conditions (Zimmer et al. 2011a). Other intrinsic factors have been found to play on the performance (foraging and breeding) of the little penguins e.g. body mass or sex (Robinson et al. 2005; Kato et al. 2008; Saraux et al. 2011a).

Previous studies have highlighted the use of little penguin as a good ecological indicator of its environment (e.g. Ropert-Coudert et al. 2009; Chiaradia et al. 2010). Variability in the timing of breeding (Chambers 2004) and reproductive success of this species reflects the variation of the environmental conditions among years (Chiaradia & Nisbet 2006; Cullen et al. 2009). Foraging and breeding success have been related to environmental fluctuations (e.g. ENSO-related fluctuations, Ropert-Coudert et al. 2009). As such, the little penguin, being at the top of a short food chain (Cullen et al. 1991), is an ideal model to study local environmental alterations. This species presents one of the smallest foraging ranges among seabirds (Collins et al. 1999) and their breeding success is strongly related to food availability (Chiaradia and Nisbet 2006). At Phillip Island, Victoria, Australia, after a mass mortality of pilchard (*Sardinops sagax*), that were key prey in the diet of little penguins (Cullen et al. 1991), between 1985-1988, an increase in mortality and a very low breeding success of little penguins were immediately observed (Chiaradia et al. 2010).

The studies that form parts of this thesis have been conducting in a colony whose birds breeding and foraging activities have been monitored for 10 consecutive years as of 2013 (see Chapter 2: General materials and methods). This biological model gives us the opportunity to study the impact of environmental variability but also the heterogeneity of the individual on foraging strategies.

IV. Problematic and Thesis Structure

Little penguins exploit a coastal environment during the breeding season, a period classically regarded as extremely costly (Gales & Green 1990). We have seen that the coastal environment is one of the most productive marine ecosystems but it is also one of the most threatened marine habitats. In this context, studying the foraging strategies of little penguins appears to be particularly relevant for understanding the responses and adaptations of this species in the context of environmental change. Foraging performance should be adjusted so that individuals keep the energy balance at the equilibrium and so limit the trade-offs between reproduction and self-maintenance (Figure 10).

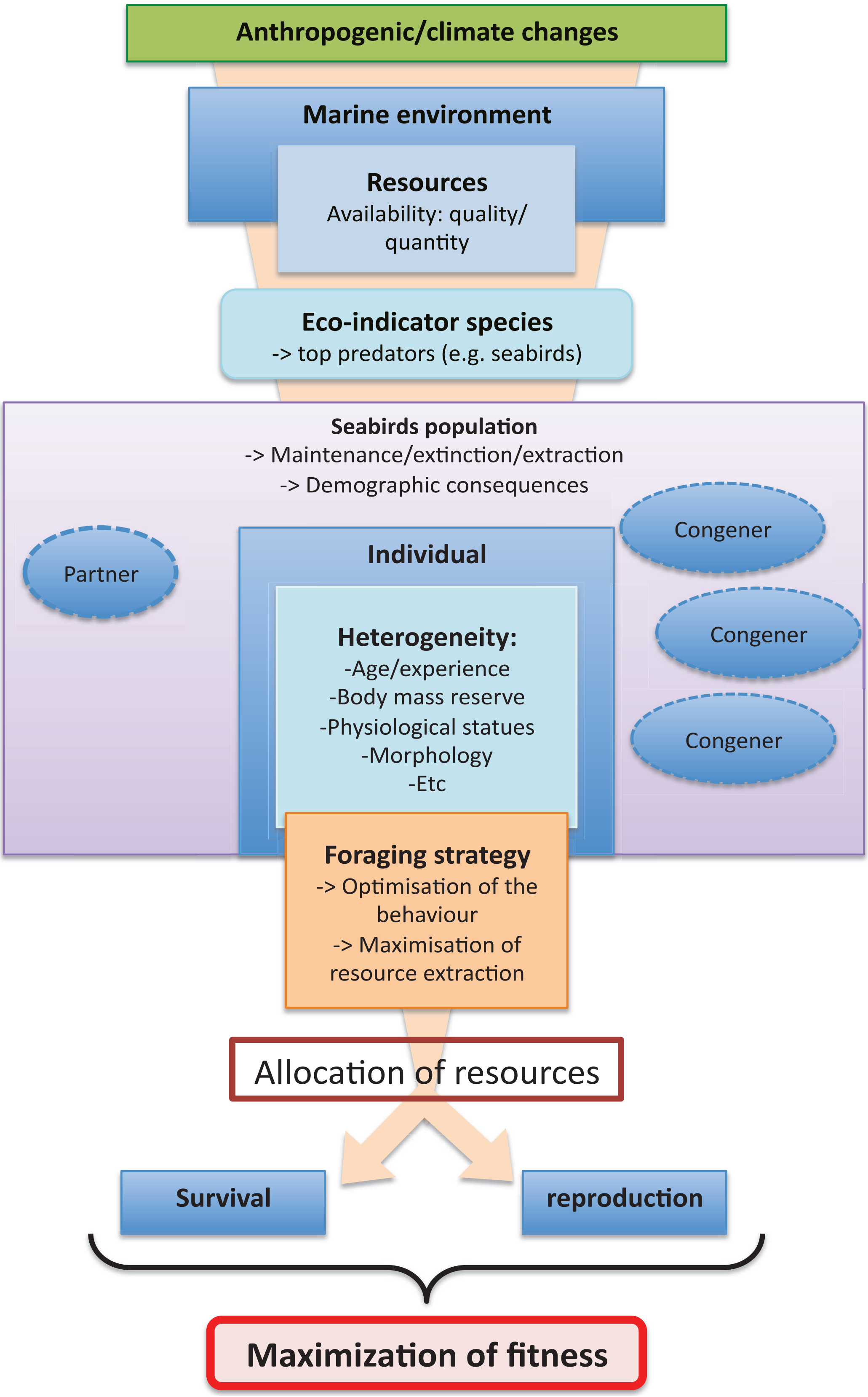


Figure 10: Simplified diagram summarizing the different factors involved in the allocation of resources between survival and reproduction in an adult individual whose purpose is to maximize its fitness.

Foraging strategies should thus reflect environmental conditions surrounding the colony of the birds. However, individuals' heterogeneity is expected to influence the foraging performance and strategies. This thesis will aim at answering the following question:

« Are little penguins good ecological indicators of their environment? »

In order to answer this question, it is necessary to understand the influence of intrinsic parameters of the individual and to separate it from the influence of extrinsic factors on the foraging behaviour. Then it would be possible to describe complex matrices that connect organisms to their environment. The aim of my thesis is thus to examine i) the influence of physical parameters of the ecosystem on the foraging activity of little penguins and ii) if the individual status of these birds plays a role on the implementation of foraging strategies. We can expect that (1) birds of different age, sex, and/or experience will adopt different behaviours and that (2) the efficiency of strategies is higher as individuals gain experience with the accumulation of breeding seasons. (3) In addition, birds would adjust their foraging strategies in function of the environmental conditions of the moment, such as the availability of prey.

I decided to focus my studies on foraging performance recorded during the guard stage, when birds were the most constrained during the breeding season. This also allowed me to compare activities between birds as they all performed a one-day foraging trip at that stage (data were thus not influenced by the trip duration). This manuscript is structured around two main axes.

At first, I investigated **how physical changes in the environment affect individuals' foraging strategies.** In this section I looked how the physical feature of the marine environment influenced the diving behaviour and success of the little penguins. In a first article (article A), I looked at the variations in the thermal structure of the water column that take place during a unique breeding season and how these can impact the foraging effort/success of the birds. In a second article (article B), I compared the foraging behaviour of birds sampled at different period of the summer over 5 consecutive breeding seasons. I looked how environmental conditions varied over these breeding seasons and how this affected the birds' foraging performance. I tried to determine of the real importance of the presence of a stratified water column for the little penguin.

A second section of the thesis shows **how individual characteristics influence the development of foraging strategies in little penguins.** This second part focuses on intrinsic factor that can influence the foraging behaviour, especially the age of the birds. In the third article (Article C) I determined how individuals of different ages organise their foraging trip (e.g. dives characteristics, spatial use…). I examined if experienced individuals exploited the foraging area more efficiently than the younger individuals. In the fourth article (Article D), I focused on how foraging performances are linked to physiological parameters, especially those that relate to the biological age of individuals, i.e. oxidative stress and telomere length.

I conclude this thesis with a general discussion that synthetise all the results and compare them to other studies published on little penguins or other species. I finish by enumerate some perspectives for futures studies on little penguins.

CHAPTER 2 : GENERAL MATERIALS AND METHODS

General Materials & Methods

I. Studies Site

All experiments were carried out on the colony of the 'Penguin Parade' at Phillip Island (38°31'S, 145°09'E), Victoria, Australia. This colony is located in the northwest of Bass Strait (Figure 1). Bass Strait is an area of shallow water (60 to 80 m on average), which separates Tasmania from Australia mainland (Murray et al., 2001; Chiaradia et al. 2007a).

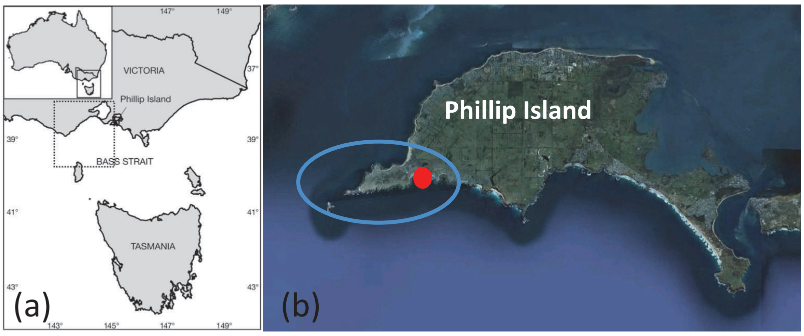


Figure 1: (a) Location of the study site (From Cullen et al. 2009). (b) Penguin colony studied marked with a red dot. Circled in blue the 'penguin reserve' (figure extracted from Google Earth).

This region is of great interest for the study of changes in a coastal marine habitat because it is influenced by different sources of marine currents from three major ocean basins: the Indian Ocean to the west, the Pacific Ocean to the east and Southern Ocean via the southwest (Figure 2). The waters of Bass Strait on the west side are under the influence of two major currents. The Leeuwin current originates in the Indian Ocean and flows down along the west coast of Australia. This stream may provide oceanographic anomalies of sea surface temperature (SST) that are driven by the Indian Ocean Dipole (Yamagata et al. 2004). The Leeuwin current is a tropical ocean current composed of warm waters with low salinity that are poor in nutrients. It influences the marine flora and fauna as well as the climate of South-western Australia and Tasmania. The second current affecting the west side corresponds to the Flinders current, which is itself under the influence of the variation of the Antarctic Circumpolar Current (Middleton et al. 2007). The Antarctic circumpolar current flows from west to east in the Southern Ocean, around the Antarctic continent. Through interactions with the bottom topography, this current generates large eddies that move north across the current. The waters of western Bass Strait are also under the influence of the East Australian current from the Pacific Ocean. This is an oceanic current of hot waters flowing between Australia and New Zealand, forming a vortex flow in the Tasman Sea (Geistdoerfer 2002). The presence of the East Australian current seems to increase the sea surface temperature (SST) to above 20°C in the Bass Strait (Cresswell 1997). The increase in SSTs has been associated with reduced body condition of penguins and longer foraging trips (Mickelson et al. 1991). From one year to another, the Bass Strait region is subjected to contrasting oceanic regimes that depend on which of these three currents is predominant.

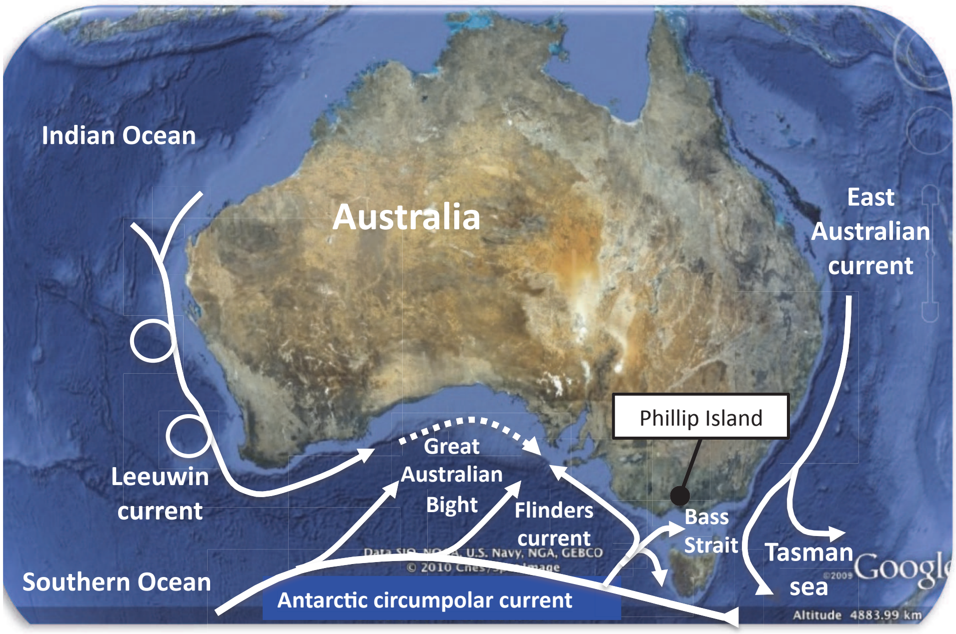


Figure 2: The major currents influencing the waters of the Bass Strait, localisation of the study site. (Modified from the original figure in Ropert-Coudert et al. 2009)

II. Biological model

1. General presentation

Little penguin is endemic to Australia and New Zealand; this penguin explores seas that are warm compared with those of cold Antarctic. It breeds in several colonies of different size in which environmental conditions and reproductive success vary (Chiaradia et al. 2007a). The little penguin is the smallest Spheniscidae measuring on average 40 cm long and weighing around 1 kg (Marchant & Higgins 1990). For penguins at Phillip Island, there exists a strong dimorphism between males and females in the bill depth making it the unique reliable morphological parameter to determine the sex of an individual, without going through the genotypic sexing (Figure 3; Arnould et al. 2004). Penguins with a bill depth <13.4 mm are considered females (Arnould et al. 2004).

Figure 3: measure of bill depth on a chick.

2. Reproductive cycle

The timing of breeding is asynchronous among individuals (Chiaradia & Kerry 1999). Individuals breeding earlier are more likely to lay a second clutch (Reilly & Cullen 1981; Chiaradia & Kerry 1999). The reproductive cycle can be decomposed into five main periods

Courtship: The start of the breeding season is variable and differs between colonies (Chambers 2004). The breeding season is considered to start at courtship when pairs are seen together for the last time before the pre-laying exodus (Chiaradia & Kerry 1999; Figure 4). Courtship starts approximately one month before egg laying. After courtship the females depart, i.e. a pre-laying exodus, which lasts 10.6±3.2 days on average (Chiaradia & Kerry 1999). The females then remain ashore for a mean 5.6±3.1 days, during which a clutch of two eggs is laid, each egg being laid at one day interval (Chiaradia & Kerry 1999).

Incubation: The incubation period started from the date of laying to the date of hatching of first egg (Chiaradia & Kerry 1999). Egg-laying starts between September-October and is not synchronous, taking place over a prolonged period that varies between 8 and 28 weeks (Stahel & Gales 1987). Some birds can replace their first lost clutches, but some pairs can also lay a second clutch after the first one had been reared successfully. Two successive clutches are more likely to occur when the first clutch is initiated early in the season (Reilly & Cullen 1981; Reilly 1994). Reilly and Cullen (1981) noted that the later the reproduction starts the lower the final breeding success. The incubation period, averaging 35 days with a range of 33-37 days, varies with the development of the chick embryo (Chiaradia & Kerry 1999). Both parents alternate to incubate the two eggs, while one bird attended the eggs (incubation shift), the other foraged at sea (foraging trip). Individual birds made on average 5.6 incubation shifts, which lasted on average of 3.4 days (Chiaradia & Kerry 1999). Foraging trips must be coordinated between partners so that one parent is available to feed the chick at hatching. This means that as incubation progresses, trip duration shortens until just before the hatching, which will mark the beginning of the chick-rearing period (Chiaradia & Kerry 1999). There is no difference between males and females in the number and length of foraging trips. However, it has been shown that successful breeders had shorter incubation shifts than failed breeders (Chiaradia & Kerry 1999). Foraging trips during incubation aim to improve body condition of the parents to prepare them for the chick rearing (Robinson et al. 2005; Kato et al. 2008). Hatching is completed within 24 hours.

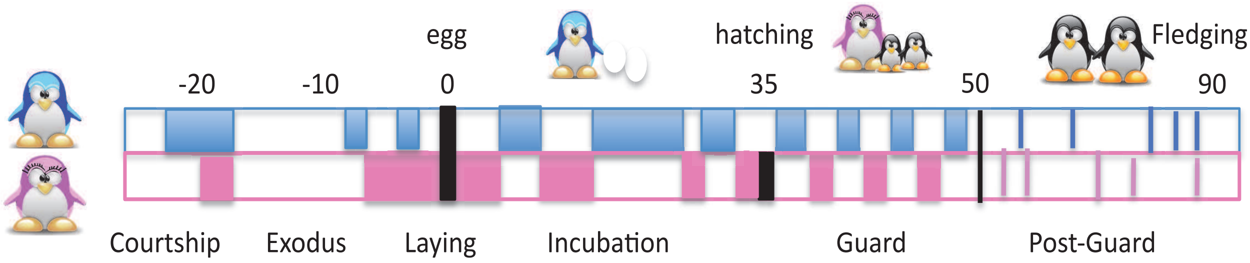


Figure 4: Simplified reproductive cycle of the little penguin, from the first arrival on the colony to the end of the chick-rearing period. The scale at the top represents the days before and after laying which is counted here as day zero. Presence ashore is in blue (for male) and pink (for female). In black is the laying and hatching date and the end of guard period. (Modified from figure in Chiaradia & Kerry 1999)

Following incubation, the chick-rearing period lasts for 56 days (8 weeks) but ranges between 48 and 59 days (Stahel & Gales 1987). Duration of chick rearing is the interval from the date of hatching of the first chick to the date of fledging of the last chick. The chick-rearing period is divided into two parts; the guard and post-guard. Failure in little penguins usually occurs during chick rearing, when the parents may struggle to rear chicks successfully if they have to forage far away from the colony (Chiaradia & Kerry 1999; Robinson et al. 2005).

Guard: Guard period is the period from hatching of the first egg to the first time neither adult is present at the nest during the day (Chiaradia & Kerry 1999; Chiaradia & Nisbet 2006). After hatching, chicks are very weak and cannot raise their head, eyes are fully open after about 1 week (Reilly 1994). Parents guard their chicks for an average of 14.5 days (range 8-25 days) until chicks becoming physically and thermally independent (Chiaradia & Kerry 1999). Very young chicks may need to be fed several times throughout the night and day due to their small stomach capacity. Like during the incubation, parents alternate phases of chick guarding at the nest with foraging trips at sea (Chiaradia & Kerry 1999; Chiaradia & Nisbet 2006). During the guard period, little penguins do not make foraging trips longer than one day (2 days trip may occur very occasionally). This compulsory one-day trip constrains them to forage in an area within a radius of 20-25 km (maximum 30 km) around the colony, which is related to the maximum speed they can attain at sea (Collins et al. 1999). The maximum speed of little penguins observed swimming underwater at Detroit Zoo, USA, was 6 km/h (Reilly 1994). Bethge et al. (1997) have recorded a mean swimming speed of free-ranging little penguins at sea of 6.5km/h, with a maximum swimming speed of 12 km/h.

In a bad season the guard period is shortened probably forcing chicks towards the end of the guard phase to spend more energy on thermoregulation rather than in development, leading to chicks growing less rapidly (Chiaradia & Nisbet 2006). It is likely that the guard period will vary considerably between seasons and between colonies depending on resource availability around the colony (Chiaradia & Kerry 1999; Chiaradia & Nisbet 2006).

Post-guard: It is the period from the end of the guard to fledging of the last chick (Chiaradia & Nisbet 2006). Both parents go to sea and alternate several short trips (one day) intended to feed the chicks and longer trip (average 4 days), which are more profitable for the adults as they allow them to restore their body reserve (Saraux et al. 2011a). The weight of the adult before the trip will determine its overall length. This stage will last on average 6 weeks. Although créching is not usual, sometimes a group of neighbouring chicks will shelter in the same burrow or each chick will wait alone outside of its burrow (Reilly 1994). Chicks are mature at 54 days (7-9 weeks) (Stahel & Gales 1991). Departure is separated by 2-3 days commonly between 2 chicks in a brood (Stahel & Gales 1991). Fledged chicks disperse widely during their first and second years of life (Reilly 1994). The little penguin can breed at two – to the earliest – or three years old (Reilly 1994; Nisbet & Dann 2009).

Moult: Once chicks are independent, adults must accumulate enough fat to be able to sustain the fasting period that correspond to their annual moult (Reilly 1994). The moult, lasting two to three weeks, generally occurs during the period of February to April (Reilly & Cullen 1981). During this time the birds are unable to feed, as they are restricted to land. Moult is essential for survival as birds renew their waterproof feathers. Before moult little penguins reach twice their normal weight (~2kg) and will loose it after moulting (Reilly 1994). With their new feathers birds finally depart to sea to restore their body reserves for the next breeding season. During winter birds remain mainly at sea and some of them return sporadically at the colony (Collins et al. 1999).

Local weather patterns and variations in ocean currents affect food availability, and may account, at least in part for the elasticity of the breeding schedule in little penguins (Stahel & Gales 1991; Reilly 1994). The timing of egg-laying of little penguins on Philip Island was significantly and negatively correlated with SST in south-eastern Australia at some times of the year (Reilly 1994; Chiaradia & Kerry 1999). Warmer temperature in late summer and autumn corresponded to earlier nesting (Chambers 2004; Cullen et al. 2009). To a lesser extent, the number of chicks fledged per pair and the body mass of chicks at fledging were positively correlated to SST (Cullen et al. 2009).

3. Diet and foraging

As the little penguin is a visual predator, diving is exclusively diurnal (Cannell & Cullen 1998). Despite its small size little penguins are excellent divers since the empirical equation of Wilson (1995), which relates to the mass of an animal (x kg) at the maximum depth achievable (y m):

$y=18x+47.6, r^2=0.81$,

predicted maximum diving depths of 65.5 m for a penguin of 1 kg. These predictions were confirmed by dives recorded from free-living penguins (69 m, Montague 1985; 66.8 m: Ropert-Coudert et al. 2006a) for a maximum duration up to 90 s (Ropert-Coudert et al. 2006a). Dive depth is correlated with dive duration, as dive depth increases the duration increases too (Ropert-Coudert et al. 2003). However, these extremes are rarely achieved and most dives of penguins are concentrated between 20 and 40 m (Ropert-Coudert et al. 2006b). More than 90% of the foraging dives are <50 m depth.

Little penguins eat fish and squid, but their diet consists mostly of small clupeiform fish that are less than 12 cm long and less than 2 years old (Cullen et al. 1992), such as sardine (*Sardinops sagax*), anchovy (*Engraulis australis*), red cod (*Pseudophycis bachus*), barracouta (*Thyrsites atun*), blue warehou (*Seriolella brama*) and cephalopods (mainly squid species), but also crustaceans (most frequently Krill, *Nyctiphanes australis*) (Cullen et al., 1992; Chiaradia et al. 2003). When there are fewer fish available, little penguin diets contain more squids, which have a lower energy content and are thus less profitable food for chicks (Cullen et al., 1991; Chiaradia et al. 2010).

III. General method

1. Monitoring breeding season

The ‘Penguin Parade’ colony contains approximately 1000 breeding pairs (Chiaradia & Kerry 1999). Since 1978 nests have been checked and the birds were banded as either chicks or as adults when they were first captured at the breeding colony and it was assumed that all adult birds were at least three years old at banding (Chiaradia & Kerry 1999). In the study area, there are approximately 100 artificial nest boxes (Figure 5). The entry and exit of penguins from this area are monitored since 1994 using an Automated Penguin Monitoring System (APMS; Kerry et al. 1993; Chiaradia & Kerry 1999), developed by the Australian Antarctic Division (Figure 5).

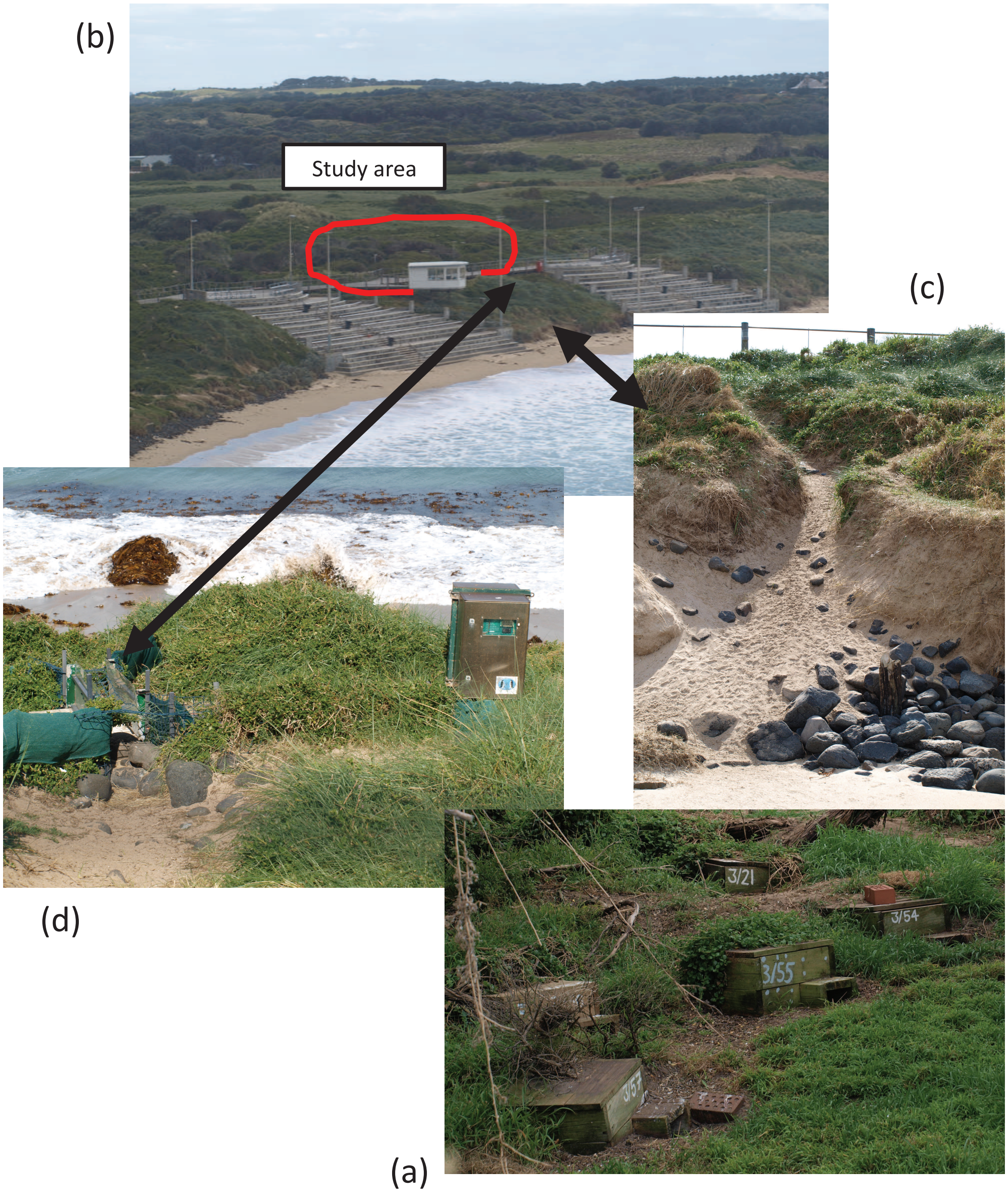


Figure 5: (a) 100 nest boxes checked in (b) the area three (study site). Penguins coming back from the sea or leaving the colony, (c) following their natural track, will pass by (d) the Automated Penguin Monitoring System. It will record the ID, the weight and date/time of the passage of the bird.

Passive transponders (Allflex, Australia) with unique numbers are injected annually under the loose skin between the shoulder blades of the chicks just before they fledge. (Figure 6; Daniel et al. 2007). The wounds are closed with surgical glue (Vetbond^{TM}, 3M worldwide) to prevent infection. This manipulation takes less than a minute. In addition to recording the time and the ID of the bird crossing, the APMS records the weight of the bird. In addition, the artificial nests are checked every two days for the presence of eggs and chicks and to establish the breeding chronology every year. A graphical representation is then performed to reconstruct visually the reproductive cycle (Annexe 1).

Figure 6: injection of a transponder to a chick.

2. Long-term study

Since 2004, the monitoring of the foraging activity of breeding birds of known age and sex has been realised using a bio-logging approach (Ropert-Coudert & Wilson 2005). Each breeding season, data loggers have been deployed to monitor the behaviour at sea during incubation, guard and post-guard. The birds in incubation and guard are captured in their nest on the day of their departure to sea. The birds in post-guard are away from the colony during the day and are therefore captured upon their return at night thanks to the APMS system.

Loggers were attached to the feathers of the midline of the back of the animal, near the tail with marine Tesa tape (Wilson et al. 1997). The unit is positioned so as to minimize the drag effect and minimize disruption during the diving behaviour (Bannasch et al. 1994; Ropert-Coudert et al. 2007). Before attachment and after logger removal the bird was weighed to the nearest 10g with a spring balance. The bird was released at the nest entrance. Each season at least 10 individuals (5 males, 5 females) were equipped with accelerometers or Time Depth Recorder (TDR) during each stage.

I conducted two fieldwork seasons: 2010/2011 and 2011/2012. During these two seasons protocols were changed compared to previous years. Global Positioning System (GPS) loggers (figure 7) were used concomitantly to the 3-axis accelerometers and in addition blood sampling was performed for various analyses (see below). GPS use a set of 24 satellites in orbit, permitting the geo-localisation of each individual. During the first season, 30 individuals in guard were equipped within 5 weeks (15 males and 15 females). In the second season birds were equipped with GPS and accelerometers during incubation (10 birds), guard (30 birds) and post-guard (30 birds) periods. In the second breeding season the deployment on birds in guard and post-guard lasted over 6 weeks.

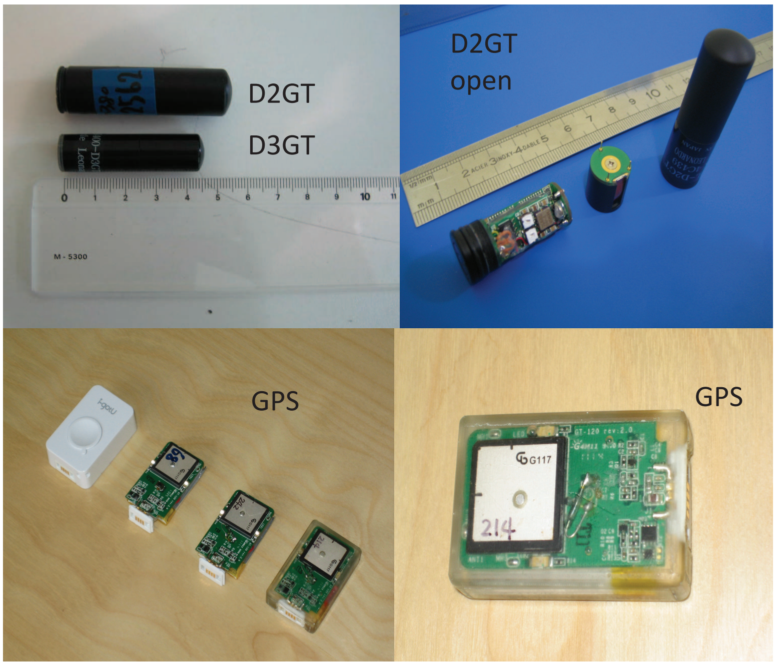


Figure 7: the loggers GPS and accelerometers

IV. Foraging behaviour monitoring

1. Bio-loggers

a. Accelerometers

Two types of accelerometers were used in the course of the long-term study, two- and three-axes accelerometers.

The two-axes accelerometer (M190-D2GT, Little Leonardo, Tokyo, Japan) is cylindrical shaped, 12-bit resolution, with 4-channels recordings, 15 mm in diameter and 52 mm long, with a weight of 16g in air (Ropert-Coudert et al. 2006b for details; Figure 7). It records the depth (with a resolution of 0.05 m) and temperature (0.01°C) every second. The logger also records the acceleration in two planes: the dorso-ventral axis (*heave*) and along the longitudinal axis (*surge*) of the animal. The acceleration of these two axes is measured between -30 and 30 m s^{-2} at a frequency of 32 or 16 Hz.

The three-axes accelerometer is smaller in size, 12 x 45 mm, and weigh 9 g (ORI400-D3GT, Little Leonardo, Tokyo, Japan). It records depth (range 0~400 m, resolution 0.1 m) and temperature (range -50~50°C, 0.1°C) every second, as well as acceleration along the longitudinal body axis (*surge*), the dorso-ventral axis (*heave*) and the lateral axis (*sway*) of the birds, between -40 and 40 m.s^{-2} at 50 Hz.

b. GPS logger

CatTraQ™ GPS loggers (16 Mb memory, 230 mA lithium-ion battery, Catnip Technologies, USA) were originally intended for the tracking of domestic cats (Figure 7). They were customized in our laboratory by the engineering team Métrologie et Instrumentation en Biologie et Environnement, in order to be used on wild, diving species. The original package was removed, the main switch button replaced by a reed switch, and the units were then moulded into a water-resistant resin. Finally, each unit was placed in a heat-shrink tube for waterproofing. Final size after customization was 14 × 35 × 70 mm with, a weight of ca. 30 g. In 2010 and 2011 for the guard stage the sampling interval was 15 sec. In 2011 GPS were also used during incubation and post-guard with a sampling interval of 3 min and 1 min, respectively.

2. Data analysis

a. Diving data

The first step in the analysis consists in correcting the drift of the pressure sensor with the temperature, i.e. adjusting the pressure value when the bird is at the surface before or after a dive to zero. This was done with the program 'WaterSurface_D2GT' from the application 'Ethographer' (Sakamoto et al. 2009) using a linear regression between the depth and the temperature recorded by the same logger.

A dive starts when depth becomes greater than 1 m and ends when depth becomes less than 1 m again. The beginning and end of the foraging trip are defined by the first dive and the last dive. Each dive is divided into three phases: the descent, the bottom and the ascent phases. A dive is followed by a period, the post-surface phase, when the bird remains at the water surface (depth <1 m) (Figure 8). This period is considered as a phase of recovery and preparation for the next dive (Wilson 2003). The bottom phase is around the maximum depth of the dive. In our studies, this phase begins and ends when the depth change rate becomes more and less than 0.25 m/s in the dive, respectively.

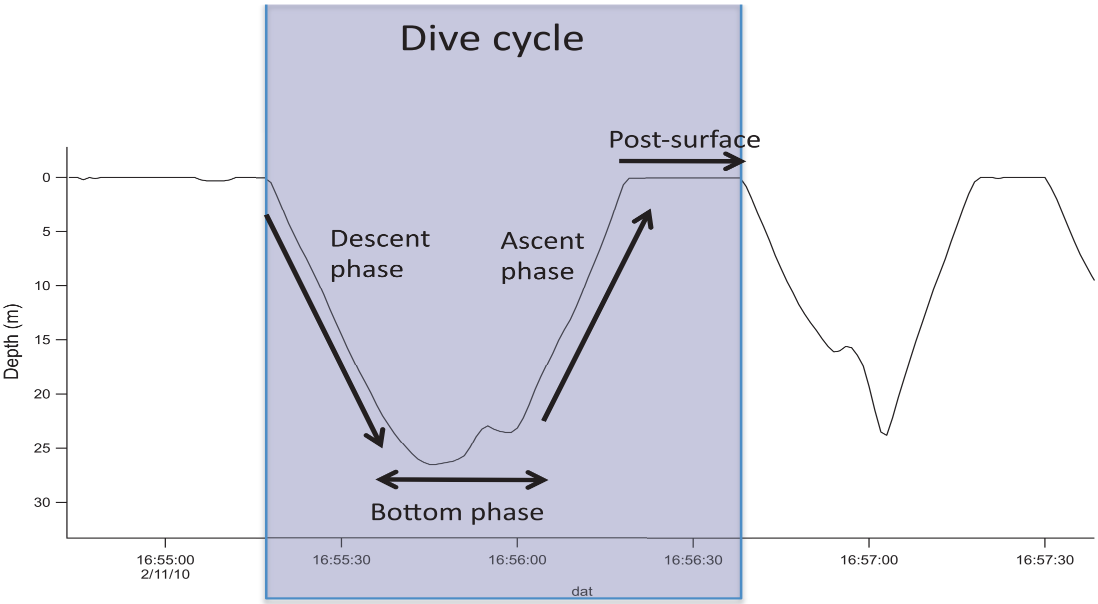


Figure 8: Example of two dives performed by a bird during a foraging trip, indication the dive parameters classically monitored.

It is during this period that penguins are known to encounter prey predominantly, as has been demonstrated in Adélie penguins (*Pygoscelis adeliae*, Ropert-Coudert et al. 2001), king penguins (*Aptenodytes patagonicus*, Ropert-Coudert et al. 2000) and little penguins (Ropert-Coudert et al. 2006b). IGOR Pro software (Wavemetrics Inc., USA, 2008, Version 6.04) was used for the analysis of data obtained by the loggers.

The script “kaiseki” written by Dr. Katsufumi Sato and modified by the Dr. Akiko Kato was used for the analysis of dive data. This script calculate automatically several parameters for each dive: the maximum depth (m), the total duration (s), the durations of the descent, bottom, ascent and post-dive phases (s), among others.

b. Accelerometer data

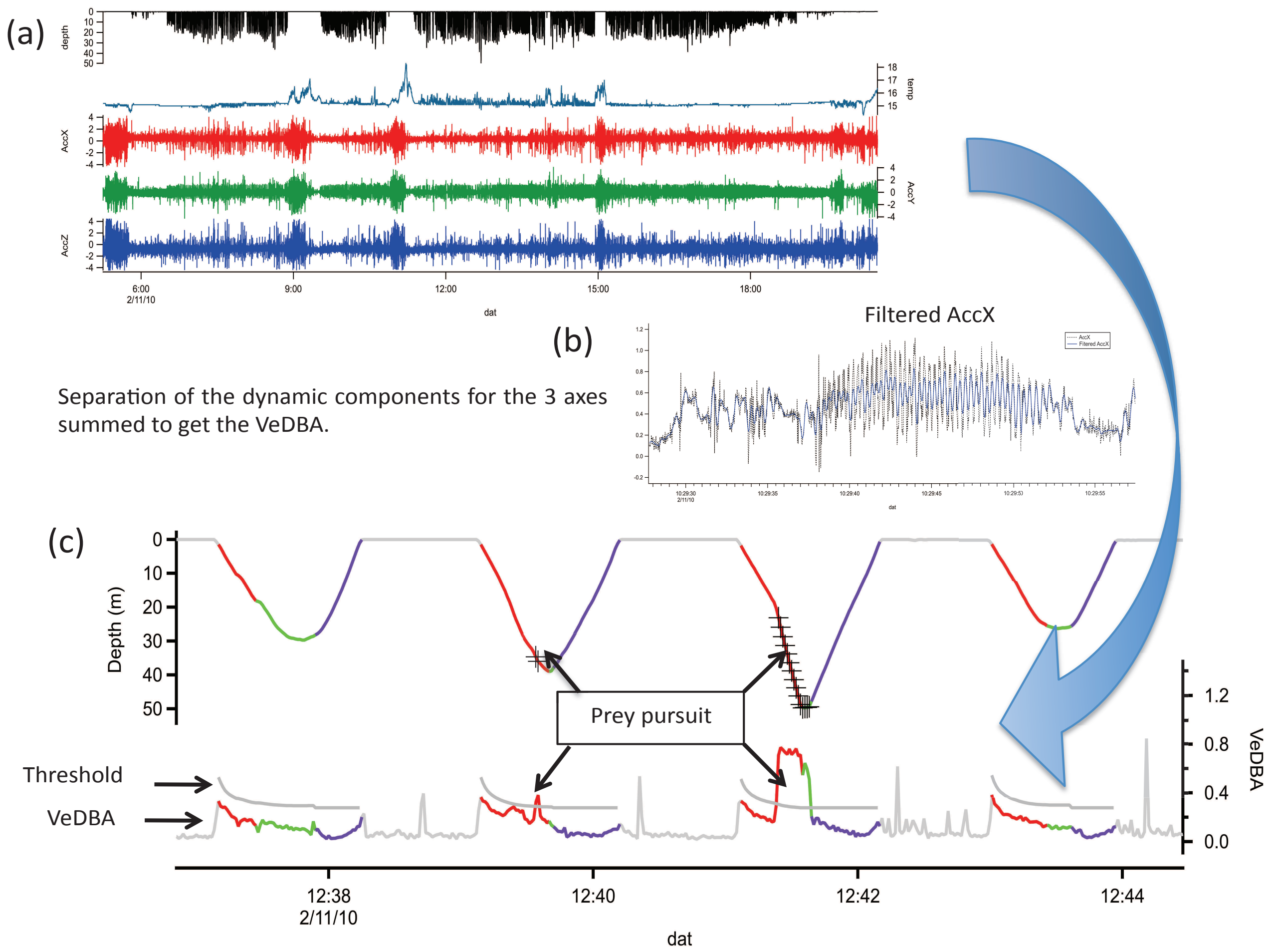
Recorders used in this study comprise an accelerometer positioned to record the components of the acceleration along two or three axes of the space. The signal delivered by the accelerometer consists in the sum of two components that are named ‘static (gravity-related) acceleration’ and ‘dynamic (specific) acceleration’, respectively. The low frequency component of the acceleration is assumed to represent the static acceleration, which reflects the body posture, i.e. angle of the body, and the high frequency component is assumed to represent the dynamic acceleration, which correspond to the rapid movement of the body, i.e. flipper beat. Dynamic and static acceleration signals were separated by a two-band low-pass filter (IFDL, Version 4, Wavemetrics).

A measure of the whole body activity can be derived from the acceleration signals recorded along the three axes (ODBA, Wilson et al. 2006, Gleiss et al. 2011). In the present study we derived from the three-axes acceleration signals the Vectorial Dynamic Body Acceleration (VeDBA; Figure 9), which is also an index of activity level but that is calculated as the vectorial sum of the accelerations along the three axes (*surge*, *heave* and *sway*).

$$\mathrm{VeDBA} = \surd\mathrm{Da}_{surge} + \surd\mathrm{Da}_{heave} + \surd\mathrm{Da}_{sway} \qquad (\mathrm{Da} = \text{dynamic acceleration})$$

From the two-axes accelerometer we calculated the Partial Dynamic Body Acceleration (PDBA, in $m.s^{-2}$ or g, see Gleiss et al. 2010; Halsey & White 2010; Wilson et al. 2006) as follows:

$$\mathrm{PDBA} = \mathrm{Da}_{surge} + \mathrm{Da}_{heave}$$



Separation of the dynamic components for the 3 axes summed to get the VeDBA.

Figure 9: (a) Raw dataset recorded by a 3-axes accelerometer, placed on a bird during the guard stage and performing a one-day foraging trip, that recorded the depth (in black), the water temperature (in blue) and the acceleration (AccX=heave, AccY=Surge and AccZ=Sway). (b) A measure of the whole body activity during a dive was derived from the acceleration along the three axes. (c) When the VeDBA exceeds the threshold the bird is chasing a prey. On the diving profile prey pursuits are visible (black cross) during the descent phase (red), the bottom phase (green) and the ascent phase (purple).

During the descent phase, the little penguins have to overcome the phenomenon of buoyancy. To this end, birds flap actively their flippers at a relatively high constant frequency throughout the descent and the bottom phase. In contrast, mainly of the ascent phases are passive (Kato et al. 2006). Birds use their floatability to reach the surface at a low cost (Kato et al. 2006). The constant flipper beating activity observed during most of the dive changes during a prey pursuit. Here, the frequency and/or amplitude of flipper beat increase (Ropert-

Coudert et al. 2006b), resulting in a peak of activity recorded in the PDBA or VeDBA (Wilson et al. 2010; Shepard et al. 2010). The accelerometer method does not give a precise quantification of the number of prey ingested. However, it gives an estimate of food availability during a trip and an accurate indication of where prey encounter events occurred during a dive. We assumed that any dynamic body acceleration signal superior to a given threshold corresponds to an encounter and a pursuit of a prey (Figure 9; see Ropert-Coudert et al. 2006b).

As the characteristics of the signal corresponding to flapping flippers differ between individuals the threshold was adjusted for each bird. To calculate the threshold, the values of PDBA or VeDBA during the descent phase were used, when birds are constrained to maintain a high level of stroke activity (Kato et al. 2006). The values of PDBA or VeDBA were plotted as a function of instantaneous depth. Clear patterns were observed on the diminution of the body activity with depth. Kernel density estimation was used to determine an upper limit containing most of the data using a regression line (Figure 10). The best regression was selected using the lowest AIC. Then, the 3 parameters (a, b and c) from the best regression were used to calculate the threshold in acceleration signal for each dive that define a prey encounter using this formula:

$$\text{Threshold} = a \times (\text{Depth} - c)^{-1} + b$$

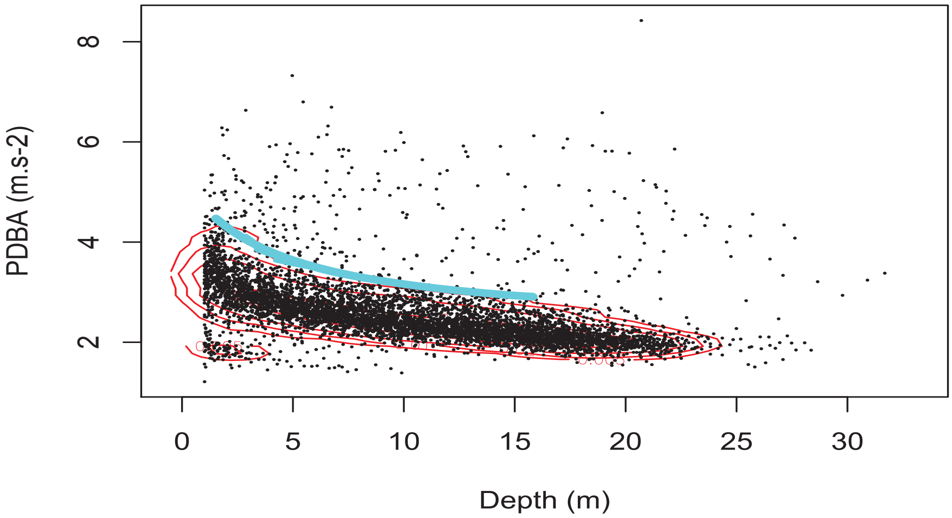


Figure 10: PDBA during the descent phase plotted against instantaneous depth. In blue the regression line calculated on R and selected with the lowest AIC, determining the parameters (a, b and c, see text) to calculate the threshold used to identify periods of prey pursuit.

The number, the duration (s) and depth (m) of each prey encounter were thus extracted. We calculated the ratio of the number of dives with prey encounter to the total number of dives during the foraging trip, as an index of hunting efficiency (Zimmer et al. 2011a,b).

c. Temperature data

The logger also recorded temperature (resolution of 0.01°C) every second. As the temperature sensor has a slow time response $T_{0.9}$=15s (i.e. it is the time to reach 90% of the temperature changes), the temperature data were corrected according to Daunt et al. (2003). This correction allowed us to examine the temperature distribution of the water column. For each hour a temperature profile was determined from the deepest dive (> 20 m) (Takahashi et al. 2008). An average of temperature was calculated every 2 m between the temperature data recorded during the descent, the bottom and the ascent phase. From hourly data, an average daily temperature profile was calculated.

d. GPS data

Data were downloaded with the software @tripPC and saved as csv and gpx files. With Igor Pro the csv files were filtered (duplicated points were deleted) automatically using a script written by Dr. Akiko Kato. Travelling speeds more than 8 km/h, which are not biologically meaningful for this species, were removed. Data were interpolated at fixed interval (60 s). The geodesic system used by the GPS was WGS 84 (World Geodetic System 1984). The geographic coordinates in latitude and longitude were converted to the Universal Transverse Mercator projection in km using the spatial analysis program in 'Ethographer' (Sakamoto et al. 2009) on Igor Pro. Distances between the colony and the farthest point were calculated.

V. Blood samples

Blood samples were collected only during the season 2010/2011 and 2011/2012. A sample of approximately 300 µL was taken from the tarsal vein following the retrieval of the loggers after a single foraging trip (Figure 11).

Figure 11: illustration of technique of blood sampling on a little penguin.

Immediately after collection, plasma and red blood cells were separated by centrifugation and placed at -20°C. These samples were transported in the Department of Ecology, Physiology and Ethology (DEPE-IPHC) where several analyses were performed on plasma and red blood cells.

1. Oxydative stress

In the cells of higher organisms, energy is produced in the form of ATP, generated in the mitochondria via electron transport chain (Figure 12; Sapra et al. 2003). Within mitochondria during energy production, aerobic metabolism results in the generation of chemical radicals derived from oxygen (Reactive Oxidative Species or ROS; Balaban et al 2005). At several sites along the cytochrome chain, electrons derived from NADH or FADH can directly react with oxygen or other electron acceptors and generate free radicals (Balaban et al. 2005). Complex I (NADH dehydrogenase) and complex III (ubiquinone-cytochrome *c* reductase) of the electron-transport chain are the major sites for ROS production (Figure 12; Chen et al. 2003; Balaban et al. 2005; Monaghan et al. 2009).

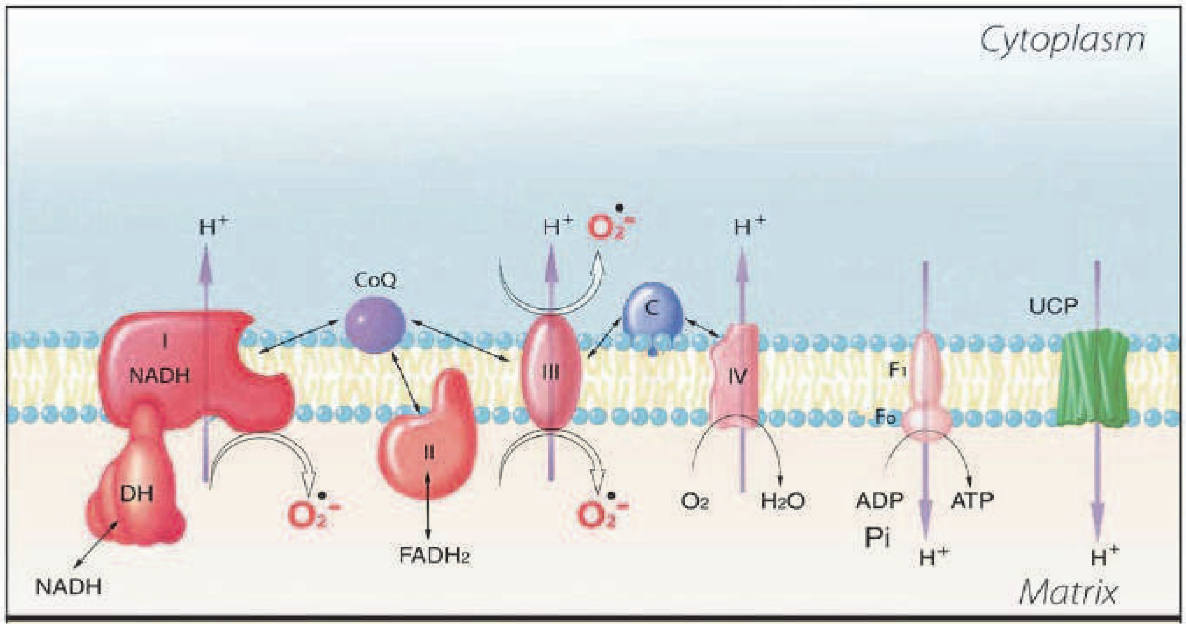


Figure 12: A Schematic Model of ROS Generation in the Mitochondria.
The production of mitochondrial superoxide radicals occurs primarily at two points in the electron transport chain: complex I (NADH dehydrogenase) and at complex III (ubiquinone-cytochrome *c* reductase). Under normal metabolic conditions, complex III is the main site of ROS production. This system lies in the formation of free radical semiquinone anion species that occurs, as an intermediate in the regeneration of coenzyme Q. free radical semiquinone can transfer electrons to molecular oxygen with the subsequent generation of a superoxide radical (From Balaban et al. 2005).

These radicals are highly reactive and unstable (Balaban et al. 2005). These may induce damages to other key biological molecules, notably DNA, lipids and proteins (Finkel & Holbrook 2000; Balaban et al. 2005; Monaghan et al. 2009). Damages generated by the attack of ROS will lead to the generation of oxygen-derived metabolites (Reactive Oxidative Metabolites or ROM).

Individuals have developed sophisticated defence mechanisms to prevent such damages: the set of defences have been termed the ‘antioxidant system’ (Surai 2002;

Monaghan et al. 2009). Many antioxidants are obtained from the diet, such as vitamin E and the carotenoids, while some of the antioxidants components are endogenous (enzymes, e.g. superoxide dismutase, glutathione peroxidase) (Finkel & Holbrook; Constantini 2008; Monaghan et al. 2009). The antioxidants form the antioxidant barrier that will turn ROS into less reactive species (Monaghan et al. 2009). The production or deployment of the antioxidants can be modulated so as to address for example an increased ROS production, i.e. stored dietary antioxidants can be mobilized and/or the endogenous production of antioxidants can also be increased (Surai 2002; Costantini 2008; Monaghan et al. 2009).

Oxidative stress assays were performed on plasma using commercial kit d-ROM test and oxy-absorbent (Diacron International). The d-ROM test measures hydroperoxides and oxy-absorbent test measures the ability to resist oxidative action of hydro-chlorine acid (HClO) (Beaulieu et al. 2010).

2. Telomere

The telomeres are generally rich in guanine, which make them much more susceptible to oxidative damage than the rest of the genome (Monaghan 2010). In vertebrates, telomeres are composed of numerous tandem repeats of TTAGGG/CCCTAA, with some variation in other taxa (Bodnar 2009; Monaghan & Haussman 2006; Monaghan 2010). This is indicating that telomeres are an ancient, highly conserved and effective system of genome protection (Epel et al. 2006; Monaghan 2010). However, the number of repeat sequences varies among chromosomes and individuals of the same species and there are also considerable inter-specific variations (Monaghan 2010). The rate of telomere loss is responsive to cell division rates and to environmental circumstances in the cell, particularly the level of oxidative stress, and this provides an important link between lifestyle and senescence (Finkel & Holbrook 2000; Monaghan & Haussmann 2005; Monaghan et al. 2009). Once some crucial threshold length is attained, the telomere becomes dysfunctional and the protective capping of chromosome ends is no longer operating (Monaghan 2010).

From 5µL of red blood cell, DNA was extracted using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen). We measured the relative size of telomeres by a quantitative PCR method (described by Criscuolo et al. 2009).

3. Stable isotopes

The isotopes are atoms, which possess the same number of protons and electrons, but a variable number of neutrons (Criss 1999; Fry 2006). When these isotopes are energetically stable, it means they do not deteriorate or are non-radioactive; stable isotopes (Michener & Lajtha 2008). An isotope is stable as soon as the number on neutrons (N) and the number of protons (Z), which constitute it, tend to be similar ($N/Z \leq 1.5$) (Criss 1999; Fry 2006). These atoms form the set of molecules of an organism, i.e. amino acids, proteins (Fry 2006). Each proteins of an organism have their own isotopic signature depending the nature of the amino acids and so its stable isotopes (Fry 2006). When a predator ingests a prey, it ingests the proteins constituting it. These proteins are fractioned, liberating the amino acids, which will be used by the predator to synthetise its own proteins (Fry 2006). In a way, the amino acids and the isotopic signature are transferred from the prey to the predator's tissue, '*we are what we eat*'. The biochemical composition of our tissues is reflecting the biochemical composition of our alimentation (Fry 2006). In the marine environment, two elements are frequently used: the Carbon (C) and the Nitrogen (N) (Forero & Hobson 2003; Layman et al. 2012). Both of them possess two stable isotope forms: ^{12}C and ^{13}C for the carbon, ^{14}N and ^{15}N for the nitrogen (Kelly 2000). For each case, the ratio of the heavy isotope to the light isotope $^{13}C/^{12}C$ ($\delta^{13}C$) and $^{15}N/^{14}N$ ($\delta^{15}N$) are calculated (Kelly 2000). The measurement of ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) is a powerful tool in ecology and is increasingly used to explore ecosystem-level energy flows and to describe diet reconstruction and animal movement patterns (Gannes et al. 1998; Hobson 1999; Kelly 2000). In seabird ecology, the stable isotopic technique was used to delineate bird-feeding habitat within and outside the breeding season and their trophic relationships (e.g. Hobson 1993; Hobson et al. 1994; Forero & Hobson 2003). Indeed, the $\delta^{15}N$ enhances from about 3‰ between each trophic level (Deniro & Epstein 1981), delivering information on the trophic level occupied by the predator within its ecosystem (Michener & Kaufman 2007). Ratio of carbon isotopes ($\delta^{13}C$) varies substantially among primary producers with different photosynthetic pathways (e.g. C3 versus C4 photosynthetic pathways in plants), but change a little with trophic transfers (DeNiro & Epstein, 1981; Inger & Bearhop, 2008; Peterson & Fry, 1987; Post 2002). Therefore, $\delta^{13}C$ can be used to determine original sources of dietary carbon, i.e. benthic/neritic vs. pelagic/oceanic (Kelly 2000). The isotopic signature of a tissue reflects the diet during the period of its synthesis (Bearhop et al. 2002). For the whole blood, the turnover rate of half-life is around a month for seabirds like penguins (Cherel & Hobson 2007). In contrast, the complete turnover for the plasma is <5 days (Cherel & Hobson 2007). This temporal integration of protein turnover allows the analysis of different tissues of the same animal, monitoring its feeding ecology at different time scales and therefore different periods of its life cycle (Hobson 1993; Cherel et al. 2008).

For this study we analysed the stable isotope from the blood sample (red cells and plasma). From 100 µL of plasma lipids were removed with cyclohexane (Cherel et al. 2010), no need for red blood cells. Stable-carbon and nitrogen isotopes assays were performed on 1 mg subsamples of homogenized materials by loading into tin cups and combusting at 1,800°C in a Robo-Prep elemental analyser. Resultant CO_2 and N_2 gases were then analysed using an interfaced Europa 20:20 continuous-flow isotope ratio mass spectrometer (CFIRMS) with every five unknowns separated by two laboratory standards. Stable isotope abundances were expressed in δ notation as the deviation from standards in parts per thousand (‰) according to the following equation:

$$\delta X=[(R_{\text{sample}}/R_{\text{standard}})-1]\times 1000$$

where X is ^{13}C or ^{15}N and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The R_{standard} values were based on the PeeDee Belemnite (PDB) for ^{13}C and atmospheric N_2 (AIR) for ^{15}N.

VI. Statistical analyses

All statistical analyses will be described in each article composing this thesis, as they are specific to each study.

CHAPTER 3: ENVIRONMENTAL CONDITIONS AND FORAGING BEHAVIOUR

ARTICLE A

Can thermoclines be a cue to prey distribution for marine top predators? A case study with little penguins

Laure Pelletier, Akiko Kato, André Chiaradia, Yan Ropert-Coudert

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Article A

Abstract in French

Est-ce que la thermocline peut être un indice sur la distribution des proies pour les prédateurs marins supérieurs?

Une étude de cas avec le manchot pygmée

Résumé :

L'utilisation des prédateurs situés au sommet des réseaux trophiques comme bio-plateformes est une approche moderne pour surveiller l'état des écosystèmes marins. Dans ce but, nous avons équipés 43 manchots pygmées, *Eudyptula minor*, d'appareils miniaturisés pour déterminer les profils thermiques de la colonne d'eau, ainsi que le comportement de nage et le taux de rencontre de proies des manchots, sur une méso-échelle de temps et d'espace. Durant la période d'élevage du poussin, les oiseaux effectuent un voyage alimentaire d'une journée dans un rayon de 20-25 km autour de la colonie. Notre étude a pour but de (1) caractériser la structure thermique de la colonne d'eau, notamment la présence/l'absence de la thermocline, (2) de lier ces caractéristiques physiques du milieu à un taux de rencontre de proies par le manchot, pour (3) conclure sur les conséquences qu'une telle relation peut avoir sur les stratégies de reproduction du manchot. Sur les 5 semaines d'enregistrements, les manchots ont mis en évidence la dynamique des caractéristiques thermiques de la colonne d'eau. Les 3 premières semaines une thermocline a été visible mais à des profondeurs différentes. L'observation d'une thermocline coïncidait avec un succès de rencontre des proies important. Une disparition de la thermocline dans les profils thermiques en parallèle d'un succès de pêche plus faible nous suggèrent que la thermocline ne s'est pas simplement enfoncée dans la colonne, hors de portée du manchot, mais que des modifications plus importantes ont eu lieues par des entrées de masses d'eaux dans le détroit. Cette dynamique temporelle de la thermocline doit avoir une incidence particulièrement importante sur les décisions de reproduction des oiseaux puisque les espèces doivent synchroniser leurs efforts de plongées avec le pic de disponibilité alimentaire qui correspondrait selon nos résultats à un état de stratification de la colonne d'eau au moment de l'élevage du petit poussin. Cela devrait nous permettre d'étudier les déterminants de la qualité individuelle, notamment comment des manchots plus expérimentés ajustent leurs stratégies de reproduction en fonction des caractéristiques thermiques de la colonne d'eau. Notre travail montre également tout le potentiel d'utiliser les manchots pygmées comme bio-plateformes indicatrices de l'impact que les changements climatiques vont induire dans le détroit de Bass.

Mots clés : Manchot pygmée, bio-plateforme, thermocline, succès de pêche, écosystème côtier

Article A

Can thermoclines be a cue to prey distribution for marine top predators? A case study with little penguins

Laure Pelletier, Akiko Kato, André Chiaradia, Yan Ropert-Coudert

Published April 2012 PLoS ONE 7(4): e31768. doi:10.1371/journal.pone.0031768

1. Abstract

The use of top predators as bio-platforms is a modern approach to understand how physical changes in the environment may influence their foraging success. This study examined if the presence of thermoclines could be a reliable signal of resource availability for a marine top predator, the little penguin (*Eudyptula minor)*. We studied weekly foraging activity of 43 breeding individual penguins equipped with accelerometers. These loggers also recorded water temperature, which we used to detect changes in thermal characteristics of their foraging zone over 5 weeks during the penguin's guard phase. Data showed the thermocline was detected in the first 3 weeks of the study, which coincided with higher foraging efficiency. When a thermocline was not detected in the last two weeks, foraging efficiency decreased as well. We suggest that thermoclines can represent temporary markers of enhanced food availability for this top-predator to which they must optimally adjust their breeding cycle.

Keywords: bio-logging, seabirds, foraging, oceanographic conditions, predator-prey interaction, match-mismatch resource

2. Introduction

During reproduction, parents have to make decisions to optimise energy acquisition to simultaneously address their own needs and that of their offspring (Shultz et al. 2009). To this end, breeding animals should optimally match their peak of food requirements with the seasonal peak of resource availability (Durant et al. 2007). A mismatching of these peaks can cause a decrease in the current reproductive output, as well as a reduction in the animal's long-term fitness (Thomas et al. 2001; Visser & Both 2005). The impact of this match-mismatch is particularly significant in marine ecosystems. The open ocean is a heterogeneous environment that is characterized by patchy prey distribution over a large time and spatial scale. As a consequence, top predators target places of high prey abundance, the hot spots which are a result of physical processes, such as up-wellings, eddies, gyres or sea-ice edges (Bost et al. 2009). These places often change seasonally, annually or on a decadal basis (Sæther 2000). Prey availability in these places can be affected by changes in oceanographic conditions, which could affect foraging success of marine top predators. These changes taking place at local foraging zones are generally influenced by large-scale processes (Georges & Le Maho 2003) and can trigger a mismatch between predators and their prey.

For diving marine animals, oceanographic conditions of the water column, such as the presence of a thermocline, can also be important for the distribution of prey (Kitagawa et al. 2000). Clupeids, an abundant food source for top predators, can aggregate around thermoclines as shown, for example, in Argentine anchovies (*Engraulis anchoita*), which distribute preferentially in the layer immediately above the thermocline (Hansen et al.2001). This is probably because thermoclines are rich in nutrients where the different levels of the food web concentrate (Franks 1992; Russell et al. 1999; Takahashi et al. 2008). For instance, the foraging behaviour of thick-billed murres (*Uria lomvia*) varies with the vertical distribution of prey, which is associated with annual variation in the intensity of the thermocline and water temperature at different depths (Kokubun et al. 2010). Another seabird, Rhinoceros auklets (*Cerorhinca monocerata*) usually dive above or around the thermocline, indicating that either the distribution of their prey is constrained by this shift in temperature (Matsumoto et al. 2008) or that the escape speed of the ectothermic prey is slowed down by the sudden change in temperature, making them easier targets to predators.

Here, we studied the foraging behaviour of the little penguin (*Eudyptula minor*), a marine diving seabird in which case the link between thermocline and foraging success has also been reported (Ropert-Coudert et al. 2009). These authors found that a reduction in thermal stratification in the water detected by data loggers in a weak El Niño year (2006) was associated with reduced foraging success of little penguins. Thus, the increase in the mixing of the water column could have resulted from an increase in the wind force and in the number of storms (Ropert-Coudert et al. 2009), although other physical factors may lead to a similar mixing. The foraging patterns of penguins suggested that their prey were dispersed widely in the presence of poorly stratified waters (Ropert-Coudert et al. 2009). In these studies (Matsumoto et al. 2008; Ropert-Coudert et al. 2009; Kokubun et al. 2010), the absence of the thermocline reduced their foraging success during chick rearing, leading to a decrease in reproductive success. While previous studies (Matsumoto et al. 2008; Ropert-Coudert et al. 2009; Kokubun et al. 2010) have looked at a composite of breeding/foraging success in relation to predominant oceanographic conditions over a whole season (Francis et al. 1998), no study, to our knowledge, has investigated the rate of prey encounter in relation to oceanographic conditions over short time scale (i.e. within a season).

In this study, we examined changes in the foraging activity and efficiency of breeding little penguins, while simultaneously monitoring changes in the vertical thermal characteristics of the water in their foraging zone. Since thermoclines can act as a boundary to prey distribution seasonally (Ropert-Coudert et al. 2009), we hypothesised that the presence of a thermocline could be a reliable signal of resource availability. We deployed miniature accelerometers on little penguins at early chick-rearing phase in a single season of high breeding success, when food supply was probably not a limiting factor (Chiaradia & Nisbet 2006). We expect the ability of penguins to match the energetically demanding chick-rearing phase (Gales & Green 1990) with the presence of a thermocline to be critical to the foraging behaviour of these diving seabirds.

3. Materials and methods

The study was conducted on the little penguin breeding colony at Phillip Island (38°31’S, 145°09’E), Victoria, Australia. We deployed data loggers on 43 adult penguins at guard phase, tending chicks aged 1 to 2 weeks. At guard phase penguins make one-day foraging trips within 20 km from the colony (Collins et al. 1999). The study period spanned 5 weeks, from 13 November to 17 December 2005. We used 12-bit, 52×15 mm, four-channel data loggers that weighed 16 g (M190L-D2GT, Little Leonardo, Tokyo, Japan) to record depth (resolution 0.05 m) and temperature (0.01°C) every second. This logger also recorded two axis accelerations along the longitudinal body axis (surging) and the dorso-ventral axis (heaving) of the bird, between -30 and 30 m s^{-2} at 32 Hz. The accelerometer measured both specific acceleration (e.g. movement) and gravity-related acceleration (e.g. posture).

Penguins were captured in their artificial nest box and loggers were attached on the lower back of the bird with Tesa tape (Wilson et al. 1997). All birds were recaptured in their nest boxes, the logger retrieved and the tape completely removed. Attachment and removal of the logger was completed within 5min from the capture, and birds were returned to their nest-boxes. All equipped birds were monitored until the end of breeding (Ropert-Coudert et al. 2007). Fieldwork protocol was approved by the Animal Experimentation Ethics Committee, Phillip Island Nature Park (PINP AEEC, number PINP AEEC 2.2004) with a research permit issued by the Department of Sustainability and Environment, Flora and Fauna (number 10003419) of Victoria, Australia.

Data were downloaded from the loggers into a computer and analysed using Igor Pro (Wavemetrics Inc., USA, 2008, Version 6.04). Given the low accuracy of the depth sensors at surface, only dives > 1m were considered for analysis (Kato et al. 2006). Dive depth, total number of dives, time spent underwater, defined as the sum of all dive durations, and proportion of time at the bottom phase were calculated for each individual. A dive started and ended when birds departed and returned to the water surface. Start and end of bottom phases were defined as the first and last time the depth change rate became < 0.25 m s^{-1} during a dive (Kato et al. 2006). It is during the bottom phase of dives that little penguins encounter most of their prey (75.4%) (Ropert-Coudert et al. 2006b).

We measured foraging efficiency using frequency and amplitude of flipper beatings, which were automatically extracted from the signal using purpose-written macro in Igor Pro (Wavemetrics Inc., USA, Version 4.02) (Zimmer et al. 2011a). The acceleration data were separated into low and high frequency components using the IFDL package from Igor (Kato et al. 2006). Each propulsive stroke was recorded on the heaving axis resulting in a forward acceleration recorded on the surging axis. The amplitude of each stroke was analysed using the heaving acceleration, which is the most sensitive signal to detect frequency of strokes (Kato et al. 2006). We could then identify periods of higher than the normal amplitude values observed during diving periods. Those periods of high amplitude were used as a proxy of prey encounter and pursuit (Ropert-Coudert et al. 2006b). Note this method does not provide a direct measure of prey consumption but prey encounter, which gives an estimate of food available to a bird during a trip. We calculated the ratio of the number of dives with prey encounter to the total number of dives during the foraging trip, as an index of hunting efficiency (Zimmer et al. 2011a). We determined prey encounter rates for depths >10 m because the high buoyancy of the birds in <10 m depth influences the flipper beating activity (Ropert-Coudert et al. 2006b, 2009).

Given that temperature sensors have a delayed time response ($T_{0.9} \approx 15$ sec) (Matsumoto et al. 2008), we corrected the temperature associated to depth following Daunt *et al.* (2003). Within a day trip, we determined a single thermal profile of the water column for each bird. For each hour of the day, we obtained a temperature profile by grouping temperatures from the same depth from both the descent and the ascent phase of the dive (Ropert-Coudert et al. 2009) during the course of the deepest dive (> 25 m) (Matsumoto et al. 2008). From the several profiles obtained from a given bird, we calculated a mean temperature every 2 meters. This resulted in one thermal profile for each penguin that we then used to determine the presence or absence of a thermocline, defined as a zone of rapid decrease of temperature in the water column (Minster 1997) in the five weeks of this study. The depth of the thermocline was visually detected from the vertical profile of temperature.

Statistical analysis of dive parameters was performed using the R software (version 2.8.1) (R Development Core Team 2009). For the hunting efficiency analyses, the sample size was only 39 birds due to missing data or excluding birds, which did not dive deeper than 10 m. We tested for normality and applied a logarithmic transformation when necessary. We used a generalized linear mixed model (GLMM) (Bates & Maechler 2009) with individuals as a random factor. For proportions, a binomial distribution was used, while a Poisson distribution was used for other variables. Subsequently, multiple comparisons were undertaken using the Tukey’s post hoc test. Unless otherwise stated, values are presented as mean ± SE with significance at 0.05.

4. Results

All equipped birds made one-day trips and succeeding in raising their chicks until fledging. During the five weeks of the study, significant changes in the thermal profiles were

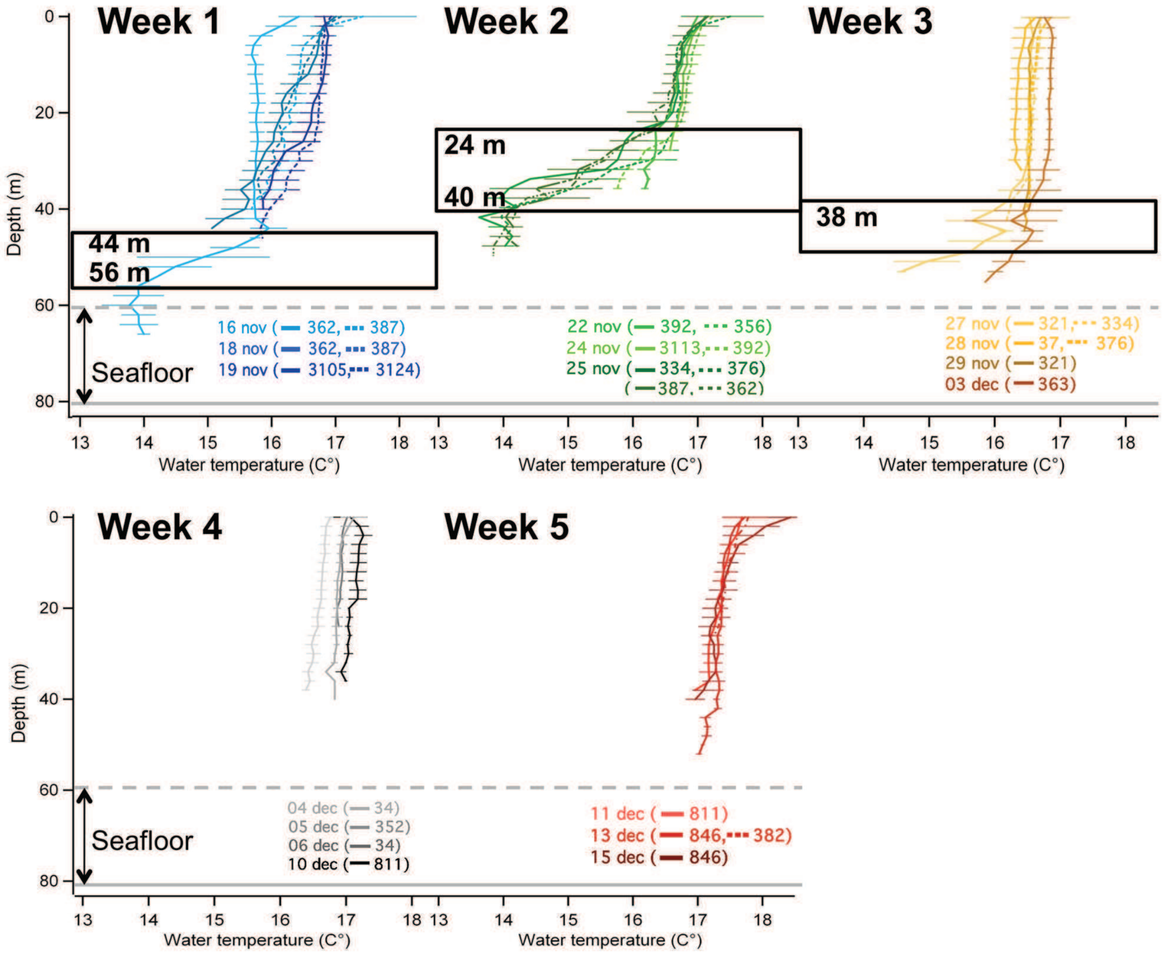

Figure 1 : Changes of the thermal profiles of the water column in Bass Strait, Australia. During five weeks in November-December 2005, as measured by little penguins equipped with data loggers during one-day trips at their guard phase of breeding. Each temperature profile corresponds to a mean water temperature (6 SD) every 2 meters calculated from several profiles obtained from each given bird (identified by date and nest number). The thermocline is framed in bold. The seabed is situated between 60 m (dotted, horizontal grey line) to 80 m (solid, solid, horizontal grey line). We only represented the dive/temperature profiles of those birds that dived deeper than 25 m (see Materials and Methods for details).

observed in the water column (Figure 1).

A thermocline was visible in the first three weeks, but not detected in the last two weeks. The thermocline was higher in the water column during the second week (24 - 40 m) compared with the first (44 - 56 m) and third weeks (38 m - the end of the thermocline being not detected). During the third week, the thermocline disappeared gradually from temperature profiles.

The mean number of dives performed by penguins during a foraging trip varied on a weekly basis (Table 1). Birds foraging during the second week made significantly less dives than individuals from other weeks (Tukey's post hoc test: all p-values <0.05, Table 1). Moreover, deep dives (> 25 m) were more frequent in the first two weeks (between 10 and 15% dives) than in the subsequent three weeks (4% of dives). A reduction in the mean dives depth was observed after the third week (Table 1).

Table 1: Comparison of different diving parameters of little penguins during one-day trips at guard phase of breeding. Values expressed in mean ± SE over the five weeks. a, b, c, d: letters indicate significant differences (at 0.05). n = number of birds.

	Week 1 (*n=6*)	Week 2 (*n=12*)	Week 3 (*n=10*)	Week 4 (*n=7*)	Week 5 (*n=8*)
Nb of dive	892 ± 127^{a}	600 ± 37^{b}	979 ± 140^{c}	1165 ± 140^{a}	1441 ± 149^{a}
Dive depth (m)	10.9 ± 0.1^{a}	10.9 ± 0.1^{a}	$8.2 \pm 0.1^{a,b}$	6.3 ± 0.1^{c}	6.1 ± 0.1^{b}
Time underwater (h)	6.6 ± 0.4^{a}	4.4 ± 0.3^{b}	$4.8 \pm 0.6^{c,d}$	$4.9 \pm 0.6^{b,c}$	5.8 ± 0.3^{d}
Bottom phase (%)	35.2 ± 1.8^{a}	28.3 ± 1.4^{b}	34.2 ± 2.4^{a}	35.7 ± 2.2^{a}	37 ± 0.7^{a}

The total time spent underwater also differed weekly (Table 1). During the first week, birds spent significantly more hours underwater than individuals in all other weeks (Tukey's post hoc test: all p-values <0.05). For the second week, birds spent on average less time underwater, but also less time (in proportion) at the bottom phase of dives (Table 1). The time spent at the bottom phase of dives was equivalent for birds foraging the other four weeks (Table 1, Tukey's post hoc test: all p-values > 0.05).

The hunting efficiency was higher for the first three weeks than for the last two (Figure 2), although the average efficiency of individuals from week 2 and week 5 was not significantly different (p-value = 0.056).

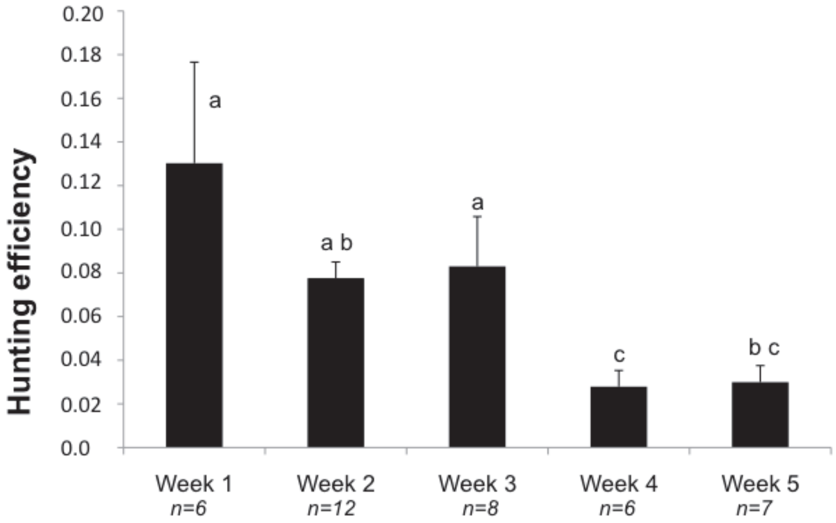

Figure 2: Changes of the hunting efficiency during the five weeks. Mean hunting efficiency 6SE (see Materials and Methods) of penguins for each week is represented. Letters a, b and c indicate significant differences (0.05) following GLMM-binomial and Tukey’s post hoc tests. n = number of birds.

5. Discussion

The thermal stratification of the water column in the foraging zone of little penguins changed over the course of the chick-rearing phase. These changes coincided with a decrease in foraging performance over time. Penguins showed higher hunting efficiency in the first 3 weeks when the thermocline was detected in the water column. Hunting efficiency declined while the total number of dives tended to increase when the thermocline weakened or was no longer detected.

No thermocline was detected in the temperature profiles recorded by the data loggers in the last two weeks of our study. A possible explanation for this is that the thermocline was deeper and not reached by penguins in the last two weeks. This is, however, unlikely given that little penguin can dive up to 70 m (Ropert-Coudert et al. 2006a), implying that they are capable of foraging throughout the whole water column of Bass Strait with the mean depth between 60-80m (Chiaradia et al. 2007a). In week 5 for instance, birds dived as deep as 70 m, without detecting a thermocline. In fact, it would be surprising if the thermocline is located below 70 m, i.e. only 10 m above the maximum seabed depth in the penguin’s foraging zone. In any case, >70 m depth would be beyond the penguin’s reach. Secondly, birds could be foraging above the thermocline so that changes in water temperature were not detected by the data loggers. However, our biological data do not support that since penguins had lower hunting efficiency in weeks 4 and 5, suggesting that their foraging conditions were similar to those observed when thermoclines were absent. For these reasons, we suggest that the temperature profile recorded by the loggers were a close to real representation of the thermal structure in penguins foraging area over the course of our study (Figure 1).

When the thermocline was present in the water column, birds showed a higher hunting efficiency than when the thermocline was absent. Thermoclines are known to aggregate marine life. For example, anchovies, a common prey for little penguins (Chiaradia et al. 2008), are known to concentrate around thermoclines (Hansen et al. 2001). While we believe prey can still be found sporadically distributed in the water column, the thermocline may act as a physical barrier, preventing prey from dispersing. The ectothermic nature of fish could be one possible explanation for this behaviour. The abrupt cooling when crossing the thermocline would reduce prey metabolism and consequently their maximum escape speeds, thus making them easy prey to predators (Kitagawa et al. 2001; Ropert-Coudert et al. 2009). Alternatively, a high concentration of fish above the thermocline could be as consequence of phytoplankton being concentrated in the upper water mass [Franks 1992; Russell et al. 1999; Takahashi et al. 2008).

Interestingly, birds foraging during week 2 had a high prey encounter with the smallest diving effort (few dives, little time spent underwater, short bottom time), which coincided with the period where the thermocline was the shallowest in the water column. This suggests that prey were probably concentrated at shallow waters on week 2 so penguins had less diving effort to capture them. In contrast, penguins increased the number of dives in the last two weeks reflecting an increase in birds' foraging effort. Despite of greater number of dives, the prey encounter was lower than the first three weeks. This lower foraging efficiency coincided with absence of a thermocline in the foraging zone of the penguins towards the end of guard phase. In the absence of a thermocline, prey were likely to be more dispersed in a mixed water column so penguins were exploiting a less optimal environment.

Many seabird species can increase their foraging range and decline foraging success as the breeding season progresses. This change in foraging behaviour can be explained by prey depletion within the foraging zones close to the colony, the so-called Ashmole's halo effect

(Ashmole 1971; Ainley et al. 2004; Gaston et al. 2007; Elliott et al. 2009). However, this energy-limitation hypothesis does not always find support in the literature (Schreiber & Burger 2001). Our results suggest an alternative explanation for a shift in foraging behaviour of diving birds during breeding. The lower prey encounter rate in the foraging area as the breeding season progresses could be explained by changes in oceanographic conditions that limit access to prey. For little penguins and perhaps for most diving marine animals, the presence and abundance of prey is not only associated with their distance from the central place and prey depletion but also with factors that affect prey distribution and availability in the water column, such as a thermocline and its change over time.

The absence of the thermocline late in the breeding season indeed led to an increase in diving effort while reducing hunting efficiency. We know that earlier breeding onset of little penguins has been related to an increase in sea surface temperature (SST) 3-6 months prior to breeding (Cullen et al. 2009). An increase in SST is precisely what can lead to the formation of a thermocline because stratification is initiated when the water surface warms up and separates from much colder deep water (Gaspar 1988). We propose here that if individuals are indeed adjusting the onset of breeding using SST information before the reproduction, then these individuals could be in a position to match their peak of food demand to peaks of food availability, as defined by the presence of thermoclines in their foraging zone. One main condition for this would be the ability of these individuals to relate those thermal regimes with prey availability and this can come through the accumulation of breeding attempts, i.e. experience (Nisbet & Dann 2009; Zimmer et al. 2011a).

In this context, future work should examine how individual's characteristics, such as age or experience, influence the ability of penguins to match their peak of food requirement to the presence of a thermocline in their foraging environment. These are important parameters to assess climatic scenarios, such as the predicted increase in El Niño events in the next decades (IPCC 2007). El Niño events could lead to a greater mixing of the water column and disappearance of thermocline, affecting the foraging patterns of marine predators that depend, as shown in this study, on these thermal structures to forage more successfully.

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Kirkwood and M. Salton. P. Fallow assisted in the field and I. Zimmer provided invaluable input during the analysis. We also thank P. Gaspar for his comments and help to improve our manuscript and P. Wasiak for proof-reading the final version. This study was funded by grants from the Japan Society for Promotion of Science, Australian Academy of Science and BHP-Billiton. Fieldwork protocol was approved by the ethics committee of the Phillip Island Nature Parks with a research permit issued by the Department of Sustainability and Environment of Victoria, Australia.

References

References have been included in the general bibliography at the end of this thesis.

ARTICLE B

Long-term linkage of the thermal structure of the water column with the foraging behaviour of a coastal seabird

Laure Pelletier, Akiko Kato, Yan Ropert-Coudert, André Chiaradia

Article in preparation – Not ready to be submitted

Article B

Abstract in French

Lien à long terme de la structure thermique de la colonne d'eau avec le comportement alimentaire d'une espèce d'oiseaux marins côtiers

Résumé :

Les espèces marines prédatrices à place centrale vont, durant la période contraignante qu'est la reproduction, utiliser des zones d'alimentation en mer connues pour être abondantes en nourriture et fiables. Les fronts marins sont des zones stables et vitales pour ces espèces, leurs permettant d'assurer leur saison de reproduction. Il est en effet important de trouver suffisamment de nourriture pour répondre aux besoins de sa progéniture, mais également assez pour palier à ses propres besoins. De nombreuses études ont récemment accentué l'importance de la relation associant les oiseaux et mammifères marin aux fronts situés à une méso-echelle d'espace, tels que les eddies, les gyres ou encore les courants marins. Cependant cette relation est plus difficile à détecter lorsqu'il s'agit d'espèces explorant un environnement à plus fine échelle. Néanmoins il a déjà été décrit que la présence d'une stratification thermique de la colonne, avec notamment la présence d'une thermocline, jouait un rôle important dans l'amélioration de l'accessibilité des proies pour le prédateur. Cela a notamment été montré chez le manchot pygmée (*Eudyptula minor*). Cependant il a été également montré dans une autre étude sur le manchot pygmée, que la thermocline était un front instable et pouvait disparaître au cours d'une saison de reproduction. Cela nous laisse donc la question de la fiabilité et de l'importance de ce front pour le manchot pygmée. Dans le but de comprendre l'importance de la présence de la thermocline lors de la période d'élevage des poussins, nous avons suivi le comportement de recherche alimentaire ainsi que le succès de pêche des manchot en utilisant des accéléromètres miniaturisés attachés de manière temporaire sur leur dos.

Ces accéléromètres nous ont également permis d'enregistrer la température de la colonne d'eau traversée par l'oiseau. Nous avons ainsi pu créer des profiles de température/profondeur de la colonne d'eau. Ce suivi a été réalisé au cours de cinq saisons de reproduction consécutives (n= 61 ; 2006-2010). Au cours de ces cinq saisons, les oiseaux ont rencontré différentes conditions thermiques de la colonne d'eau. A partir de ces profils de températures il a été défini 4 types de structures thermiques de la colonne d'eau explorés par les oiseaux. La première catégorie correspond à une colonne d'eau froide (avec des températures de surface <15.5°C), mais également totalement mixée, c'est à dire peu de différence de température entre la surface et la profondeur maximale atteinte par les manchots. La deuxième catégorie correspond à une colonne d'eau chaude et avec une diminution de la température partant de la surface. La troisième marque la présence d'une thermocline et la dernière catégorie est caractérisée par la présence d'une colonne d'eau mixée et chaude. Nous avons montré que le comportement de recherche alimentaire changeait en fonction de la structure de la colonne d'eau. La présence d'une thermocline notamment a grandement influencé ce comportement en affectant la distribution des proies dans la colonne d'eau. En effet, les oiseaux ont réalisé des plongées peu profondes, recherchant activement les proies dans le haut de la colonne d'eau. Le fait de réaliser des plongées peu profondes a permis aux oiseaux de passer plus de temps dans la phase de fond des plongées et de rencontrer plus de proies. Au contraire, en l'absence de thermocline, les manchots ont plongé, de manière significative, plus profondément dans la colonne d'eau mixée et ils ont dû passer plus de temps à poursuivre une proie lors d'une plongée. La thermocline a un rôle important pour le manchot pygmée. Elle permet aux manchots d'optimiser leur comportement de recherche de nourriture. Cependant l'absence de thermocline dans la zone d'alimentation des oiseaux ne résulte en rien en un échec de reproduction, comme les manchots semblent ajuster leur comportement à un niveau 'sous optimal'. Le comportement qu'ils adoptent dans leurs limites physiologiques et morphologiques, lors de l'absence de thermocline, leur permet d'augmenter leur chance d'obtenir un succès de pêche convenable.

Mots clés : Thermocline, manchot pygmée, comportement de recherche alimentaire, conditions océanographique, synchronisation, variabilités inter et intra saisons.

Article B

Long-term linkage of the thermal structure of the water column with the foraging behaviour of a coastal seabird

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Article in preparation – Not ready to be submitted

1. Abstract

For central place foragers, having a stable and reliable marine front during the constraining period of breeding seems vital in order to secure enough food for their offspring and themselves. Many studies have recently emphasized the relationship of mesoscale marine fronts such as eddies, gyres or currents and foraging success of large marine predators, although such a relationship is harder to detect for those species with a small foraging zone. Nevertheless the presence of thermocline is an important frontal zone to assist prey encounter for an inshore top-predator like little penguins (*Eudyptula minor*). Further studies on little penguin have also highlighted the instability of this structure that is the thermocline. In order to understand the importance of this front for this species, foraging activity and success were monitored using miniaturized accelerometers placed on birds' lower back over five consecutive breeding seasons of the little penguin (n=61; 2006-2010). During this period birds explored different water column conditions, e.g. cold and mixed water column or warmer with a gradual decrease of temperature from the surface or presence of a thermocline. The presence of a thermocline influenced the foraging behaviour of the little penguin by affecting the distribution of prey in the water column. Indeed birds dove more often as they performed shallow dives. They spent more time at the bottom of their dives and encountered more prey. In absence of thermoclines, penguins made deeper dives and increased the duration of prey pursuit per dive in both warm and cold mixed water column. The thermocline had an important role for the little penguin allowing them to optimise their foraging behaviour. However, the absence of a thermocline in the foraging area of the birds did not result in a complete breeding failure, as penguins seem to have adjusted their behaviour to sub-optimal conditions so as to increase their chance to obtain prey.

Keywords: Thermocline, seabirds, foraging, oceanographic conditions, synchronization, inter- and intra-seasonal variability

2. Introduction

In the marine environment, physical processes affect the abundance and distribution of oceanic organisms (Pollard et al. 2002; Bost et al. 2009). Discontinuity and heterogeneity of physical processes offer suitable areas for development of foodweb by grouping all abiotic conditions for the development of the primary and secondary productions (Lima et al. 2002; Moloney et al. 2011). The increase of marine productivity and biomass with respect to inter-frontal waters masses, often characterize frontal zone (Bost et al. 2009). These marine fronts are known to be associated with predators as where they can find favourable feeding conditions (Hyrenbach et al. 2006; Bost et al. 2009). Localisation and stability of these fronts are more predictable at a large or meso-scale, permitting top-predators to forage actively and become specialized in their exploitation (Bost et al. 2009). For example, king penguins (*Aptenodytes patagonicus*) travel hundreds of kilometres towards oceanic fronts or mesoscale (100-1000 km) eddies using local current and temperature gradient to locate these features (Charrassin & Bost 2001; Cotté et al. 2007). Some species can target zones with specific gradients of temperature, e.g. thermocline. Thermocline is the layer of water separating warmed mixed surface water from cold deeper water and which is characterized by rapid temperature changes (Fiedler 2010). The thermocline is commonly referred to as an ecological boundary since nutrient or oxygen fluxes can be dramatically reduced across strong thermoclines (Fielder 2010). As such, persistent and strong thermocline can concentrate nutrients and prey above or under them (Gray & Kingsford 2003). Thermocline is known to be ecologically important for many top-predators (e.g. Northern fur seals, *Callorhinus ursinus*, Kuhn 2011; Thick-billed murres, *Uria lomvia*, Takahashi et al. 2008, Kokubun et al. 2010; king penguins, Scheffer et al. 2012; southern elephant seals, *Mirounga leonina*, Biuw et al. 2007). In shallow coastal waters, perturbations in the depth and intensity of thermoclines are frequent, making them less important in determining the vertical distribution of species such as mesozooplankton and fish larvae than those thermoclines that are found in pelagic waters (Gray & Kingsford 2003). Hence, at smaller scales (i.e. coastal environment), this relationship between foraging activities and marine physical processes is less clear (Bost et al. 2009).

Previous studies have shown that differences in breeding and foraging success of an inshore seabird, the little penguin (*Eudyptula minor*) between two seasons associated to the presence and absence of thermocline in relation to the El Niño events (Ropert-Coudert et al. 2009). In addition, the thermocline is not always present within a breeding season, and its disappearance had influenced the foraging success of the little penguin within a single breeding season (Pelletier et al. 2012). These studies suggested that thermoclines are crucial for little penguins to face optimum foraging conditions. However, to which extent thermoclines can result in foraging failure and ultimately breeding failure remain unclear. To understand how individuals evolve their foraging behaviour in contrasted environment we examined the potential influence of physical parameters (e.g. water surface temperature, presence of thermocline) that could affect prey distribution and availability for little penguins over several breeding seasons by monitoring their foraging behaviour. Compared with previous studies on the topic (Ropert-Coudert et al. 2009; Pelletier et al. 2012) the present study is looking simultaneously at the dynamic evolution of the thermal features of the water column at a large temporal scale, i.e. between breeding seasons, and at a finer scale, i.e. within a breeding season. The aim of our study is to examine the oceanographic patterns leading to the formation of a stratified water column in the Bass Strait region around Phillip Island and to investigate the persistence of the thermocline in the foraging zones of little penguins. We concomitantly examine the birds' foraging efficiency in response to the dynamics of the ecosystem, notably in an attempt to see if they are able to match their peak of food requirement (i.e. chicks rearing) with the peak of food availability.

3. Materials and Methods

The study was conducted in a breeding colony of little penguins' at Phillip Island (38°31'S, 145°09'E), Victoria, Australia. Foraging activities of male and female birds rearing 1 or 2 young chicks, where monitored over 5 consecutive breeding seasons (2006: 3 males – 3 females; 2007: 5 males – 3 females; 2008: 6 males – 5 females; 2009: 4 males – 5 females; 2010: 14 males – 13 females). This was done during the guard stage when birds alternate one-day foraging trip and one-day nesting (Chiaradia & Nisbet 2006). During this period they foraged in a radius of 20 km around the colony (Collins et al. 1999). Penguins were captured at their nest and accelerometers were attached on the lower back of birds with waterproof Tesa tape. After one trip the logger and the tape were removed and the nest were checked until the chicks fledged. A previous study has already examined the potential effects of using devices without finding any difference in breeding performance between instrumented and control individuals (Ropert-Coudert et al. 2006b, 2007; Agnew et al. 2013). We used 12-bit, 52×15 mm, four-channel data loggers that weighed 16g (M190L-D2GT, Little Leonardo, Tokyo, Japan) to record depth (range 0~190 m, resolution 0.05 m) and temperature (range -20~50°C, resolution 0.01°C) every second from 2006 to 2009. In 2010, we used 12 x 45 mm, five-channel data loggers that weighed 9 g (ORI400-D3GT, Little Leonardo, Tokyo, Japan) to record depth (range 0~400 m, resolution 0.1 m) and temperature (range -50~50°C, accuracy 0.1°C) every second. Both loggers also recorded both specific acceleration (dynamic) and gravity-related acceleration (static) along the longitudinal body axis (surge) and the dorso-ventral axis (heave) of the penguins, between -30 and 30 m s^{-2} at 32 Hz for the M190L-D2GT and between -40 and 40 $m.s^{-2}$ at 50 Hz for the ORI400-D3GT. Data from the accelerometer loggers were downloaded into a computer and analysed with purpose-written software in Igor Pro (Wavemetrics Inc., USA, 2008, Version 6.22A, see Kato et al. 2006). Specific and gravity-related acceleration signals were separated by a two-band low-pass filter (IFLD, Version 4, Wavemetrics) on each acceleration axis. The sum of the 2 axes' specific accelerations was calculated to give the Partial Dynamic Body Acceleration (PDBA in g or $m.s^{-1}$) as an index of the whole body activity underwater (Wilson et al. 2006; Gleiss et al. 2010; Shepard et al. 2010). During prey pursuit, the frequency and amplitude of flipper beat increases (Ropert-

Coudert et al. 2006b), resulting in a peak in PDBA (Wilson et al. 2006; Shepard et al. 2010; Wilson et al. 2010). Burst of activity recorded, i.e. different from a normal activity recorded during the dive, was defined as a prey pursuit (Ropert-Coudert et al. 2006b). Depth at which prey pursuits occurred and duration of each prey pursuit were determined. The percentage of dives with prey pursuit (number of dives with prey encounter/total number of dives) was used as an index of hunting efficiency, it is not a quantitative measure of prey ingestion but just reflect resource availability (Ropert-Coudert et al. 2006b). Depth data were corrected for surface drift and according to resolution of the loggers, only dives >1 m were considered for analysis (Kato et al. 2006). We obtained information concerning the number of dives performed, depth (m), duration (s), and duration of bottom phase (s) for each dive. A dive started and ended when birds dived from and returned to the water surface. The start and the end of bottom phases were defined as the first and last time in a dive when the depth change rate become <0.25 m.s^{-1} (Kato et al. 2006). The total vertical distance travelled during the one-day foraging trip was calculated for each bird (sum of all dive depth × 2; in km). Since the temperature sensors had slow time responses ($T_{0.9} \approx 15$ sec) (Matsumoto et al. 2008), we corrected the temperature associated to depth following Daunt et al. (2003). For each bird, a daily average temperature/depth profile was calculated using the temperature recorded during the deepest dive (>25m) performed for each hour throughout the day trip (Kokubun et al. 2010; Pelletier et al. 2012). These temperature/depth profiles allowed us to visually determine the structure of the water column, i.e. the presence/absence of a thermocline.

The sampling periods were not the same between years: September in 2009, October in 2008, end of October to end of November in 2007 and end of November to mid-December in 2006. In order to see a general trend in 'environmental conditions' between the different seasons we used data of sea surface temperature covering the region from 35°to 45°S and 138° to 152°E (for more information see Cullen et al. 2009). It allowed us to observe the complete evolution, over the whole summer (i.e. breeding period) for the 5 consecutive years, of the sea surface temperature but also the difference of temperature between surface and 50 m of depth (ΔT).

Based on the temperature/depth profiles obtained thanks to the data recorded by accelerometer we defined categories of thermal structure of the water column (see figure 1). We visually determined four categories of thermal structure of the water column: i) cold and mixed water column, ii) warm surface temperature with a gradual decrease of water temperature from the surface, iii) the presence of a stratified water column with a clear thermocline and iv) a warm and mixed water column (Figure 1). Water was considered as cold when the temperature at the surface is < to 15.5°C. This is the temperature from which we observed changes in temperature/depth profiles in the upper part of the water column.

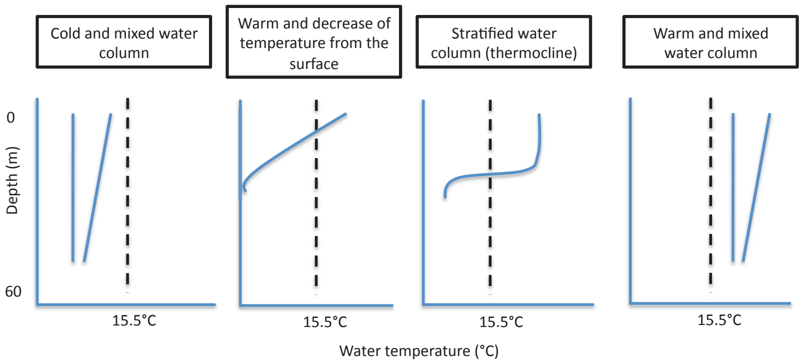

Figure 1: The 4 categories of water column defined from the Figure 3 and used for the statistical analysis.

We compared the birds' diving parameters when they were foraging in the different categories of water column using Generalised Linear Mixed Modelling (GLMM) with the *nlme* package (Pinheiro et al. 2013). Individuals were treated as random factor given that some individuals were equipped more than once. Then a post-hoc Tukey's test used with the package *Multcomp* (Hothorn et al. 2008). The sex of the bird was tested for any influence on diving parameter and as no influences were found the sex was not included in the statistical models. All analyses were conducted using R 2.11.1 (R Development Core Team 2009). Results are expressed as mean ± SE and significance level set at α=0.05.

4. Results

Environmental conditions

Data obtained by satellite showed similar trend in the evolution of the surface temperature and in the ΔT over the summer in the 5 breeding seasons, i.e. an increase in the SST and ΔT as the season progresses (Figure 2). In all years, the increase is progressive and of small amplitude early in the season but inter-annual differences are observed from around mid-October: in 2007 and 2009 a sudden and abrupt increase in the SST occurred, with the temperature increasing by nearly 2°C in a few days; the SST increased also in 2010 but this warming phase took place over a longer period of time; finally 2006 and 2008 showed no clear increase in the SST over the whole season. In parallel, the ΔT did not strongly increase in 2008 and 2006 compare to the other years. The most elevated ΔTs were associated with the highest SST (Figure 2).

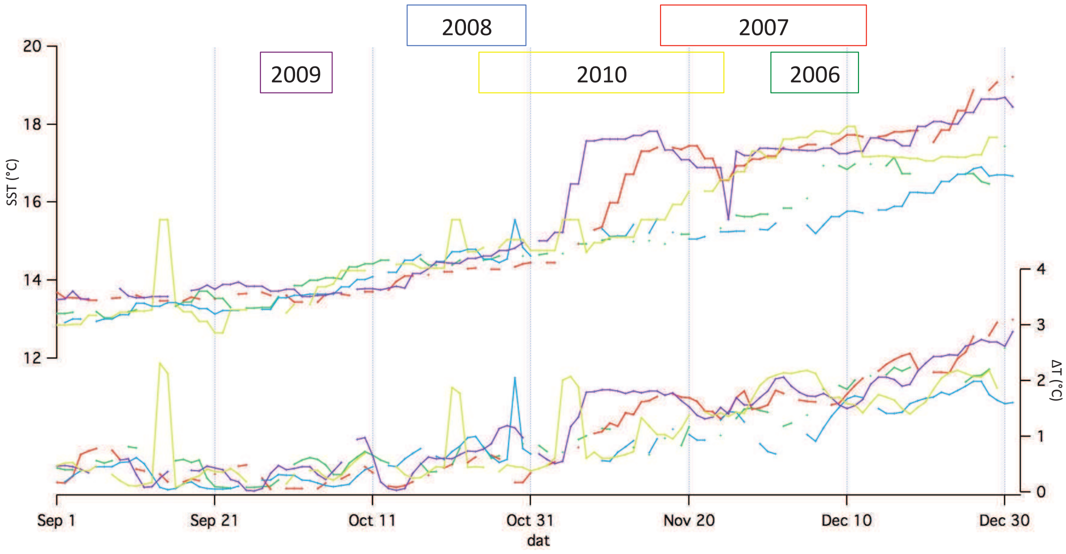

Figure 2: Observation of the evolution of the sea surface temperature (SST) and the difference of temperature between the surface and 50 m (ΔT) over 5 breeding seasons. Boxes defined the sampling interval for each year.

However, a high ΔT was not necessarily marking the presence of a thermocline, as temperature/depth profiles that had a gradual decrease of temperature from the surface also had high ΔT values (Figure 3)

The evolution of the thermal structure of the water column as recorded by the loggers mounted on the birds showed a similar trend (Figure 3). From these profiles, birds in

2009, 2008 and, to some extent in 2010, foraged in a cold and mixed water column (n=30). As season progressed, some birds of 2010 foraged in a warmer surface temperature with a gradual decrease of water temperature from the surface (n=10). In 2007, birds encountered first a profile of the same type, i.e. warm surface temperature column with a gradual decrease of water temperature from the surface, before a change occurred in the foraging area leading to formation of a stratified water column, i.e. birds exploited a water column with a thermocline (n=6) later in the season. The rest of the birds (n=23) exploited a warm and mixed water column (in 2006 and 2010).

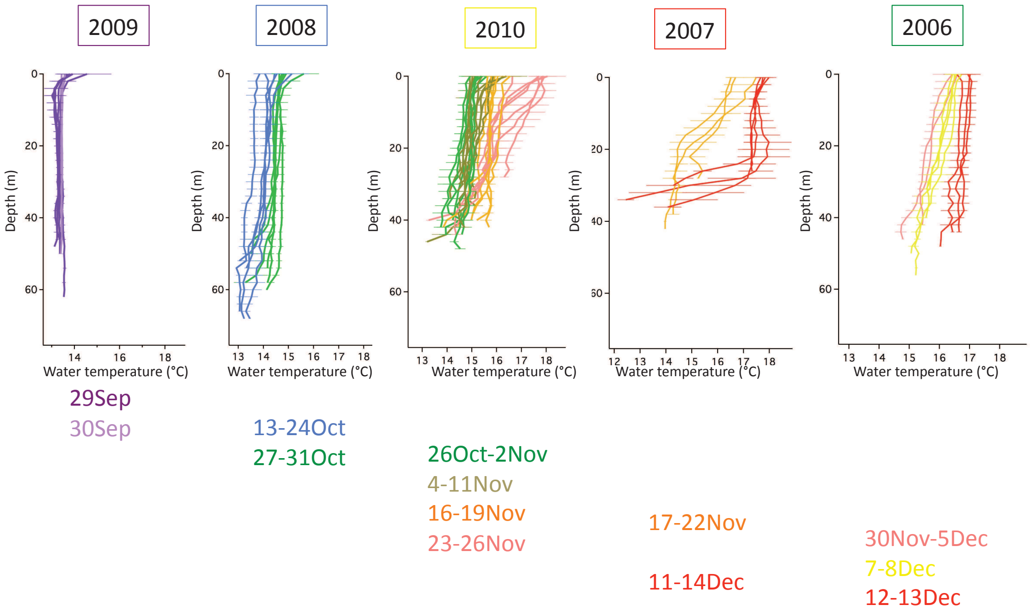

Figure 3: Temperature/depth profiles calculated with the accelerometer temporally attached to the lower back of little penguin over the 5 breeding seasons. Data were presented in function of the timing in a season to see evolution of the structure of a water column.

Diving behaviour

The number of dives performed during the one-day foraging trip was strongly and positively influenced by the presence of a thermocline (Figure 4). Birds performed less dives in mixed water column, regardless if waters were warm or cold (Figure 4).

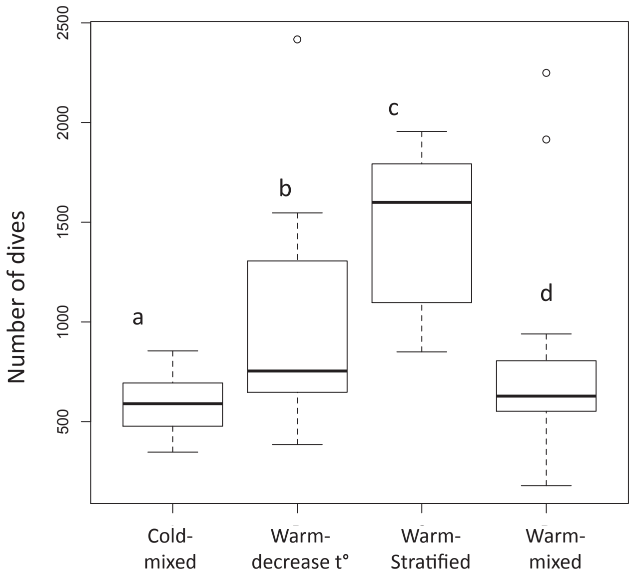

Figure 4: The number of dives performed by the birds in the different water column conditions. The different letters indicates significative differences (0.05)

However, the total vertical distance performed by the birds was significantly different between the cold and warm mixed water column, with birds in a colder water column travelling more distance (Table 1). No differences were observed with the two other categories (Table 1). The same pattern was observed for the proportion of time spent underwater, birds foraging in a cold mixed water column spent more time underwater than birds in a warm and mixed water column (Table 1).

Table 1: Average±SE of the different foraging parameters in the different water column conditions. GLMM and post-hoc Tukey's test were used. The different letters indicates significative differences (0.05)

	Cold-Mixed	Warm-decrease of temperature	Warm-stratified	Warm-mixed
Nb of dives with prey pursuit	213±12^{a}	301±53^{b}	656±92^{c}	233±25^{d}
Total vertical distance (km)	20.1±0.9^{a}	15.8±1.4^{a,b}	19.8±1.8^{a,b}	15.4±1^{b}
Time underwater (%)	43±1.6^{a}	36.5±3.4^{a,b}	41.5±4.3^{a,b}	34.1±1.9^{b}
Time bottom (%)	26.3±1.1^{a}	32.3±2.3^{b}	41.3±4.8^{c}	29.9±1.6^{a,b}
Median dive depth prey encounter (m)	19.6±1.2^{a}	11.6±1.8^{b,c}	6.7±0.9^{b}	13.1±0.9^{c}
Dive prey pursuit descent (%)	24.6±1.3^{a}	18.9±2.5^{a}	10.5±2.2^{b}	23.7±1.7^{a}
Dive prey pursuit bottom (%)	40.5±1.1	39.3±2.2	47.8±5.4	41.1±1.6
Dive prey pursuit ascent (%)	34.9±1.1^{a}	41.5±2.7^{b}	42±3.7^{b}	35.1±1.7^{a}

In presence of a thermocline birds performed shallow dives compared to a water column totally mixed (Figure 5). In cold and mixed water columns birds dove deeper than the other categories (Figure 5). Birds increased the proportion of time spent at the bottom of dives with the presence of a thermocline and, conversely, this proportion decreased in cold and mixed water columns (Figure 6).

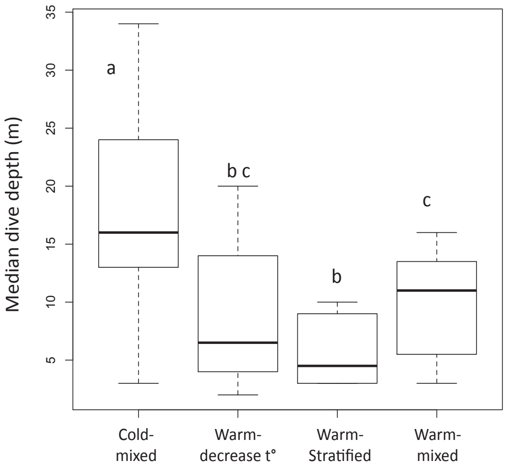

Figure 5: The median of dives depth performed by the birds in the different water column conditions. The different letters indicates significative differences (0.05)

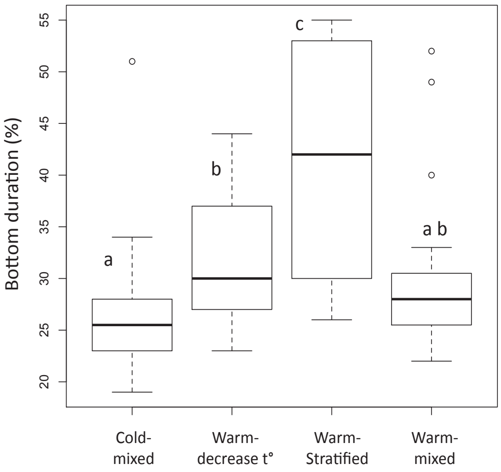

Figure 6: proportion of time spent at the bottom of dives in the different water column conditions. The different letters indicates significative differences (0.05)

Prey pursuit

Birds in presence of a thermocline dove more often and also had a number of dives with prey pursuit that was greater than birds foraging in a mixed water column (Table 1). During the foraging trip, the sum of prey pursuit duration and the hunting efficiency differed according to the environmental conditions (Figure 7, 8). Birds exploiting a water column with a thermocline had a better hunting efficiency and spent more time pursuing prey (Figure 7, 8). Moreover the duration of prey pursuit per dive was much shorter in a stratified water column than in a mixed one (Figure 9). Dives with prey pursuits were shallower when a thermocline was present (Table 1). In a stratified water column, birds showed a decrease in proportion of dives with prey pursuit during the descent phase (Table1). However, the proportion of dives with prey pursuit during the ascent phase of the dive increased, as was also observed in birds that exploited a water column with warm surface temperature and a gradual decrease of water temperature from the surface (Table 1). The proportion of dives with prey pursuit during the bottom phase was not different between the 4 categories of water column (Table 1).

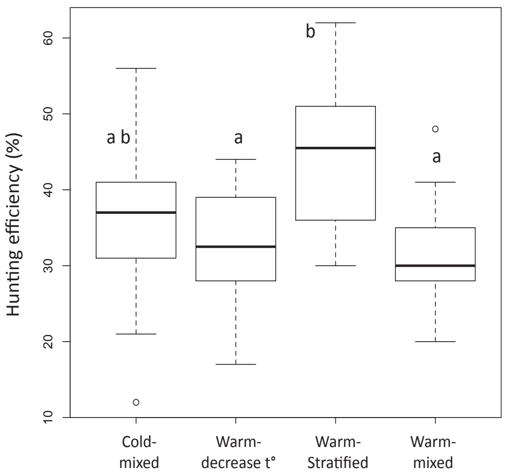

Figure 7: Hunting efficiency in the different water column conditions. The different letters indicates significative differences (0.05)

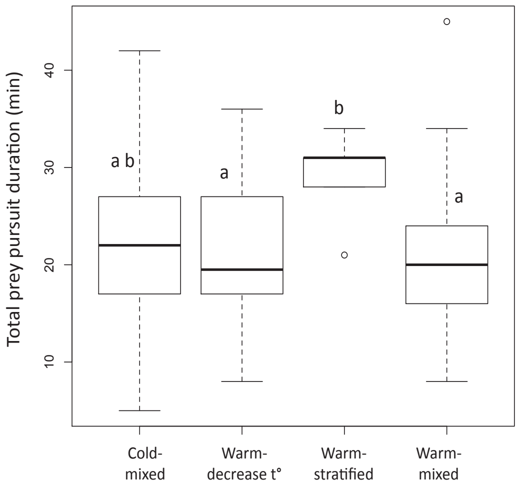

Figure 8: The total duration of prey pursuit in a one-day foraging trip (in min) in the different water column conditions. The different letters indicates significative differences (0.05)

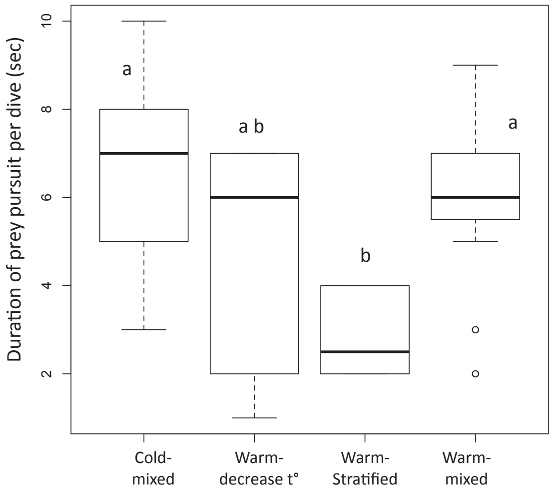

Figure 9: The average duration of a prey pursuit during a dive (in sec) in the different water column conditions. The different letters indicates significative differences (0.05)

5. Discussion

Within season and between years, little penguins exploited water columns with different thermal structures. From September to late October the inter-annual variability between years in the SST and the ΔT was low. From early November, the situation changed dramatically. A sudden increase in SST was observed in 2007 and 2009, whereas in 2010 this increase was progressive but reaching values comparable to 2007 and 2009, while it was even more gradual in 2008 and 2006. Nonetheless, we observed an increase of the surface temperature followed by an increase of ΔT in all years. Birds breeding early in a season, i.e. September-October foraged in a cold and mixed water column (2009, 2008 and 2010). With the increase of the sea surface temperature the upper of the water column started to be modified (end of October – November). As season progresses, increase in air temperature would cause the warming of SST (Gaspar 1988). The increase of temperature of the surface layer with the low wind condition would lead to the formation of thermoclines (Gaspar 1988). The thermocline generally forms at depth where wind forces are close to zero, accounting for the lower limit of the surface mixing (Gaspar 1988). Temperature at the surface would continue to increase leading to a greater difference of temperature between the surface and the deep mixed layer, i.e. to the formation of a stronger thermocline (Gaspar 1988; Fielder 2010). In that sense, thermocline could represent a predictable feature of the little penguins' environment. From our results we can hypothesise that a thermocline is likely to be found around the end of November to early December when the SST became warmer. Yet, in some years, drastic changes apparently took place, i.e. there was also some unpredictability in the system as events like storms, or sudden water masses entry in the Bass Strait, etc. led to a sudden change in SST and occasionally in the ΔT. The thermocline was only detected in the profile of some of the birds monitored in early December 2007, suggesting that the formation of this structure is temporary and limited in time (see also Pelletier et al. 2012). The absence of thermocline in early December 2006 further suggests that the timing of the appearance of the thermocline is year/condition-dependent. In 2006, a water column warm and mixed was observed in the temperature/depth profiles. The surface temperature conditions were different between 2006 and 2007.with a higher SST in 2007 coupled to a gradual decrease of temperature. Maybe the absence of thermocline in 2006 during the sampling period was related to strong wind, storm or also current that are important in this region (Ropert-Coudert et al. 2009).

Following the change in the thermal profile of the water column (from cold mixed to more warm and stratified), the diving behaviour has also changed. The number of dives increased with the stratification of the water column. This is possible because birds reduced the dive depth. We saw that it was the physical presence of thermocline that affected the most the diving behaviour. Thermocline was a physical barrier allowing the little penguins to easily find their prey. Penguins in presence of a thermocline encountered better food availability that consequently increased their foraging success. The presence of a thermocline probably means that prey were more accessible, i.e. in the upper of the water column as the birds' shallower diving activity suggest. When dives are shallow birds can dive more often per day, which in turn is concomitant with an increase in the number of dives with prey encounter and an increase in the time spent at the bottom phase of the dives where prey are more likely to chased (Ropert-Coudert et al. 2006b). In addition, birds spent less time chasing a prey during a dive, which further suggests that prey availability was higher in years with thermocline. Overall, birds foraging in a stratified water column were more efficient than birds foraging in a warm water column (both mixed or with a gradual decrease of temperature), as well as birds exploiting cold mixed water, as in the latter they seem to produce a greater diving effort than birds foraging in a stratified water column to achieve the same success. Birds exploiting a cold, mixed water column had to dive deeper in order to catch a prey and increased the duration of prey pursuit during a dive.

The birds exploiting a water column where a decrease of temperature from the surface was observed had similar behaviours than birds exploiting a stratified water column but they were less successful in encountering prey. These birds exploiting a water column with a gradual decrease of temperature from the surface exhibited an intermediate behaviour between birds exploiting a stratified and a cold mixed water column. They started to reduce the depth of their dives and to increase their number, but also to reduce the duration of a prey pursuit during a dive. The strategies of birds exploiting a cold or warm mixed water column were not the same. In both situations they dove less often but birds foraging in cold water performed deeper dives, at the end of the foraging trip the total vertical distance travelled was greater and so they spent more time underwater. Nevertheless, they had the same hunting efficiency. When there was not thermocline in the water column, prey inclined to disperse in the water column forcing birds to adapt their behaviour (Ropert-Coudert et al. 2009; Pelletier et al. 2012). Diving behaviour of the king penguins was also correlated with the thermal structure of the water column (Scheffer et al. 2012). Wiggles occurrence (i.e. prey encounter) was correlated with strong thermal gradients. This study underlines the crucial role of the thermocline for foraging king penguins breeding at South Georgia (Scheffer et al. 2012).

It seemed evident that little penguins were more successful with the presence of the thermocline, as they may increase prey consumption. However, on the five breeding seasons studied, thermocline has been observed only during one season and not for all birds. Conditions allowing the formation of a water column stratified were not met the four others seasons, at least during the sampling periods considered. We cannot ascertain that thermoclines formed later in the breeding seasons of 2009 and 2008 although the evolution of the water column in 2010 and 2007 seems to suggest that this should be the normal evolution for the zone in the vicinity of Phillip Island. The absence of observation of a thermocline in 2006 suggests that this is, however, an evolution that is subjected to change according to phenomena occurring at a larger spatial and temporal scale.

If thermoclines are indicative of an optimum for prey availability, why did birds in guard phase – the peak of food demand – foraged at a moment of the breeding cycle when no thermoclines were present in their environment in most of the seasons? The first conclusion that comes to mind is that little penguins cannot read oceanographic conditions to adjust the demanding chick-rearing period to the peak of food availability, i.e. the period when a thermocline is present in the foraging zone. This idea is further re-enforced by the fact that some birds in our study were monitored across different seasons. This means that these birds were sampled during a guard phase, which took place at different times in the year, from late September to late December, i.e. in highly different environmental conditions. Such an inter-annual difference in the timing of breeding for the same individuals suggests that these birds did not succeed/try to match the peak of food demand with the presence of a thermocline. Said differently, birds apparently did not anticipate the presence of a thermocline in the foraging area. However, such an inability to interpret early oceanographic condition is contradictory with a previous study that linked variations in SST earlier in the years with the onset of breeding in the little penguins (Cullen et al. 2009). These authors concluded that the SST provided an accurate prediction of mean laying date with 53% of the variance explained by their models. In the light of our results, we can explain such a high predictability by the fact that oceanographic conditions observed between September to end of October, roughly the incubation phase, are not much variables among years (Stahel & Gales 1987). The SST and ΔT evolved in a same manner during this period over the 5 breeding seasons that we monitored. We can thus expect penguins to 'anticipate' relatively stable conditions around this period of the breeding cycle and thus be able to 'read' better early (March-April) signs of the oceanographic conditions they are likely to meet later (September-October) in the year. Interestingly, the predictions of Cullen et al. (2009) were less accurate when it comes to correlate the number of chicks produced per pair (with only 22% of variance explained) with early oceanographic conditions. Predictions were even worst regarding the mean chick mass at fledging (16% of the variance). Again, our data can help explain this trend: the high inter-annual variability in the oceanographic condition that takes place during the rearing period would make it difficult for birds to predict the situation several months in advance. We saw that the SST increased rapidly in 2009 and 2007 early November and that this increase arrived later in 2010, for examples. While birds can expect that there are high probabilities for a thermocline to be present during this part of their breeding cycle, the differences observed from one year to another actually augurs for the low predictability which is found in models of Cullen et al. (2009).

The presence of thermocline allowed birds to increase their foraging success. However, the thermocline cannot be considered as a reliable marine front, as little penguins had not the possibility to profit of it every year and within a season (Ropert-Coudert et al. 2009; Pelletier et al. 2012). Even without thermocline, birds adapted their behaviour in order to be the most successful in their one-day foraging trip. During a one-day foraging trip, birds did all they could do in their physiological and morphological limits. The consequence would be less food brought to the nest and the chicks' growth pattern would be more likely to be influenced (Chiaradia & Nisbet 2006). Future studies would have to look at when birds make longer foraging trip (incubation and post-guard) and how environmental conditions could play on their foraging behaviour but also at their localisation at sea.

References

References have been included in the general bibliography at the end of this thesis.

CHAPTER 4 : INDIVIDUAL CHARATERISTICS AND FORAGING BEHAVIOUR

ARTICLE C

Fine-scale spatial segregation in the limited foraging area of an inshore seabird species; the effect of age and sex

Laure Pelletier, André Chiaradia, Akiko Kato, Yan Ropert-Coudert

Article to be submitted to Ecology

Article C

Abstract in French

La ségrégation spatiale à fine échelle dans une zone d'alimentation limitée d'une espèce d'oiseaux marins côtiers; l'effet de l'âge et le sexe

Résumé :

Les individus vivant en colonie bénéficient d'une diminution du risque de prédation et d'un meilleur choix du partenaire. Cependant la réunion de ces individus qui ont les mêmes besoins va conduire à une compétition pour les ressources en limitant ainsi leurs accès. La compétition pour les ressources de nourriture peut résulter à une ségrégation spatiale entre les individus d'une même espèce. Il a beaucoup été montré que cette ségrégation était due à un phénomène d'exclusion compétitive. En général les individus montrant un fort dimorphisme dans la taille se retrouvaient à exploiter des niches différentes. Ces études portaient en général sur des espèces ayant un fort dimorphisme sexuel ou encore sur des juvéniles face à des reproducteurs. Ces espèces exploitaient une large zone de prospection alimentaire. Peu d'études se sont concentrées sur les effets combinés du sexe et de l'âge chez une espèce qui exploite une faible zone de recherche alimentaire. Dans cette étude nous avons examiné l'utilisation de l'espace, en trois dimensions, d'une espèce exploitant une zone de prospection alimentaire limitée. Au cours d'une saison de reproduction 29 manchots pygmée (*Eudyptula minor*) d'âge et de sexe connus, ont été suivis au cours d'un voyage alimentaire. Pour cela, nous avons équipé les oiseaux d'un appareil

GPS et d'un accéléromètre. Ainsi nous avons pu suivre leur déplacement, mais également nous avons enregistré leur comportement sous l'eau. De plus, des prises de sang ont été effectuées à leur retour de voyage. Grâce à ces échantillons de sang nous avons retrouvé la composition de leur alimentation par l'utilisation des isotopes stables. Nous avons contrôlé tous les facteurs pouvant être confondant dus à la durée des voyages alimentaires et de la disponibilité des proies en échantillonnant uniquement des adultes qui étaient en période de garde. A cette période le mâle et la femelle d'un même nid vont alterner un voyage d'une journée en mer avec une journée au nid. Tous les oiseaux ont donc réalisé des voyages alimentaires d'une seule journée. Notre étude a été réalisée pendant une année de forte réussite en terme de succès reproducteur (1.6 poussins à l'envol par femelle) – signe de l'abondance des ressources autour de la colonie durant cette saison. Nos résultats ont montré une ségrégation spatiale dans la zone d'alimentation entre les oiseaux d'âge moyen (entre 5-11 ans) et les oiseaux plus âgés (>11 ans). Les manchots les plus âgés vont rechercher leurs proies du côté de la côte dans des zones d'eau moins profondes que celles exploitées par les manchots d'âge moyen. Bien que les oiseaux de différentes catégories d'âge et de sexe utilisent différentes zones d'alimentation, ils ont apparemment ciblé le même type de proie. On observe des variations au sein même des groupes de la niche isotopique, mais pas de différence entre ces derniers. De plus, il n'a pas été mis en évidence de différence dans le comportement de recherche alimentaire (distance parcourue et comportement de plongée) et du succès de pêche.

Nous avons émis l'hypothèse que cette exploitation différentielle des zones de recherche alimentaire est de manière prédominante déterminé par un « effet identitaire » qui conduirait les oiseaux partageant les mêmes histoire de vie (i.e. ayant éclos et s'étant dispersés autour de la même année) ou partageant des caractéristiques physiologiques similaires (i.e. la même expérience passée) à rechercher de manière préférentielle leur nourriture ensemble ou encore car ces individus font aussi face aux mêmes limitations dans la recherche alimentaire.

Mots clés : Elevage du poussin, compétition intraspécifique, oiseau marin côtier, zone de recherche alimentaire, ségrégation

Article C

Fine-scale spatial segregation in the limited foraging area of an inshore seabird species; the effect of age and sex

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Article to be submitted to Ecology

1. Abstract

Competition for food resources can result in spatial segregation among individuals from the same species. Few studies have looked at the combined effect of sex and age in species with short foraging ranges. In this study we examined the 3D spatial use of the environment in a species with a limited foraging area of 29 little penguins (*Eudyptula minor*) of known age, sex and breeding output in 2010. We equipped birds with GPS and accelerometer loggers and collected information on stable isotope (diet) from blood samples. We controlled for confounding factors of foraging trip length and food availability by sampling adults at guard stage when parents only make one-day trips. Our study was conducted in a year of high breeding success (1.6 chicks fledged per female) – an index of high food availability. We observed a spatial segregation between old (>11 year old) and middle-aged penguins (between 5-11 year old) in the foraging area. Old penguins foraged closer to the shore, in shallower water. Although birds of different ages used different foraging zones we could not identify any apparent, immediate benefits as we found no differences in the foraging effort (i.e. diving effort) and success (i.e. foraging efficiency) between age classes and sexes and birds were apparently targeting similar prey. We hypothesize that this differential exploitation is predominantly determined by an "identity effect" that would lead individuals sharing a common life history (i.e. having fledged and disperse around the same year) or sharing similar physiological characteristics (i.e. of similar past experience) to forage preferentially together or have similar foraging limitations.

Keywords: Chick-rearing, Intra-specific competition, Inshore seabird, Foraging 'hot-spots', Segregation

2. Introduction

Colonial living confers benefits as it may provide protection against predators or could enhance mate-choice (reviewed in Andersson 1994). However, a large aggregation of individuals, whether it consists of a single species or several species using similar habitats, can lead to resource limitation and may limit the available space for breeding. Also, if individuals in the aggregation have similar energy requirements and exploit the same trophic niche, this may constrain their access to food resources (Schoner 1974; Keddy 1989; Furness & Birkhead 1984; Chapman, Wrangham & Chapman 1995; Koenig 2002).

Central-place foragers commute between their breeding site and the foraging grounds on a regular basis and are limited in their foraging range by their travelling capacities. Competition for food can be particularly acute for central-place foragers that live in dense colonies. This competition for resources may affect an individual's foraging – and consequently reproductive – success, ultimately impacting their survival (Schoener 1986). In the presence of resource limitation, animals should thus modify their foraging behaviour to reduce competition in order to maximize their fitness (Pianka 1981; Bolnick et al. 2003). To reduce the extent of competition, species, population and/or individuals can partition the use of resources by exploiting habitats that differ in location (Schoener 1974), by exploiting habitats at different times (e.g. Alanärä, Burns & Metcalfe 2001; Kronfeld-Schor & Dayan 2003), and/or by modifying their dietary preferences (e.g. Bocher, Cherel & Hobson 2000).

Individual characteristics (e.g. sex, age, body condition, etc,) will favour the partition of resources in space, time or diet segregation among different species. Among the characteristics, sex and body sizes are probably features that have received most attention (Phillips et al. 2004; Page et al. 2006; Stauss et al. 2012).

Segregation often results from social dominance and competitive exclusion or from niche specialisation (Pianka 1981). Sex-specific segregation may occur when differences in morphology (sexual dimorphism) confer a higher manoeuvrability like in the smaller males of American kestrel *Falco sparverius* (Ardia & Bildstein 1997), or when sex-specific reproductive roles command for different foraging strategies like in the females of New Zealand fur seals *Arctocephalus forsteri* that use a different habitat than the males during their relatively brief foraging trips (Page et al. 2006). In these marine diving top predators, including seabirds, the spatial segregation can further expand to the vertical dimension as individuals can use different portions of the water column in relation to differential diving capacities that are related to difference in body size. For instance, larger male cormorants, *Phalacrocorax* spp., dive deeper than smaller females to access higher trophic level prey items (Kato et al. 2000; Bearhop et al. 2006). Age-specific foraging skills could also lead to foraging segregation as i) age-specific requirements could result in a specialization in food resources; ii) younger individuals may not be skilful enough to track high quality food resources; or iii) younger individuals could be excluded from using the best feeding sites by the older and more experienced individuals (Wunderle 1991; Sol, Santos & Cuadrado 2000).

A combination of size, sex and age could result in a spatial (horizontal or vertical) and/or dietary segregation but this has been rarely examined, at least in long-lived marine vertebrates. The influence of age on spatial segregation has been explicitly demonstrated in the wandering albatrosses *Diomedea exulans* and their large foraging range (Lecomte et al. 2010). We can expect competition for resources to be more acute for species with a restricted foraging range but little information is available for species with shorter foraging range.

In this study we investigated whether seabirds exploiting inshore foraging zones use segregation strategies to avoid competition for resources and determined which of the individual's characteristics were involved in these strategies. We used a diving seabird with a restricted foraging range, the little penguin *Eudyptula minor* as a model. During the chick-rearing phase when little penguins conduct one-day foraging trips (Chiaradia & Kerry 1999), they forage in a radius of about 30 km around their breeding site (Collins, Cullen & Dann 1999). As a visual hunters (Cannell & Cullen 1998), they are further restricted to hunting during daylight hours (Chiaradia et al. 2007b). We expect these constraints to force foraging segregation between individual little penguins, even in a year of high food availability, as it was the case in the present study.

3. Materials and methods

The study was conducted in a breeding colony of little penguins' at Phillip Island (38°31'S, 145°09'E), Victoria, Australia. Birds from this colony were identified with passive RFID transponders (Allflex, Australia). The transponders (23 × 3.8 mm), supplied in individually packed sterilized needles, were injected under the loose skins on the back of the penguin between the shoulders and the wound was closed with surgical glue (Vetbond^{TM}, 3M worldwide) to prevent infection (details in Chiaradia and Kerry 1999). The transponders have been used at Phillip Island since 1994 and no negative effects have been noted, i.e. no tissue damage or transponder migration from injected site (Daniel et al. 2007). The Birds were sexed by bill measurements (Arnould et al. 2004)

From October to November 2010, we monitored the foraging activity of 29 known-age penguins (15 males and 14 females) guarding 1 or 2 chicks aged from 1 to 19 days. These birds are part of a study site consisted of 100 nests where experimental penguins (equipped) and non-experimental penguins (control) were monitored continuously until the end of the breeding season. We found no effect of loggers on breeding success between experimental and control penguins.

Birds were caught in their artificial nest boxes and weighed to the nearest 10 g using a spring balance. Adult ages ranged from three to eighteen years. For the analyses, penguins were grouped in three age classes following Daniel et al. (2007): young (3-4 years, n=3), middle-aged (5-11 years, n=11) and old penguins (12-18 years, n=15). A GPS receiver and an accelerometer data loggers were attached to the middle and lower back of the penguin using waterproof Tesa tape preserving the integrity of the plumage and allowing us to minimize the handling time (Wilson et al. 1997). The devices weighed ca. 39 g in total, which is 3.4±0.3% of the penguins' mean body weight and 4.1% of the lightest bird. Logger attachment was completed within 5 minutes, and penguins were returned to their nest boxes (details in Zimmer et al 2011a). After a single foraging trip, penguins were recaptured, loggers and tape removed, weighed again and a 150µL blood sample was taken from the tarsal vein (see Chiaradia et al. 2010 for blood sampling details). The fieldwork protocol was approved by the Phillip Island Animal Experimentation Ethics Committee (all procedures were conducted under permits 2.2010 and 3.2011) with a research permit issued by the Department of Sustainability and Environment of Victoria, Australia (number 10006148).

Foraging activity measurement

We used miniature accelerometer logger, 12 x 45 mm, five-channel data loggers that weighed 9 g (ORI400-D3GT, Little Leonardo, Tokyo, Japan) to record depth (range 0~400 m, resolution 0.1 m) and temperature (range -50~50°C, accuracy 0.1°C) every second. This logger also recorded both specific acceleration (dynamic) and gravity-related acceleration (static) along the longitudinal body axis (surge), the dorso-ventral axis (heave) and the lateral axis (sway) of the penguins, between -40 and 40 $m.s^{-2}$ at 50 Hz. Location was recorded by CatTraQ^{TM} GPS loggers (16 Mb memory, 230 mA lithium-ion battery, Catnip Technologies, USA).

Data from the accelerometer loggers were downloaded into a computer and analysed with purpose-written software in Igor Pro (Wavemetrics Inc., USA, 2008, Version 6.22A, see Kato et al. 2006). Depth data were corrected for surface drift caused by the temperature. Based on the accuracy of the depth sensor and wave effect at the surface (Kato et al. 2006), dives started when a penguin descended at least 1 m from the water surface and ended once the penguin surfaced again. Using this method we calculated the total number of dives performed during the foraging trip, the total vertical distance travelled (the sum of the maximum dive depth × 2), as well as the proportion of time spent underwater during the one-day foraging trip.

The Vectorial Dynamic Body Acceleration (VeDBA), a measure of the whole body activity, was derived from the acceleration along the three axes (Gleiss, Wilson & Shepard 2011). Specific and gravity-related acceleration signals were separated by a two-band low-pass filter (IFLD, Version 4, Wavemetrics) on each acceleration axis (surge, heave and sway). The gravity-related acceleration was subtracted from the total acceleration in order to obtain the specific acceleration. The vectorial sum of the 3 axes' specific accelerations was calculated to give VeDBA. During prey pursuit, the frequency and amplitude of flipper beat increases (Ropert-Coudert et al. 2006b), resulting in a peak in VeDBA (Wilson et al. 2010; Zimmer et al. 2011a). Note this method does not provide a direct measure of prey consumption but an estimate of food available to a penguin during a trip. We calculated the ratio of the number of dives with prey pursuit to the total number of dives during the foraging trip, as an index of hunting efficiency (Zimmer et al. 2011a,b).

Spatial analysis

GPS loggers were modified in our laboratory (IPHC–DEPE, Strasbourg, France) for underwater use. The initial packaging was removed, the main switch button replaced by a reed switch, and the units were then moulded into a resin. After sampling protocols were uploaded, each unit was put in a heat-shrink tube for waterproofing before deployment. Dimensions of customized unit were 14×35×70 mm, weighing ca. 30 g. The GPS loggers were programmed to run from 3 am to 9 pm local time with a sampling interval of 15 sec. All GPS tracks were analysed using IGOR Pro with the '*Spatial analysis'* function of the *Ethographer* (version 2.0; Sakamoto et al. 2009) and R software (R Development Core Team 2009). Geographic coordinates from GPS loggers were transformed in UTM (Universal Transverse Mercator) values. Tracks were linearly interpolated at a rate of one point per minute. The maximum foraging distance and major heading directions were calculated as the distance (in km) and bearing (degree from north) to the furthest point in the trip from the colony. We also calculated the total distance travelled during the foraging trip as the cumulative distance between each point (in km).

Using data from the accelerometer loggers combined with GPS data we plotted the locations of prey pursuits onto the tracks of each penguin. To determine overall patterns of spatial foraging distribution, we calculated Kernel density (Worton 1989) to determine the zone of prey pursuit for each age class and each sex using the *KernelUD* function in R package *adehabitat* (Calenge 2006). To allow comparisons between age classes and sexes we used the same smoothing factor and grid cell (7 and 2 km, respectively). Areas of high density of prey encounter for the 25% (core area) and 50% (focal region) utilization distributions were calculated for each age class and each sex (Hyrenbach, Fernandez & Anderson 2002; Kappes et al. 2011). Calculation of the overlap between the groups, on the 25 % and 50 % of the foraging range, was performed using the R command *Kerneloverlap* with *VI* method (Fieberg & Kochanny 2005). This method computes the proportion of the volume of intersection between groups. We calculated the surface area (in km^2) used by each group, at 25% and 50%, using the *kernel.area* command in R package *adehabitat* (Worton 1989; Calenge 2006). Because to the limitation of statistical analysis for small sample size the group of young penguins was not used in the Kernel density analysis.

We then used published bathymetry data (Whiteway 2009) to determine the depths in the penguins' foraging zones.

Stables isotopes

Blood samples were centrifuged and the red cells and plasma were frozen (-20°C) separately until further analyses in the laboratory (IPHC-DEPE, Strasbourg, France). The stable isotopic ratios of carbon ($\delta^{13}C$, $^{13}C/^{12}C$) and nitrogen ($\delta^{15}N$, $^{15}N/^{14}N$) estimate the penguins' foraging locations (high levels of $\delta^{13}C$ are related to benthic/inshore prey) and the trophic position of the predator (high values of $\delta^{15}N$ are related to prey consumed with higher trophic values), respectively (Hobson & Clark 1993; Cherel & Hobson 2007). The ratios were measured on the plasma, which provides composite of prey consumed in the last 5 days (Hobson & Clark 1993). Lipids were extracted from plasma using cyclohexane solvent (Cherel et al. 2010). Each sample was lyophilized (48h), powdered, and weighed with a microbalance (0.22-0.95 mg) and the material was loaded into tin cups for analysis. Results are presented in the δ notation (‰) following international standards (PeeDee Belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$). Replicate measurements of internal laboratory standards indicate measurement errors of ± 0.19‰ and ± 0.08‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. To quantify isotopic niche width and overlap, we used metrics based on standard ellipses, comparable to standard deviation in univariate cases, to measure dietary similarity among groups using the program SIBER in the *SIAR* package in R (Jackson et al. 2011). We calculated the Standard Ellipse Area corrected for small sample sizes (SEAc) (Jackson et al. 2011). As a minimum of 3 individuals per group was required, young individuals (2 males and 1 female) were excluded from the SEA analyses (47). We compared 4 groups: the middle-aged females (n=7) and males (n=3) and the old-aged females (n=4) and males (n=9). In order to statistically compare the size of standard ellipses, we calculated Bayesian standard ellipses (SEA_B) for each groups and compared their 95% credible limits using

Markov chain Monte Carlo simulation with 10^4 iterations for each groups (Jackson et al. 2011).

Statistics

To identify any horizontal, vertical and isotopic diet segregation in relation to the effect of age, sex and/or the interaction between sex*age, we tested these parameters on the bearing and the stable isotope values using Generalised Linear Model (GLM). For maximum depth of dives with prey pursuit we used Generalised Linear Mixed Modelling (GLMM) with individuals as random factor given that each individual performed several dives within the one-day foraging trip. All analyses were conducted using R 2.11.1 (R Development Core Team 2009) with the *nlme* package (Pinheiro et al. 2013). Results are expressed as mean ± SE and significance level set at $\alpha=0.05$.

We used the following foraging parameters to characterise the foraging effort: the number of dives performed during the foraging trip, the percentage of time spent underwater, the total vertical distance, the maximum distance reached from the colony and the total horizontal distance travelled. To define the efficiency of a foraging trip we used the index of hunting efficiency (see above). We then tested the effect of the following individual parameters on the aforementioned diving parameters: age, sex and body mass, as well as chick's growth stage, which although not a characteristic of the individual adults *per se*, is known to be related to their foraging effort (Zimmer et al. 2011b).

We used model selection to choose the best-fitted models with GLM, using AICc and Akaike weight (w_i) (Burnham & Anderson 2002; Richards 2005). As no single model was strongly weighted (i.e., no $w_i > 0.90$) we did a model averaging to calculate the relative importance of each explaining variable and the potential interactions (Burnham & Anderson 2002; Richards 2005; Symonds & Moussalli 2011). All models with a $\Delta\text{AICc} \leq 6$ were used in the model averaging with the package MuMIn in R (Burnham & Anderson 2002; Symonds & Moussalli 2011). For each variable we summed the w_i from each model containing this specific variable in order to get its relative importance (Burnham & Anderson 2002; Symonds & Moussalli 2011).

4. Results

We obtained foraging locations and diving records from 29 individuals and stable isotope values from plasma of 26 samples from these individuals. All penguins headed southeast of Phillip Island, travelling 20 ± 4 km (max. 30 km) away from the colony and foraging in shallow waters along the continental shelf (55 ± 14 m). The penguins performed 658 ± 27 dives per trip, of which 37 ± 1 % contained prey pursuits (Table 1).

Table 1: Average ± SE of the different foraging parameters for each age classes and sexes.

	Young		Middle		Old	
	Female (n=1)	Male (n=2)	Female (n=7)	Male (n=4)	Female (n=6)	Male (n=9)
Diving parameter						
Max distance (km)	30	20 ± 4	21 ± 2	20 ± 2	20 ± 2	19 ± 1
Total distance (km)	71	49 ± 11	54 ± 3	54 ± 4	51 ± 2	52 ± 3
Vertical distance (km)	11	18 ± 4	15 ± 2	17 ± 3	15 ± 2	18 ± 1
Time underwater (%)	28	41 ± 6	34 ± 4	37 ± 6	36 ± 4	41 ± 2
Number of dives	496	783 ± 104	613 ± 46	667 ± 73	634 ± 48	696 ± 59
Hunting efficiency (%)	46	39 ± 5	36 ± 2	38 ± 3	33 ± 5	38 ± 3
Horizontal segregation						
Bearing (°)	133.34	115.18 ± 5.56	134.55 ± 3.71	125.26 ± 5.04	119.36 ± 6.43	120.50 ± 3.33
Vertical segregation						
Depth median (m)	14.0	14.0 ± 3.0	14.1 ± 2.0	14.5 ± 2.4	13.5 ± 1.3	14.5 ± 1.1
Diet segregation						
$\delta^{15}N$	14.96	15.37 ± 0.57	15.50 ± 0.38	15.73 ± 0.33	15.22 ± 0.40	15.37 ± 0.25
$\delta^{13}C$	-20.3	-20.64 ± 0.27	-20.44 ± 0.25	-20.63 ± 0.06	-20.79 ± 0.18	-20.56 ± 0.13
SEA_c	-	-	2.16	0.39	1.17	1.01

Spatial segregation

There was a clear segregation in prey pursuit distribution between the middle-aged (n=11) and old groups (n=15) (Figure 1). The middle-aged and old groups did not overlap in the core areas, with only 3% overlap in their focal area. Old penguins exploited the shallower water closer to the shore while middle-aged penguins went further offshore (Figure 1; GLMM on the sea depth data: estimate=-0.165, S.E. =0.07, z=-2.3, p=0.02). Middle-aged penguins used a surface area 43% to 45% larger than old penguins (focal area: 66 km^2 vs. 38 km^2 and core area: 27 km^2 vs. 15 km^2, respectively).

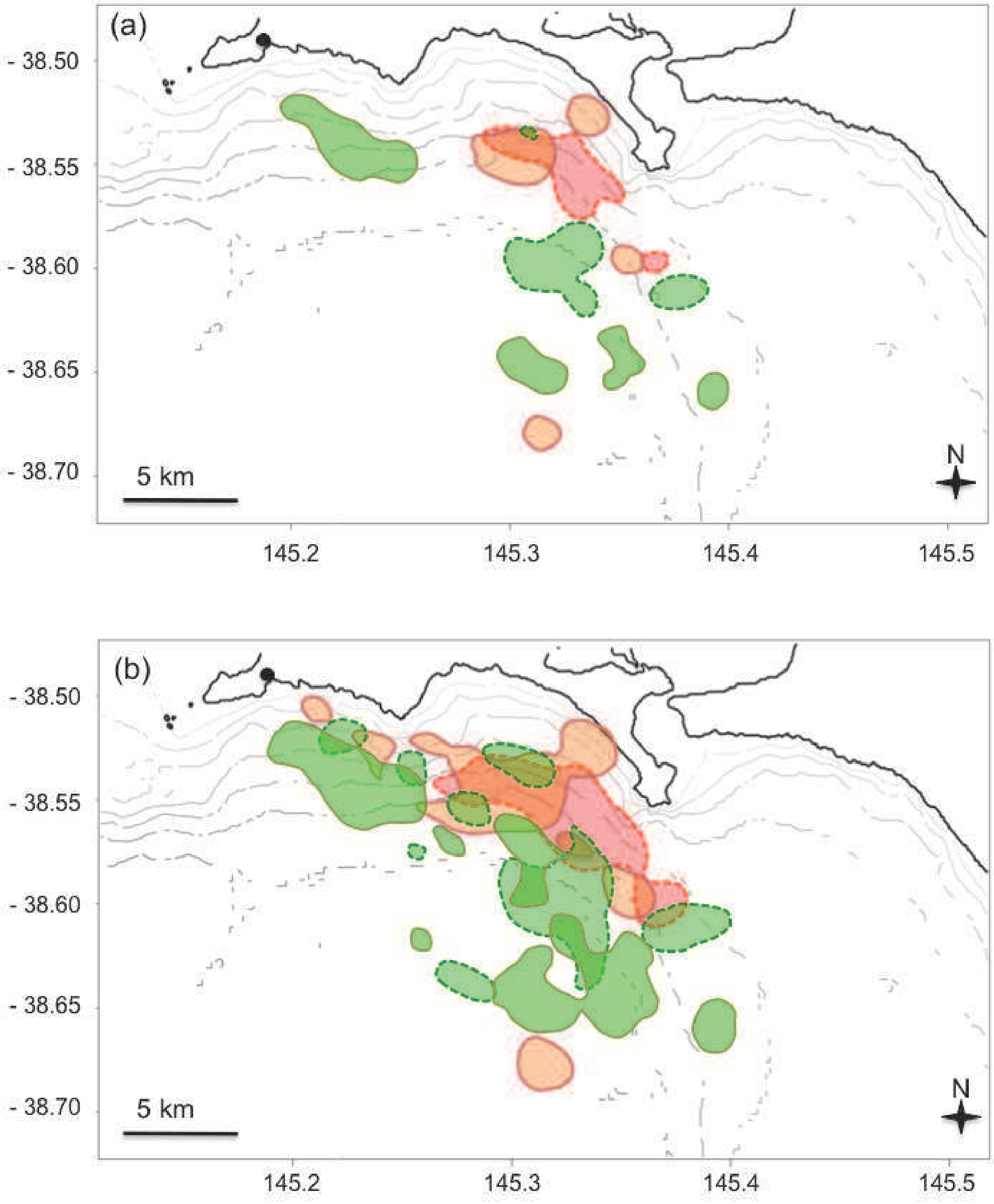

Figure 1: Distribution of little penguins in their foraging zone according to age and sex. Kernel density estimates zone of high prey encounter, (a) 25% and (b) 50% of the foraging area in relation to middle (green) and old (red) age class. The dot and the full line represent males and females, respectively. Grey lines represent the seabed depth by 10m intervals from the coastlines. Black dots mark the position of the colony.

Table 2: Percentages of area overlap between males and females according to middle and old age classes at (a) 25 % and (b) 50 % of the foraging area, using the 'VI' method (see methods).

(a)		Middle		Old	
		Female (n=7)	Male (n=4)	Female (n=6)	Male (n=9)
Middle	Female	-	0%	0%	0%
	Male		-	0.4%	0.2%
Old	Female			-	6%
	Male				-

(b)		Middle		Old	
		Female (n=7)	Male (n=4)	Female (n=6)	Male (n=9)
Middle	Female	-	5%	1.5%	0%
	Male		-	5.6%	4.8%
Old	Female			-	18.4%
	Male				-

There was a significative difference in bearings between the old and the middle-aged groups (GLM: estimate=0.0013, S.E. =0.0003, t=4.139, p=0.0005), with middle-aged penguins heading on average towards 131±3° against 120±3° for the old penguins. The major direction followed by penguins was neither affected by the interaction sex*age (GLM: estimate=0.005, S.E.=0.003, t=1.681, p=0.109) nor sex (GLM: estimate=-0.0008, S.E.=0.0004, t=-1.704, p=0.105). Old males and females showed higher overlap in the core and focal foraging areas than middle-aged males and females (Table 2; Figure 1).

Females exploited a greater surface area than males in both the core and focal foraging areas of both middle-aged and old groups (Table 3). For both male and female groups, middle-aged penguins occupied a larger foraging surface area than old penguins (Table 3).

We did not find significant differences in the vertical segregation between age groups (Table 1). There was no effect of sex (GLMM: Estimate=-0.012, S.E.=0.189, z=-0.062, p=0.95), adult age (GLMM: Estimate=-0.003, S.E.=0.012, z=-0.225, p=0.822) and the interaction sex*age (GLMM: Estimate=0.008, S.E.=0.016, z=0.497, p=0.619) on the maximum depth of foraging dives. For both groups few dives with prey encounter were within 5 m of the seabed (2.15% for the old and 1.17% for the middle-aged birds).

Table 3: Surface area in km^2 used by males and female from the different age classes at 25 % and 50 % of the kernel density.

	Middle		Old	
	Female (n=7)	Male (n=4)	Female (n=6)	Male (n=9)
25 %	22.3	14.8	13.8	12.7
50 %	57.3	42.3	40.5	29.8

Dietary segregation

There was no significant difference in the stable isotope values of both $\delta^{13}C$ and $\delta^{15}N$ between sexes and age classes. The interaction sex*age of the penguin was not significant for both $\delta^{13}C$ and $\delta^{15}N$ (All $p > 0.05$).

The different age/sex groups showed significant degrees of isotopic niche overlap (Figure 2). Old males occupied the largest niche compared to middle-aged (larger SEAc; Table 1). The pattern was the opposite for females: middle-aged female occupied a larger niche than old ones. Both old and middle-aged females had wider isotopic niche compared to males (Figure 2; Table 1).

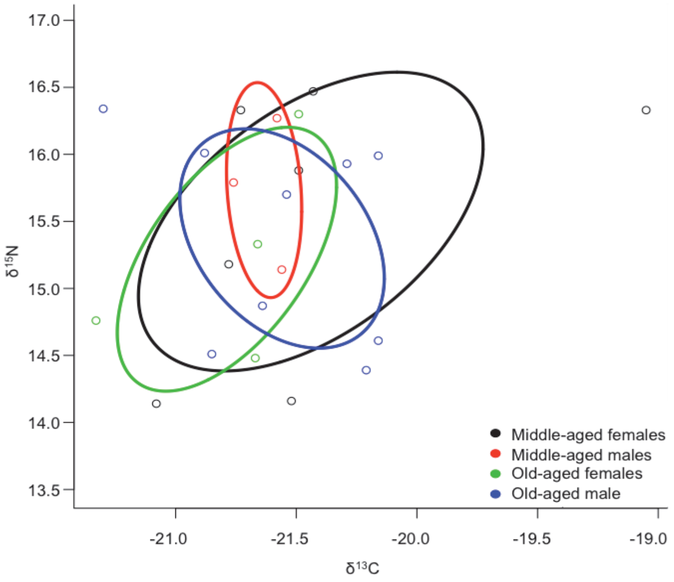

Figure 2: Niche occupancy of little penguins during guard stage at Phillip Island grouped by age and sex. The biplot depicting $\delta^{13}C$ and $\delta^{15}N$ isotope ratios. Solid lines represent standard ellipses and represent the isotopic niche width of 40% of typical individuals within the group based on bivariate normal distribution following Jackson et al. 2011.

The middle-aged females displayed the largest isotopic niche while the middle-aged males had the smallest one (Figure 2; Table 1). Old males and females had a similar SEAc (Table 1). From the Bayesian approach (SEA_B), there were no significant differences of the ellipse area between groups (Figure 3). The middle-aged males exhibited the largest uncertainty probably associated with smaller sample size (Figure 3).

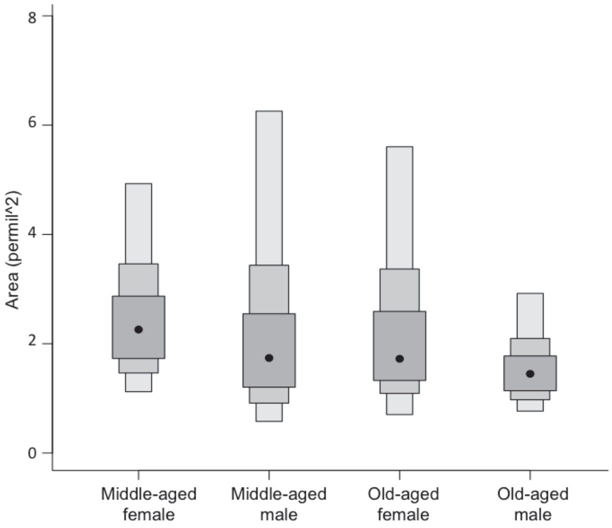

Figure 3: The credible interval for the estimated ellipse area estimate posterior distribution of little penguins during guard stage at Phillip Island grouped by age and sex. Each bar shows the range of Bayesian standard ellipse areas. Black dots represent the mode, and shaded boxes represent the 50%, 75% and 95% credible interval.

Foraging effort and efficiency

For all parameters of foraging effort and success, no models had an Akaike weight (w_i) exceeding 30% (see Annexes 2 to 7). Following Burnham & Anderson (2002), we included all models output from the MuMIn R package in the model averaging to avoid bias when low weight models are eliminated in the model selection.

Compared with adult age, sex and body mass, chick's age was the best predicator variable for most of the foraging parameters, especially for the maximum (79%) and total distance (87%) travelled (Table 4). Parents travelled closer to the colony, covering smaller distance, as chicks grew older. The adult age was also relatively important to explain the total distance travelled. It was the sole foraging parameter with an interaction showing a relative importance of 47% with the age of the chick (Table 4).

Table 4: Relative importance of the predictor variables (including interactions). Calculated as a sum of the Akaike weights over all of the models in which the parameter of interest appears. Chick=age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

	age	bm	chick	sex	age:bm	age:chick	age:sex	bm:chick	bm:sex	chick:sex
Maximum distance	0.35	0.26	**0.79**	0.33	0.02	0.14	0.00	0.02	0.01	0.07
Total distance	**0.60**	0.26	**0.87**	0.17	0.02	0.47	0.00	0.04	0.00	0.00
Nb of dives	0.19	0.33	0.25	0.49	0.00	0.00	0.01	0.04	0.02	0.03
% Underwater	0.24	0.26	**0.51**	0.48	0.00	0.01	0.02	0.03	0.02	0.05
Vertical distance	0.22	0.25	0.48	0.42	0.00	0.01	0.01	0.03	0.01	0.04
Hunting efficiency	0.19	0.22	0.30	0.26	0.00	0.00	0.00	0.02	0.00	0.05

The age of the chicks was also an important predicator of the proportion of time spent underwater (51%), followed by sex (48%), both parameters accounting for more than twice the contribution of age and body mass of adults (Table 4). None of the predicator variables exhibited a relative importance over 50% for the number of dives, although sex at 49% was twice the contribution of the other variables. A similar trend was observed for the vertical distance, where chick age and sex of the adult explained up to 48% and 42% of the variability, respectively (Table 4). The hunting efficiency had the lowest response, with no predicator variables showing a relative importance higher than 30% (Table 4).

For the maximum distance, the vertical distance and the proportion of time spent underwater, the best-fit models contained only the age of the chick (Table 5). As chick got older, the maximum distance decreased when the vertical distance and the proportion of time spent underwater increased (Table 5). The best-fit model for the total distance contained age of the chick, adult age and the interaction age of the chick age*adult age. Young penguins travelled 11% farther than the old penguins (58±7 km and 52±2 km, respectively), while there was no difference between the middle and old penguins or between young and middle-aged group (54±2 km for the middle-aged penguins). The statistical model with the lowest AICc for the total number of dives performed by birds only included the variable sex with male performing more dives (700±31 dives) than females (613±31 dives) (Table 5).

Table 5: Parameters present in the statistical model with the lowest AICc/QAICc.

Independent variable	Age	Sex	Body mass	Age of the chick	Estimate±SE	GLM	*P*
Maximum distance (km)	-	-	-	X	-0.019±0.01	*z*=-2.03	0.042
Total distance (km)	X	-	-	X	Age: -0.029±0.011 Chick age:-0.033±0.01 Interaction:0.003±0.001	z=-2.59 z=-3.175 z=2.475	0.0096 0.0015 0.013
Vertical distance (km)	-	-	-	X	0.02±0.01	t=1.81	0.08
Time underwater (%)	-	-	-	X	0.03±0.02	t=1.808	0.082
Number of dives	-	X	-	-	0.13±0.07	z=1.73	0.0827
Hunting efficiency (%)	-	-	-	-			

5. Discussion

Here, little penguins showed distinct spatial segregation in the 'hot-spots' of their foraging areas in relation to adult age, and to a lesser extent sex, during their one-day trip at sea. Unlikely other studies, we found a spatial horizontal segregation at a restricted scale. Horizontal segregation has generally been observed at spatial scales much larger than our study. For example black-browed (*Thalassarche melanophrys*), and grey-headed albatrosses, (*T. chrysostoma*) displayed a spatial segregation during far-away trips of the incubation phase but not when they foraged at small spatial scales during the chick-rearing stage (Phillips et al. 2004). Surprisingly, horizontal segregation was not associated with a vertical or dietary segregation. Further, niche occupancy was not accompanied by a difference in foraging effort or efficiency. In other words, the old and middle-aged groups used different foraging zones to target similar prey without any apparent difference in hunting efficiency and in diving activity. That suggests adult penguins were preying on the same food resources, using the same strategies but simply in different foraging areas according to their age.

The absence of dietary segregation can be attributed to high food availability within foraging zone. The high breeding success of little penguins, as observed in this study, is highly correlated with prey abundance (Chiaradia & Nisbet 2006). This high prey availability could indicate that small pelagic fish schools, a common prey for little penguins (Chiaradia et al 2003), could be readily available with their limited foraging zone, at least during the short period of the guard stage. Here, isotopic niches were large for each group of birds and overlapped greatly. In years of low food availability or over a long period of the chick rearing, a dietary segregation could become significant as observed in previous diet studies (Chiaradia et al 2010, 2012).

In contrast, penguins used different foraging areas even under apparent good foraging conditions as discussed above. Thus, this little penguin foraging segregation could result from intrinsic individual characteristics, such as adult age rather than a response to competition to food resources. Indeed, such segregation was evident in this study when the chicks' demands for energy required for growth and development peaked (Chiaradia & Nisbet 2006), i.e. when the penguins are limited in the maximum distance they can reach from the colony within one-day trip (Collins, Cullen & Dann 1999). Pattern of segregation driven by the age has been reported in other species (Sol et al. 2000; Field et al. 2005; Lecomte et al. 2010). Age-specific foraging skills have been suggested to be at the origin of spatial segregation: individuals could specialize in a given food resource or could lack the specific skills required to track high quality food resources, thus being excluded from the best feeding sites by older and/or more experienced individuals (Wunderle 1991; Sol, Santos & Cuadrado 2000). Previous studies on little penguin differences in age class have favoured breeding success (Nisbet &Dann 2009), group formation (Daniel et al 2007) and diving efficiency (Zimmer et al 2011a), clearly benefitting middle-aged penguins. In contrast to this trend, different age groups did not show any noticeable gain from segregating foraging zones in the present study. In other words, the segregation pattern we observed was not related to resource limitation but depended on the age of the birds, i.e. on individual characteristics (cf. Schelling 1971; Hemelrijk & Kunz 2004). Contrary to old birds, young and perhaps less experienced birds continue to travel farther while increasing the vertical distance too as chicks get older. As chick demand increases, birds could maintain their ratio foraging cost/gain low or neutral as they reduced the (horizontal) travel costs while maintaining the same foraging success. Such a situation would probably only exist when prey availability is not a limiting factor. With increasing competition for resources, we would expect age-based differences in foraging ability to be enhanced. During a year of strong prey depletion, this age-based segregation strategy could lead to one age class of individuals to perform better – in terms of both foraging and reproductive success.

In our study, middle-aged and old penguins attained the maximum distance that is physically and biologically achievable for this species during the chick-rearing stage (i.e. 25-30km; Collins, Cullen & Dann 1999; Hoskins et al. 2008). Chick age was actually the main factor influencing the foraging behaviour of little penguins as chick age appeared in most of the best-fit models. As chicks grew, adults may change their behaviour by increasing their vertical movement (by probably increasing their number of dives and dive depth) and reducing the horizontal movement (the maximum and the total horizontal distance), a pattern consistent with previous studies on the same species (Zimmer et al. 2011b). In wandering albatrosses (Lecomte et al. 2010) senescence was at the origin of differences in the foraging activity and reproductive investment of males. Yet, none of these hypotheses apply to our case: the different age groups did not gain anything noticeable from segregating. We can infer from our data that the quality and density of prey patches did not influence the observed age-specific segregation and in such conditions, individuals should be randomly spread, which is not the case here. In addition, our birds did not show an ‘energy-saving strategy’ like albatrosses. In other words, the segregation pattern we observed is not explained by a difference in needs due to, for example, different physiological aspect caused by the senescence, but depends on the age of the birds, i.e. on individual characteristics (cf. Schelling 1971; Hemelrijk & Kunz 2004). In the light of this result, and following the ‘dynamic model of segregation’ of Schelling (1971), which proposes that segregation proceeds from the preference for one’s neighbours that share similar characteristics than one’s own, we suggest that the origin of the little penguins segregation we observed is based on a consensus that leads individuals that either are closely related or share a set of individual characteristics linked to age, to travel together and exploit the same foraging zones. The consensus could result from an attraction process, i.e. middle-aged birds prefer to forage with middle-aged birds, or from a discriminatory process, i.e. middle-aged birds may avoid birds from a different age groups. We believe the former to be the most plausible explanation: individuals would naturally follow the birds that share the same characteristics as they can expect them to share also the same needs and the same physiological constraints. Cooperative foraging has been described in other penguin species (e.g. Wilson et al. 1986; Tremblay & Cherel 1999; Takahashi et al. 2004; Copeland 2008) and this would seem likely that such cooperation develops among individuals that are closely related. This idea is further reinforced by the fact that little penguins form groups – or rafts – of 5 to 10 individuals before leaving the colony as it gives them a sense of security before crossing the empty spaces of the beaches (Chiaradia 1999). These groups are formed randomly as birds from a group share similar characteristics, especially age which was found to be a significant factor entering in the composition of groups (Daniel et al. 2007). It would thus be particularly interesting, in future studies, to try and track birds from these groups and examine whether they retain these bounds while at sea.

From our results it seems clear that to understand the mechanisms behind ecological segregation one needs take into account, not only the spatial and temporal variability of the environment, but also the heterogeneity of the population studied as the characteristics of individuals and social interactions may play a vital role in explaining resource partitioning.

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References have been included in the general bibliography at the end of this thesis.

ARTICLE D

The cost of having chicks: foraging and oxidative stress in a freely-living seabird increase as chicks grow

Laure Pelletier, Yan Ropert-Coudert, Akiko Kato, Antoine Stier, Sophie Reichert and André Chiaradia

Article to be submitted to Functional Ecology

Article D

Abstract in French

Le coût d'avoir des poussins: une augmentation du stress oxydant et de la recherche de nourriture chez des oiseaux marins lors de la croissance des poussins

Résumé :

Le coût de la reproduction est souvent déterminé par un changement dans la condition corporelle des parents. Il y a peu d'investigation réalisée sur les mécanismes physiologiques qui sous tendent les compromis entre l'investissement dans un évènement de reproduction et le maintien de l'organisme (la survie). Récemment, le stress oxydant a été décrit comme un indicateur du coût de la reproduction. Le stress oxydant correspond à un déséquilibre entre la production de radicaux libres dérivés de l'oxygène (*reactive oxygen species*) et la barrière antioxydante. Le stress oxydant est impliqué dans les mécanismes du vieillissement. En effet, il a été montré que la production d'espèces réactives de l'oxygène agissait sur le raccourcissement des télomères. Les télomères sont des parties non-codantes de l'ADN situées à l'extrémité des chromosomes et qui ont pour rôle de protéger et de stabiliser le génome. Lors de la réplication cellulaire, un défaut dans la réplication fait que les extrémités se réduisent à chaque division. Lorsque les télomères atteignent une taille limite, ils ne sont plus fonctionnels et la cellule va rentrer en apoptose (i.e. mort de la cellule). On peut en conclure que les télomères jouent le rôle de 'chronomètre biologique' et

refléteraient donc l'âge biologique des individus.

L'utilisation du stress oxydant comme mécanisme impliqué dans les compromis évolutifs n'a cependant été que largement étudié en laboratoire. Les rares études faites en milieu naturelles ont utilisé un protocole de manipulation de l'effort reproducteur (exemple, une manipulation de la nichée). De plus, l'ensemble de ces études montre des résultats contradictoires. Dans certain cas l'effort de reproduction n'est pas accompagné par une augmentation du stress oxydant chez les parents et si il y a une augmentation celle-ci reste minime. La question concernant l'action du stress oxydant comme indicateur du coût de la reproduction reste encore ouverte.

Dans cette étude nous avons cherché à connaître l'impact de l'investissement parental sur la balance oxydative chez une espèce d'oiseau marin côtier, le manchot pygmée (*Eudyptula minor*). Nous avons regardé comment la croissance du poussin (i.e. l'augmentation en demande d'énergie), en utilisant l'âge de ces derniers comme un indicateur, influencerait les stratégies de recherche alimentaire des parents (i.e. leurs activités) et quels seraient les conséquences sur le statut oxydant de 18 manchots adultes. Nous avons pour cela équipé les parents avec un accéléromètre et un GPS (des *loggers*) lors de la période de garde. Cette période débute au moment de l'éclosion et se termine lorsque les deux parents laissent les poussins seuls au nid. Pendant cette période les parents vont alterner un voyage en mer d'une journée avec une journée au nid à prendre soin des poussins. Après un unique voyage en mer, en même temps que la récupération des *loggers* nous avons effectué des prises de sang sur ces mêmes oiseaux.

Les résultats ont montré, qu'avec l'âge croissant des poussins, les parents ont parcouru une plus grande distance verticale, correspondant à un plus grand nombre de plongées et/ou des plongées plus profondes. Cependant les parents ont également changé d'autres comportements. Avec des poussins plus âgés les parents ont retardé leur départ de la colonie et ont par conséquent parcouru une plus faible distance horizontale. Ce changement dans l'activité des parents suivant l'âge des poussins est concomitant avec une augmentation des dégâts oxydants. L'activité augmentant avec un nombre de plongées plus important, cela fait que les oiseaux subissent un plus grand nombre de cycles d'hypoxie et de re-perfusion des organes. Cela a pour conséquence de produire un plus grand nombre d'espèces réactives de l'oxygène et de causer des dégâts oxydants. Ces dégâts apparaissent car la barrière antioxydante des individus n'a pas changé. En effet, les individus n'ont pas investi dans l'augmentation

de leurs défenses. Aucun des paramètres individuels tels que l'âge, le sexe, la longueur des télomères ou le masse des animaux n'ont influencé leur comportement de recherche alimentaire ainsi que leur statut oxydant. Cependant il a été trouvé que les oiseaux avec un poids plus important avaient des défenses plus importantes. Le poids va représenter une meilleure condition de l'individu, une meilleur réserve de l'énergie et donc plus de capacité à se défendre contre les impacts négatifs de la reproduction.

Notre étude a donc mis en évidence que la demande des poussins en croissance va pousser les parents à changer leur comportement de recherche alimentaire. Cela a pour conséquence un changement dans leur statut oxydant, avec la production de dégât oxydatif qui n'est pas contre balancé par une augmentation des défenses conduisant à un stress oxydant. Cela confirme que le stress oxydant est un reflet des compromis réalisés entre l'investissement dans la reproduction et le maintien de l'organisme.

Mots clés : la croissance des poussins, l'investissement parental, la recherche alimentaire, oiseau marin côtier, le stress oxydant

Article D

The cost of having chicks: foraging and oxidative stress in a freely-living seabird increase as chicks grow

Laure Pelletier, Yan Ropert-Coudert, Akiko Kato, Antoine Stier, Sophie Reichert and André Chiaradia

Article to be submitted to Functional Ecology

1. Abstract

The cost of reproduction is often assessed through changes in parental body condition with little investigation of the underlying physiological mechanisms that regulate the trade-off between parental investment and self-maintenance. Recently, oxidative stress has been used as a good proxy of cost of reproduction. However, the use of oxidative stress has focused on laboratory experiments and brood manipulation in the wild have largely come to the somewhat unexpected conclusion that reproduction causes little or no increase in parental levels of oxidative damage. Here we investigated the impact of parental investment on the oxidative balance of an inshore seabird, little penguins (*Eudyptula minor*). We looked at the relationship between chick's growth, using chick age as a proxy – and the foraging strategies and their oxidative status of 18 adult penguins. We equipped chick-rearing birds with accelerometer and GPS loggers in the wild. Following a single-day foraging trip, we collected blood sample to investigate the oxidative status and telomere length of each individual. The total vertical distance travelled and the total number of dives increased as chick's age increased. In contrast, adults left the colony later and decreased the horizontal distance, as the chicks grew old. This change in foraging behaviour was concomitant with an increase of the oxidative damage (as activity is increasing). However, there were no changes in the total plasma antioxidant capacity. None of the individual parameters (age, sex, telomere length or body mass) influenced foraging activity and the oxidative status, except the body mass that was strongly related to the antioxidant defences. Our study showed that the increasing demand of growing chicks might have forced parents to change their foraging behaviour accordingly. This change had consequences to their physiological status as the production of reactive oxygen metabolites was boosted, due to the fact that the birds were more active and did not adjust their anti-oxidant defence, confirming that oxidative stress is a good reflection of the physiological cost of parental investment.

Keywords: Chick's growth demand, Parental investment, foraging, inshore seabird, oxidative stress

2. Introduction

One of the major trade-off for an individual is the amount of energy that can be allocated to reproduction, as this energy is no longer available for self-maintenance (Stearns 1992). As such, the investment rate in a reproductive event has a potential impact on the survival and the future reproduction (Cox et al. 2010) as production of gametes, courting, mating and eventually parental cares require extra energy (Zera & Harshman 2001). Parental care aims to improve an individual's fitness by increasing its offspring survival rates (Trivers & Willard 1973; Reynolds 1996). Parental investment is mainly accompanied by an increase in activity in several species (Wilson et al. 2012), which leads to an increase in the metabolic rate (Murray et al. 2009).

Oxidative stress has been defined as an indicator of the proximal cost of reproduction (see Salmon et al. 2001; Alonso-Alvarez et al. 2004, 2010; Garrat et al 2011). Alonso-Alvarez et al. (2004) reported an increase in oxidative stress following an increase in parental investment in an experimental increase of the brood size in captive zebra finch (*Taeniopygia guttata*). However, relatively few studies have examined this relationship in the wild (Bergeron et al. 2011; Isaksson et al. 2011; Marko et al. 2011; Heiss and Schoech 2012; Wilson et al. 2012). However these studies have led the authors to question the basis for the hypothesis that oxidative stress is a mechanism underlying the cost of reproduction (Metcalfe & Monaghan 2013).

Oxidative stress is defined as the imbalance between endogenous free radical production and the ability to absorb oxidative damage (antioxidant capacity; OXY), and represents an important cause of cellular damage (Finkel & Holbrook 2000). Metabolic activity continuously generates highly reactive pro-oxidant compounds, e.g. reactive oxygen species (ROS) that can provoke oxidative damage to all biological molecules generating reactive oxygen metabolites, ROMs (Finkel & Holbrook 2000). Reactive oxygen species are also thought to cause mutations in DNA and to accelerate telomere erosion (Monaghan et al. 2009), considered as the principal proximate mechanism explaining cell senescence (Beckman & Ames 1998). Telomere length is considered as the possible reflection of the biological age of an individual (Bize et al. 2009). At the whole individual level, senescence, or ageing, is defined as an inevitable and irreversible accumulation of damages with age that leads to loss of function and eventually death (Monaghan et al. 2008).

For most seabirds, chick rearing is the most energetically expensive period when parents have to forage to provide food to their chicks and feed for themselves at the same time (Gales & Green 1990). The period of growth from hatching to fledging determines the survival of the chicks after fledging; chicks must attain a size and body mass sufficient to ensure their survival (Magrath 1991). Thus, the chick-rearing period corresponds to the peak of the parent-offspring conflict over the allocation of resources (Riou et al 2012). While the cost of reproduction has often been assessed mainly through changes in parental body condition (Drent & Daan 1980; Tveraa et al. 1998; Weimerskirch & Lys 2000), physiological mechanisms underlying the trade-off between parental investment and self-maintenance remain poorly understood.

Thus, foraging efficiency is a major determinant of individual fitness as limiting the trade-off. The increase in breeding performances age-dependants could be explained by the improvement of foraging skills with age (Curio 1983). In wandering albatrosses (*Diomedea exulans*), age influences foraging behaviour and reproductive performance with a decline in foraging activity with age, suggesting that older albatrosses can adopt an “energy-saving” strategy (Lecomte et al. 2010). In little penguins (*Eudyptula minor*) too, age is strongly related to breeding success and foraging performance. Middle-aged penguins are more efficient foragers (Zimmer et al. 2011a) and more successful breeders (Nisbet & Dann 2009) than young and old ones. Young individuals may not have fully developed yet their foraging skills and old ones may be affected by senescence (Zimmer et al. 2011a). Foraging is further constrained in little penguins because parents are alternating guarding chicks and foraging at sea daily during chick-guard phase and foraging during the day light only (Cannell & Cullen 1996, Chiaradia & Kerry 1999) in a limited foraging area, one the smallest among seabirds (Collins et al. 1999, Chiaradia et al 2007). These constrains make little penguins a good model to study physiological mechanisms during parental provisioning and self-maintenance.

To our knowledge, no study has looked at the effect of chicks' growing needs on the foraging activity of the parent and its physiological consequences in a wild, long-lived seabird species. In the present study, we investigated (1) how the chicks' growing need (using chick age as a proxy) is related to the foraging activity and the oxidative status of the parents, and (2) whether and how individual characteristics (age, sex, body mass and telomere length) affect the parents' behavioural and physiological responses. We expect an increase in the foraging effort and oxidative stress of the parents with chick growth. Following ageing theories (Kirkwood & Austad 2002) we also expect significant differences in the foraging activity between old and young parents, with an accumulation of damages with age.

3. Materials and Methods

Fieldwork

The study was conducted at the little penguin breeding colony of Phillip Island, Victoria, Australia (38°31'S, 145°09'E). From October to November 2010, we recorded the foraging activity of 18 known-aged birds (11 females and 7 males) during a one-day trip. Penguins were equipped with miniature accelerometers (ORI400-D3GT, Little Leonardo, Japan) and GPS loggers (CatTraQ^{TM}, Catnip Technologies, USA) in guard stage. Birds were rearing one or two chicks aged from 1 to 15 days. The 2010 breeding season was a successful one for the whole colony as the protracted guard stage and high breeding success indicate (see Chiaradia & Nisbet 2006 for details on breeding success). Birds were caught in their nest boxes and weighed to the nearest 10g using a spring balance. The GPS logger and accelerometer were attached to the middle and lower back of the penguin using waterproof tape, respectively (Wilson et al. 1997). The attachment of the loggers was completed within 5 min and birds were returned inside the nest box. After a single one-day foraging trip, birds were recaptured in the nest boxes and loggers removed. A blood sample (0.1 ml) was taken from the tarsal vein using capillary tubes (see details in Chiaradia et al 2010). After centrifugation, plasma and red blood cells were separated in Eppendorf tubes and stored at -20°C until laboratory analyses.

Bio-logging devices

The miniature accelerometer (12 x 45 mm) weighed 9 g and was used to record depth (range 0~400 m, resolution 0.1 m) every second. This logger also recorded acceleration between -40 and 40 $m.s^{-2}$ at 50 Hz along three axes: the longitudinal body axis (surge), the dorso-ventral axis (heave) and the lateral axis (sway) of the birds. Data from the accelerometer were downloaded into a computer and analysed with purpose-written software in Igor Pro (Wavemetrics Inc., USA, 2008, Version 6.22A). Depth data were corrected for surface drift and according to the accuracy of the depth sensor; all dives > 1 m were considered for analysis (Kato et al. 2006). A dive started when the bird descended from the water surface and ended once the bird surfaced. The number of dives and total vertical distance travelled (sum of maximum dive depth × 2; in km) were calculated. As birds tend to leave the colony at variable times before sunrise we calculated a departure time relative to sunrise as the time elapsed between the departure of the colony and the sunrise (in min) – hereafter termed departure time – as this variable was subsequently found to correlate with the birds' at-sea activity budgets.

The accelerometer measured both specific (dynamic) acceleration (e.g. movement) and gravity-related acceleration (e.g. posture). The Vectorial Dynamic Body Acceleration (VeDBA), a measure of the whole body activity during a dive, was derived from the acceleration along the three axes (Wilson et al. 2006, Gleiss et al. 2011). VeDBA was calculated by isolating the static component of the total acceleration in each acceleration axis (surge, heave and sway) using a two-band low-pass filter (IFLD, Version 4, WaveMetrics). The static component was subtracted from the total raw data in order to obtain the dynamic component. The dynamic values were squared and summed on the 3 axes and the square root was calculated to produce VeDBA (Elliot et al. 2012). During a prey pursuit, the frequency of flipper beat increases (Ropert-Coudert et al. 2006b), resulting in a peak of activity in VeDBA (Wilson et al. 2010; Shepard et al. 2010). Note this method does not provide a direct measure of prey consumption but an estimate of food available to a bird during a trip. We calculated the ratio of the number of dives with prey encounter to the total number of dives during the foraging trip, as an index of hunting efficiency (Zimmer et al. 2011a).

The CatTraQ^{TM} GPS loggers (16 Mb memory, 230 mA lithium-ion battery, Catnip Technologies, USA) were customized in our laboratory (IPHC–DEPE, CNRS UMR7178, Strasbourg, France). The original casing was removed, button switch was replaced by a reed switch, and the unit was moulded in epoxy resin. The GPS loggers were programmed to run from 3am to 9pm with a sampling frequency of 15 sec. Finally, each unit was put in a heat-shrink tube for waterproofing resulting in a final size of 14 × 35 × 70 mm and a total weight of 30 g. All GPS tracks were analysed using IGOR Pro with the '*Spatial analysis'* function of the *Ethographer* (version 2.0, Sakamoto et al. 2009) and R (version 2.11.1). The maximum distance (in km) was calculated as the distance to the furthest point in the trip from the colony.

Laboratory analyses

Analyses of oxidative stress and telomere length were carried out at the IPHC-DEPE. Oxidative stress was measured in plasma samples by using the d-ROMs test and the OXY-Adsorbent tests (Diacron International, Italy) (for details on the procedure see Costantini et al. 2008; Beaulieu et al. 2010; 2011). The d-ROM test measures the plasmatic concentration of hydroperoxyde (ROM) resulting from the attack of ROS on organic substrates. The concentration of ROM was then calculated by comparison with a standard solution whose oxidative activity on the chromogen is equivalent to the activity of H_2O_2 (0.08 mg dl^{-1}). Measurements were therefore expressed as mg dl^{-1} H_2O_2 equivalents. Intraplate coefficient of variation was 3%.

The OXY-Adsorbent test measures the total plasma antioxidant capacity (OXY). This test evaluates the ability of the plasma antioxidant barrier to cope with the oxidant action of hypochlorous acid (HOCl) solution. We measured the excess of

HOCl in plasma and transformed it into a pink derivative. The intensity of the coloured complex is inversely related to the antioxidant power. Measurements were expressed as mmol^{-1} HOCl neutralized. Intraplate coefficient of variation was 2.8%.

DNA was extracted from 5 µl of red blood cells using a commercial kit (DNeasy® Blood & Tissue kit, Qiagen). Telomere measurements were conducted following the quantitative Polymerase Chain Reaction (qPCR) procedure described by Criscuolo et al. (2009) and adapted to seabird samples. This method is based on the measurement of a number of amplification cycles (Ct) necessary to detect a lower threshold of fluorescent signal. The number of cycles necessary is proportional to the telomere length (T), or the length of a control gene (S). A ratio (T/S) of telomere repeated copy number (T) to a control gene copy number (S) is then calculated for each sample that will reflect relative inter-individual differences in telomere length and has been validated by measurements made on the same samples using qPCR method and telomere restriction fragment method. As control gene, we used the human 18S ribosomal RNA gene, with the primer sequences: 18S-F (5'-GAGGTGAAATTCTTGGACCGG-3') and 18S-R (5'-CGAACCTCCGACTTTCGTTCT-3'). Telomere primers were: Tel1b (5'-CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT-3') and Tel2b (5'-GGCTTGCCTTACCTTACCCTTACCCTTACCCTTACCCT-3'). Primers were used at a final isodilution of 100nM. Telomere and control gene PCR conditions were 10 min at 95°C followed by 30 cycles of 1 min at 56°C and 1 min at 95°C. Amplification efficiencies for the telomere reached 103% while those measured for control gene were 90%. To take into account the slight variation of efficiencies (E) between telomere and 18S amplifications, we calculate relative telomere length using the method suggested by Pfaffl (2001). The mean values were used to calculate the relative T/S ratios using the formula:

$$\frac{T}{S} = \frac{(1 + E\ \text{telomere})^{\Delta \text{Ct telomere (control – sample)}}}{(1 + E\ \text{GAPDH})^{\Delta \text{Ct GAPDH (control – sample)}}}$$

Intraplate variations were 1.7% and 2.3% for the telomere and the control gene Ct values, respectively.

Statistics analysis

In order to test the effect of individual characteristics (age of the bird, telomere length, body mass and sex) and chick's age on foraging parameters and oxidative status, we used a Generalised Linear Model (GLM) with the *nlme* package in R (Pinheiro et al. 2013). As our data did not follow the normality even after a log transformation, we used a Gamma family to test the different parameters, excepted for the hunting efficiency where we used a binomial family.

We did a model selection with the function 'dredge' from MuMIN package of R and as no single model was strongly weighted (Akaike weight >0.9) we did a model averaging in order to get the relative variable importance using the MuMIn package (Burnham and Anderson 2002). We then used principal component analysis (PCA) to assist on explaining the variability among individuals by meeting the variables that exhibit similarity or differences (Jolliffe 2005). On the basis of the correlation coefficient among all variables present, the principal axis was built. The PCA allowed us a direct interpretation by factor rotation with the first two dimensions; it provided a trend of similarities or differences between the available variables. This means that parameters are positively correlated when the angle between variables is small (<45°) and negatively correlated when this angle is large (>135°). The closer the arrows to the circle, the better the variables are explained by the first or the second dimension (Jolliffe 1986; Zimmer et al. 2011b). We tested the interaction between the following group of variables: foraging effort, which is represented by the number of dives, total vertical distance travelled and the maximum distance; trip organisation, which is given by the relative departure time; foraging success, which is represented by the hunting efficiency; individual parameters that are represented by the adults' age, body mass, telomere length, and oxidative status (decomposed into OXY and ROM, cf. above); the energy demand of the chicks, which is approximated by its stage of growth. This analyse was conducted in R with the ade4 package (Dray & Dufour 2007). All analyses were conducted using R 2.11.1 (R development Core Team 2009). Results are expressed as means ± SD.

4. Results

We obtained GPS track, accelerometer data and a blood sample for all 18 birds. Birds departed the colony on average 139 ± 46 minutes before the sunrise (Table 1). During their one-day foraging trip they performed between 385–845 dives and reached a maximum distance from the colony of 21.0 ± 4.5 km (Table 1). From the blood sample individuals showed a large range of OXY, ROM and telomere length (Table 1).

Table 1: Descriptive summary of the various parameters measured on the 18 little penguins.

Parameters	Average ± SD	Range
Max distance (km)	21.0±4.5	9 - 30
Departure time (min)	139±46	59 - 224
Number of dives	658±143	385 - 845
Vertical distance (km)	16.0±5.0	6 - 25
Hunting efficiency (%)	36.9±7.7	13.5 – 46.8
ROM (mg dl^{-1} H_2O_2)	3.2±0.8	1.95 - 4.35
OXY ($mmol^{-1}$ HOCl)	232.6±30.3	155.6 – 289.7
Telomere length (T/S ratio)	0.85±0.46	0.34 – 1.65

Relative Variable Importance

Interestingly, the age of the chick had a relative importance of over 50% in explaining most of the adults' diving activities, i.e. departure time, maximum distance and vertical distance, while the individual characteristics of the adults showed relative importance values that did not exceed 34% (Table 2). The number of dives and the hunting efficiency were the only diving variables for which the age of the chick did not show any strong influence (Table 2). Similarly, no parameters could explain more than 20% of the hunting efficiency.

For the oxidative status of the birds the age of the chicks had the highest relative importance on the ROM production with 65% (Table 2). Conversely, the OXY level was influenced by the body mass (89%) and the age of the adult (59%), i.e. the adult's individual parameters were more important in explaining the OXY level than was the chick age (Table 2).

Table 2: Relative variable importance resulting from the model averaging (with telomere length, age of the parent, body mass, sex and chick age). Relative importance values > 50% are indicated in bold.

	Adult age	Body mass	Sex	Telomere	Chick age
Departure time	11%	12%	11%	11%	**72%**
Max distance	21%	19%	15%	28%	**71%**
Vertical distance	17%	31%	34%	10%	**90%**
Number of dives	21%	15%	16%	13%	30%
Hunting efficiency	12%	12%	12%	20%	12%
ROM	22%	41%	17%	14%	**65%**
OXY	**59%**	**89%**	31%	47%	16%

PCA

The first four components (Eigenvalue >1) explained 76% of the variation. The first two components represented 53.3% of the variation (Figure 1). The parameters representing the foraging effort, as well as the ROM production and chick age, explained the variation of the first component (Table 3). The chick age accounted for the variation of the second component but most of the variation was explained by the adult's body mass and its OXY levels (Table 3). The number of dives and the vertical distance were positively correlated and clearly in opposition to the horizontal displacement (the maximum distance) and the departure time, which were both also positively correlated (Table 3; Figure 1). The maximum distance was negatively correlated with chick age, which in turn influenced positively the number of dives and vertical distances (Table 3; Figure 1). In other words, the adults produced a greater diving effort as the chick's demand increased, although this meant they travelled less far and left later after the sunrise Parameters describing the diving effort were also strongly related to the ROMs' production (Table 3; Figure 1), i.e. the greater the diving effort the greater the oxidative damages. The consequence of the positive relationship between ROMS and diving effort was that ROMs were also positively related to chick age (Figure 1).

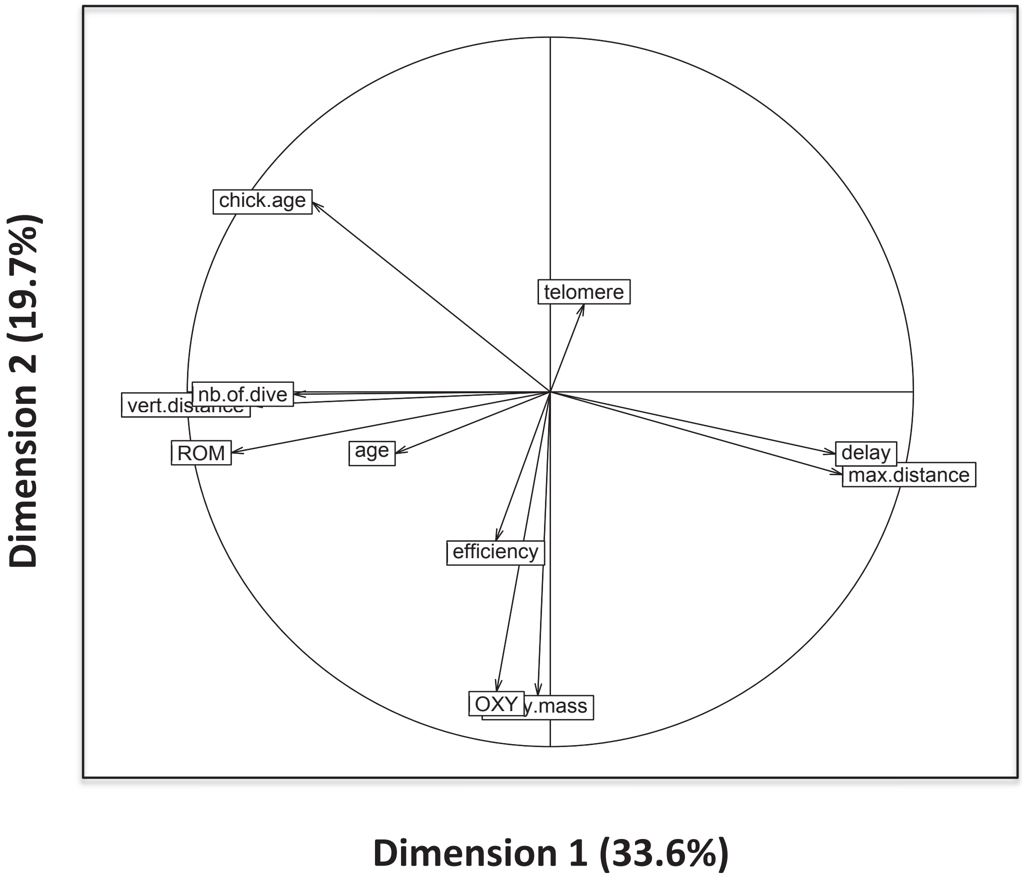

Figure 1: PCA circle with the first two component loadings, presenting the interactions between all available variables: with information concerning the foraging effort and success; individual characteristics and the chick's age as an indicator for chick food demand
Chick.age= age of the chick; *telomere*= relative telomere length; *body.mass*= body mass of the adult; *OXY*= antioxidant capacity of the adult; *ROM*= oxidative damages of the adult; *ver.distance*= total vertical distance; *nb.of.dive*= total number of dives; *efficiency*= hunting efficiency; *delay*=relative departure time; *max.distance*= maximum distance reached by the bird; *age=age of the adult.*

Unexpectedly, the ROMs (damages) and OXY (defences) were not related (Table 3; Figure 1) and consequently, OXY was not related to the diving effort and chick age but had a strong positive relationship with adults' body mass (Table 3; Figure 1). Hunting efficiency is apparently positively influenced by adults' body mass and OXY but the distances between the perimeter of the circle and the tip of the arrows for these parameters indicate that the correlation is non-significant, and that hunting efficiency explained most of the variation of the third component (Table 3).

The variation of the third axis was accounted by the age of the adult, its telomere length and hunting efficiency (Table 3), while the variation of the last component was only explained by the telomere length (Table 3).

The hunting efficiency was not related to any other foraging parameter or the chick age. Even if the hunting efficiency was inversely related to telomere length both arrows were too distant from the perimeter of the circle to conclude to a significant correlation between them.

Table 3: Component loadings on four axes of the principal component analysis (PCA) for the foraging parameters of little penguin from Phillip Island during the 2010 guard period. The four axes collectively explained 76.2% of the total variance. Parameters accounting for most of the variation in each principal component are shown in bold.

	Comp1	Comp2	Comp3	Comp4
Adult age	-0.43	-0.17	**0.58**	0.47
Body mass	-0.03	**-0.86**	0.30	-0.10
Max distance	**0.80**	-0.23	-0.28	0.17
OXY	-0.15	**-0.84**	-0.35	-0.07
ROM	**-0.88**	-0.17	-0.06	0.06
Chick age	**-0.66**	**0.53**	-0.08	0.27
Vertical distance	**-0.83**	-0.04	-0.12	-0.17
Number of dives	**-0.71**	-0.006	-0.03	-0.08
Hunting efficiency	-0.15	-0.42	**-0.58**	-0.16
Departure time	**0.79**	-0.17	0.37	0.18
Telomere length	0.09	0.25	0.53	**-0.75**

5. Discussion

This study focused on the first stage of chick rearing, the guard stage which is extremely energy demanding stage when parents have to alternate on a daily basis on the nest; one parent brood their chick(s) while another forage at sea (Chiaradia & Kerry 1999). We found that foraging activity of a parent performing one single trip at guard was closely related to its chick's growth: As guard stage progressed, parents departed later and decreased the horizontal distance travelled while increasing their diving effort (i.e. vertical distance travelled and number of dives). This strategy had negative repercussions at the physiological level with an increase in the production of ROMs, i.e. oxidative damages. The level of the antioxidant barrier (OXY) did not change. Together, this suggests that there was an increase of oxygen reactive species (ROS) that was not followed by an increase of defence (OXY), hence resulting in a state of oxidative stress. Diving species are sensitive to an increase in oxidative damages when diving effort increases, e.g. Adélie penguins (*Pygoscelis adeliae*) and emperor penguins (*Aptenodytes forsteri*) were more susceptible to produce reactive oxygen species in comparison with non-diving Antarctic seabirds (e.g. south polar skua, *Catharacta maccormicki*, and snow petrel, *Pagodroma nivea*) (Corsolini et al 2001). During dives the blood flow is selectively redirected towards the vital organs, which lead to a restriction of blood and therefore oxygen (ischemia/hypoxia) in some tissues (Kooyman & Ponganis 1998). The restoration of the blood flow and the oxygenation of the tissues (reperfusion/re-oxygenation) are associated with a dramatic increase in oxy-radical generation (McCord 1985). Following ischemia, the formation of reactive oxygen species increases as oxygen flows again, due to the accumulation of electrons in the electron transport chain (Kelvin et al. 2005). In this respect, frequent ischemia/reperfusion cycles (dive cycles) can expose the organism to enhanced formation of oxidative damages. Elevated values of oxidative damages observed could be related to vigorous exercise that occurs during pursuit and hunting in the little penguins, for it is known that ROS are produced in response to exercise (Dekkers et al. 1996; DiMeo & Venditti 2001). In homing pigeon (*Columba livia*) long flights resulted in an increase in oxidative damage and a drop of antioxidant capacity causing an increase in oxidative stress (Constantini et al. 2008).

Growing chicks influenced the foraging activity and decisions of the adults, and we have shown that this enhanced effort led to an increase in the oxidative stress. However, we expected that individual characteristics, especially the age of the adult, could also influence foraging activities and the oxidative status. The trade-off between self-maintenance and reproduction has indeed been thoroughly related to the evolution of ageing (Kirkwood & Austad 2000). In long-lived species, senescent individuals should reduce their reproductive output to preserve their self-maintenance and maximise their fitness (Selman et al. 2012). For instance, older red-legged partridges (*Alectoris rufa*) produced less offspring and endured higher levels of oxidative damage than did middle-aged individuals and older birds showed higher plasma total antioxidant status than younger birds (Alonso-Alvarez et al. 2010). However, adult age had no influence on the foraging activities of little penguins in our study. We also investigated the effect of telomere length, as it is a parameter linked to individual biological age and an important parameter in senescence process (von Zglinicki et al. 1995). We expected to see a decrease of telomere length as the adults aged but this was not the case in our study. The absence of telomere shortening with age can partly be explained by the processes of telomere reparation thanks to the activity of the telomerase for instance (Monaghan et al. 2009; Monaghan 2010). Given that telomere lengths were suggested to reflect individual quality (Pauliny et al. 2006), we also expected individuals with different telomere lengths to adopt specific behaviour to further reduce the physiological damages that an intensive foraging effort could incur. In our study, however, the telomere length did not correlate with foraging behaviour and the oxidative status. High prey availability during this particular season might have reduced inter-individual variability both in terms of foraging activity and anti-oxidant components (Zimmer et al. 2011a).

However, we found a positive relationship between the level of anti-oxidant capacities and the body mass. Birds in good condition probably had a better capacity to defend themselves against the harmful repercussions of the reproduction. Like for most birds, little penguins' body condition during incubation is a reliable indicator of the breeding success during a good year (Chiaradia 2001; Robinson et al. 2005). During incubation little penguins increase their body condition during long trips that allow birds to accumulate enough resources in preparation for the demanding chick-rearing period (Robinson et al. 2005; Kato et al. 2008). It suggests that the birds with better body condition have not only more energy reserves but also higher physiological capacity against oxidative stress, allowing for more energy to be invested into the reproductive effort and leading consequently to high reproductive success.

Several recent papers have found no evidence that reproduction increases oxidative damages, leaving the question of oxidative stress as a mediator of the trade-off between reproduction and self-investment (Metcalfe & Monaghan 2013). However, Fletcher et al. (2013) found in North American red squirrels (*Tamiasciurus hudsonicus*) an increase in daily energy expenditure during lactation accompanied by an increase in plasma protein oxidative damages, corroborating our findings. These authors also found that access to abundant resources can reduce the physiological cost of reproduction by increasing antioxidant capacity.

This increase in oxidative damages without a concomitant increase in the level of the antioxidant barriers can have an accumulative effect, which may force parents to go for longer trips. Short one-day trips at guard are beneficial to chicks (Chiaradia and Nisbet 2006) but at the expense of the parents' body condition (Saraux et al 2011). Like in other seabirds, short trips improve the frequency of food delivery to the chicks, while longer trips allow parents to restore their body condition (Chaurand & Weimerskirch 1994; Weimerskirch et al. 1994). Indeed, 92% of the first post-guard trips, following the travel-intensive period of the guard, are long trips (Saraux et al. 2011a). Our results suggest that this crucial change in foraging strategy from short trips (guard) to long trips (post-guard stage) may be triggered by the accumulation of oxidative damages. Future works should try and assess if long trips also help restoring the balance between oxidative damages and defences.

Our results show the overall balance in oxidative damage and defences could influence changes in foraging strategies. We advocate that the accumulation of oxidative damages may play an important role in defining the costs of reproduction in a wild species and mediates life-history trade-offs.

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References

References have been included in the general bibliography at the end of this thesis.

CHAPTER 5 : GENERAL DISCUSSION & CONCLUSION

General Discussion & Conclusion

In this thesis I explored intrinsic and extrinsic parameters that could influence the foraging behaviour and success of the little penguin. The first part was dedicated to the importance of environmental parameters on the birds' behaviour. Penguins responded to the presence of a thermocline in the water column by adjusting their behaviour and it allowed the birds to increase their foraging success. In the absence of thermocline, however, birds adjusted their behaviour at sea in order to optimize their hunting. The second part aimed to analyse the influence of individual parameters on the foraging behaviour. I found a spatial segregation in the foraging area according to age (i.e. experience) without change in diet composition and foraging effort. In addition, the age, the sex or the telomere length of individuals did not influence any of the parameters describing the diving activities. The demand for food from growing chicks seems to be the main driving force that shapes the little penguin's foraging behaviour, which in turn increases the adults' oxidative damages.

I. Is the thermocline crucial for little penguin?

The articles A and B focused on the structure of the water column with a specific attention on the presence or the absence of thermocline. Previous work has showed the importance of thermoclines in the water column for the little penguins (Ropert-Coudert et al. 2009). Between two reproductive seasons the foraging and breeding successes of little penguins were higher the year when a thermocline was present in the foraging areas of the birds. In the first part of this thesis, I investigated this matter further, confirming the importance of the structure of the water column on the foraging behaviour of the breeding birds but also showing that thermoclines can disappear – or rather not appear at all during the whole breeding season – with a concomitant change in foraging behaviour and decrease in the foraging efficiency of the birds.

The presence of the thermocline in the water column was the unique element permitting the birds to express behavioural plasticity (Articles A and B). The presence of a thermocline coincided with the birds' optimal foraging behaviour, as they decrease their effort while maintaining or even increasing their foraging success (Articles A and B).

The article A looked at the variation of environmental condition and the foraging behaviour at a finer scale, within a single breeding season (2005). Here, changes in foraging behaviour were also closely related to the structure of the water column. Birds seem to benefit from the presence of thermocline. I showed a clear decrease in foraging efficiency the weeks when no thermocline was found in the temperature profiles recorded by the birds.

Although similar in essence, the behavioural responses to the presence of a thermocline differ in the studies of articles A and B. In article B, in 2007 when a thermocline was observed in the records, birds made more dives than in other years. They performed shallow dives, which allowed them to increase the bottom duration, and therefore to optimize prey encounter. Moreover the duration of prey pursuit per dive was shorter, reflecting suitable conditions to improve prey capture. Contrarily, when thermocline disappeared, birds increased their number of dives (foraging effort) while their hunting efficiency (foraging success) decreased, compared to weeks when thermocline was present (Article A). The absence of the thermocline cannot be the sole responsible for this decrease in hunting efficiency. We could indeed expect that, at least, the hunting efficiency could be maintained at high levels since, in article B, the absence of thermocline had no dramatic impact on the hunting efficiency (proportion of dives with prey encounter). For central place foragers, prey abundance around the colony will decrease as season progresses (Birt et al. 1987). In that situation, the presence of a thermocline would be very helpful to assist the birds in capturing prey, as it will act as a physical barrier to the dispersal of the fish (e.g. Kitagawa et al. 2000; Table 1). The combined presence of a stratified water column and enhanced resource abundance would have contributed to the increased foraging performance of little penguins (Table 1). However, the disappearance of the thermocline later in the season, associated to a decrease of food resource around the colony, can have a greater negative impact on birds' foraging performances (Table 1). In contrast, birds foraging in a mixed water column and with low resource abundance would need to increase their foraging effort if they are to maintain their breeding investment as high as birds foraging in optimum conditions, or alternatively they could choose to decrease their breeding investment (Table 1).

Table 1: Hypotheses on the foraging effort/success of little penguins in high/low resource abundance and in presence or absence of thermocline. Based on the results from the article A, B and Ropert-Coudert et al. 2009.

Resource abundance	**High**	**High**	**Low**	**Low**
Thermocline presence	**Yes**	**No**	**Yes**	**No**
Foraging effort	Low	Middle	Middle	High
Foraging success	High	Middle	Middle	Low

It seems crucial for the birds to synchronize their peak of food requirement with the peak of food availability as prescribed in the temporal match-mismatch concept (Cushing 1990; Edwards & Richardson 2004; Frederiksen et al. 2004; Durant et al. 2005). By breeding early in the season, birds would have greater opportunities to meet suitable conditions of prey abundance but they would face a cold mixed water column in which prey are apparently able to disperse deeper, forcing the birds to increase their diving effort in return. Later in the breeding season, the water column would warm up, leading to the appearance of a thermocline that could balance the decrease in food resource abundance by improving prey accessibility.

Within a season I have also showed that the position of the thermocline in the water column affected the foraging behaviour of the birds. Moreover, the intensity of the thermocline probably plays an important role, as birds were more likely to have higher foraging efficiency when thermocline had stronger gradient and intensity. The thermocline would act as a physical barrier to nutrients and thus create a productivity layer that potentially attracts small fishes as showed in several studies (Derenbach et al. 1979, Hansen et al. 2001, Gray & Kingsford 2003). Thermocline would thus represent a strong cue of enhanced prey availability for foraging top predators, like has been shown in Southern elephant seals, *Mirounga leonina* (Boyd & Arnbom 1991; Bailleul et al. 2010; Dragon et al. 2010), Northern elephant seals, *M. angustirostris* (Hakoyama et al. 1994), Pacific bluefin tuna, *Thunnus orientalis* (Kitagawa et al. 2000), king penguins, *Aptenodytes patagonicus* (Charrassin & Bost 2001), and Brünnich's guillemots, *Uria lomvia* (Watanuki et al. 2001, Ito et al. 2010, Kokubun et al. 2010). Depending on the type of prey, predators may have to feed at different localisation in the water column. For example, one of little penguins' preferred prey, anchovies, is often the most abundant just above the thermocline (Hansen et al. 2001) and little penguins are more likely to forage above or within the thermocline rarely under it (Ropert-Coudert et al. 2009; Article A and B). Conversely, thick-billed murres are more likely to dive just below the thermocline (Takahashi et al. 2008). Similarly, southern elephant seals from Macquarie Island dove well below the thermocline depth in the Polar Front Zone Water and Antarctic Water Masses (Field et al. 2001). Seabirds in the Gulf of California showed a decrease in the season of sardine consumption even when there was no evidence of decreasing in the fishery catch, indicating that other components may be responsible for a decline in sardine available to seabirds (Velarde et al. 2013). In the little penguins' case, availability may further decline when the thermocline is located deeper, which would means that prey may distribute deeper in the water column, i.e. out of reach for divers with limited diving capacities (Velarde et al. 2013). As little penguins are small birds their diving capacity is indeed limited (Schreer & Kovacs 1997; Watanuki & Burger 1999) to a maximum of 90s underwater and a maximum depth of 70 m (Ropert-Coudert et al. 2006a).

The absence of thermocline may not only affect the 'quantity' of prey available but also the type of prey composing the diet of the predator. When the principal prey was not accessible, little penguins targeted prey that are more accessible and had a more diversified diet (Chiaradia et al. 2010; Figure 1).

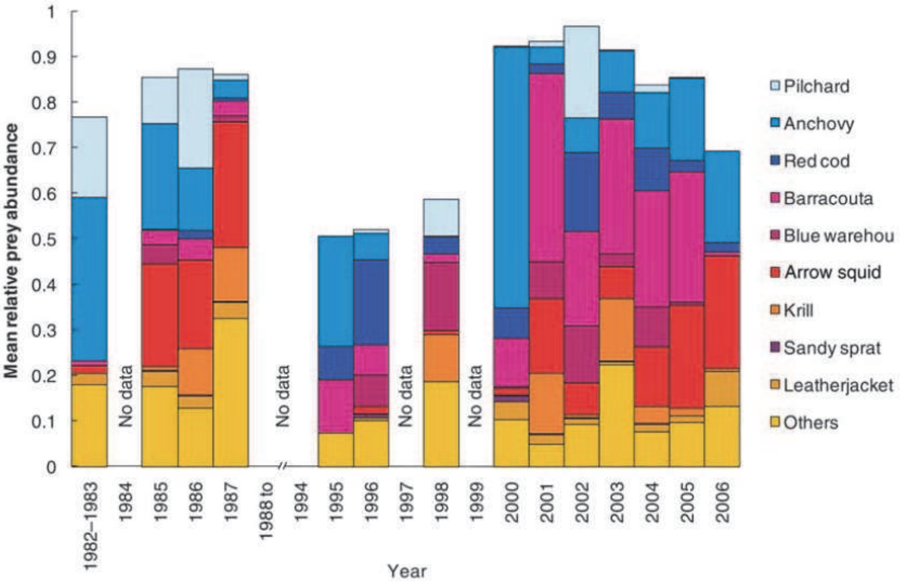

Figure 1: Mean relative prey abundance in the diet of little penguins from 1982 to 2006. (Figure from Chiaradia et al. 2010)

Thermocline would play an important role during the season, especially when prey quantity is low and difficult to access (e.g. at the end of the season) by improving the prey accessibility. The increase in foraging activity by king penguins has been correlated to strong thermal gradients (Scheffer et al. 2012). Southern elephant seals from Kerguelen Island showed a higher probability of switching from migrating state into resident state if they encounter a zone where the thermocline strengthened, even temporarily (Bestley et al. 2013). First passage time analysis (Fauchald & Tveraa 2003) on tracking data of northern fur seals, *Callorhinus ursinus*, revealed that foraging hotspots are linked to thermocline depth and occur near sub-mesoscale surface fronts (eddies and filaments) (Nordstrom et al. 2012). Although the significance of fronts to top-predators differs strongly between the southern oceans and the tropics (Ballance & Pitman 1999), thermocline depth also plays a major role in prey aggregation in the tropics and in the structuring habitat (Bost et al. 2009). Planktivorous species are more likely to respond to the presence of fronts than piscivorous ones, as prey will be more readily available to the former, while a time lap may occur for the prey of the latter (Spear et al. 2001). The best habitat model of plankton-feeding seabirds corresponds to areas of well-mixed surface layer with much shallower thermocline (<30m) that concentrate prey at the surface (Balance et al. 2001; Spear et al. 2001; Bost et al. 2009).

Predators rely extensively on fronts that are predictable in time and space and correlated with higher resource predictability (Bost et al. 2009). Birds that are not constrained by the necessity to return to their nest to provision small chicks may show different association with fronts and other physical features (Weimerskirch et al. 1994; Nel et al. 2001). Marine predators with a large foraging range are specialized in the exploitation of fronts, especially diving predators like seals and penguins that exploit the water column in its three dimensions, which is not the case for non-diving, flying birds (Bost et al. 2009). Wherever the location of the breeding colonies, king penguins exploit frontal zones in a consistent way, especially the polar front (Bost et al. 2009). For diving predators, the disappearance or displacement of marine front away from their travelling range would have negative consequences on their foraging performance (Koudil et al. 2000; Cotté et al. 2007; Péron et al. 2012). As an example of this, and following the climate models of the Intergovernmental Panel on Climate Change (Solomon et al. 2007), the distances that king penguins from Crozet Archipelago would have to travel to reach their optimal foraging zones would double by 2100, as the warming of surface waters would lead to a gradual southward shift of the polar front, the main feeding area for these birds (Péron et al. 2012). It is far beyond the usual foraging range of this bird and would negatively affect the population on the long term (Péron et al. 2012), making this species a good ecological indicator of environmental changes.

II. Using little penguins as an ecological indicator

Previous studies already linked little penguins' performance with environmental variability (See Table 2).

Table 2: Summary of impact of intrinsic and extrinsic factors as well as chick growth on foraging and breeding performances of little penguins. ? Means need to be confirm, empty cell no study confirming any trends

	Intrinsic	Extrinsic	Chick growth
Dive characteristics (number, depth, duration,…)	• Age in females (Zimmer et al. 2011a,b) • Sex during incubation (Kato et al. 2008)	• Structure of the water column (Articles A and B) • Season progress (Zimmer et al. 2011; Articles A, B) • Localisation of the colony e.g. bathymetry (Chiaradia et al. 2007; Preston et al. 2007; Hoskins et al. 2008)	• Increase diving effort with chick age (Articles C and D; Zimmer et al. 2011b)
Foraging tactics (localisation in the dive of prey chased)	Age of females (Zimmer et al. 2011a)	Pursuit of prey influenced by structure of the water column (Article B)	
Trip length	• Body mass (incubation: Kato et al. 2008; post-guard: Saraux et al. 2011a) • ? Physiological parameters	• Bad season increase post-guard trip length (Chiaradia & Nisbet 2006)	
Spatial use	• Age of the adult Article C	?	• Total and maximum distance (Articles C and D)
Hunting efficiency		• Thermal structure of the water column (Articles A and B; Ropert-Coudert et al. 2009) • Localisation of colony affect diving efficiency (Chiaradia et al. 2007a) • Advancing season (Zimmer et al. 2011b; Articles A, B)	• ? Increase meal size with chick age (Chiaradia & Nisbet 2006)
Diet		• Localisation of the colony (Chiaradia et al. 2012) • Environmental condition (Chiaradia et al. 2010)	• Reproductive stage (Chiaradia et al. 2010)
Breeding decision	• Early breeding related to age (Nisbet & Dann 2009) • ? Start of breeding season linked to body mass & physiological parameter • ? Transition guard/post-guard linked to physiological parameter	• Environmental condition affected guard length, duration of post-guard foraging trip length…(Chiaradia & Kerry 1999; Chiaradia & Nisbet 2006) • SST (Chamber 2004; Cullen et al. 2009)	
Breeding success	• Age of the adult (Nisbet & Dann 2009) • Body condition during incubation in good year (Robinson et al. 2005) • Body size in males (Miyazaki & Wass 2003)	• Localisation of the colony (Fortescue 1999; Chiaradia et al. 2007) • SST (Cullen et al. 2009) • Thermal structure of the water column (Ropert-Coudert et al. 2009) • Diet diversity prey abundance (Chiaradia et al. 2010) • Late/early breeders (Reilly & Cullen 1981; Chiaradia & Kerry 1999)	

For example, if sea or air temperatures were to increase during summer or autumn we could expect earlier mean laying dates the next season for little penguins (Chambers 2004). This has been seen in other species as well: for example, there is a general tendency towards later arrival and egg laying for the entire community of seabirds in East-Antarctica (Barbraud & Weimerskirch 2006). This delayed phenology is linked to a decrease in sea-ice extent, underlying the contrasted effects of global climate change on seabirds' species (Barbraud & Weimerskirch 2006). For little penguins, egg-laying date, chick mass and the number of chicks fledged per pair were correlated with SST in the first 3 months of the year preceding the breeding season (Cullen et al. 2009). When SSTs in Bass Strait are warmer than average in March an earlier egg-laying date is predicted, together with a heavier chick mass at fledging and more chicks produced (Cullen et al. 2009). However, the SST variation early in the year only explained few variances in the number and the mass of fledging chicks (Cullen et al. 2009). This is certainly due to the unpredictability and the big variation between years of the oceanographic conditions later in the breeding season (Article B). The different fish species that compose the diet of little penguins may respond differently to changes in sea-surface temperature (Chambers 2004). These modifications occurring along the food web would affect breeding and foraging performance of little penguins (Chiaradia et al. 2010). The low breeding success and underweight chicks are indeed used as indicators of poor food availability (Chiaradia & Nisbet 2006; Chiaradia et al. 2010). In the 1990's, the disappearance of the main prey of little penguins (pilchards) forced the birds to target the next-most profitable, or available, prey so as to be able breed successfully (Chiaradia et al. 2010). The diet diversity is increasing towards the end of the breeding season, matching the changes in foraging conditions that usually deteriorate (Chiaradia & Nisbet 2006; Chiaradia et al. 2010; Article B). In years of poor breeding success, little penguins made longer trips to deliver the same amount of food to their chicks (Chiaradia & Nisbet 2006). As such, the average length of the guard stage is a good indicator of the breeding success of the colony, with a long guard stage being a sign of good food availability around the colony (Chiaradia & Nisbet 2006). Similarly, oceanographic conditions around the breeding colonies influence breeding success (Fortescue 1999). For example, little penguins breeding in colonies surrounded by shallow waters (90% of the zone <50m) have a lower diving effort and a high fledging success (Chiaradia et al. 2007a). In other words, the phenology of little penguins, their breeding performance and foraging behaviour are reflecting changes in environmental conditions. All these suggest that little penguins could be good indicators of the environment.

Yet, to conclude on the relevance of little penguins as an ecological indicator it is important to determine the influence of intrinsic characteristics on the foraging behaviour. For example, it is known that the sex of individuals could affect foraging activity in seabirds (e.g. Japanese cormorant *Phalacrocorax filamentosus*, Kato et al. 2001). In this thesis, no obvious differences were observed between males and females. This was rather unexpected, as physiological needs and capacities can differ between sexes. Several studies highlighted differences in foraging activity caused by a large sexual dimorphism (e.g. northern giant petrels, *Macronectes halli*, González-Solís et al. 2000; black-browed albatrosses, *Thalassarche melanophrys*, grey-headed albatrosses, *T. chrysostoma*, Phillips et al. 2004; gentoo penguins, *Pygoscelis papua*, macaroni penguins, *Eudyptes chrysolophus*, South Georgian shag, *Phalacrocorax georgianus*, Kerguelen shags, *P. verrucosus*, Bearhop et al. 2006; red-footed boodies, *Sula sula*, Weimerskirch et al. 2006). In little penguins, sex difference in body size is rather small (Arnould et al. 2004), while variations in body mass among individuals is large (Zimmer et al. 2011b). In the present thesis, the body mass of adults did not influence dive characteristics (Articles C and D), but was a good indicator of the physiological condition of the birds (Article D). My results, therefore, agree with those of Zimmer et al. (2011b), where no influence of adult body mass on foraging parameters was found. Yet, it should be noted that the link between individuals' characteristics and foraging activity was only investigated in a single year (2010) when food availability was high and no large variation in body mass between individuals was observed. However, early body condition is more likely to determine the timing of breeding and the breeding success (Robinson et al. 2005), as birds must have enough body stores to face the energetically demanding reproductive attempt (Article D). In a colony of little penguins in New Zealand, body size (estimated by head length) was related to some reproductive parameters in males; larger males produced eggs and chicks earlier in the breeding season, and had chicks that grew more quickly than chicks in nest defended by small males (Miyazaki & Waas 2003). When prey availability is low, the effect of body mass and/or sex difference could be more pronounced in foraging and reproductive parameters.

Reproductive performances have been related to age of the birds with an increase in breeding performance with age early in the life of individuals, before these performances start to decline in old birds, providing strong evidence for senescence (Nisbet & Dann 2009). The quadratic relationship in breeding performance with age has been supported by the hypothesis that this trend is mediated by an improvement of foraging behaviour with age (Desrochers 1992). Such age-specific differences in foraging performance were already observed in female little penguins: Zimmer et al. (2011a) found that diving behaviour of middle-aged birds differed from young and old birds. Dive durations of middle-aged females were shorter than that of young and old birds, while their dive effort (the ratio of dive to post-dive duration) was lower than that of young ones, suggesting that middle-aged birds were in better physical condition than other ones and were thus more efficient divers (Zimmer et al. 2011a). I thus expected to find differences in foraging behaviour among the little penguins' age classes, mirroring in some ways the age-related breeding success pattern. In this thesis, the most surprising result was that age had little – if not – influence on the diving behaviour (Articles C and D) although we observed a clear pattern of spatial segregation between old and middle-aged birds (Article C). In Zimmer et al. (2011), as in my studies, there were no differences in prey pursuit frequency or duration between age classes, but hunting tactics differed. More experienced bird chased prey around and after reaching the maximum depth of dives, suggesting middle-aged penguins foraged more efficiently than young or old ones as they made a better use of the time spent underwater.

However, the tactics of prey pursuit during the different parts of the dive were influenced by the thermal structure of the water column (Article A). In the paper of Zimmer et al. (2011a) no information were given about the environmental condition. I wonder if the phenomenon observed in Zimmer et al. (2011a) was really due to age of the birds or if it was not an artefact due to the possible changes occurring in the water column structure (like in article B). Alternatively, there could also be a bias in the study of article B, with the age of the birds influencing the results. It can also be a combination of both effects, i.e. the interaction between age and environmental conditions. A similar case was found for the king penguins, where no differences in dive characteristics (i.e. mean maximum dive depth, duration, proportion of bottom phase, post-dive duration…) were observed between young and old birds (Le Vaillant et al. 2012). However, differences in the dynamic body acceleration, i.e. the overall activity of the birds, according to depth and speed, were found between both age groups, with old king penguins exerting a greater effort for a given speed during the initial part of the descent than younger penguins, but these latter individuals worked harder in relation to performance at greater depths (Le Vaillant et al. 2012). Again, could the environmental condition, i.e. the thermal structure of the water column, have influenced the results of Le Vaillant et al. (2012), knowing that king penguins target marine fronts to forage (Koudil et al. 2000; Cotté et al. 2007; Bost et al. 2009; Scheffer et al. 2012)?

Several authors have envisioned that the interaction between age and environmental conditions on foraging performance would become more apparent when prey availability is reduced (e.g. Sydeman et al. 1991; Barbraud & Weimerskirch 2005; Lescroël et al. 2009). I can thus expect to find little differences in diving behaviour between age classes when food supply is not limited as was the case in the studies of articles C and D, whose data were collected in a year of high food availability, i.e. when little penguins showed high body mass and breeding success. Birds return to the colony at age of two or three years old (Dann & Cullen 1990). Before the first breeding we can suppose that they have enough time to improve their foraging ability. Differences between age classes could be more pronounced when the food availability is low, as the young would have never encountered such situation before (i.e. to simultaneously collect enough food for itself and for the chicks in a bad environment). I could hypothesize that older and more experienced individuals would respond more rapidly to any environmental changes compared with young and non-experienced birds. In European shags (*Phalacrocorax aristotelis*) differences between young/naive (first reproduction) and old/experienced breeders (more than one reproduction experience) in foraging performance were more marked when conditions deteriorated later in the breeding season. Experienced parents had higher foraging efficiency than the young parents. In addition, experienced parents adjusted their foraging effort to the environmental conditions as early in the season experienced birds made less effort than the naive ones. Yet, under poorer conditions, i.e. in the late breeding season, experienced parents increased their effort while the naive parents were unable or unwilling to do so (Daunt et al. 2007).

In this thesis, I also explored another parameter, which has been suggested could be used as a good indicator of individual quality, namely the telomere length (Bauch et al. 2013). In king penguins, telomere length is a better predicator of proximate foraging performance than other individual characteristics, as individuals with longer telomeres had greater dive efficiency (Le Vaillant et al., unpublished data). For the little penguins, the telomere length was not a good predicator of foraging performance, but more in-depth studies are still needed to explore potential relationship between telomere and performance (breeding and foraging).

My work showed that all diving characteristics were mainly related to environmental parameters, and also depended on the foraging tactics adopted to face changes in prey distribution (i.e. in which part of the dive birds are more likely to chase their prey). Biomechanical performances (e.g. stroke frequency and amplitude or change in buoyancy) would be more related to intrinsic factors (Zimmer et al. 2011a; Le Vaillant et al. 2012), but it may also be related to environmental components (Table 2). It is not contradicting behaviour as birds go where their prey are and they are more likely to follow physical features of the environment (Table 2). Birds dove deeper in mixed water column whereas with a thermocline birds performed shallower dives (Article B). Prey followed thermal gradient of the water column and so did the little penguin.

Zimmer et al. (2011b) noted that for females little penguin, the relationship between variation in prey availability and hunting effort can change at a fine temporal scale, i.e. within a breeding season, with no strong evidence that individual characteristics (as age and body mass) play a major role. This is another evidence that adds to the articles presented in this thesis, confirming that the foraging behaviour is more likely to be dependent on the environmental conditions (e.g. the resource availability) than on individual characteristics. In this context, I would conclude which may characterise little penguins as a good ecological indicator (Table 2). Among all individual parameters tested none of them influenced the foraging behaviour, contrary to the food demand of the growing chick (i.e. chick age).

III. How parents respond to chick growth

All studies conducted in this thesis were focused on the period of guard stage, in order to compare foraging trip of each bird as they made a one-day trip. This period is the shortest stage (14 days) compared to the period of incubation (35 days) or post-guard (42 days; Chiaradia & Kerry 1999) and it seems that this period has a great influence on the capacities of birds (physiological and behavioural). In the articles C and D, I included chick age in the different statistical models in addition to other individual parameters. Indeed, a previous study has already demonstrated that the diving effort of female little penguins varied with the chick age (Zimmer et al. 2011b). However, the relationship between foraging activities and chick growth was not as much important in Zimmer et al. (2011b) as it was in my study. I found that the chick growth had the strongest effect on foraging behaviour compared with any other individual characteristics of the adult birds (Table 2; Articles C and D). At such small spatial and temporal scales, parents adjusted their behaviour to the chicks' growing requirement (Articles C and D). An increase in feeding activity with chick age has been reported during the first part of the nestling period that requires substantial amount of energy to be delivered to the chicks as their growth rate is peaking at that time (Chiaradia & Nisbet 2006; Barba et al. 2009; Steen et al. 2012). Under the influence of the growing chick demand the organisation of the one-day foraging trip was modified. For example, the number of dives and vertical movements in a trip increased when birds left the colony later in the morning, while they reduced the horizontal distance travelled as the chicks grew older (Article D).

The increase of breeding effort, through the foraging effort, has for rapid repercussion to affect the oxidative balance of the parents (Article D). Increase in parental investment lead to the production of more oxidative damage. During this period parents invest more energy in reproduction to the detriment of their own maintenance. Rearing chicks can also affect parental body condition, like a decline in body mass (Weimerskirch & Lys 2000). In red-footed boobies, the transition from the non-parental to the parental phase is accompanied by an increase in corticosterone, whereas body mass remained stable (Lormée et al. 2003; Figure 2).

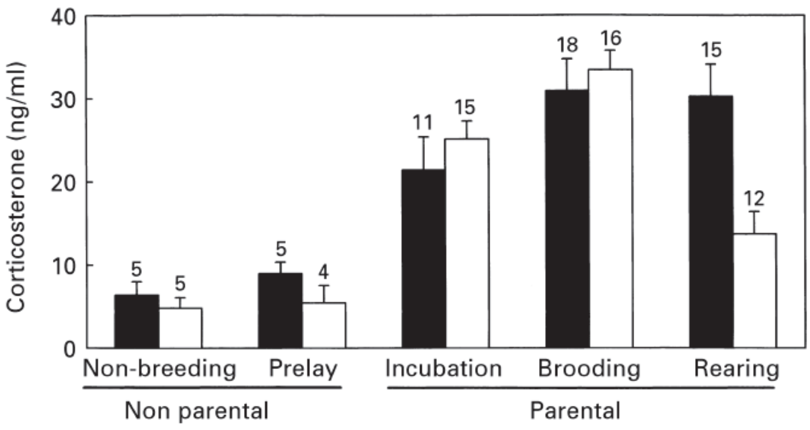

Figure 2: Baseline corticosterone levels in male (solid bars) and female (open bars) Red-footed Boobies *Sula sula* in relation to reproductive stage. (Figure from Lormée et al. 2003)

This augmentation of the level of corticosterone reflects the higher foraging activity imposed by chicks' growing needs (Lormée et al. 2003). In article D, I have hypothesized that the end of the guard stage would be trigger by the physiological condition of the parents. During guard stage, Adélie penguins (*Pygoscelis adeliae*) show metabolic alkalosis and recover from it during the crèche (=post-guard) period (Sakamoto et al. 2010). I suggested that adults can accumulate a certain amount of oxidative damage but after a while it could be too much deleterious which may trigger changes in foraging strategy. Modification in reproductive investment can be mediated by the oxidative stress (Figure 3; Monaghan et al. 2009), in a similar fashion as corticosterone modulates foraging strategies in favour of maintenance or reproduction (Angelier et al. 2007; 2008). Glucocorticoids (e.g. corticosterone) are often employed as indices of physiological condition, and there is evidence that the glucocorticoid stress response is adaptive. Moreover, an increase in glucocorticoids is known to be concomitant to an increase in oxidative stress in rodents, humans and birds (see Haussmann & Marchetto 2010).

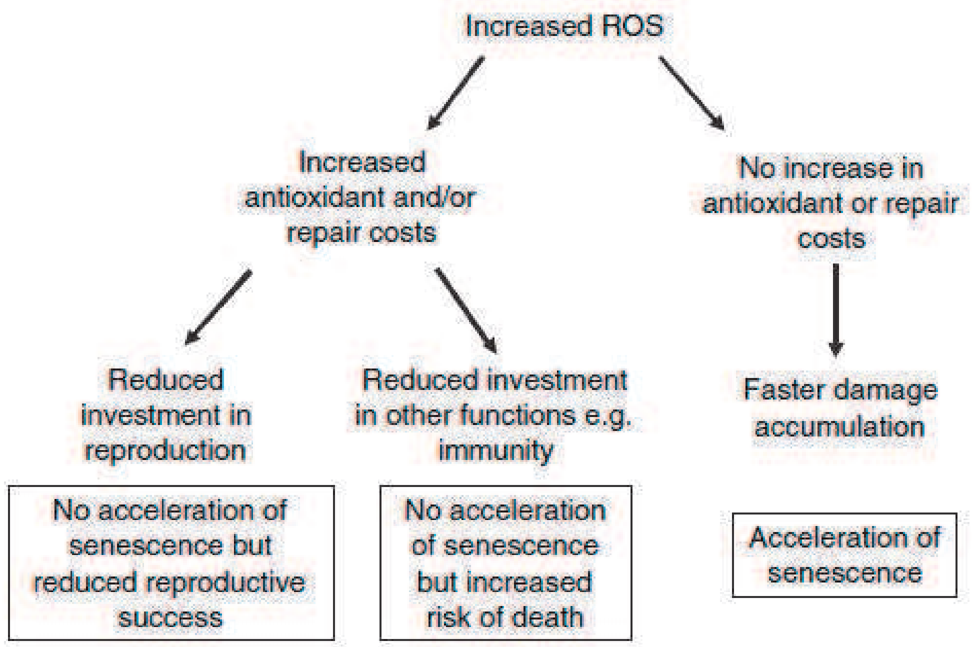

Figure 3: Possible consequences of increased reactive oxygen species (ROS) for life histories, depending on the organisms response. In the boxes at the bottom are shown consequences for fitness. Following the increased of ROS levels, animals can invest more in antioxidant defences. However, this will have consequences for other traits, as this response is costly. The optimal level of investment in antioxidant defence will depend on the fitness consequences of reducing oxidative damage. (Figure from Monaghan et al. 2009)

IV. Perspectives

We saw that individual parameters may not have so much importance on foraging variability during guard stage (Articles C and D). However, physiological condition of the adult is different between the start and the end of the guard stage. We can expect more drastic changes in foraging behaviour during the post-guard. In post-guard, chicks can be left alone and adults can thus be more flexible in their foraging investment to respond to their own need (e.g. by performing longer foraging trip). Moreover, during the post-guard period, male and female probably make independent decision on their foraging trip duration, unlike during incubation when they have to synchronise attendance with each other to incubate eggs. No study, to my knowledge, focused on the foraging behaviour (i.e. diving activity) during the post-guard and comparing long vs. short foraging trip in little penguins so far. During the post-guard adult birds will certainly invest less in parental care and will be more attentive to their physiological needs (e.g., during long foraging trip; Saraux et al. 2011a). During post-guard, birds will also explore larger area and experience different environmental conditions especially during long trips. These long trips could permit the birds to target further marine zones that offer optimum foraging conditions (e.g. zone of shallower depth) with maybe also less competition, a feat that is not possible when birds are limited to a small foraging range. Individual foraging strategies could also be observed during incubation trip, as the influence of the chicks is absent. This can be illustrated by the fact that three different foraging strategies were observed in habitat use of male Adélie penguins at Dumont d'Urville during their first long incubation trip (Cottin et al. 2012). Individuals specifically target open-ocean areas and followed the currents of two persistent eddies, others foraged close to the Antarctic shelf slope at the limit of the pack ice; and some individuals covered a smaller distance (Cottin et al. 2012). The body condition of the birds before their departure at sea was the characteristic limiting their foraging range (Cottin et al. 2012). Body condition of little penguins seems to be also an important factor influencing trip duration during incubation (Kato et al. 2008). Birds leaving the colony with lower body condition were more inclined to initiate longer foraging trip in order to increase body condition (Kato et al. 2008). Differences between male and females strategies were also found (Kato et al. 2008). During long trip (>1 day) females performed more dives than males but the time spent underwater was not different. However, during one-day trip, males dove as many times as females but spent more time underwater. Birds making longer trip increased their number of dives and the proportion of dives with prey encounter the second and subsequent days (Kato et al. 2008). In future studies, it would be interesting to study little penguins' movement in parallel to diving effort, and see if they target specific foraging areas that are beyond their reach during the guard stage.

In bi-parental species investment of both parents during reproduction is essential but, in some instances, an unequal parental care can be observed, with a parent working less, forcing the other to compensate to breed successfully. Compensatory behaviours have already been studied in several seabird species (e.g. Velando & Alonso-Alvarez 2003; Paredes et al. 2005; Beaulieu et al. 2009; Bijleveld & Muller 2009). When manipulating the flying ability of one parent in a breeding couple of Cape gannets (*Morus capensis*), the handicapped birds doubled their foraging trip duration and reduced nest attendance, while their partners showed behavioural compensation via increased nest attendance (Bijleveld & Muller 2009). However, in Adélie penguins, handicapped birds increase their foraging effort without any compensation from their partners (Beaulieu et al. 2009).

All studies composing this thesis did not take into account the behaviour of the partner. It would be interesting to look at the investment of the both parents, as in the little penguins during post-guard stage was found an unequal parental care in chick provisioning (Saraux et al. 2011b). Most pairs (72% of breeding birds; Figure 4) exhibited unequal parental effort, meaning that one individual of the pairs contributed more than the other one (one parent of a

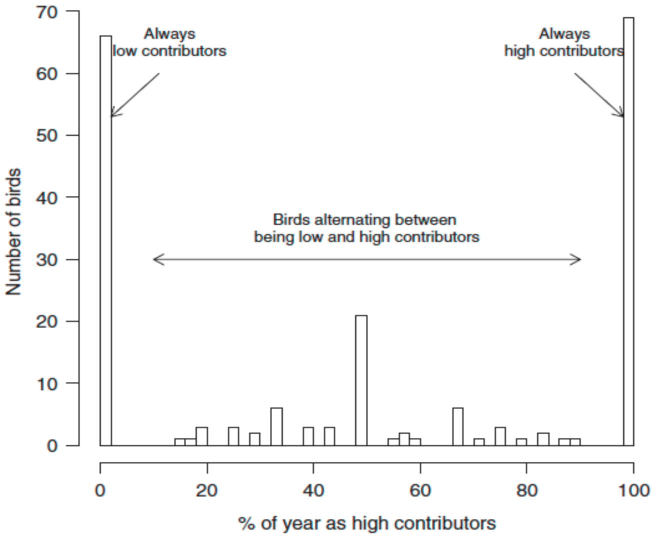

Figure 4: Number of little penguins displaying low or high contribution to their parental investment (Figure from Saraux et al. 2011b).

little penguins' pair was found to perform 7 more trips on average and bring back 4% more food to the chick than its low contributing partner), independently of number of chicks (Saraux et al. 2011b). This pattern of non-equal parental care is not gender specific or driven by experience (age) of the birds (Saraux et al. 2011b). It would be interesting to look at physiological component of the low contributing individual. One could expect, for example, that low contributors may have a poorer oxidative status and/or shorter telomere length than the better contributors. It would be equally interesting to see how these foraging strategies evolve for an individual during the entire breeding season according to particular physiological parameters such as oxidative status, for a better understanding of the mechanism involved in the trade-off between survival and reproduction. It seems necessary to investigate physiological mechanism leading little penguins to better adapt to environmental variation.

Many studies have examined differential investment by the parent in function of the sex ratio of their offspring. If fitness benefits gains from producing male or female offspring differ, parents should adjust their level of investment according to the sex of the offspring (Trivers & Willard 1973; Charnov 1982). In common guillemots (*Uria aalge*), the sex ratio was biased toward daughters in years of higher food availability, which was a year of higher body mass and earlier hatching date (Kristensen et al. 2013). Female chicks seemed to be more costly to breed than male chicks. Adults that were in good body condition invested more (relative mass loss per day) in reproduction and had higher probability of producing a daughter (Kristensen et al. 2013). No studies explored differential investment function of the sex ratio of offspring in the little penguins. They can raise a clutch of two chicks during a breeding season (even more if they invest in a second clutch). The investment by the parent could be different according to the sex ratio of the clutch and to the environmental conditions. During bad seasons, the little penguins' strategy is to invest only in one of their two chicks (Chiaradia & Nisbet 2006). In general, they invest in the first chick to be hatched, which is also the bigger of the two (Chiaradia & Nisbet 2006). It is something that I wish to investigate in future studies: how the sex ratio of offspring and the potential differential investment by the parents to their offspring change in good and bad environmental conditions.

V. Conclusions

This thesis revealed important intrinsic and extrinsic characteristics playing on the foraging behaviour. However, it seems obvious that the foraging behaviour responds more rapidly to environmental changes than following individuals' characteristics (i.e. sex, age, body mass, telomere length), at least at guard stage, making little penguins a good sensitive ecological indicator of the Bass Strait marine system.

Understanding how individuals respond to environmental changes can help us set up conservation measures to protect and manage this iconic species of Australia. However, environmental conditions and breeding success can vary drastically amongst colonies (Fortescue 1999; Chiaradia et al. 2007a, 2012). Birds in different colonies may have specific responses and show differential adjustments to environmental variations. If productivity around these colonies differ it may force individuals to implement specific foraging strategies (Chiaradia et al. 2007a). In addition, the potential impacts of climate change on seabirds suggest that projected changes in ocean temperature and ENSO type activity are likely to reduce prey availability during future breeding seasons (Chambers et al. 2009). Changes in temperatures, ocean currents and ocean stratification, as well as in wind patterns, storms and precipitations occurring in Australia, will affect seabird species (Chambers et al. 2009, 2011). A lack of knowledge on species' reactions when facing environmental change limits the development of conservation actions (Chambers et al. 2009). To resolve this, increasing research and long-term monitoring, as the studies presented in this thesis, are required. This thesis could play a part in a management project aiming at protecting this seabird species, with notably the designation and the potential set up of marine protected areas. Our results have indeed brought new insights on how the little penguins explored horizontally and vertically their foraging area. The creation of marine protected areas would have to take into account such data that provide information on the zones that are ecologically significant to top predators like little penguins. This thesis brings an additional level of information on the effort that birds invest in different condition, notably with the vertical structure of the water column. These areas would allow or restrict different anthropogenic activity in order to manage the protection of biodiversity and/or a specific species (Schofield et al. 2013). For the little penguins, during breeding season, the use of some areas (e.g. foraging area) may require seasonal protection. The distribution of these protected zones would be determined by a set of fixed features (e.g. bathymetry) and oceanographic features (e.g. temperature) (Hyrenbach et al. 2000; Ardron et al. 2008; Schofield et al. 2013). However, we have seen that the oceanographic characteristics reflecting the important area of penguins were transient, highlighting the difficulties to implement this kind of area. Hence, a population might not necessarily frequent the zones designed to protect them. To remediate at these problems, long terms field monitoring, especially the tracking animals at the different stages of their breeding cycle, are of the first importance to connect individuals to their environment use.

This thesis brings new insights on biology and ecology of the little penguin. Environmental variability can strongly affect foraging behaviour on a one-day foraging trip, as well as the chicks' growing need. Articles present in this thesis highlight the importance of the chicks' growing needs compare to individual parameters on the foraging behaviour. However, segregation at sea based on individual phenotype emphasizes the possibility of specific needs and abilities of birds of the different age classes. Understanding the factors that affect breeding success of the little penguins and being able to predict population trends are particularly important for providing scientific advices in management response to climate change.

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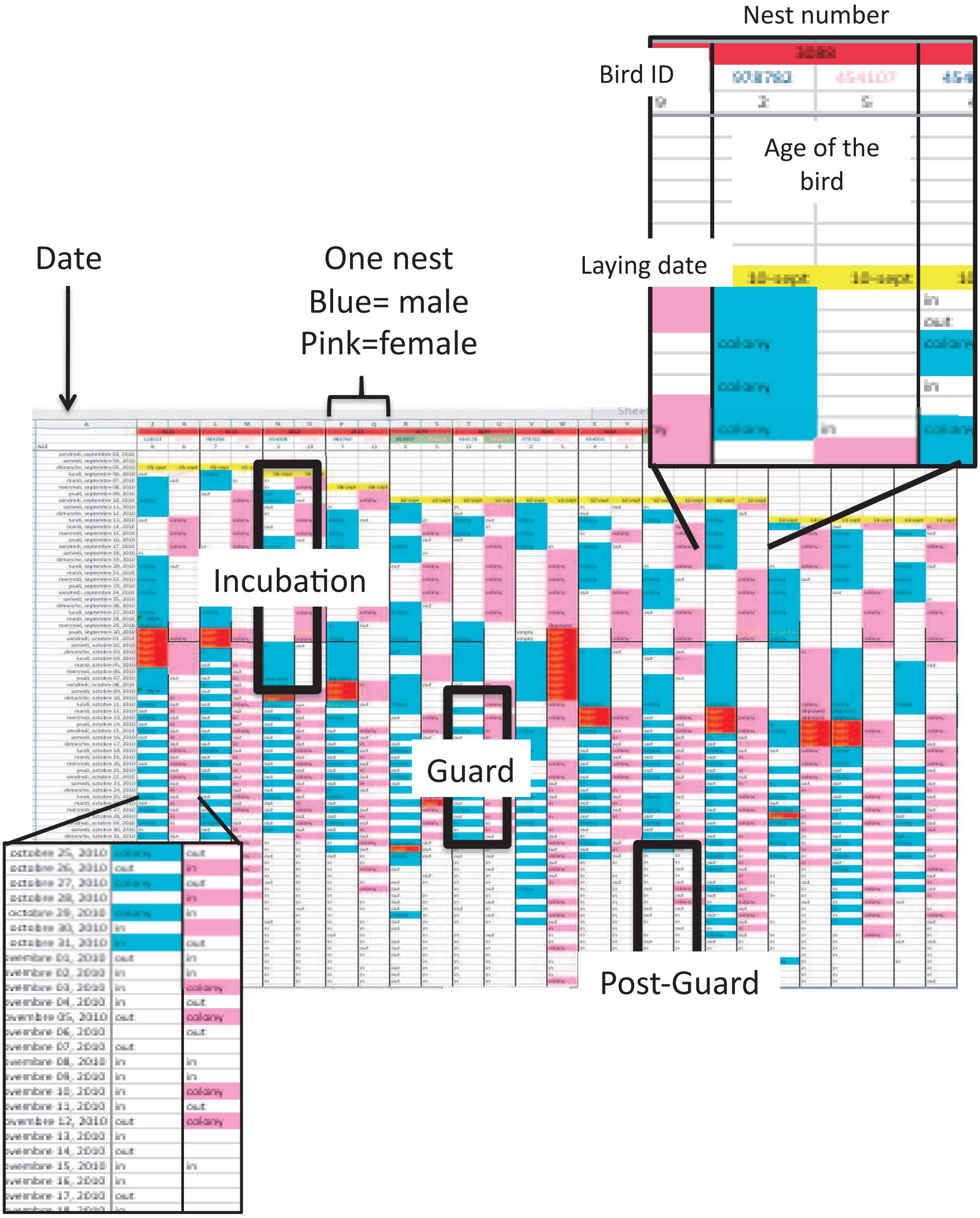
ANNEXES

ANNEXE 1 : Materials & Method



Example of graphical representation of the reproductive cycle of some birds in the colony with data from the APMS and the checking of the colony.

In yellow the laying date (start of the incubation). In colour period spent in the nest by the birds. In red bird at sea equipped by a logger.

The colony is checked every 2 days and the presence of bird in the nest was noted “colony”. “In” and “Out” are information from the APMS.

Annexes 2 to 7

Supporting Information

For the Article C

Fine-scale spatial segregation in the limited foraging area of an inshore seabird species; the effect of age and sex

Laure Pelletier, André Chiaradia, Akiko Kato, Yan Ropert-Coudert

Annexe 2: Results from the model selection (with a ΔAICc < 6) on the variable “maximum distance” using GLMs with a Poisson family. Chick= age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	AICc	ΔAICc	w_i
chick	2	-82.71	169.87	0.00	0.19
age+chick+age:chick	4	-80.83	171.33	1.46	0.09
chick +sex	3	-82.23	171.41	1.54	0.09
bm+chick	3	-82.31	171.59	1.71	0.08
age+chick	3	-82.35	171.67	1.80	0.08
(Null)	1	-84.80	171.75	1.88	0.08
chick +sex+chick:sex	4	-81.51	172.69	2.82	0.05
sex	2	-84.18	172.82	2.95	0.04
age	2	-84.52	173.50	3.63	0.03
age+chick +sex	4	-81.93	173.52	3.65	0.03
age+bm+chick age	4	-82.05	173.78	3.90	0.03
age+chick +sex+age:chick	5	-80.68	173.96	4.09	0.03
bm+chick +sex	4	-82.17	174.00	4.12	0.02
bm	2	-84.79	174.03	4.16	0.02
bmi+chick +bm:chick	4	-82.20	174.07	4.20	0.02
age+bm+chick +age:chick	5	-80.82	174.25	4.37	0.02
bm+sex	3	-83.80	174.55	4.68	0.02
age+bm+chick +age:bm	5	-81.09	174.79	4.92	0.02
age+sex	3	-83.94	174.83	4.96	0.02
bm+chick+sex+bm:sex	5	-81.26	175.13	5.26	0.01
age+chickA+sex+chick:sex	5	-81.40	175.40	5.53	0.01
bm+chick+sex+chick:sex	5	-81.47	175.54	5.67	0.01

Annexe 3: Results from the model selection (with a ΔAICc < 6) on the variable "total distance" using GLMs with a Poisson family. Chick= age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	AICc	ΔAICc	w_i
age+chick+age:chick	4	-95.74	201.15	0.00	0.30
chick	2	-99.10	202.66	1.50	0.14
bm+chick	3	-98.51	203.99	2.83	0.07
age+chick+sex+age:chick	5	-95.73	204.08	2.92	0.07
age+bm+chick+age:chick	5	-95.74	204.09	2.94	0.07
(Null)	1	-101.11	204.36	3.21	0.06
age+chick	3	-98.74	204.43	3.28	0.06
chick+sex	3	-99.00	204.96	3.80	0.05
age	2	-100.83	206.12	4.96	0.03
age+bm+chick	4	-98.26	206.18	5.03	0.02
sex	2	-100.94	206.33	5.18	0.02
bm+chick+sex	4	-98.47	206.61	5.45	0.02
bm	2	-101.11	206.68	5.52	0.02
bm+chick +bm:chick	4	-98.51	206.68	5.53	0.02
age+chick age+sex	4	-98.66	206.98	5.83	0.02
age+bm+chick age+age:chick age+bm:chick	6	-95.60	207.01	5.86	0.02
age+bm+chick age:bm+age:chick age	6	-95.63	207.09	5.93	0.02

Annexe 4: Results from the model selection (with a ΔQAICc < 6) on the variable "vertical distance" using GLMs with a Quasi-poisson family. Chick= age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	QAICc	ΔQAICc	w_i
chick	2	-86.85	103.36	0.00	0.16
(Null)	1	-89.22	103.49	0.13	0.15
sex	2	-87.30	103.85	0.49	0.13
chick+sex	3	-85.55	104.62	1.26	0.09
bm+sex	3	-86.49	105.66	2.30	0.05
bm+chick	3	-86.56	105.74	2.38	0.05
age	2	-89.01	105.75	2.39	0.05
age+chickA	3	-86.62	105.80	2.44	0.05
bm	2	-89.22	105.98	2.62	0.04
chick+sex+chick:sex	4	-84.38	106.26	2.90	0.04
age+sex	3	-87.11	106.35	2.99	0.04
bm+chick+bmi:chick	4	-85.13	107.09	3.73	0.03
age+chick+sex	4	-85.32	107.30	3.94	0.02
bm+chick+sex	4	-85.51	107.52	4.16	0.02
bm+sex+bm:sex	4	-86.15	108.23	4.87	0.01
age+bm+sex	4	-86.23	108.31	4.95	0.01
age+chick+age:chick	4	-86.27	108.35	5.00	0.01
age+bm	3	-88.99	108.44	5.08	0.01
age+bm+chick	4	-86.35	108.45	5.09	0.01
age+sex+age:sex	4	-87.04	109.21	5.85	0.01

Annexe 5: Results from the model selection (with a ΔAICc < 6) on the variable "number of dives" using GLMs with a Poisson family. Chick= age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	AICc	ΔAICc	w_i
sex	3	-182.82	372.60	0.00	0.21
(Null)	2	-184.25	372.96	0.36	0.18
bm	3	-183.41	373.78	1.18	0.12
bm+sex	4	-182.67	375.01	2.41	0.06
age+sex	4	-182.81	375.29	2.70	0.05
chick+sex	4	-182.82	375.30	2.70	0.05
chick	3	-184.24	375.45	2.85	0.05
age	3	-184.25	375.45	2.85	0.05
bm+chick	4	-183.11	375.89	3.29	0.04
bm+chick+bm:chick	5	-181.73	376.07	3.48	0.04
age+bm	4	-183.40	376.46	3.87	0.03
chick+sex+chick:sex	5	-181.94	376.49	3.89	0.03
bm+sex+bm:sex	5	-182.60	377.82	5.22	0.02
bm+chick+sex	5	-182.63	377.87	5.27	0.02
age+bm+sex	5	-182.66	377.93	5.33	0.01
age+sex+age:sex	5	-182.72	378.04	5.44	0.01
age+chick	4	-184.24	378.15	5.55	0.01
age+chick+sex	5	-182.81	378.23	5.63	0.01

Annexe 6: Results from the model selection (with a ΔQAICc < 6) on the variable “proportion of time spent underwater” using GLMs with a Quasi-binomial family. Chick=age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	QAICc	ΔQAICc	w_i
chick	2	-114.92	71.47	0.00	0.15
sex	2	-115.48	71.79	0.31	0.13
(Null)	1	-120.01	71.83	0.36	0.13
chick+sex	3	-111.70	72.37	0.90	0.10
bm+sex	3	-113.71	73.50	2.03	0.05
age+chick	3	-114.06	73.70	2.22	0.05
bm+sex	3	-114.17	73.76	2.29	0.05
age	2	-119.15	73.85	2.38	0.05
age+sex	3	-114.71	74.06	2.59	0.04
chick+sex+chick:sex	4	-109.78	74.23	2.76	0.04
bm	2	-120.00	74.33	2.86	0.04
age+chick+sex	4	-110.90	74.87	3.39	0.03
bm+chick+bm:chick	4	-111.04	74.95	3.47	0.03
bm+chick+sex	4	-111.61	75.27	3.79	0.02
bm+sex+bm:sex	4	-112.68	75.86	4.39	0.02
age+bm+sex	4	-112.72	75.89	4.42	0.02
age+bm+chick	4	-113.43	76.29	4.82	0.01
age+chick+age:chick	4	-113.84	76.51	5.04	0.01
age+bm	3	-119.12	76.54	5.07	0.01
age+sex+age:sex	4	-114.48	76.87	5.40	0.01
age+chick+sex+age:sex	5	-109.57	77.33	5.86	0.01
age+sex+chick+chick:sex	5	-109.58	77.33	5.86	0.01
bm+chick+sex+chick:sex	5	-109.66	77.38	5.91	0.01
bm+chick+sex+bmi:chick	5	-109.81	77.46	5.99	0.01

Annexe 7: Results from the model selection (with a ΔQAICc < 6) on the variable "hunting efficiency" using GLMs with a Quasi-binomial family. Chick=age of the chick, age=age of the adults, bm= body mass of the adults, sex=sex of the adults.

Models	K	logLik	QAICc	ΔQAICc	w_i
(Null)	1	-110.15	78.87	0.00	0.30
Chick	2	-109.21	80.73	1.86	0.12
sex	2	-109.38	80.85	1.98	0.11
bm	2	-109.79	81.12	2.25	0.10
age	2	-109.91	81.21	2.34	0.09
chick+sex+chick:sex	4	-103.60	82.59	3.72	0.05
bm+chick	3	-108.06	82.66	3.80	0.05
chick+sex	3	-108.68	83.08	4.21	0.04
age+chick	3	-108.97	83.28	4.41	0.03
age+sex	3	-109.13	83.38	4.52	0.03
bm+sex	3	-109.35	83.53	4.66	0.03
age+bm	3	-109.50	83.63	4.77	0.03
bm+chick+bm:chick	4	-106.66	84.65	5.79	0.02

ANNEXE 8

Article E

Fractal time in foraging sequences of a diving seabird

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Temporal fractals in seabird foraging behaviour: diving through the scales of time

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Animal behaviour exhibits fractal structure in space and time. Fractal properties in animal space-use have been explored extensively under the Lévy flight foraging hypothesis, but studies of behaviour change itself through time are rarer, have typically used shorter sequences generated in the laboratory, and generally lack critical assessment of their results. We thus performed an in-depth analysis of fractal time in binary dive sequences collected via bio-logging from free-ranging little penguins (*Eudyptula minor*) across full-day foraging trips (2^{16} data points; 4 orders of temporal magnitude). Results from 4 fractal methods show that dive sequences are long-range dependent and persistent across ca. 2 orders of magnitude. This fractal structure correlated with trip length and time spent underwater, but individual traits had little effect. Fractal time is a fundamental characteristic of penguin foraging behaviour, and its investigation is thus a promising avenue for research on interactions between animals and their environments.

Fractal structure characterizes a diverse array of natural systems, from coastlines, DNA sequences, and cardio-pulmonary organs, to temporal fluctuations in temperature, heart rate, and respiration[1–11]. Spatial and temporal patterns of animal behaviour have also been described as fractal, exhibiting self-similarity or self-affinity across a range of measurement scales. For example, fractal movements (a.k.a. Lévy walks) are super-diffusive and thus theoretically adaptive in heterogeneous and unpredictable environments where they can enhance the probability of resource encounters over Brownian (random) movements (Lévy Flight Foraging Hypothesis)[12–15]. In the temporal domain, various physiological impairments or other challenges can lead to complexity loss in behavioural sequences, i.e. increased periodicity or stereotypy[16–21]. The latter is congruent with studies of altered physiology in stress and disease in humans, which have underpinned the hypothesis that fractal structure is adaptive because it is more tolerant to variability extrinsic to the biological or physiological system producing it[4,6,11,22,23]. Fractal analysis can thus help us understand the structure and function of animal behaviour.

However, while exploring fractal properties in spatiotemporal data is currently a hot topic in the movement ecology literature, less attention has been paid to strictly temporal fluctuations in behaviour, despite that the first studies of fractal time appeared nearly two decades ago[19,24–26] and that temporal complexity has been linked to individual quality or health (see above). There are two main obstacles to assessing fractal time in behaviour sequences. First, generating sufficiently long time series to perform meaningful analyses is no easy task because accurately recording behaviours continuously is difficult, particularly under natural conditions; all but 3 studies of fractal time were experimental[16,17,27]. There is debate about whether fractal analyses apply to shorter sequences because scaling is theoretically asymptotic[28–31], and while the methods used may be sensitive to long-range dependence they may not always be specific, i.e. one can always find a higher order short-range correlated model to describe apparently fractal patterns[32,33]. Furthermore, irrespective of sequence length, single values produced by fractal analysis to characterize observed sequences by their long-range correlative properties (i.e. scaling exponents) may not represent the entire range of measurement scales examined; scaling exponents may be scale-dependent rather than scale-independent as theoretically predicted[34,35]. While scale-dependency can undoubtedly provide useful information about animal responses to salient features at various scales[36–39], multiple scaling regions means that single exponents cannot accurately characterize their behaviour. Alternatively, log-log plots of fluctuation as a function of scale, upon which calculation of scaling exponents is typically based, may appear linear even in the absence of scaling[35,40,41]. Unfortunately, few – if any – studies of fractal time in animal behaviour have critically addressed these issues *sensu*[34,35], leaving questions about the robustness of their results.

In this study, we address these issues by applying fractal analysis to binary sequences of foraging behaviour (i.e. diving and the gaps between successive dives) collected via bio-logging from a marine predator. Bio-logging can be described as the use of animal-attached devices to investigate "phenomena in or around free-ranging organisms that are beyond the boundary of our visibility or experience"[42]. This approach is indispensable for monitoring behaviours of animals that cannot be systematically observed because accurate records of various behavioural parameters can be attained at fine time scales over long periods[43,44]. In addition to increasing the robustness of fractal results, such lengthy sequences allow us to better assess the fit of the regression line in the double logarithmic plot and thereby test for its accuracy and the potential for multiple scaling regions. In one of the first investigations of fractal time, the authors note that identifying fractal scaling in the behaviour of their study subjects (*Drosophila melanogaster*) was only possible following the development of technology capable of accurately recording behaviour at previously unavailable resolutions (i.e. 0.1 s in this case)[25]. Bio-logging technology offers similar advantages for the study of fractal properties in temporal sequences of wild animal behaviour, and we expect this merger of techniques to yield valuable information about general qualitative properties in sequences of animal behaviour *in situ*.

We were able to use behaviour sequences of little penguins (*Eudyptula minor*) spanning complete foraging trips, ca. 50,000 data points at 1 second sampling intervals ($2^{15} \sim 2^{16}$ points across ca. 15 hours); among the longest continuous binary sequences of animal behaviour that have been used in studies of fractal time. Such waveform behaviour sequences can mitigate some issues concerning sequence length because data can be recorded at very fine resolutions (e.g. <1 s)[18,25,45]. Previous studies using this approach have examined behavioural sequences with 2^{11} or 2^{12} total data points[16,17,27,46,47], but total observation periods have typically remained in the range of ca. 30–60 minutes, i.e. 2048–4096 data points, with few exceptions[27]. Short sequences such as these can be problematic under natural conditions because animal activity patterns tend to occur in rhythms with strong temporal variation in behavioural performance. Context-specific (e.g. within bout) analyses of complexity can provide useful information[16], but they do not allow us to assess correlational properties at larger time scales incorporating multiple bouts and modes of behaviour.

We employed 4 fractal analytical methods to avoid potentially misleading results that can occur when relying on any single method[48,49], including Detrended Fluctuation Analysis (DFA; both linear- and bridge-detrended versions), the Hurst Absolute Value method, and the Box-counting method to determine whether temporal sequences of penguin behaviour are consistent with patterns expected if they were generated by a long-memory process characterized by scaling. We examine whether a single scaling exponent can characterize entire foraging sequences, whether scaling is restricted to a certain range of scales within these sequences, or whether multiple scaling regions must be considered. We then use the scaling exponents generated to test whether general differences exist in relation to individual traits (age, sex, chick age, and body mass) and whether the various methods produce consistent results across individuals. Finally, we compare these results with those generated by more traditional, frequency-based approaches commonly used to quantify marine animal foraging behaviour.

Results

Frequency-based dive parameters. During the study period, little penguin foraging trips lasted for a mean ± s.d. of 14.8 ± 0.9 hours (range: 12.1–16.9). Within each foraging trip, penguins spent 35.4 ± 10.6% of the time underwater (range: 21.3–55.4). Individual dives within the sequence lasted for a mean ± s.d. of 29.8 ± 6.3 seconds (mean range: 20.0–39.9), with mean dive depths of 12.3 ± 3.0 meters below the surface (mean range: 4.9–16.8). Correlations between these dive parameters are shown in Fig. 1a.

Scaling exponents. All fractal measures point to the existence of temporal scaling in observed sequences of penguin foraging behaviour. The mean ± s.d. scaling exponents were: $\alpha_{DFA} = 0.88 \pm 0.06$; $\alpha_{DFAb} = 1.89 \pm 0.05$; $H_{AV} = 0.80 \pm 0.06$; $D_b = 1.10 \pm 0.07$. Examination of α_{DFA} shows that the original binary sequences (example shown in Fig. 2a) were characteristic of fractional Gaussian noise (fGn: $\alpha_{DFA} \in (0,1)$), which was confirmed by the fact that the integrated sequences (examples shown in Fig. 2b) measured via DFA$_b$ produced $\alpha_{DFAb} \in (1,2)$, characteristic of fractional Brownian motion (fBm). Furthermore, our estimates of the Hurst exponent H using α_{DFA} and α_{DFAb} are in agreement with the expected theoretical relationships ($\alpha_{fGn} \approx \alpha_{fBm-1}$), and the Pearson correlation coefficient of 0.93 for values of α_{DFA} and α_{DFAb} further confirms their compatibility (Fig. 1b). Agreement between other measures was fair, ranging between absolute values of $|0.66|$ and $|0.89|$ for all other combinations. Negative correlations involving D_b were predicted by the inverse relationship expected between Hurst and fractal dimension estimates. Finally, that $0.5 < H < 1$ for all estimates of H clearly suggests that little penguin foraging sequences are characterized by persistent long-range dependence (positive autocorrelation); i.e. behavioural patterns tend to persist across long time frames and scale accordingly, although they did not persist across all scales examined (see below). Note that all scaling exponents presented above were calculated using the best scaling region which is derived in the next section.

Validation of scaling regions. A closer examination of the log-log plot of $F(n)$ versus n in DFA shows that scaling does not persist across all scales examined (Fig. 3). The R^2 – SSR procedure demonstrates that the best scaling region lies between $2^7 \sim 2^{12}$, ca. 128 ~ 4096 s or 2.1 ~ 68.3 min (Fig. 3A, B). However, the compensated slope procedure places values at the 2 largest scales within the range of variation expected given some element of noise (Fig. 3C), and thus scaling may persist to 2^{14}, 16384 s or 273.1 min, spanning more than 2 orders of magnitude; i.e. a similar correlation structure is found at all of these measurement scales. To be conservative, we calculated scaling exponents using only the range of scales included in the best scaling region by both methods, i.e. $2^7 \sim 2^{12}$. If on the other hand we relied only on R^2 values as many previous studies have done, we might have included all scales in this region given that all values were greater than 0.997 in DFA across sequences using all scales examined (Fig. 3), and given the similar mean values of α_{DFA} using the best and full range of scales (0.877 and 0.865, respectively).

Increasing the sampling resolution from 1 s to a maximum of 30 s did not significantly alter resultant αDFA values, despite that total sequence lengths decreased from a mean of 54000 data points to ca. 10800, 5400, 2700, and 1800 for 5, 10, 20 and 30 s intervals, respectively. Values of αDFA were 0.88 ± 0.06, 0.88 ± 0.06, 0.87 ± 0.07 and 0.84 ± 0.08 when using the best scaling regions from each set of sequences, respectively. Pearson correlation coefficients for comparisons between these and values from the 1 s interval sequences were 0.88, 0.86, 0.84 and 0.87. There was also considerable overlap in their best scaling regions. However, while scaling was found to begin at ca. 2 min when using the higher-resolution 1 s sequences, the lower-bound limits of the scaling region were higher in all of these lower-resolution sequences (range: ca. 4–5 min). Conversely, the R^2 – SSR procedure included slightly larger upper-bound limits for the 5, 10 and 20 s interval sequences, extending to ca. 85 min in each case (respectively 1024, 512 and 256 data points) as opposed to the ca. 68 min scaling limit (4096 data points) for 1 s intervals. Perhaps

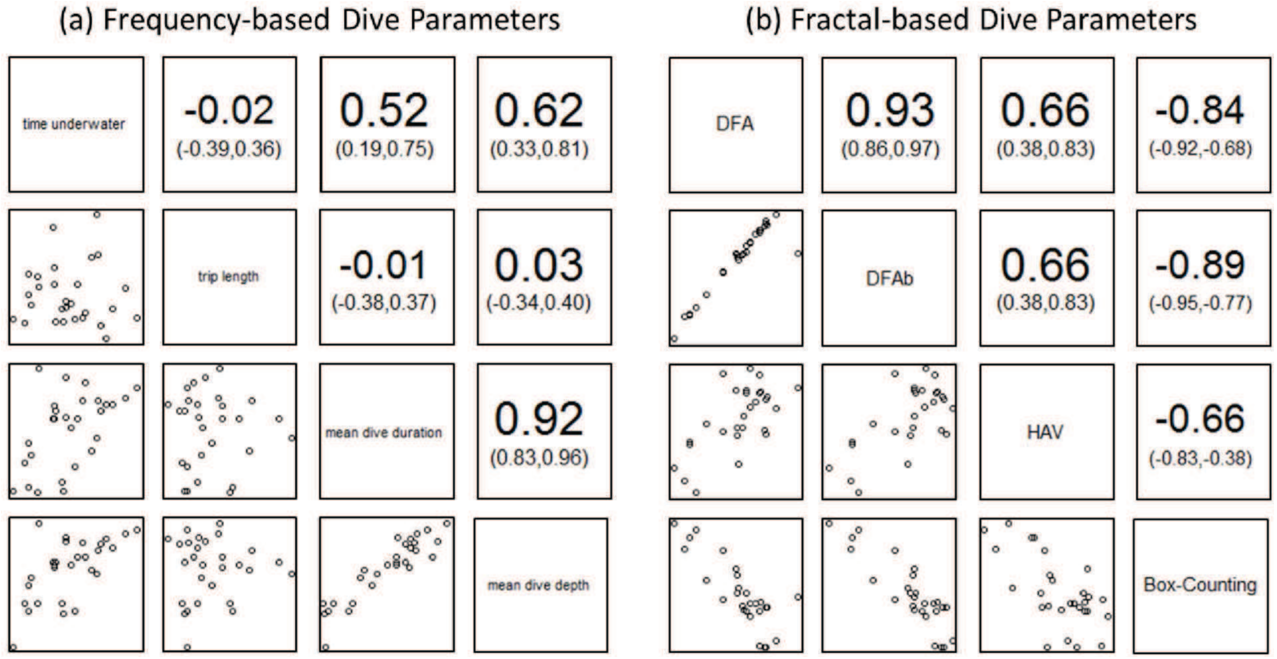

Figure 1 | Correlations between diving parameters for both (a) frequency-based and (b) fractal measures. Lower-left panels show correlation scatterplots while upper-right panels give Pearson's correlation coefficients along with their respective confidence intervals. Measurement types are shown diagonally between these panel blocks.

because of the considerably shorter sequence lengths, scaling regions in the 30 s interval sequences capped at ca. 64 min (128 data points), as did the 1 s interval sequences. Like the original results, the compensated-slope procedure applied to these sequences also included all of the largest scales in the best scaling region, pushing the potential upper-bound limit of the scaling region to over 340 min from the 273 min estimated above.

Variation in scaling exponents and frequency-based dive parameters. Individual differences between study subjects could not explain any significant portions of the variation in either scaling exponents (Table 1) or summary statistics (Table 2) from observed foraging trips, with one exception: initial body mass was positively associated with scaling exponents generated by DFA_b. Because α_{DFAb} is inversely related to fractal dimension, this result suggests that birds with greater body mass at the beginning of the foraging trip performed less temporally complex dive sequences than did initially lighter birds. However, none of the three other fractal measures produced similarly significant relationships between these variables, although the effect size was indeed largest for body mass in all cases. Therefore, while the relationship between body mass and complexity must remain equivocal until further data can be examined, we observed fair agreement across measures in the effects of these four variables on complexity.

We observed a number of associations between frequency-based dive parameters and scaling exponents (Table 3). First, total time spent underwater had a significant positive effect on exponents measured by DFA and DFA_b, and the inverse effect on the box-counting dimension; i.e. complexity increased with dive time in all three cases. Given that time spent underwater ranged from ca. 21–55% across foraging trips, these results mimic those from the simulated sequences in which α_{DFA} increased as the probability function diverged from 50–50 (see Supplentary Table S1). However, since randomized surrogate sequences analysed by DFA_b and box-counting all produced $H = 0.5$ (see Supplentary Information), the effect of total time spent underwater on these scaling exponents cannot be explained completely by such altered distributional characteristics. Second, trip durations were positively associated with both DFA_b and H_{AV}; i.e. complexity decreased with sequence length using these indices. Finally, we found a negative association between mean dive duration and α_{DFAb}, but no relationship between this summary statistic and other fractal measures. Similarly, mean dive depth, which was highly correlated with mean dive duration (0.9 in all model correlation matrices), was unrelated to observed scaling exponents. Note that despite the strong positive correlations between these two variables, we found no evidence for excessive variance inflation ($<$10 in all cases) and have therefore left all terms in the statistical models. The weight of evidence therefore suggests that dive sequence complexity may well be associated with total time spent underwater and foraging trip duration, but is generally independent of both mean dive duration and depth.

Discussion

We demonstrated that binary sequences of little penguin foraging behaviour resemble patterns expected if they were produced by a long memory process characterized by temporal scaling; binary sequences were consistent with fractional Gaussian noise (fGn: $0 < H < 1$) while integrated sequences were consistent with fractional Brownian motion (fBm: $1 < H < 2$). Scaling exponents of all analyses fit their expected theoretical relationships, fell in the range $0.79 < H < 0.9$, and therefore suggest strong persistence in dive sequences. In other words, any given region within a dive sequence is dependent upon patterns that occurred much earlier in the sequence, more so than would be expected of stochastic or even short-range dependent sequences. The persistence of the autocorrelation means that long diving events tend to be followed by long diving events, and vice versa. Upon closer examination of the local slopes produced by the log-log plot of fluctuation as a function of scale using DFA, the data indicate that the best-scaling region ranged from windows of 2^7 (2 min) $\sim 2^{12}$ (68 min) or 2^{14} (273 min), which in the latter case would constitute temporal scaling across more than 2 orders of magnitude, a rarity among behavioural studies. Within this range of scales, therefore, there is no single scale at which dive sequences can be fundamentally measured or distinguished.

In this way, our results correspond well with the many recent studies that have demonstrated fractal patterns in the Lévy-like movement paths of various marine animals. This indicates a search

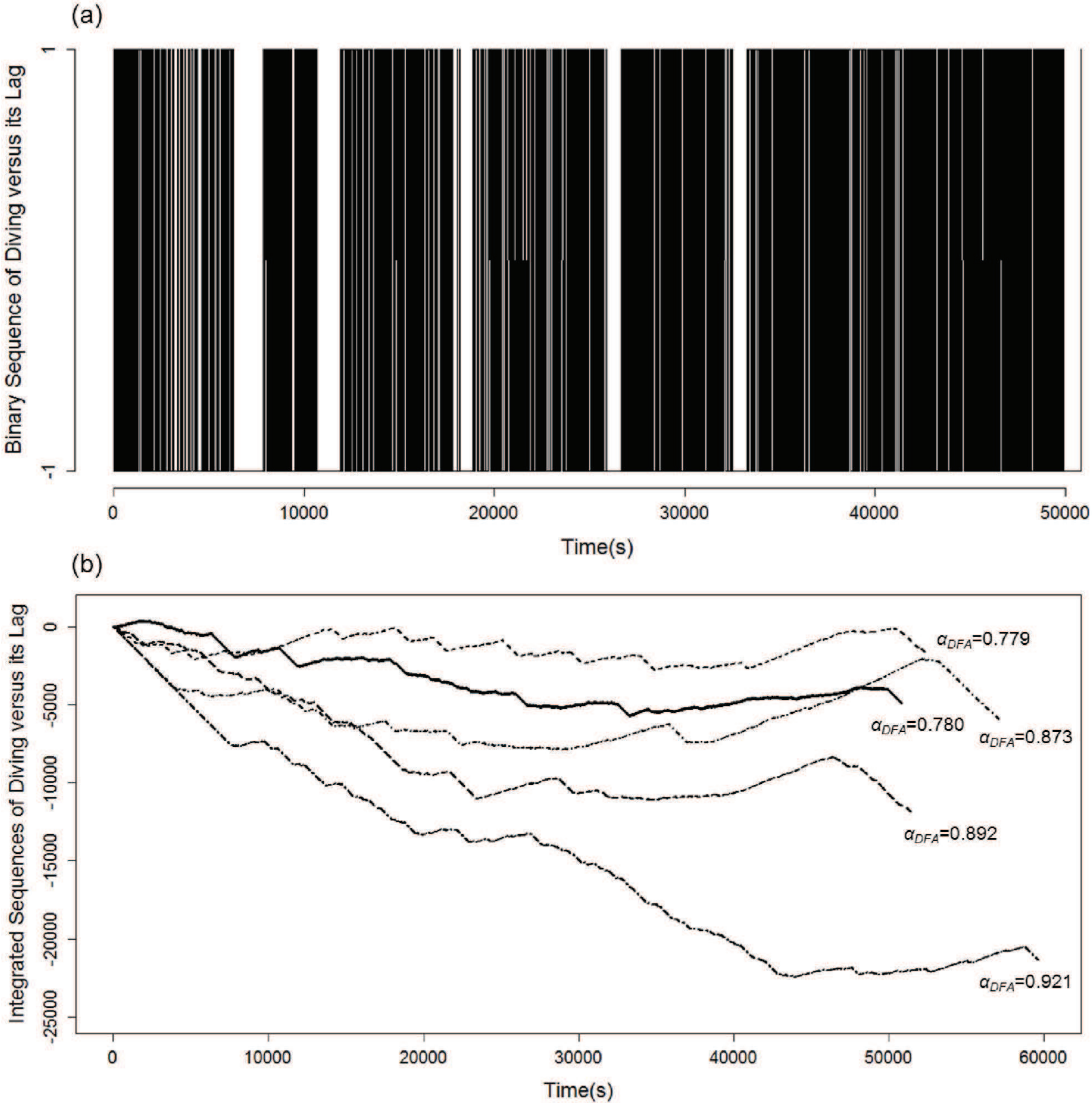

Figure 2 | Example of (a) a single little penguin female's binary foraging sequence denoted 1 for diving and −1 for lags between successive dives and (b) integrated (cumulatively summed) dive sequences from 5 different little penguin females showing variation in foraging patterns and resultant changes in α_{DFA} values. The bold solid line indicates the integrated dive sequence corresponding to the binary sequence shown above.

strategy which approximates theoretically optimal behaviour, allowing an organism to maximize its encounter rates with resources under heterogeneous conditions[13–15,50,51]. Thus we return full circle to the original studies of fractal time in animal behaviour which first demonstrated the link between resource distribution and temporal complexity[25,26]. Further investigation in the temporal domain is now warranted on at least two key accounts. First, studies such as ours, while admittedly ignoring the spatial location of an organism, focus on behavioural performance itself, in this case the sequential distribution of diving inclusive of changes in behavioural state, which may better address actual prey search and pursuit than does simple spatial data. In fact, fractal patterns in the distribution of any relevant behaviour through time can be investigated using this approach, and to date, studies have investigated not only foraging and movement but also vigilance, posture, and even reproductive and social behaviours[16–18,20,27,52]. The fact that animals do engage in multiple behaviours may make simple interpretation of sequential patterns in any given behaviour problematic. In this respect, seabird foraging behaviour provides a particularly useful model system for studies of fractal time, with switches between periods that crudely consist of prey search/pursuit and surface recovery[53,54]; two clearly contrasting and mutually exclusive behaviours *sensu*[55] and characteristic of behavioural intermittence[56]. Still, for marine birds and pinnipeds, temporal patterns of behaviour must arise not only as the outcome of prey encounters and distributions, but also of physiological limitations related to respiration[57]. Scaling exponents therefore represent behavioural complexity in a global rather than specific sense, resulting from multiple interacting variables, which would be expected of any complex adaptive system.

Second, there is a growing body of evidence suggesting that various stressors can lead to loss of complexity in behaviour sequences, i.e. the progression of behaviour through time becomes more

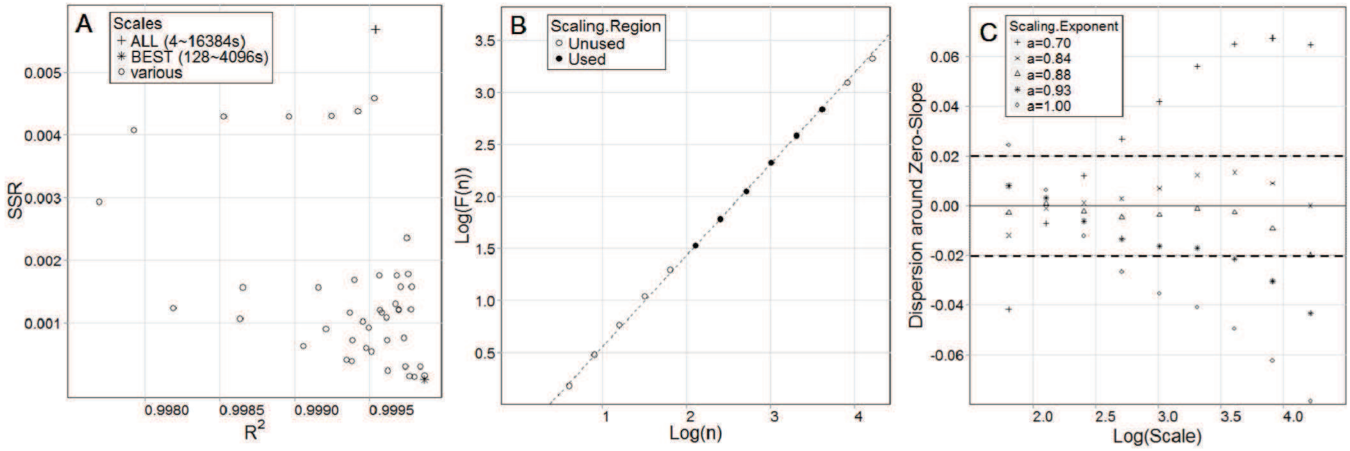

Figure 3 | Validation of scaling regions in sequences of diving behaviour from little penguins. (A) The R^2 – SSR procedure determines the values of log(scale) that maximize the coefficient of determination and minimize the sum of squared residuals (*), corresponding to the range of scales across which the data reflect strong scaling behaviour (filled circles shown in (B)). Note that when all scales are used (+) the coefficient of determination remains comparable to that of the best scaling region, indeed all regression fits produced R^2 values greater than 0.997, but the sum of squared residuals increases dramatically. In this case, the estimates of α_{DFA} for the best scaling region and the full range of scales are also comparable at 0.877 and 0.865, respectively. (C) The compensated slope procedure allows testing the effect that varying the scaling exponent has on dispersion around a ''zero-slope'' (solid line), the point at which the scaling exponent is a true representation of the sequence. The scaling exponent derived from the best scaling region produces values that best approximate a zero-slope (Δ), with all points examined falling within the 95% confidence intervals (dotted lines) generated by 1000 simulations of random variation around a zero-slope. Therefore, these observed sequences do exhibit fractal structure with power-law scaling behaviour, i.e. strong linearity in the log-log plot of fluctuation as a function of scale, at least across the scales outlined in (B).

periodic or stereotypical. This also suggests that scaling exponents can be used to characterize some aspect of individual or environmental quality. A number of studies have tested this hypothesis, showing that impairments or challenges ranging from parasitic infection through toxic substance exposure to increased exposure to anthropogenic disturbance[16–21,27,58] are all associated with such complexity loss[16–19,21,58]. This may have major implications concerning the viability of individuals operating in a sub-optimal state. It will be interesting to determine whether similar examples of complexity loss can be demonstrated using spatial data collected from challenged individuals. Although the animal movement ecology literature on statistical patterns of search is growing with reference to optimality and response to environmental cues, little attention has been paid to intrinsic factors that might cause variation in scaling across individuals under the same ecological conditions[55]. Indeed, there seems to be a divide in the current literature in which spatial patterns (animal locations through time) and temporal patterns (behavioural changes through time) are generally discussed in relation to extrinsic (e.g. landscape variables) and intrinsic (e.g. health states) control mechanisms, respectively. Integration of these two domains and the framework through which their results are interpreted should therefore be a goal of future research, to further our understanding of how animals respond to scales in both time and space, and to investigate whether complexity loss is a feature of both.

Table 1 | Results of linear mixed-effects models examining influence of individual traits on variation in scaling exponents from little penguin foraging sequences

Model	Predictor	est.	s.e.m.	df	*t*	Pr(>|*t*|)
DFA	(Intercept)	0.652	0.168	13	3.884	0.002
	Age	−0.001	0.002	10	−0.604	0.560
	Sex (male)	−0.046	0.027	10	−1.677	0.125
	BM	0.0002	0.0001	10	1.750	0.111
	Chick Age	−0.003	0.003	10	−0.829	0.426
$\mathrm{DFA_b}$	(Intercept)	1.611	0.145	13	11.124	0.000
	Age	0.000	0.002	10	−0.188	0.855
	Sex (male)	−0.040	0.023	10	−1.730	0.114
	BM	0.0003	0.0001	10	2.238	0.049
	Chick Age	−0.001	0.003	10	−0.442	0.668
$\mathrm{H_{AV}}$	(Intercept)	0.530	0.171	13	3.100	0.008
	Age	0.0001	0.002	10	0.026	0.979
	Sex (male)	−0.037	0.028	10	−1.302	0.222
	BM	0.0002	0.0001	10	1.588	0.143
	Chick Age	−0.002	0.003	10	−0.516	0.617
Box Count	(Intercept)	1.354	0.163	13	8.320	0.000
	Age	−0.001	0.002	10	−0.374	0.716
	Sex (male)	0.030	0.027	10	1.120	0.289
	BM	−0.0002	0.0001	10	−1.615	0.137
	Chick Age	0.002	0.003	10	0.743	0.475

BM refers to initial body mass at time of logger deployment.

Table 2 | Results of linear mixed-effects models examining influence of individual traits on variation in frequency-based dive parameters from little penguin foraging sequences

Model	Predictor	est.	s.e.m.	df	*t*	$\Pr(>\lvert t\rvert)$
Dive Trip Duration	(Intercept)	52915.520	7613.679	13	6.950	0.000
	Age	46.980	91.300	10	0.515	0.618
	Sex (male)	−980.990	1264.637	10	−0.776	0.456
	BM	1.400	6.408	10	0.219	0.831
	Chick Age	−99.560	138.918	10	−0.717	0.490
Dive Duration	(Intercept)	36.188	18.474	13	1.959	0.072
	Age	0.195	0.227	10	0.861	0.409
	Sex (male)	0.793	2.962	10	0.268	0.794
	BM	−0.009	0.015	10	−0.592	0.567
	Chick Age	0.249	0.334	10	0.746	0.473
Dive Depth	(Intercept)	16.067	9.085	13	1.768	0.100
	Age	0.022	0.111	10	0.202	0.844
	Sex (male)	0.618	1.459	10	0.424	0.681
	BM	−0.005	0.008	10	−0.651	0.530
	Chick Age	0.184	0.164	10	1.124	0.287
Underwater Time	(Intercept)	0.482	0.228	13	2.111	0.055
	Age	−0.001	0.003	10	−0.348	0.735
	Sex (male)	0.054	0.038	10	1.413	0.188
	BM	−0.0002	0.0002	10	−0.783	0.452
	Chick Age	0.005	0.004	10	1.244	0.242

BM refers to initial body mass at time of logger deployment.

A key result of our study, however, is that scaling did not persist across all scales examined, a common limitation of using this approach to characterize complete sequences. Indeed, this has been a major criticism of using fractal analysis in studies of animal behaviour in the past[59,60], although this criticism has been rebutted persuasively[34], largely because previous studies had not critically assessed the data upon which their scaling exponents were based. In the present study, the lack of clear scaling at smaller scales likely reflects a combination of: (1) the influence of the mean individual dive durations, which were larger than most of these smaller scales at 29.4 ± 20.6 s; (2) decay of the strong short term autocorrelation; and, (3) mathematical error when small numbers of data points are used in regression analyses. At the largest scales, it is impossible to determine whether the bias in scaling is due to its absence or simply the paucity of available windows, i.e. an artefact of finite sequence length. This can only be answered by collecting sequences of greater length, which should continue to be a goal in future studies. What is promising, however, is that changing the resolution of the data did not lead to significant changes in the fractal properties of observed sequences, although our results do suggest that higher- and lower-resolution sequences may be better at detecting the presence of scaling at small and large scales, respectively.

In addition to these measurement-related issues, there may be biological reasons to expect changes in the correlation structure of foraging sequences at certain scales. This may in part reflect certain habitat characteristics and how animals interact with their environments at different scales. For example, the tortuosity of foraging paths in wandering albatross (*Diomedea exulans*) differs across three scaling regions: patterns at the smallest scales (~100 m) reflect adjustment to wind currents, at medium scales (1–10 km) food-search behaviour, and at the largest scales (>10 km) long-distance movement between patches and change in local weather

Table 3 | Results of linear mixed-effects models examining relationship between scaling exponents and frequency-based dive parameters from little penguin foraging sequences

Model	Predictor	est.	s.e.m.	df	*t*	$\Pr(>\lvert t\rvert)$
DFA	(Intercept)	0.796	0.156	13	5.093	0.000
	Trip Duration	5.2E-06	2.8E-06	10	1.865	0.092
	Underwater Time	−0.396	0.111	10	−3.568	0.005
	Dive Duration	0.003	0.003	10	0.830	0.426
	Dive Depth	−0.010	0.007	10	−1.482	0.169
DFA_b	(Intercept)	1.810	0.104	13	17.480	0.000
	Trip Duration	5.0E-06	1.8E-06	10	2.716	0.022
	Underwater Time	−0.357	0.073	10	−4.921	0.001
	Dive Duration	0.003	0.002	10	1.250	0.240
	Dive Depth	−0.011	0.005	10	−2.331	0.042
H_{AV}	(Intercept)	0.299	0.200	13	1.491	0.160
	Trip Duration	1.1E-05	3.6E-06	10	2.994	0.014
	Underwater Time	0.016	0.145	10	0.109	0.915
	Dive Duration	0.002	0.004	10	0.486	0.638
	Dive Depth	−0.014	0.009	10	−1.597	0.141
Box Count	(Intercept)	1.099	0.125	13	8.787	0.000
	Trip Duration	3.9E-06	2.3E-06	10	−1.736	0.113
	Underwater Time	0.471	0.095	10	4.974	0.001
	Dive Duration	3.0E-04	2.4E-03	10	0.126	0.902
	Dive Depth	0.004	0.005	10	0.821	0.431

conditions[61]. For central place foragers like the penguins studied here, travel between foraging sites and the colony, which can constitute a considerable portion of the total sequence length (trip duration), may lead to scaling breaks at large scales. However, this cannot explain the deviation from scaling we observed at large scales because our sequences began only when the first instances of diving were observed, eliminating any effects of such movement types. Landscape heterogeneity can also affect scaling in animal movements, such as in American martens (*Martes americana*) where movement paths are determined by microhabitat features at small ($<$3.5 meters) but not larger scales[38], and in grazing ewes where such features affected movements at scales greater than a threshold value (5 meters)[39]. While addressing variation in microhabitat structure is beyond the scope of the current study, prey locations are likely to have contributed strongly to deviations from scaling at small scales. This would be compounded in seabirds by the necessary pauses in foraging as animals return to the surface to breathe[57]. Strong short-term autocorrelation can result from bouts in which animals dive to similar depths in pursuit of prey within a patch and then surface to replenish oxygen reserves before repeating the process[62].

In addition to the distinction between real and perceived scaling breaks, another difficulty in inferring fractal structure is that it is generally always possible to find a short-range correlated model of higher order and complexity to fit any sequence with finite length[33]; i.e. all real world data. For example, DFA failed to distinguish between a real long-range dependent process and a short-range one generated by the super-position of three first-order autoregressive processes[32]. In real-world data, simple autoregressive models have been used to predict the correlation structure of sequential dive depths in macaroni penguins (*Eudyptes chrysolophus*) with some precision[63]. However, other recent evidence also using successive measurements of dive depths strongly suggests that such sequences are rather consistent with long memory processes in most cases[15,50,51]. Indeed, short-range correlations cannot adequately model many biological and physical phenomena found in nature, which is why more parsimonious models of long-range dependence were developed[33]. Our study is among the first to examine binary time series of animal behaviour with lengths up to 2^{16}, sequences spanning 4 orders of magnitude and thus nearing and in some cases even exceeding those used in many simulation studies. Therefore, our results offer compelling support for long-range fractal structure in penguin dive sequences.

Ultimately, while describing the scaling exponents of behavioural sequences accurately is a fundamental component of this research, what may be of more interest to many researchers is the next step; the ability to apply such quantifiable properties in distinguishing between the behaviours of various groups of individuals or taxa. In this regard, our study did not show any clear differences in the complexity signatures of individuals in relation to age, sex, or chick age, and produced only weak evidence for an effect of initial body mass. Similarly, these variables also did not affect any of the summary statistics measured. There is considerable variation across studies in the impacts of such biological factors on seabird behaviour[64–67], so it remains difficult to make any strong inferences based on as small a data set as that used here. However, it is clear that certain aspects of foraging behaviour such as time spent underwater and trip duration can be correlated with dive sequence complexity. Our simulated data show that DFA can be sensitive to variation in the probability distribution of dives, but further analysis of the scaling region easily distinguished between simulated and observed behaviour (see Supplementary Information). Furthermore, reshuffling the dives produced random sequences in 3 of our 4 fractal methods, all of which correlated well with DFA, particularly DFA$_b$. Together, these results suggest that time spent underwater and trip duration cannot on their own explain the variation in fractal scaling observed. It is also notable that neither mean dive duration nor depth was related to a sequence's fractal properties. Therefore, the sequential distribution of dives within a sequence is ultimately the key factor, adding weight to our assertion that temporal fractal analyses provide a metric that describes a fundamental property in animal behaviour: fractal time[26].

In conclusion, we show here that penguin dive sequences exhibit a complex fractal structure through time, and relate this structure to a combination of extrinsic (environmental) and intrinsic (self) organizational control elements. The application of fractal tools to temporal sequences of animal behaviour should be explored further, particularly in, though far from limited to, organisms that are often used as indicator species for climate and environmental change, like the penguins examined here and many other top predators in marine ecosystems. The merger of bio-logging and fractal analysis represents an important opportunity to do so, promising to advance our understanding of the many interactions that occur between animals and the environments in which they are found.

Methods

Study site & subjects. This study was conducted during the guard stage of the 2010 breeding period (October 26 – November 26) with free-living little penguins (*Eudyptula minor*) at the Penguin Parade, Phillip Island (38°31′S, 145°09′E), Victoria, Australia. Birds from this colony were marked with injected passive RFID transponders (Allflex, Australia) as chicks[68]. We collected diving data consisting of single full-day foraging trips from 28 penguins, 14 males and 14 females, guarding 1- to 2-week-old chicks. Each penguin's age was determined from the date of transponder injection. Sex was determined using bill depth measurements[69]. We captured the birds in artificial wooden burrows and fitted them with time-depth data loggers (ORI400-D3GT, Little Leonardo, 12 × 45 mm, 9 g) set to record depth to a resolution of 0.1 m with an accuracy of 1 m (range: 0–400 m) at one-second intervals. Devices were attached using waterproof Tesa® tape (Beiersdorf AG, Hamburg, Germany) along the median line of the lower back feathers to minimize drag[70] and facilitate rapid deployment and easy removal upon recapture[71]. After a single foraging trip, each bird was recaptured in its nest box and the logger and tape were removed. All birds were weighed before and after logger attachment. Fieldwork was approved by the Phillip Island Animal Experimentation Ethics Committee (2.2010) and the Department of Sustainability and Environment of Victoria, Australia (number 10006148).

Frequency-based dive parameters. We first characterized dive sequences during each foraging trip with commonly-used summary statistics, including: (1) trip length; (2) mean dive duration; (3) mean dive depth; and, (4) total dive time, i.e. total time spent below the surface during a trip. After recovery, data were downloaded from the loggers and analysed using custom-written programs in IGOR Pro, version 6.22A (Wavemetrics, Portland, Oregon). We consider diving to have occurred only when the depth at a given sampling interval was greater than 1 m. We include Pearson correlation tests to examine relationships between these parameters.

Fractal analyses. We applied 4 methods to estimate the scaling behaviour of observed dive sequences. We emphasize Detrended Fluctuation Analysis or DFA[2] because it has become a mainstream method for examining scaling behaviour in time series data and remains the only method used to examine binary sequences of animal behaviour, though it is not without its critics[72]. For comparison, we used two variants of DFA (see below), but also two other measures in the Hurst Absolute Value (H_{AV}) method[73,74] and the box-counting method[75]. We performed DFA and H_{AV} using the package 'fractal'[76], and box-counting with the package 'fractaldim'[77], in R statistical software v.2.15.0[78].

Signal class. A critical first step in examining fractal structure in any data set for which the signal class is not *a priori* known is to determine whether the sequences reflect fractional Gaussian noise (fGn) or fractional Brownian motion (fBm). Choosing an appropriate scaling exponent estimator and correctly interpreting the results require knowledge about the class of the original signal[30,31,41]. We therefore tested the signal class of these sequences to determine whether they reflect fGn or fBm by examining the scaling exponent calculated by DFA (α_{DFA}), with $\alpha_{DFA} \in (0,1)$ indicating fGn and $\alpha_{DFA} \in (1,2)$ indicating fBm.

Detrended fluctuation analysis (DFA). DFA is a robust method used to estimate the Hurst exponent[79,80], i.e. the degree to which time series are long-range dependent and self-affine[30,73]. The method is described in[9], and its application to binary sequences of animal behaviour can be found in[16,18,27]. Other names for this method include linear detrended scaled windowed variance[30] and residuals of regression[73]. The following description of DFA is taken from the above studies.

First, we coded dive sequences as binary time series [$z(i)$] in wave form containing diving (denoted by 1) and lags between diving events (denoted by -1) at 1 s intervals to length *N*. Diving behaviour was recorded at all *t* during which the subject was submerged to a depth greater than 1 m. Series were then integrated (cumulatively summed) such that

$$y(t)=\sum_{i=1}^{t} z(i)$$

where $y(t)$ is the integrated time series.

After integration, sequences were divided into non-overlapping boxes of length n, a least-squares regression line was fit to the data in each box to remove local linear trends ($\hat{y}_n(t)$), and this process was repeated over all box sizes such that

$$F(n)=\sqrt{\frac{1}{N}\sum_{i=1}^{N}\left(y_n(t)-\hat{y}_n(t)\right)^2}$$

where $F(n)$ is the average fluctuation of the modified root-mean-square equation across all scales ($2^2, 2^3, \ldots 2^n$). The relationship between F and n is of the form

$$F(n)\sim n^{\alpha}$$

where α is the slope of the line on a double logarithmic plot of average fluctuation as a function of scale. Like all estimators of the Hurst exponent, $\alpha_{DFA}=0.5$ indicates a non-correlated, random sequence (white noise), $\alpha_{DFA}<0.5$ indicates negative autocorrelation (anti-persistent long-range dependence), and $\alpha_{DFA}>0.5$ indicates positive autocorrelation (persistent long-range dependence)[9]. Theoretically, α_{DFA} is inversely related to the fractal dimension, a classical index of structural complexity[81], and thus smaller values reflect greater complexity (see Theoretical Relationships between Scaling Exponents below).

In addition to the standard (linear) form of DFA, we also used a bridge detrending method in our analysis, which is reportedly more appropriate to fBm signals[49] and sequences of lengths greater than 2^{12}[30]. Bridge-detrended Fluctuation Analysis (hereafter DFA_b) differs in two distinct ways from the linear form. Bridge-detrended Fluctuation Analysis (hereafter DFAb) differs in two distinct ways from the linear form. First, rather than using the regression line that best fits all data points in each window to detrend the sequence, the slope of the line bridging only the first and last points in each window is calculated[30]. Second, since it was suggested to work well with fBm rather than fGn sequences[49], and assuming that original binary sequences in this study were of the class fGn, we first integrated our time series before applying DFAb, meaning that observed sequences were integrated twice during application of DFAb but only once during DFA. We refer to the scaling exponent generated by this analysis as α_{DFAb}.

Hurst absolute value method (H_{AV}). We calculated the Hurst exponent H directly using the Absolute Value method. While fractal dimension estimates theoretically provide information about both memory and self-similarity or self-affinity, a previous study has shown that DFA, while giving robust estimates of long-range dependence (serial correlation), fails to capture the self-similarity parameter in data with certain non-Gaussian distributional characteristics[74]. The same study showed that the absolute value method, on the other hand, captured both parameters. Using this method, time series of length N are divided into smaller windows of length m and the first absolute moment is calculated as

$$\delta^{(m)}=\frac{1}{N/m}\sum_{k=1}^{N/m}\left|X^{(m)}(k)-\langle X\rangle\right|$$

where $X^{(m)}$ is a window of length m and $\langle X\rangle$ is the mean of the entire series. The variance δ scales with the window size m as

$$\delta^{(m)}=m^{H_{AV}-1}$$

where H_{AV} is the scaling (absolute value) exponent. Note that while DFA first integrates the time series before calculation, H_{AV} is calculated from the original time series, which in this case is the binary sequence of dives and their lags.

Box-counting dimension. We also employ a classical measure of fractal dimension to measure sequence complexity; box-counting[75,82]. The principle behind box-counting is simple. First, the integrated curve of the time series is placed within a single box, which is subsequently divided into smaller and smaller equally-sized boxes of size n. We use the entire range of scales from total sequence length down to the resolution of the data (i.e. 1 s). At each value of n, the number of boxes required to cover the curve is counted, with the expected relationship

$$N(n)=kn^{-D_b}$$

where n is the box size, $N(n)$ represents the number of boxes required to cover the curve at each box size, k is a constant, and D_b is the box-counting dimension, which is estimated from the slope of the least squares regression line on the log-log plot of $N(n)$ as a function of n.

Validation of scaling region. We use various methods to ensure the validity of our DFA results. There are algorithmic reasons why values diverge from scaling at small and large scales in a given analysis, and some of these are specific to the method used. For example, omitting some of the smallest and largest scales from the analysis is recommended when using DFA and DFA_b; excluding the largest scales can reduce variance but increase bias, whereas excluding the smallest scales reduces bias but increases variance[30]. The range of scales used should therefore be selected to maximize the fit of the regression line, i.e. minimize the mean squared error, on the double logarithmic plot[30]. Similarly, excluding scales smaller than 1/5 of the total sequence length as well as the two largest scales is recommend when using box-counting[75]. Alternatively, multiple scaling regions may also exist for biological reasons as a response of an organism to temporal or spatial scale[34,36–38]. Therefore, we independently determined the appropriate range(s) of scales within which strong scaling behaviour existed in our observed sequences using two procedures described in detail in[35].

The R^2 – SSR procedure involves the creation of a series of regression windows in which the number of data points (scales) ranges from a minimum of 5 (for valid regression analysis) to the maximum number of scales examined, 14 in our case. Each window was then slid across the entire data set so that the smallest windows provided 8 regression estimates, the next window size 7, and so on until only a single regression was performed on the largest window covering all scales. For fractal sequences, there should be a point at which, on a plot of the coefficient of variation (R^2) versus the sum of squared residuals (SSR), points converge to maximize the former and minimize the latter. This allows for the identification of the best scaling regions to be used in the calculation of scaling exponents in observed sequences. We performed this analysis on the mean values of $F(n)$ and n across all observed sequences, and therefore do not test for variation in scaling regions across individual birds.

The compensated-slope procedure uses a scaling factor c to 'compensate' the scaling behaviour such that, in the case of DFA,

$$F(n)=n^c * n^{-D_f}$$

where $F(n)$ is the fluctuation about the box size n as described above, c is the compensation exponent taking values of $c\in(0,1)$ for self-affine curves such as those examined here, and D_f is the fractal dimension estimate for the sequence. By varying c between 0 and 1, we can find the value at which our dimension estimate (based on the range of scales determined via the R^2 – SSR procedure) and compensated slope converge to 0 to produce a straight line (if scaling exists) with slope zero on the plot of Log($n^{c}*n^{-D_f}$) versus Log(n). Here, we used 5 values for c, the lowest (0.70) and highest (1.00) of which for illustrative purposes and the middle three values representing the minimum, best, and maximum estimates of α_{DFA} derived from the sliding windows used in the R^2 – SSR procedure. We then bootstrapped 1000 simulations to determine whether variation from this zero slope in observed sequences could be explained by noise, i.e. data points fall within the 95% confidence intervals, or whether scaling was simply unlikely given the fractal dimension estimate produced.

While the procedures described above are robust, many previous studies have relied on less convincing measures to support their results, such as high coefficients of variation for the slope of the double logarithmic plot and showing that surrogate sequences in which observed data points have been shuffled to break any serial correlation results in the expected relationship $\alpha_{DFArandom}=0.5$[16,27,46]. We also present R^2 values in our study, and take the mean of 10 surrogate sequences for each observed sequence (i.e. $N = 28*10 = 280$), but additionally apply the R^2 – SSR and compensated-slope procedures to these randomized sequences for comparison with observed sequences. Furthermore, we computed α_{DFA} for simulated random binary sequences of various lengths ($2^{11}\sim 2^{16}$ s) and distributions of diving behaviour (100 simulations for each of 5 binary probability distributions, i.e. diving versus its lag, at 0.25, 0.33, 0.50, 0.66, and 0.75) for comparison with observed and surrogate data. The results of these analyses are presented as Supplementary Information online.

Finally, in addition to the original 1 s interval sequences, we also applied the linear form of DFA to sequences sampled at 5, 10, 20 and 30 s intervals to determine whether the same scaling relationship would hold given different data resolutions. We also applied the R^2 – SSR and compensated-slope procedures to these sequences to determine whether their scaling regions corresponded to those in the high-resolution 1 s interval sequences.

Theoretical relationships between scaling exponents. Most scaling exponents and other fractal dimension estimates are theoretically related. For example, α_{DFA} provides a robust estimate of the Hurst exponent H[30,73], such that

for fGn: $H=\alpha_{DFA}$

for fBm: $H=\alpha_{DFA}-1$

In addition, H itself is inversely related to fractal dimension, here the box-counting dimension, such that for one-dimensional time series like those examined here

$$D_f=2-H$$

While these measures are theoretically related, in practice the various methods often lead to different results, either because of mathematical differences or nonlinearity in the series themselves[73,83,84]. Therefore, we estimated each of these parameters separately using the methods described above for a more robust interpretation of the results. We include an analysis of Pearson correlation coefficients to test for agreement between the four measures used.

Statistical analyses. Using the scaling exponents estimated via the above methods as Gaussian-distributed response variables (X^2 goodness-of-fit tests, $P>0.05$), we constructed general linear mixed-effects (LME) models to determine whether age, sex, initial body mass and the age of the young chicks being guarded were associated with variation in penguin dive sequence complexity ($N = 28$). We could not use final body mass to calculate mass gain during trips because measurements were taken hours after birds had returned to the nest and had already fed their chicks. We used the same approach to test whether these individual factors could explain variance observed in the summary statistics for each foraging trip, which were also Gaussian-distributed across individuals (X^2 goodness-of-fit tests, $P > 0.05$). For all models, we set the date on which data were collected for each individual as a random factor in our analyses to control for temporal variation. All LME models were run using the nlme package[85] in R. Models were fit by restricted maximum likelihood, using all factors and covariates in a single full model to estimate the parameter effects. Finally, we used a general linear model (GLM) to test whether the summary statistics themselves could explain variation in the observed scaling exponents. In all models, we tested for variance inflation caused by correlation between fixed effects using the car package in R[86]. If the variance inflation factor exceeded 10, we arbitrarily removed one of the 2 correlated variables and ran the model again. We set the alpha level for all statistical analyses at 0.05.

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Author contributions

A.M., Y.R.-C. and A.K. conceived of the experiment. L.P. collected the data and analysed the frequency-based measures presented. A.C. managed the field site and data collection. A.K. analysed and converted the raw data from the loggers and arranged the data set. A.M. conducted all fractal analyses and wrote the manuscript. All authors contributed to manuscript discussion and revision.

Additional information

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Résumé

Pour étudier l'impact des changements environnementaux dans un écosystème marin côtier, il est nécessaire d'utiliser des espèces indicatrices, comme les espèces situées au sommet des chaînes alimentaires qui intègrent les variations de l'environnement dans leur écologie (indicateurs écologiques). Pourtant, il est essentiel de distinguer les performances de recherche de nourriture ou de reproduction qui résultent des changements qui se produisent dans l'environnement de la performance qui dépend des caractéristiques de l'individu. Dans ce contexte, l'objectif de ma thèse était d'étudier l'influence des facteurs intrinsèques et extrinsèques sur l'activité de recherche alimentaire des manchots pygmée (*Eudyptula minor*) pour tester si cette espèce peut être considérée comme un éco-indicateur fiable de l'état du détroit de Bass situé dans le sud de l'Australie.

J'ai démontré que la colonne d'eau thermiquement stratifiée influençait positivement le comportement de recherche alimentaire et la réussite des manchots au cours de leurs voyages en mer d'une journée au sein d'une saison et sur plusieurs années. La thermocline a permis aux oiseaux d'approcher le comportement optimal (c'est à dire de réduire leur effort de recherche alimentaire tout en augmentant leur succès). Cependant, la thermocline est un élément instable dans le temps et j'ai montré que sa présence dans la zone de recherche de nourriture des oiseaux est imprévisible d'une année à l'autre. Étonnamment, je n'ai trouvé aucun effet de l'âge, du sexe et des caractéristiques physiologiques des individus sur leur comportement et le succès. Cependant, l'influence de la croissance des poussins sur l'activité en mer des parents est importante. Mon travail suggère que les conditions environnementales sont les principaux facteurs qui influencent le comportement des manchots pygmée, me permettant de conclure que les manchots pygmée sont de bons indicateurs écologiques de leur environnement

Mots clés : Stratégies de recherche alimentaire, manchots pygmée, caractéristique individuelle, conditions océanographiques, thermocline, plongée, télomères, stress oxydant

Abstract

To study the impact of environmental changes in a coastal marine ecosystem, it is necessary to use indicator species, such as those species situated at the top of the food chains that integrate the variations of the environment in their ecology (i.e. ecological indicators). Yet, it is crucial to disentangle the foraging or breeding performances that proceed from changes occurring in the environment from the performance that depends on the individual's characteristics. In this context, the aim of my thesis was to examine the influence of intrinsic and extrinsic factors on the foraging activity of the little penguins (*Eudyptula minor*) to test if this species can be considered as a reliable ecological indicator of the state of Bass Strait located in the south of Australia.

I demonstrated that a thermally stratified water column influenced positively the diving behaviour and foraging success of little penguins during their one-day foraging trips within a season and across several years. The thermocline allowed birds to approach optimal behaviour (i.e. reducing their foraging effort while simultaneously increasing their success). However, the thermocline is an unstable element in time and I showed that its presence in the foraging zone of the birds is unpredictable from one year to the next. Surprisingly, I did not find any effect of the age, sex and physiological characteristics of the individuals on their foraging behaviour and success. However, chick growth's influence on the foraging activity of parents was paramount. My work suggests that environmental conditions are major factors that will influence the behaviour of little penguins, allowing me to conclude that little penguins are good ecological indicators of their environment.

Keywords: Foraging performance, little penguins, intrinsic factors, oceanographic conditions, thermocline, diving, telomeres, oxidative stress