# UNIVERSITÉ DE STRASBOURG

*ÉCOLE DOCTORALE DES SCIENCES DE LA VIE ET DE LA SANTE*

**Institut Pluridisciplinaire Hubert Curien**

# THÈSE présentée par :


## Anne-Mathilde THIERRY


soutenue le : **13 septembre 2013**

pour obtenir le grade de : **Docteur de l'université de Strasbourg**

Discipline/ Spécialité : Sciences du Vivant

# Statut endocrinien et effort de reproduction chez un oiseau marin longévif, le manchot Adélie, dans un environnement changeant

**THÈSE dirigée par :**

**Dr. ROPERT-COUDERT Yan** — Chargé de recherche, Université de Strasbourg

**RAPPORTEURS :**

**Dr. BIZE Pierre** — Maître assistant, Université de Lausanne

**Dr. GREMILLET David** — Directeur de recherche, CNRS Montpellier

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**AUTRES MEMBRES DU JURY :**

**Pr. GENDRAULT Jean-Louis** — Professeur, Université de Strasbourg

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Thèse présentée pour obtenir
le grade de docteur de l'Université de Strasbourg

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*Spécialité : Physiologie et Biologie des Organismes – Populations – Interactions*

Par
**Anne-Mathilde Thierry**

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Devant le jury composé de :

| | | |
|---|---|---|
| Dr. Pierre Bize | Maître assistant, Université de Lausanne | Rapporteur |
| Dr. David Grémillet | Directeur de recherche, CNRS Montpellier | Rapporteur |
| Pr. Jean-Louis Gendrault | Professeur, Université de Strasbourg | Examinateur interne |
| Dr. Yan Ropert-Coudert | Chargé de recherche, CNRS Strasbourg | Directeur de thèse |
| Dr. Frédéric Angelier | Chargé de recherche, CNRS Chizé | Membre invité |
| Dr. Thierry Raclot | Chargé de recherche, CNRS Strasbourg | Membre invité |

A ceux qui ne reviendront jamais du continent blanc
*(in memoriam)*

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Je tiens également à remercier Frédéric Angelier, Pierre Bize, Jean-Louis Gendrault et David Grémillet d'avoir accepté d'évaluer ce travail.

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Parce qu'elles ont réussi à trouver un peu de temps entre un bateau à voile et un chien de traîneau pour se consacrer à d'autres projets, merci à Pascaline Bourgain et Camille Robineau pour l'organisation de la première semaine polaire en France et la création du comité national français de l'Association of Polar Early Career Scientists qui s'en est suivie. Merci également à Pascale Klein pour m'avoir aidé, dans le cadre de l'exercice NCT® - Nouveau Chapitre de la Thèse, à élargir le champ des possibilités de l'après-thèse.

Merci à Laure, Sophie et Antoine pour leur soutien dans les dernières semaines de la rédaction de nos manuscrits *(This is the end)*, et à Mathilde, Mutti & Papitou pour leurs relectures attentives.

Enfin, pour m'avoir suivie dans cet endroit qui lui est hostile – qui contrairement aux apparences n'est pas l'Antarctique, pour m'avoir supportée pendant les périodes parfois fatigantes de la thèse, à Strasbourg ou à Dumont d'Urville, et pour être une véritable boule de tendresse :-), merci Rominou.

## Liste des études sur lesquelles ce travail est basé

Etude 1 Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins. Anne-Mathilde Thierry, Sylvie Massemin, Yves Handrich et Thierry Raclot. **Publié dans Hormones and Behavior**, 2013, 63: 475-483 doi: 10.1016/j.yhbeh.2012.12.011

Etude 2 Decreased prolactin levels reduce parental commitment, egg temperatures, and breeding success of incubating male Adélie penguins. Anne-Mathilde Thierry, Sophie Brajon*, Sylvie Massemin, Yves Handrich, Olivier Chastel et Thierry Raclot. **Publié dans Hormones and Behavior**, 2013, doi: 10.1016/j.yhbeh.2013.06.003

Etude 3 Hormonal treatment reveals reduced behavioral sensitivity to elevated testosterone levels during incubation in a polar seabird, the Adélie penguin. Anne-Mathilde Thierry, Olivier Bles*, Morgane Couchet*, Yves Handrich, Sylvie Massemin et Thierry Raclot. **Soumis à Hormones and Behavior.**

Etude 4 On the relationship between weather conditions, incubation parameters, and hatching success in Adélie penguins over five breeding seasons. Anne-Mathilde Thierry, Michaël Beaulieu et Thierry Raclot. **En préparation.**

Etude 5 Elevated corticosterone levels decrease reproductive output but do not affect chick mass at fledging in chick-rearing Adélie penguins. Anne-Mathilde Thierry, Yan Ropert-Coudert et Thierry Raclot. **Publié dans Conservation Physiology**, 2013, 1 doi: 10.1093/conphys/cot007

Etude 6 Differential effects of corticosterone on the behaviour and the reproductive output of chick-rearing Adélie penguins. Anne-Mathilde Thierry, Sophie Brajon*, Marion Spée et Thierry Raclot. **Soumis à Animal Behaviour.**

Etude 7 On the effects of a low long-term elevation of corticosterone levels on the behaviour and the breeding success of male Adélie penguins. Anne-Mathilde Thierry, Aurélie Gebus*, Françoise Amélineau et Thierry Raclot. **En préparation.**

Etude 8 On the relationship between endocrine status and environmental conditions in Adélie penguins at the onset of the reproductive season. Anne-Mathilde Thierry, Yan Ropert-Coudert et Thierry Raclot. **En préparation.**

Etude 9 Harsh environmental conditions stress the importance of good physiological parameters to survive in Adélie penguin chicks. Anne-Mathilde Thierry, Françoise Amélineau, Matthieu Boureau, Barbara Class*, François Criscuolo et Thierry Raclot. **En préparation.**

* Etudiants de Licence ou Master encadrés au cours de la thèse.

## Article présenté en annexe

Antioxidant defences reflect penguin population trends. Michaël Beaulieu, Anne-Mathilde Thierry, Daniel González-Acuña et Michael J. Polito. Publié dans **Conservation Physiology**, 2013. 1 doi: 10.1093/conphys/cot004

## Autres études en cours

On the effects of increased testosterone levels on oxidative status of Adélie penguins. *En collaboration avec Morgane Couchet* et Thierry Raclot.*

On the heritability of telomere length in Adélie penguins. *En collaboration avec François Criscuolo, Thierry Raclot et Sophie Reichert.*

On the relationships between population trends and foraging areas in Pygoscelid penguins using stable isotopes. *En collaboration avec Michaël Beaulieu, Daniel González-Acuña et Michael J. Polito.*

## Communications orales présentées dans le cadre de la thèse

- "Stress hormone affects incubation behaviour of male Adélie penguins *(Pygoscelis adeliae)*", Anne-Mathilde Thierry, Sylvie Massemin, Yves Handrich, Yvon Le Maho, Akiko Kato et Thierry Raclot. **4th International Science Symposium on Bio-logging**, Hobart (Australie), mars 2011.

- "Effects of stress hormone and severe weather conditions on the incubation behaviour of Adélie penguins *(Pygoscelis adeliae)*", Anne-Mathilde Thierry, Sylvie Massemin, Yves Handrich et Thierry Raclot. **8th European Ornithologists' Union Conference**, Riga (Lettonie), août 2011.

- "Hormone de stress, perturbations météorologiques et investissement parental au cours de l'incubation chez le manchot Adélie", Anne-Mathilde Thierry, Sylvie Massemin, Yves Handrich, Yvon Le Maho et Thierry Raclot. **7èmes journées scientifiques du Comité National Français des Recherches Arctiques et Antarctique**s (CNFRA), Paris, mai 2011.

- "Hormones et comportement chez le manchot Adélie en fonction des conditions environnementales", Anne-Mathilde Thierry, Sophie Brajon* et Thierry Raclot. **8èmes journées scientifiques du CNFRA**, Brest, mai 2012.

- "L'école des pôles, une initiative de vulgarisation des sciences polaires sur le plan national", Anne-Mathilde Thierry, Camille Robineau et Pascaline Bourgain. **9èmes journées scientifiques du CNFRA**, Paris, mai 2013.

- "Growing in Antarctica: effects of extended sea-ice conditions on Adélie penguin chicks", Anne-Mathilde Thierry, Françoise Amélineau, Matthieu Boureau, Barbara Class*, Morgane Couchet*, Sandrine Zahn, François Criscuolo et Thierry Raclot. **XIth SCAR International Biology Symposium**, Barcelone (Espagne), juillet 2013.

- "Linking weather conditions and hatching success in Adélie penguins using dummy eggs recording incubation parameters". Anne-Mathilde Thierry, Michaël Beaulieu et Thierry Raclot. **8th International Penguin Conference**. Bristol (Royaume-Uni), septembre 2013.

## Posters présentés dans le cadre de la thèse

- "Let's fish krill, or how to present ecological concepts to young students in a playful way". Céline Bret, Laure Pelletier, Sophie Reichert, Floriane Rudwill, Antoine Stier et Anne-Mathilde Thierry. **Journée de l'Ecole Doctorale Vie et Santé**, Strasbourg, décembre 2012.

- "When penguins make the best of a bad situation: how severe environmental conditions affect Adélie penguin chick growth, physiology, and survival", Anne-Mathilde Thierry, Françoise Amélineau, Matthieu Boureau, Barbara Class*, Morgane Couchet*, Sandrine Zahn, François Criscuolo et Thierry Raclot. **Journée de l'Ecole Doctorale Vie et Santé**, Strasbourg, décembre 2012. **9th Ecology & Behaviour meeting**, Strasbourg, avril 2013.

- "Country-specific actions and international cooperation to better communicate polar science in communities and classrooms", Anne-Mathilde Thierry, Pascaline Bourgain, Sarah Crowley, Camille Robineau, Heidi Roop, Teresa Valkonen et Janet Warburton. **XIth SCAR International Biology Symposium**, Barcelone (Espagne), juillet 2013.

- "Linking stress hormone levels, behaviour, and reproductive output in a polar seabird, the Adélie penguin: insights from experimental studies" Anne-Mathilde Thierry et Thierry Raclot, **11th INTECOL Congress,** Londres (Royaume-Uni), août 2013.

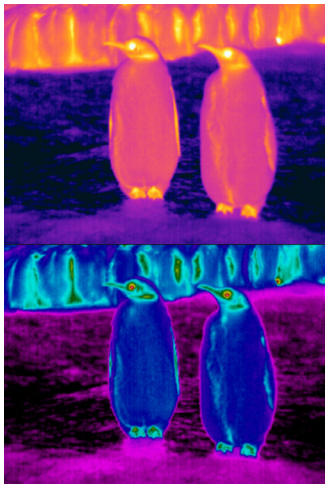
## Vulgarisation scientifique

- Participation à la conception et la présentation du jeu “A la pêche au krill” pour la Fête de la science d’octobre 2012.

- Interviews pour la presse écrite régionale (20 minutes Strasbourg, Courrier de l’Eure), pour des radios locales (RBS Strasbourg et France Bleue Haute-Normandie) et nationales (RTL) et pour la télévision locale (France 3 Alsace et UTV, la télévision de l’Université de Strasbourg) dans le cadre de la médiatisation de la bourse L’Oréal France – UNESCO – Académie des Sciences *Pour les Femmes et la Science* reçue en octobre 2012.

- Initiation et organisation de la première semaine polaire française en avril 2013, en collaboration avec l’APECS *(Association of Polar Early Career Scientists)* et PEI *(Polar Educators International).* Différentes activités ont été proposées à des classes de primaires et collège, dont des échanges par e-mail avec des scientifiques polaires et des webinars (présentations en ligne d’environ une heure sur une thématique définie au préalable). Cette première semaine polaire a conduit à la création du comité national français de l’APECS. La prochaine semaine polaire aura lieu du 30 septembre au 4 octobre.

- Vulgarisation des résultats de deux études scientifiques :
  - Des discussions avec Michel Legrand du LGGE lors d’une campagne d’été en Antarctique ont abouti à la mise en évidence de l’émission de composés organiques volatils par les colonies de manchot Adélie (Legrand *et al.* 2012). Cette étude a été mentionnée dans de nombreux sites internet, notamment le blog de l’American Geophysical Union.
  - Une partie du travail que j’ai effectué dans le cadre d’un hivernage en Terre Adélie a permis de mettre en évidence que le plumage des manchots empereurs est plus froid que l’air extérieur (McCafferty *et al.* 2013). Cette étude réalisée en collaboration avec Dominic McCafferty de l’Université de Glasgow et André Ancel de l’IPHC a été mentionnée sur de nombreux sites internet (BBC, New Scientist, Wired, …) et dans de nombreux journaux. J’ai été en charge de la diffusion des thermographies infrarouges à la demande de journalistes de différents pays (Allemagne, Australie, Belgique, Etats-Unis, France, Pologne, …).



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## Avant-propos

Pendant ma thèse, je me suis intéressée aux relations entre hormones, effort reproducteur et conditions environnementales chez un oiseau marin polaire. Si le sujet prévoyait initialement de s'intéresser uniquement à la corticostérone, principale hormone dite de stress chez les oiseaux, il s'est vite étendu à d'autres hormones. Ce travail de thèse s'inscrit dans le cadre d'un programme de recherche sur les capacités d'adaptation comportementales et physiologiques des manchots face aux changements de leur environnement (programme 137/ECOPHY puis ECOPHY-ANTAVIA sous la responsabilité d'Yvon Le Maho), programme soutenu et financé par l'Institut polaire français Paul-Emile Victor. Parmi les différentes espèces de manchots étudiées par les scientifiques du programme 137, les études sur le manchot Adélie ont été initiées il y a une dizaine d'années.

Avant la thèse, j'ai pu participer à la récolte de données sur le manchot Adélie dans le cadre d'un Volontariat Civil à l'Aide Technique (VCAT) effectué entre octobre 2007 et février 2009 sur la base Dumont d'Urville en Terre Adélie. J'ai également traité une partie de ces données lors d'un stage de Master 2 sous la direction de Thierry Raclot. Pendant la thèse, j'ai eu l'opportunité d'effectuer deux campagnes d'été sur la base Dumont d'Urville en Antarctique pour y récolter les données. Le travail de terrain a consisté au suivi d'une centaine de couples de manchots chaque été, de la pariade jusqu'à quelques jours avant le départ des poussins en mer. Les nids étaient contrôlés toutes les 2 à 3 heures, 24 heures sur 24 et 7 jours sur 7. Ce suivi, ainsi que les captures, prélèvements sanguins et mesures biométriques ont été réalisés avec l'aide de plusieurs VCATs dont la motivation a été très appréciée. En dehors de ces mois sur le terrain, j'ai partagé mon temps entre du travail en laboratoire et des analyses de données. Pendant cette période, j'ai eu la chance d'encadrer plusieurs étudiants, qui ont tous pris à cœur d'analyser une partie des données physiologiques et comportementales récoltées sur les manchots. Le temps restant a été consacré à la rédaction d'articles scientifiques en anglais qui ont été ou seront soumis à des revues internationales à comité de lecture. Ce manuscrit de thèse incorpore ces articles, qui sont à différentes étapes du processus de publication, en préparation, en révision ou publiés. Du fait de la nécessité d'un effort de publication important, obligeant les doctorants à valoriser leur travail le plus tôt possible, j'ai choisi de présenter ce travail de thèse sous la forme d'un manuscrit dit « sur articles ». Après une introduction générale plaçant le travail de thèse dans un contexte plus général, le modèle d'étude et les différentes méthodes de travail seront présentées. Les articles seront suivis d'une discussion générale, intégrant les limites et les perspectives à ce travail. La bibliographie a été placée à la fin du manuscrit afin d'éviter la répétition des références communes aux différentes études. En annexe se trouve le NCT (Nouveau Chapitre de la Thèse) rédigé dans le cadre d'un travail de bilan et valorisation de compétences proposé par l'ABG-Intelli'agence et l'Université de Strasbourg.

Cette thèse a été réalisée à l'Institut Pluridisciplinaire Hubert Curien (IPHC, UMR 7178, CNRS – Université de Strasbourg), au sein du Département Ecologie, Physiologie et Ethologie (DEPE), dans l'Equipe Ecologie Fonctionnelle et rattachée à l'école doctorale des Sciences de la Vie et de la Santé de l'Université de Strasbourg. La thèse a été financée par une allocation de recherche de l'Ecole Normale Supérieure de Cachan. Une bourse de la fondation anglaise Antarctic Science a également financé une partie des analyses en laboratoire. Enfin, une bourse L'Oréal France – UNESCO – Académie des Sciences Pour les Femmes et la Science a aidé à la valorisation de la dernière année de thèse et la recherche d'un post-doctorat.

# Introduction

# 1. Introduction

## 1.1 Contexte général

> *Since an organism is inseparable from its environment, any person who attempts to understand an organism's distribution must keep constantly in mind that the item being studied is neither a stuffed skin, a pickled specimen, nor a dot on a map. It is not even the live organism held in the hand, caged in the laboratory, or seen in the field. It is a complex interaction between a self-sustaining physico-chemical system and the environment.*
>
> *George A. Bartholomew (1958, p. 83)*
>
> *The role of physiology in the distribution of terrestrial vertebrates.*
> *In Zoogeography, pp. 81–95. AAAS, Washington, D.C.*

### 1.1.1 Un environnement changeant

Tout biologiste doit prendre en compte que son objet d'étude n'est pas un organisme en dehors de tout contexte, mais un organisme dans son environnement (Bartholomew, 1958). Cette remarque est d'autant plus valable que les organismes vivent dans un environnement changeant. Les animaux peuvent faire face aux variations prévisibles ou non prévisibles de cet environnement en ajustant leur morphologie, leur physiologie et leur comportement (Piersma & Van Gils, 2011).

Cependant, de nombreuses études rapportent une augmentation de la variabilité des conditions environnementales associée aux changements climatiques actuels (IPCC, 2007). Les changements climatiques actuels, dont la réalité et le caractère anthropique ne sont plus à démontrer (Cook *et al.* 2013), sont notamment associés à une augmentation de la fréquence et de l'intensité d'événements climatiques extrêmes (Stenseth *et al.* 2002) et à des modifications de la saisonnalité des principaux paramètres environnementaux (IPCC, 2007).

Les travaux visant à déterminer l'impact des changements climatiques sur les organismes, les populations, les communautés et les écosystèmes ont été nombreux au cours des dernières décennies (Parmesan, 2006). Il a notamment été rapporté que l'augmentation de la variabilité environnementale associée aux

changements climatiques actuels peut affecter les populations animales de façon drastique (Walther *et al.* 2002 ; Stenseth *et al.* 2003). Par exemple, certaines populations animales ont fortement diminué en réponse à de tels changements (McCarthy *et al.* 2001 ; Croxall *et al.* 2002 ; Both *et al.* 2006). Afin de mieux prédire les conséquences écologiques des changements climatiques sur les populations et les écosystèmes, il est important de bien comprendre les capacités d'adaptation des animaux aux changements rapides de leur environnement, en étudiant à l'échelle des individus les mécanismes physiologiques associés (Pörtner, 2002 ; Chown & Gaston, 2008 ; Pörtner & Farrell, 2008 ; Chown *et al.* 2010 ; Fuller *et al.* 2010).

### 1.1.2 Stress, allostasie et surcharge allostatique

Le terme de stress est souvent utilisé dans la littérature associée à l'étude des changements climatiques sur la physiologie animale. Il n'est pas inutile de proposer quelques définitions associées à ce terme, d'autant plus que sa définition est sujette à controverses. Le terme de stress fait en effet référence à trois notions :

- un événement stressant, c'est-à-dire un stimulus potentiellement néfaste non prévisible
- la réponse au stress, c'est-à-dire les modifications physiologiques et comportementales déclenchées par un événement stressant
- le stress chronique, c'est-à-dire l'état pathologique qui se met en place lorsque la réponse au stress est maintenue pendant de longues périodes (Romero, 2004)

Ainsi, une augmentation prévisible de la demande énergétique au cours de la reproduction n'est pas considérée comme un événement stressant, puisque prévisible (Jacobs & Wingfield, 2000 ; Wingfield, 2003 ; Wingfield 2005).

La notion d'allostasie a été proposée pour prendre en compte que les organismes répondent à des variations à la fois prévisibles et non prévisibles de leur environnement (McEwen & Wingfield, 2003 ; Wingfield 2005 ; McEwen & Wingfield, 2010) et correspond au maintien d'un état interne stable, ou homéostasie (sensu Cannon, 1932). Ce concept intègre donc les événements stressants mais aussi les événements prévisibles à l'origine de variations de la demande et de la disponibilité énergétique (Jacobs & Wingfield, 2000 ; Wingfield, 2005). Par exemple, la migration des oiseaux est associée à des modifications morphologiques, physiologiques et comportementales qui ne sont pourtant pas essentielles au maintien de l'homéostasie. Un ensemble de processus physiologiques et comportementaux permet aux organismes de maintenir un état interne stable (McEwen, 2000 ; McEwen & Wingfield, 2003 ; Wingfield, 2005). Lorsque la dépense énergétique est trop élevée et/ou les apports énergétiques trop réduits, un animal peut entrer dans un état dit de surcharge allostatique. Cette surcharge allostatique, qui arrive lorsque la capacité de réaction de l'organisme est dépassée par les perturbations, est équivalente au stress chronique. Parmi les potentiels médiateurs de l'allostasie, les glucocorticoïdes, ou hormones dites de stress, sont facilement mesurables, y compris sur des animaux sauvages en milieu naturel.

## 1.2 Décisions de reproduction et effort reproducteur

*The individual animal does not sit there with an evolution textbook and a calculator. This is not an arena of conscious strategizing by the animal. Instead, phrases like "The animal wants to do this, decides that this is the time to", and so on are a shorthand for the more cumbersome "Over the course of evolution, animals of this species, who at least in part through genetically influenced mechanisms, are better able to optimize the timing of this (…) behavior leave more copies of their genes, thus making this attribute more prevalent in the population". The personifying is just an expository device agreed upon to keep everyone from falling asleep during conferences.*

*Robert M. Sapolsky (2006, p. 41)*

*Monkeyluv and other essays on our lives as animals*

### 1.2.1 Quelques définitions

Au cours de leur vie, les animaux prennent des 'décisions' de reproduction, comme se reproduire ou non, quand se reproduire ou encore combien de temps et d'énergie investir dans la reproduction en cours. Les être vivants disposent de ressources limitées en temps et en énergie qui sont allouées à différentes fonctions biologiques, telles que la reproduction, la croissance et la survie (Maynard-Smith, 1978). Il en résulte des compromis entre ces différentes fonctions biologiques (Stearns, 1992). Les organismes répondent à ces compromis en maximisant leur fitness (ou valeur sélective), c'est-à-dire la capacité d'un individu à transmettre son patrimoine génétique aux générations futures (Williams, 1966 ; Stearns, 1992).
L'effort reproducteur correspond au temps et à l'énergie alloués à la reproduction (Trivers, 1974). Il se décompose en investissement sexuel (part de la ressource parentale consacrée sous forme de temps et d'énergie à la recherche d'un partenaire, à l'éloignement des rivaux et à l'accouplement) et en investissement parental (temps et énergie investis dans les soins parentaux apportés aux jeunes, qui augmentent les chances de survie d'un descendant aux dépens de la capacité des parents à investir dans d'autres descendants : Trivers, 1974 ; Clutton-Brock, 1991). Le coût de la reproduction est défini comme l'impact négatif de l'investissement parental en cours sur la survie et donc le succès reproducteur à venir (Williams, 1966).

### 1.2.2 Les oiseaux marins polaires, un modèle d'étude des décisions de reproduction dans un environnement changeant

Les oiseaux apparaissent comme des modèles appropriés pour étudier les mécanismes sous-jacents aux compromis entre les différents traits d'histoire de vie et à la régulation de l'effort reproducteur (Ricklefs & Wikelski, 2002). En effet, ils ont été étudiés de façon intense pour comprendre la grande variété des cycles biologiques et des traits d'histoires de vie dans un contexte naturel (Stearns, 1989 ; Ricklefs, 2000 ; Ricklefs & Wikelski, 2002). Pour la majorité des espèces, les deux parents prennent part à l'incubation et/ou à l'élevage des poussins, fournissant un effort reproducteur important (Lack, 1968 ; Orians, 1969 ; Cockburn, 2006 ; Kokko & Jennions, 2008). S'il existe des espèces qui ne prodiguent aucuns soins parentaux, comme dans les familles des Mégapodidés et des Cuculidés, les comportements parentaux sont particulièrement importants pour les espèces dont les poussins sont nidicoles, c'est-à-dire entièrement dépendants de leurs parents après l'éclosion.

L'étude des mécanismes sous-jacents aux variations de l'effort de reproduction chez les oiseaux marins, et particulièrement chez les oiseaux marins polaires, est très intéressante dans le contexte actuel des changements climatiques. En effet, les prédateurs supérieurs que sont les oiseaux marins peuvent être utilisés comme indicateurs de changements environnementaux (Cairns, 1987 ; Piatt *et al.* 2007), du fait de leur position dans les réseaux trophiques et de zones de prospection alimentaires étendues. Ce sont des espèces longévives, qui prodiguent des soins biparentaux et alternent entre des séjours en mer pour se nourrir et des séjours à terre pour couver les œufs et nourrir les poussins. Les animaux qui vivent et se reproduisent dans les régions polaires sont inféodés à un environnement extrême et variable, auquel ils sont adaptés. Pourtant, bien que les écosystèmes polaires soient relativement éloignés de la grande majorité des activités humaines, ils sont très sensibles aux changements globaux. Il est donc important d'étudier ces oiseaux pour mieux comprendre leurs capacités d'adaptation aux variations et parfois aux dégradations de leur environnement (Kitaysky *et al.* 2006 ; Grémillet & Boulinier, 2009).

### 1.2.3 Régulation de l'effort reproducteur

L'effort reproducteur et les décisions de reproduction sont susceptibles de varier en fonction des espèces, des populations, des individus, et également au cours de la vie d'un individu. Par exemple, les espèces longévives sont décrites comme étant des parents prudents, puisqu'elles limitent les risques de mortalité pendant la reproduction tout en maximisant les possibilités de reproductions futures (Drent & Daan 1980).

*FACTEURS EXTRINSÈQUES AUX PARENTS*

Le principal facteur extrinsèque aux parents participant à la régulation de l'effort reproducteur est le niveau de ressources disponibles, qui dépend de la saisonnalité, de l'abondance, et de l'accessibilité des ressources alimentaires (Durant *et al.* 2007). Lorsque les ressources sont insuffisantes, les parents peuvent ne pas se reproduire et attendre la prochaine saison de reproduction (Drent & Daan, 1980), décaler la saison de reproduction (Barbraud & Weimerskirch, 2006), ou se reproduire sans changer la phénologie, au risque de ne pas disposer de suffisamment de ressources ou en changeant la nature des ressources (Croxall *et al.* 1999 ; Miller & Trivelpiece, 2008 ; Nicol *et al.* 2008). Lorsque les individus se reproduisent malgré des ressources alimentaires insuffisantes, les parents peuvent diminuer l'approvisionnement de la progéniture tout en maintenant leur condition corporelle, ou maintenir un certain niveau de nourrissage aux dépens de leur condition corporelle, ou encore présenter un intermédiaire entre ces deux situations (Gaston & Hipfner, 2006). D'autres facteurs extrinsèques sont également susceptibles d'intervenir dans la régulation de l'effort reproducteur, comme l'effort reproducteur du partenaire chez les espèces biparentales (Williams, 1966 ; Trivers, 1972), le risque de prédation (Ghalambor & Martin, 2000), la demande de la progéniture (Ottosson *et al.* 1997), ou encore la valeur de la couvée (Ukegbu & Huntingford, 1988).

*FACTEURS INTRINSÈQUES AUX PARENTS*

De nombreux facteurs intrinsèques aux parents sont également susceptibles de réguler l'effort reproducteur. La notion d'histoires de vie état-dépendantes a été proposée par des théoriciens du fait de l'importance de l'hétérogénéité individuelle dans l'expression des stratégies d'histoires de vie (McNamara & Houston, 1996). L'état d'un individu intègre différents paramètres, tels que la qualité de son territoire, ses réserves énergétiques, sa physiologie, ses performances de recherche alimentaire, sa charge parasitaire, ou encore l'état de son système immunitaire. Les hormones jouent un rôle particulièrement important dans la régulation de l'effort reproducteur et dans les décisions de reproduction, en permettant aux organismes d'ajuster leur physiologie et leur comportement face aux modifications prévisibles ou non de leur environnement (Ricklefs & Wilkeski, 2002).

## 1.3 Mécanismes hormonaux sous jacents aux décisions de reproduction

> *Comparative endocrinologists attempt to measure hormones in the field and document how animals respond to the exigencies of their particular environment. This has only been made possible through the spectacular development of assay techniques that now permit hormones to be measured in drops of blood, rather than buckets! (…) When I started we needed a litre of plasma for a single measurement using a very tedious double-isotope derivative method with paper chromatography, but after 18 months we had this down to 2 mL and this soon fell to 20 µL with the introduction of competitive protein binding assays and radio-immunoassays.*
>
> *Don Bradshaw, 2007*
>
> *Proceedings of the 23rd Conference of European Comparative Endocrinologists*

Les hormones sont des messagers chimiques sécrétés par des cellules endocrines en réponse à des stimuli internes ou environnementaux. Elles agissent sur des cellules cibles après avoir été transportées par le système circulatoire sanguin (ou dans une moindre mesure lymphatique), en se liant à des récepteurs spécifiques, pour induire une réponse physiologique ou comportementale. La mise au point de dosages précis nécessitant de faibles volumes de plasma a permis le développement d'une discipline dite d'endocrinologie environnementale qui cherche à comprendre comment les hormones modulent et contrôlent la physiologie des animaux dans leur environnement naturel (Bradshaw, 2007). Par exemple, le fonctionnement des ovaires et de l'oviducte chez les femelles et le développement des testicules chez les mâles sont contrôlés par un ensemble de processus neuroendocriniens dans lesquels les hormones stéroïdiennes jouent un rôle important, processus qui intègrent des informations environnementales (Deviche *et al.* 2011 ; Ubuka & Bentley, 2011 ; Williams, 2012). Comme le montre la Figure 1-1, de nombreuses hormones autres que les stéroïdes sexuels interviennent dans le contrôle de la reproduction chez les oiseaux (voir aussi Ramenosky, 2011 ; Riters & Alger, 2011 ; Vleck & Vleck, 2011).

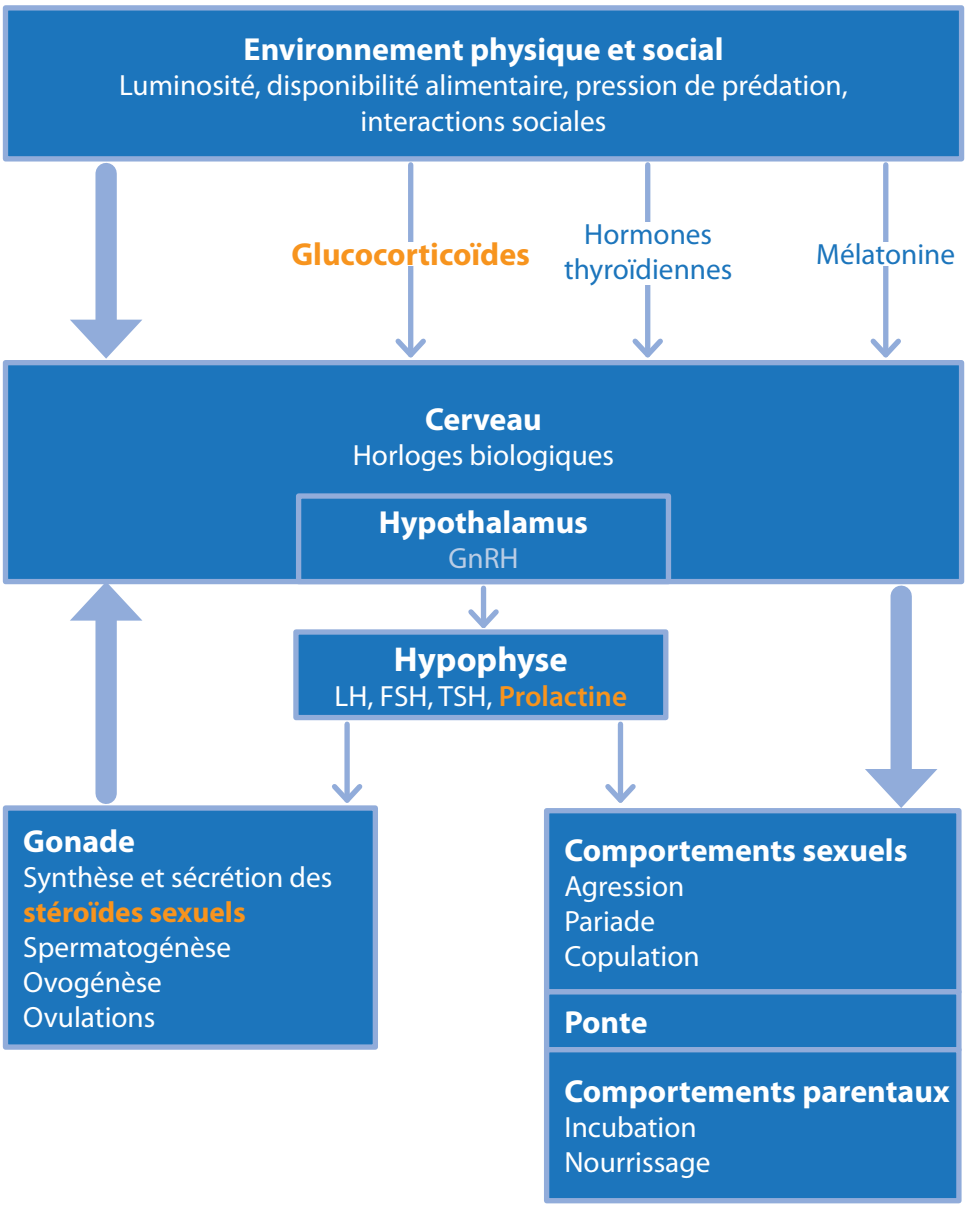



**Figure 1-1 :** Contrôle neuroendocrinien de la reproduction chez les oiseaux (adapté d'Ubuka & Bentley, 2011)

Le cerveau est considéré comme une boîte noire ne faisant pas l'objet de ce travail de thèse. Les hormones étudiées dans la thèse sont indiquées en orange. GnRH: Gonadotropin Releasing Hormone ou hormone de libération des gonadotrophines hypophysaires, LH: Luteinizing Hormone ou hormone lutéinisante, FSH: Follicle Stimulating Hormone ou hormone folliculo-stimulante, TSH: thyroid-stimulating hormone ou thyréostimuline

A quelles hormones faut-il s'intéresser dans le cadre des mécanismes sous-jacents aux variations de l'effort reproducteur ? Parmi les différentes hormones d'intérêt, la corticostérone est connue pour être un médiateur important de l'allostasie et la prolactine est décrite pour réguler les soins parentaux, bien que ses rôles biologiques ne soient pas aussi bien décrits chez les oiseaux que chez les mammifères (Williams, 2012). En gardant à l'esprit que l'effort reproducteur se décompose en investissement sexuel et en investissement parental, il est important de considérer une troisième classe d'hormones que sont les stéroïdes sexuels, fortement impliqués dans l'expression des comportements sexuels. De plus, glucocorticoïdes, prolactine et stéroïdes sexuels sont susceptibles d'interagir. Par exemple, la prolactine et les stéroïdes sexuels semblent agir en synergie pour développer la plaque incubatrice des oiseaux (Jones, 1971 ; Lea & Klandorf, 2002).

### 1.3.1 La corticostérone

La corticostérone est le principal glucocorticoïde chez les oiseaux. Les glucocorticoïdes sont sécrétés par les glandes corticosurrénales en réponse à l'activation de l'axe hypothalamo-hypophyso-surrénalien. Les niveaux basaux de glucocorticoïdes jouent un rôle important dans la régulation de la balance énergétique (Strack *et al.* 1995 ; Dallman *et al.* 2004) et sont régulés selon des cycles circadiens (Dickmeis, 2009) et circannuels (Romero, 2002). Une augmentation importante et rapide des niveaux de glucocorticoïdes a lieu en réponse à une perturbation ou à un événement stressant (Wingfield *et al.* 1998 ; Sapolsky *et al.* 2000), tel qu'une diminution de la disponibilité alimentaire (Kitaysky *et al.* 1999), un fort risque de prédation (Scheuerlein *et al.* 2001) ou de mauvaises conditions météorologiques (Romero *et al.* 2000). Les glucocorticoïdes activent les récepteurs de cellules cibles, modifiant la physiologie et le comportement de différentes manières, dont une mobilisation du glucose par néoglucogenèse (Dallman *et al.* 1996), une augmentation de la lipogenèse (Landys *et al.* 2004) et du stockage de lipides (Dittami *et al.* 2006 ; Yuan *et al.* 2008), une diminution voire un abandon de l'effort reproducteur (Silverin, 1986 ; Wingfield & Silverin, 1986 ; Love *et al.* 2004 ; Criscuolo *et al.* 2005 ; Spée *et al.* 2010 ; Ouyang *et al.* 2012), l'induction de comportements de fuite (Breuner *et al.* 1998 ; Wingfield *et al.* 1998 ; Breuner & Hahn, 2003), une augmentation de l'activité locomotrice, de la recherche alimentaire, et de la prise alimentaire (Landys *et al.* 2006). Les glucocorticoïdes jouent donc un rôle important à la fois dans le maintien de la balance énergétique, dans la médiation des réponses physiologiques et comportementales à des événements stressants et également dans la médiation de l'allostasie (Wingfield & Romero, 2010).

Trois niveaux d'activité des glandes surrénales ont été décrits (Fig. 1-2, Wingfield *et al.* 1998 ; McEwen & Wingfield, 2003). Le niveau A correspond aux concentrations minimales nécessaires pour le maintien de l'homéostasie, en particulier pour la régulation des niveaux de glucose et de minéraux. Le niveau B intègre les variations journalières et saisonnières et dépend du ratio entre demande énergétique et énergie disponible (Landys *et al.* 2006). Le niveau C correspond à des pics transitoires permettant un ajustement rapide de l'état physiologique de l'organisme en réponse à une perturbation et l'activation d'un état d'urgence (Wingfield & Ramenofsky, 1999 ; Sapolsky *et al.* 2000 ; McEwen & Wingfield, 2003). De

plus, les effets d'une augmentation des niveaux de glucocorticoïdes dépendent de leurs concentrations plasmatiques. En effet, deux types de récepteurs fixent l'hormone, un avec une grande affinité activée par de faibles taux, et un avec une faible affinité, activé uniquement par des taux élevés de l'hormone (Reul *et al.* 1987 ; de Kloet *et al.* 1993 ; Kalman & Spencer, 2002).

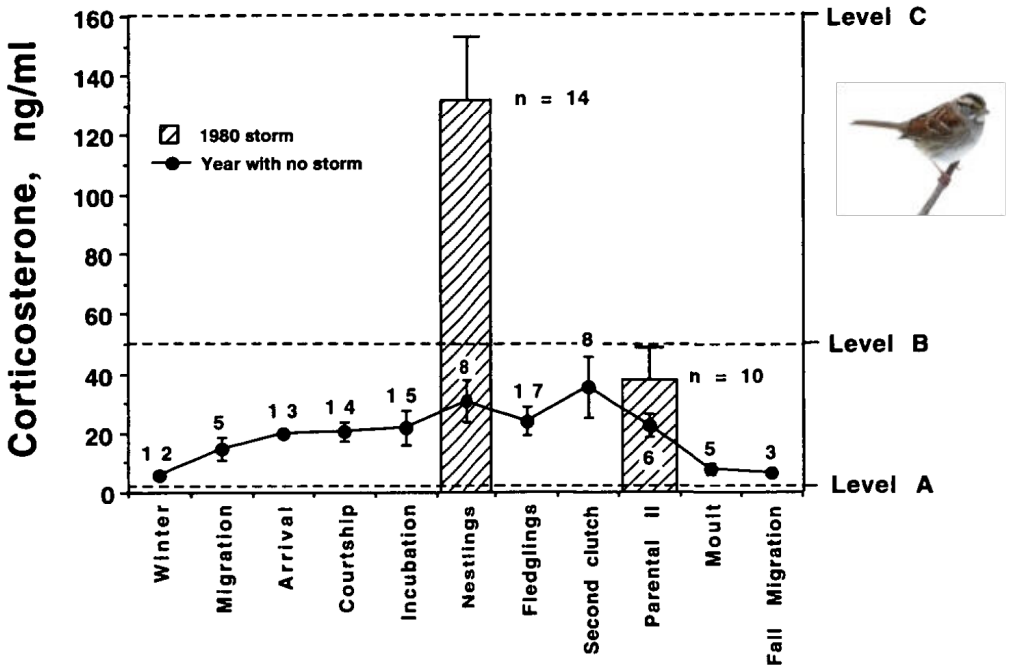



**Figure 1-2 :** Variations des niveaux plasmatiques de corticostérone chez le bruant à couronne blanche *(Zonotrichia leucophrys pugetensis)* au cours de deux saisons de reproduction (d'après Wingfield *et al.* 1998)

Les deux saisons correspondent à une saison de reproduction normale (courbe) et une saison pendant laquelle a eu lieu une tempête (barres hachurées) qui entraîna l'abandon des nids et territoires.

De nombreuses études se sont intéressées aux relations entre les glucocorticoïdes et la fitness (Bonier *et al.* 2009a). Des niveaux élevés de corticostérone ont été associés à une diminution du succès reproducteur, et parfois à un abandon de la reproduction en cours, chez différentes espèces d'oiseaux (Silverin, 1986 ; Criscuolo *et al.* 2005 ; Spée *et al.* 2010 ; Ouyang *et al.* 2012). D'autre part, l'augmentation de la demande énergétique au cours de la reproduction est associée à une augmentation des niveaux de corticostérone, du fait du rôle majeur de l'hormone dans le contrôle de la balance énergétique et de la prise alimentaire (Love *et al.* 2004 ; Bonier *et al.* 2009b). Plusieurs études montrent d'ailleurs une relation positive entre les niveaux basaux de corticostérone et le succès reproducteur (Bonier *et al.* 2009b ; Ouyang *et al.* 2011), suggérant une relation complexe entre la corticostérone et l'effort reproducteur. Busch et Hayward (2009) proposent en effet une relation log-quadratique plutôt que linéaire entre les niveaux de glucocorticoïdes et la fitness.

### 1.3.2 La prolactine

La prolactine est une hormone protéique antéhypophysaire dont le nom vient de sa capacité à promouvoir l'allaitement chez les mammifères (Riddle *et al.* 1933). Il a été montré que l'hormone est impliquée dans différents aspects de la reproduction, mais également dans le développement, l'immunité et le métabolisme chez les mammifères (voir la revue de Freeman *et al.* 2000). Cependant, les effets biologiques de variations des niveaux de prolactine chez les oiseaux sont moins bien connus. Les études concernant la prolactine chez des oiseaux sauvages en milieu naturel ont en effet été principalement corrélatives, décrivant les variations des niveaux plasmatiques de l'hormone et leurs possibles relations avec les comportements parentaux (Vleck *et al.* 1991 ; Garcia *et al.* 1996 ; Lormée *et al.* 1999 ; Lormée *et al.* 2000 ; Vleck *et al.* 2000 ; Chastel & Lormée, 2002 ; Criscuolo *et al.* 2002 ; Setiawan *et al.* 2006 ; O'Dwyer *et al.* 2006 ; Spée *et al.* 2010). La sécrétion de la prolactine est initiée au moment de la ponte et maintenue pendant l'incubation chez la majorité des oiseaux (Vleck, 2002). Les niveaux de prolactine décroissent rapidement au moment de l'éclosion pour les espèces dont les poussins sont mobiles et émancipés thermiquement dès l'éclosion. Pour les espèces dont les poussins sont thermiquement dépendants des parents, les niveaux de prolactine restent élevés pendant l'élevage des poussins, au moins jusqu'à l'indépendance thermique de la progéniture (Sharp *et al.* 1988 ; Goldsmith, 1991 ; Vleck, 1998 ; Lormée *et al.* 2000). Chez certaines espèces comme les manchots, la sécrétion de prolactine serait programmée de façon endogène et peu influencée par des stimuli externes (Garcia *et al.* 1996 ; Lormée *et al.* 1999 ; Vleck *et al.* 2000). Chez ces oiseaux, les niveaux de prolactine augmentent du début de la pariade jusqu'au milieu de la période d'incubation, et restent élevés jusqu'à la fin de l'élevage des poussins (Vleck *et al.* 1999), y compris pour les individus qui échouent dans leur reproduction (Lormée *et al.* 1999 ; Vleck *et al.* 2000). Ce maintien endogène de niveaux élevés de prolactine permettrait aux parents de garder une motivation à revenir au nid malgré de longues absences lors des voyages alimentaires en mer loin de la colonie au cours de la reproduction (Lormée *et al.* 1999).

La majorité des études expérimentales qui se sont intéressées aux effets de la prolactine sur les soins parentaux chez les oiseaux ont été réalisées sur des espèces domestiques (Buntin, 1996). Par exemple, la perfusion intracrânienne de prolactine ovine chez des dindes pendant la période de ponte induit le comportement d'incubation, définit comme une augmentation du temps passé au nid (Youngren *et al.* 1991). Toujours chez des dindes, une immunisation active dirigée contre la prolactine diminue les niveaux de prolactine et empêche l'expression des comportements d'incubation (Crisóstomo *et al.* 1998). Des stimuli tactiles et visuels tels que la présence d'œufs sur le nid stimulent la sécrétion de prolactine chez des dindes et des poules (El Halawani *et al.* 1980 ; Richard-Yris *et al.* 1998). Une diminution des niveaux de prolactine peut également être induite en empêchant des poules naines d'accéder au nid et est accompagnée d'une diminution de la propension à couver (Sharp *et al.* 1988). Ces travaux, probablement motivés par des intérêts économiques, ont principalement cherché à comprendre comment augmenter la production d'œufs et diminuer la propension à couver (Riddle *et al.* 1935 ; Sharp *et al.* 1988 ; Sharp, 1997). De plus, les Galliformes prodiguent peu de soins parentaux et les espèces domestiques ont été artificiellement sélectionnées pour différents caractères, dont une propension à couver réduite (Sossinka,

1982). Des conclusions différentes quant au rôle de la prolactine sur les soins parentaux pourraient donc être tirées d'études d'oiseaux non domestiques. Il faut également noter que la prolactine est une hormone protéique, dont la structure varie selon les espèces, alors que la structure d'une hormone stéroïdienne comme la corticostérone est strictement identique d'une espèce à l'autre.
Si les premières études ont établi que la prolactine jouait un rôle dans la régulation des comportements parentaux chez les oiseaux (Eisner, 1960 ; Lehrman & Brody, 1961), le rôle de la prolactine dans l'induction et le maintien du comportement d'incubation semble principalement reposer sur la relation temporelle entre les variations des niveaux de prolactine et le cycle de reproduction (revue de Williams, 2012). Pourtant, les vachers à tête brune *(Molothrus ater)*, des oiseaux parasites qui pondent leurs œufs dans les nids d'autres oiseaux et ne prodiguent aucuns soins parentaux, présentent des variations saisonnières des niveaux de prolactine, avec un pic pendant la période de reproduction (Dufty *et al.* 1987). Les preuves expérimentales d'un lien causal entre l'hormone et les comportements d'incubation chez des oiseaux sauvages restent rares (Sockman *et al.* 2006) et le rôle de la prolactine dans les comportements parentaux pendant la période d'élevage des poussins reste très peu étudié (Pedersen, 1989 ; Adkins-Regan, 2005).

Plusieurs études suggèrent que les niveaux de prolactine peuvent diminuer en réponse à des perturbations (Gratto-Trevor *et al.* 1991 ; Maney *et al.* 1999 ; Chastel *et al.* 2005 ; Angelier & Chastel, 2009 ; Riou *et al.* 2010). Aussi, une diminution des niveaux de prolactine, accompagnée d'une augmentation des niveaux de corticostérone, a été décrite chez les manchots royaux *Aptenodytes patagonicus* et les manchots Adélie *Pygoscelis adeliae* lors de l'abandon du nid consécutif à une déplétion des réserves énergétiques (Cherel *et al.* 1994 ; Groscolas *et al.* 2008 ; Spée *et al.* 2010). Il a également été montré qu'une augmentation expérimentale des niveaux de corticostérone est susceptible de diminuer les niveaux de prolactine (Buyse *et al.* 1987 ; Criscuolo *et al.* 2005 ; Angelier *et al.* 2009). De plus, les niveaux de corticostérone et de prolactine avant la reproduction sont susceptibles de prédire les différences individuelles en termes de succès reproducteur (Ouyang *et al.* 2011). Pourtant, les études considérant les effets de modification des niveaux de prolactine seuls, c'est-à-dire sans variation des niveaux de corticostérone, sur les soins parentaux d'espèces sauvages en milieu naturel restent rares.

### 1.3.3 Les stéroïdes sexuels

Les hormones stéroïdiennes sexuelles sont majoritairement secrétées par les gonades et sont fortement impliquées dans l'expression des comportements sexuels et dans la physiologie de la reproduction (Riters & Alger, 2011). Chez les vertébrés mâles, la testostérone, principale hormone stéroïde sexuelle, est principalement produite dans les testicules. Elle participe grandement au développement et au maintien des caractères sexuels secondaires, et est impliquée dans le compromis entre les comportements sexuels et parentaux chez les vertébrés mâles (Ketterson & Nolan, 1992 ; Wingfield *et al.* 2001 ; Hau 2007). Les niveaux de testostérone sont maximum au début de la saison de reproduction, pendant l'acquisition d'un territoire et la pariade, et diminuent ensuite jusqu'à des niveaux proches de ceux atteints en dehors de

la saison de reproduction (Wingfield *et al.* 1990 ; Vleck *et al.* 1999). Des niveaux élevés de testostérone ont été associés à une augmentation du succès reproducteur, via les comportements sexuels lors de la pariade, comme la défense du territoire et du partenaire, les copulations hors couple, le développement des caractères sexuels secondaires et la production de sperme (voir Horton *et al.* 2010). Mais des niveaux élevés de cette hormone plus tard dans la saison de reproduction sont également associés à une diminution du succès reproducteur du fait de la suppression des soins parentaux chez les mâles (Lynn, 2008), par exemple en diminuant le taux de nourrissage de la progéniture ou en perturbant l'incubation (McDonald *et al.* 2001). De plus, de hauts niveaux de testostérone sont associés à une suppression du système immunitaire (Ketterson & Nolan, 1999 ; Owen-Ashley *et al.* 2004), à un déséquilibre de la balance énergétique (Wikelski *et al.* 1999 ; Lynn *et al.* 2000) et à une augmentation du stress oxydant (Alonso-Alvarez *et al.* 2007).

Chez les femelles, les comportements sexuels sont sous le contrôle de plusieurs stéroïdes sexuels : œstrogènes, dont l'œstradiol, et progestérone (Riters & Alger, 2011 ; Vleck & Vleck 2011). Blas et Hiraldo (2010) se sont intéressés aux variations saisonnières des hormones stéroïdes sexuelles chez le milan noir *Milvus migrans*. Si les niveaux de testostérone ne diffèrent pas significativement entre mâles reproducteurs et non reproducteurs à une même période de la saison de reproduction, les femelles non reproductrices ont des taux plasmatiques d'œstradiol plus faibles que les femelles reproductrices. En plus de leur rôle dans la gamétogénèse et dans la ponte (Williams *et al.* 2004), ces hormones interviennent également dans l'induction des comportements d'incubation (Buntin, 1996 ; El Halawani *et al.* 1986) mais pas dans le maintien de ces comportements dans le temps (Ramesh *et al.* 1995 ; Lea *et al.* 1996).

## 1.4 Objectifs de la thèse

> *In the early days of exploration Antarctica lay far from the centre of world affairs. I sometimes wonder if, during the first decade of the [twentieth] century, my father or any of the pioneer explorers imagined that, before the end of the century, their chosen region would attain such far-reaching global significance – as the scene of intense, large-scale scientific research, and the subject of a remarkable international treaty for peaceful research. (…) Perhaps they did: as visionaries who looked beyond the horizon. (…)*
>
> *Lord Keith Shackleton (1991, p. x)*
>
> *Foreword. In Antarctica and Global Climate Change, Edited by C. Harris and B. Stonehouse, London: Bellhaven Press.*

**L'objectif général de ce travail de thèse a été de préciser les relations entre le statut endocrinien, les conditions environnementales et l'effort reproducteur d'un oiseau marin polaire, le manchot Adélie.** Les travaux menés au cours de cette thèse ont combiné des approches comparatives et expérimentales.

### 1.4.1 Hormones et effort reproducteur dans un environnement changeant

Nous avons vu précédemment l'intérêt d'étudier les oiseaux marins polaires et de mieux décrire les mécanismes physiologiques sous-jacents aux décisions de reproduction et aux variations de l'effort reproducteur. De plus, il est important de prendre en compte les conditions environnementales et leur potentielle variabilité dans ce type d'étude. En effet, une augmentation de la charge allostatique au sens de McEwen & Wingfield (2003 ; 2010), c'est-à-dire des coûts associés au maintien de l'allostasie lorsque les ressources énergétiques sont limitées et/ou la dépense énergétique augmentée, similaire au concept de *wear and tear* (usure) au sens de Romero *et al.* (2009), augmente la susceptibilité à des perturbations.

Breuner et Hahn (2003) ont proposé un modèle décrivant les effets des interactions entre les conditions météorologiques, la disponibilité alimentaire, la condition corporelle, et la physiologie du stress sur l'initiation de l'abandon du site de reproduction (Fig. 1-3). Une augmentation expérimentale des niveaux de corticostérone a retardé le retour au nid de bruants à couronne blanche *Zonotrichia leucophrys oriantha* après l'abandon du territoire consécutif à une tempête de neige. Mais une augmentation seule des niveaux de corticosterone n'a pas entraîné l'abandon du nid lorsque les conditions météorologiques étaient favorables, tout en augmentant la zone d'activité autour du site de reproduction.

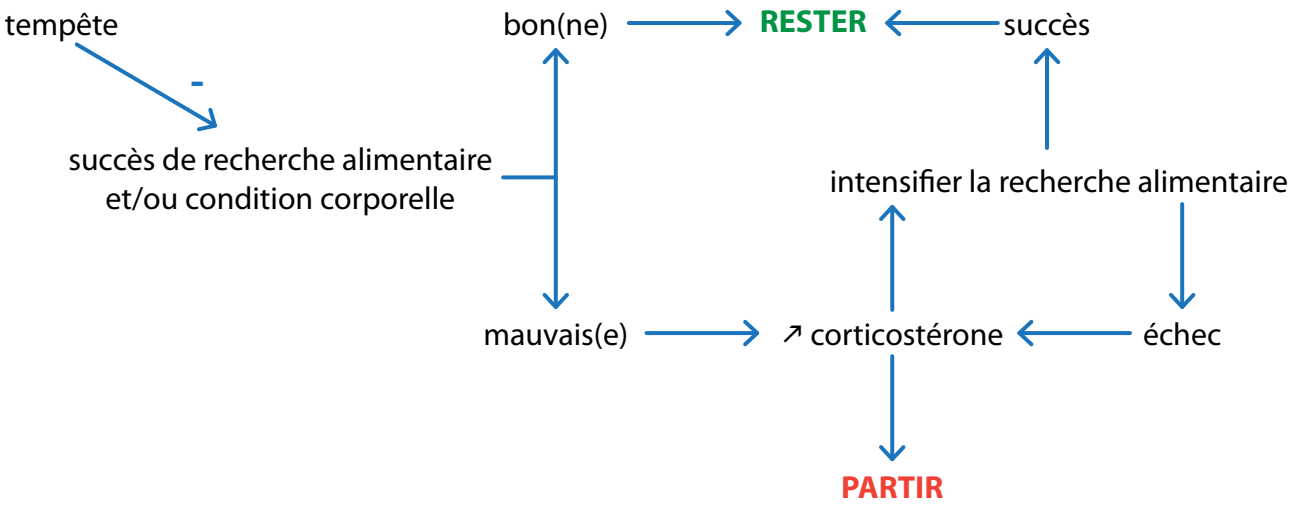



**Figure 1-3 :** Facteurs physiologiques et environnementaux susceptibles de participer à l'induction de l'abandon facultatif du nid (d'après Breuner & Hahn, 2003)

Pourtant, peu d'études ont essayé de déterminer dans quelle mesure des variations du statut endocrinien et des conditions météorologiques peuvent ensemble affecter le comportement et l'investissement parental dans la reproduction en cours.

### 1.4.2 Modèle d'étude : le manchot Adélie

Parmi les différentes espèces d'oiseaux marins polaires intéressantes à étudier dans le contexte de variations et de perturbations environnementales, mes travaux se sont concentrés sur le manchot Adélie *Pygoscelis adeliae*. Les études concernant cette espèce sont nombreuses et constituent une base bibliographique importante. Il est ainsi bien connu que le manchot Adélie est une espèce associée à la glace de mer (Ainley, 2002). Plusieurs études ont notamment décrit la dynamique des populations de manchots Adélie en réponse à des changements dans l'étendue ou la concentration de glace de mer (Wilson *et al.* 2001 ; Jenouvrier *et al.* 2006 ; Emmerson & Southwell, 2008) ou à la présence humaine (Giese, 1996 ; Carlini *et al.* 2007 ; Bricher *et al.* 2008).

L'évolution du statut endocrinien au cours du cycle de reproduction du manchot Adélie est également connue (corticosterone : Vleck *et al.* 2000 ; corticosterone et stéroïdes sexuels : McQueen *et al.* 1999 ; prolactine, testostérone et œstradiol : Vleck *et al.* 1999). Des conditions environnementales différentes sont également susceptibles d'affecter le statut endocrinien des oiseaux en début de saison de reproduction, avec une augmentation des niveaux de corticostérone et une diminution des niveaux de testostérone et d'œstrogènes lorsque d'une saison avec une extension importante de la glace de mer (Ninnes *et al.* 2011). En revanche, une débâcle précoce de la banquise n'était pas associée à une modification des niveaux

de corticostérone (Beaulieu *et al.* 2009). Plusieurs études ont abordé les mécanismes physiologiques susceptibles d'affecter l'investissement parental chez cette espèce mais ont été soient uniquement corrélatives (Vleck & Vleck, 2002 ; Angelier *et al.* 2008) soient spécifiques de l'abandon du nid consécutif à une déplétion extrême des réserves énergétiques (Spée *et al.* 2010 ; Spée *et al.* 2011a).

Dans le cadre de cette thèse, je me suis principalement intéressée aux effets de modifications du statut endocrinien sur l'effort reproducteur de manchots Adélie mâles. S'il eût été intéressant d'étudier de façon détaillée mâles et femelles, cela n'aurait pu se faire, du fait de contraintes de temps et d'effectifs lors de la récolte des données, qu'aux dépens de l'étude de différentes hormones et/ou de différentes étapes du cycle de reproduction. De plus, des études réalisées précédemment au sein du laboratoire permettaient d'établir des prédictions quant aux effets d'une augmentation expérimentale des niveaux de corticostérone sur l'investissement parental des manchots Adélie mâles (Cottin *et al.* 2011 ; Spée *et al.* 2011a).

### 1.4.3 Objectifs détaillés de la thèse

Les effets d'une modification expérimentale du statut endocrinien sur le comportement et le succès reproducteur de manchots Adélie mâles ont été mesurés à différentes périodes du cycle de reproduction. Trois hormones ont été considérées pendant l'incubation la corticostérone, la principale hormone dite de stress chez les oiseaux, la prolactine, fortement impliquée dans le contrôle des soins parentaux, et la testostérone, associée aux comportements sexuels. Seule la corticostérone a été considérée pendant l'élevage des poussins. Enfin, les effets de modifications dans les paramètres environnementaux, à la fois en termes de conditions météorologiques et d'étendue de la glace de mer ont été considérés, seuls ou en interaction avec une modification du statut endocrinien.

#### Statut endocrinien, conditions météorologiques et effort reproducteur pendant l'incubation

Cette première partie vise à déterminer les effets d'une modification du statut endocrinien sur l'investissement parental pendant l'incubation. Différents outils ont permis d'étudier de façon détaillée le comportement des manchots au cours de l'incubation : des œufs factices enregistreurs de températures et de mouvements de rotation et un suivi vidéo pour déterminer le budget temps comportemental et la position des manchots sur le nid.

- Etude 1 : Corticostérone, conditions météorologiques et incubation
  *(Etude expérimentale)* Evaluation des effets d'une augmentation expérimentale des niveaux de corticostérone et d'un épisode de blizzard sur l'investissement parental de manchots Adélie mâles pendant l'incubation.

- Etude 2 : Prolactine et incubation
  *(Etude expérimentale)* Evaluation des effets d'une diminution expérimentale des niveaux de prolactine sur l'investissement parental de manchots Adélie mâles pendant l'incubation.

- Etude 3 : Testostérone et incubation
  *(Etude expérimentale)* Evaluation des effets d'une augmentation expérimentale des niveaux de testostérone sur le comportement de manchots Adélie mâles pendant l'incubation.

- Etude 4 : Conditions météorologiques et incubation
  *(Etude comparative)* Etude des relations entre le comportement d'incubation, le succès à l'éclosion et les conditions météorologiques.

## Corticostérone et investissement parental à différentes périodes du cycle de reproduction et différentes doses

Cette seconde partie s'est intéressée aux effets d'une augmentation des niveaux de corticostérone pendant l'élevage des poussins sur le comportement de manchots Adélie mâle et le succès reproducteur, et aux conséquences d'une faible élévation des niveaux de corticostérone pendant l'ensemble de la saison de reproduction sur l'effort de reproduction.

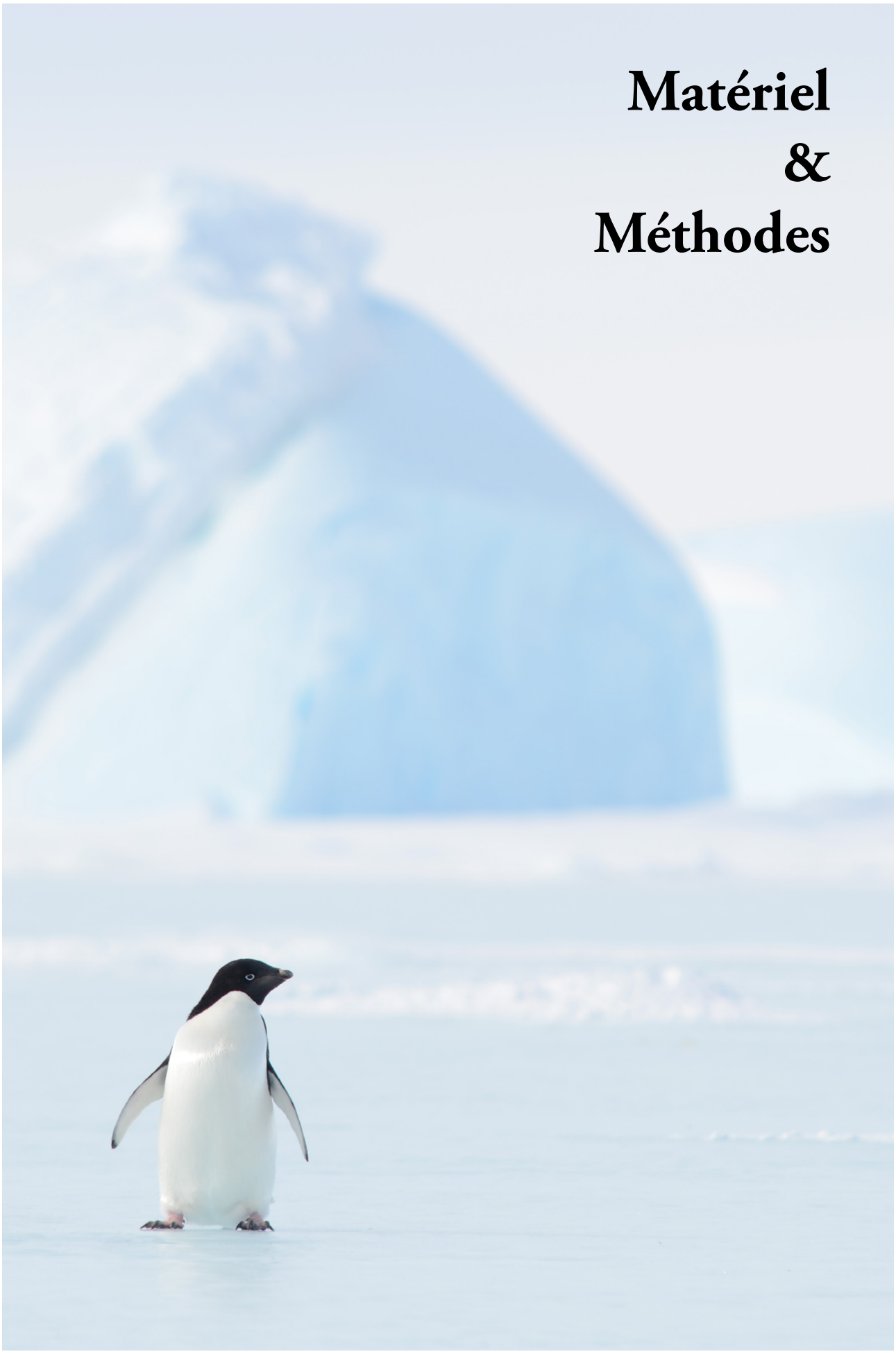
- Etude 5 : Corticostérone et élevage des poussins - I
  *(Etude expérimentale)* Evaluation des effets d'une augmentation expérimentale des niveaux de corticostérone pendant l'élevage des poussins sur le succès reproducteur.

- Etude 6 : Corticostérone et élevage des poussins - II
  *(Etude expérimentale)* Evaluation des effets d'une augmentation expérimentale des niveaux de corticostérone pendant l'élevage des poussins sur le comportement au nid et sur le succès reproducteur.

- Etude 7 : Corticostérone légèrement augmentée pendant l'ensemble de la saison de reproduction
  *(Etude expérimentale)* Mesure des effets d'une faible augmentation des niveaux de corticostérone sur l'effort reproducteur tout au long de la saison de reproduction.

D'autres travaux sont également en cours et seront rapidement présentés dans la discussion générale et les perspectives de la thèse, et détaillés en annexe. Ils concernent les conséquences de variations des conditions environnementales sur le statut endocrinien en début de saison de reproduction (Etude 8), et les effets de conditions environnementales contrastées (conditions inhabituelles de glace de mer) sur la croissance, la physiologie et la survie de poussins de manchot Adélie (Etude 9).

# Matériel & Méthodes



# 2. Matériel & Méthodes

## 2.1 Modèle d'étude : généralités

Il existe 18 espèces de manchots appartenant tous à la famille des Sphéniscidés. Avec le manchot empereur, le manchot Adélie *Pygoscelis adeliae* est le seul à se reproduire uniquement en bordure du continent Antarctique. Les effectifs sont estimés à 2,5 millions de couples nicheurs répartis en 161 colonies (Ainley, 2002). Les études récentes de dynamique des populations montrent des tendances contrastées en fonction des zones géographiques étudiées. En Antarctique de l'Est et dans la mer de Ross, les populations sont en légère augmentation, certaines zones étant plus accessibles (Taylor & Wilson, 1990 ; Taylor *et al.* 1990 ; Southwell & Emmerson, 2013). Dans la péninsule Antarctique, les manchots Adélie sont petit à petit remplacés par des manchots à jugulaire. Ce changement a été associé avec le recul des glaces dans cette zone (Trivelpiece *et al.* 1987 ; Forcada *et al.* 2006 ; Hinke *et al.* 2007).

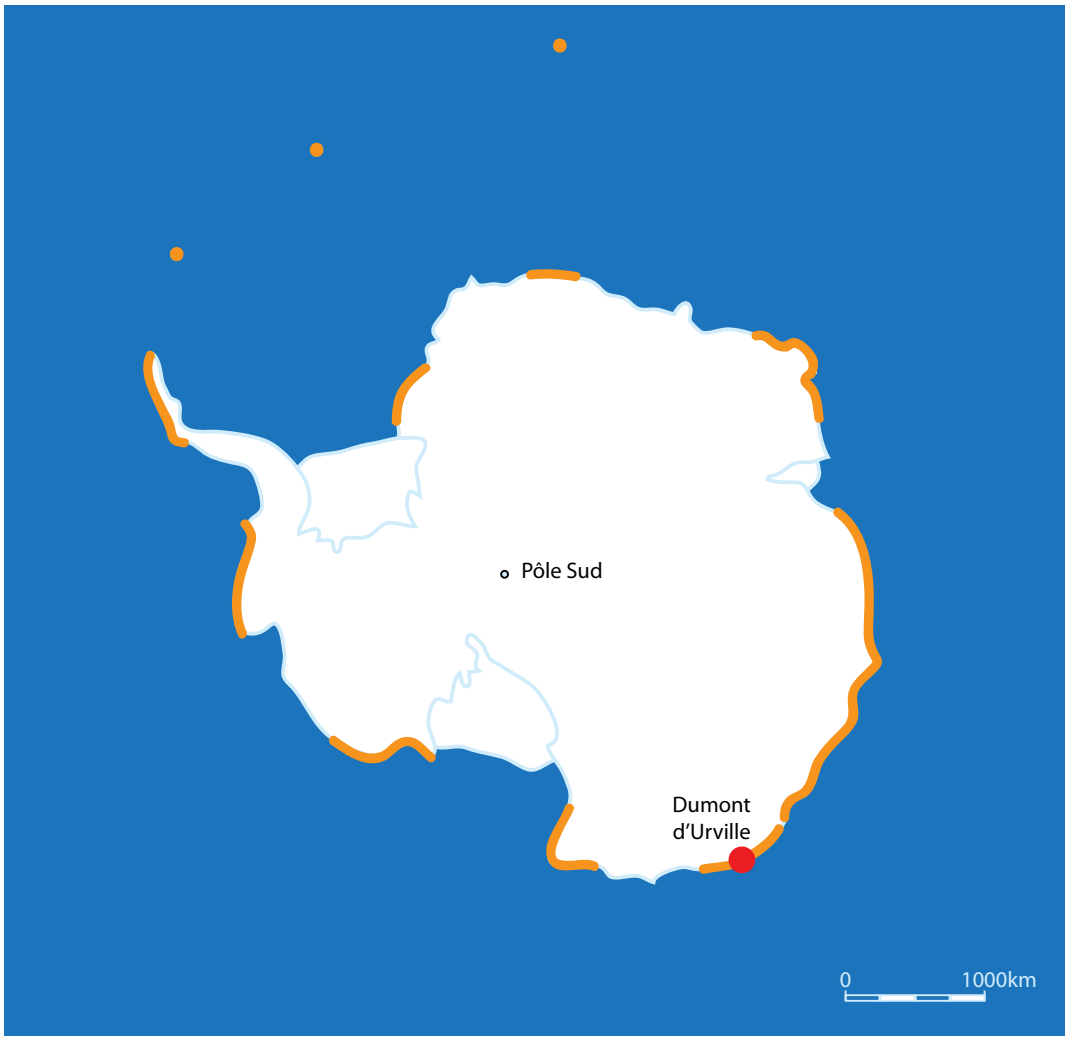



**Figure 2-1 :** Zone de reproduction du manchot Adélie (d'après Marchant *et al.* 1990) et localisation du site d'étude (point rouge)

Cet oiseau marin longévif et monogame (un seul partenaire par saison de reproduction) se reproduit au cours de l'été austral (octobre à mars). Les mâles arrivent sur le site de reproduction à partir de mi-octobre et sont suivis des femelles quelques jours plus tard. Après la période de pariade pendant laquelle un nid est construit à partir de cailloux, les manchots s'accouplent. La femelle pond le plus souvent deux œufs (mi-novembre) avant de partir se réalimenter en mer. Le mâle assure la première partie de l'incubation jusqu'au retour de la femelle, faisant face à une période de jeûne qui peut durer plusieurs semaines (jusqu'à 50 jours, Vleck & Vleck, 2002). Dès le retour de la femelle, le mâle part à son tour en mer pour environ 10 jours. Mâles et femelles alternent ensuite séjours d'alimentation en mer et de jeûne à terre pour couver les œufs puis élever les poussins. Un ou deux œufs éclosent à la fin du mois de décembre, après 30 à 39 jours d'incubation (Ainley, 2002). Le stade de garde est caractérisé par la présence obligatoire d'un des deux parents au nid. Une fois l'indépendance thermique des poussins acquise, les parents continuent à les nourrir mais les laissent seuls pour des périodes de plus en plus longues jusqu'à la mue et au départ en mer pour l'hiver (Sladen, 1958 ; Ainley, 2002).

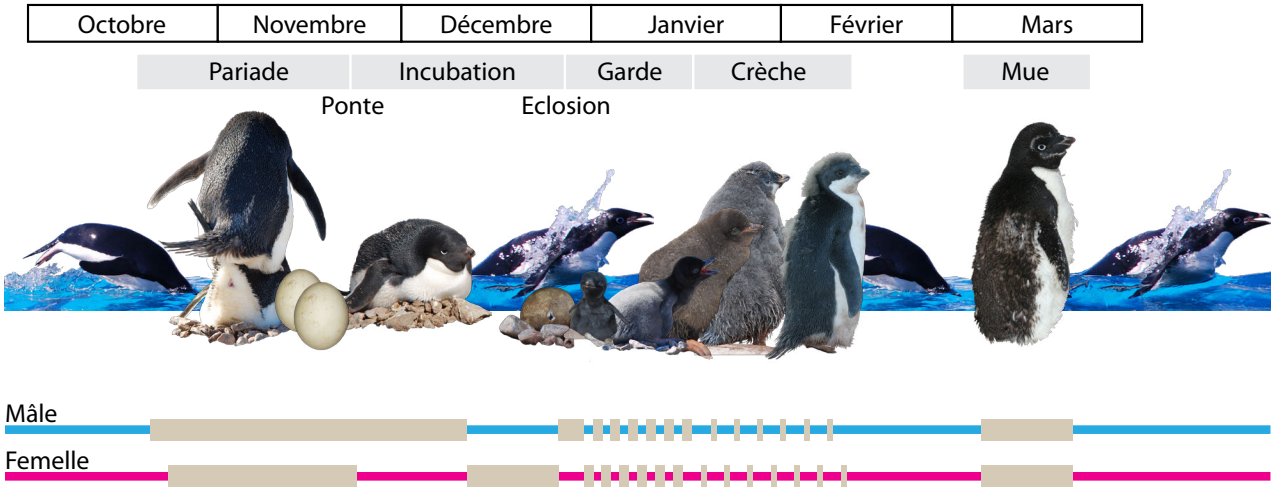



**Figure 2-2 :** Cycle de reproduction du manchot Adélie

Les rectangles beiges correspondent aux périodes où les manchots sont à terre.

## 2.2 Site d'étude

Les données analysées dans le cadre de ce travail de thèse ont été récoltées au cours de plusieurs campagnes d'été effectuées entre 2006 et 2012 sur la base polaire française Dumont d'Urville en Terre Adélie, Antarctique (66°40'S, 140°01'E). J'ai participé à quatre de ces campagnes, dont deux dans le cadre de la thèse (2010-2011 et 2011-2012). La base Dumont d'Urville est située sur l'île des Pétrels, dans l'archipel de Pointe Géologie.

**Figure 2-3 :** Vue aérienne de la base Dumont d'Urville située sur l'île des Pétrels dans l'archipel de pointe Géologie en Terre Adélie

L'archipel de pointe Géologie abrite près de 45 000 couples de manchots Adélie chaque année, dont 15 000 couples sur l'île des Pétrels (Micol & Jouventin, 2001 ; Jenouvrier *et al.* 2006). L'accès au site de reproduction des manchots Adélie est donc particulièrement aisé. Les études ont toutes été menées sur des oiseaux nichant à proximité du laboratoire de biologie marine, permettant de suivre de façon très régulière les nids d'études et d'utiliser les infrastructures en cas de mauvaises conditions météorologiques. Un même nid n'était pas étudié deux années de suite (sauf protocole particulier) de façon à limiter le dérangement des animaux.

**Figure 2-4 :** Localisation du laboratoire de biologie marine et des colonies d'études sur l'île des Pétrels

## 2.3 Protocole expérimental

Les études réalisées dans le cadre de cette thèse ont été approuvées par le comité d'éthique de l'Institut polaire français Paul-Emile Victor (IPEV) et autorisées par les Terres Australes et Antarctiques Françaises (TAAF). Le détail des méthodes et de l'analyse des données relatives à chaque étude est décrit dans la section "Matériel & Méthodes" de l'article correspondant.

*ETHIQUE*

Le manchot Adélie est, comme toutes les autres espèces autochtones des Terres Australes et Antarctiques Françaises, intégralement protégé (arrêté ministériel du 14 août 1998). Tout au long du travail de récolte de données en Antarctique, nous avons fait en sorte de limiter au maximum le dérangement potentiel causé par ces études en milieu naturel. Pour ce faire, il s'est agit de former le personnel et de suivre un certain nombre de règles sur le terrain : pose d'une cagoule sur la tête de l'oiseau pendant la contention, limitation du temps de contention, volume de prise de sang adapté à l'âge et au statut de l'animal. Par exemple, le volume des prises de sang effectuées chez des jeunes poussins était particulièrement réduit : 200µL maximum pour les poussins de 5 jours qui pèsent 200 à 300g.

*PROCÉDURE GÉNÉRALE*

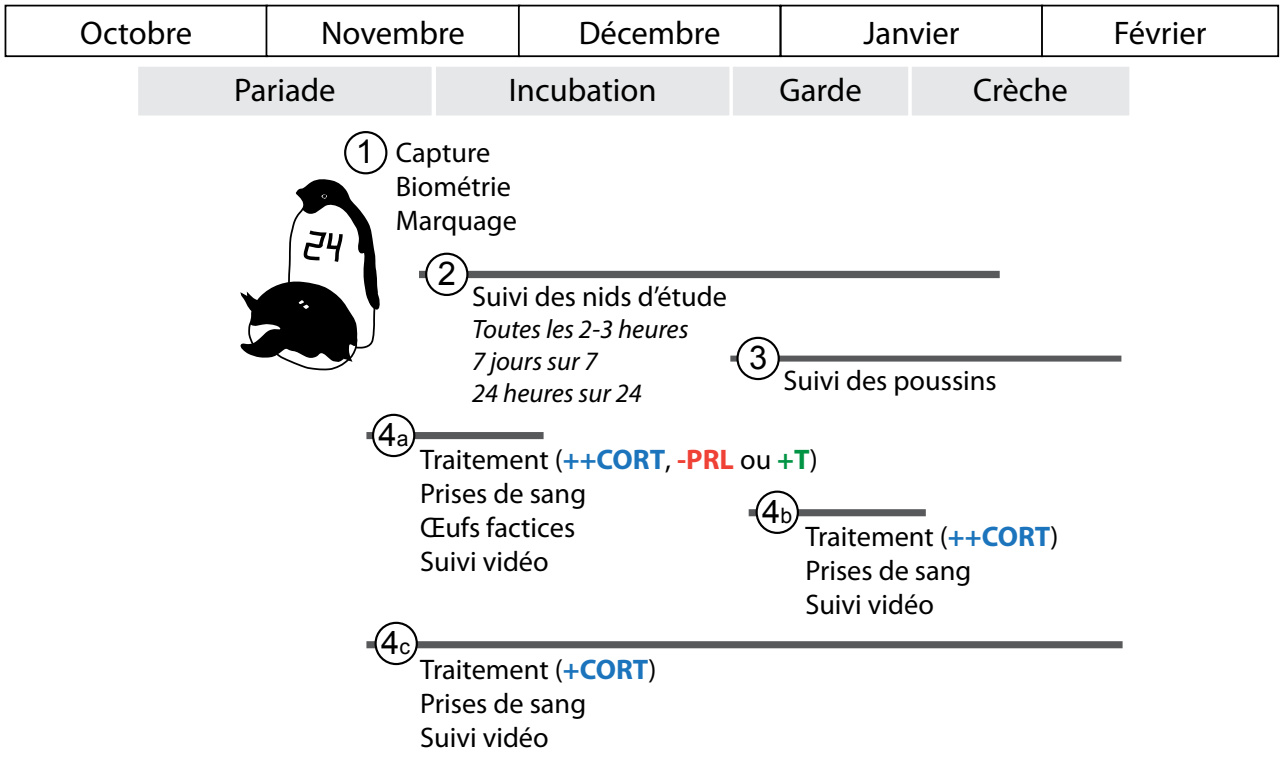



**Figure 2-5 :** Procédure générale relative aux différentes études

++CORT : augmentation expérimentale des niveaux de corticostérone, -PRL : diminution expérimentale des niveaux de prolactine, +T : augmentation expérimentale des niveaux de testostérone et +CORT : faible augmentation expérimentale des niveaux de corticostérone.

*CAPTURE*

Au début de la saison de reproduction, des nids ont été aléatoirement sélectionnés au sein d'une partie d'une colonie choisie avant le début du travail de terrain. Après la formation des couples et en règle générale quelques jours avant la ponte du premier œuf, les deux partenaires de chaque couple ont été capturés sur leur nid. Une cagoule est posée sur la tête de l'animal dès la capture de façon à limiter le stress de contention (Cockrem *et al.* 2008). Afin de minimiser le dérangement ainsi que l'impact possible de la capture sur la stabilité du couple, les deux partenaires ont été capturés à quelques jours d'intervalle. Une capture trop précoce dans la saison de reproduction risquerait d'entraîner la formation d'un nouveau couple pendant la capture de l'un des partenaires.

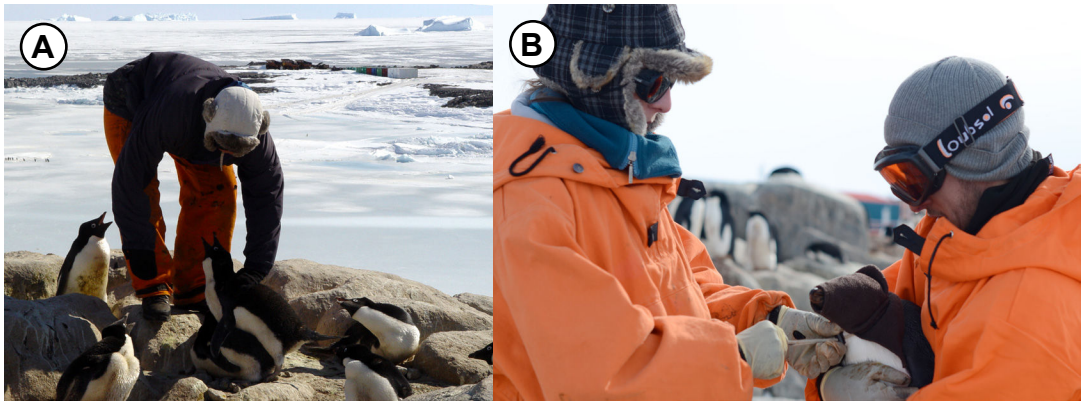



**Figure 2-6 :** Capture d'un manchot Adélie sur son nid (**A**, © Benjamin Friess) et pose d'une cagoule sur la tête d'un oiseau après la capture (**B**)

*MARQUAGE*

Chaque oiseau est identifié avec un numéro peint sur le thorax avec de la teinture brune (Nyanzol®) et deux étiquettes de scotch marin de couleur (Tésa®) insérées entre les plumes du dos. Ce marquage permet d'identifier chaque oiseau quelque que soit sa position sur le nid. Les deux partenaires d'un même couple sont marqués avec le même numéro. Un trait est peint au niveau du cou du deuxième partenaire capturé, et des étiquettes d'une couleur différente sont utilisées. Dans le cadre de certaines études, les manchots ont également été marqués de façon permanente par l'injection sous-cutanée d'un transpondeur (0,8 g ; 31,2 x 3,8 mm ; Texas Instruments TIRIS) entre la patte gauche et la queue.


**Figure 2-7 :** Marquage d'un manchot Adélie pour identification à distance (**A**, © Benjamin Friess) et manchot Adélie « 21 barre » (partenaire du manchot 21) à proximité de son nid (**B**)

*PRISE DE SANG*

Lorsque le protocole le prévoyait, une prise de sang a été réalisée dans les minutes suivant la capture (seringue sèche de2 mL et aiguille 25G). Le chronomètre était déclenché aux premiers signes du manchot lors de l'approche du nid afin de pouvoir effectuer la prise de sang en moins de cinq minutes après la capture, temps recommandé pour mesurer les concentrations plasmatiques basales de corticostérone (Vleck *et al.* 2000). Pendant qu'une personne tenait le manchot, une ou deux personnes réalisaient la prise de sang au niveau de la veine alaire ou d'une veine de la patte, avant de transvaser le sang dans des tubes prétraités avec 25µL d'héparine sodique ou d'EDTA (1M) puis centrifugation (10 min, 5000 rpm, 4°C). Le plasma et les globules rouges ont été congelés et stockés à -20°C jusqu'aux analyses en laboratoire effectuées à Strasbourg.

Un même oiseau a été prélevé une à trois fois (maximum) pendant toute la saison de reproduction, les prises de sang étant espacées entre mi-novembre et mi-janvier

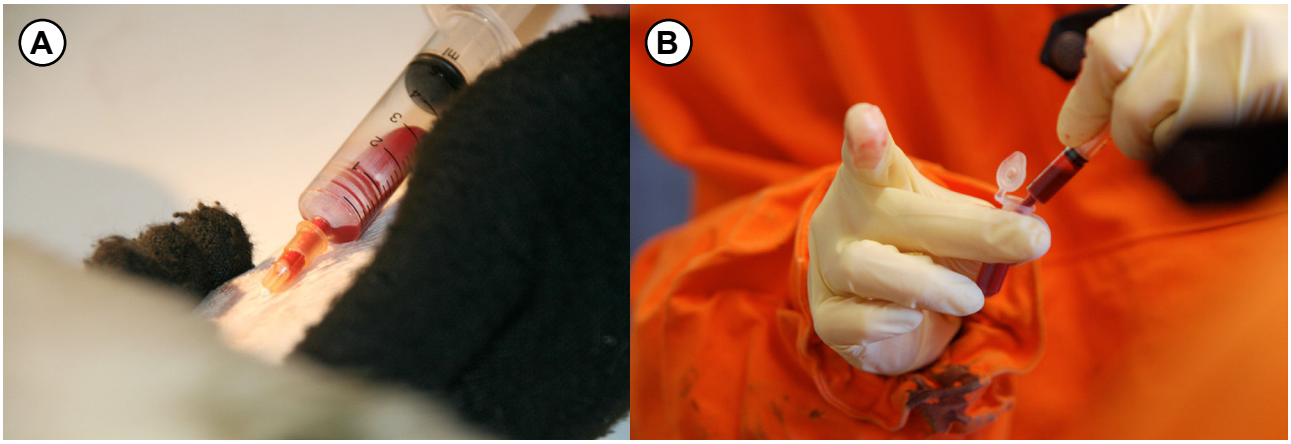



**Figure 2-8 :** Prise de sang (**A**) et transfert du prélèvement sanguin dans un tube prétraité avec un anticoagulant (**B**)

*PESÉE & BIOMETRIE*

Sauf exception, les animaux sont systématiquement pesés lorsqu'ils sont capturés (balance électronique Ohaus, ± 2g ou peson mécanique Pesola ± 50g). La longueur de l'aileron gauche et du culmen sont également mesurés lors de la première capture (aileron : réglet ± 1mm ; culmen : pied à coulisse ± 0.2mm).

*SUIVI DES NIDS D'ÉTUDE*

Un suivi régulier et quotidien a été réalisé afin de noter la présence de chaque partenaire sur le nid, toutes les 2 à 3 heures, 24 heures sur 24, 7 jours sur 7. A l'approche de la ponte et de l'éclosion, l'effort d'observation a été augmenté afin de déterminer dans la mesure du possible la date de ponte de chaque œuf et la date d'éclosion de chaque poussin. Le suivi a pu être légèrement moins fréquent pendant les deux premiers shifts d'incubation, lorsque les années où le personnel était réduit (tous les jours de 6h à 1h). Tout comportement particulier ainsi que le nombre de poussins étaient également notés sur la feuille de suivi. Dans les cas où il n'a pas été possible de déterminer la date de ponte, la date de départ de la femelle peut être utilisée comme indicateur de la date de ponte, car fortement corrélée à celle-ci (Ainley, 2002).

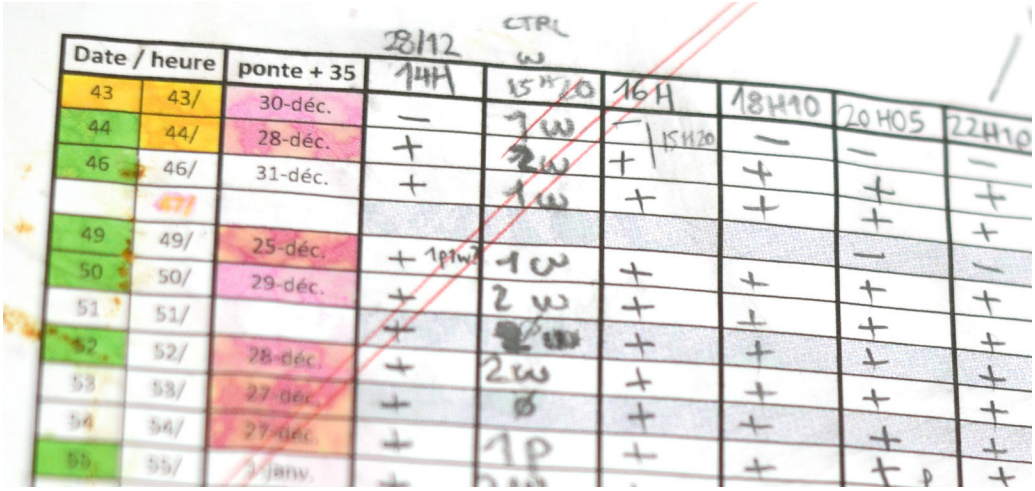



**Figure 2-9 :** Extrait d'une feuille de suivi des nids d'étude du 28 décembre 2011, pendant la période des éclosions

- désigne le manchot marqué avec une barre (par exemple 43/ pour la première ligne) et + le partenaire sans barre (43). 1w correspond à un oeuf, 2w à deux oeufs et 1p à un poussin.

*SEXAGE*

Les manchots ont été sexés à l'aide de plusieurs paramètres : inspection cloacale (le cloaque des femelles est dilaté avant la ponte), critères morphologiques et comportementaux, observation des copulations et alternance des shifts d'incubation (le mâle assure généralement la première partie de l'incubation). En cas de doute, un sexage moléculaire a pu être réalisé au laboratoire à partir d'un prélèvement sanguin.

*TRAITEMENT HORMONAL*

Le statut hormonal de certains oiseaux a été modifié expérimentalement pour les besoins de certaines études. Des implants sous-cutanés de corticostérone, bromocriptine (inhibiteur de la sécrétion de prolactine) ou de testostérone fabriqués par Innovative Research of America (Sarasota, Floride, Etats-Unis) ont été utilisés. Ces implants libèrent de façon passive l'hormone d'intérêt et sont biodégradables. La quantité d'hormone contenue dans chaque type d'implant, la durée de diffusion et les effectifs de manchots reproducteurs ayant reçu chaque type d'implant sont précisés dans le tableau suivant :

**Tableau 2-1 :** Récapitulatif des traitements hormonaux utilisés et des études correspondantes

| Hormone | Dose (mg) | Durée de diffusion (jours) | Période du cycle de reproduction | Effectifs d'oiseaux manipulés | Etude correspondante |
|---|---|---|---|---|---|
| **Corticostérone** | 100 | 21 | Incubation | 23 | Etude 1 |
| | | | Elevage | 17 | Etudes 5 et 6 |
| | | 90 | Incubation et Elevage | 18 | Etude 7 |
| **Bromocriptine** | 25 | 21 | Incubation | 30 | Etude 2 |
| **Testostérone** | 100 | 21 | Incubation | 12 | Etude 3 |

La procédure pour l'implantation sous-cutanée de ces substances bioactives est décrite dans la figure suivante :

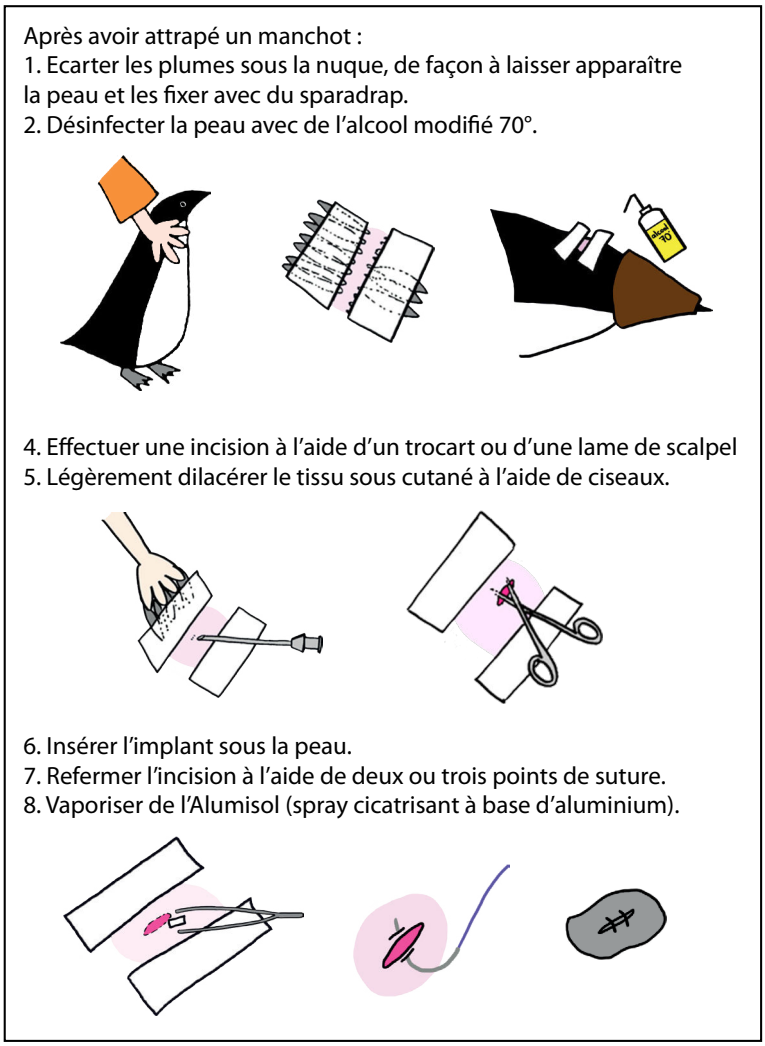



**Figure 2-10 :** Descriptif de l'implantation sous-cutanée de substances bioactives

Nous n'avons pas utilisé d'implant placebo dans nos différentes études. Une étude précédente de notre équipe n'avait pas montré de différences en termes de comportement et de physiologie entre deux groupes de manchots, l'un implanté avec des placebos et l'autre ayant subi la même procédure sans implantation (Spée *et al.* 2011b).

*OISEAUX CAPTIFS EN ÉCHEC DE REPRODUCTION*

Afin de valider l'efficacité des différents implants hormonaux et de mesurer la cinétique de diffusion des implants, un petit groupe d'oiseaux en échec de reproduction a été étudié entre mi-décembre et mi-janvier. Ces oiseaux ont été capturés et placés dans un enclos grillagé (5,2 x 2,4 m) adjacent au laboratoire de biologie marine. Le nombre de manchots présents dans l'enclos en même temps n'a pas dépassé 8 individus. A leur capture, les oiseaux ont été pesés et mesurés. Après deux jours de captivité, une prise de sang a été effectuée et un implant hormonal a été inséré en position sous-cutanée selon la procédure

décrite précédemment. Cette prise de sang a été exceptionnellement réalisée sous anesthésie gazeuse ($O_2$ médical et isoflurane).

**Tableau 2-2 :** Récapitulatif des traitements hormonaux utilisés sur des oiseaux captifs en échec de reproduction

| Hormone | Dose (mg) | Durée de diffusion (jours) | Effectifs d'oiseaux manipulés | Etude correspondante |
|---|---|---|---|---|
| Corticostérone | 100 | 21 | 8 | Spée *et al.* 2011b |
| | | 90 | 0 | - |
| Bromocriptine | 25 | 21 | 15 | Etude 2 |
| Testostérone | 100 | 21 | 3 | Etude 3 |

Les manchots ont été pesés tous les deux jours et des prélèvements sanguins effectués régulièrement pour évaluer la cinétique de diffusion des différents implants. Les oiseaux ont été relâchés avant d'atteindre une masse corporelle minimum de 3,5kg. La masse critique d'un manchot Adélie mâle à l'abandon est de 3,2 – 3,4kg (Spée *et al.* 2010). Les réserves corporelles au relâcher étaient donc suffisantes pour qu'un manchot prolonge son jeûne pendant quelques jours sur la colonie avant de partir se réalimenter en mer.

*COMPORTEMENT*

Le comportement des manchots pendant leurs différents séjours à terre a été évalué de différentes façons. Des œufs factices enregistreurs de températures et de mouvements de rotation ont été utilisés pendant l'incubation. Pour certaines études, les manchots ont également été filmés afin de déterminer le budget temps comportemental de ces oiseaux à différentes étapes du cycle de reproduction.

Les œufs factices utilisés ont été fabriqués à l'IPHC et sont de même forme, taille et couleur que les œufs de manchot Adélie. Ils sont remplis d'huile de vaseline de façon à avoir des propriétés thermiques proches de celles des vrais œufs. Ils sont munis de six sondes externes réparties de façon équidistante sur un même axe autour de l'œuf et d'une sonde interne de température. Ils contiennent également un accéléromètre deux dimensions qui mesure les projections de la gravitation terrestre sur les axes de rotation de l'œuf. Les sondes externes de température répondent en quelques secondes et la sonde interne en environ 20 minutes à un changement de la température de 5°C. La résolution est de 0,1°C et le coefficient de variation de 1,4% ± 0,2 entre les sondes (moyenne ± se, n=14 œufs, 8 heures d'enregistrement à température constante) et de 1,2% ± 0,1 entre les œufs (calculé à partir de la température moyenne enregistrée par toutes les sondes externes d'un œuf, n=14 œufs). Les œufs artificiels ont été posés après le départ de la femelle (œufs configurés avec le logiciel VBlog pour enregistrer chaque donnée toutes les 10 secondes). Les véritables œufs ont été conservés à l'étuve (34°C, rotation toutes les 4 heures, hygrométrie contrôlée) (2007-2008 et 2008-2009, Etude 1) ou confiés à des couples alentours (2009-2010, Etude 2 ; 2011-2012, Etude 3)

le temps de l'enregistrement. Dès que les œufs artificiels ont été récupérés, les vrais œufs ont été replacés sur le nid. Les données enregistrées ont alors été déchargées et sauvegardées sur support informatique. Le détail de l'analyse des données enregistrées par ces œufs factices est présenté dans la partie suivante.

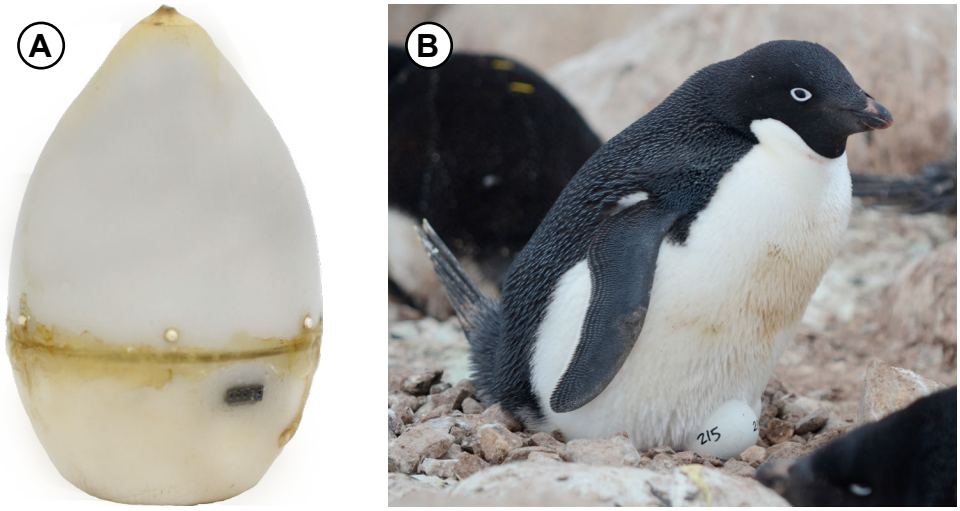



**Figure 2-11 :** Œuf factice enregistreur de températures et de mouvements de rotations utilisé dans les études 1 à 4 (**A**) et manchot couvant un œuf marqué provenant d'un des nids d'étude (**B**)

Un caméscope Sony HDR X550 a été utilisé pour filmer les manchots au cours de certaines études. Le caméscope était posé sur un trépied, fixé sur une passerelle métallique ou immobilisé à l'aide de rochers. En 2011-2012 (Etude 3), la caméra a été placée dans un caisson étanche afin de pouvoir filmer malgré les mauvaises conditions météorologiques. Les nombreuses heures de film ont été dépouillées à Strasbourg (voir Traitement des Données).

*DONNÉES MÉTÉOROLOGIQUES*

Les données horaires de température (°C), rayonnement global ($J/m^2$), vent moyen sur 10 minutes et vent maximum instantané (vitesse en m/s et direction) ont été recueillies par la station météorologique de Dumont d'Urville (Météo France). Les données de précipitations sont seulement enregistrées pendant les horaires d'ouverture de la station météorologique (9h à 22h, heure locale).

*MARQUAGE DES POUSSINS*

Lorsque les poussins avaient entre 15 et 20 jours, ou au plus tard à l'émancipation, ils ont été marqués avec deux "fishtags". Un fishtag est un petit marqueur en plastique de couleur dont l'extrémité est insérée sous la peau du dos du poussin. La combinaison d'une couleur de fishtag et d'un morceau de scotch marin collé à l'extrémité du fishtag permet de s'assurer de l'identité du poussin lors de la capture. Les fishtags sont retirés lorsque les poussins ont entre 40 et 45 jours, avant leur départ en mer. A chaque capture, les poussins sont pesés et mesurés.


**Figure 2-12 :** Poussins d'un même nid marqués à l'aide de fishtags (**A**) et contention d'un poussin pour la mesure de la longueur tête-bec (**B**)

*CROISSANCE DES POUSSINS*

La croissance des poussins a été suivie dans le cadre de certaines études. Lors des campagnes 2010-2011 et 2011-2012, les poussins ont été pesés et mesurés (aileron gauche et longueur tête-bec) à plusieurs reprises, notamment à 5, 20 et 42 jours après l'éclosion. Une prise de sang a également été effectuée dans les quelques minutes qui ont suivi la capture. Lorsque deux poussins étaient présents sur le nid, le plus âgé des deux a été légèrement marqué à l'aide d'un marqueur indélébile sur les ongles.

## 2.4 Analyses en laboratoire

Les concentrations plasmatiques d'hormones stéroïdiennes (corticostérone et testostérone) ont été déterminées par méthode immuno-enzymatique (ELISA, *Enzyme-Linked Immunosorbent Assay*) en utilisant des kits issus du commerce. Ce type de dosage repose sur le principe de compétition entre deux antigènes, l'un marqué et l'autre non (la molécule à doser), pour les sites de liaison d'un anticorps spécifique de la molécule dont on cherche à déterminer la concentration. L'affinité des antigènes pour ces anticorps étant égale et la quantité d'anticorps disponible dans le milieu réactionnel étant limitée, tous les antigènes ne participent pas à la formation de complexes anticorps-antigènes. La quantité d'antigènes marqués est connue et constante, seule la concentration de l'antigène non marqué est susceptible de varier. Après avoir éliminé les antigènes qui n'ont pas formés de complexes, l'ajout d'un anticorps secondaire qui se fixe spécifiquement à l'antigène marqué et/ou d'une enzyme spécifique de l'antigène marqué, associée à un chromogène, permettra d'obtenir un produit coloré. L'intensité de la coloration, mesurée par un spectrophotomètre, est inversement proportionnelle à la concentration de l'hormone, qui est alors déterminée en comparaison avec une gamme étalon (Figure 2-13). Les dosages ont été réalisés à l'IPHC à Strasbourg selon la procédure décrite par les fournisseurs. Les différents fournisseurs de ces kits de dosage sont précisés dans les articles correspondants.

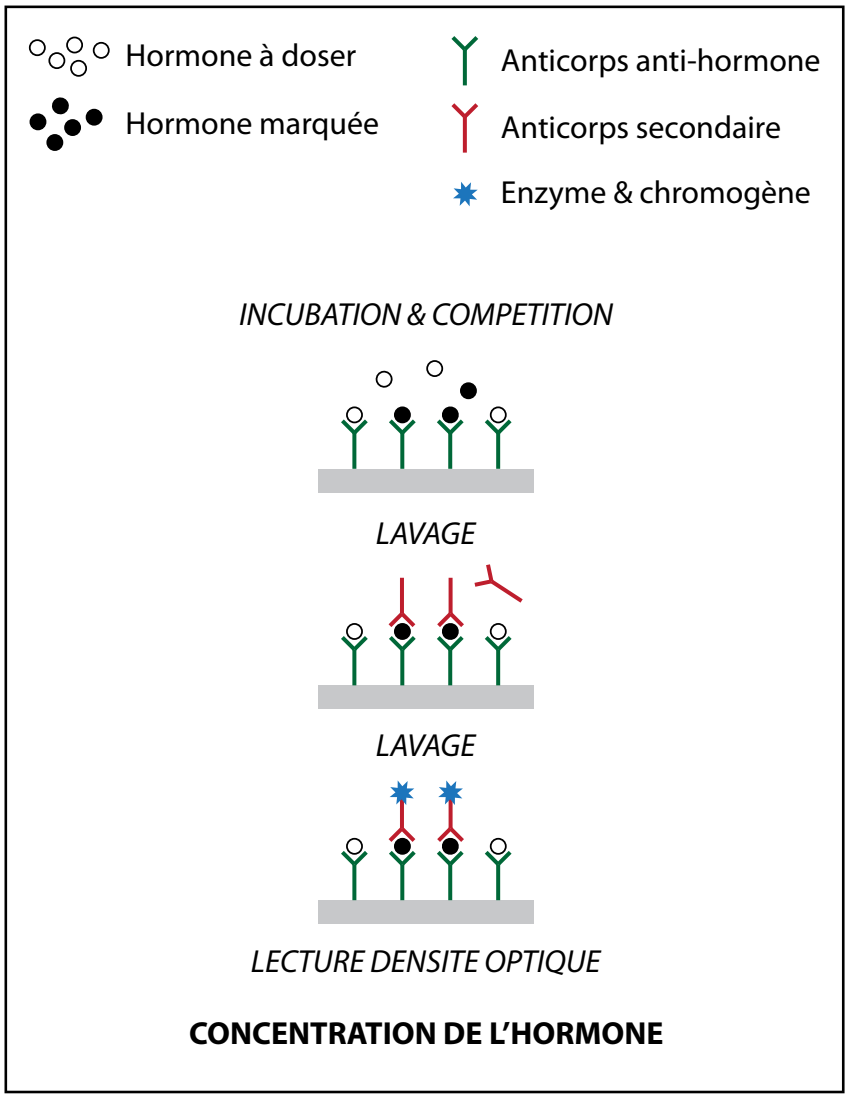



**Figure 2-13 :** Principe du dosage des hormones stéroïdiennes par ELISA

Le dosage des niveaux plasmatiques de prolactine a été réalisé par dosage radio-immunologique (RIA) au Centre d'Etudes Biologiques de Chizé dans le cadre d'une collaboration avec le Dr. Olivier Chastel selon le protocole décrit par Lormée *et al.* (2000). La prolactine étant une hormone protéique, son dosage est spécifique à chaque espèce. De plus, il n'existe pas de kit pour le dosage de la prolactine chez l'oiseau en général, et le manchot en particulier, dans le commerce. Ne disposant pas d'anticorps spécifiques de la prolactine du manchot, ce dosage hétérologue est réalisé en utilisant de la prolactine de poulet et des anticorps anti-prolactine de poulet (Fig. 2-14). La prolactine dans l'échantillon à doser est en compétition avec de la prolactine de poulet marquée avec un isotope radioactif (iode 125) pour la formation de complexes anticorps - antigène. La concentration inconnue de l'hormone à doser (antigène froid) est déterminée en fonction du nombre de complexes anticorps - antigène froid par rapport aux complexes anticorps - antigène marqué, en comparaison avec une gamme étalon.

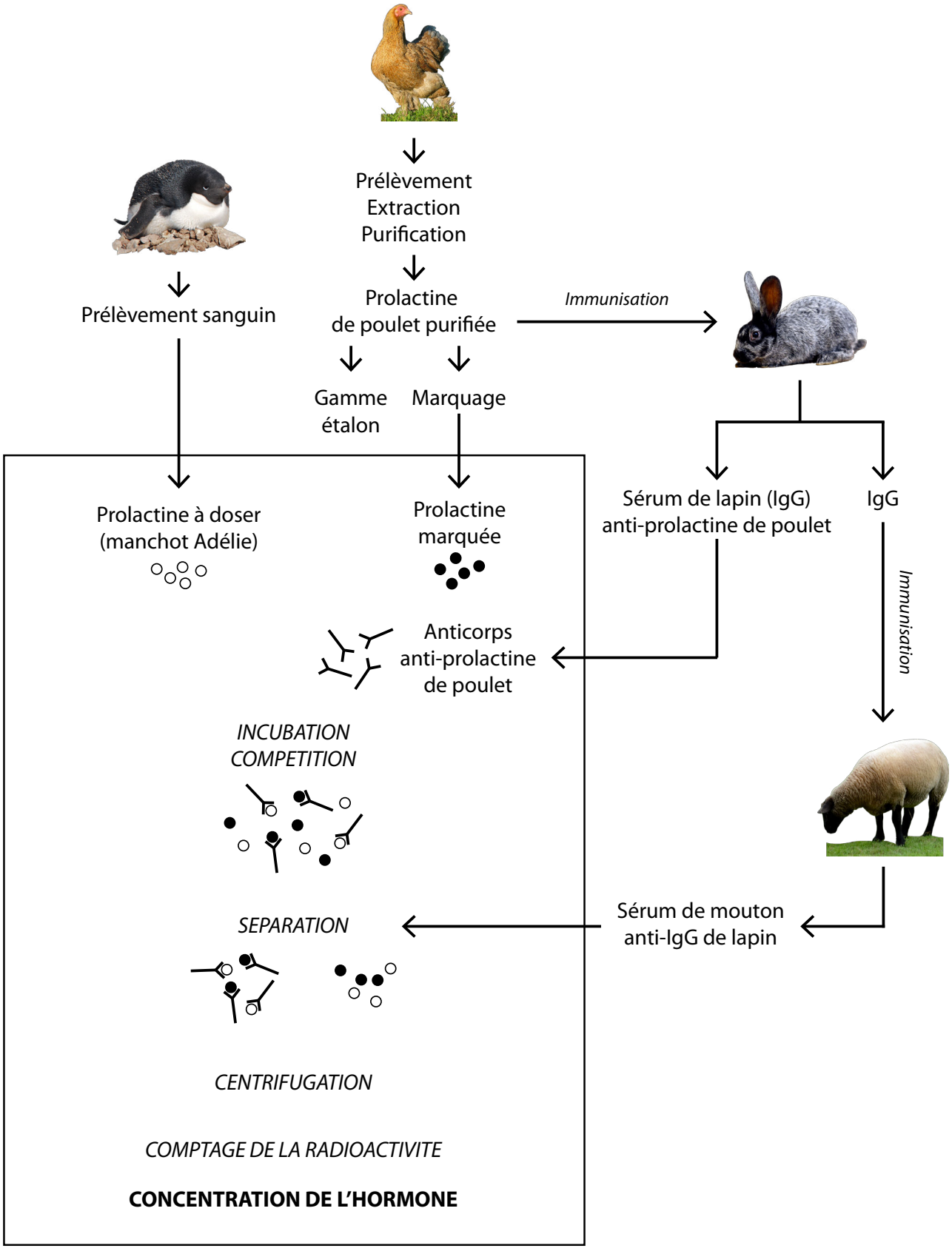


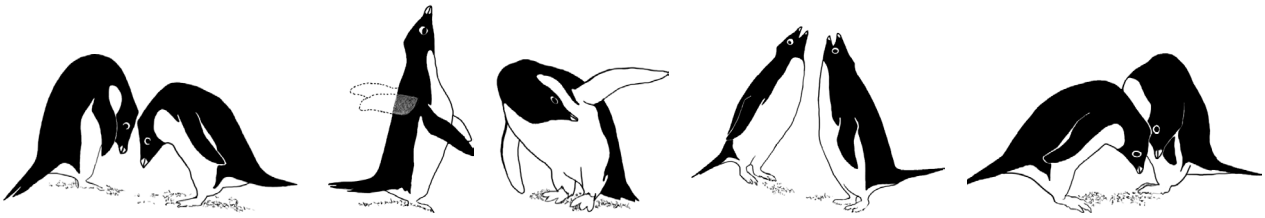

**Figure 2-14 :** Principe du dosage de la prolactine par RIA (d'après Angelier 2006)

## 2.5 Traitement des données

*COMPORTEMENT*

Dans le cadre du stage de Master 2 de Sophie Brajon (2011), les éthogrammes des manchots Adélie pendant l'incubation et pendant l'élevage des poussins ont été définis de façon détaillée. Un éthogramme est le répertoire comportemental d'une espèce dans un contexte donné. Plusieurs séquences de 15 ou 30 minutes (selon les études) ont été observées pour déterminer le budget temps comportemental détaillé de différents manchots. Sauf exception, un observateur unique a travaillé sur l'ensemble d'une étude. Les observateurs ont été formés à partir de séquences tests, et les observations considérées comme correctes lorsque le pourcentage de concordance entre deux observateurs était de plus de 95%. Les observateurs ont, en plus du détail du comportement, observé la position des manchots sur le nid (couché ou debout), la présence du partenaire, le nombre de poussins, et tout événement particulier.

*Interactions sociales positives :*




*Interactions sociales agonistiques :*

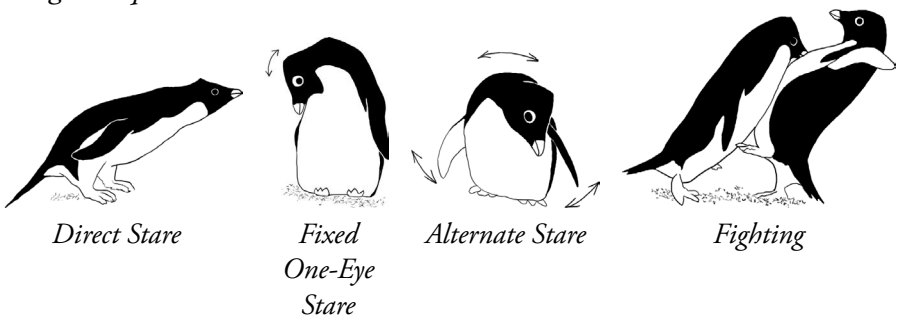



**Figure 2-15 :** Illustrations de quelques comportements typiques du manchot Adélie (d'après Marchant *et al.* 1990)

*PARAMÈTRES D'INCUBATION*

La durée d'incubation a été définie comme le nombre de jours écoulés entre la ponte du premier œuf et l'éclosion du premier poussin. L'analyse des données enregistrées par les œufs factices a été réalisée avec les logiciels R (R Development Core Team, 2011) et IgorPro (Wavemetrics) et est illustrée dans la figure 2-16. Les données des trois premières heures d'enregistrement ont été supprimées de façon à ce que la température interne de l'œuf artificiel ait atteint des températures correspondant aux températures d'incubation.

*Température*

La température externe maximale a été calculée à partir des données enregistrées par les œufs artificiels. Les maxima horaires de cette température externe maximale ont été calculés. Cette mesure est la plus proche mesure de la température de la poche incubatrice. Les moyennes horaires et quotidiennes des différentes températures ont été calculées. Une période pendant laquelle la température externe maximale était inférieure à 30°C a été définie comme une hypothermie transitoire, dans la mesure où le développement de l'embryon est inhibé pour des températures inférieures à 30°C (Weinrich & Baker, 1978). Une macro a été écrite dans IgorPro pour définir et dénombrer ces événements d'hypothermies, calculer leur durée et le temps qui sépare deux hypothermies.

*Mouvements de rotation*

L'accéléromètre 2D situé à l'intérieur des œufs artificiels a été paramétré pour enregistrer les composantes x et y de la gravité toutes les 10 secondes. L'angle correspondant a été calculé par la fonction atan2(y,x) dans R, puis le changement d'angle entre deux enregistrements a été évalué. L'importation des données de rotation dans IgorPro a permis de visualiser les variations d'angle au cours du temps. Un grand nombre de points correspondent à un bruit de fond de rotation. Le 99ème percentile de l'ensemble des données de rotation de 15 manchots est de 14,7°. Cet angle est proche de l'angle minimum de rotation de 15° choisi par Beaulieu *et al.* (2009). Une macro a été écrite dans IgorPro pour définir et compter les mouvements de rotations, qui correspondent à une période pour laquelle l'angle est supérieur à 15°. On considère qu'il s'agit d'un même mouvement de rotation lorsque deux valeurs supérieures au seuil sont séparées de moins de 2 minutes. Cette durée a été définie en calculant le nombre de rotations pour l'ensemble de l'enregistrement en faisant varier le seuil temporel entre 10 et 650 secondes. La droite de régression qui passe par les trois premiers points coupe l'axe des abscisses à 127 secondes. La macro permet de distinguer chaque événement de rotation et de définir sa durée, l'angle maximum de rotation et la vitesse de rotation (angle cumulé de rotation divisé par la durée). Le nombre de rotations par heure et par jour ont été calculés.

*ANALYSES STATISTIQUES*

L'ensemble des analyses statistiques a été réalisé avec le logiciel R. Le détail des analyses est décrit dans chaque article.

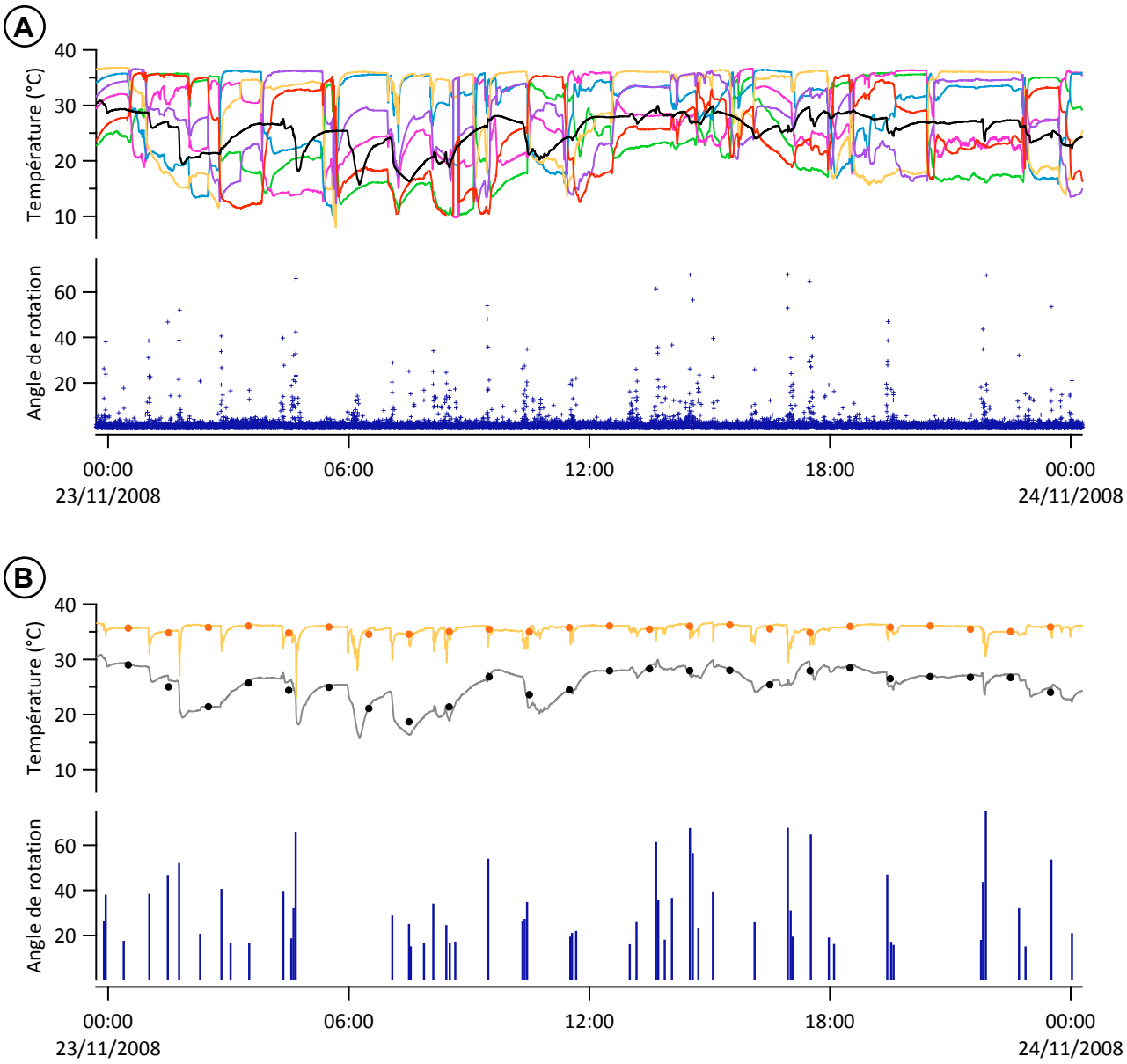
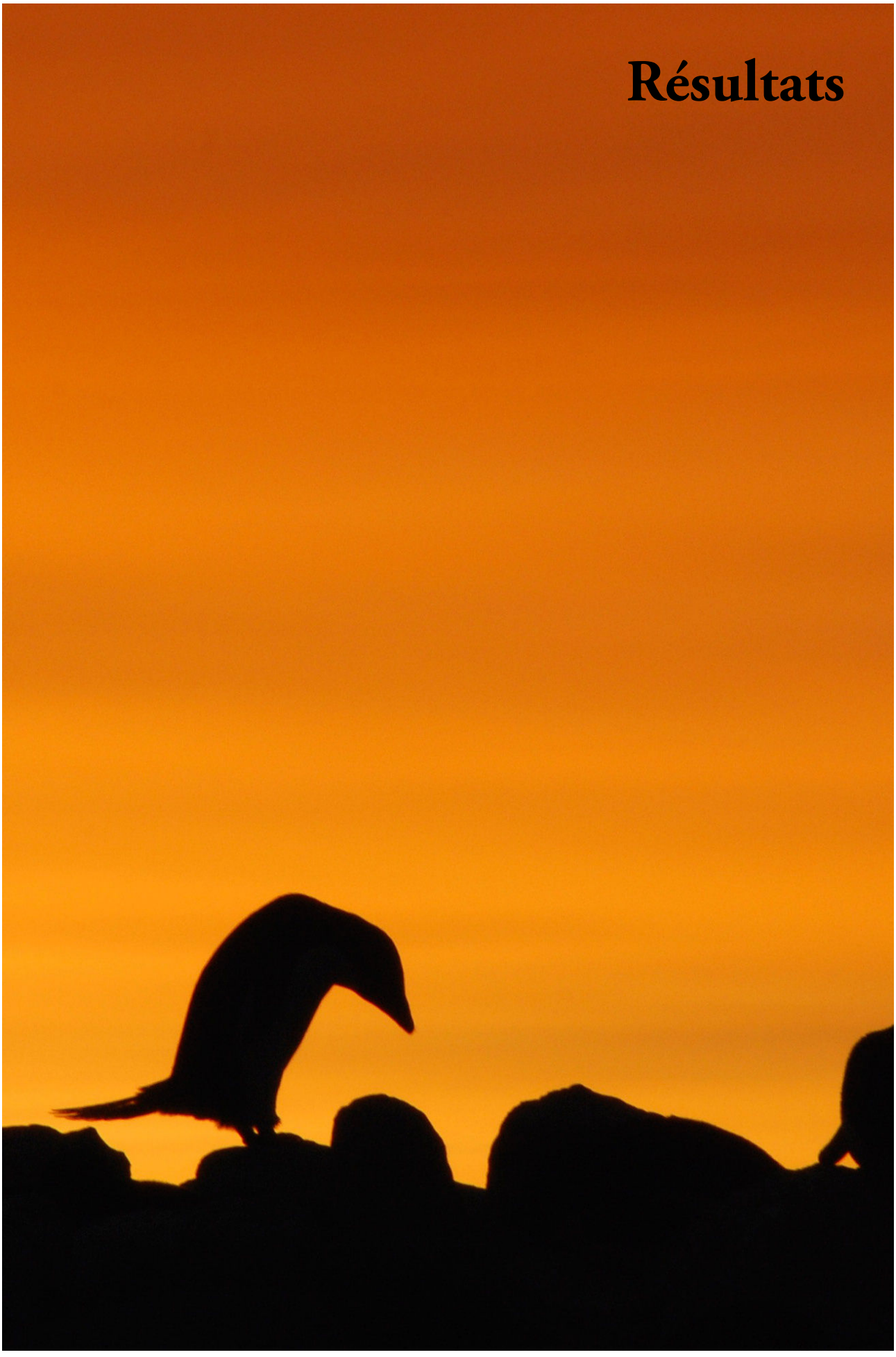



**Figure 2-16 :** Exemple de données enregistrées par un œuf artificiel couvé le 23 novembre 2008 par un manchot témoin (**A**) et illustration du traitement des données (**B**)

A : Données brutes : températures interne (courbe noire) et externes (courbes colorées) enregistrées toutes les 10 secondes, angle de rotation (points bleus) calculé à partir des valeurs d'accélérométrie.
B : Données après traitement : températures interne (courbe noire) et maximale externe (courbe orange), angle maximum par événement de rotation. Les points correspondent aux moyennes horaires de températures.

# Résultats



## Rappel des objectifs

L'objectif général de ce travail de thèse a été de préciser les relations entre le statut endocrinien, les conditions environnementales et l'effort reproducteur d'un oiseau marin polaire, le manchot Adélie, à l'aide d'approches comparatives et expérimentales.

Les effets d'une modification expérimentale du statut endocrinien sur le comportement et le succès reproducteur de manchots Adélie mâles ont été mesurés à différentes périodes du cycle de reproduction. Trois hormones ont été considérées pendant l'incubation : la corticostérone – principale hormone dite de stress chez les oiseaux, la prolactine – fortement impliquée dans le contrôle des soins parentaux, et la testostérone – associée aux comportements sexuels. Les conséquences de variations de certains paramètres environnementaux, notamment les conditions météorologiques pendant l'incubation, ont été considérées, seules ou en interaction avec une modification du statut endocrinien. Les effets d'une augmentation des niveaux de corticostérone pendant l'élevage des poussins, et d'une faible élévation des niveaux de corticostérone pendant l'ensemble de la saison de reproduction ont également été considérés.

# Incubation

# 3. Statut endocrinien et investissement parental pendant l'incubation

*If you march your Winter Journeys you will have your reward, so long as all you want is a penguin's egg.*

*Apsley Cherry-Garrard, The Worst Journey in the World (1922)*

Cette première partie décrit les effets d'une modification du statut endocrinien sur l'investissement parental pendant l'incubation chez le manchot Adélie. L'incubation est une période intéressante du cycle de reproduction des oiseaux. En effet, le maintien des œufs à des températures favorables au développement de l'embryon nécessite de l'énergie, alors que la présence au nid n'est généralement pas compatible avec des activités de recherche alimentaire permettant de fournir cette énergie. Cette période peut se terminer de façon prématurée lorsque les réserves énergétiques sont épuisées (Ainley *et al.* 1983 ; Aldrich & Raveling, 1983 ; Groscolas *et al.* 2000 ; Spée *et al.* 2010) ou que les conditions environnementales sont sévères (Davis & McCaffrey, 1986 ; Astheimer *et al.* 1995 ; Conway & Martin, 2000). Les coûts énergétiques liés à l'incubation sont particulièrement importants pour les espèces qui se reproduisent dans des environnements inhospitaliers comme les déserts, les hautes altitudes, et dans les régions froides (Tinbergen & Williams, 2002 ; Piersma *et al.* 2003). Parmi ces oiseaux, les manchots sont des modèles intéressants pour étudier les variations de l'investissement parental pendant l'incubation : ils jeûnent pendant de longues périodes et se reproduisent dans des zones où les conditions météorologiques sont fréquemment sévères. Pourtant, peu d'études se sont intéressées au comportement d'incubation des manchots Adélie (Derksen, 1977 ; Beaulieu *et al.* 2010b) ou d'autres espèces de manchots (Handrich, 1989 ; Groscolas *et al.* 2000). Le comportement de manchots Adélie mâles en réponse à une modification expérimentale de leur statut endocrinien lors de l'incubation a été mesuré de façon détaillée à l'aide d'œufs factices enregistreurs de températures et de mouvements de rotation et d'un suivi vidéo permettant de déterminer a posteriori le budget temps comportemental et la position des manchots sur le nid.

## 3.1 Etude 1 : Corticostérone, conditions météorologiques, et incubation

**Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins**

Anne-Mathilde Thierry, Sylvie Massemin, Yves Handrich, Thierry Raclot

*Hormones and Behavior*

2013
63: 475-483

Cette étude expérimentale s'est intéressée aux effets d'une augmentation expérimentale des niveaux de corticostérone sur l'investissement parental de manchots Adélie mâles pendant l'incubation. L'effet cumulé de l'augmentation des niveaux de corticostérone et de mauvaises conditions météorologiques (blizzard) a également été considéré. La durée d'incubation et le succès reproducteur de 60 manchots ont été suivis de la fin de la pariade jusqu'à l'éclosion. Les couvées de certains oiseaux ont été remplacées par des œufs factices enregistreurs de température et de mouvements de rotation, permettant un suivi détaillé du comportement d'incubation.

**Abstract**

Corticosterone, the main stress hormone in birds, mediates resource allocation, allowing animals to adjust their physiology and behaviour to changes in the environment. Incubation is a time and energy-consuming phase of the avian reproductive cycle. It may be terminated prematurely, when the parents' energy stores are depleted or when environmental conditions are severe. In this study, the effects of experimentally elevated baseline corticosterone levels on the parental investment of incubating male Adélie penguins were investigated. Incubation duration and reproductive success of 60 penguins were recorded. The clutches of some birds were replaced by dummy eggs, which recorded egg temperatures and rotation rates, enabling a detailed investigation of incubation behaviour. Corticosterone levels of treated birds were 2.4-fold higher than those of controls 18 days post treatment. Exogenous corticosterone triggered nest desertion in 61% of the treated birds; consequently reducing reproductive success, indicating that corticosterone can reduce or disrupt parental investment. Regarding egg temperatures, hypothermic events became more frequent and more pronounced in treated birds, before these birds eventually abandoned their nest. The treatment also significantly decreased incubation temperatures by 1.3 °C and lengthened the incubation period by 2.1 days. However, the number of chicks at hatching was similar among successful nests, regardless of treatment. Weather conditions appeared to be particularly important in determining the extent to which corticosterone levels affected the behaviour of penguins, as treated penguins were more sensitive to severe weather conditions. This underlines the importance of considering the interactions of organisms with their environment in studies of animal behaviour and ecophysiology.

## Introduction

Understanding how animals manage their energy reserves is a central topic in ecology and physiology as energy constrains many aspects of animal behaviour and life history strategies. Life history theory predicts that individuals should allocate time and available energy for self-maintenance and reproduction in order to maximise their fitness (reviewed in Stearns, 1992). This is particularly true for long-lived species, which should behave as 'prudent parents' (Drent & Daan, 1980) because their lifetime reproductive success is mainly a factor of adult survival (Williams, 1966). A number of parameters can affect both the energy available to an animal and its breeding decisions: environmental conditions such as weather conditions (Fisher *et al.* 2004), food availability (Weimerskirch *et al.* 2001), or predation risk (Fontaine & Martin, 2006), and the parents' state, including, among others, body condition, age and breeding experience (McNamara & Houston, 1996).

In breeding vertebrates, when environmental conditions deteriorate dramatically and/or when the parent's energetic state reaches a minimum threshold, an emergency life-history stage is expressed, causing the individual to abort the current breeding attempt, to ensure survival of the perturbation (Wingfield, 2003; Wingfield & Kitaysky, 2002; Wingfield *et al.* 1998). Hormones play an important role in enabling birds to adjust their behaviour to both predictable (seasonal) and unpredictable regimes of environmental variation (Jacobs & Wingfield, 2000; Wingfield & Silverin, 2009). In particular, glucocorticoids are released under life-threatening situations, such as food shortage (Kitaysky *et al.* 1999), severe weather conditions (Romero *et al.* 2000), predation risk (Cockrem & Silverin, 2002), or disturbance (Fowler, 1999; Romero and Remage-Healey, 2000), and can trigger the emergency stage (Landys *et al.* 2006; Wingfield *et al.* 1998). Glucocorticoids are usually maintained at relatively low levels, fluctuating on a daily and annual basis to regulate energy availability and utilisation (Dallman *et al.* 1994). Elevated glucocorticoid levels affect the physiology and behaviour of animals in a variety of ways, by controlling energy metabolism through glucose mobilisation, increasing lipogenesis and fat deposition, increasing locomotor activity, foraging behaviour and food intake, promoting escape behaviour, and finally, leading to a reduction in or abandonment of the reproductive effort (see Breuner, 2011; Landys *et al.* 2006 for review). In birds, corticosterone (CORT) is the main glucocorticoid. High baseline CORT levels are associated with low reproductive success and a high potential for abandonment of reproduction (Bonier *et al.* 2009a ; Groscolas *et al.* 2008 ; Ouyang *et al.* 2012; Silverin, 1986; Spée *et al.* 2010; Wingfield, 2003). High CORT levels are suggested to tip the trade-off between reproduction and survival in favour of survival, so that under stressful conditions birds will mobilise energy for self-maintenance rather than for reproduction (Angelier *et al.* 2007a; Landys *et al.* 2006; Spée *et al.* 2010; Wingfield *et al.* 1998).

Numerous studies have investigated the hormonal basis of the activation of the emergency life-history stage in recent years, in particular in the context of behavioural and physiological responses to environmental perturbations (Breuner, 2011; Wingfield *et al.* 1998; Wingfield & Ramenofsky, 1997). Poor body condition has been suggested to cause the greatest and most rapid increase in circulating CORT, and also

the most pronounced behavioural response to inclement environmental conditions, possibly accelerating nest desertion (Breuner & Hahn, 2003). To date, too few studies have considered how elevated baseline CORT levels and severe weather conditions could both affect behaviour and parental investment in the current breeding attempt (Breuner & Hahn, 2003). Breuner and Hahn proposed a model to describe the interactions of weather, food availability, body condition, and stress physiology in initiating departure from the breeding site. Their study showed that exogenous CORT delayed the return of white-crowned sparrows *(Zonotrichia leucophrys oriantha)* to their breeding site, when the territory was abandoned after a snow storm. However, exogenous CORT alone did not induce territory abandonment when weather conditions were favourable, but it increased the activity range around the breeding site (Breuner & Hahn, 2003). This suggests that an animal with elevated CORT levels prior to a severe weather event may be more sensitive to these stressful conditions than an individual with low CORT levels. Yet, the only study considering at the same time stress physiology, weather conditions, and reproductive behaviour cited above has been carried out on income breeders. However, the physiological and behavioural responses to both elevated CORT levels and severe weather conditions may be amplified for capital breeders, relying upon stored energy reserves to support reproductive output through prolonged fasting (Drent & Daan, 1980; Jönsson, 1997).

Incubation is an interesting stage of the avian reproductive cycle since it poses conflicting difficulties for adults. On the one hand, high rates of heat production require energy, while nest attendance usually prevents animals from foraging to replenish their energy stores. Incubation may be prematurely terminated, when the parents' energy stores are depleted (Ainley *et al.* 1983; Aldrich & Raveling, 1983; Groscolas *et al.* 2000; Spée *et al.* 2010) or when environmental conditions are severe (Astheimer *et al.* 1995; Conway & Martin, 2000; Davis & McCaffrey, 1986). Incubation costs are especially high for species breeding in inhospitable environments such as deserts, at high altitudes, and in cold regions, as they potentially lose heat rapidly (Piersma *et al.* 2003; Tinbergen & Williams, 2002). Among these birds, penguins are valuable models to investigate parental investment during incubation, and enable us to integrate stress physiology with environmental conditions and behaviour. Penguins are long-lived animals which undergo spontaneous fasting while on land, and breed in areas with a high incidence of severe weather. However, few studies have investigated the incubation behaviour of Adélie penguins (Beaulieu *et al.* 2010b; Derksen, 1977) or of any other penguin species (Groscolas *et al.* 2000; Handrich, 1989).

Previous studies have reported that harsh environmental conditions such as unusual sea ice conditions are correlated with CORT levels in Adélie penguins (Cockrem *et al.* 2006), and also that exogenous CORT can trigger nest desertion in the same species (Spée *et al.* 2011a). An investigation that combines stress physiology and behaviour of a free-living fasting seabird with weather conditions has, to our knowledge, not yet been conducted. Therefore, the objective of this study was to investigate the effects of both elevated baseline CORT levels and inclement weather conditions on the incubation behaviour of a capital breeder, the Adélie penguin. CORT is described as adjusting life-history strategies to environmental conditions as well as physiological state (Wingfield & Sapolsky, 2003). Because increased levels of CORT redirect behavioural and metabolic processes from high energy demanding to emergency activities to the benefit

of survival (Wingfield *et al.* 1998), we predicted that high CORT levels would decrease parental care of incubating birds and decrease the reproductive success at hatching, notably when penguins are exposed to severe weather conditions.

To better understand the role of elevated CORT levels on the reproductive behaviour of male Adélie penguins, we monitored how experimentally elevated CORT levels affected nest attendance, hatching success, and incubation duration, as well as incubation behaviour (incubation temperature and egg rotation rates, which were taken as a proxy of the birds' investment in the current breeding attempt). The extent to which CORT levels affected the behaviour of penguins during severe weather conditions was also considered. Finally, to evaluate potential long-term detrimental effects of the CORT-treatment to the penguins, we monitored their return rate the year following experimentation.

## Materials and Methods

### *STUDY SITE AND SPECIES*

The study was carried out at the French polar station Dumont d'Urville (66°40'S, 140°01'E, Adélie Land, Antarctica) during three consecutive austral summers (2007-2010). The protocol was approved by the ethical committee of the French Polar Institute Paul-Emile Victor. Adélie penguins are long-lived seabirds that breed during the short summer in colonies around Antarctica, from late October to early March. Adélie penguins were monitored during courtship and incubation period, from mid-November until the end of December. Females usually depart to sea to refeed as soon as they complete their clutch by laying the second egg (Sladen, 1958). Males then start to incubate the egg(s) until females return from sea, on average after 12 to 15 days. Under normal incubation routine, mates alternate between foraging trips at sea and incubation bouts on land (Fig. 1). The incubation period for an Adélie penguin egg ranges between 30 and 39 days (Ainley, 2002).

### *EXPERIMENTAL PROCEDURE*

#### *Capture, handling, and nest monitoring*

Adélie penguins were first handled at the end of the courtship period (between November 10 and 20). A total of 60 nests were randomly selected (20 per breeding season). Males were marked using black dye (Nyanzol-D) on chest feathers and stickers, which were inserted between feathers on their back. Penguins were sexed by a combination of parameters including copulatory position, cloacal inspection and incubation routine (Sladen, 1958). They were weighed with an electronic balance (Ohaus, Giessen, Germany, resolution: ± 2 g), and the length of the left flipper was measured to calculate a scaled mass index (Peig & Green, 2009). To acquire incubation routine data (i.e. determine which partner is present on the nest), nests were checked from a distance of 10 to 30 m every two to three hours (between 6 am and 1 am) for the entire study period.

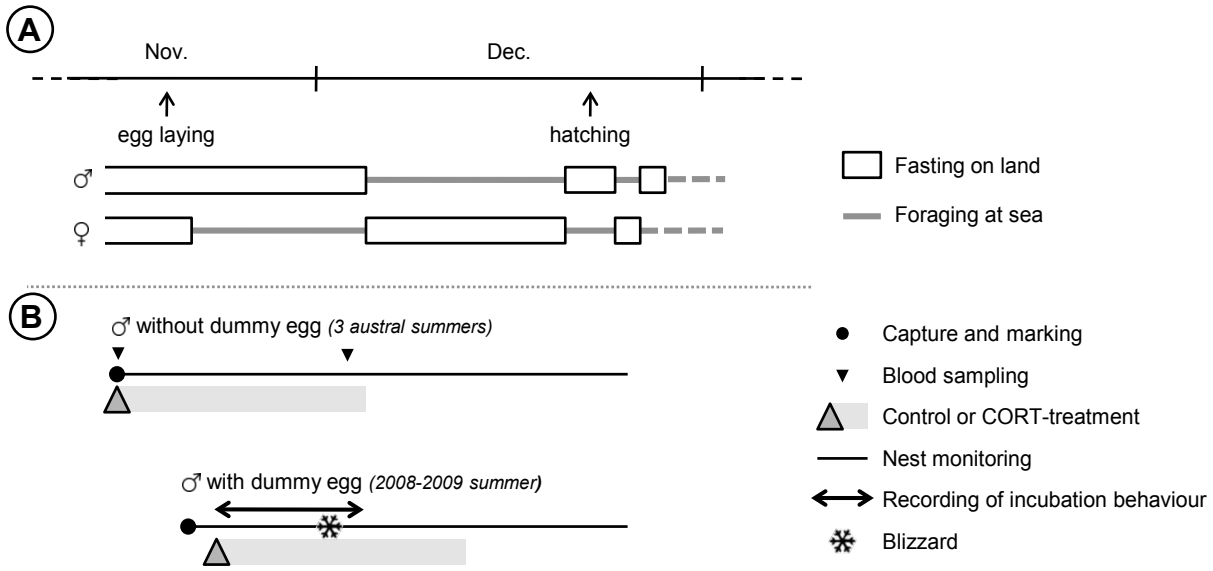



**Figure 1:** Patterns of nest attendance during the incubation period (**A**) and study protocol (**B**)

Male Adélie penguins were separated into four groups depending on treatment (CORT or control) and whether their clutch was replaced by a dummy egg to record incubation behaviour.

*Experimental manipulation of corticosterone levels and nest success: birds without dummy eggs*

During initial handling, a blood sample was collected from the alar vein (2 mL syringe, 25-gauge needle) in 29 males within 3 to 5 min of capture. To minimise handling stress, a bird's head was covered with a hood (Cockrem *et al.* 2008). Handling times of up to 5 min have been shown to have no effect on CORT levels in Adélie penguins (Vleck *et al.* 2000). Blood samples were transferred into tubes pre-treated with 25 µL EDTA and centrifuged (10 min at 5000 rpm and 4 °C). Plasma was kept at -20 °C until laboratory analyses.

23 males received a corticosterone pellet (G-111, 100 mg, 21-day release) obtained from Innovative Research of America (Sarasota, FL, USA). The pellet was inserted subcutaneously in the nape of the neck. Similar pellets have been used successfully in previous studies on eider ducks (Bourgeon & Raclot, 2006) and Adélie penguins (Spée *et al.* 2010; Spée *et al.* 2011b). The skin was disinfected with rubbing alcohol and a small incision was made in the nape of the neck, using a scalpel blade. After positioning the pellet subcutaneously with tweezers, the incision was closed with sterile suture and Alumisol® spray (CEVA, Libourne, France) was applied topically. A further 23 males were sham-operated. They underwent the same procedure during initial handling, including skin incision and sterile suture, without actual implantation of a pellet (Table 1, Fig. 1). Handling time averaged 15 min (range: 11-23 min). A previous study carried out on captive Adélie penguins showed no significant difference in behavioural and hormonal responses of birds, which were either implanted with a placebo pellet or sham-operated and not implanted (Spée *et al.* 2011b). 27 birds were recaptured before leaving the colony, either during nest relief by their partner or following nest desertion. Birds were weighed and another blood sample was taken to assess CORT levels post treatment. The number of eggs and/or chicks was recorded once a day during nest monitoring to determine laying and hatching dates. Nest success was determined as the percentage of successful nests at

the end of incubation. Incubation duration was defined as the difference between the laying date of the first egg and the hatching date of the first chick.

*Experimental manipulation of corticosterone levels and incubation behaviour: birds with dummy eggs*

During the 2008-2009 austral summer, the clutches of 14 males were replaced by dummy eggs that recorded temperature and egg position every 10 s to examine incubation behaviour in detail. Five birds received a CORT pellet on November 19, and their clutches were replaced by dummy eggs to record incubation behaviour until November 28. Due to the limited number of dummy eggs, and in order to increase sample size, four other birds were CORT-treated on November 28, and incubation behaviour recorded until early December. Five controls birds underwent the same procedure between November 20 and 25, without actual implantation of a pellet (Table 1). Incubation behaviour of all birds was recorded at the same incubation stage, zero to three days following female departure. For these 14 birds, no blood sample was taken in order to minimise handling duration. The penguins were also implanted with a subcutaneous passive transponder tag (0.8g, Texas Instrument TIRIS, Dallas, TX, USA) under the skin of their leg. The nests occupied by the birds and the adjacent nests were checked with a manual antenna at the end of November 2009 (during the first incubation shift of the males) to assess the birds' return rate one year after the experiment, Adélie penguins having very high nest fidelity (Ainley, 2002). Dummy eggs were produced at the IPHC-DEPE (Strasbourg, France) and were the same size and shape as genuine penguin eggs. Seven temperature probes (six external probes, located equidistantly around the mid-shell, and one internal probe) and a bi-axial accelerometer recorded egg temperatures and egg position every 10 s, respectively. Time constants were 1.5 s and 8.7 min (3.5 s and 20 min to reach 90% of the temperature equilibrium) for the external and internal temperature probes, respectively, with a resolution of 0.1 °C. Further technical details of the dummy eggs are provided in Beaulieu *et al.* (2010b). During the first incubation shift of a male penguin, its two real eggs were replaced by one single dummy egg. The real penguin eggs were incubated in the laboratory (at 32-34 °C, 180° rotation every 4 to 6 h) and returned to the nest at the end of the male's incubation shift.

*WEATHER DATA*

Weather data were collected at Dumont d'Urville by Météo France. Snowfall data were only available for 6 h periods, and were only recorded from 9 a.m. to 10 p.m., local time. At 10:00 a.m. on November 30 2008 a strong blizzard started, which lasted for 20 h,with both snow and freezing rain, followed by warm temperatures on the next day. This was the only blizzard that occurred during the incubation period of the 2008-2009 season. To investigate the effect of severe weather conditions on the incubation behaviour of control and CORT-treated birds, we defined three distinct 24-h-periods between Nov. 29 10:00 a.m. and Dec. 2 9:59 a.m., which were considered as 'before', 'during' and 'after' the blizzard. Mean air temperatures during these three periods were -2.5 ± 0.4 °C (range: -5.2 to 0.1), -2.7 ± 0.3 (range: -4.9 to -0.6) and -0.5 ± 0.2 (range: -2.1 to 0.6).

*CORTICOSTERONE ASSAYS (birds without dummy eggs)*

Plasma CORT levels were determined in duplicate by immunoassay according to guidelines provided by the manufacturer (AssayMax Corticosterone ELISA Kit, EC3001-1, AssayPro, St. Charles, MO, USA). Plasma samples were diluted 1:4 into EIA diluent before assay. Intra- and inter-assay variations for CORT were 9% and 15%, respectively. Minimum detectable dose of CORT was 0.3 ng/mL.

*DATA ANALYSIS (birds with dummy eggs)*

Incubation behaviour data recorded with the dummy eggs were analysed using R (R Development Core Team, 2011) and IgorPro Software (Wavemetrics, Las Oswego, OR, USA). To remove potential differences in initial dummy egg warming between birds, data recorded during the first 3 h were discarded. Maximum egg temperature recorded by one of the six external probes was calculated (in selecting the sensor that recorded the highest temperature) and averaged daily (0:00 a.m. to 11:59 p.m.). Hourly maxima of maximum temperature (i.e. the single highest value during one hour) were also calculated as an estimate for the temperature of the brood patch and averaged daily. Periods when maximum temperature fell below 30 °C were defined as transitory hypothermia, as incubation temperatures below 30 °C have been shown to inhibit the embryonic development in Adélie penguin eggs (Weinrich and Baker, 1978). The total number of hypothermic events per day was calculated. Rotation events were counted to compare egg rotation rates between control and CORT-treated birds. In the following, 'egg temperatures' refer to the means of maximum temperature recorded by the six external probes, while 'maximum egg temperatures' refer to maxima of maximum temperature, reflecting brood patch temperature. The bi-axial accelerometer located inside the dummy eggs recorded the two perpendicular components of gravity. The corresponding angle was calculated using the 'atan2' function in R. Per definition, a rotation event occurred, when two criteria were fulfilled: (1) the change in egg position between two recordings was above a threshold defined by the 99th percentile of all rotation data for control birds (15°), which corresponded to a value above the background noise level; (2) two consecutive changes in egg angle over the 15° threshold were separated by less than 2 min. To define the time threshold, we first calculated the number of rotation events using time thresholds of 10 and 20 s. Plotting the number of calculated rotation events against the corresponding time threshold used showed that the resulting curve intersected the x-axis at 2 min. Using this 2 min threshold, a macro was written to calculate the number of rotation events per day, as well as maximum rotation angle and rotation speed.

*STATISTICAL ANALYSIS*

All statistical analyses were performed with R 2.13.3. Results are expressed as means ± SE and differences were considered as statistically significant when $p<0.05$.

*Birds without dummy eggs*

Since data were not distributed normally, we used a Wilcoxon test to compare date of implantation, body mass and scaled mass index at the beginning of the experiment, laying date, and clutch size of control birds with that of CORT-treated birds. Wilcoxon tests were also used to compare baseline CORT levels

between control and CORT-treated birds at the time of the treatment and at the end of the male incubation shift, and to compare pre-treatment baseline CORT levels between years. The proportion of successful nests between groups was compared with Fisher's exact tests. Differences were first tested between years for control birds. As there were no differences, the proportion of successful nests for control vs. treated birds was compared. Differences in incubation duration were assessed using a two-way ANOVA with treatment, year, and interaction between treatment and year as factors, after testing for normality and homogeneity of variances using the Shapiro–Wilkes and Bartlett tests, respectively. Differences in number of chicks at hatching were assessed with a Generalised Linear Model with a Poisson distribution, with treatment, year, and interaction between treatment and year as factors. Model selection was performed by excluding non-significant interactions. Tukey Honest Significant Differences were calculated when required.

*Birds with dummy eggs*

To take into account the spatial and temporal correlation structure of the incubation behaviour data, Generalised Estimating Equations (GEE) were used (Højsgaard *et al.* 2006; Yan, 2002; Yan and Fine, 2004). Because consecutive measures of incubation temperatures for each bird lack independence, GEE (Hardin and Hilbe, 2007; Liang and Zeger, 1986) were used for the regression, assuming an autoregressive (AR[1]) correlation structure to more appropriately estimate regression coefficient variances. The *geeglm* function of the *geepack* package in R was used to compare incubation behaviour, with repetition on penguin identity, treatment as a fixed factor, day (number of days since November 19), air temperature, and mean wind speed as covariates, and interaction between treatment and day. We tested if exogenous CORT and the covariates affected egg temperatures, frequency of transitory hypothermia, and egg turning rates. For the penguins that incubated during a blizzard (five control and four CORT-treated birds), incubation behaviour (hourly mean of egg temperature and number of egg rotation per hour) was compared during the 24 h before, during, and after the blizzard to determine the potential cumulative effect of severe weather conditions and elevated CORT levels on incubation behaviour. Models were compared using ANOVA in *geepack* (Zuur *et al.* 2009). Model selection was performed by excluding non-significant interactions first, and then all non-significant covariates. Finally, statistical analyses were also performed with incubation data of CORT-treated birds separated in two data sets according to treatment date. These analyses gave similar results to those reported below with CORT-treated birds as a single group.

## Results

*BIRDS WITHOUT DUMMY EGGS*

*Pre-treatment*

Date of initial handling and implantation did not differ between control and CORT-treated birds whose clutch was not replaced by a dummy egg (Wilcoxon, W=297.5, p=0.473, Table 1). At the time of implantation, these birds had a similar body mass (Wilcoxon, W=204.5, p=0.191, Table 1) and scaled

mass index (Wilcoxon, W=215.5, p=0.287). Laying date (Wilcoxon, W=230, p=0.451, Table 1) and clutch size (Wilcoxon, W=277, p=0.625, Table 1) were also not significantly different between these groups.

**Table 1:** Study layout and breeding parameters for 60 male Adélie penguins

| | **Without dummy eggs** | | **With dummy eggs** | |
|---|---|---|---|---|
| | **Control** (n=23) | **CORT** (n=23) | **Control** (n=5) | **CORT** (n=9) |
| Date of implantation | 15-Nov ± 0.59 | 14-Nov ± 0.54 | 23-Nov ± 1.64 | 23-Nov ± 1.58 |
| Male body mass at implantation (kg) | 4.98 ± 0.08 | 5.13 ± 0.08 | 4.74 ± 0.13 | 4.75 ± 0.11 |
| Laying date | 18-Nov ± 0.50 | 18-Nov ± 0.53 | NA | NA |
| Female departure date* | 21-Nov ± 0.58 | 22-Nov ± 0.55 | 22-Nov ± 0.97 | 22-Nov ± 1.19 |
| No. of eggs per nest | 1.91 ± 0.06 | 1.83 ± 0.10 | 2 ± 0.0 | 2 ± 0.0 |
| Start of incubation behaviour recording | NA | NA | 23-Nov ± 0.73 | 23-Nov ± 1.58 |
| End of incubation behaviour recording | NA | NA | 5-Dec ± 0.72 | 30-Nov ± 0.88 |
| Male departure date | 6-Dec ± 0.64 | 3-Dec ± 1.16 | 5-Dec ± 0.81 | 2-Dec ± 0.95 |
| Hatching date | 22-Dec ± 0.55 | 27-Dec ± 0.75 | NA | NA |
| Return rate** | NA | NA | 4/5 | 9/9 |

* Female departure date can be used as a proxy of laying date (Sladen, 1958, Ainley 2002).
** Proportion of males that returned to their nest site the year following experimentation.

Birds were separated into four groups depending on treatment (Control vs. CORT) and the presence or absence of a dummy egg on the nest. Results are expressed as means ± SE.

*Plasma corticosterone levels*

At the time of implantation, baseline CORT levels did not differ between control and CORT-treated penguins (Wilcoxon, W=82, p=0.126, Fig. 2A). Year had no effect on pre-treatment CORT levels (Wilcoxon, W=74, p=0.934). CORT levels significantly increased between the time of implantation and departure to sea in CORT-treated birds (Wilcoxon, W=23, p=0.010, Fig. 2A). CORT levels of CORT-treated birds were 2.4-fold higher than those of controls at the time of departure to sea, 17.7 days post treatment (18.3 ± 3.7 vs. 7.6 ± 1.1 ng/mL; Wilcoxon, W=23, p=0.016, Fig. 2A).

*Incubation success*

Nest success differed significantly between control (89.3%) and CORT-treated birds (39.3%) (Fisher's exact test, p<0.001, Fig. 2B). The lower nest success of CORT-treated birds can be attributed to a higher proportion of males that deserted their nest during this phase, when compared with control penguins (see also Spée *et al.* 2011a). Incubation duration could only be measured for successful nests. Year had a significant effect on incubation duration (ANOVA, $F_{2,25}$=3.96, p=0.032), incubation duration being 1.9 days shorter during the 2009-2010 austral summer compared to 2007-2008 (TukeyHSD, p=0.032). Incubation duration in CORT-treated birds was significantly 2.1 days longer than in control birds

(ANOVA, $F_{1,25}$=6.94, p=0.014, Fig. 2C). Among the successful nests, the number of chicks at hatching was similar between years and treatment (GLM with a Poisson distribution, p>0.425).

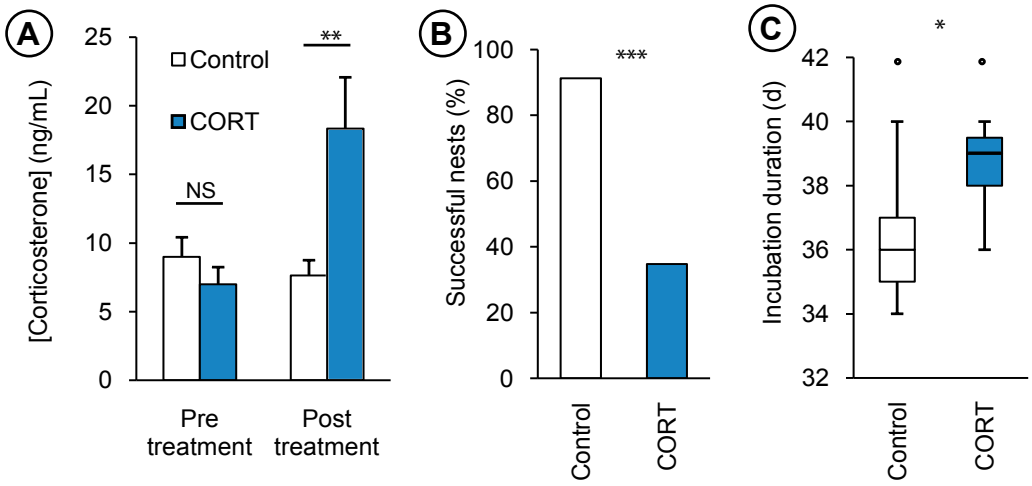



**Figure 2:** Effects of CORT-treatment on corticosterone levels (**A**), incubation success (**B**), and incubation duration (**C**) for birds whose clutch was not replaced by a dummy egg

Corticosterone levels of control and CORT-treated male Adélie penguins (mean ± SE, n=29, 15 control and 14 treated birds) are shown at the time of implantation (Pre treatment) and at the end of the first incubation shift (Post treatment). Values for nest success (n=46, 23 control and 23 treated birds) and incubation duration (n=29, 21 control and 9 treated birds) were averaged (mean ± SE) over three summers for birds, whose clutch was not replaced by a dummy egg. White circles correspond to extreme values. NS: Non Significant. * p<0.05, ** p<0.01, *** p<0.001.

*BIRDS WITH DUMMY EGGS*

*Pre-treatment*

Date of implantation did not differ between control and CORT-treated birds whose clutch was replaced by a dummy egg (Wilcoxon, W=25, p=0.781, Table 1). Control and CORT-treated birds did not differ in body mass (W=29.5, p=0.386, Table 1) or scaled mass index at the time of implantation (W=30.5, p=0.317).

*Return rate*

Return rate of male Adélie penguins one year after the experiment was not affected by treatment (Fisher's exact test, p=1, Table 1).

*Incubation behaviour*

Individual egg temperature profiles varied greatly between birds (Fig. 3A). In general, incubation temperatures in CORT-treated birds were lower than in control birds. In particular, eggs of a CORT-treated bird that eventually deserted its nest experienced several severe decreases in incubation temperature. These hypothermic events became more frequent and pronounced over time, before definitive nest abandonment (Fig. 3A).

We tested whether exogenous CORT affected egg temperatures and rotation rates in incubating birds. Egg temperatures were significantly lower for CORT-treated birds, when compared with control penguins (Fig. 3B). Egg temperature of control birds was 35.19 ± 0.23 °C, while that of CORT-treated

birds was 1.27 °C lower (GEE, Wald=35, p<0.001). Maximum egg temperatures were also significantly lower for CORT-treated birds, when compared with controls (GEE, Wald=36.72, p<0.001). Day, air temperature, mean windspeed and interaction between day and treatment had no significant effect neither on egg temperature (GEE, p>0.106) nor on maximum egg temperature (p>0.130). There was a significant increase in the number of transitory hypothermic events per day in CORT-treated birds, when compared with controls (GEE, Wald=4.4, p=0.036, Fig. 3C). However, the duration of hypothermic events did not differ between groups (GEE, Wald=1.97, p=0.16). Egg rotation rates were not affected by CORT-treatment (GEE with a Poisson distribution, Wald=1.32, p=0.251, Fig. 3D). The interaction between day and treatment, as well as mean wind speed, significantly reduced egg rotation rates (GEE, Wald=4.41, p=0.036; Wald=6.90, p=0.009, respectively). There was no difference between groups for the other rotation parameters determined (maximum rotation angle, cumulative rotation angle, duration of rotation events).

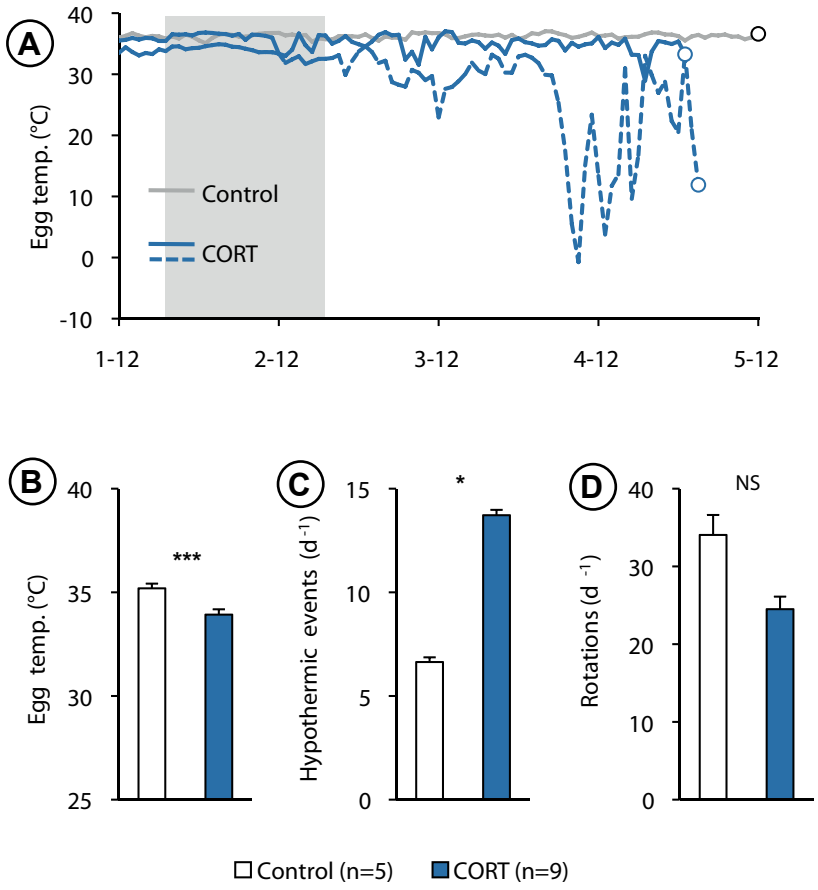



**Figure 3:** Parameters of incubation behaviour recorded from control and CORT-treated male Adélie penguins. Shown are egg temperatures (**A, B**), number of transitory hypothermic events per day (when egg temperature fell below 30°C) (**C**), and number of egg rotations (**D**)

Fig. 3A corresponds to egg temperatures recorded from one control and two CORT-treated birds. These birds exemplify the pattern seen in their respective groups. The grey solid line shows the egg temperature of a control bird, while the black solid line corresponds to the egg temperature of a CORT-bird that did not desert the nest. By contrast, the black dashed line shows the egg temperature of a CORT-bird that deserted its nest on December 4. The shaded area indicates the period during which penguins were exposed to a blizzard. White circles represent the end of incubation behaviour recordings. Values shown in Fig. 3B, 3C, and 3D, are means ± SE (n=14; 5 control, 9 CORT-treated birds; for incubation temperatures and egg rotation rates: 58 vs. 50 daily observations). NS: Non Significant, * p<0.05, *** p<0.001.

*SEVERE WEATHER CONDITIONS*

Incubation behaviour of five control and four CORT-treated birds was recorded during a blizzard. Incubation temperatures were markedly decreased in CORT-treated birds during and after the blizzard event, when compared with control birds (Fig. 4A). Egg temperatures were significantly affected by both CORT treatment (GEE, Wald=4.64, p=0.031) and blizzard (before vs. during, Wald=0.03, p=0.866; before vs. after, Wald=3.87, p=0.049; during vs. after, Wald=2.30, p=0.130). Interaction between CORT treatment and blizzard periods was also significant ($\chi^2$=6.67, p=0.036). The number of egg rotations did not differ between control and CORT-treated birds (GEE, Wald=0.17, p=0.680, Fig. 4B), but was significantly lower during the blizzard than before or after in both groups (GEE, Wald=10.46, p=0.001).

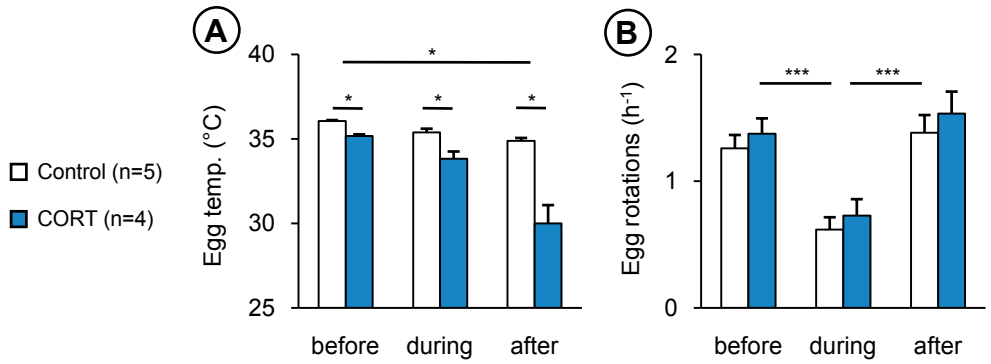



**Figure 4:** Effects of a blizzard on incubation behaviour. Shown are the egg temperatures (**A**) and the number of egg rotations per hour (**B**) before, during, and after a blizzard in control (n=5, 360 hourly observations) and CORT-treated (n=4, 250 hourly observations) male Adélie penguins

Values shown are means ± SE. * p<0.05, ***p<0.001.

## Discussion

The purpose of this study was to investigate the effects of increased CORT levels and severe weather conditions on the parental behaviour of incubating male Adélie penguins. The experimental increase in baseline CORT levels led to either incubation failure or a lengthening of the incubation period. Lower incubation temperatures were recorded in CORT-treated birds, especially when associated with harsh weather conditions.

*METHODOLOGICAL CONSIDERATIONS*

Self-degradable pellets are an efficient tool to artificially elevate circulating hormone levels in field studies, requiring one single intervention. However, the pellets do not strictly work in birds as reported by the manufacturer (Müller *et al.* 2009). The use of CORT pellets in birds may result in peak-like elevation of circulating CORT during a shorter period than expected. The increase in CORT levels and the time-

course over which CORT levels changed in treated birds are in agreement with the previous results obtained from eider ducks (Bourgeon & Raclot, 2006) and Adélie penguins (Spée *et al.* 2011a; Spée *et al.* 2011b) using similar pellets.
The effects of circulating CORT are assumed to be similar to those observed in birds whose clutch was not replaced by a dummy egg, which were actually monitored for CORT levels. We expect no major differences in behavioural or hormonal responses based on the difference of timing of CORT-treatment between birds with and without dummy eggs, mainly because all birds had been fasting for several weeks.

*PARENTAL INVESTMENT IN THE INCUBATION*

Previous studies have reported that elevated baseline CORT levels can reduce, or even disrupt, parental care (Love *et al.* 2004; Silverin, 1986; Spée *et al.* 2011a; Wingfield & Kitaysky, 2002). Our results support this finding, since 60.7% of the CORT-treated penguins deserted their nest (Fig. 2B). Similar observations have been reported previously for the same species (Spée *et al.* 2011a). Reproductive success drops to zero when birds ultimately abandon their nest and such behaviour may appear mal adaptive. However, it increases the chances for individuals to survive the perturbation, in our case elevated CORT levels, so that the prospect of future reproductive attempts is preserved. Hence, nest desertion can be seen as an abandonment of the current breeding attempt in favour of survival in long-lived species.

In our study, abandoning penguins returned to the colony after nest desertion and the return rate in the following year was not affected by the experimental treatment. However, we are unable to draw any meaningful conclusion concerning the effects of CORT on survival, since our sample size was rather small and unbalanced for such investigation (5 control and 9 CORT-treated birds). Many studies have focused on the effects of elevated CORT levels on reproduction, while studies evaluating the link between CORT and survival in adult birds are still rare (Goutte *et al.* 2010a; Love & Williams, 2008). Using an experimental manipulation of CORT levels in black-legged kittiwakes *(Rissa tridactyla)* in combination with capture-mark-recapture models, Goutte and colleagues (2010a) observed a significantly lower apparent annual survival rate in CORT-implanted birds than in controls. These authors suggested that the hormonal treatment may have down-regulated the endogenous secretion of CORT through prolonged negative feedback. Consequently, a high apparent mortality rate of CORT-treated kittiwakes could have resulted from birds failing to trigger an appropriate stress response, which, as a result, were unable to adjust their behaviour and physiology towards immediate survival. The consequences of the CORT treatment for endogenous CORT secretion in Adélie penguins warrant further investigation.

A total of 39.3% of the CORT-treated penguins incubated successfully (Fig. 2B), but the incubation period was longer (Fig. 2C). Lower incubation temperatures in CORT-treated penguins could explain the extended incubation period, reflecting a slower embryonic development (Hepp *et al.* 2006). Adélie penguin embryos appear to be resilient, as eggs incubated at lower temperatures nevertheless hatched. However, lower incubation temperatures could affect the body condition of chicks at hatching, as well as their stress physiology (DuRant *et al.* 2010).

Lower incubation temperatures caused by exogenous CORT are unlikely the result of a down-regulation of the body core temperature set point. Instead, glucocorticoids are understood to play a permissive role in the induction of fever (Ben-Hur *et al.* 2001). A 'stress-induced hyperthermia' has been observed in rats exposed to novel environments or during handling, with a rise in body temperature of as much as 2°C (reviewed in Kluger, 1991). Glucocorticoids have been characterised as impairing vasodilatation (reviewed in Ullian, 1999). Accordingly, lower incubation temperatures observed in CORT-treated penguins could have been induced by a decrease in the peripheral vasodilatation of the brood patch. The brood patch is formed when the skin on the ventral surface is defeathered, vascularised, and oedematous. This facilitates heat transfer from the incubating bird to the clutch. In many species, the formation of the brood patch is under the control of prolactin (reviewed in Lea and Klandorf, 2002). Decreased prolactin levels have been reported in incubating CORT-treated Adélie penguins (Spée *et al.* 2011a), which might lead to structural changes of the brood patch, thus, decreasing the amount of heat transferred to the eggs.

CORT has also been shown to stimulate locomotor activity and food searching in white-crowned sparrows (Astheimer *et al.* 1992), and to enhance foraging behaviour in chick-rearing black-legged kittiwakes (Kitaysky *et al.* 2001). Similarly, exogenous CORT induced a rise in locomotor activity in captive CORT-treated Adélie penguins (Spée *et al.* 2011b). Consequently, free-living treated birds may either move more on the nest or move away from the nest, resulting in higher or lower egg rotation rates, respectively (Fig. 3D). Whether egg rotation rates can be used reliably as a proxy of adult locomotor behaviour is not certain, however, and detailed behavioural analyses are required to properly assess the time-activity budget of penguins following CORT-treatment. We expect that CORT-treated birds might be more vigilant to external events and consequently, spend less time resting and caring for the eggs.

*BEHAVIOURAL AND HORMONAL CORRELATES OF NEST DESERTION*

Birds may desert their nest, either temporarily or definitively, because of deteriorating environmental conditions and/or when body condition reaches a minimum threshold. The use of dummy eggs, which enabled us to record incubation behaviour in great detail, showed that in nest deserting CORT-treated Adélie penguins transitory abandonments (each causing a severe drop in egg temperature) preceded definitive nest desertion (Fig. 3A). Such transitory abandonments have been reported previously for incubating king penguins undergoing a prolonged fast (Groscolas *et al.* 2000). In that study, transitory abandonments began on average 36 ± 5 h before definitive abandonment. This was accompanied by a significant decrease in egg temperature, which started two days before definitive egg abandonment. In a recent study on captive Adélie penguins, it was shown that the experimental increase in CORT levels caused hormonal and behavioural changes that mimicked the late stage of fasting (phase III) (Spée *et al.* 2011b). In our study, CORT-treatment lead to an immediate decline in incubation temperature, while it took several days before transitory abandonments and eventual nest desertion occurred. Hence, the effect of CORT on incubation behaviour is gradual. This is in agreement with previous studies and suggests that while high CORT levels are a pre-requisite, they alone are not sufficient to induce nest desertion in Adélie penguins.

In the wake of studies on free-living fasting penguins, the existence of a refeeding signal has been proposed, which enables animals to sense the low level of their energy reserves and precedes nest desertion (Cherel *et al.* 1988; Groscolas & Robin, 2001; Robin *et al.* 1998). CORT appears to play an important role in this refeeding signal (Groscolas *et al.* 2008; Spée *et al.* 2011a). However, in our study, not all the CORT-treated penguins deserted their nest (Fig. 2B), confirming as previously explained that the decision to abandon the nest does not exclusively depend on the level of CORT. Several, not mutually exclusive hypotheses can be put forward to explain the high percentage of nest abandonments in CORT-treated penguins. Birds that deserted their nests might have been less experienced breeders and/or they might have been in poorer body condition than the birds which did not desert their nest. Incubating fasting common eider ducks with experimentally elevated CORT levels did not desert their nests, until energy reserves reached a minimum threshold level leading to the proteolytic stage of fasting (Criscuolo *et al.* 2005). The birds might also have had lower prolactin levels or protein use might have been increased in these birds. Prolactin is the most important hormone for the regulation of parental care in birds (Buntin, 1996). Spée *et al.* (2010) showed that Adélie penguins which deserted their nest not only had elevated CORT levels but also decreased prolactin levels and an increased protein breakdown. In a further study, the same authors showed that CORT-treated penguins which were relieved by females had higher initial and final prolactin levels than CORT-treated birds which deserted their nest (Spée *et al.* 2011a). Even though the respective roles of CORT and prolactin in triggering nest desertion have been highlighted recently (Spée *et al.* 2010), it would be of great interest to further assess the effects that a decrease in prolactin alone might have on the parental behaviour of incubating Adélie penguins. This would enable us to distinguish the effects of prolactin from those of CORT.

*SEVERE WEATHER CONDITIONS*

Weather is known to influence incubation rhythms in various bird species (see Hébert, 2002 for a review on the effects of ambient temperature on incubation). This is especially true for species living in extreme environments. In Adélie penguins, weather conditions during incubation and early chick-rearing period seem to be important in determining hatching and breeding success. Incubation duration in Adélie penguins has been positively correlated with windspeed and snowdays (Emmerson *et al.* 2011). During a particularly snowy summer (2010-2011), we noticed many unhatched Adélie penguin eggs at the end of the incubating stage (A.-M. Thierry, unpublished data).

Inclement weather is also known to induce an increase in circulating CORT levels. Dark eyed juncos *(Junco hyemalis)* were shown to have significantly higher CORT levels during a snow storm than before or after (Rogers *et al.* 1993). Similarly, circulating CORT levels of white-crowned sparrows during a severe storm were also highly elevated, causing birds to abandon their nests and territories (Wingfield *et al.* 1983). Birds were seen renesting, when CORT levels had returned to lower levels (Wingfield *et al.* 1983). We did not collect blood samples from our penguins before or after the blizzard, but we expect that similar changes in CORT levels occurred (i.e. an increase during and a decrease after the blizzard). The blizzard and the following warm phase (i.e. temperatures close to 0 °C) affected both egg temperatures and egg rotation rates of our incubating Adélie penguins. Similar to the findings of Breuner and Hahn (2003),

exogenous CORT affected the behaviour of our birds during both good and inclement weather (Figs 3 and 4). Egg temperatures were particularly decreased after snowfall followed by warm temperatures in CORT-treated birds (Fig. 4A).

It can be reasonably proposed that the large decline in incubation temperatures in CORT-treated birds may also have been a result of a lower body condition induced by the pellet independently of any changes in weather conditions. Indeed, Spée and colleagues (2011a) showed that CORT-treated Adélie penguins had a 20% higher loss in daily body mass than controls, while CORT-treatment also altered metabolism. The incubation behaviour of four CORT-treated birds was recorded during the blizzard, which happened four days after implantation, while incubation parameters of five other CORT-treated birds were recorded up to eight days post-treatment during good weather conditions. Thus, if the lower body condition did trigger the further decrease in egg temperatures reported above, we should also have recorded it in birds that did not incubate during a blizzard, which was not the case.

A detailed study investigating the relationship between incubation behaviour and weather conditions would allow determining what conditions may be called severe for incubating Adélie penguins. For snow petrels *(Pagrodoma nivea)*, which breed in similar locations than Adélie penguins, snowfall followed by warm temperatures, causing water run-off, appears to be more problematic than snow and ice (Chastel *et al.* 1993; Wingfield *et al.* 2011). Climate models predict overall increases not only in temperature for Antarctica but also in precipitation (Bracegirdle *et al.* 2008). The resulting water run-off is expected to affect the reproductive success of many Antarctic seabirds (Wingfield *et al.* 2011). In the current study, we present new insights into the responses of a polar seabird species to severe Antarctic weather conditions, i.e. snow followed by warm temperatures; indicating that birds with elevated CORT levels are more sensitive to such conditions.

*CONCLUSION*

Our study shows that elevated baseline corticosterone levels in incubating Adélie penguins can 1) reduce or disrupt parental investment (i.e. increased nest desertion rates and more frequent and pronounced phases of egg hypothermia, before the definitive nest abandonment); 2) decrease incubation temperatures and lengthen the incubation period; and 3) increase the sensitivity of penguins to severe weather conditions. Interestingly, weather conditions were shown to be crucial in determining the extent to which corticosterone levels affected the incubation behaviour of penguins. This underlines the importance of considering the interactions of organisms with their environment in studies of animal behaviour and ecophysiology. In further comparative and experimental studies, it would be interesting to examine the relationships between the hormonal status of parents, their incubation behaviour and environmental conditions on the body condition of chicks at hatching, and, ultimately, on chick growth until departure to sea.

**Acknowledgements**

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## 3.2 Etude 2 : Prolactine et incubation

**Decreased prolactin levels reduce parental commitment, egg temperatures, and breeding success of incubating male Adélie penguins**

Anne-Mathilde Thierry, Sophie Brajon, Sylvie Massemin,
Yves Handrich, Olivier Chastel, Thierry Raclot

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Cette étude a cherché à déterminer les effets d'une diminution expérimentale des niveaux de prolactine sur le comportement d'incubation de manchots Adélie mâles. Si les premières études sur la prolactine chez les oiseaux ont établi que l'hormone jouait un rôle dans la régulation des comportements parentaux (Eisner 1960 ; Lehrman & Brody, 1961), le rôle de la prolactine dans l'induction et le maintien du comportement d'incubation semble principalement reposer sur la relation temporelle entre les variations des niveaux de prolactine et le cycle de reproduction (Williams, 2012). Les effets d'une diminution expérimentale des niveaux de prolactine sur les températures d'incubation, taux de rotation des œufs, budget temps comportemental et position sur le nid ont été mesurés. Enfin, une possible corrélation entre les variations individuelles des niveaux de prolactine et l'investissement parental, dont les températures et taux de rotation pendant l'incubation sont un indice, a également été évaluée.

**Abstract**

Hormones regulate many aspects of an individual's phenotype, including various physiological and behavioral traits. Two hormones have been described as important players in the regulation of parental investment in birds: the glucocorticoid hormone corticosterone and prolactin, a pituitary hormone, widely involved in mediating parental behavior. In comparison with corticosterone, the role of prolactin on parental investment remains poorly documented, and most studies so far have been correlative. In this study, the effects of an experimental decrease of prolactin levels on the incubation behavior of a long-lived seabird species were assessed. Male Adélie penguins were treated with self-degradable bromocriptine pellets, inhibiting prolactin secretion. Filming and subsequent video analysis allowed determination of a behavioral time budget for birds and their position on the nest, while dummy eggs recorded incubation parameters. Incubation duration and breeding success at hatching were also monitored. As expected, bromocriptine-treatment significantly decreased plasma prolactin levels, but did not affect corticosterone levels. The behavioral time budget of penguins was not affected by the treatment. However, treated birds spent significantly more time in an upright position on the nest. These birds also incubated their eggs at lower temperatures and turned their eggs more frequently than controls, resulting in a lengthened incubation period. Despite this, the treatment was insufficient to trigger nest desertion and eggs of treated birds still hatched, indicating that several endocrine signals are required for the induction of nest abandonment. We suggest that the decreased prolactin levels in treated birds offset their timeline of breeding, so that birds displayed behavior typical of early incubation.

**Introduction**

Animals face trade-offs in terms of how energy and resources are allocated to different biological functions, including self-maintenance and reproduction (Stearns, 1992). Although previously disputed, it is now clear that the incubation period of avian reproduction is considerably energy demanding (Nord & Nilsson, 2012; Nord *et al.* 2010), despite the fact that parents remain relatively inactive during this period. The energetic stress that parents experience then is a consequence of the restricted foraging opportunities during incubation (Reid *et al.* 2002). Incubation costs are especially high for species breeding in inhospitable environments, such as polar regions, where eggs may lose heat rapidly (Piersma *et al.* 2003; Tinbergen & Williams, 2002). An emergency life history stage (ELHS) is expressed in breeding vertebrates when the immediate survival is threatened by poor energetic conditions (Wingfield *et al.* 1998). This EHLS shifts energy investment away from reproduction and redirects it towards immediate survival, which is especially true for long-lived species, which should behave as 'prudent parents' (Drent and Daan, 1980). Accordingly, incubation may be prematurely terminated, when energy reserves reach a critical point of exhaustion (Ainley *et al.* 1983; Aldrich & Raveling, 1983; Groscolas *et al.* 2000; Groscolas *et al.* 2008; Spée *et al.* 2010) or when environmental conditions are severe (Astheimer *et al.* 1995; Conway & Martin, 2000; Davis & McCaffrey, 1986).

Activation of the ELHS is known to be mediated by several endocrine mechanisms (Wingfield, 2003; Wingfield & Kitaysky, 2002; Wingfield *et al.* 1998). The reduction or suppression of parental effort is associated with the activation of the hypothalamo-pituitary-adrenal axis and the resulting secretion of glucocorticoids (reviewed by Landys *et al.* 2006). In birds, the ELHS is activated by a release of the stress hormone corticosterone. For instance, elevated corticosterone levels have been reported to reduce brood provisioning and even trigger nest desertion in birds (Love *et al.* 2004; Silverin, 1986; Spée *et al.* 2011a; Wingfield & Kitaysky, 2002). Exogenous corticosterone triggered nest desertion in 60 % of treated incubating Adélie penguins *Pygoscelis adeliae* (Spée *et al.* 2011a; Thierry *et al.* 2013b), indicating that this hormone plays a major role in regulating the outcome of the trade-off between reproduction and survival, but is not necessarily sufficient on its own. Glucocorticoids not only play a major role in mediating behavioral and physiological responses to stress, but are also vital in the maintenance of a daily homeostatic balance (Sapolsky, 2000). Indeed, elevated baseline corticosterone levels have been linked with increased fitness (Bonier *et al.* 2009b; Wingfield & Sapolsky, 2003). For example, female macaroni penguins *Eudyptes chrysolophus* with experimentally elevated corticosterone levels increased their foraging activity with positive consequences in terms of reproductive output (Crossin *et al.* 2012). How elevated corticosterone levels affect the expression of parental care and may trigger nest abandonment remains to be clarified (Angelier *et al.* 2009; Wingfield & Kitaysky, 2002).

One popular hypothesis is that corticosterone affects other endocrine mechanisms that mediate the expression of parental care. In particular, exogenous corticosterone has been shown to decrease circulating prolactin levels (Angelier *et al.* 2009; Buyse *et al.* 1987; Criscuolo *et al.* 2005). Several studies suggest

that the suppressive effects of corticosterone on parental investment may be mediated through a negative effect on prolactin levels (Angelier & Chastel, 2009; Angelier *et al.* 2009). However, studies on the effects of a decrease in prolactin levels alone, i.e. without any concomitant changes in corticosterone concentrations, are rare, precluding conclusions about the respective role of these two hormones in the regulation of parental care. The pituitary hormone prolactin has been widely described as being involved in the initiation and the maintenance of avian parental (reviewed in Buntin *et al.* 1996; Sockman *et al.* 2006) and alloparental behavior (Angelier *et al.* 2006a). Prolactin has been indicated to play an important role in incubation behavior (egg defense and thermoregulation) in birds (Buntin *et al.* 1996; El Halawani *et al.* 1986; Lormée *et al.* 1999). Yet, the role of prolactin on the onset and maintenance of incubation behavior seems to mostly rely on the broad temporal relationship between changes in prolactin levels and the reproductive cycle (reviewed in Williams, 2012), with little experimental evidence of a causal link between hormone levels and the onset of incubation (Sockman *et al.* 2006).

Most of the experimental work investigating cause and effect relationships between prolactin and parental care in breeding birds has been conducted with domesticated species. Motivated by economic interests, these correlative and experimental studies have focused on the understanding of how egg production can be increased and broodiness inhibited (Riddle *et al.* 1935; Sharp, 1997; Sharp *et al.* 1988). However, the role of prolactin in these domesticated species might differ from that in many other bird species. In fact, galliform birds show little parental care, and domesticated species have been artificially selected for different parameters, in particular reduced broodiness (Sossinka, 1982). Studies in free-living birds have mostly been correlative, describing patterns of prolactin secretion and their putative relationship with parental behavior (e.g., Garcia *et al.* 1996; Lormée *et al.* 2000; Setiawan *et al.* 2006). From these studies it is clear that prolactin secretion increases steadily during egg laying and incubation. Its highest level is usually reached during incubation, before it declines again, either immediately following hatching in precocial species, or at the end of the brooding period in altricial species (Vleck, 1998). In penguins, prolactin secretion is endogenously programmed and poorly influenced by external stimuli (Garcia *et al.* 1996; Lormée *et al.* 1999; Vleck *et al.* 2000). In these birds, prolactin levels increase during courtship, peak in mid-incubation, and remain elevated until the end of chick brooding (Vleck *et al.* 1999). A decrease in prolactin levels, accompanied by elevated corticosterone levels, has been reported in king penguins *Aptenodytes patagonicus* and in Adélie penguins that deserted their nest when energy stores were extremely depleted (Cherel *et al.* 1994; Groscolas *et al.* 2008; Spée *et al.* 2010). Such a drop in prolactin levels might decrease the incubation drive and favor nest abandonment. Besides, prolactin levels were not correlated with corticosterone levels, foraging activity, and parental effort in macaroni penguins (Crossin *et al.* 2012).

Further studies are required to better assess the role of prolactin alone in nest desertion and incubation behavior. Indeed, the extent to which prolactin determines parental investment during incubation remains unclear and needs further investigation in free-living bird species. Experimental studies allow establishing cause and effect relationships between prolactin and parental behavior. However, studies on the effects of a modulation of prolactin levels alone on the breeding behavior of wild birds are still rare. Seabirds, and

notably penguins, are ideal species to address these issues. Hormonal profiles during incubation, including prolactin levels, are well established for these species (Vleck *et al.* 1999). Furthermore, corticosterone-induced effects on parental behavior have been examined recently in incubating male Adélie penguins (Spée *et al.* 2011a; Thierry *et al.* 2013b).

In this study, we evaluated the effects of a decrease in prolactin levels alone on parental investment during incubation, using self-degradable bromocriptine pellets in male Adélie penguins. Because the effects of bromocriptine-treatment on glucocorticoid levels have not been well described (Curlewis *et al.* 1988; Kan *et al.* 2003), especially in birds, prolactin and corticosterone levels were both measured in treated birds and controls. Incubation behavior of both groups was monitored by several means: (1) dummy eggs were used to record incubation temperatures and egg rotation rates, while (2) filming and subsequent video analysis allowed assessing the detailed behavioral time budget, as well as the position of birds on the nest. Direct observation of incubation routine allowed monitoring incubation duration and hatching success. We predicted that birds with low prolactin levels would be less committed to incubate than controls. Accordingly, these birds should be less attentive to the nest, which would potentially manifest itself in decreased vigilance and a decreased frequency of nest rearrangements. Consequently, we expected that egg temperatures, egg rotations rates, and hatching success in this group would be decreased.

## Materials and Methods

This study was conducted at the French station Dumont d'Urville (66°40'S, 140°01'E), Terre Adélie, Antarctica, during the 2008-2009 and 2009-2010 austral summers. The study was approved by the ethical committee of the French Polar Institute Paul-Emile Victor and authorized by the French Southern and Antarctic Lands (Terres Australes et Antarctiques Françaises).

### *STUDY SPECIES*

Adélie penguins are long-lived seabirds, which breed during the short austral summer in colonies around Antarctica from late October to early March. After arriving at the colony in spring, male Adélie penguins fast for five to six weeks, while they establish territories, proceed through courtship, and then incubate for about two weeks. Females usually depart to sea to refeed as soon as they complete their clutch by laying the second egg (Sladen, 1958), after a fast of two to three weeks. The incubation period for an Adélie penguin egg ranges between 30 and 39 days (Ainley, 2002), with mates alternating between foraging trips at sea and incubation bouts on land (Fig. 1).

While studying the relationships between hormones and parental care in both sexes is of importance, previous studies on the role of corticosterone in incubation behavior and nest desertion have been mainly conducted on male Adélie penguin due to their extended fasting period (Spée *et al.* 2011a; Thierry *et al.*

2013a). Thus, only male Adélie penguins were considered in our study in order to get comparable data sets.

*SAMPLING PROCEDURE AND MONITORING*

A total of 58 Adélie penguin pairs were studied from courtship through to hatching (22 in 2008-2009 and 36 in 2009-2010). Males were first handled at the end of the courtship period, between pair formation and egg-laying (November 8 to November 23). Sex was determined by a combination of parameters, including copulatory position, cloacal inspection, and observation of the incubation routine (Sladen, 1958). To minimize handling stress, a bird's head was covered with a hood (Cockrem *et al.* 2008). A blood sample was collected from the alar vein (2 mL syringe, 25-gauge needle) within three to five minutes of capture (to obtain baseline corticosterone levels, as recommended by Romero and Reed, 2005). Handling durations not exceeding five minutes have been demonstrated to have no effect on baseline corticosterone levels in Adélie penguins (Vleck *et al.* 2000). In a few birds, handling time exceeded the one recommended to assess baseline corticosterone levels. Consequently, samples from these birds were not assayed for hormone levels. All blood samples were transferred into pre-treated tubes (25 µL Na-heparin or EDTA) and centrifuged at 4 °C for 10 min (5000 rpm). Aliquots of plasma were kept at -20 °C until further analyses. Birds were then weighed with an electronic balance (Ohaus, Giessen, Germany, resolution: ± 2 g), and wing length was measured to the nearest mm to calculate a scaled mass index (Peig & Green, 2009). To allow identification, penguins were individually marked using black dye (Nyanzol-D) on their chest feathers and Tesa tape that was inserted between back feathers, before they were released on their nest. Nests were checked from a distance of 10 to 30 m every two to three hours between 6 am and 1 am for the entire study period. Adélie penguins were monitored during courtship and incubation period, from mid-November until the end of December. Detailed incubation routine data were acquired by observing which partner was present on the nest. 42 out of 58 males were recaptured, blood sampled, and weighed before leaving the colony, either during nest relief by their partner or following nest desertion. A blood sample was collected to assess prolactin and corticosterone levels at the end of the incubation shift, and treated as reported above.

*EXPERIMENTAL MANIPULATION OF PROLACTIN LEVELS*

*Breeding penguins*

During initial handling, 30 males (11 in 2008-2009 and 19 in 2009-2010) received a bromocriptine mesylate pellet (C-231, 25 mg, 21-day release, 6.4 mm diameter) purchased from Innovative Research of America (Sarasota, FL, USA). Bromocriptine is a dopamine agonist, and has been successfully used in birds to reduce plasma prolactin levels (Angelier *et al.* 2006a; Reddy *et al.* 2006). To insert the pellet subcutaneously in the nape of the neck, the skin was disinfected with 70% alcohol and a small incision was made, using a scalpel blade. After positioning the pellet subcutaneously with tweezers (disinfected with povidone-iodine), the incision was closed with sterile suture and a topical application of Alumisol® spray (CEVA, Libourne, France) was given. Handling time averaged 18 min (range: 12-24 min). During initial handling, 28 other males (11 in 2008-2009 and 17 in 2009-2010) underwent the same procedure

including skin incision and positioning with tweezers, without actual implantation of a pellet (Table 1, Fig. 1).

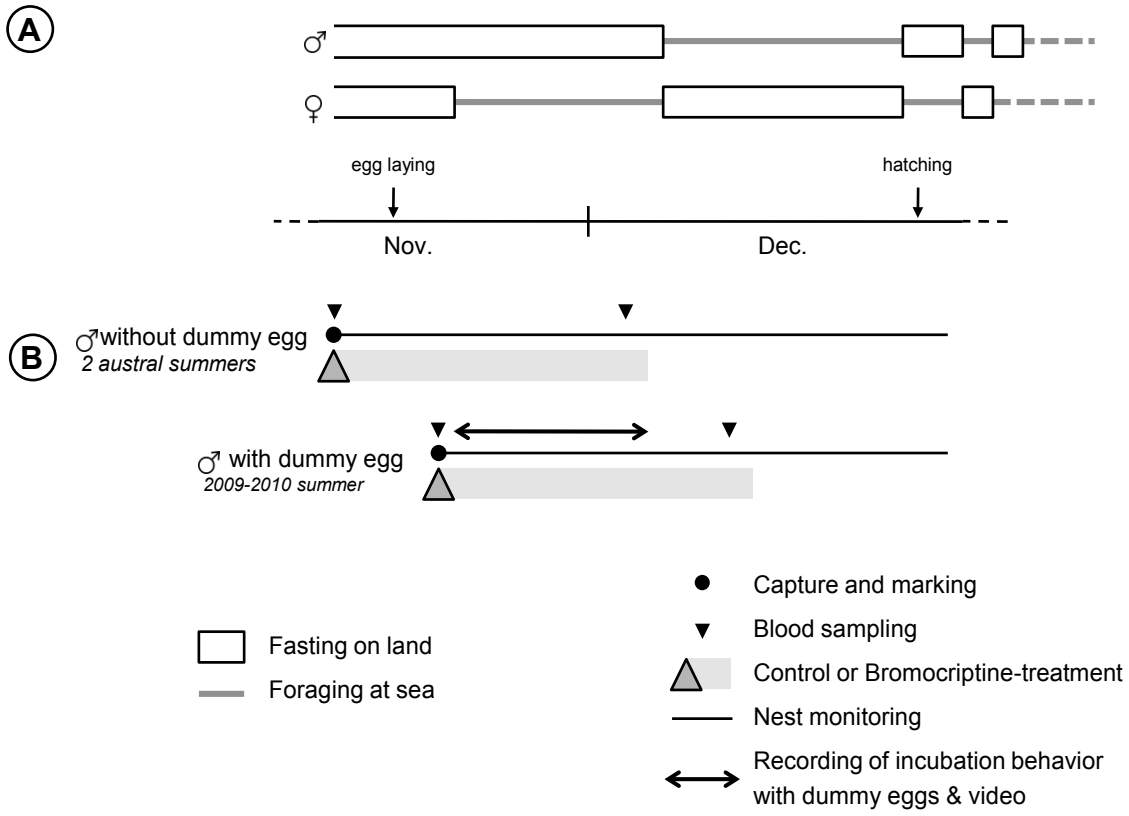


**Figure 1:** Schematic view of nest attendance during the incubation period (**A**) and study protocol (**B**)

Male Adélie penguins were separated into four groups depending on treatment (control or bromocriptine-treated) and whether their clutch was replaced by one dummy egg to record incubation behavior or not.

*Captive penguins*

To confirm the efficacy of the bromocriptine-treatment to reduce prolactin levels, and to assess the kinetics of prolactin secretion over the course of the treatment, a further 21 birds were captured and kept in a pen (5.2 x 2.4 m) during the 2008-2009 and 2009-2010 austral summers. These captive birds were failed breeders because of predation and/or bad weather conditions, and their sex could not be determined in the field. The number of birds in the pen at any time never exceeded eight individuals. After a few days in captivity, birds were captured in the pen, blood sampled, and implanted as mentioned above with a bromocriptine pellet (treated birds, n=15) or no pellet (control birds, n=6). All captive birds were weighed every two days. Blood samples were collected at different times during a bird's captive period: on the day of implantation (day 0; before implantation), several days after implantation, and on the last day of captivity (days 7–17, depending on the initial body mass and daily body mass loss of individuals). All captive penguins were released on the edge of the breeding colony (and, thus, close to sea, which is free of sea ice at this period of the year), with a body mass average of 3.5kg. For comparison, body mass in free-living breeding Adélie penguins can be as low as 3.2-3.3kg in males and 2.8kg in females (Spée *et al.* 2010).

*INCUBATION*

*Nest success and incubation duration (Birds without a dummy egg)*

During nest monitoring, the number of eggs and/or chicks was observed from the periphery of the colony at least once daily to determine laying and hatching dates, and nest success at hatching. Nest success was defined as the percentage of successful nests at hatching. Incubation duration was calculated as the difference between the hatching date of the first chick and the laying date of the first egg.

*Incubation behavior (Birds with a dummy egg)*

During the 2009-2010 austral summer, the clutches of 14 males were replaced by dummy eggs containing several temperature probes and an accelerometer, to register key parameters of incubation behavior, i.e. incubation temperature and egg rotation rate. Eight of these males also received a bromocriptine pellet (see above for details). Dummy eggs, produced at the IPHC-DEPE (Strasbourg, France), were of the same size and shape as genuine penguin eggs, and were easily accepted and incubated by penguins (Beaulieu *et al.* 2010b; Thierry *et al.* 2013b). Clutch replacement occurred at the same incubation stage for all birds, between zero and two days following female departure. Egg temperature and egg position were recorded every 10 s by seven temperature probes (six external probes, located equidistantly around the mid-shell, and one internal probe) and a bi-axial accelerometer, respectively. Further technical details regarding the dummy eggs are provided in Beaulieu *et al.* (2010) and Thierry *et al.* (2013a). The two real eggs of each clutch were replaced by one dummy egg between November 20 and 24 (mean: November 22). The real penguin eggs were marked and placed in surrounding nests for incubation. They were returned to the original nest at the end of the first male incubation shift, i.e. between November 30 and December 11 (mean: December 3). Two dummy eggs were predated by South Polar skuas *(Catharacta maccormicki)* on November 21 and 22, soon after the beginning of the recording. Fortunately, the eggs were found close to the colony and data were successfully downloaded.

To obtain detailed behavioral data during incubation, five control and six treated penguins with a dummy egg were filmed in the afternoons (between 1:00 p.m. and 8:00 p.m.) from November 22 to December 1 for a total recording of ~48 hours. Camera's field of view allowed filming two to four birds at once. Video sessions of 30 minutes each were chosen randomly and analyzed using an ethogram recognizing the following behaviors: rest, vigilance, comfort, social positive interactions, agonistic interactions, and nest rearrangement and egg turning. Behaviors were defined as follows: during rest, birds were sleeping or otherwise inactive for at least five seconds, with birds either lying down or standing up, head still. Vigilance consisted of alert movements, with bird watching other birds or the surroundings, head moving for at least five seconds. Comfort corresponded to stretching, preening, head-shaking, ruffle-shake, swallowing, and yawning (McKinney, 1965). Social positive interactions included ecstatic display, bill-to-axilla display, bowing, loud and quiet mutual display (Marchant *et al.* 1990). Agonistic interactions included threat displays (direct, fixed one-sided and alternate stare, crouch), as well as fighting, charge and pecks. Nest rearrangement and egg turning encompassed nest-scraping, stone rearrangement, and clutch movements, with beak, body and/or feet.

*HORMONE ASSAYS*

Hormone concentrations were measured as previously reported by Spée and colleagues (2010). All samples were analyzed in duplicates, except for prolactin levels of captive birds. Plasma prolactin levels were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé (CEBC, France). This prolactin assay has previously been validated for this species by the CEBC, as previously reported (see Spée *et al.* 2010; Spée *et al.* 2011a). Intra and inter-assay variations were 5% and 13%, respectively. The minimal detectable level of prolactin was 0.43 ng/mL. Plasma corticosterone levels were determined by a quantitative competitive sandwich enzyme immunoassay technique (AssayMax Corticosterone ELISA Kit, EC3001-1, AssayPro, St. Charles, MO, USA). The corticosterone assay was run in two assays at the IPHC-DEPE according to guidelines provided by the manufacturer. Before assaying, plasma samples were diluted 1:4 into EIA diluent. Intra- and inter-assay variations for corticosterone were 9% and 15%, respectively. The minimal detectable level of corticosterone was 0.06 ng/mL.

*DATA ANALYSIS*

Incubation behavior data recorded with the dummy eggs were analyzed using R 2.13.2 (R Development Core Team, 2011) and IgorPro Software (Wavemetrics, Lake Oswego, OR, USA). Data recorded during the first three hours of deployment were discarded to remove potential differences in initial dummy egg warming between birds. Maximum egg temperature, recorded by one of the six external probes, was calculated by selecting the sensor that recorded the highest temperature for each measurement (every 10s) and was averaged daily (from 0 to 24 hours). In the following, 'egg temperature' refers to the mean value of the maximum temperature recorded by the six external probes. Hourly maxima of maximum temperatures (i.e. the single highest value during one hour) were averaged daily and served as an estimate for the temperature of the brood patch, hereafter called 'maximum egg temperature'. Rotation events were counted to compare egg rotation rates between treated birds and controls (see Thierry *et al.* 2013b for a detailed description of the calculations regarding egg temperature and rotation rate).

*STATISTICAL ANALYSIS*

All statistical analyses were performed with R 2.13.2. Results are expressed as means ± SE. Differences were considered as statistically significant when $p<0.05$. The number of birds in each group according to the different study parameters is described in Table 1.

**Table 1:** Number of birds in each group depending on treatment (control vs. bromocriptine-treated), year (2008-2009 vs. 2009-2010) and the presence or absence of a dummy egg in the nest for the different parameters measured in the study

| | | Without a dummy egg | | | | With a dummy egg | |
|---|---|---|---|---|---|---|---|
| | | Control | | Treated | | Control | Treated |
| | | 2008-2009 | 2009-2010 | 2008-2009 | 2009-2010 | 2009-2010 | 2009-2010 |
| Breeding parameters | Body mass and scaled mass index at implantation, female departure date, no. eggs per nest | 11 | 11 | 11 | 11 | 6 | 8 |
| | Laying date | 11 | 11 | 8 | 11 | 0 | 0 |
| | No. chicks at hatching | 11 | 11 | 11 | 11 | 0 | 0 |
| | Incubation duration | 11 | 10 | 8 | 7 | 0 | 0 |
| Hormone levels | Pre-treatment prolactin levels | 11 | 10 | 11 | 11 | 5 | 8 |
| | Prolactin levels at departure | 5 | 10 | 7 | 9 | 5 | 6 |
| | Pre-treatment corticosterone levels | 5 | 9 | 6 | 8 | 5 | 6 |
| | Corticosterone levels at departure | 5 | 9 | 6 | 8 | 5 | 6 |

*Birds without a dummy egg*

Differences in body mass and scaled body mass at the time of implantation between birds whose clutch was not replaced with a dummy egg were assessed using a two-way ANOVA with treatment, year, and interaction between treatment and year as factors after testing for normality and homogeneity of variances using the Shapiro-Wilkes and Bartlett tests, respectively. Laying date, female departure date, and the number of eggs per nest before the treatment were compared with generalized linear models (GLM) with a quasi-Poisson distribution with treatment, year, and the interaction between treatment and year as factors. ANOVAs were also used to compare prolactin and corticosterone levels between control and bromocriptine-treated birds at the time of implantation (pre-treatment) and at the end of the male incubation shift (post-treatment). Corticosterone levels were log-transformed to meet the assumptions of normality. The differences in the change in hormone levels between implantation and departure were assessed with paired t-tests. The proportion of successful nests was compared with Fisher's exact tests. Differences in incubation duration and in the number of chicks at hatching between control and treated birds were assessed using a GLM with a quasi-poisson distribution, with treatment and year as factors.

*Birds with a dummy egg*

Due to the small sample size (6 controls and 8 treated birds), Wilcoxon tests were used to compare the different bird characteristics, including hormone levels, between groups for the subset of birds whose clutch was replaced with a dummy egg. Generalized estimating equations (GEEs, *geeglm* function of the *geepack* package in R) were used to compare incubation behavior data between groups, taking into account the spatial and temporal correlation structure of the data (Hardin and Hilbe, 2007; Liang and Zeger, 1986). An autoregressive (AR[1]) correlation structure was assumed to estimate regression

coefficient variances more appropriately, as consecutive measures of incubation temperatures for each bird lacked independence. The *geeglm* function was used to compare incubation temperatures, rotation rates, behavioral time budget, and position on the nest between treated birds and controls, with repetition on penguin identity. Models comparing incubation temperatures and rotation rates had treatment, day since implantation, and interaction between treatment and day since implantation as fixed factors. Models comparing behavioral time budget and position on the nest only included treatment as a fixed factor.
Spearman correlation coefficients between hormone levels at departure and mean egg temperature or the number of egg rotations during the 24 hours prior to the blood sample were calculated to assess the relationship between hormone levels and incubation behavior.

*Birds without vs. birds with a dummy egg*
Initial handling dates between birds without vs. with a dummy egg were compared using a GLM with a quasi-poisson distribution.

*Captive penguins*
GEEs were used to assess the effect of the treatment on prolactin levels over the course of the captive period, with repetition on penguin identity, treatment, day since implantation, and interaction between treatment and day since implantation as fixed factors.

**Results**

*PRE-TREATMENT BIRD CHARACTERISTICS*
*Birds without a dummy egg*
Control and treated birds, whose clutch was not replaced by a dummy egg, had a similar body mass and scaled mass index (Table 2, ANOVA, $F_{1,40}$=0.00, p=0.978 and $F_{1,40}$=0.22, p=0.638, respectively). Year and the interaction between treatment and year had no effect on body mass (year: $F_{1,40}$=0.50, p=0.486, treatment x year: F1,40=0.29, p=0.593) and scaled mass index (year: $F_{1,40}$=1.65, p=0.206, treatment x year: $F_{1,40}$=0.78, p=0.382). There was no difference in laying date between groups (GLM with a quasi-poisson distribution, treatment: t=0.08, p=0.937; year: t=1.42, p=0.164; treatment x year: t=-1.32, p=0.194), as well as in female departure date (treatment: t=-0.91, p=0.366; year: t=0.27, p=0.788; treatment x year: t=-0.59, p=0.559). Finally, the number of eggs per nest did also not differ significantly between control and treated birds and between years (treatment: t=-0.56, p=0.578; year: t=-0.56, p=0.578; treatment x year: t=-0.02, p=0.985).

**Table 2:** Study design and breeding parameters for 58 male Adélie penguins

| | Without a dummy egg | | With a dummy egg | |
|---|---|---|---|---|
| | **Control** (n=22) | **Treated** (n=22) | **Control** (n=6) | **Treated** (n=8) |
| Date of implantation | Nov. 12 ± 0.44 | Nov. 12 ± 0.29 | Nov. 21 ± 0.60 | Nov. 21 ± 0.50 |
| Male body mass at implantation (kg) | 5.09 ± 0.09 | 5.09 ± 0.11 | 4.53 ± 0.23 | 4.66 ± 0.16 |
| Laying date | Nov. 17 ± 0.66 | Nov. 16 ± 0.52 | NA | NA |
| Female departure date* | Nov. 21 ± 0.51 | Nov. 20 ± 0.47 | Nov. 21 ± 0.79 | Nov. 22 ± 0.64 |
| No. of eggs per nest | 1.95 ± 0.21 | 1.86 ± 0.47 | 2.00 ± 0.00 | 2.00 ± 0.00 |

Birds were separated into four groups, depending on treatment (control vs. bromocriptine-treated) and the presence or absence of a dummy egg in the nest. Results are expressed as means ± SE.

* Female departure date can be used as a proxy of laying date (Ainley, 2002; Sladen, 1958)

*Birds with a dummy egg*

For birds whose clutch was replaced by a dummy egg, there were no statistical differences in body mass, body condition, female departure date, and number of eggs per nest between groups (Table 2, Wilcoxon test, body mass: W=19, p=0.573; scaled mass index: W=19, p=0.573; female departure date: W=24, p=1). All females from this group laid two eggs.

*Birds without vs. birds with a dummy egg*

Initial handling dates were later for birds whose clutch was replaced by a dummy egg (GLM with a quasi-Poisson distribution, t=11.40, p<0.001), regardless of the bromocriptine-treatment (t=0.56, p=0.579) and the interaction between the two factors (t=-1.19, p=0.851). Consequently, body mass and scaled mass index at implantation was lower for these birds when compared with birds without a dummy egg (ANOVA, $F_{1,54}$=11.26, p=0.001; F1,54=5.85, p=0.019, respectively), regardless of the bromocriptine-treatment ($F_{1,54}$=0.00, p=0.958; F1,54=0.01, p=0.943) and the interaction between the two factors ($F_{1,54}$=0.16, p=0.687; $F_{1,54}$=1.10, p=0.300). However, after correcting body mass of these birds for a daily mass loss of 50g per day (Spée *et al.* 2010), the estimated body mass on November 12 did not differ between both groups ($F_{1,54}$=0.04, p=0.833; scaled mass index: $F_{1,54}$=0.71, p=0.402).

*EFFECT OF BROMOCRIPTINE ON HORMONE LEVELS*

*Free-living penguins without a dummy egg*

Before the treatment, prolactin levels were different between groups of birds whose clutch was not replaced by a dummy egg (Fig. 2, ANOVA, treatment: $F_{1,39}$=7.41, p=0.010; year: $F_{1,39}$=3.51, p=0.069; treatment x year: $F_{1,39}$=8.34, p=0.006). Prolactin levels were still significantly different between groups at departure from the colony (treatment: $F_{1,27}$=17.27, p<0.001; year: $F_{1,27}$=0.99, p=0.329; treatment x year: $F_{1,39}$=0.07, p=0.791). The overall change in prolactin levels between implantation and departure was significantly different between groups ($F_{1,26}$=24.85, p<0.001; year: $F_{1,27}$=0.07, p=0.797; treatment x year: $F_{1,39}$=0.02, p=0.897). In control birds, prolactin levels significantly increased between initial handling

and departure from the colony, which occurred on average 19.5 days after implantation (paired t-test, t=-5.29, p<0.001). By contrast, in treated birds prolactin levels tended to decrease between these two stages (t=2.02, p=0.061).

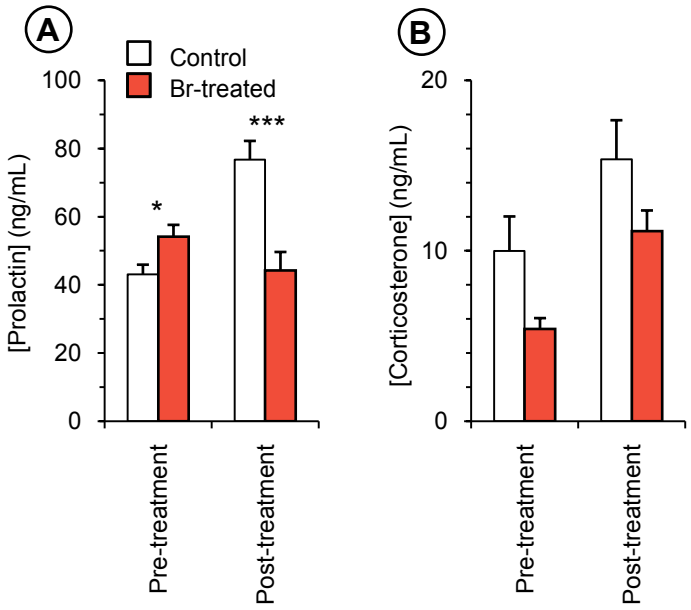


**Figure 2:** Effects of bromocriptine-treatment on prolactin (**A**) and corticosterone (**B**) levels in male Adélie penguins whose clutch was not replaced with a dummy egg

Hormone levels of control and treated birds (means ± SE) are shown at the time of implantation (Pre-treatment) and at the end of the first incubation shift (Post-treatment). *p<0.05, *** p<0.001

Corticosterone levels were not significantly different between groups before the treatment (ANOVA, log-transformed for normality, treatment: $F_{1,24}$=3.18, p=0.087; year: $F_{1,24}$=0.05, p=0.827; treatment x year: $F_{1,24}$=0.00, p=0.977) and at departure (treatment: $F_{1,24}$=1.51, p=0.231; year: $F_{1,24}$=2.85, p=0.104; treatment x year: $F_{1,24}$=0.86, p=0.363). The change in corticosterone levels was not affected by the treatment, the year, nor the interaction between treatment and year (treatment: $F_{1,24}$=0.27, p=0.610; year: $F_{1,24}$=1.66, p=0.209; treatment x year: $F_{1,24}$=0.42, p=0.521).

*Free-living penguins with a dummy egg*

Prolactin and corticosterone levels before the treatment were similar in birds whose clutch was replaced with a dummy egg (prolactin: 54.8 ± 9.5 ng/mL for controls vs. 57.9 ± 5.2 for treated birds, W=20, p=1; corticosterone: 7.3 ± 3.7 vs. 9.8 ± 7.5, respectively, W=16, p=0.931). At departure, prolactin levels were decreased in treated birds compared to controls (63.7 ± 6.1 vs. 41.0 ± 7.3, respectively, W=27, p=0.030), while corticosterone levels were not affected by the treatment (17.4 ± 4.0 vs. 12.5 ± 2.7, respectively, W=20, p=0.429). The change in prolactin levels between implantation and departure was 0.51 ± 0.57 in controls vs. -1.36 ± 0.89 ng/mL in treated birds and did not differ significantly between the two groups (W=23, p=0.178, 5 controls and 6 treated birds). The change in corticosterone levels between the two sampling dates was similar between the two groups (W=17, p=0.792).

*Captive penguins*

Pre-treatment prolactin levels in captive penguins did not differ significantly between control and treated birds (Fig. 3; 26.7 ± 4.5 ng/mL and 32.6 ± 2.4, respectively; W=33, p=0.381). However, pre-treatment prolactin levels of captive birds were significantly lower than those of free-living penguins (Figs. 2-3; ANOVA, treatment: $F_{1,58}$=3.10, p=0.084; reproductive status: $F_{1,58}$=27.58, p<0.001; treatment x reproductive status $F_{1,58}$=0.47, p=0.496). The significantly decreased prolactin levels in treated birds (GEE, Wald=26.3, p<0.001) and the significant interaction between treatment and day (Wald=3.92, p=0.048) illustrate the negative effect of the bromocriptine treatment on prolactin levels, with prolactin levels at a minimum two days after implantation. Number of days since implantation had no effect on prolactin levels (Wald=0.08, p=0.784).

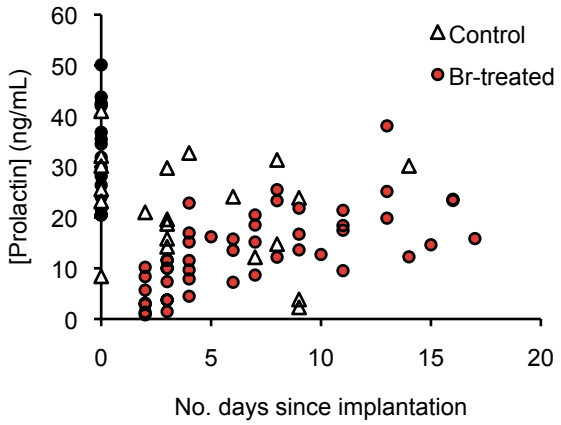


**Figure 3:** Effects of bromocriptine-treatment on prolactin levels in captive control (n=6) and treated (n=15) Adélie penguins between the day of implantation (0) and up to 17 days post-treatment

*INCUBATION BEHAVIOUR (BIRDS WITH A DUMMY EGG)*

The behavioral time budget did not differ significantly between treated birds and controls (Fig. 4A, GEE, rest: Wald=1.64, p=0.20; vigilance: Wald=1.9, p=0.17; nest rearrangement and egg turning: Wald=0.12, p=0.73; comfort: Wald=0.02, p=0.89; agonistic interactions: Wald=1.01, p=0.31; positive social interactions: Wald=3.39, p=0.065). However, treated birds spent a significantly greater proportion of time in the upright on the nest rather than controls, while the latter spent most of their time on the nest in a prone position (Fig. 4B, GEE, Wald=9.64, p=0.002). Two treated birds (out of eight) had their dummy egg predated by a skua, while all six control birds incubated their dummy egg for the entire incubation bout.

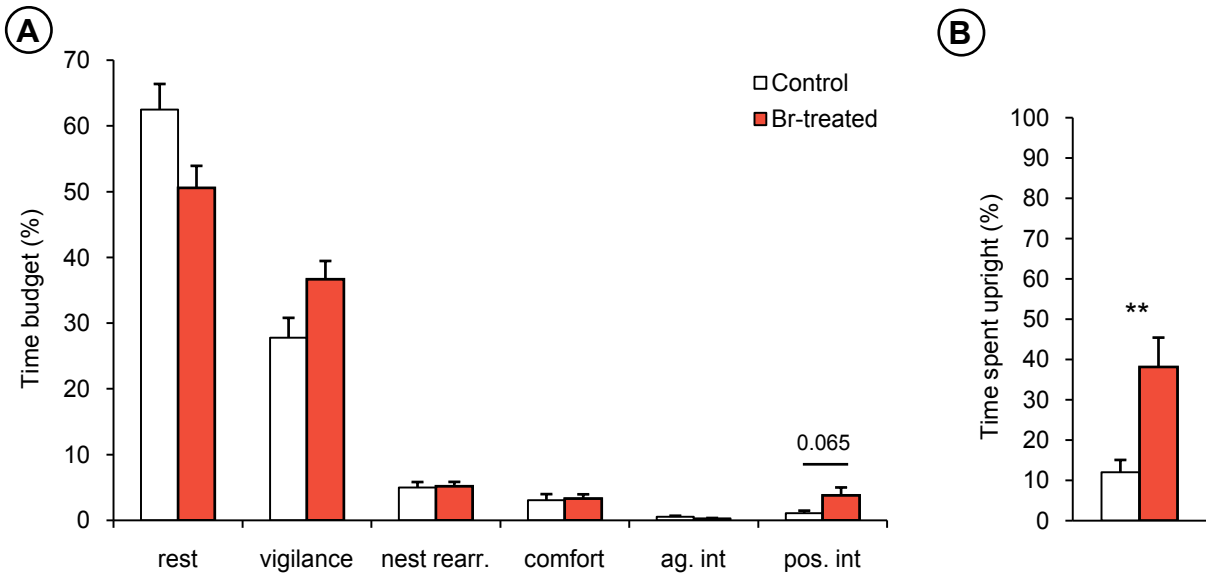


**Figure 4:** Behavioral time budget (**A**) and percentage of time spent standing in the upright position on the nest (**B**) of control and bromocriptine-treated male Adélie penguins

The ethogram recognized the following behaviors: rest, vigilance, nest rearrangement and egg turning (nest rearr.), comfort, agonistic interactions (ag. int.), and social positive interactions (pos. int.). The behavioral time budget of 11 birds (5 controls and 6 treated) was analyzed, for a total of 103 video sessions of 30 minutes. ** $p<0.01$

Egg temperatures of treated penguins were significantly lower than those of control birds, differing on average by 7.1 °C (Fig. 5A, Table 3, $p<0.001$). Maximum egg temperatures were also significantly lower in treated birds (by 5.2 °C), when compared with controls ($p<0.001$). However, there was a significant increase in the egg temperature of treated birds over the course of the treatment (Fig. 5B, $p=0.017$), while egg temperature of controls remained stable. By contrast, egg rotation rates were significantly higher in treated birds, when compared with controls (Fig. 5D, $p<0.001$). Egg rotation rates in treated birds also changed significantly over the course of the treatment period (Fig. 5E, $p<0.001$). However, while egg temperatures increased over time, egg rotation rates declined.

There was a significant correlation between egg temperatures during 24 hours prior to departure and prolactin levels at departure (Fig. 5C, $S=64.58$, $\rho=0.71$, $p=0.015$) but not between egg temperatures and corticosterone levels ($S=196.38$, $\rho=-0.19$, $p=0.599$). There was no significant correlation between egg rotation rates and hormone levels at departure (prolactin: Fig. 5F, $S=320.69$, $\rho=-0.46$, $p=0.157$; corticosterone: $S=138.84$, $\rho=0.16$, $p=0.662$).

**Table 3:** Results of the GEEs, comparing egg temperatures and egg rotation rates between control and bromocriptine-treated incubating male Adélie penguins

| | Egg temperature | | Maximum egg temperature | | Egg rotations | |
|---|---|---|---|---|---|---|
| | Wald | p | Wald | p | Wald | p |
| Intercept | 5490.3 | <0.001 | 9513.2 | <0.001 | 2241.2 | <0.001 |
| **Treatment** | 43.14 | <0.001 | 34.29 | <0.001 | 49.31 | <0.001 |
| Day* | 5.75 | 0.017 | 4.33 | 0.037 | | NS |
| **Treatment** x Day | 21.50 | <0.001 | 18.91 | <0.001 | 19.03 | <0.001 |

* Day refers to the number of days since implantation

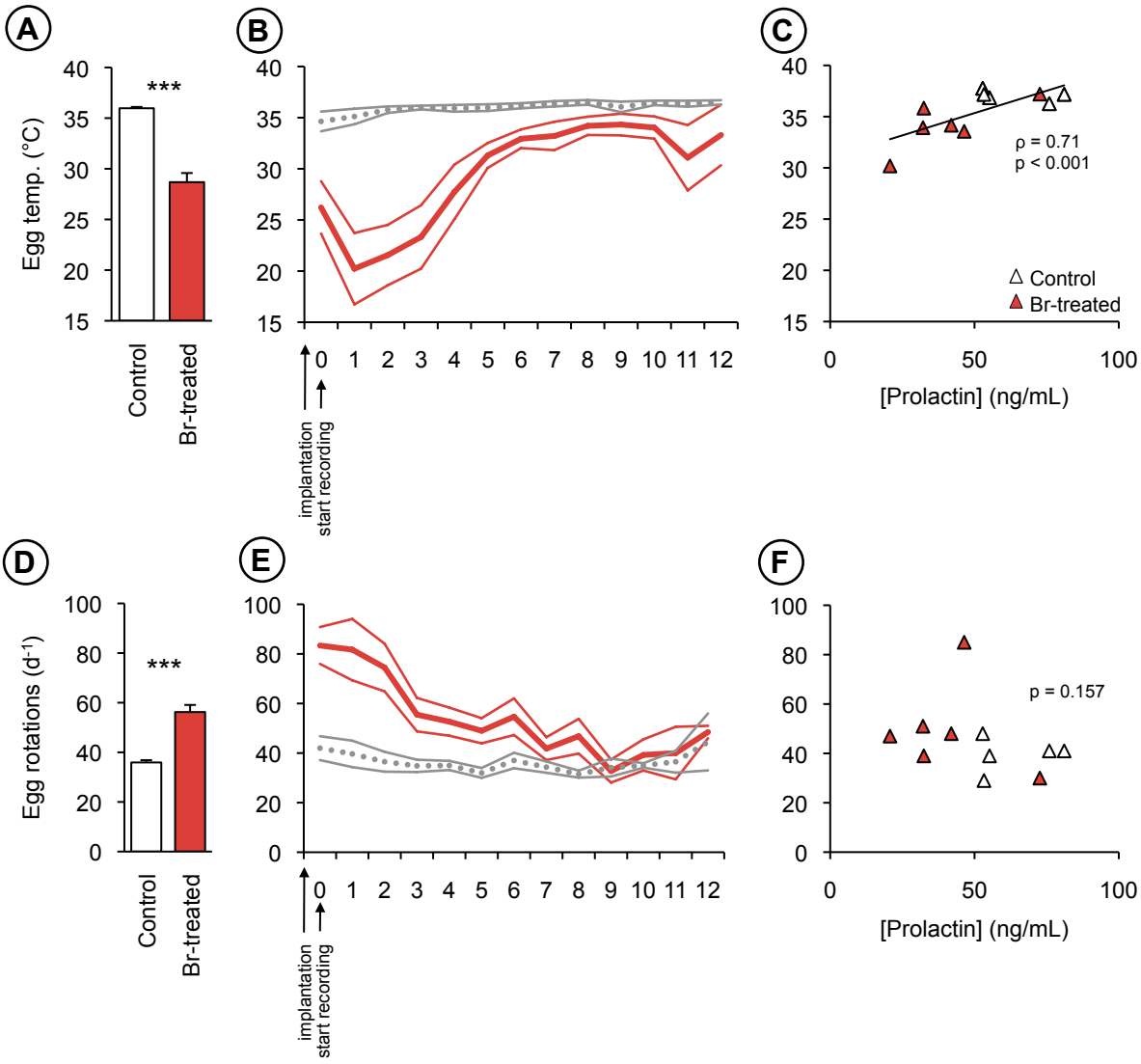


**Figure 5:** Incubation behavior recorded from control and bromocriptine-treated male Adélie penguins. Parameters shown are egg temperatures (**A, B, C**) and egg rotations rates (**D, E, F**). **C** and **F** show the relationship between prolactin levels at departure and incubation parameters during the 24 hours prior to departure

Values are means ± SE (n=14; 6 control and 8 Br-treated birds; 71 vs. 65 daily observations). White bars, grey lines, and white triangles refer to control birds; while red bars, red lines, and red triangles refer to treated birds. ***p<0.001

*NEST SUCCESS AND INCUBATION ROUTINE (Birds without a dummy egg)*

The proportion of successful nests at the end of the incubation period did not differ significantly between control (95.5%) and treated birds (68.2%) (Fisher's exact test, p=0.505). Incubation duration was not different between years (GLM with a quasi-Poisson distribution, t=-0.20, p=0.841) and was significantly longer in treated birds (on average by 2.1 days; Fig. 6A, t=2.52, p=0.017). While the number of chicks at hatching did not differ between years (t=-0.34, p=0.739), it was significantly lower in treated birds (Fig. 6B, t=-2.23, p=0.031).

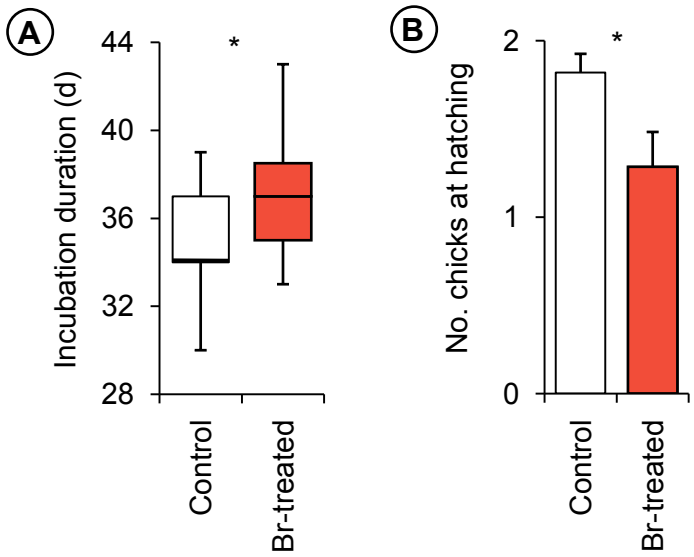


**Figure 6:** Effects of bromocriptine-treatment on incubation duration (**A**) and breeding success at hatching (**B**) in Adélie penguins

Incubation duration values are plotted as median, 25th and 75th percentiles, minima and maxima. Breeding success values are means ± SE. Data (n=44, 22 control and 22 treated birds) were averaged over two consecutive breeding seasons for birds whose clutch was not replaced by a dummy egg. * p<0.05

## Discussion

The purpose of this study was to investigate the endocrine and behavioral consequences of experimentally decreased prolactin levels in incubating male Adélie penguins. This was achieved by monitoring the hormonal status, behavioral time budget, position on the nest, incubation parameters, and breeding success of bromocriptine-treated and control birds. The experimental decrease in prolactin levels reduced incubation commitment of male Adélie penguins and modified subsequent reproductive success, independently of any changes in corticosterone levels.

*METHODOLOGICAL CONSIDERATIONS*

Experimental studies assessing the role of prolactin in non-domesticated bird species are rare, which is partially explained by the difficulty to pharmacologically manipulate the pituitary hormone. Bromocriptine

acts as an agonist of dopamine D2 receptors which are notably localized on the hypothalamic VIP neurons (Chaiseha *et al.* 2003). Hence, bromocriptine reduces prolactin secretion by inhibiting the stimulation of cells secreting prolactin (Benker *et al.* 1990). Subcutaneous, self-degradable pellets, such as the ones used in our study, have been found to rarely deliver the hormones for the period claimed by the manufacturers when used in birds (Müller *et al.* 2009). In our study of free-living incubating Adélie penguins, prolactin levels were still significantly decreased in bromocriptine-treated birds 19.5 days after implantation, when compared with control birds (Fig. 2A). The significant difference in pre-treatment prolactin levels might be explained by inter-individual variability, despite the fact that birds were randomly assigned to the control and treated groups. Repeated blood sampling of captive treated and control birds (i.e. the failed breeders) allowed assessing the kinetics of prolactin secretion consecutive to pellet implantation. Prolactin levels were at their minimum two days following implantation and increased slowly thereafter (Fig. 3). It seems that there was no longer a difference in prolactin levels between control and treated birds 6-8 days post-treatment but this could not be assessed statistically. This shows a reduced efficiency of the pellets that were designed to continuously release bromocriptine during 21 days. Yet, among incubating Adélie penguins, whose prolactin levels were higher at implantation than those of captive birds, prolactin levels were still significantly reduced in treated birds compared to controls 19.5 days post-treatment. These self-degradable pellets are, thus, an effective tool for artificial manipulation of hormonal levels in field studies. However, repeated blood sampling at different times and hormone assays are required to validate the efficacy and the time-course of the treatment for each species and life-history stage.

Subcutaneous administration of bromocriptine in poultry has been shown to decrease prolactin levels, reduce broodiness (Bedrak *et al.* 1983), and increase egg production (Reddy *et al.* 2006). Other studies on mammals (Roberts *et al.* 2001; see also Schradin & Anzenberger, 1999 for a review) and fish (Kindler *et al.* 1991) have successfully used bromocriptine to decrease prolactin levels and were instrumental in establishing cause and effect relationships between prolactin and parental care. More recently, bromocriptine-treatment has also been used in emperor penguins *Aptenodytes forsteri* to demonstrate that kidnapping behavior observed in these birds is the result of high levels of prolactin in failed breeders (Angelier *et al.* 2006a). To our knowledge, our study is the first contribution that assessed the effects of an experimental decrease of prolactin levels, using bromocriptine implants, on the detailed incubation behavior of non-domesticated breeding birds in the wild, monitoring both prolactin and corticosterone levels.

*DECREASED PROLACTIN LEVELS AND BEHAVIOUR*

Prolactin is often described as the main hormone involved in parental care in birds. Accordingly, we expected that, low prolactin levels would reduce nest attentiveness. Surprisingly, however, the bromocriptine-treatment did not induce any changes in the behavioral time budget of incubating male Adélie penguins per se (Fig. 4A). In particular, treated birds spent the same amount of time being vigilant and rearranging their nests than control birds, contrary to our expectations. Yet, experimental studies on poultry species have demonstrated that a decrease in prolactin levels can reduce and even suppress incubation behavior. For example, in one study, active immunization against prolactin led to decreased

prolactin levels and prevented the expression of incubation behavior in turkey hens (Crisóstomo *et al.* 1998). In another study, nest deprivation in bantam hens induced a decrease in prolactin levels within two to three days, which was paralleled by the disappearance of incubation readiness (Sharp *et al.* 1988). Several hypotheses can be advanced to explain the absence of a decrease in nest attentiveness in our treated penguins: (1) birds could be insensitive to decreased prolactin levels at this time of the breeding cycle, (2) hormonal changes might have been insufficient to affect behavior, and/or (3) other hormones might play a role in the control of incubation behavior. Certainly, hormones such as progesterone have been shown to play a role in the regulation of nest attentiveness and parental care. For example, prolactin injection was found to be ineffective for inducing incubation behavior in the ring dove *Streptopelia risoria*, when compared with progesterone injection (Lehrman & Brody, 1961). It has also been reported that only a combination of estrogens and progesterone administration followed by prolactin administration was able to induce incubation behavior in female ovariectomized turkeys, while administration of prolactin alone was able to maintain incubation behavior after its induction (El Halawani *et al.* 1986). Accordingly, this justifies the sole consideration of prolactin in our study. Nevertheless, to better understand the induction of incubation behavior and its control in penguins, the role of other hormones should be examined.

While the treatment did not affect the behavioral time budget of penguins, birds with experimentally decreased prolactin levels spent more time in the upright position on the nest (Fig. 4A). In Adélie penguins, standing in the upright position on the nest is characteristic for the early incubation phase (Derksen, 1977), when birds incubate their first egg, possibly to delay development, before the second egg is laid (Spurr, 1975). Later on, however, eggs are typically incubated between their feet and an exposed brood patch on their belly, while birds are in a prone position. The change in incubation posture we observed in treated birds probably enhanced the chances for eggs to be predated, as was the case with two dummy eggs, which were predated by skuas. One could interpret the greater proportion of time that treated birds spent in the upright position, together with their tendency to engage more often in positive social interactions, as an offset in the timing of their breeding. Accordingly, treated birds displayed behavior that is more typical for their courtship phase or early incubation, rather than their incubation period. Prolactin secretion is endogenously programmed in penguins (Vleck *et al.* 2000), and could therefore transmit information regarding the timing of breeding. However, the transition from egg laying to incubation in birds is not only associated with changes in the plasma level of prolactin but also with changes in steroid hormones levels (Vleck & Vleck, 2011; Wingfield & Goldsmith, 1990). Hence, in addition to decreased prolactin levels, variations in the secretion patterns of steroid hormones may also be influential and help to understand the suggested change in the timing of breeding of treated birds. While bromocriptine-treatment has no androgenic effects in fish (Kindler *et al.* 1991) and mammals (Taverne *et al.* 1982), it has been shown to increase progesterone and estradiol levels in hens (Reddy *et al.* 2006). Consequently, to better understand the hormonal control and the timing of incubation behavior in male Adélie penguins, further studies should examine the possible links between prolactin and steroid hormones.

*DECREASED PROLACTIN LEVELS AND INCUBATION PARAMETERS*

As a consequence of the expected decrease in nest attentiveness in bromocriptine-treated penguins, we predicted a decrease in egg temperatures and egg rotation rates. However, since we found that nest

attentiveness was similar in treated birds and controls, these incubation parameters should also have been similar. Contrary to that, treated penguins, with consequently decreased prolactin levels, incubated their eggs at lower temperatures (Fig. 5A) and turned their eggs more frequently (Fig. 5D) than control birds. These changes might have resulted from the higher proportion of time treated birds spent in an upright position on the nest (Fig. 4B). Standing upright during incubation might be associated with a frequent a rapid chilling of eggs, when birds move eggs with their feet, while changing position (Derksen, 1977). Lower incubation temperatures observed in treated penguins could also be the result of decreased peripheral vasodilatation in the brood patch, as suggested by the observed decrease in maximum egg temperature (Table 3). The formation of the brood patch, i.e. defeathered, vascularized, and edematous skin, is under the control of prolactin in many species (reviewed in Lea & Klandorf, 2002). Hence, decreased prolactin levels might have induced structural changes to the brood patch, therefore decreasing the amount of heat transferred to the eggs.

The differences in egg temperatures and egg rotation rates between treated birds and controls were maximal soon after implantation, and decreased over time (Fig. 5, Table 3). In our captive penguins, prolactin levels of treated birds were at a minimum two days after implantation and increased afterwards. On the other hand, prolactin levels at departure were significantly correlated with egg temperatures (Fig. 5C) but not with egg rotation rates (Fig. 5F) prior to the blood sampling. This lends support to the idea that parental investment of incubating Adélie penguins correlates negatively with prolactin levels: birds with elevated prolactin levels (80-100 ng/mL) incubate optimally, while birds with decreased prolactin levels (40-60 ng/mL) incubate their eggs sub-optimally, at lower temperatures. Lower incubation temperatures can be harmful to embryos by reducing growth efficiency and increasing in ovo mortality (Feast *et al.* 1998; Nord & Nilsson, 2011; Olson *et al.* 2006). Incubation duration was lengthened in our treated birds (Fig. 6A), and since avian embryos are essentially ectothermic (Webb, 1987), it is very likely that the developmental rate was slowed in eggs, which were incubated by treated birds.

*DECREASED PROLACTIN LEVELS AND BREEDING SUCCESS*

Incubation temperatures together with a detailed behavioral time budget can be considered to directly reflect parental investment in nestling development. The bromocriptine-treatment was expected to reduce breeding success because of reduced parental investment. Accordingly, we found that treated birds had a lower breeding success at hatching than controls (Fig. 6B). In ring doves, declining prolactin levels have been linked to the termination of incubation of infertile eggs, and to the termination of brooding of young chicks (Silver, 1984), while in penguins low prolactin levels have been associated with nest abandonment (Groscolas *et al.* 2008; Spée *et al.* 2010). However, several studies concerning free-living species, such as the semi-palmated sandpiper *Calidris pusilla* (Gratto-Trevor *et al.* 1990) and the pied flycatcher *Ficedula hypoleuca* (Silverin & Goldsmith, 1990), have failed to reveal a relationship between plasma prolactin levels and a decline in brooding. Furthermore, in black-legged kittiwakes *Rissa tridactyla*, a short-term increase in corticosterone levels led to a long-term decrease in prolactin levels, which was associated with lower brooding commitment and a decreased reproductive success (Angelier *et al.* 2009).

There is growing evidence that prolactin has to reach a threshold concentration, below which the drive to incubate is inhibited (Boos *et al.* 2007; Spée *et al.* 2010; Spée *et al.* 2011a). A prolactin levels below threshold, together with increased corticosterone levels, seems necessary to induce nest desertion in Adélie penguins (Spée *et al.* 2010; Spée *et al.* 2011a). The post-treatment prolactin levels of birds in our study (40 ng/mL) were similar to post-treatment prolactin levels of corticosterone-treated Adélie penguins (Spée *et al.* 2011a). They were also similar to prolactin levels of non-treated Adélie penguins, which deserted their nests, i.e. about 30 ng/mL (vs. 80 ng/mL for non-treated birds, which did not desert their nest) (Spée *et al.* 2010). On the other hand, corticosterone levels in our study were not affected by the bromocriptine-treatment (Fig. 2B). Furthermore, nest desertion rate did not differ in our study between treated males and controls, which is unlike the situation in Adélie penguins treated with corticosterone pellets (Spée *et al.* 2011a; Thierry *et al.* 2013b). The current study therefore demonstrates that experimentally decreased prolactin levels alone are insufficient to trigger nest desertion in Adélie penguins, but instead decrease incubation commitment, which ultimately reduces breeding success.

*CONCLUSION AND PERSPECTIVES*

The experimental decrease in prolactin levels alone did not affect the birds' behavioral time budget. However, treated birds spent significantly more time in the upright position during incubation than controls. Our study supports the view that prolactin is one - but not the only - physiological agent controlling parental care in birds. Most studies investigating the hormonal control of incubation behavior have been carried out on females of domesticated bird species. To what extent their conclusions are valid for male birds in the wild remains to be examined. We found that decreased prolactin levels alone were not sufficient to induce nest desertion in Adélie penguins, but reduced incubation commitment, especially during the early stages of the embryonic development. Our treatment led to a decrease in incubation temperature and a higher frequency of egg rotation, which was paralleled by a lengthening of the incubation period. However, the observed decrease in incubation temperature (i.e. several degrees) did not lead to a higher in ovo mortality, but instead slowed the rate of embryonic development. It would be of great interest to investigate the physiological consequences of such lengthened incubation period on chick physiology, growth, body condition at fledging, and ultimately, survival.

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## 3.3 Etude 3 : Testostérone et incubation

**Hormonal treatment reveals reduced behavioral sensitivity to elevated testosterone levels during incubation in a polar seabird, the Adélie penguin**

Anne-Mathilde Thierry, Olivier Bles, Morgane Couchet,
Yves Handrich, Sylvie Massemin, Thierry Raclot

Soumis à *Hormones & Behavior*
(en révision)

La testostérone est un important médiateur du compromis entre comportements sexuels et soins parentaux, mais des niveaux élevés de testostérone sont associés à des coûts physiologiques. Les passereaux qui se reproduisent pendant une saison courte et/ou fournissent des soins parentaux essentiels au bon déroulement de la reproduction, sont généralement insensibles à des niveaux élevés de testostérone en dehors de la période de pariade. Seules deux études ont évalué les effets d'une augmentation expérimentale des niveaux de testostérone sur le comportement d'oiseaux marins, ne mettant pas en évidence d'insensibilité comportementale à la testostérone chez deux espèces de goélands. Les manchots Adélie faisant face à une saison de reproduction particulièrement courte, il a été intéressant de mesurer les effets d'une augmentation des niveaux de testostérone sur le comportement de manchots Adélie mâles pendant l'incubation.

**Abstract**

Testosterone is an important mediator of the trade-off between sexual and parental behavior in male vertebrates. However, the behavioral and physiological benefits of elevated testosterone levels early in the breeding season are balanced with the costs of maintaining high hormone levels throughout the season. The parental and aggressive behaviors of birds are usually affected by an experimental testosterone treatment. However, birds that face a short reproductive season and/or require essential paternal care are behaviorally insensitive to testosterone during later phases of the breeding season. Understanding the generality of the hypotheses posed to explain behavioral insensitivity to testosterone requires experimental studies in long-lived species, as previous studies have been biased towards songbirds. To test the behavioral sensitivity to testosterone of a polar seabird, the Adélie penguin *(Pygoscelis adeliae)*, testosterone levels of incubating males were experimentally elevated and their behavioral and physiological effects monitored. Pre- and post-treatment testosterone levels were measured and hatching success was monitored. Birds were filmed to establish a behavioral time budget and dummy eggs were used in a subset of birds to record incubation parameters. The treatment significantly increased testosterone but affected neither hatching success nor aggressive and sexual behaviors. However, treated birds spent more time standing on the nest, their eggs were incubated at lower temperatures, and their chicks tended to be smaller and lighter at hatching. Despite being highly constrained in the timing of their breeding and providing essential paternal care, we show that male Adélie penguins are behaviorally sensitive to testosterone.

## Introduction

Hormones play an important role in mediating behavioral, physiological, and morphological traits, and provide a mechanistic basis underlying breeding decisions, such as whether to breed, when to breed, or how much energy to invest into a breeding episode. In particular, testosterone (T) has been described as an important mediator of the life-history trade-off between sexual and parental behavior in male vertebrates (Hau, 2007; Ketterson & Nolan, 1992; Wingfield *et al.*, 2001). Manipulating the hormone levels of free-living individuals is an interesting tool to study hormone-mediated traits and their adaptive variation (Ketterson *et al.*, 1996). For instance, experimental elevation of T levels in free-living birds results in changes in many traits correlated with fitness, decreasing some components of reproductive success while enhancing others (Ketterson and Nolan, 1999; Reed *et al.*, 2006).

The steroid hormone increases reproductive success by promoting courtship and sexual behaviors (e.g. territorial aggression, mate guarding, extra-pair copulations, the development of secondary sexual characters, and sperm production) (see Horton *et al.*, 2010 for a partial review). However, T often decreases fitness simultaneously through the suppression of parental care in males (see Lynn, 2008 for a review), for example by decreasing nestling feeding rates and by disrupting the incubation routine (McDonald *et al.*, 2001). Furthermore, high T levels are associated with a suppression of the immune system (Ketterson & Nolan, 1999; Owen-Ashley *et al.*, 2004), an altered energy balance (Lynn *et al.*, 2000; Wikelski *et al.*, 1999), and increased levels of oxidative stress (Alonso-Alvarez *et al.*, 2007). These conflicting effects of T on fitness most probably lead to strong stabilizing selection related to T levels (McGlothlin *et al.*, 2010).

T levels of males are usually at their maximum at the beginning of the breeding season (i.e. during territory acquisition and courtship) and decline during the provision of parental care (Vleck *et al.*, 1999; Wingfield *et al.*, 1990), but not necessarily (see Goymann *et al.*, 2004; Hau *et al.*, 2008 for studies relating to tropical songbirds where these patterns do not apply). Temporal variations of T levels in birds are believed to reflect a balance between the behavioral and physiological benefits of elevated levels of the hormone early in the breeding season and the potential costs of maintaining T at high levels throughout the remainder of the breeding season (see Wingfield *et al.*, 2001 for a review). Sensitivity to T can be defined as the behavioral responsiveness to an experimental hormone treatment, in terms of sexual and aggressive behavior and/or parental care (Lynn, 2008). Experimental studies (see Lynn, 2008 for a review; see also Lynn *et al.*, 2002) showed that some birds retain the ability to respond behaviorally to experimental elevations of T late in the breeding season. Treated birds usually show significant increases in sexual and aggressive behavior together with decreased parental investment (Lynn, 2008). However, high T levels do not depress the expression of paternal care nor increase aggressive behavior in males of several passerine species: e.g. Lapland longspurs *(Calcarius lapponicus)* (Hunt *et al.*, 1997), chestnut-collared longspurs *(Calcarius ornatus)* (Lynn & Wingfield, 2008), great tits *(Parus major)* (Van Duyse *et al.*, 2002).

Two ecological constraints may potentially drive the insensitivity to T after the eggs hatch: 1) a short

breeding season, and 2) a need for paternal care to ensure reproductive success (Lynn, 2008). The "short season" hypothesis emphasizes that a short breeding season limits breeding opportunities and, consequently, reduces the benefits of sexual behavior late in the breeding season. By contrast, the "essential paternal care" hypothesis (Hunt *et al.*, 1999; Lynn *et al.*, 2002) suggests that reduced assistance by males in response to high T levels might lead to a significant reduction in reproductive success. The necessity of paternal care and a short breeding season may thus be strong selective factors driving behavioral insensitivity to T during the parental phase.

There have been numerous studies examining the relationship between testosterone and breeding behavior in songbirds (see Lynn, 2008 for a review). For example, T-treated male rufous whistlers *(Pachycephala rufiventris)* significantly reduced their incubation effort when compared with controls (McDonald *et al.*, 2001). Similar results were reported in studies of T-treated male European starlings *(Sturnus vulgaris)* (De Ridder *et al.*, 2000), blue-headed vireos *(Vireo solitarius)* (Van Roo, 2004), and dark-eyed juncos *(Junco hyemalis)* (Schoech *et al.*, 1998). T-treated incubating male spotted sandpipers *(Actitis mecularia)* either deserted their nest (30%) or exhibited reduced incubation constancy (50%), while controls incubated normally (Oring *et al.*, 1989).

Yet, understanding the generality of the hypotheses posed to explain behavioral insensitivity to testosterone requires experimental studies in different species: species of different orders of birds to allow phylogenetic comparisons (presumed independent evolution of behavioral insensitivity to T), as well as species with different life-histories. In contrast to the numerous studies concerning passerine birds, few studies have considered the effects of elevated T levels on the reproductive behavior of long-lived birds, which differ from shorter-lived species in their life-history trajectories. Seabirds are long-lived animals with obligate biparental care. Moreover, given the short reproductive season, polar seabirds such as penguins are typically unable to attempt a second brood. Hence, in the context of the ecological constraints that potentially drive the behavioral insensitivity to T in breeding birds, penguins are ideal subjects to study. To our knowledge, only two studies have experimentally altered T levels in seabird species (Alonso-Alvarez, 2001; Kazama *et al.*, 2011). These studies reported behavioral sensitivity to T, with increased sexual behavior and decreased parental investment (less time spent incubating and decreased egg temperatures) in T-treated yellow-legged gulls *Larus michahellis* (Alonso-Alvarez, 2001), and increased courtship and parental behaviors but no effect on aggressiveness and chick feeding rates in T-treated black-tailed gulls *Larus crassirostris* (Kazama *et al.*, 2011).

Here we investigated the effects of experimentally elevated T on the hormones and behavior of male Adélie penguins during incubation. We administered exogenous T at the beginning of the incubation period in male Adélie penguins. These birds spontaneously fast for a long period, from the courtship until the end of the first incubation shift, while the female went at sea to refeed after egg laying. We monitored the levels of total T and free T, as well as corticosterone, and dehydroepiandrosterone (DHEA). Indeed, an increase in T levels could also affect the secretion and/or action of other hormones, such as corticosterone (Viau, 2002) and DHEA, an androgen precursor (Labrie *et al.*, 1995), possibly leading to confounding

behavioral effects. Incubation temperatures and egg rotation rates were recorded with dummy eggs that replaced the clutches of some birds. Birds were also filmed to assess the effects of T-treatment on the birds' behavioral time budget, considering both sexual and aggressive behavior (song, positive social interactions, and agonistic interactions) and parental care (nest attentiveness and incubation). Because polar seabirds face a greater constraint with respect to the timing of breeding than gulls, we predicted that male Adélie penguins would display a low sensitivity to elevated T levels during incubation.

## Materials and Methods

*STUDY SITE*

Adélie penguins were studied at the French research station Dumont d'Urville (66°40'S, 140°01'E, Adélie Land, Antarctica) during two breeding seasons between November and December of 2010 and 2011. In 2010, the main objective was to assess the efficacy of the treatment to elevate T levels in incubating Adélie penguins. The protocol was approved by the ethics committee of the French Polar Institute Paul-Emile Victor (IPEV) and authorized by the Terres Australes et Antarctiques Françaises (French Southern and Antarctic Lands).

*BREEDING BIRDS*

Males were captured by hand on their nests at the end of the courtship period or the beginning of incubation (between November 25 and 27; this was after the female had left the nest in 2010 and on average one day after their partner laid the first egg in 2011; range: between two days before and three days after their partner laid the first egg in 2011). Blood samples were collected to determine plasma androgen levels prior to implantation. A total of 25 birds were studied during the incubation period (9 in November 2010 and 16 in November 2011). 12 of these males (5 in November 2010 and 7 in November 2011) were implanted with a testosterone pellet (A-151, 100mg, 21-day release) obtained from Innovative Research of America (Sarasota, FL, USA), which has been used successfully in a number of species (e.g. in golden-collared manakins *Manacus vitellinus*, Day *et al.*, 2006; Fusani *et al.*, 2007; Bengalese finches *Lonchura striata domestica*, Ritschard *et al.*, 2011). After skin disinfection with 70° alcohol, a small incision was made in the nape of the neck, using a scalpel blade. The pellet was positioned subcutaneously with tweezers. The incision was then closed with sterile suture, and sprayed with Alumisol® (CEVA, Libourne, France). The remaining 13 males underwent the same procedure, including handling, sampling and skin incision, but without actual implantation of a pellet. A previous study conducted with Adélie penguins found no differences in behavior and hormonal status between birds treated with a placebo pellet and birds that were sham-manipulated only, without actual pellet implantation (Spée *et al.*, 2011).

After handling, birds were weighed with an electronic balance (Ohaus, Giessen, Germany, resolution: ± 2 g), and the length of the left flipper was measured to the nearest mm. From this, a scaled body mass index was calculated according to Peig and Green (2009). For identification purposes a number was

painted on the birds' chests using black dye (Nyanzol-D®), before birds were released near their nest. At the end of the male incubation shift, 22 birds (11 controls and 11 T-treated birds) were recaptured for blood sampling and weighing. In January 2012, 15 birds (9 controls and 6 T-treated birds) were captured a third time to assess androgen levels after the end of the treatment. Sample sizes differ between groups as some birds were not recaptured when they left the colony because the nest relief happened very quickly.

*CAPTIVE BIRDS*

To study how androgenic hormone levels changed over time following the treatment, six failed breeders were captured between December 22, 2010 and January 2, 2011 and were kept in a pen (5.2 x 2.4m) on average for 14.2 ± 1.5 days. Two days after capture, three birds were blood sampled and implanted with a testosterone pellet to monitor hormone kinetics after implantation. Three control birds underwent the same procedure (handling, surgical procedure, and sampling) but were not implanted with a pellet. In our time course, day 0 corresponds to the date of implantation. Birds were blood sampled at day 0, day 2, day 4, and before being released from the pen, which was ~day 12 (range: day 8 to day 19). During the surgical procedure on day 0, birds were briefly anesthetized with isoflurane in pure O2 administered via a hood, placed over the birds' head. Birds were weighed every two days and released immediately, when body mass was 3.79 ± 0.06 kg. Previous studies found that the minimum mass threshold at which male Adélie penguin desert their nest, i.e. 3.2-3.5 kg, is below our chosen threshold (Cockrem *et al.*, 2006; Spée *et al.*, 2010).

*HORMONE ASSAYS*

Blood samples were collected from the alar vein, on average within four minutes of capture (4 min ± 0.01, range: 2 to 7 minutes), using a 2mL syringe and a 25G needle. In a few birds, handling time exceeded the time recommended to assess baseline corticosterone levels. Consequently, these samples were discarded from further analyses. Blood samples were transferred into tubes pre-treated with 25µL EDTA. Plasma was separated by centrifugation (4 °C, 5000rpm, 10 min) and kept frozen at -20°C until analyses at the IPHC (Strasbourg, France).

Total testosterone, free testosterone, DHEA, and corticosterone levels in the plasma were determined by immunoassay, according to guidelines provided by the manufacturer (ELISA kits, GenWay Biotech, San Diego, CA, USA; total testosterone: 40-052-115040, free testosterone: 40-056-205042, DHEA: 40-056-205045; AssayMax corticosterone ELISA Kit, EC3001-1, AssayPro, St. Charles, MO, USA). Because free T and DHEA kits were designed for assay of human serum, free T and DHEA levels are only used as proxies of these hormone levels to adjust for possible confounding effects of the T-treatment on the bird behavior. 10µL, 25µL, 50µL undiluted plasma samples were used to assay total T, free T, and DHEA levels, respectively. 25µL plasma samples diluted 1:4 into EIA were used to assay corticosterone levels, as previously validated for this species (Spée *et al.*, 2010). Intra-assay and inter-assay variations for total T were 10.0% and 8.9%, respectively, For free T these values were 14.7% and 8.3%, respectively. Intra-assay variations for DHEA and corticosterone were 8.4% and 12.4%, respectively. The minimum detectable doses for total T, free T, DHEA, and corticosterone were 0.05 ng/mL, 0.17 pg/mL, 0.082 ng/mL, and 0.3

ng/mL, respectively. Undetectable samples were assigned the detection limit.

*INCUBATION BEHAVIOR (2011-2012 ONLY)*

During the 2011-2012 austral summer, the second egg of 14 clutches (7 controls and 7 T-treated males) was replaced by a dummy egg that recorded incubation temperatures and egg position every 10 sec. This was done after the female left the nest, on average 2.2 ± 0.5 after implantation (range: 0.3 – 7.0 days). For a detailed description of these dummy eggs (characteristics, deployment, and data analysis), see Thierry *et al.* (2013b). In brief, from the recordings we calculated the maximum egg temperature every 10 sec and averaged it per day (hereafter referred to as 'egg temperature'). Egg temperature is a meaningful response variable to assess how hormonal treatment and/or male behavior affects offspring. We also defined rotation events, allowing calculation of the number of egg rotations per day. Incubation duration was calculated as the difference between the hatching date of the first chick and the laying date of the first egg. The first hatched chick of each clutch was weighed and the left flipper was measured five days after hatching. Hatching success was defined as the percentage of nests that successfully hatched at least one chick. One dummy egg was lost, most probably predated by a South Polar skua *(Catharacta maccormicki)* and two dummy eggs did not record data for the whole period, resulting in data from 7 controls and 6 T-treated males for an average of 10.2 ± 1.2 days per clutch (range: 1.8 – 17.1 days) .

In addition, we recorded incubation behavior of birds using a HD video camera (Sony XR550VE), placed in a waterproof container and mounted on a tripod that was positioned within a few meters from the nest. During analysis, a single observer watched randomly selected 30 min periods of the recorded video material from five T-treated and seven control birds and noted their behavior. Sample sizes differ between behavioral analyses because the number of dummy eggs was limited and because some nests could not be filmed from a distance. Birds were observed between Nov. 27 and Dec. 13 2011, i.e. between 2.5 ± 0.2 (range: 2 – 4) and 14.0 ± 0.6 (range: 10 – 17) after implantation. Bird behavior was separated into the following categories: rest, vigilance, egg turning and nest rearrangement, comfort, agonistic interactions (aggression), and positive social interactions (including songs), as defined in Thierry *et al.* (2013b). Besides from the detailed behavioral time budget, the position of the bird on the nest, i.e. standing or lying, was also observed. In Adélie penguins, standing in the upright position on the nest is typical for the early incubation period (Derksen, 1977), when birds incubate their first egg, possibly to delay development, before the second egg is laid (Spurr, 1975). Later on, however, eggs are typically incubated between their feet and an exposed brood patch on their belly, while birds are in a prone position.

*STATISTICAL ANALYSES*

Statistical analyses were performed with R 2.13.3 (R Development Core Team, 2011). Differences were considered as statistically significant when $p<0.05$. Results are expressed as means ± SE.

Wilcoxon tests were used to compare the following parameters between controls and T-treated birds: body mass and scaled mass index at the onset of incubation (beginning of the experiment), pre-treatment and post-treatment androgen levels, after checking that there were no differences between years in these

parameters. Generalized Linear Models (GLMs) with a Poisson distribution were used to compare laying date and clutch size (no. eggs per nest and no. chicks at hatching) between groups. Pre- and post-treatment hormone levels of the subset of birds with dummy eggs, and hormone levels of captive birds at the time of implantation were also compared with Wilcoxon tests. Generalized Estimating Equations (GEE) were used to compare post-treatment hormone levels between controls and T-treated birds, with treatment, number of days since implantation, and the interaction between treatment and day as factors, and repetition on penguin identity (geeglm function of the geepack package in R).

GEEs were also used to compare egg temperature and egg rotation rate between controls and T-treated birds. An autoregressive (AR[1]) correlation structure was assumed for egg-temperatures because consecutive measurements of incubation temperature lack independence. A GEE with a Poisson distribution was used to compare egg rotation rates between groups. The models included T-treatment, the number of days since implantation, and the interaction between treatment and day as factors, and repetition on penguin identity. Model selection was performed by excluding non-significant interactions first, followed by the removal of all non-significant factors. Models were compared using ANOVA (Zuur *et al.*, 2009) and QIC (Quasi-likelihood under the Independence model Criterion, Pan, 2001). Spearman correlation coefficients between post-treatment androgen levels and mean egg temperature during the day of the blood sample, and between post-treatment androgen levels and the number of rotations on that day were calculated to assess the relationship between endocrine status and incubation behavior.

The differences in the global behavioral time budget of males depending on their treatment were assessed with a multivariate analysis of variance (MANOVA). As recommended by Pezzanite and colleagues (2005) (see also Sokal & Rohlf, 1995), time budget proportions were angularly transformed (arcsin(proportions1/2)) prior to analyses to stabilize variances and eliminate estimation convergence problems associated with the proportions summing to 1. GEEs with binomial distribution were used for the behavioral time budget and bird position comparisons, with T-treatment, the number of days since implantation, and the interaction between treatment and day as factors, and repetition on penguin identity.

## Results

*PENGUIN STATUS AND BODY CONDITION*

There was a significant difference in body mass and scaled mass index at initial handling between years, with lighter birds in 2010-2011 (body mass: W=26, p=0.010, scaled mass index: W=32, p=0.025) despite the fact that initial handling dates were similar (GLM with a Poisson distribution, z=0.65, p=0.513). The two austral summers during which data was collected were different in terms of sea-ice conditions and arrival dates of Adélie penguins on their colonies (later arrival in 2010, Thierry A.M., unpublished data), possibly explaining the significant difference in body mass: birds had probably been fasting for a longer period. Hence, we only present results from the 2011-2012 austral summer in the following paragraph.

Date of initial handling and implantation did not differ between control and T-treated penguins (GLM

with a Poisson distribution, z=-0.05, p=0.96, Table 1). Body mass (W=19, p=0.203, Table 1) and scaled mass index (W=22, p=0.341) were similar in all birds prior to implantation. At the time of implantation, clutch size was not significantly different between experimental groups (GLM with a Poisson distribution, z=0.05, p=0.963, Table 1). Similarly, post-treatment body mass and scaled mass index (10 days after implantation) did not differ between groups (body mass: W=16, p=0.182, Table 1; scaled mass index: W=19, p=0.325).

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**Table 1:** Profiles of control and T-treated incubating male Adélie penguins

| | **Control** | | **T-treated** | |
|---|---|---|---|---|
| | 2010-2011 (n=4) | 2011-2012 (n=9) | 2010-2011 (n=5) | 2011-2012 (n=7) |
| Date of implantation | 27/11 ± 0.00 | 26/11 ± 0.18 | 27/11 ± 0.20 | 25/11 ± 0.20 |
| Male body mass at implantation | 4.35 ± 0.12 | 4.64 ± 0.11 | 4.33 ± 0.13 | 4.89 ± 0.16 |
| Female departure date | NA | 27/11 ± 0.36 | NA | 28/11 ± 0.97 |
| No. eggs per nest | 2.00 ± 0.00 | 1.89 ± 0.11 | 2.00 ± 0.00 | 1.86 ± 0.14 |
| Male body mass at departure | 3.87 ± 0.13 | 4.14 ± 0.11 | 3.89 ± 0.22 | 4.38 ± 0.15 |
| Daily mass loss during incubation (g/d) | 57.9 ± 4.2 | 50.3 ± 1.7 | 50.9 ± 1.3 | 48.2 ± 1.6 |
| Incubation duration (d) | NA | 32.9 ± 0.6 (n=7) | NA | 34.5 ± 0.8 (n=6) |
| Hatching success (%) | NA | 77.8 (7/9) | NA | 85.7 (6/7) |
| No. chicks at hatching | NA | 1.22 ± 0.28 | NA | 1.00 ± 0.22 |
| Chick mass 5 days after hatching (kg) | NA | 0.315 ± 0.05 (n=5) | NA | 0.097 ± 0.03 (n=3) |
| Chick flipper length 5 days after hatching (cm) | NA | 4.9 ± 0.2 (n=5) | NA | 3.7 ± 0.3 (n=3) |

Values are means ± SE.

*HORMONE LEVELS*

*Time course effects of T implants*

Androgen levels did not differ between control and T-treated captive birds at the time of implantation (total T: W=3, p=0.7; free T: W=6, p=0.7; DHEA: W=5, p=1), and reached their maximum two days after the beginning of the treatment (Fig. 1), indicating that testosterone diffusion from implants was high during the first two days following implantation. T-treatment had a significant effect on total T, free

T, and, to a lower extent, on DHEA levels (Table 2). T levels were negatively correlated with the number of days since implantation (Table 2), but remained elevated during the last days of captivity.

When compared to free-living birds, the androgen levels of captive birds were partly different (total T: W=30.5, p=0.023; free T: W=69, p=0.677; DHEA: W=68, p=0.648), most probably because the timing of the first blood sampled differed (end of November for the free-living birds vs. end of December for the captive birds) and/or they had a different breeding status (breeding vs. failed breeders, respectively).

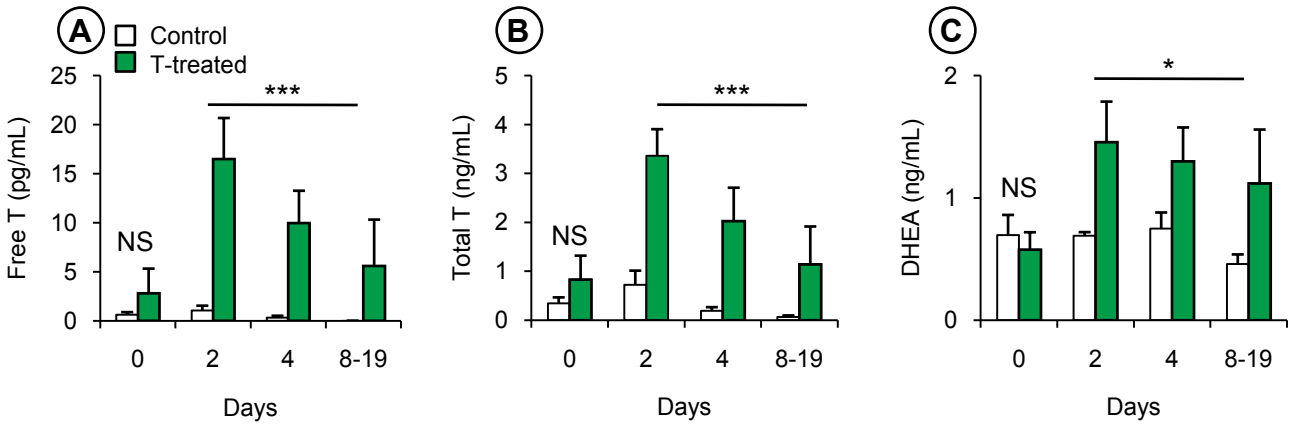


**Figure 1:** Effects of testosterone-treatment on free testosterone (**A**), total testosterone (**B**), and dehydroepiandrosterone (DHEA) levels (**C**) in captive male Adélie penguins (3 control and 3 T-treated birds) between the day of implantation (0) and up to 19 days post implantation

NS: Not Significant, *p<0.05, ***p<0.001.

**Table 2:** GEE results concerning the effects of T treatment, number of days since implantation, and their interaction on androgen levels in control and T-treated captive birds

| | Intercept | | | **T-treatment** | | | No. days since implantation | | | **T-treatment** x No. days | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Est. | Wald | p | Est. | Wald | p | Est. | Wald | p | Est. | Wald | p |
| Total T | 0.90 | 11.69 | <0.001 | 1.83 | 14.82 | <0.001 | -0.09 | 4.63 | 0.031 | | | NS |
| Free T | 0.86 | 13.15 | <0.001 | 14.85 | 34.74 | <0.001 | -0.05 | 17.84 | <0.001 | -0.79 | 12.18 | <0.001 |
| DHEA | 0.77 | 75.29 | <0.001 | 0.65 | 5.06 | 0.024 | -0.02 | 4.07 | 0.044 | | | NS |

NS: Not Significant

*Incubating penguins*

Pre-treatment testosterone and DHEA levels did not significantly differ between years (total T: W=38.5, p=0.061; free T: W=46, p=0.131; DHEA: W=25.5, p=0.122; Table 3). However, there was a significant difference in pre-treatment corticosterone levels between years, with higher corticosterone levels in 2010 compared to 2011 (W=95, p<0.001; Table 3).

Pre-treatment hormone levels did not differ between control and T-treated birds (total T: Wilcoxon, W=98, p=0.288; free T: W=85.5, p=0.691; DHEA: W=66, p=0.224; corticosterone: W=44, p=0.460,

Table 3). T implants effectively increased total T and free T levels. Accordingly, 10 days after implantation, total and free T levels differed significantly between control and T-treated penguins (total T: W=1.5, p<0.001; free T: W=1, p<0.001, Table 3). By contrast, DHEA and corticosterone levels were not different between T-treated and control birds at this point in time (DHEA: W=26, p=0.230, corticosterone: W=73, p=0.218; Table 3). In January 2012, penguins were re-captured to assess androgen levels after the end of the treatment. Total and free T levels did not differ significantly between groups at that time (total T: W=12.5, p=0.250; free T: W=10, p=0.123; Table 3). Similar results were found when comparing hormone levels for the subset of birds with a dummy egg (e.g. for total T: at implantation W=11.5, p=0.195; 10 days after implantation W=0, p=0.003; in January 2012: W=11, p=0.834).

**Table 3:** Hormone levels of control and T-treated male Adélie penguins

| | Pre-treatment levels (25-27 Nov.) | | Post-treatment levels (5-7 Dec.) | | Post-treatment levels (16-20 Jan.)* | |
|---|---|---|---|---|---|---|
| | **Control** n=13 | **T-treated** n=12 | **Control** n=11 | **T-treated** n=11 | **Control** n=9 | **T-treated** n=6 |
| Total Testosterone (ng/mL) | 0.35 ± 0.10 | 0.34 ± 0.14 | **0.14 ± 0.02** | **3.25 ± 0.62** | **0.31 ± 0.15** | **0.76 ± 0.33** |
| Free Testosterone (pg/mL) | 0.62 ± 0.17 | 0.86 ± 0.45 | **0.29 ± 0.12** | **22.16 ± 5.05** | **1.00 ± 0.63** | **2.51 ± 0.97** |
| DHEA (ng/mL) | 0.79 ± 0.15 | 0.62 ± 0.12 | 0.67 ± 0.08 | 0.83 ± 0.10 | NA | NA |
| Corticosterone (ng/mL) 2010-2011 | 10.0 ± 2.2 | 12.6 ± 0.1 | 14.1 ± 7.0 | 8.7 ± 3.4 | NA | NA |
| Corticosterone (ng/mL) 2011-2012 | 4.3 ± 0.6 | 4.7 ± 0.6 | 8.4 ± 1.8 | 6.1 ± 0.8 | NA | NA |

* Only available in 2011-2012
Values are means ± SE. NA: Not available. Figures shown in bold correspond to statistically different values between control and T-treated birds.

*INCUBATION PARAMETERS AND HATCHING SUCCESS*

Egg temperatures were significantly lower in T-treated birds (by 1.5 °C) when compared with control penguins (Fig. 2A-B, GEE, Wald $\chi^2$=3.84, p=0.050). Egg temperature and egg rotation rates (Fig. 2D-E) were not affected by the treatment (Wald $\chi^2$=2.72, p=0.099 and Wald $\chi^2$=1.07, p=0.301, respectively). However, egg rotation rates were negatively correlated with the number of days since implantation (Wald $\chi^2$=9.3, p=0.002). There was a significant correlation between T levels and egg temperatures on the day the blood was sampled, i.e. 10.6 ± 0.1 days after implantation (range: 10.0 – 11.0) (Fig. 2C, Total T: S=377.4, $\rho$=-0.72, p=0.013; Free T: S=371.4, $\rho$=-0.69, p=0.019) but not between DHEA or corticosterone levels and egg temperature (p>0.620). There was no correlation between the levels of the different hormones and the number of rotations on the day of the blood sample (Fig. 2F, p>0.267).

Incubation duration was not affected by the treatment (W=11, p=0.166, Table 1) and hatching success did not differ significantly between control and T-treated birds (Fisher's exact test, p=1, Table 1). We found a tendency for chicks hatching from eggs incubated by T-treated birds to be 69 % lighter and 24% smaller than those hatching from eggs incubated by control birds. However, these differences in chick mass and flipper length between the two groups were not significant (chick mass: W=14, p=0.071,

n=5; flipper length: W=14, p=0.072, n=3; Table 1). A power analysis suggests that a sample size of eight chicks per group would have been sufficient to detect significant differences in mass or size. However, this analysis does not allow taking into account the fact that such data may not be independent as two chicks may originate from a same nest.

*BEHAVIORAL TIME BUDGET AND BIRD POSTURE ON THE NEST*

The global behavioral time budget of incubating Adélie penguins was not affected by the T-treatment (Fig. 3A; MANOVA, Wilks's $\lambda$=0.060, p=0.073; GEE for each behavioral category, p>0.18). However, treated birds spent more time standing on the nest than controls (Fig. 3B; Wald $\chi^2$=9.7, p=0.002). There was no significant effect of the interaction between date and treatment for the different behaviors. Time spent in agonistic and positive social interactions decreased with the number of days since initial handling in all birds (Wald $\chi^2$=8.7, p=0.003 and Wald $\chi^2$=144.3, p<0.001, respectively).

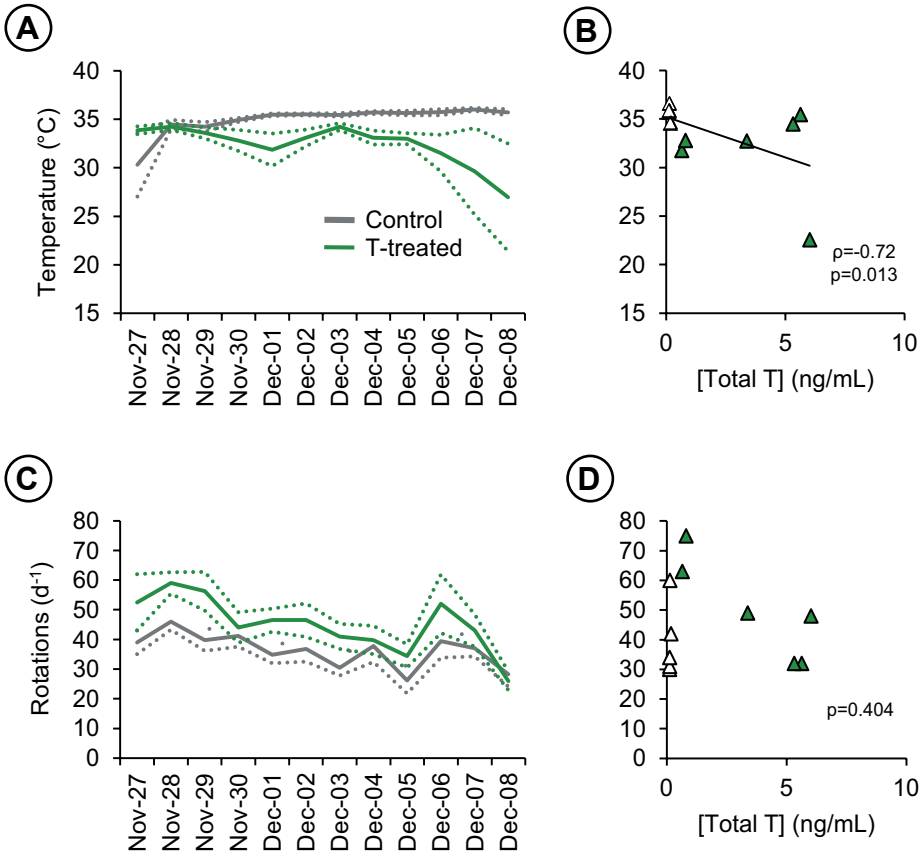


**Figure 2:** Incubation behavior recorded from control and testosterone-treated male Adélie penguins using dummy eggs. Parameters shown are egg temperatures (**A**, **B**), egg rotations rates (**C**, **D**) and the correlation between incubation parameters and total testosterone levels (**B** and **D**).

Grey lines and white triangles refer to control birds while plain black lines and black triangles refer to T-treated birds. Values are daily means ± SE (**A** and **B**). (n=13; 6 controls and 7 T-treated birds). NS: Not Significant, *p<0.05.

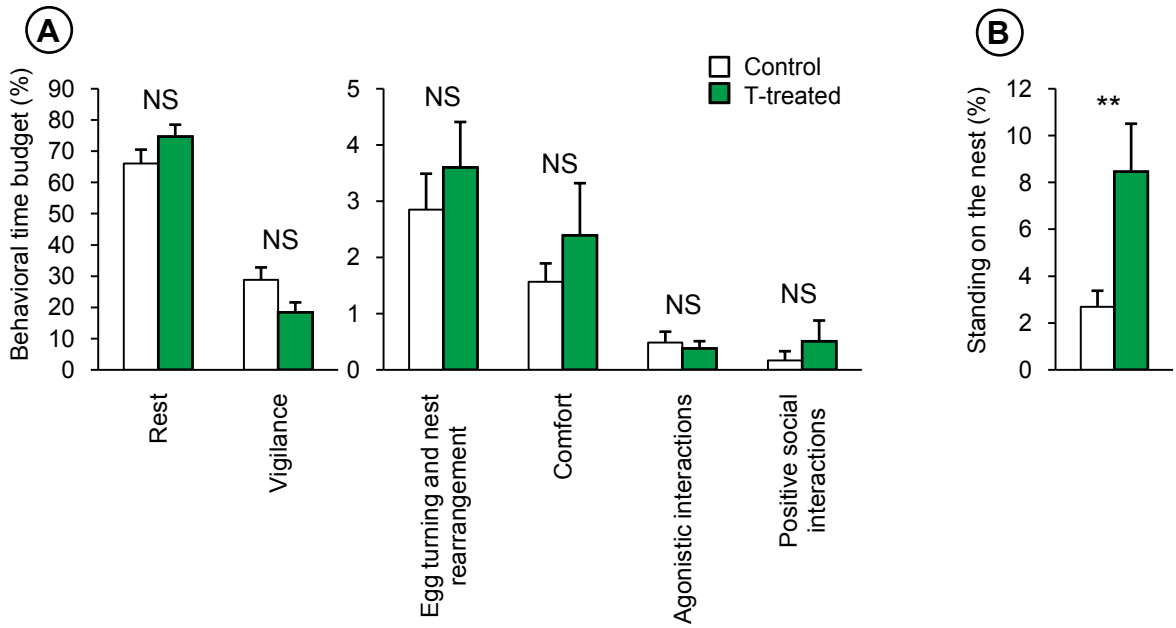


**Figure 3:** Behavioral time budget (A) and percentage of time spent standing in the upright position on the nest (B) for control and testosterone-treated male Adélie penguins (n=11; 6 controls and 5 T-treated birds, for a total of 38 hours of observations)

NS: Not Significant, **p<0.01.

## Discussion

We investigated the effects of increased T levels on the incubation behavior of male Adélie penguins and subsequent hatching success. Our study reveals a partial behavioral sensitivity to elevated T levels in a polar seabird at this stage of the reproductive cycle.

*BEHAVIORAL SENSITIVITY TO TESTOSTERONE IN A POLAR SEABIRD*

Behavioral insensitivity to T is expected to arise when males can benefit more from allocating care to their own nestlings than from enhancing sexual behavior in response to elevated T (Hunt *et al.*, 1999; Lynn *et al.*, 2002). Species with either essential paternal care and/or breeding during a short reproductive season are predicted to show this pattern. Our study shows that incubating male Adélie penguins treated with testosterone implants are sensitive to high T levels after the courtship. The experimental treatment decreased parental care, but did not affect aggressive and sexual behavior. There was also no effect of the treatment on hatching success. Post-treatment total T levels of treated birds averaged 3.3 ng/mL in our study, which are within the physiological range of T levels (2 to 8 ng/mL) that have been measured in Adélie penguins during courtship (McQueen *et al.*, 1999; Vleck *et al.*, 1999).

While there have been many studies on the relationship between T and paternal behavior in passerine birds, the only studies concerning seabirds were conducted with a Japanese and a Mediterranean gull species (Alonso-Alvarez, 2001; Kazama *et al.*, 2011). In both studies, T implants induced changes in the birds' behavior. This concerned particularly their sexual behavior, so that copulation frequency was increased (Alonso-Alvarez, 2001; Kazama *et al.*, 2011). Aggressiveness against egg-predators and against conspecifics was assessed in one of these studies and was not affected by the treatment (Kazama *et al.*, 2011). There are certain aspects in the life histories of Adélie penguins and gulls that are similar, for example an essential paternal care and a long life span. However, Adélie penguins are much more constrained in the timing of their reproduction than gulls. For example, egg laying is very synchronous in Adélie penguins, where half of all eggs are typically laid within a six-day period (Ainley, 2002). By contrast, the first eggs of black-tailed gulls are laid within a period of two to three weeks (K. Kazama, pers. comm.), and yellow-legged gulls lay their three eggs between mid March and early May. This comparison between gulls and Adélie penguins suggests that in seabirds the constraints imposed by a very short season available for breeding may be a stronger selective force than the necessity of paternal care in driving a reduced behavioral sensitivity to T.

Interestingly, results from studies concerning passerine birds suggest the opposite and favor the necessity of paternal care as the stronger selective force (Lynn *et al.*, 2005). Male snow buntings, which are constrained by a short breeding season, while paternal care is not essential for the survival of nestlings, changed their behavior in response to an experimental elevation of T (Lynn *et al.*, 2005). Similar experiments carried out with closely related longspur species *(Calcaricus spp.)*, for which paternal care is essential for nestling survival, demonstrated a behavioral insensitivity of these birds to experimental increases in T (Lynn *et al.*, 2005). However, in blue-headed vireos, a strictly monogamous songbird with biparental incubation, T

treatment depressed male parental behavior (Van Roo, 2004). Testing the behavioral sensitivity to T in a long-lived bird such as the Adélie penguin, which present both essential paternal care and a short breeding season, and a partial behavioral response to increased T, allows suggesting that Lynn's hypotheses may not generally applicable. The differences in behavioral responsiveness to T between songbirds and seabirds probably arise from the fact that these species notably differ from shorter-lived species in their life-history trajectories. Behavioral insensitivity to T may have evolved several times in birds. Further studies on species with different life-history traits and across a variety of breeding habitats are required to better understand the role of proximate and ultimate factors in shaping the behavioral sensitivity to T in birds.

*PHYSIOLOGICAL COSTS OF ELEVATED TESTOSTERONE LEVELS*

High T levels are associated with physiological costs such as changes in metabolic rate and altered energy balance (Lynn *et al.*, 2000; Wikelski *et al.*, 1999). For example, Feuerbacher and Prinzinger (1981) showed that elevated levels of T caused a decrease in the body temperature of Japanese quail (Coturnix coturnix japonica), possibly associated with changes in metabolism (Hänssler and Prinzinger, 1979). Hourly maximum of egg temperatures (data not shown), which can be used as a proxy for the temperature of the brood patch, was not affected by the treatment, suggesting that the treatment did not lead to a decrease in the body temperature of Adélie penguins. Metabolic rates were not measured in our study. However, we found no differences in body mass between groups, neither pre-treatment nor post-treatment, and the same was true for body mass loss. This suggests that changes in the resting metabolic rate of our fasting penguins were either minor or similar in both groups. Further work is required to improve our understanding of the links between T and energy metabolism.

The physiological effects of T-treatment are most likely due to direct and/or indirect actions of free T on target tissues. T implants effectively increased both total T and free T levels of male Adélie penguins in our study. However, an increase in T levels could also affect the secretion and/or action of other hormones, such as corticosterone. For example, in male dark-eyed juncos, T-treatment increased corticosterone and corticosterone-binding globulin (CBG) levels, as well as the responsiveness of the hypothalamic-pituitary-adrenal axis to stressors (Schoech *et al.*, 1999). Both corticosterone and T can bind to CBG (Deviche *et al.*, 2001); a change in CBG levels could alter the levels of both hormones in their unbound and physiologically active form (Siiteri *et al.*, 1982). Corticosterone levels were not increased in T-treated penguins. One cannot for certain exclude changes in free corticosterone levels in our study, even if T treatment did not affect free levels of corticosterone in male juncos (Breuner and Orchinik, 2002). Finally, DHEA, one of the main hormones associated with aggressive behavior in birds (outside the breeding season, when circulating testosterone levels are low) (Soma *et al.*, 2008), was not greatly affected by the T treatment of our incubating Adélie penguins. In our study, the absence of detectable effects of T implants on corticosterone and DHEA levels may result from complex regulation of both hypothalamic-pituitary-adrenal and -gonadal axes, even if down-regulation of hormone secretion by elevated T levels could have occurred (Labrie *et al.*, 1995).

*TESTOSTERONE AND THE TIMING OF BREEDING*

T is generally believed to play an important role in the regulation of the trade-off between sexual and parental behavior. Since plasma levels of T drop markedly after laying in male Adélie penguins (Davis *et al.*, 1995), the steroid hormone might potentially convey some information to birds regarding the timing of incubation. Our results lend support to this suggestion, as we observed behavioral patterns in T-treated birds that are more typical for the early incubation period, i.e. spending more time in the upright position on the nest. Furthermore, increased T levels did not affect the behavioral time budget of penguins per se, in particular with respect to sexual and parental behavior. We therefore suggest that male Adélie penguins display a partial behavioral sensitivity to high T levels, and that increased T potentially misled birds with respect to the timing of breeding.

Postural changes observed in our T-treated penguins during incubation quite possibly explain the lowered temperatures we measured for their eggs (by 1.5 °C), as eggs chill more rapidly when penguins stand upright (Derksen, 1977). Chicks hatching from eggs incubated by T-treated birds tended to be smaller and lighter than chicks incubated by control birds (measured five days after hatching). However, this difference was not significant ($p=0.07$), most likely because of the small sample size ($n=3$ and 5, respectively). In a study concerning rufous whistlers, McDonald and colleagues (2001) showed that T-treatment disrupted the incubation behavior of males. While T-treated males reduced the amount of time they engaged in egg incubation, control birds maintained their incubation effort. Unfortunately, since the authors of that study did not collect any information regarding the reproductive output or chick body condition at hatching, we do not know how increased T levels might have affected these parameters at this particular stage.

Other hormones, such as prolactin, play an important role with respect to the timing of breeding in Adélie penguins (Spée *et al.*, 2010; Thierry *et al.*, 2013a). Prolactin can affect incubation temperatures through differences in brood patch development, and might also play a role in the control of the timing of breeding in incubating male penguins (Thierry *et al.*, 2013a). Prolactin is generally associated with incubation, parental care, and brood patch development (Buntin, 1996; Etches *et al.*, 1979; Lea and Klandorf, 2002), behavior displayed after egg laying. Further studies are needed for a better understanding of the endocrine mechanisms underlying the timing of breeding in these polar seabirds.

1989) and in dark-eyed juncos (Schoech *et al.* 1998). To what extent this also applies to T-treated Adélie penguins remains to be determined. Clearly, for a better understanding of the physiological mechanisms underlying the timing of breeding in these polar seabirds, further studies are needed. This is especially true for an examination of the relationships between these various reproductive hormones in incubating Adélie penguins.

*CONCLUSIONS*

Our study shows that male Adélie penguins display a partial behavioral sensitivity to high T levels during incubation. Indeed, increased T levels did not affect the behavioral time budget of birds per se, but

changed their behavior towards patterns more typically observed during early incubation. The results also suggest that T might convey information to birds regarding the timing of breeding, with high T levels misleading birds towards pattern of early incubation. Our study provides new insights into the role of testosterone in the control of parental behavior in a long-lived seabird, helping to understand the generality of the hypothesis posed to explain behavioral insensitivity to T in birds. It would be interesting to further evaluate how changes in the endocrine status of parents affect embryonic development, chick body condition at hatching, and chick survival. Further studies should also examine the relationships between the different reproductive hormones in incubating Adélie penguins, so that a better understanding of the physiological mechanisms underlying the timing of breeding in these polar seabirds will be possible.

**Acknowledgments**

The French Polar Institute Paul-Emile Victor (IPEV) provided logistic support through the 137/ ECOPHY research program. We thank F. Amélineau, M. Boureau, and M. Debin for their great help in the field. We also thank S. Brajon for her help in establishing an ethogram of the behaviors of incubating Adélie penguins.

## 3.4 Etude 4 : Météorologie et incubation

**On the relationships between weather conditions, incubation parameters, and hatching success in Adélie penguins over five breeding seasons**

Anne-Mathilde Thierry, Michael Beaulieu, Thierry Raclot

*Données non publiées*

Dans l'étude 1, nous avons pu mettre en évidence que des conditions météorologiques sévères, telles qu'un épisode de blizzard suivi d'une période de fonte de la neige, pouvait affecter le comportement d'incubation des manchots Adélie, en particulier pour les manchots avec des niveaux élevés de corticostérone (Thierry *et al.* 2013b). Les oiseaux marins polaires sont adaptés aux conditions météorologiques extrêmes typiques de ces milieux, telles que des températures basses, des vents violents et de la neige (Moss, 1988). Pourtant, des conditions météorologiques changeantes peuvent avoir des effets désastreux sur le succès reproducteur d'espèces adaptées à cet environnement polaire (Wingfield *et al.* 2011). Les effets biologiques de mauvaises conditions météorologiques sont susceptibles de différer selon la période à laquelle les oiseaux y font face, avec des conséquences particulièrement néfastes pendant l'incubation et dans les jours suivant l'éclosion. De plus, les modèles climatiques prédisent une augmentation des températures mais également des précipitations en Antarctique (Bracegirdle *et al.* 2008). La fonte de neige lors de périodes chaudes a des effets plus néfastes qu'une accumulation de neige et de glace pour le pétrel des neiges *Pagrodoma nivea,* un autre oiseau marin se reproduisant autour du continent Antarctique (Chastel *et al.* 1993, Wingfield *et al.* 2011). Les relations entre les conditions météorologiques et le comportement au nid des manchots Adélie n'ont jamais été décrites de façon détaillée. L'objectif de cette étude est de comparer les données de comportement d'incubation enregistrées au cours de plusieurs saisons de reproduction et de succès à l'éclosion avec les conditions météorologiques.

## Matériel & Méthodes

Un total de 167 nids ont été suivis de la ponte à l'éclosion pendant cinq saisons de reproduction consécutives, de 2007-2008 à 2011-2012. La date de ponte n'ayant pas été observée pour tous ces nids, la date de départ en mer de la femelle après la ponte a été utilisée comme proxy de la date de ponte (Ainley, 2002). Pour ces nids sont également connus le nombre d'œufs pondus, l'issue de l'incubation (succès ou échec), et pour les nids en succès la date d'éclosion et le nombre de poussins éclos. Enfin, le nombre de jours pendant lesquels il a neigé entre le départ de la femelle et l'éclosion du premier poussin et différents autres paramètres météorologiques tels que la température, la vitesse et la direction du vent et le rayonnement solaire ont été enregistrés à la station Météo France de Dumont d'Urville.

Des œufs factices enregistreurs de températures et de mouvements de rotation ont également été utilisés pendant les étés 2008-2009, 2009-2010 et 2011-2012 (Fig. 1). Le comportement d'incubation de 18 mâles et 4 femelles ont été enregistrés (durée moyenne d'enregistrement : 11,5 jours par mâle et 1,5 jour par femelles). La description détaillée de l'analyse des données enregistrées par les œufs factices est disponible dans le paragraphe Paramètres d'incubation (pages 34-35).

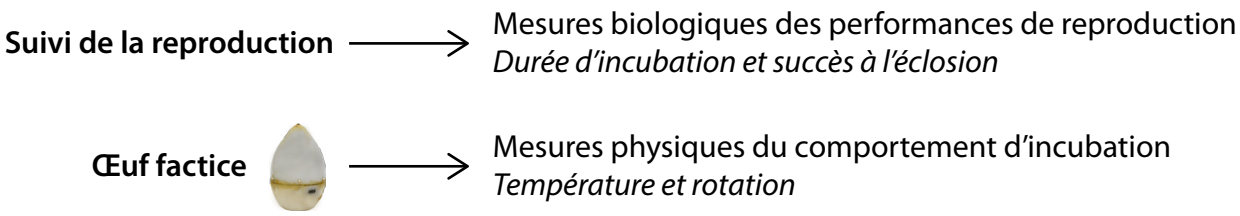


**Figure 1 :** Résumé des méthodes utilisées

## Résultats préliminaires

La durée d'incubation diffère en fonction des années (ANOVA suivie d'un test de Tukey, $F_{4,111}$=11.82, p<0.001), avec une durée d'incubation plus longue en 2010-2011 et 2011-2012 (Fig. 2A). Il y a eu significativement moins de poussins à l'éclosion en 2011-2012 (vs. 2007-2008 p=0.079, vs. 2008-2009 : p=0.003, vs. 2009-2010 : p<0.001, vs. 2010-2011 : p=0.127, GLM avec une distribution quasi-Poisson). Il y a globalement peu de différence en termes d'échecs pendant l'incubation entre les années (GLM binomial suivi de comparaisons multiples, p>0.237), sauf pour 2011-2012 où l'on observe significativement plus d'échecs qu'en 2008-2009 (p=0.046). Les échecs tendent également à être plus fréquents en 2011-2012 qu'en 2007-2008 (p=0.076).

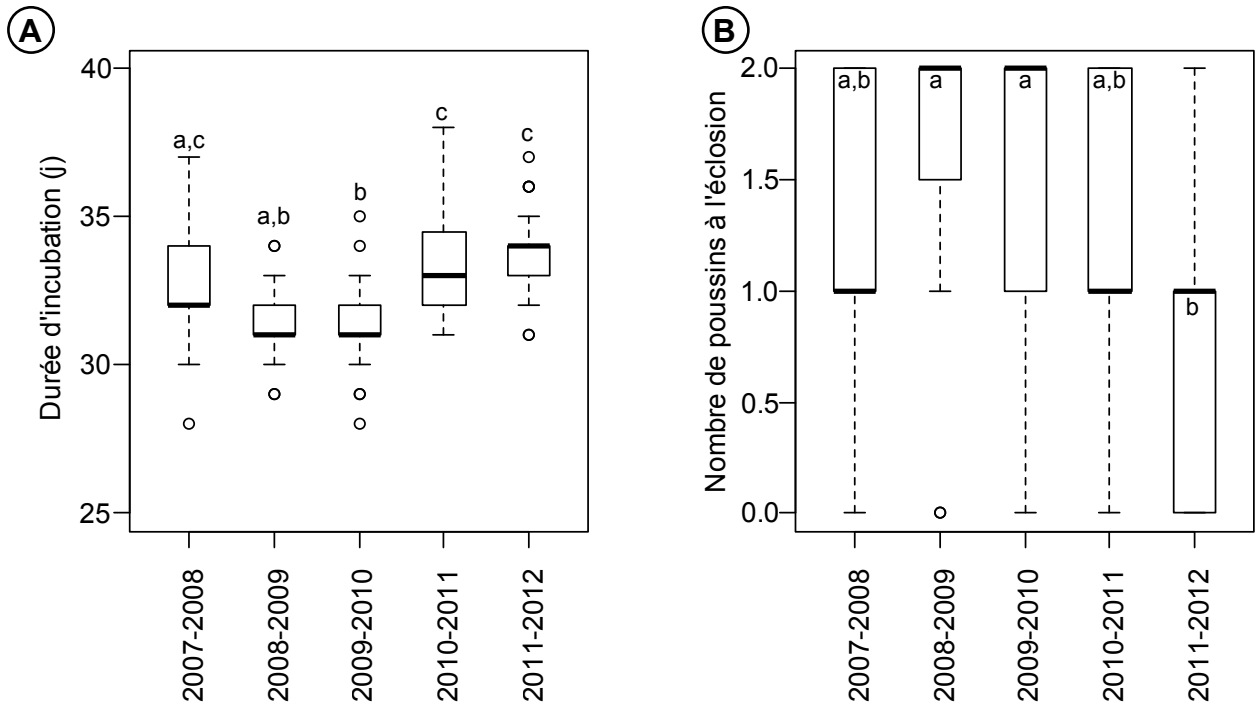


**Figure 2 :** Durée d'incubation (**A**) et succès à l'éclosion (**B**) en fonction des années

Des lettres différentes signalent une différence significative à p<0,05.

Il existe une corrélation positive significative entre le nombre de jours où il a neigé pendant l'incubation et la durée d'incubation (Fig. 3A, corrélation de Spearman, t=4.61, p<0.001, ρ=0.44). Il existe également une relation positive significative entre la vitesse moyenne du vent pendant la période d'incubation et la durée d'incubation (Fig. 3B, t=187271, p=0.002, ρ=0.28), mais pas entre la température extérieure moyenne et la durée d'incubation (t=252031, p=0.740).

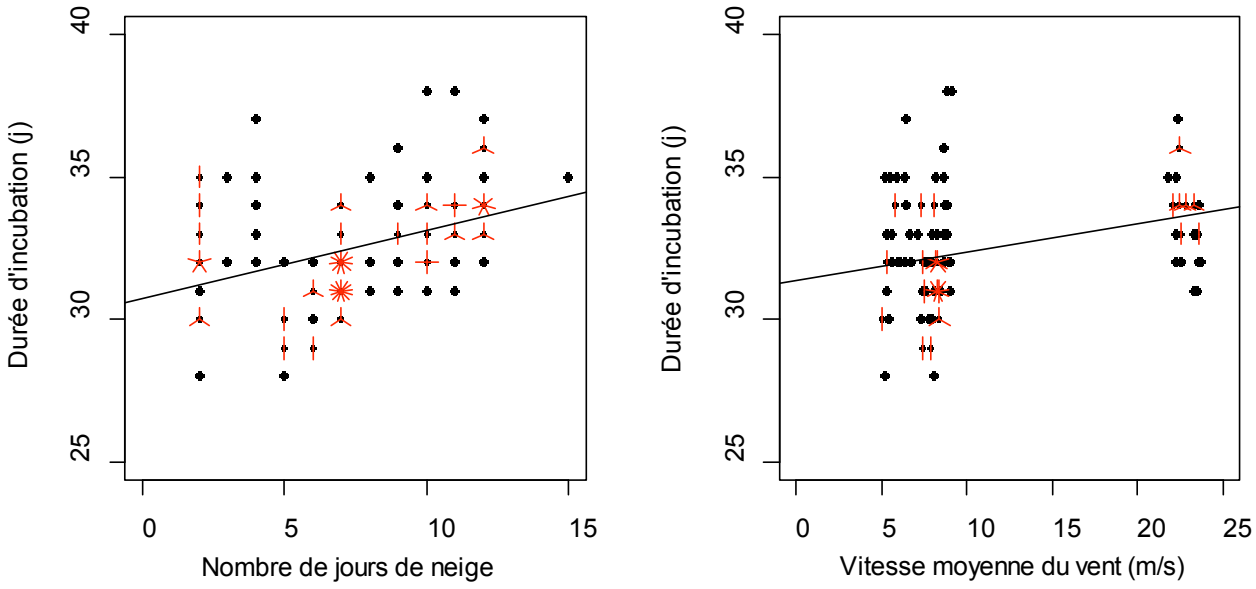


**Figure 3 :** Relation entre la durée d'incubation et le nombre de jours de neige (**A**) ou la vitesse moyenne du vent (**B**)

Lorsque plusieurs observations prennent la même valeur, ces observations sont représentées par des traits rouges autour du point correspondant.

Les paramètres d'incubation enregistrés lors des étés 2008-2009, 2009-2010 et 2011-2012 ont été mis en relation avec les conditions météorologiques. Le sexe du manchot a également été pris en compte dans les modèles. Les résultats sont présentés dans la figure et la table suivantes :

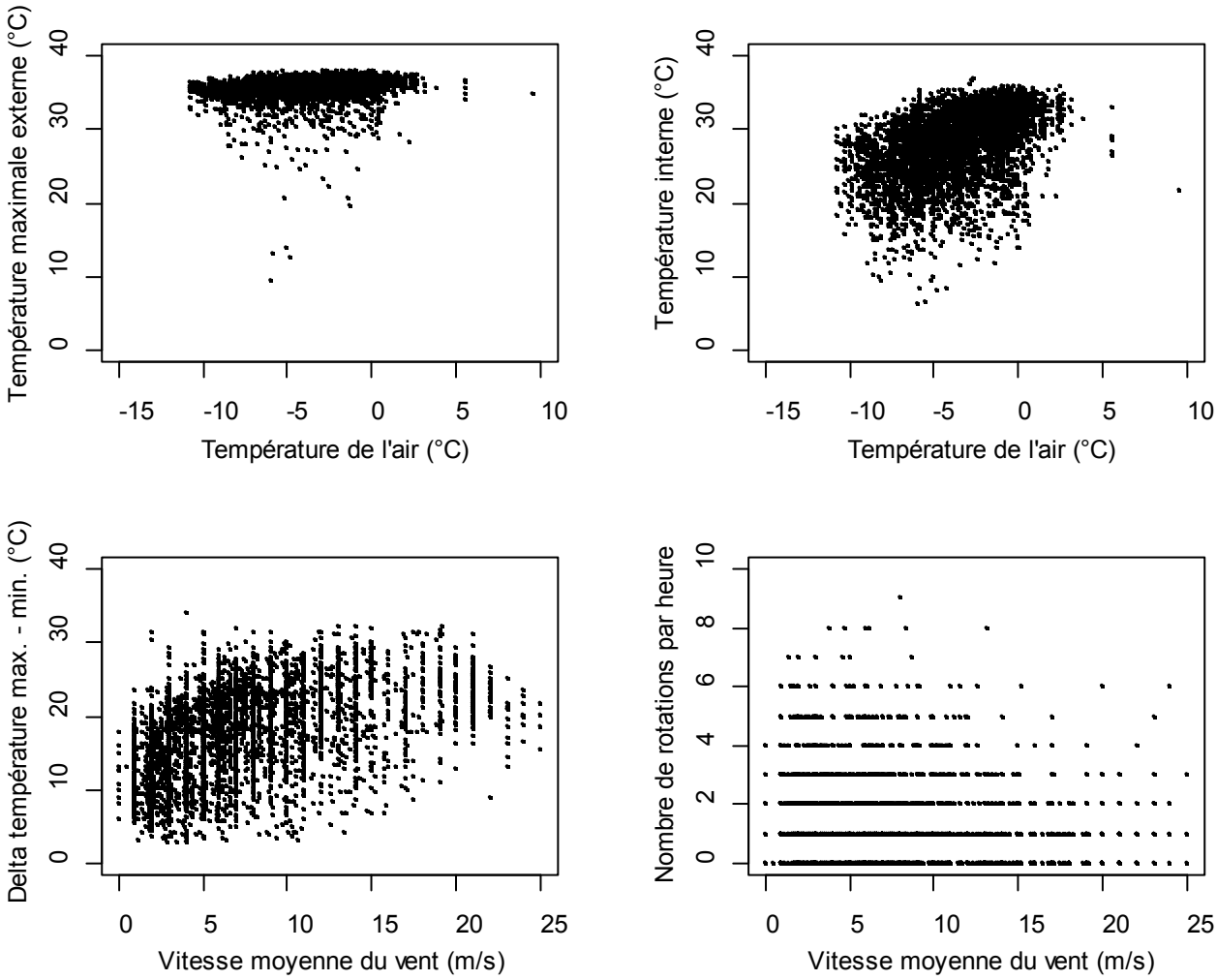


**Figure 4 :** Représentation de quelques paramètres d'incubation enregistrés par les œufs factices en fonction des conditions météorologiques

**Tableau 1 :** Relations entre paramètres d'incubation enregistrés par les œufs factices (données horaires), sexe et conditions météorologiques (GEE, matrice de corrélation de type ar(1), distribution de Poisson pour le modèle concernant le nombre de rotations)

| | **Sexe** | **Température extérieure (°C)** | **Vent moyen (m/s)** | **Humidité** |
|---|---|---|---|---|
| Température externe maximale | NS | NS | NS | NS |
| Maximum horaire de la température maximale | NS | + (<0.001) | NS | NS |
| Température externe minimale | NS | + (<0.001) | - (<0.001) | NS |
| Différence entre températures externes maximale et minimale | + (0.001) | - (<0.001) | + (<0.001) | - (0.018) |
| Température interne | NS | + (0.007) | - (<0.001) | NS |
| Nombre de rotations | NS | + (0.024) | - (<0.001) | - (0.033) |

**Discussion**

Les résultats montrent que les conditions météorologiques sont susceptibles de modifier les paramètres d'incubation des manchots Adélie et la durée de l'incubation. Les tendances observées sont en accord avec une étude modélisant les relations entre phénologie et conditions environnementales chez le manchot Adélie, montrant une relation positive entre durée d'incubation et nombre de jours de neige, et entre durée d'incubation et vitesse du vent (Emmerson *et al.* 2011). Les observations montrent également que certains paramètres d'incubation diffèrent entre mâles et femelles. Il conviendrait donc d'étudier la contribution des femelles à l'incubation avec plus de détails, et les possibles différences dans les réponses des manchots à des mauvaises conditions météorologiques en fonction de leur sexe. D'autre part, les relations entre conditions météorologiques, paramètre d'incubation et développement de l'embryon sont encore à préciser. Des températures d'incubation diminuées peuvent augmenter la durée d'incubation, reflétant ainsi un développement embryonnaire ralenti (Hepp *et al.* 2006). Les embryons de manchots Adélie semblent relativement résistants, dans la mesure où des œufs couvés à des températures réduites ont éclos (Etudes 1 et 2), mais une diminution des températures est susceptible d'affecter la condition corporelle et les niveaux de corticostérone des poussins à l'éclosion (DuRant *et al.* 2010). Une augmentation de la durée d'incubation peut également avoir des conséquences indirectes sur le succès reproducteur des parents, dans la mesure où la période de reproduction est courte et l'accès aux ressources alimentaires restreint.

Chez le pétrel des neiges, un oiseau se reproduisant dans des conditions comparables à celles du manchot Adélie, une augmentation des températures et la fonte conséquente de la neige a des conséquences plus néfastes qu'une accumulation de neige et de glace (Chastel *et al.* 1993 ; Wingfield *et al.* 2011). Dans quelle mesure l'augmentation des températures et des précipitations en Antarctique prédite par les modèles climatiques (Bracegirdle *et al.* 2008) peuvent-elles affecter la durée et la qualité de l'incubation des manchots ? Si notre étude a bien mis en évidence les relations entre conditions environnementales et durée d'incubation, il serait intéressant de déterminer l'effet de conditions d'incubation variables sur les paramètres d'incubation et le succès à l'éclosion, ce que ne permet pas le jeu de données dont nous disposons. De plus, de mauvaises conditions météorologiques dans les quelques jours suivant l'éclosion sont également susceptibles d'avoir des effets néfastes sur le succès reproducteur puisque les jeunes poussins ne sont pas émancipés thermiquement et sont donc à considérer dans le cadre de l'étude des relations entre conditions météorologiques et succès reproducteur.

Contrairement aux études précédentes, cette étude n'a pas considéré le statut endocrinien des oiseaux. Pourtant, certains paramètres tels que les niveaux de corticostérone sont susceptibles de modifier les effets de conditions environnementales particulières sur le comportement d'incubation (Etude 1). Un événement stressant ponctuel, du type d'une tempête de neige, peut également interrompre la reproduction du pétrel des neiges, en particulier celle des jeunes individus qui présentent une réponse au stress plus importante que les individus plus âgés (Angelier *et al.* 2007b ; Goutte *et al.* 2010b). De prochaines études

pourraient s'intéresser aux effets de mauvaises conditions météorologiques et de la période à laquelle elles se produisent sur le statut endocrinien des manchots.

Si le manchot Adélie est une espèce intéressante pour étudier le comportement d'incubation d'un animal sauvage dans un environnement polaire changeant, les relations entre paramètres d'incubation, développement de l'embryon et succès à l'éclosion sont difficiles à appréhender du fait que le manchot couve un ou deux œufs seulement. L'étude d'oiseaux incubant des couvées plus grandes permettrait de préciser la contribution de variations individuelles du comportement d'incubation au succès à l'éclosion, la répétabilité de ces variations, les aspects du phénotype d'un oiseau qui déterminent cette variation individuelle ainsi que les effets de modifications de conditions d'incubation sur les poussins à l'éclosion (e.g. études réalisées chez le canard carolin *Aix sponsa* Hepp *et al.* 2006 ; DuRant *et al.* 2010 ; 2012).

**Remerciements**
Nous remercions Akiko Kato pour son aide dans l'utilisation d'IgorPro pour analyser les données enregistrées par les œufs loggers, ainsi qu'Elise Devos et Marjory Thomas pour leur participation à la mise en relation des paramètres d'incubation avec les données météorologiques dans le cadre d'un stage de Master 1.

# Corticostérone

# 4. Corticostérone et investissement parental à différentes périodes du cycle de reproduction et différentes doses

La partie précédente s'est intéressée à l'incubation. En particulier, l'étude 1 a considéré les effets d'une augmentation des niveaux de corticostérone sur l'investissement parental pendant l'incubation. Dans cette seconde partie des résultats de la thèse, trois études concernant les effets d'une manipulation des niveaux de corticostérone sur l'effort de reproduction à différentes doses et périodes du cycle de reproduction sont présentées. Les études 5 et 6 concernent les effets d'un traitement similaire à celui de l'étude 1 sur le succès reproducteur et le comportement de manchots Adélie. L'étude 7 a considéré les conséquences d'une faible élévation des niveaux de corticostérone sur l'effort reproducteur pendant l'ensemble de la saison de reproduction.

## 4.1 Etude 5 : Corticostérone et élevage des poussins – I : effets sur le succès reproducteur

**Elevated corticosterone levels decrease reproductive output of chick-rearing Adélie penguins but do not affect chick mass at fledging**

Anne-Mathilde Thierry, Yan Ropert-Coudert, Thierry Raclot

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Jusqu'alors, nous avions considéré les conséquences d'une modification du statut endocrinien sur l'investissement parental de manchots Adélie mâles pendant l'incubation, lorsque les manchots sont à jeun. Il convient de s'intéresser à la période d'élevage des poussins, pendant laquelle les manchots alternent entre des séjours à terre pour nourrir les poussins et des voyages alimentaires en mer. Si le statut nutritionnel des animaux n'est pas le même entre l'incubation et la période d'élevage des poussins, il faut également prendre en compte que la valeur de la nichée (brood value) augmente au fur et à mesure de la saison de reproduction et peut moduler les conséquences d'une modification du statut endocrinien. Cette étude expérimentale préliminaire a consisté à mesurer les effets d'une augmentation des niveaux de corticostérone sur le succès reproducteur des manchots Adélie au cours de l'élevage. L'objectif de ce travail est de mieux comprendre le rôle de cette hormone dans le compromis entre reproduction et survie à une période du cycle de reproduction particulièrement coûteuse en énergie.

**Abstract**

Studying physiological mechanisms can help understand how animals respond to changing environmental conditions. In particular, stress hormones (i.e. glucocorticoids such as corticosterone) are described as mediating resource allocation, allowing animals to adjust their physiology and behaviour to predictable and unpredictable changes in the environment. In this study, we investigated the effects of an increase in baseline corticosterone levels on the breeding effort and the reproductive output of chick-rearing male Adélie penguins (*Pygoscelis adeliae*). The number of chicks per nest, their body mass, and their size were monitored throughout the study. Direct observations allowed measuring the time spent foraging at sea and caring for the young on the nest. At the end of the treatment, blood samples were collected for isotope analysis. Although all birds raised at least one chick, reproductive output was decreased by 42 % in corticosterone-treated birds compared with controls. The increase in corticosterone levels during the guard stage neither affected the mass of surviving chicks nor the brood mass at fledging. Corticosterone-treated males spent on average 21 % more time at the nest than controls. However, foraging trips duration was similar between both groups. In addition, similarity of isotopic signatures suggests that both groups foraged at similar locations and ingested the same preys. The detailed on-land behaviour of birds should be examined in further studies to clarify the possible links between corticosterone levels, brooding time, and reproductive output. Understanding the relationships between glucocorticoids, fitness, and ultimately population dynamics is fundamental to conservation physiology as a discipline being successful in helping to manage species of conservation concern.

**Introduction**

Organisms live in a changing environment, which they deal with by adjusting their morphology, physiology, and behaviour to face current conditions. Many studies report that increases in environmental variability associated with climate change affect wildlife drastically (Stenseth *et al.* 2003; Walther *et al.* 2002). For instance, cases of animal population decline in response to such changes are increasingly reported (Both *et al.* 2006; Croxall *et al.* 2002; McCarthy *et al.* 2001). Animals face trade-offs in terms of how they allocate energy to different biological functions, such as reproduction and survival (Stearns, 1992). Although changes in key life-history trade-offs are thought to be at the heart of these population declines (e.g. Acevedo-Whitehouse & Duffus, 2009; Woodhams *et al.* 2008), we still know little about the mechanisms underlying these declines. In order to better understand how organisms respond to changing environmental conditions, physiological mechanisms must be considered (Chown & Gaston, 2008; Chown *et al.* 2010; Fuller *et al.* 2010; Pörtner, 2002; Pörtner & Farrell, 2008). In particular, stress hormones (i.e. glucocorticoids) are described as mediating resource allocation, allowing animals to adjust their physiology and behaviour to both predictable and unpredictable regimes of environmental variations (Jacobs & Wingfield, 2000; Wingfield & Silverin, 2009).

Glucocorticoids are acutely released under life-threatening situations, such as food shortage (Kitaysky *et al.* 1999), severe weather conditions (Romero *et al.* 2000), and acute predation risk (Cockrem & Silverin, 2002). The activation of the hypothalamic-pituitary-adrenal axis and the subsequent release of glucocorticoids trigger the emergency life-history stage, i.e. when an individual aborts the current breeding attempt in order to survive the perturbation (Landys *et al.* 2006; Wingfield, 2003; Wingfield & Kitaysky, 2002; Wingfield *et al.* 1998). Elevated glucocorticoid levels can affect the physiology and behaviour of animals in a variety of ways. In particular, stress hormones control energy metabolism and fuel utilisation and may promote escape behaviour through glucose mobilisation and increased locomotor activity, finally leading to a reduction in or abandonment of the reproductive effort (see Breuner, 2011; Landys *et al.* 2006 for review). Elevated glucocorticoid levels also enhance activities related to foraging behaviour and food intake. In birds, elevated baseline levels of corticosterone (the main avian glucocorticoid, hereafter CORT) generally observed during reproduction might facilitate reproductive effort (Love *et al.* 2004; Romero, 2002), especially allowing individuals to supply their offspring through its positive effect on foraging activity (Angelier *et al.* 2008; Angelier *et al.* 2007c; Crossin *et al.* 2012; Koch *et al.* 2002; Koch *et al.* 2004; Miller *et al.* 2009).

On the other hand, high CORT levels are suspected to disrupt parental behaviour, as they are often associated with abandonment of reproduction in birds (Groscolas *et al.* 2008; Silverin, 1986; Spée *et al.* 2010; Wingfield & Sapolsky, 2003). These contrasting effects seem to be driven by extrinsic factors: during unfavourable environmental conditions, when organisms cope with high energetic constraints, CORT could redirect energy allocation from chicks provisioning to the benefit of self-maintenance (reviewed in Wingfield *et al.* 1998). Furthermore, it is often assumed, despite little direct evidence,

that the acute adrenocortical response to stress favours self-maintenance behaviour at the expense of current reproduction (see Breuner *et al.* 2008 for a detailed review of the relationships between the acute adrenocortical response and fitness). Growing evidence suggests that modulating baseline CORT levels participates in the mediation of trade-offs between current reproductive output (parental investment) and self-maintenance through foraging activities (Angelier *et al.* 2008; Angelier *et al.* 2007a; Horton & Holberton, 2009; Kitaysky *et al.* 2001; Landys *et al.* 2006). Subcutaneous CORT implants modulating baseline CORT levels may help better understand the mechanisms linking glucocorticoids and reproductive effort, and hence allow establishing conservation measures in species facing changes in their environment.

Seabirds respond to food shortages by increasing circulating baseline corticosterone levels. For example, a three-fold increase in stress hormone levels was measured in food-deprived black-legged kittiwakes *(Rissa tridactyla)* adults and chicks (Kitaysky *et al.* 2001). Artificially increasing CORT level could, to some extent, mimic the effect of an environmental stressor. The objectives of this study were hence to examine the consequences of an experimental increase in baseline CORT levels on the parental effort and reproductive output of control and CORT-treated Adélie penguins *(Pygoscelis adeliae).*

Polar ecosystems are relatively pristine environments (Bargagli, 2004). Yet, recent climate change poses a new challenge to the survival of Arctic and Antarctic wildlife. For example, Adélie penguin populations are increasing in the Ross Sea region and decreasing in the Antarctic Peninsula, with an overall increase of the net global population (Ainley *et al.* 2010). However, the species is expected to undergo a 30 % population decline over the next three generations due to the effects of projected climate change, in particular in relation with a decrease in sea-ice concentration (Ainley *et al.* 2010). Loss of sea-ice can be seen as a major stressor for Adélie penguins and other top predators. Indeed, sea-ice is the preferred habitat of Antarctic krill *Euphausia superba*, the main food source of penguins, leading to a strong dependency of Adélie penguins to sea-ice (Adélie penguins as "creature of the pack ice", see Ainley, 2002). There can be important inter-annual variations in environmental conditions in Antarctica, in particular regarding sea-ice extent and the timing of its sea-ice retreat. These changes can have major consequences on the breeding success of Adélie penguins (Emmerson & Southwell, 2008), corticosterone levels (Cockrem *et al.* 2006), and foraging trip durations (Beaulieu *et al.* 2010a). The species has recently been uplisted from Least Concern to Near Threatened on the IUCN Red List for these reasons (BirdLife International, 2012). Future climatic changes remain largely uncertain, and further work is required to determine how they will impact penguins. As such, we need to rapidly establish a benchmark for future investigations and understand the factors affecting Adélie penguins' breeding success.

Exogenous CORT induced nest abandonment of fasting incubating male Adélie penguins (Spée *et al.* 2011a), together with decreased incubation temperatures and a lengthened incubation period (Thierry *et al.* 2013b). We could hence expect the CORT treatment to increase the nest desertion rate of chick-rearing male Adélie penguins, resulting in decreased reproductive output. In this case, individuals would tend to allocate their energy to self-maintenance at the expense of the current reproduction. On the other hand, CORT implants in female macaroni penguins *Eudyptes chrysolophus* were found to positively affect

foraging behaviour, parental care, and chick growth (Crossin *et al.* 2012). Consequently, positive effects of the treatment on the reproductive effort could also be expected in our study. To distinguish between these contrasting predictions, we manipulated the CORT levels and examined the effects of the treatment on the parental effort of chick-rearing Adélie penguins.

## Materials and Methods

### *STUDY SITE AND SPECIES*

The study was carried out at the French research station Dumont d'Urville (66°40'S, 140°01'E), East Antarctica, during the 2008-2009 austral summer. Adélie penguins reproduce once a year. Their breeding cycle comprises four distinct stages from mid-October to mid-February: courtship, incubation, guard stage, and crèche stage. This study focuses on the guard stage, when both parents alternate between foraging at sea and chick attendance at the nest.

Adélie penguins weigh 3.2-8kg, depending on the life-history stage. Females usually lay two eggs (mean clutch size: 1.8), and 1.6 chicks per nest hatch. About one chick is fledged per breeding pair, with a mean weight at fledging of about 3 kg (Ainley, 2002). Although in Adélie penguins, like in most seabird species, both parents care for their young, previous studies on stress hormones in Adélie penguins have mostly considered male birds (Spée *et al.* 2011a; Thierry *et al.* 2013b) because males can fast for up to 40-50 days at the beginning of the breeding season (Vleck & Vleck, 2002). In order to get data comparable with these previous studies and because treating both partners could induce confounding effects or be deleterious for the current reproduction, only male Adélie penguins were studied here.

### *STUDY PROTOCOL*

The protocol was approved by the ethic committee of the French Polar Institute (IPEV, Institut Paul-Emile Victor) and authorised by the French Southern and Antarctic Territories (TAAF, Terres Australes et Antarctiques Françaises).

Thirty randomly selected pairs were captured on their nest at the end of the courtship (mid-November). Each member of the pair was identified with a Nyanzol-D number painted on the chest feathers and stickers inserted between the back feathers (Beaulieu *et al.* 2010a), allowing easy identification in the colony. Penguins were sexed by a combination of parameters including cloacal inspection before egg laying and observations of incubation routine (Beaulieu *et al.* 2010a; Kerry *et al.* 1993). Visual observations of the 30 nests were made from a distance every 2 to 3 hours a day during the entire study period, to observe laying, hatching, presence of each partner on the nest, and to measure foraging trip duration.

At the beginning of the guard stage, pairs were randomly assigned to control (n=7) and experimental groups (n=7) among the 30 pairs marked during the courtship, which were synchronised in their breeding cycle (similar hatching dates and number of foraging trips made before implantation). All males were

captured on two occasions (see Fig.1). To minimise stress, a bird's head was covered with a hood (Cockrem *et al.* 2008) and chicks were kept safe.

All 14 males were captured at the beginning of the guard stage (December 28 to January 2). Half of them (experimental group) were implanted with a self-degradable CORT releasing pellet, while the others (control group) underwent the same procedure without actual implantation of a pellet. For both groups, a small area of skin around the nape of the neck was disinfected with 70 % alcohol, incised on ca. 1 cm and finally (after implantation for experimental group) closed with a sterile stitch and sprayed with Alumisol® (healing external suspension). CORT pellets (100 mg, 21 days release; Innovative Research of America, Sarasota, FL, USA) were implanted subcutaneously in the nape of the neck. Previous studies showed that these pellets led to a 2-4 times increase of CORT levels in free-living male Adélie penguins (Spée *et al.* 2011a; Thierry *et al.* 2013b).

At the end of the guard stage/early crèche stage, 19 ± 2 days after the first capture (January 16 to 19), males returning from a foraging trip were recaptured together with their chicks. A small blood sample (about 0.3 mL) was collected from the alar or tarsus vein and subsequently kept at -20 °C until stable isotope analysis. For 4 (3 control-chicks and 1 CORT-chick) of the 18 chicks (12 control-chicks and 6 CORT-chicks), blood sampling was not performed due to severe weather conditions during the sampling period. A control pair placed next to a crèche had no attributable chick, so that only 13 chicks were included for blood analyses. Adult male and chick mass and flipper length were measured using a Pesola spring balance (5 kg ± 0.3% for chicks, 10 kg ± 0.3% for adults) and a ruler (± 1mm), respectively. In adult penguins, the flipper length has been considered as a good indicator of body size, because they do not grow after fledging (Mínguez *et al.* 1998). A scaled mass index was calculated as previously reported (Peig & Green, 2009). Chicks were also weighed and measured 39-43 days after hatching, when their weight is at a maximum (Ainley, 2002).

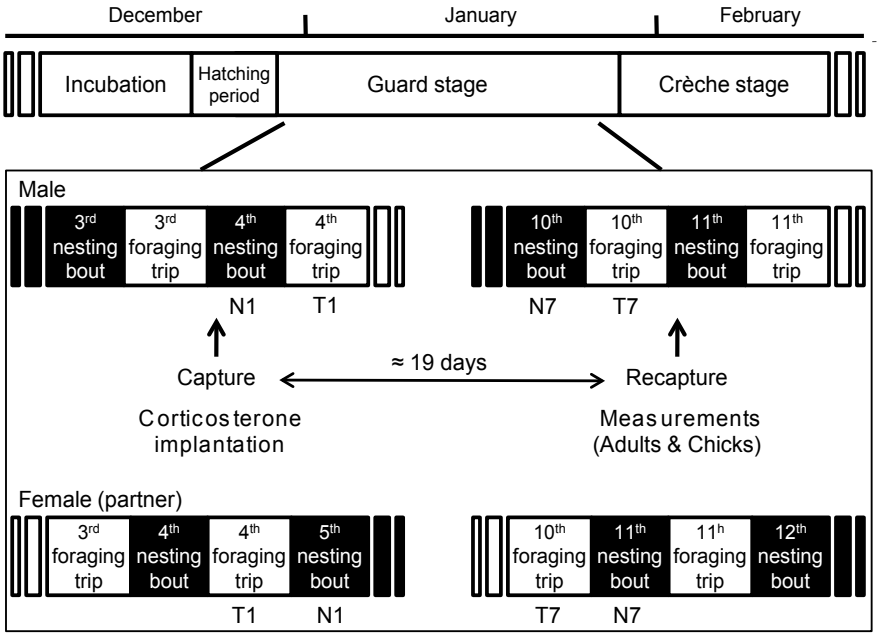


**Figure 1:** Breeding phenology of Adélie penguins and study protocol during the chick-rearing period (guard stage)

The studied male penguins were captured twice and monitored throughout this period

*REPRODUCTIVE OUTPUT*

During the study period, the number of chicks was checked thoroughly (by gently pushing the adult present on the nest when needed) on several occasions: before treatment (Dec. 27), at capture devoted to CORT-implantation or sham-manipulation, during treatment (Jan. 9), at the end of the study during recapture, and 39-43 days after hatching. This was done to assess the reproductive output, defined as the number of chicks per nest, chicks body weight, and brood mass.

*STABLE ISOTOPE ANALYSES*

In Adélie Land, penguins are known to principally feed on a mix of krill and fish (Wienecke *et al.* 2000). The stable isotope signatures have been evaluated by Cherel (2008) for Antarctic krill (*Euphausia superba,* $\delta^{13}C$ = -25.4 ± 0.6, $\delta^{15}N$ = 5.3 ± 0.5; sampling: summer 2002), ice krill (*Euphausia crystallorophias,* $\delta^{13}C$ = -25.4 ± 0.4, $\delta^{15}N$= 6.8 ± 0.7; sampling: summer 2002), and Antarctic silverfish (*Pleuragramma antarcticum,* $\delta^{13}C$ = -24.7 ± 0.4, $\delta^{15}N$ = 10.6 ± 0.3, sampling: winter/spring 2002). The stable isotope analysis of Adélie penguins' diet is known to be relatively consistent with analyses of prey found in their stomach content (Tierney *et al.* 2008).

Tissue isotopic signature mirrors the diet throughout the period of tissue synthesis (Bearhop *et al.* 2002), and according to Cherel & Hobson (2007), whole blood has a one-month turnover in large birds. Thus, we assume that our isotopic measure integrates the diet of adult males over the whole treatment period. Because chicks are unable to feed by themselves, their isotopic signature depends largely on the food brought by their parents. Stable carbon and nitrogen assays were carried out at the Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés, L'Houmeau, France. Replicate measurements showed coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide of 0.34 % and 9.61 %, respectively. Values are expressed in the usual δ notation (‰) relative to PeeDee Belemnite (PDB) for $\delta^{13}C$ and atmospheric nitrogen ($N_2$) for $\delta^{15}N$.

*DATA ANALYSIS*

All statistical analyses were performed with R 2.13.2 (R Development Core Team, 2011). Results are expressed as means ± SE. Differences were considered statistically significant when $p<0.05$. Because of small sample sizes, Wilcoxon tests were used to compare adult male body mass, scaled mass index, hatching date between groups, initial and final capture dates, and the number of chicks per pair before and at the end of the treatment (control vs. CORT). Generalised Estimating Equations (GEE) were used to compare time budget (time spent at sea and time spent at the nest), reproductive output (number of chicks per nest), and chick mass between controls and CORT-treated birds. GEE were computed using the *geeglm* function of the *geepack* package in R (Højsgaard *et al.* 2006). Model selection was performed by excluding non-significant interactions first, and then all non-significant factors. Statistics and p-values of non-significant factors and/or interactions are reported before removal from model. Regarding time budget analysis, the first nesting bout (N1) of males and the first foraging trip of females (T1) of the experiment were removed from analyses because first capture and treatment were carried out during this period. In addition, to homogenise the number of individuals per groups, only the first seven foraging

trips after treatment were considered for the analyses (see Fig. 1), i.e. during the time the treatment was efficient. Treatment, foraging trips (T1, T2, etc.) or nesting bouts (N1, N2, etc.) ranks, and the interaction between treatment and foraging trip rank or between treatment and nesting bout rank were taken into account as fixed factors. Data obtained from males and females (partners) were analysed separately because only males were CORT-treated. Wilcoxon tests were used to compare the number of foraging trips made between 1 and 15 days after implantation and between 1 and 21 days after implantation, and the total time spent at the nest during these periods between groups. Relationships between adult mass (or scaled mass index) and brood mass at the end of the treatment were estimated with Spearman correlation tests. Multivariate analysis of variance (MANOVA) with Wilk's lambda statistics was used to compare overall isotopic signatures between groups, according to the condition of residuals normality (assessed by Shapiro-Wilk test). Differences in $\delta^{13}C$ and $\delta^{15}N$ between control males and their chicks and between CORT-treated males and their chicks were assessed with Wilcoxon tests.

**Results**

There was no difference in the date birds were captured and treated (control vs. CORT) at the beginning of the chick-rearing period (Wilcoxon, W=29, p=0.627). Control and CORT-treated birds were recaptured on similar dates at the end of the guard stage/early crèche stage (W=17, p=0.333).

*TIME BUDGET*

Time budget of control birds was similar between sexes, with foraging trips (GEE, Wald $\chi^2$=0.496, p=0.481) and nesting bouts (Wald $\chi^2$=1.47, p=0.225) lasting on average 1.2 days for both males and females (Figs. 2 and 3).

For males, time spent at sea was not affected neither by the treatment (Fig. 2, Wald $\chi^2$=0.008, p=0.930), the foraging trip rank (Wald $\chi^2$=0.707, p=0.400), nor the interaction treatment*foraging trip rank (Wald $\chi^2$=0.733, p=0.733). However, nesting bouts were significantly longer in CORT-treated males compared with control birds (Wald $\chi^2$=4.747, p=0.029, Fig. 2). Neither the nesting bout rank (Wald $\chi^2$=0.113, p=0.737) nor the interaction treatment*nesting bout rank (Wald $\chi^2$=0.849, p=0.357) had a significant effect on the time spent on the nest. Consequently, the number of foraging trips tended to be lower, although not significantly, in CORT-treated birds compared with controls (5.9 ± 0.3 vs. 6.6 ± 0.3 foraging trips, respectively, after 15 days, W=11.5, p=0.087; 8.1 ± 0.4 vs. 9.1 ± 0.5, respectively, after 21 days, W=13.5, p=0.169). CORT-treated males spent on average 21 % more time at the nest and 17 % less time at sea than controls between 1 and 15 days after pellet implantation (8.6 ± 0.5 days at the nest vs. 7.1 ± 0.4 for CORT-treated birds and controls, respectively, W=40, p=0.055; 7.6 ± 0.3 vs. 6.3 ± 0.4 days at sea, respectively, W=9, p=0.055).

For females, the time spent on the nest was neither affected by the treatment of their mates (Wald $\chi^2$=1.637, p=0.201), the nesting bout rank (Wald $\chi^2$=0.944, p=0.331), nor the interaction of these two factors (Wald $\chi^2$=0.357, p=0.550). In addition, female foraging trip duration was neither affected by the partner treatment (Wald $\chi^2$=0.902, p=0.342), the foraging trip rank (Wald $\chi^2$=0.105, p=0.746), nor the interaction of partner treatment*foraging trip rank (Wald $\chi^2$=1.779, p=0.182). Because CORT-treated males spent more time at the nest while their partners did not perform longer trips, treated pairs spent more time together at the nest (Wald $\chi^2$=8.901, p=0.003). The nesting bout rank (Wald $\chi^2$=0.000, p=0.988) and the interaction treatment*nesting bout rank (Wald $\chi^2$=0.002, p=0.966) had no significant effect on the time spent together at the nest.

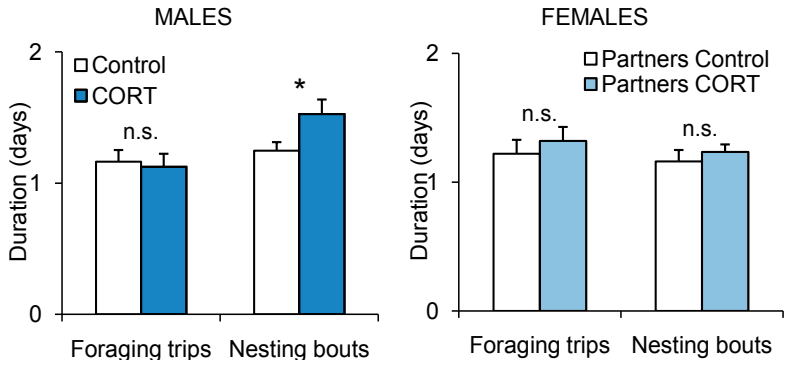


**Figure 2:** Mean duration (± SE) over the study period of foraging trips and nesting bouts for treated and control male Adélie penguins and their partners (n=7/group)

* indicates significant difference (p<0.05) between the two treatments. n.s.: non-significant.

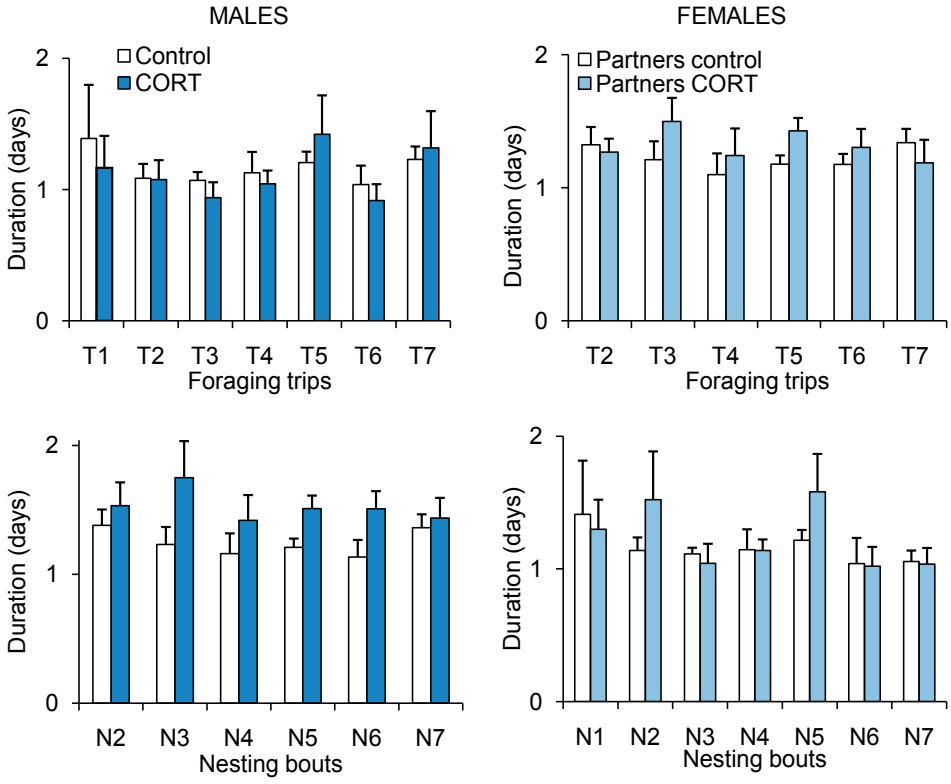


**Figure 3:** Duration (means ± SE) of successive foraging trips and nesting bouts (following the experimental treatment) for treated and control male Adélie penguins and their partners (n=7/group)

*REPRODUCTIVE OUTPUT AND BODY CONDITION*

There was no difference in the number of chicks per pairs between control and CORT-treated birds at the beginning of the experiment (W=31.5, p=0.334). The CORT treatment resulted in a significant 42% decrease of the number of chicks per nest (Wald $\chi^2$=9.122, p=0.003). Neither the period at which the number of chicks was checked (Wald $\chi^2$=2.047, p=0.152) nor the interaction period*treatment (Wald $\chi^2$=2.637, p=0.104) affected this parameter (Fig. 4). At the end of the experiment, all birds were successful breeders, but CORT-treated males raised only one chick, while control birds raised the same number of chicks than at the beginning of the experiment (W=42, p=0.009).

No differences in the adult male body mass and scaled mass index were observed between the two groups (Table 1), and the CORT treatment did not induce nest abandonment.

**Table 1:** Profiles of control and CORT-treated male Adélie penguins and hatching date of the first chick

| | | **Control** (N=7) | **CORT** (N=7) | **W** | **p** |
|---|---|---|---|---|---|
| **Adult males** | Body mass (kg) | 4.692 ± 0.172 | 4.703 ± 0.149 | 25 | 1 |
| | Flipper length (cm) | 19.16 ± 0.27 | 19.56 ± 0.21 | 15 | 0.248 |
| | Scaled mass index | 4.69 ± 0.14 | 4.71 ± 0.17 | 26 | 0.902 |
| **Eggs** | Hatching date of first chick | 22-Dec. ± 0.96 | 24-Dec. ± 0.59 | 12 | 0.119 |

Results are expressed as means ± SE. See text for further details.

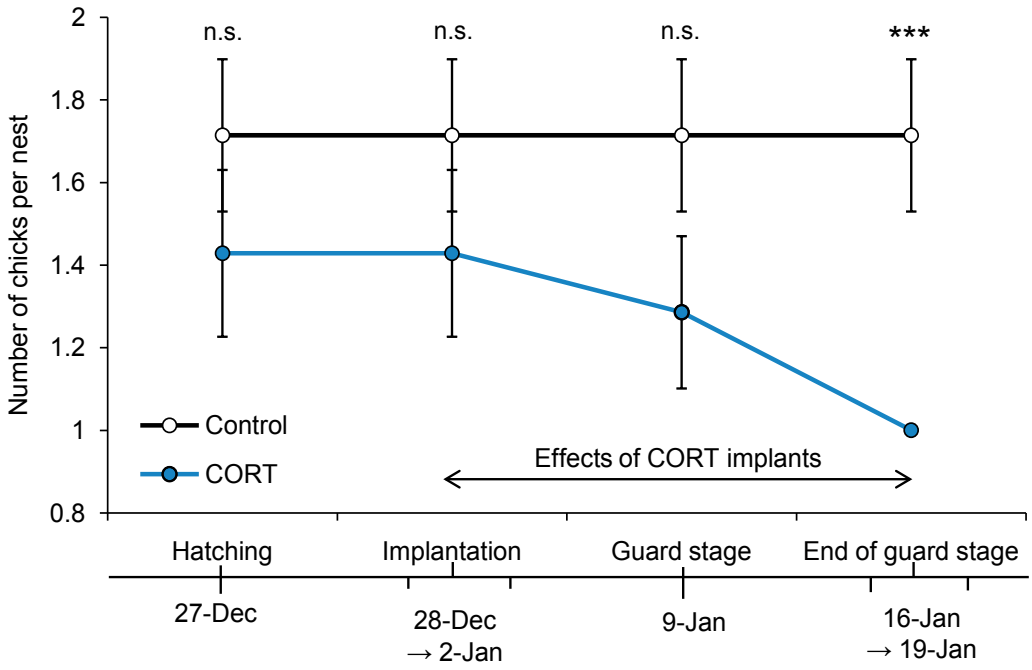


**Figure 4:** Reproductive output of Adélie penguin pairs according to experimental treatment along the study period

Values are means ± SE. *** indicates significant difference (p<0.001) between the two treatments. n.s.: non-significant.

Because of the greater chick mortality, CORT-treated birds had to feed a 42 % lower brood mass

compared to control birds at the end of the experiment (brood massCORT=2.22 ± 0.12 kg vs. brood massControl=3.80 ± 0.43 kg, W=36, p=0.035). The surviving chicks were not affected by the treatment of the male since there were no differences in their body masses and flipper lengths compared to control chicks (Table 2, Fig. 5). Indeed, chick mass was not significantly different between control and CORT-treated birds at the end of the experiment, i.e. 21-31 days after hatching (Wald $\chi^2$=0.002, p=0.969), and 39-43 days after hatching (Wald $\chi^2$=1.393, p=0.238). Brood mass did not differ between groups after the experiment, when chicks were 39-43 old (W=31, p=0.181).

There was also no relationship between adult males body mass and brood mass in both groups at the end of the experiment (Spearman correlation, control: p=0.333, CORT: p=1) and between adult scaled mass indexes and brood mass (Spearman correlation, control: p=0. 302, CORT: p=1).

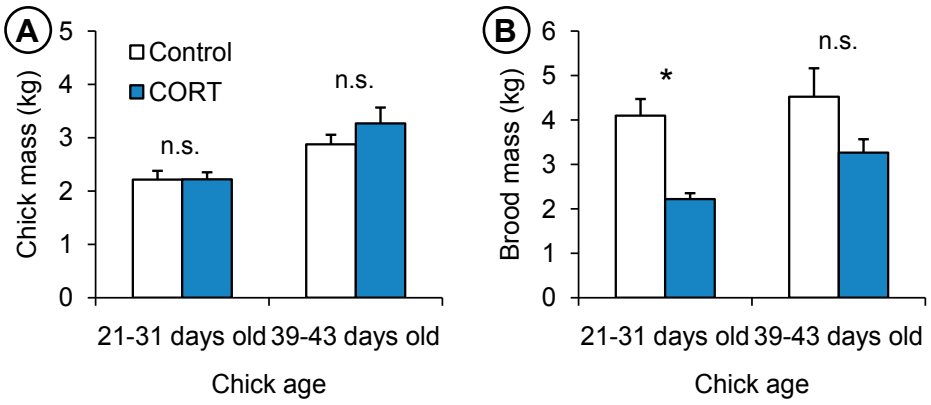


**Figure 5:** Chick body mass (**A**) and brood mass (**B**) of control and CORT-treated Adélie penguin pairs according to chick age along the study period

Values are means ± SE. * indicates significant difference (p<0.05) between the two groups. n.s.: non-significant.

**Table 2:** Profiles of chicks from control and CORT-treated Adélie penguins along the study period

| | | **Control** | **CORT** | Wald $\chi^2$ | **p** |
|---|---|---|---|---|---|
| **Chicks at 21-31 days** | N ($N_{chicks}/N_{pairs}$) | 12/7 | 7/7 | | |
| | date | 16-Jan. ± 0.3 | 17-Jan. ± 0.3 | 1.215 | 0.270 |
| | Age (days) | 25.1 ± 0.9 | 24.0 ± 0.6 | 0.628 | 0.428 |
| | Body mass (kg) | 2.13 ± 0.12 | 2.22 ± 0.13 | 0.002 | 0.969 |
| | Flipper length (cm) | 15.5 ± 0.5 | 15.3 ± 0.6 | 0.073 | 0.787 |
| **Chicks at 39-43 days** | N ($N_{chicks}/N_{pairs}$) | 11/7 | 7/7 | | |
| | date | 01-Feb. ± 0.8 | 03-Feb. ± 0.7 | 2.678 | 0.102 |
| | Age (days) | 41.2 ± 0.4 | 41.8 ± 0.4 | 2.022 | 0.155 |
| | Body mass (kg) | 2.88 ± 0.18 | 3.27 ± 0.30 | 1.393 | 0.238 |
| | Flipper length (cm) | 18.6 ± 0.3 | 18.7 ± 0.5 | 0.120 | 0.729 |

Results are expressed as means ± SE. See text for further details.

*DIET COMPOSITION*

The overall isotopic signature of control and CORT-treated adult males (Fig. 6, $F_2$=0.123, p=0.885), and that of their respective chicks (F2=0.018, p=0.982) did not differ significantly. However, there were differences in overall isotopic signatures between adults and chicks ($F_2$=36.906, p<0.001, Fig. 6). Control adult males exhibited higher $\delta^{13}C$ values than their chicks (adult males: $\delta^{13}C_{control}$: -25.44 ± 0.09 ‰, chicks: $\delta^{13}C_{control}$: -26.07 ± 0.08 ‰, W=61, p=0.002). There were no significant differences of $\delta^{15}N$ values between chicks and adults males (W=47, p=0.114). Similar trends were obtained between CORT-treated adult males and their chicks ($\delta^{13}C$: W=35, p=0.003, $\delta^{15}N$: W=27, p=0.141).

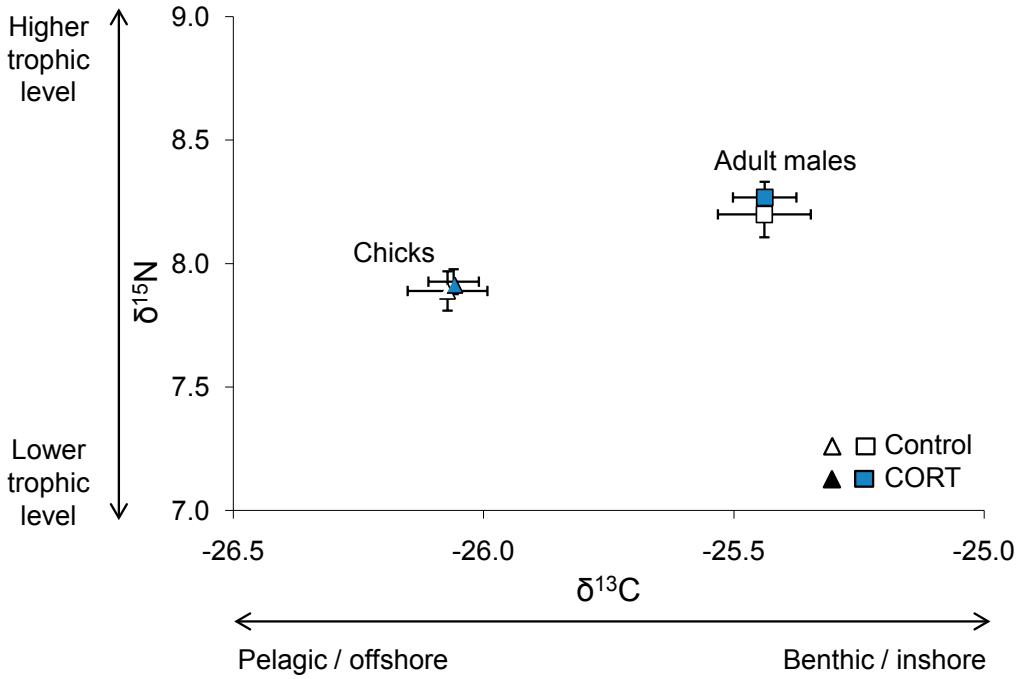


**Figure 6:** $\delta^{15}N$ and $\delta^{13}C$ values (means ± SE) of CORT-treated and control male Adélie penguins and their chicks during the guard stage

## Discussion

In this study, we investigated the effects of increased CORT levels on the breeding effort and the reproductive output of chick-rearing male Adélie penguins.

*EXPERIMENTAL MANIPULATION OF CORT LEVELS*

Small quantities of blood were sampled in the current study to minimise handling time and disturbance. These small samples were not sufficient for CORT assays to be performed. Yet, whether the treatment increased CORT levels within baseline ranges or to acute stress-induced levels, both within normal physiological range, may have different effects (Landys *et al.* 2006). CORT levels of treated chick-rearing

male Adélie penguins were measured in a similar experiment with the same study protocol (Thierry *et al.* in revision). Blood was sampled on the day of implantation and an average of 17.9 days ± 0.2 after pellet implantation and plasma kept for CORT assay. Pre-treatment CORT levels did not differ between groups (3.9 ng/mL ± 1.0 and 4.7 ± 0.5 for controls and CORT-treated birds, respectively). CORT levels had returned to initial levels by the time of the second blood sample (4.9 ± 0.5 and 4.6 ± 0.8 for controls and CORT-treated birds about 18 days post-implantation, respectively). The return to baseline levels 18 days after CORT implantation is also in agreement with the study of Spée *et al.* (2011a) using similar pellets in Adélie penguins. A two- to four-fold increase in CORT levels was measured in CORT-treated incubating male Adélie penguins (Spée *et al.* 2011a; Thierry *et al.* 2013b), i.e. below those reached during a capture stress (Cockrem *et al.* 2009), and within the physiological range of this species.

CORT pellets have been described to induce a peak-like elevation of circulating CORT levels and to temporarily remove the endogenous response to an acute stressor (Müller *et al.* 2009). In particular, highly elevated levels of CORT trigger different responses than slightly elevated levels (Wingfield *et al.* 1997; Wingfield *et al.* 1998). Intermediate CORT levels may increase food searching in a free-living animal, while higher levels may restrict movement (Breuner *et al.* 1998; Breuner & Wingfield, 2000; Crossin *et al.* 2012). In the current study, the lack of marked changes in the behaviour of male Adélie penguins on land (Raclot T., visual observations), suggests that CORT did not drastically affect the time-budget of birds. Self-degradable pellets remain an efficient tool to experimentally elevate hormone levels in field studies.

Whether baseline and/or stress-induced CORT levels are good predictors of fitness in free-living animals is not clear yet (Bonier *et al.* 2009a; Breuner *et al.* 2008). A negative relationship between baseline glucocorticoids levels and fitness measures, mediated by environmental conditions or challenges is described under the CORT-fitness hypothesis, with partial empirical support. Yet, baseline CORT levels vary consistently among individuals, depending on environmental conditions and life-history stages, making a unidirectional link between CORT and fitness unlikely (Dingemanse *et al.* 2010). The CORT-adaptation hypothesis expands the definition of environmental challenges to include challenges associated with reproduction (Bonier *et al.* 2009b), which predicts that increased reproductive demand causes increases in baseline CORT levels. But the nature of the relationship between CORT and fitness, and the validity of the CORT–adaptation hypothesis, depend on the magnitude of reproductive investment, and may thus vary among species and individuals with different reproductive strategies. Understanding how CORT relates to fitness becomes even more complex when considering that behaviour, size, and morphology also affect how organisms perceive environmental signals (Kearney, 2006), and may hence affect CORT levels.

*CORTICOSTERONE AND TIME SPENT AT THE NEST*

Negative effects of elevated CORT levels on parental care in birds have been described in many studies (Almasi *et al.* 2008; Angelier *et al.* 2009; Criscuolo *et al.* 2006; Groscolas *et al.* 2008; Horton & Holberton, 2009; Silverin, 1986; Thierry *et al.* 2013b; Wingfield & Sapolsky, 2003). Yet, the CORT-adaptation hypothesis predicts that high CORT levels support increased foraging activity and parental effort (Bonier

*et al.* 2009a). Indeed, increased CORT levels induced an increase in foraging activity and parental care in female macaroni penguins (Crossin *et al.* 2012). Interestingly, CORT-treated male Adélie penguins had a decreased reproductive output, while spending more time at their nest. Their females apparently did not compensate for this, as they did not modify their time budget. Similarly, female Adélie penguins were not affected by the temporary handicapping of their partner (forcing them to conduct longer trips), and had a decreased reproductive output (Beaulieu *et al.* 2009).

CORT-treatment is known to have a negative effect on body mass which can be explained, at least partly, by a shift in fuel utilisation (Spée *et al.* 2011b). Although CORT-treated penguins fasted for longer while at the nest, they maintained a similar body condition than that of controls and provided enough food to their remaining chicks, which had similar body mass and size than control chicks (Table 1). Because CORT-treated birds had a lower number of chicks to supply and knowing that foraging at sea is more costly than guarding the chicks (Chappell *et al.* 1993), spending more time at the nest probably allowed CORT-treated birds to maintain a similar body mass than that of controls. Our results support the idea that increasing CORT levels may have induced birds to allocate the available energy to the benefit of body maintenance at the expense of current reproduction.

CORT-treated penguins had a 42 % decreased reproductive output, but all birds raised at least one chick. Criscuolo *et al.* (2005) found that exogenous CORT increased the rate of egg loss of incubating female common eiders *(Somateria mollissima)* through a reduction in nest attentiveness associated with decreased prolactin levels. CORT-treated chick-rearing Adélie penguins may have been less attentive to their nest, leading to a lesser efficient protection of the chicks against cold or predators. Visual observations in the field do not support this idea: predators are few on this part of the Dumont d'Urville colony and adverse weather did not occur much during the study period.

Moreover, the quality or quantity of chick provisioning may have been reduced in CORT-treated birds. Horton and Holberton (2009) found an inhibitory effect of elevated CORT levels on chicks provisioning frequency in male white-throated sparrows *(Zonotrichia albicollis)*. Because the growth rate of Adélie penguin chicks is positively affected by the feeding frequency (Takahashi *et al.* 2003), the lower reproductive output of CORT-treated males was likely caused by a reduced chick-provisioning rate. Further studies are needed to examine the timing and the causes of chick mortality (e.g. in using video recording and regularly weighing chicks) in treated birds in order to better understand how increased CORT levels affect the reproductive output.

*CORTICOSTERONE AND FORAGING TRIPS*

While CORT-treated penguins spent more time at their nest, they did not spend more time foraging at sea. Similarly, CORT-treatment did not affect foraging trip durations of female macaroni penguins relative to controls, but diving behaviour differed between the two groups (Crossin *et al.* 2012). Telemetry showed that dive parameters, such as the number of dives, the mean depth, and the number of foraging events per dive differed between control and CORT-treated birds, with an overall positive effect of the

CORT-treatment of foraging behaviour and diving activity, independently of the time spent away from colony on trips. Although we did not study the foraging behaviour of our birds here, we may expect the CORT-treatment to affect the birds' foraging behaviour as suggested by a preliminary study using time-depth recorders on CORT-implanted Adélie penguins (Cottin *et al.* 2011). As for Crossin *et al.* (2012), Adélie penguins implanted with CORT pellets seem to increase their diving effort although trip duration did not change (Cottin *et al.* 2011).

In a recent correlative study, Angelier *et al.* (2008) showed that un-manipulated Adélie penguins with elevated pre-trip CORT levels spent less time at sea and foraged closer to the colony compared to individuals with low CORT levels. Because elevated CORT levels were associated with active (high effort) short trips and led to a low mass gain, the authors suggested that elevated CORT levels may help the birds support the increased energetic demands associated with chick-rearing by stimulating foraging activity at the expense of the adult body reserves. On the contrary, an experimental elevation of baseline CORT levels led treated black-legged kittiwakes to increase their foraging activities at the expense of guarding their chicks at the nest (Kitaysky *et al.* 2001). These different studies highlight the complexity of the physiological mechanisms driving changes in foraging behaviour among species. Four non-exclusive explanations can be put forward to explain the absence of CORT effect on foraging duration:

- As mentioned above, the trip duration is not affected but foraging parameters within the foraging trip window may be modified (see Cottin *et al.* 2011; Crossin *et al.* 2012), i.e. regulation takes place at the level of the diving effort but not at the larger-scale level of the foraging trip. This seems plausible as a CORT increase induces an increase in locomotor (corresponding here to diving) activity (Spée *et al.* 2011b).
- CORT has an inverted U-shaped dose-response curve: only intermediate levels of CORT activate behaviour, while low and high levels have no effect (Breuner & Wingfield, 2000). The CORT pellets used in our study were found to mimic metabolic, hormonal, and behavioural changes of long-term fast in birds (Spée *et al.* 2011b). There was a 2.5-fold increase in locomotor activity shortly after implantation in these failed-breeders kept in captivity and treated with 100mg of CORT as in our study. Yet, the CORT dose may have been too high to affect foraging behaviour and did not seem to affect birds' behaviour at the nest (Raclot T., personal observations).
- The effects of CORT on foraging behaviour might depend on the nutritional status of the birds. For example, CORT implantation in fed white-crowned sparrows *(Zonotrichia leucophrys)* did not affect food intake, while fasting CORT-implanted birds increased their foraging activity (Astheimer *et al.* 1992). In our study, the nutritional status of birds was difficult to assess. Although birds were regularly feeding at sea, they regurgitated part of the food they ingested to feed their chicks. However, Adélie penguins can fast for several weeks before they reach a low threshold in their body reserves, which precedes nest abandonment to refeed at sea (Spée *et al.* 2010). Hence it is likely that CORT-treated birds did not reach a late stage of fasting in this study.
- Other physiological mechanisms, such as negative feedback processes, the type and the distribution of receptors within target tissues, the concentration of corticosteroid-binding globulins (Almasi *et al.* 2009) may have prevented effects of exogenous CORT on foraging trip duration. Other endocrine

factors could also modulate foraging trip durations. For example, prolactin, the main hormone involved in parental care in birds (Buntin, 1996), has recently been suggested to be involved in the mediation of the trade-off between the reproductive effort and self-maintenance (Angelier & Chastel, 2009). CORT may indirectly affect this trade-offs via a stress-induced effect on prolactin, which would then affect the trade-off between chick provisioning and self-maintenance. Besides, recent studies suggest that CORT could more directly affect prolactin levels (Angelier *et al.* 2009; Criscuolo *et al.* 2005; Spée *et al.* 2011a), although such a link is not found in all species (e.g. Crossin *et al.* 2012).

*CONCLUSIONS AND PERSPECTIVES*

We showed that experimentally elevated CORT levels increased the time birds spent at their nest, did not affect foraging trip durations, foraging sites, and diet quality in terms of isotopic signature. Interestingly, while the treatment decreased reproductive output (the number of chicks fledged per nest), it did not affect the growth of the surviving chicks. The detailed behaviour of treated birds should be examined in further studies to understand how elevated baseline CORT levels increased chick mortality. Because seabirds are central-place foragers, feeding at sea and foraging activities also needs to be monitored to test whether CORT-treated individuals could be more or less efficient in terms of prey capture and thus more or less efficient in terms of chick provisioning. Birds have been used extensively as models to explore the ecological bases of stress and the underlying endocrine mechanisms. Ecologists and conservation biologists also use stress hormones levels as an indicator of physiological stress in wild animals, and in extension as a correlate of the 'health status' of a population. Yet, the relationships between glucocorticoids, fitness, and ultimately population dynamics are not fully understood and remain controversial depending on species, constraints, and changes in the environment. Studies of stress hormones using both correlative and experimental approaches are of great interest and are fundamental to conservation physiology as a discipline to be successful, hence helping to manage species of conservation concern facing detrimental conditions in a rapidly changing environment.

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## 4.2 Etude 6 : Corticostérone et élevage des poussins – II : effets sur le comportement et le succès reproducteur

**Differential effects of corticosterone on behaviour at the nest and reproductive output of chick-rearing Adélie penguins**

Anne-Mathilde Thierry, Sophie Brajon, Marion Spée, Thierry Raclot

Soumis à *Behavioral Ecology and Sociobiology*

L'étude précédente a permis de montrer qu'une augmentation des niveaux de corticostérone pendant l'élevage des poussins entraînait une diminution du succès reproducteur, mais pas d'abandon du nid comme cela était le cas au cours de l'incubation (Etude 1). Afin de déterminer les effets d'une augmentation des niveaux de corticostérone sur les soins parentaux pendant l'élevage de poussins et d'essayer d'expliquer cette diminution du succès reproducteur, nous avons étudié le budget temps comportemental au nid de manchots ayant reçu un implant de corticostérone pendant la période d'élevage des poussins. Cette étude vise à mieux comprendre les relations entre corticostérone, performances de reproduction et fitness.

**Abstract**

Hormones are important mediators of the interactions between environment, physiology, and behaviour. In particular, glucocorticoids (GCs) are important mediators of physiological and behavioural responses to stress. Many studies have evaluated the environmental, behavioural, or physiological correlates of GCs and their effects on reproductive performance. Yet, further work is needed to clarify the relationship between GCs and fitness. Assessing the effects of increased GC levels on the detailed behaviour of breeding animals should help understanding how GCs affect parental care and reproductive performance. In this experimental study, we measured the effects of an experimental increase in corticosterone (CORT, the main avian GC) levels on the detailed on-land behaviour of male Adélie penguins *(Pygoscelis adeliae)* and the indirect consequences on their partners and chicks. We show that increased corticosterone levels in males moderately affected their behavioural time budget and also had consequences on the behaviour of their partners. However, chick begging and feeding rates were not affected by male treatment. Penguins with increased CORT levels also had a delayed decreased reproductive output. Comparisons with previous studies in the same species carried out during either different life-history stages or year support the idea that the relationship between CORT levels and reproductive performance is context-dependent. These context-dependent effects, as well as the potential delay in the effects of an increase in CORT levels on the reproductive performance, should be taken into account when studying the behaviour of free-living animals in relation to stress-induced situations.

## Introduction

Animals permanently adjust their physiology and behaviour to both predictable and unpredictable changes in their environment. Studying endocrine mechanisms is of particular interest because of the major role hormones play in mediating interactions between the environment, an animal's physiology, and its behaviour (Ricklefs & Wikelski, 2002; Wingfield & Sapolsky, 2003). Indeed, hormones may be used as an informative index of the potential for populations to adapt to environmental change (Meylan *et al.* 2012). In particular, glucocorticoid (GC) hormones are important regulators of energy balance (Strack *et al.* 1995), as well as mediators of physiological and behavioural responses to stress (Wingfield & Romero, 2010). GCs are acutely released from the adrenal gland under life-threatening situations, such as food shortage (Kitaysky *et al.* 1999) or severe weather conditions (Romero *et al.* 2000), and are also regulated seasonally (Romero, 2002). GCs are increasingly used in conservation-related field studies as physiological indices of stress and of health status of individuals and populations. However, it is now clear that GCs do not systematically correlate with survival or reproductive success, resulting in a complex relationship between GCs and reproductive performance (Bonier *et al.* 2009a; Busch & Hayward, 2009). For example, increased levels of corticosterone (CORT), the main avian GC, have been associated with reduced reproductive success and potential nest desertion in different bird species (Criscuolo *et al.* 2005; Ouyang *et al.* 2012; Silverin 1986; Spée *et al.* 2010). Yet, CORT levels are also expected to increase to meet the energy and behavioural demands of breeding because of the major role the hormone plays in regulating energy balance and food intake (Bonier *et al.* 2009b; Love *et al.* 2004). Several studies have indeed shown positive relationships between CORT levels and reproductive success (Bonier *et al.* 2009b; Ouyang *et al.* 2011). A recent review highlighted the fact that the CORT-fitness relationship is complex, and neither always present nor consistent (Bonier *et al.* 2009a). For example, an experimental increase in CORT levels in chick-rearing female macaroni penguins *(Eudyptes chrysolophus) l*ed to an apparent fitness benefit in terms of increased chick mass together with increased foraging effort (Crossin *et al.* 2012). Conversely, a similar treatment decreased the reproductive output of chick-rearing male in a closely related species, the Adélie penguin *(Pygoscelis adeliae)* (Thierry *et al.* 2013c).

Most studies have evaluated environmental, behavioural, or physiological correlates of GC secretion and the effects of elevated GC levels on intermediate measures of reproductive performance, which may enhance or restrict reproductive success (see Breuner, 2011 for a review). Regarding the relationships between GCs and behaviour, experimentally increased CORT levels were associated with reduced responses to standardised simulated territorial intrusion in male song sparrows *(Melospiza melodia)* (Wingfield & Silverin, 1986) and with decreasing song complexity in common diving petrel *(Pelecanoides urinatrix)* (Smith *et al.* 1994). GCs are also known to participate in the regulation of foraging behaviour (Astheimer *et al.* 1992; Koch *et al.* 2002; Lõhmus *et al.* 2006), generally suggesting a facilitating effect on food intake (Dallman *et al.* 2004), and of chick feeding rate in different bird species (Almasi *et al.* 2008; Silverin, 1986). Yet, experimental studies investigating the effects of increased CORT levels on both the detailed on-land behaviour of breeding birds in the wild and on the associated reproductive output

remain scarce. If it is now clear that CORT plays a role in the triggering of the emergency life history stage and subsequent nest desertion (Groscolas *et al.* 2008; Landys *et al.* 2006; Silverin 1986; Spée *et al.* 2010; Wingfield *et al.* 1998), more studies are required to determine the role of the hormone in regulating individual variation in parental care, and hence to better describe the relationship between CORT levels and fitness.

The goal of this study was to assess the effects of increased CORT levels on the detailed on-land behaviour of control and CORT-treated birds, of the indirect behavioural effects of this treatment on the birds' female partners, and on their reproductive output. To do so, we experimentally increased levels of circulating CORT in chick-rearing male Adélie penguins and filmed the birds to determine their detailed activity budget on the nest. Physiologically relevant changes induced by the CORT-treatment could include a redirection of behaviour from chick care (Almasi *et al.* 2008; Silverin, 1986) to comfort behaviour (Ainley 1974; Kralj-Fišer *et al.* 2010) or agonistic (Holmes, 2007) behaviour. Captive Adélie penguins treated with similar CORT pellets were shown to increase their locomotor activity (Spée *et al.* 2011b). Thus, during chick-rearing, CORT-treated birds could hence be more agitated on their nest, which would lead to less time spent resting. Moreover, more time spent preening, shaking, or stretching might induce an increase in energy expenditure (e.g. Viblanc *et al.* 2011) in CORT-treated birds. In species where both parents provide parental care, there is a conflict of interest between partners as they cooperate to breed while both expect to minimise their current reproductive investment (Houston *et al.* 2005). A reduction in the parental investment of one bird might result in compensatory behaviour of its partner (Navarro & Gonzalez-Solis, 2007; Paredes *et al.* 2005) but not necessarily (Beaulieu *et al.* 2009; Kitaysky *et al.* 2001), depending on whether birds are working at some physiological maximum and/or are unwilling to pay the costs of increased parental investment in terms of future survival. Thus, we assessed whether the on-land behavioural time budget of the females might be indirectly affected by the treatment of their partner. The effects of male CORT-treatment on the offspring were also assessed by measuring the amount of time chicks of each clutch spent begging for food, and the responses of male adult penguins to chick begging. The fact that CORT-treated chick-rearing black-legged kittiwakes *(Rissa tridactyla)* did not decrease their chick feeding rates, allows suggesting that there should be no changes in chick begging and feeding rates between groups. Finally, the effects of increased CORT levels on the number and body mass of chicks were monitored. The predicted changes in the activity budget of CORT-treated birds, in particular decreased parental care, should decrease their reproductive output (number of chicks and/or chick mass at the end of the study period).

## Methods

### *STUDY SITE AND SPECIES*

The study was carried out on Ile des Petrels, Pointe Géologie Archipelago, next to the French polar station Dumont d'Urville (66°40'S, 140°01'E, Adélie Land, Antarctica) during the 2009-2010 austral summer.

Adélie penguins are long-lived seabirds, which breed during the short austral summer (from late October to early March) in colonies around Antarctica. After egg laying and a 30 to 39 days incubation period (Ainley, 2002), mates alternate between foraging trips at sea and incubation bouts on land (Sladen, 1958). Chicks hatch at the end of December and are always attended by a parent during the guard stage (ca. 22 days; range: 16-34, Ainley, 2002). The crèche stage corresponds to the period during which chicks are left unattended in the colony until about mid-February, before fledging (ca. 51 days after hatching, range: 41-56, Ainley, 2002). During this period, they form groups know as crèches and are regularly fed by the parents, which forage at sea simultaneously.

*EXPERIMENTAL PROCEDURE*

Adélie penguins were first handled at the end of the courtship period (between November 16 and 21, see Fig. 1). Birds of 40 nests were marked using black dye (Nyanzol-D) on breast feathers and Tesa-tape stickers inserted between feathers on their back. Penguins were sexed by a combination of parameters including copulatory position, cloacal inspection, and incubation routine (Sladen, 1958). Nests were checked from a distance of 10 to 30 m every two to three hours for the entire study period to determine which partner was present on the nest, and thus acquire data on incubation routine and foraging trip durations. Adélie penguins were monitored during the whole breeding season, from mid-November until mid-February. The number of eggs and/or chicks was regularly recorded during nest monitoring to determine laying and hatching dates, and reproductive output.

Birds were recaptured at the beginning of the guard stage, between December 26 and December 31 (mean: December 29). Ten males were treated with a 100 mg corticosterone subcutaneous pellet (G-111, 21-day release, Innovative Research of America, Sarasota, FL, USA). Ten other males underwent the same procedure during initial handling, without actual implantation of a pellet. Before being released near their nest, males were weighed using an electronic balance (Ohaus, Giessen, Germany, resolution: ± 2 g), and the length of the left flipper was measured to calculate a scaled mass index (Peig & Green 2009). Chicks were also captured in order to prevent predation during handling of the parent, and weighed on that occasion. The global activity budget of birds (i.e. the time spent by each partner on the nest and at sea) during the 15 days following implantation was evaluated using nest monitoring observations.

A blood sample was also collected from the alar vein (2 mL syringe, 25-gauge needle) within three to five minutes following capture. Indeed, CORT levels are not significantly affected by handling times of up to five minutes in Adélie penguins (Vleck *et al.* 2000). In a few birds, blood sampling duration exceeded the time recommended to assess baseline corticosterone levels. Consequently, samples from these birds were not assayed for hormone levels. Tubes pre-treated with 25µL EDTA were used to collect and centrifuge blood samples (5000 rpm during 10 min at 4 °C). Plasma was kept at -20 °C until further analysis.

Nine CORT-treated and 9 control birds were recaptured around Jan. 7 (range: Jan. 6-9, ca. 9 days after implantation) to assess body mass following treatment. Seven CORT-treated and eight control birds were also recaptured during nest relief around Jan. 15 (range: Jan. 12-17, ca. 17 days after implantation), before leaving the colony. They were weighed and another blood sample was taken to assess post-treatment

CORT levels. Their respective chicks were also weighed on these two occasions.

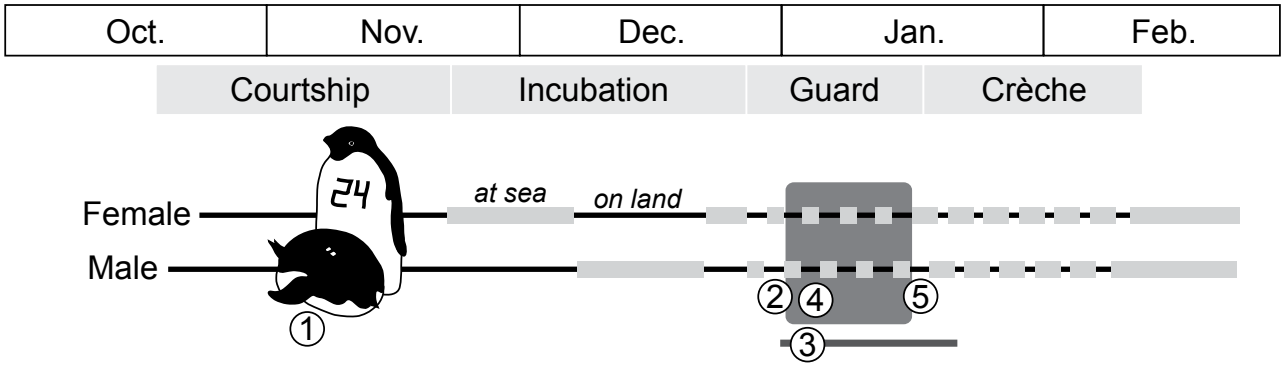


**Figure 1:** Study protocol

1: identification; 2: blood sample, implantation, weighing; 3: CORT-treatment; 4: video monitoring; 5: blood sample, weighing

*ETHICAL NOTE*

The protocol was approved by the ethics committee of the French Polar Institute Paul-Emile Victor and the study was authorised (decrees 2009-57 and 2009-59) by the French Southern and Antarctic Lands (Terres Australes et Antarctiques Françaises). Adélie penguin nests were randomly sampled from the perimeter of the colony in order to minimise disturbance. Birds were caught by hand at their nests and quickly moved away from the colony to a shed where the experimental procedure was carried out. Chicks were captured to avoid any risks of predation by South Polar skuas *(Catharacta maccormicki)* during adult handling. Nests were then protected with an upturned bucket to avoid stealing of nest materiel by conspecifics. During handling, the bird's head was covered with a hood to minimise handling stress (Cockrem *et al.* 2008). No adverse effect of the marking such as increased pecking by conspecifics or preening was observed in this study and in previous studies (Beaulieu *et al.* 2009; Spée *et al.* 2010; Spée *et al.* 2011a; Thierry *et al.* 2013a, 2013b). Regarding hormonal manipulation, self-degradable corticosterone pellets (diameter: 0.85 cm, thickness: 2mm, weight: 0.3g) were inserted subcutaneously in the nape of the neck. Implantation was performed by a qualified and experienced scientist (T. Raclot, licence no. 67-226 of the French Veterinary Services) and a vet, who wore gloves disinfected with rubbing alcohol. Surgical tape was used to clear a small area of skin from feathers in the nape of the neck, skin was then disinfected with rubbing alcohol and a small (~1 cm) incision was made using a scalpel blade. After positioning the pellet subcutaneously with sterile tweezers, the incision was closed with sterile suture, cleaned with povidone-iodine, and Alumisol® spray (CEVA, Libourne, France) was applied topically. Handling time averaged 23 min (range: 20-29 min), including bird capture, blood collection, pellet implantation, weight determination, and bird release. Birds directly returned at their nests when released. No anaesthetics or analgesics were used, because general anaesthesia would result in increased handling time as breeding birds need to be fully awake when released at their nests, and local analgesics might interfere with hormone release by the pellet (T. Raclot, personal observations). These corticosterone pellets are self-degradable, and hence do not need to be removed, avoiding a second subcutaneous surgery. Similar pellets were successfully used in previous studies of the same species, showing that they do not

have long-term consequences on Adélie penguin return rate to the colony one year after the experiment (Thierry *et al.* 2013b) and that hormone levels were increased within the natural range of the species (Spée *et al.* 2011a; Spée *et al.* 2011b; Thierry *et al.* 2013b).

*CORTICOSTERONE ASSAYS*

Plasma CORT levels were determined by immunoassay according to guidelines provided by the manufacturer (Corticosterone EIA Kit, EA66, Oxford Biomedical Research, Oxford, MI, USA). Intra-assay coefficient of variation was 9 %. Minimum detectable dose of CORT was 0.01 ng/mL.

*BEHAVIOUR*

A HD video camera (Sony XR550VE) was placed a few meters away from the nests to film penguins from January 1 to 15, except on January 10 and 11 because of bad weather conditions. Behavioural data of 10 CORT-treated birds, 7 controls, and their respective partners was analysed during 15 min periods. Males were observed during a total of 81 hours and females during 20 hours. Behavioural categories are the following: rest, vigilance, comfort, agonistic interactions (aggressiveness), social positive interactions (ecstatic display, bill-to-axilla display, bowing, loud and quiet mutual displays, and associated vocalisations), and interactions with chicks (attentive to the chick(s), sings with chick(s), threatens chick(s), feeds chick(s)), and movements from the nest. A detailed description of the different behaviours is available in the Supplementary Materials (see also Marchant *et al.* 1990). During the period males were observed, chicks begged for a total of 2.5 hours. Begging duration and male answers to chick begging were analysed.

*STATISTICAL ANALYSES*

Statistical analyses were performed with R 2.13.2 (R Development Core Team 2011). Differences were considered as statistically significant for $p<0.05$. Results are expressed as means ± SE. Because of small sample size, Wilcoxon tests were used to compare penguin body mass and scaled mass index at implantation (beginning of the guard stage) and 15 days post-implantation, pre-treatment and post-treatment CORT levels, brood mass at different times, and the time spent at sea and on the nest during the 15 days following implantation between control and treated birds. Generalised Linear Models (GLM) with a quasi-Poisson distribution were used to compare the number of chicks per nest at different times between groups and to compare the number of foraging trips made during the 15 days following implantation.

Generalised Estimating Equations (GEE) were used (*geeglm* function of the *geepack* package) to compare chick mass at the time of implantation and after, and foraging trip and nesting bouts duration between groups, with nest and penguin as identity, respectively. The differences in the global behavioural time budget of males depending on their treatment, of females depending on their partner treatment, as well as the differences between males and females, were assessed with a multivariate analysis of variance (MANOVA) with male treatment or sex as factor. As recommended by Pezzanite and colleagues (2005) (see

also Sokal & Rohlf 1995), time budget proportions were angularly transformed (arcsin(proportions1/2)) prior to analyses to stabilise variances and eliminate estimation convergence problems associated with the proportions summing to 1. GEEs with a Poisson distribution were performed to compare the behavioural time budget of control and CORT-treated birds and their partners (i.e. the amount of time birds spent at different behaviours), and to assess the effect of the CORT-treatment on the time chicks spent begging. Models included male treatment, number of chicks, age of the first chick, number of hours since last return from sea, and the interaction between treatment and the time since last return from sea as factors. Model selection was performed by excluding non-significant interactions first, and then all non-significant factors. Models were compared using ANOVA in *geepack* (Zuur *et al.* 2009) and QIC (Pan, 2001).

## Results

*BODY CONDITION*

Control and CORT-treated birds did not differ in body mass (Table 1, Wilcoxon, W=40, P=0.481, $N_{control}=N_{CORT}=10$) and scaled mass index (W=56, P=0.705) on the day of implantation, ca. 9 days after implantation (body mass: W=40, P=1, scaled mass index: W=53, P=0.297, $N_{control}=N_{CORT}=9$), and ca. 17 days after implantation (body mass: W=19, P=0.937, scaled mass index: W=15, P=0.699, $N_{control}=N_{CORT}=6$).

**Table 1:** Body mass of control and CORT-treated male Adélie penguins at implantation and during treatment, and global activity budget of both partners during this period

| | **Control** (n=10) | **CORT-treated** (n=10) |
|---|---|---|
| **Body condition** | | |
| Male body mass at implantation (kg) | 4.70 ± 0.13 | 4.80 ± 0.13 |
| Male body mass ca. 9 days after implantation (kg)* | 4.13 ± 0.09 | 4.08 ± 0.13 |
| Male body mass ca. 17 days after implantation (kg)* | 4.45 ± 0.15 | 4.42 ± 0.13 |
| **Global activity budget** | | |
| Time spent by males at sea (d) | 8.1 ± 0.2 | 6.1 ± 0.5 |
| No. foraging trips made by males | 5.0 ± 0.3 | 5.4 ±0.3 |
| Mean male at sea bouts duration during the study period (d) | 1.6 ± 0.1 | 1.2 ± 0.1 |
| Time spent by males on the nest (d) | 7.1 ± 0.3 | 8.9 ± 0.5 |
| Mean male on-land bouts duration during the study period (d) | 1.6 ± 0.1 | 1.7 ± 0.1 |
| Time spent by females at sea (d) | 5.8 ± 0.2 | 8.5 ± 0.5 |
| No. foraging trips made by females | 4.5 ± 0.3 | 4.9 ± 0.2 |
| Mean female at sea bouts duration during the study period (d) | 1.6 ± 0.1 | 1.6 ± 0.1 |
| Time spent by females on the nest (d) | 7.7 ± 0.2 | 6.5 ± 0.5 |
| Mean female on-land bouts duration during the study period (d) | 1.6 ± 0.1 | 1.3 ± 0.1 |

*CORTICOSTERONE LEVELS*

Pre-treatment and post-treatment CORT levels did not differ between groups (pre-treatment CORT levels: 3.9 ± 1.0 in controls vs. 4.7 ± 0.5 ng/mL in CORT-treated birds, W=39, P=0.232, $N_{control}$=8, $N_{CORT}$=7; post-treatment CORT levels: 4.9 ± 0.5 vs. 4.6 ± 0.8, respectively, W=29, P=0.955, $N_{control}$=8, $N_{CORT}$=7).

*GLOBAL ACTIVITY BUDGET*

During the 15 days following implantation, CORT-treated males spent significantly less time at sea (Wilcoxon, W=85.5, P=0.008) and more time on the nest (W=18.5, P=0.019) than controls (Table 1, $N_{control}$=$N_{CORT}$=10). The pattern was opposite in females: female partners of CORT-treated males spend significantly more time at sea (W=21, P=0.031, $N_{control}$=$N_{CORT}$=10) than females of control birds, and also spent less time on the nest, though not significantly (W=74, P=0.075; Table 1). The number of foraging trips performed by males and females during this period was not affected by male treatment (GLM with a Poisson distribution, males: z=0.39, P=0.695; females: z=0.41, P=0.68). However, foraging trip duration decreased in CORT-treated males (GEE, Wald $\chi^2$=4.61, P=0.032, $N_{control}$=$N_{CORT}$=10, 3 to 6 foraging trips per bird), but the treatment did not significantly affect the nesting bout durations (males: Wald $\chi^2$=0.28, P=0.599; females: Wald $\chi^2$=3.67, P=0.055) nor the foraging trip duration of their female partners (Wald $\chi^2$=0.00, P=0.967).

*DETAILED BEHAVIOURAL TIME BUDGET*

The global behavioural time budget of male Adélie penguins was not affected by the treatment (Fig. 2A, MANOVA, Wilks's $\lambda$=0.634, P=0.496, $N_{control}$=7, $N_{CORT}$=10). Similarly, the male-treatment did not globally affect the behaviour of their partner (Fig. 2B, Wilks's $\lambda$=0.366, P=0.496, Ncontrol=7, NCORT=10). On the whole, the behaviour of male and female Adélie penguins during chick-rearing was significantly different regardless whether males were treated or not (Wilks's $\lambda$=0.226, P<0.001, Nmales=17, Nfemales=17). Comparisons of detailed behavioural time budgets of adults depending on male-treatment are presented in Table 2 (see also Supplementary Materials). In particular, CORT-treated males spent more time in comfort behaviours (P=0.046), positive social interactions (P=0.003) and vocalisations (P=0.035), and less time being attentive to the nest (P=0.003). There was no significant difference in the proportion of time spent at each type of positive social interactions between groups (MANOVA, Wilks's $\lambda$=0.916, P=0.801, $N_{control}$=7, $N_{CORT}$=10). The behavioural time budget of the females was also affected by the male-treatment, with decreased vigilance (P=0.011) and agonistic interactions (P=0.024), and increased chick threatening (P=0.004) and vocalisations (P=0.016). The CORT-treatment did not affect the amount of time chicks spent begging (Fig. 3A, GEE, Wald $\chi^2$=0.061, P=0.805), nor did it affect the behavioural responses of males to chick-begging (Table 3, Fig. 3B).

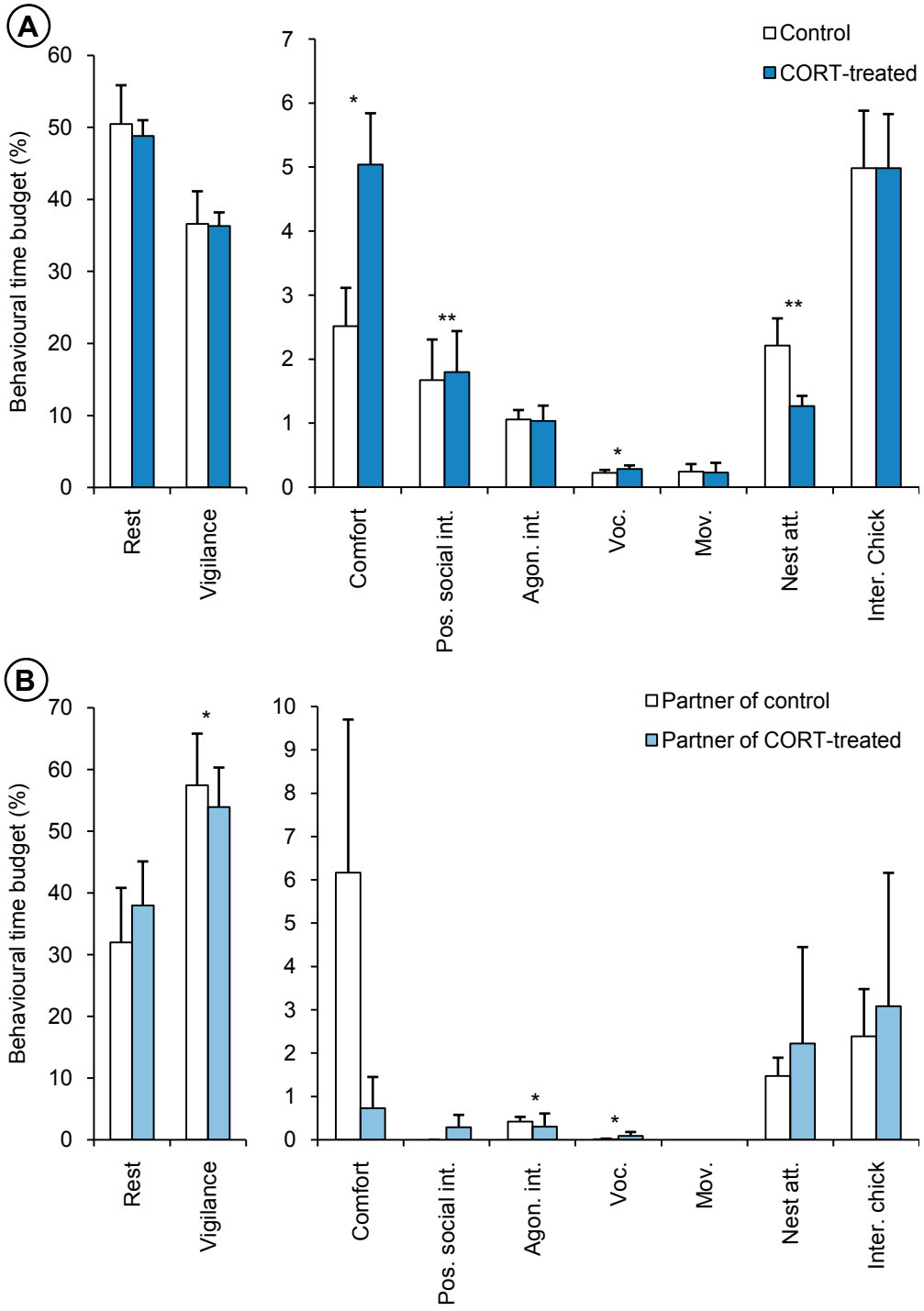


**Figure 2:** Effects of male CORT-treatment on the behavioural time budget of male Adélie penguins (**A**) and their partners (**B**)

* P<0.05, ** P<0.01

**Table 2:** Effects of male treatment and several covariates on the behavioural time budget of chick-rearing male Adélie penguins and their partners, assessed with Generalised Estimating Equations

| | **Male CORT-treatment** | No. chicks | Age of the first chick | No. hours since last return from sea (LRFS) | **Male treatment** x No. hours since LRFS |
|---|---|---|---|---|---|
| **Males** | | | | | |
| Rest | NS | NS | - (0.044) | + (0.002) | NS |
| Vigilance | NS | NS | NS | NS | NS |
| Comfort | + (0.046) | NS | NS | NS | NS |
| Positive social interactions | + (0.003) | - (0.039) | NS | + (<0.001) | - (<0.001) |
| Agonistic interactions | NS | NS | NS | NS | NS |
| Vocalisations | + (0.035) | NS | NS | + (0.003) | - (0.002) |
| Movements from nest | NS | + (<0.001) | + (<0.001) | NS | NS |
| Nest attentiveness | - (0.003) | NS | NS | NS | + (0.016) |
| Interactions with chick | NS | NS | NS | - (<0.001) | NS |
| - Feeding | NS | NS | NS | - (<0.001) | NS |
| - Threatens chick | NS | + (0.048) | NS | NS | NS |
| Females | | | | | |
| Rest | NS | NS | + (0.038) | NS | NS |
| Vigilance | - (0.011) | NS | - (0.029) | - (<0.001) | + (<0.001) |
| Comfort | NS | NS | NS | + (0.002) | - (0.001) |
| Positive social interactions | *Not enough observations* | | | | |
| Agonistic interactions | - (0.024) | NS | NS | - (0.006) | + (0.004) |
| Vocalisations | + (0.016) | - (0.002) | NS | - (<0.001) | NS |
| Movements from nest | *Not enough observations* | | | | |
| Nest attentiveness | NS | NS | NS | NS | NS |
| Interactions with chick | NS | NS | NS | - (0.004) | NS |
| - Feeding | NS | NS | NS | NS | NS |
| - Threatens chick | + (0.004) | + (<0.001) | + (0.045) | NS | NS |

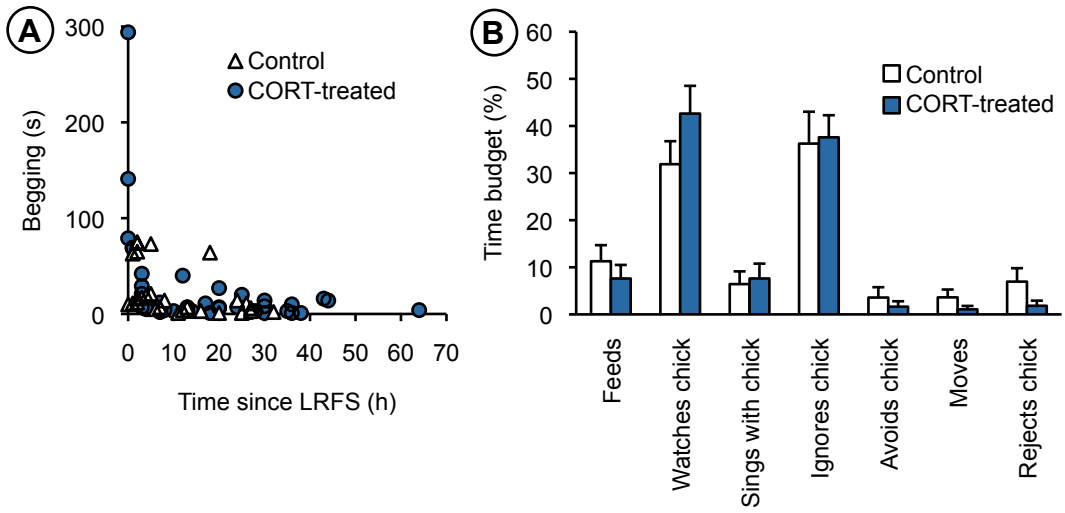


**Figure 3:** Effects of male CORT-treatment on chick begging duration (**A**) and on male responses to chick begging (**B**)

Begging duration is plotted against the time since male last returned from sea (LRFS). White triangles and bars correspond to control birds and their chicks, while black circles and bars refer to CORT-treated birds and their chicks.

**Table 3:** Behavioural responses of male Adélie penguins to chick begging depending on male treatment, number of chicks on the nest, age of the first chick, and number of hours since the male last returned from sea (GEEs)

| | **Male CORT-treatment** | No. chicks | Age of the first chick | No. hours since LRFS |
|---|---|---|---|---|
| Feeds chick | NS | NS | NS | NS |
| Watches chick | NS | - (0.017) | NS | NS |
| Sings with chick | NS | NS | NS | NS |
| Threatens chick | NS | + (0.006) | NS | NS |
| Movements from nest | NS | + (<0.001) | + (<0.001) | NS |

*REPRODUCTIVE OUTPUT*

All birds had two chicks on the day of implantation. All chicks had a similar body mass at this time (Fig. 4, GEE, Wald $\chi^2$=0.95, P=0.33, $N_{control}$=$N_{CORT}$=20 chicks on 10 nests). The brood mass was also similar between nests (W=70, P=0.143). The number of chicks per nest was not significantly different between control and CORT-treated birds during the 21 days pellets are designed to continuously release the hormone (Fig. 4A, GLM with a quasi-Poisson distribution for each day of the treatment, P>0.144, $N_{control}$=$N_{CORT}$=10 nests). The number of chicks per nest 30 and 35 days after implantation was significantly decreased in CORT-treated birds when compared to controls (t=2.55, P=0.020). Chick mass and brood mass did not differ between groups when chicks were 17 to 21 days old, ca. 17 days after implantation (chick mass: GEE, Wald $\chi^2$=2.71, P=0.100, $N_{chicks\ control}$=12, $N_{chicks\ CORT}$=8; brood mass: W=28, P=0.284) and at the end of guard stage, before fledging (chick mass: Wald $\chi^2$=1.35, P=0.246, brood mass: W=28, P=0.955, $N_{chicks\ control}$=11, $N_{chicks\ CORT}$=6).

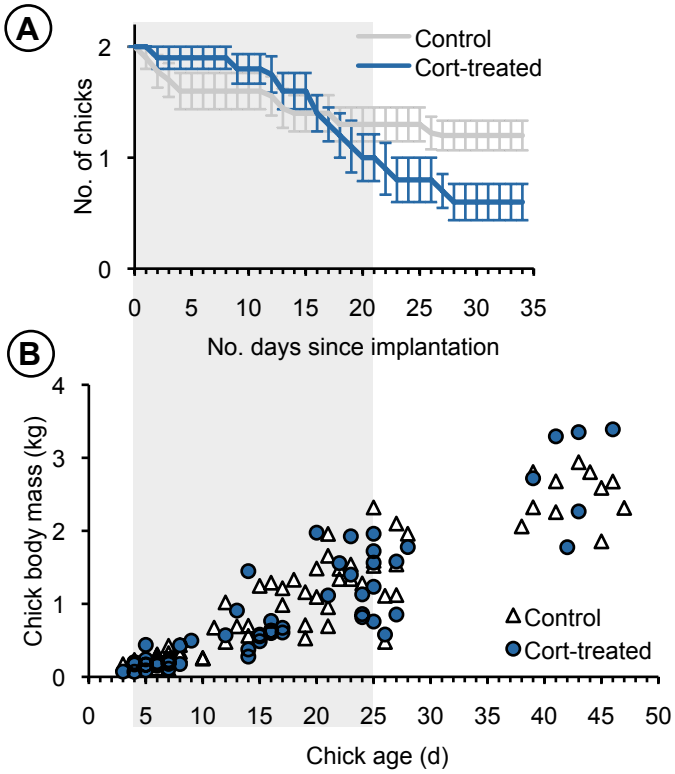


**Figure 4:** Effects of male CORT-treatment on number of chicks per nest and chick mass

Grey line and white triangles show data from control birds, and blue line and circles from CORT-treated birds. The grey square corresponds to the period over which CORT levels were manipulated according to the manufacturer.

## Discussion

We investigated whether and how an experimental increase of CORT levels modified the on-land behaviour of chick-rearing male Adélie penguins and the potential consequences on the behaviour of their partner and on the reproductive output. The behaviour of males on their nest as well as that of the females was only slightly affected by treatment, but penguins with increased CORT levels had a decreased reproductive output. Interestingly, the decrease in the number of chicks produced per nest occurred later than the period over which CORT levels were manipulated, indicating a decoupling between the increase in CORT levels and their effects on the offspring of breeding birds.

### *EXPERIMENTAL MANIPULATION OF CORTICOSTERONE LEVELS*

The use of self-degradable pellets to modify CORT levels has been previously validated on incubating male Adélie penguins, in which a 2- to 4-fold increase in CORT levels was measured until 16-18 days after implantation (Spée *et al.* 2011a; Thierry *et al.* 2013b). In the current study, CORT levels had returned to

initial levels 17 days after implantation. Because we wanted to limit the disturbance of monitored birds, the study protocol did not allow monitoring detailed variations in CORT levels following implantation. Results from previous work (Spée *et al.* 2011b) suggest that levels of up to 30-40 ng/mL (i.e. up to a 5-fold increase) might have been reached in CORT-treated birds. The earlier return of CORT levels to initial levels probably results from the fact that birds were regularly fed in our study, whereas incubating penguins had been fasting for several weeks in the former study. Due to higher activity at sea and on the nest, chick-rearing birds might have had increased energy expenditure (Chappell *et al.* 1993) and/or blood flow when compared to fasting incubating birds. This could contribute to explain a quicker release of the hormone than that provided by the manufacturer or recorded in our previous studies.

*CORT AND BEHAVIOUR*

Because of the major involvement of CORT in mediating physiological and behavioural responses to stress and of its important role in the regulation of energy balance, we expected the treatment to induce marked changes in the behavioural time budget of birds on their nest, and in particular decreased parental care. Surprisingly, the behaviour of male Adélie penguins was only slightly affected by the CORT-treatment (Table 2). The treatment decreased nest attentiveness, and increased vocalisations, positive social interactions, and comfort behaviours in CORT-treated birds when compared to controls. Our observations are in agreement with the fact that greylag geese *(Anser anser)* injected with adrenocorticotrophic hormone (ACTH), which stimulates GC synthesis and secretion, spent more time body-shaking, wing-flapping, and preening than sham-treated birds (Kralj-Fišer *et al.* 2010). When approached by humans, Adélie penguins shook their head and stretched their body (Ainley 1974) and magellanic penguins (Spheniscus magellanicus) showed increases in defensive head turns (Walker *et al.* 2006). The behavioural responses of chick-rearing gentoo *(Pygoscelis papua)*, royal *(Eudyptes schlegeli)*, and king penguins *(Aptenodytes patagonicus)* to pedestrian visitation, which are associated with increased CORT secretion (Fowler 1999; but see Walker *et al.* 2006), included increased vigilance and agonistic interactions (Holmes 2007). Such behavioural changes were not observed in our study, but short-term human disturbance might not be directly comparable to an increase in CORT levels over several days even if repeated short-term disturbances such as snowmobile activity increased GC levels of wolves and elks (Creel *et al.* 2002).

Different hypotheses can be put forward to explain the absence of major changes in the behavioural time budget of chick-rearing CORT-treated birds when compared to incubating CORT-treated birds, which incubated their eggs at lower temperatures (Thierry *et al.* 2013b) and for some of them deserted their nest (Spée *et al.* 2011a). (1) The quicker release of the hormone mentioned above in regularly fed birds might have increased CORT levels sufficiently to mask major behavioural effects. CORT is indeed known to have dose-dependent effects, with an inverted-U-shaped dose-response curve (Breuner & Wingfield 2000). (2) Only free CORT, i.e. the fraction of CORT unbound to corticosterone-binding globulins (CBG), is thought to be biologically active (Breuner & Orchinik 2002; Hammond 1995; Rosner 1990). CBG levels decreased in white-crowed sparrows exposed to short-term fasting while total CORT was unchanged, resulting in increased free CORT levels (Lynn, Breuner & Wingfield 2003). Variations of CBG levels between fasted and fed penguins may also result in differences in free CORT levels, possibly

explaining the minor behavioural changes induced by the CORT treatment in chick-rearing penguins. (3) The fact that the brood value of chick-rearing birds is higher than that of incubating birds could also explain the absence of major changes in our study. Indeed, the "brood value hypothesis" proposes that GC reactivity should be modulated as a function of the relative importance of current reproductive output and lower in life-history stages of higher brood value (Bókony *et al.* 2009; Breuner 2011).

While it would have been interesting to treat both males and females to assess the direct effects of increased CORT levels on both sexes, such a study would have required the handling of a large number of birds and could have had strong deleterious effects on reproductive output. As Adélie penguins show biparental care, it is important to study both partners when investigating the physiological mechanisms underlying variation in parental investment as one can compensate for the other. Interestingly, the activity budget of females was indirectly affected by the CORT-treatment of the male, in terms of increased time spent at sea (Table 1) and variations in their behavioural time budget (Table 2). However, in a study on the same species, Beaulieu and colleagues found that temporarily handicapped penguins supported the whole additional foraging costs (longer foraging trips) without any compensatory behaviour from their partners (2009). Further studies should evaluate whether and how partners might exchange information regarding their endocrine or nutritional status during nest relief and the potential costs of such compensation.

*CORT AND REPRODUCTIVE OUTPUT*

The relationship between CORT levels and reproductive performance has been examined in various bird species (see Bonier *et al.* 2009a for a review on baseline cort levels and fitness; Breuner, Patterson & Hahn 2008 for stress-induced cort levels and fitness). The link between CORT and reproductive performance is often controversial, depending on species (Bonier *et al.* 2009a), sex (Angelier *et al.* 2010; Bonier *et al.* 2007), reproductive strategy (Lancaster *et al.* 2008), environmental conditions (Breuner & Hahn 2003), and individuals (Dingemanse, Edelaar & Kempenaers 2010; Koolhaas *et al.* 1999). In our study, CORT-treated chick-rearing Adélie penguins had a decreased reproductive output, in terms of the number of chicks produced per nest (Fig. 4A). However, chick mass was not affected by the treatment (Fig. 4B). Penguins with high CORT levels might preferentially raise one chick because they are not able to raise two. This trade-off between chick quality and quantity does not necessarily result from direct male decisions but may be an indirect effect of sibling competition for limited resources.

Interestingly, this decrease in the reproductive output of treated birds did not happen during the period over which CORT levels were manipulated, but 30 days after implantation. This result indicates a decoupling process between the effects of the CORT-treatment on adults and the effects on the survival of their offspring. Several non-exclusive hypotheses may explain such a decoupling. First, the delayed effect of increased CORT levels on the decrease in the reproductive output of chick-rearing penguins (this study), as well as on the delayed induction of nest desertion about 14 days after implantation in CORT-treated incubating penguins (Spée *et al.* 2011a), highlights the fact that CORT is only one component of a complex physiological mechanism regulating parental care in birds. The CORT-treatment might have affected other hormones such as prolactin, a pituitary hormone widely involved in mediating parental care. Indeed, decreased prolactin levels have been reported in CORT-treated seabirds (Angelier *et al.*

2009; Criscuolo *et al.* 2005; Spée *et al.* 2011a). Secondly, the CORT treatment was associated here with significantly less time spent at sea for males and shorter foraging trips durations during the 15 days following implantation (Table 1), in agreement with a previous study (Thierry *et al.* 2013c). Decreased foraging effort was also recently reported in CORT-treated chick-rearing male Adélie penguins, using a similar protocol and time-depth recorders (Cottin *et al.* 2011). Thus, changes in both time spent at sea and foraging behaviour might have resulted in decreased food quantity and/or quality and consequently decreased reproductive output, despite the fact that feeding rates were not directly affected by the treatment (Fig. 3B). This idea ties in with the hypothesis that penguins with high CORT levels face a trade-off between chick quantity and quality, which may originate from lower performance at sea and consecutive limited resources.

One of the goals of our study was to better understand how parental behaviour on the nest could explain the negative effects of increased CORT levels on the reproductive output of Adélie penguins. For example, CORT-treated pied flycatchers *(Ficedula hypoleuca)* fed their nestlings less frequently and produced significantly fewer fledglings than controls (Silverin 1986). Reduced nestling provisioning rates were also reported in CORT-treated male barn owls *(Tyto alba)* (Almasi *et al.* 2008). We only observed minor changes in the parental behaviour of CORT-treated male Adélie penguins, including decreased nest attentiveness in males, increased chick threatening and decreased vigilance in females, and no changes in chick-feeding rates (Table 2). These changes did not translate to decreased chick growth or survival during the time-course of the treatment (Fig. 4A). Furthermore, the level of solicitation has been described as a true reflection of offspring need because begging for food is energetically costly (Godfray 1991). Feeding and chick begging rates are highly correlated in Adélie penguins (Beaulieu *et al.* 2009), and were not affected by the CORT-treatment in our study (Table 2, Fig. 3A). This is consistent with the fact that chick body mass did not differ between groups in our study. As expected, there was also no effect of the treatment on the behavioural responses of males to chick begging over the period during which birds were observed (Fig. 3B).

*CONTEXT-DEPENDENT EFFECTS OF CORT*

Differences in the effects of the CORT-treatment on the parental behaviour of male Adélie penguins depending on the timing of the treatment (incubation vs. chick-rearing) are in agreement with the fact that GC reactivity depends on the life history stage (Breuner *et al.* 2008). The birds' sensitivity to increased CORT levels might depend on several other parameters such as body condition and environmental factors. For example, severe weather conditions such as snow storms were important in determining the effects of increased CORT levels on the behaviour of breeding birds (Breuner & Hahn 2003; Thierry *et al.* 2013b). Our observations support the more general idea that the influence of CORT on behaviour is context-dependent (Landys *et al.* 2006; Orchinik 1998). Indeed, a previous study following a similar experimental design also showed a decreased reproductive output after CORT treatment in Adélie penguins, but the decrease occurred during the treatment (Thierry *et al.* 2013c). The two studies were carried out during two consecutive austral summers. Differences in environmental conditions possibly leading to differences in food availability at sea and thus food provisioning to the chicks might have affected the timing of the decrease in reproductive output measured in CORT-treated birds from one year to another. This

hypothesis warrants further examination, notably by considering the body condition and metabolic status of birds. For instance, CORT-treated black-legged kittiwakes in good condition spent more time flying and foraging than control ones, but this was not the case for males in poor body condition (Angelier *et al.* 2007a). These context-dependent effects, as well as the potential delay in the effects of an increase in CORT levels on the reproductive performance, must be taken into account when studying the responses of free-living animals to changes in their environment.

**Acknowledgments**

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## 4.3 Etude 7 : Conséquences d'une faible élévation des niveaux de corticostérone pendant l'ensemble de la saison de reproduction chez le manchot Adélie

**On the effects of a low long-term increase of corticosterone levels on the behaviour and the breeding success of male Adélie penguins**

Anne-Mathilde Thierry, Aurélie Gebus, Marion Debin, Françoise Amélineau, Thierry Raclot

Données non publiées

Les études 1, 5 et 6 se sont intéressées aux effets physiologiques et comportementaux d'une modification des niveaux de corticostérone pendant différentes périodes ciblées de la reproduction du manchot Adélie, et aux conséquences de cette manipulation sur le succès reproducteur. Les implants utilisés diffusaient 100mg de l'hormone pendant théoriquement 21 jours. Les effets biologiques de la corticostérone sont connus pour être dose-dépendants, avec une courbe dose-réponse dite en U inversé, c'est-à-dire des effets maximaux pour des concentrations intermédiaires (Breuner & Wingfield, 2000). Une étude précédente avait en effet montré que des implants de différentes doses de corticostérone (10 à 200mg diffusant pendant 21 jours) avaient des effets variables sur l'activité locomotrice de manchots Adélie en échec de reproduction, avec un effet maximum des implants de 50 et 100mg de corticostérone (Spée *et al.* 2011b), correspondant à des valeurs physiologiques élevées. Pourtant, peu d'études ont mesuré les conséquences d'une faible élévation des niveaux de corticostérone pendant une longue période, plus susceptible de mimer une situation naturelle, sur le comportement et la reproduction d'un animal en milieu naturel. Les objectifs de cette étude ont été de déterminer les effets d'une telle augmentation modérée des niveaux de corticostérone sur l'effort reproducteur de manchots Adélie en utilisant des implants diffusant 100mg de l'hormone pendant 90 jours. Par comparaison avec les implants utilisés dans les études précédentes, de tels implants seraient potentiellement équivalents à des implants diffusant 20 à 30mg de l'hormone pendant une période de 21 jours. Un tel traitement pourrait mimer une modification des niveaux de corticostérone en réponse à des conditions environnementales défavorables pendant l'ensemble de la saison de reproduction (Wingfield & Kitaysky, 2002 ; Kitaysky *et al.* 2007). L'étude s'est intéressée aux modifications du budget temps comportemental au nid pendant l'incubation et à l'évolution du nombre d'œufs, du nombre et de la masse des poussins pendant le traitement, et donc de l'effort reproducteur des adultes. Les effets à plus long terme ont également été considérés, en contrôlant le taux de retour des manchots un an après l'expérimentation.

**Matériel & Méthodes**

Cette étude a été réalisée au cours des étés austraux 2010-2011 et 2011-2012 selon le protocole général décrit précédemment (pages 23-27). Les deux partenaires de 36 couples de manchots Adélie ont été marqués, prélevés, pesés et mesurés entre le 7 et le 12 novembre 2010. Avant d'être relâchés, les mâles ont été identifiés avec un transpondeur (Texas Instruments TIRIS ; 32,2 x 3,8mm ; 0,8g) implanté sous la peau lâche entre la queue et la patte gauche. Vers la fin de la pariade, entre le 20 et le 26 novembre, les mâles de ces couples ont été de nouveau capturés. La moitié d'entre eux a reçu un implant sous-cutané de corticostérone (NG-111, Innovative Research of America, Sarasota, Floride, Etats-Unis), décrit pour libérer 100 mg de l'hormone pendant une période de 90 jours. Plusieurs prises de sang ont été réalisées après l'implantation afin de mesurer les niveaux de corticostérone au cours du traitement (Fig. 1) : lors du retour du manchot après son premier voyage en mer (10 au 26 décembre) et lors de la période d'élevage des poussins (17 au 21 janvier).

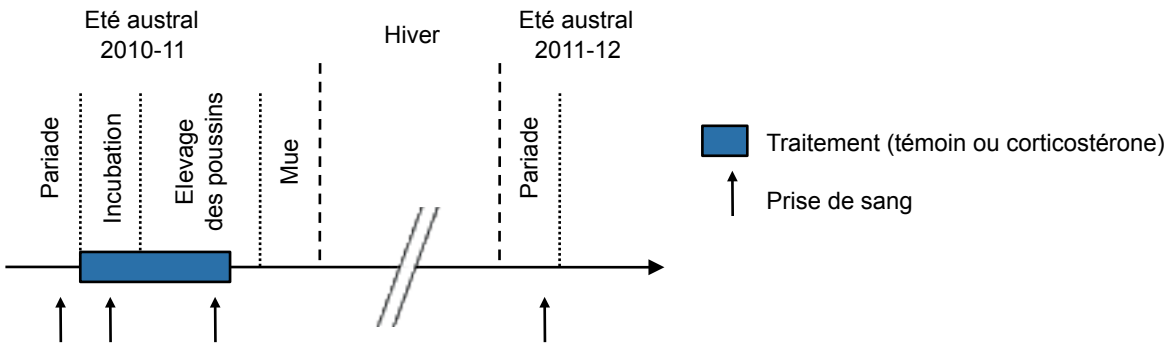


**Figure 1 :** Protocole expérimental

Le protocole de suivi est décrit dans les études précédentes, ainsi que dans le descriptif général des matériels et méthodes de la thèse (pages 23-27). En résumé, les nids ont été suivis toutes les deux à trois heures, de la première capture en novembre jusqu'à la fin de la période de garde en janvier afin de déterminer la durée des séjours à terre et des voyages alimentaires en mer. Les nids ont également été filmés pendant 4 à 6 heures par jour durant l'après-midi lors de l'incubation, sauf lorsque les conditions météorologiques ne le permettaient pas. Tous les individus étudiés n'étant pas dans le champ de la caméra, les observations comportementales ont porté sur 8 individus témoins et 8 traités, pour 9 séances d'observations de 30 minutes par individu en moyenne. En novembre 2011, les nids précédemment manipulés et les nids alentours ont été contrôlés avec un lecteur de transpondeur afin de déterminer le taux de retour des manchots dans la colonie un an après. Quelques jours après ce contrôle, les manchots ont été capturés, prélevés et pesés.

## Résultats préliminaires

### *EFFETS DU TRAITEMENT SUR LES NIVEAUX DE CORTICOSTÉRONE ET LA CONDITION CORPORELLE*

Les manchots témoins et traités CORT 90j avaient des profils similaires au début de la saison de reproduction (Tab. 1) en termes de masse des mâles (ANOVA : $F_{1,34}=0,00$ ; $p=0,967$), masse des femelles ($F_{1,34}=1,08$ ; $p=0,307$), date de ponte (GLM avec une distribution de Poisson : $z=-0,34$ ; $p=0,731$), date d'implantation ($z=-0,14$ ; $p=0,889$) et nombre d'œufs ($z=-0,49$ ; $p=0,628$).

**Tableau 1 :** Profils des couples étudiés en début de saison de reproduction

| | **Témoins** (n=18) | **CORT 90j** (n=18) |
|---|---|---|
| Masse des mâles pendant la pariade (kg) | 5,52 ± 0,09 | 5,50 ± 0,07 |
| Masse des femelles pendant la pariade (kg) | 4,62 ± 0,08 | 4,74 ± 0,08 |
| Date de ponte | 20/11 ± 0,7 | 20/11 ± 0,7 |
| Date d'implantation | 22/11 ± 0,5 | 22/11 ± 0,4 |
| Nombre d'œufs | 2,00 ± 0,00 | 1,78 ± 0,10 |

Les niveaux plasmatiques de corticostérone des mâles témoins et traités étaient similaires avant le traitement (Fig. 2A, ANOVA : $F_{1,32}$=0,70 ; p=0,409) et significativement augmentés lors du premier retour de mer après l'incubation (ANOVA sur données log-transformées : $F_{1,27}$=8,68 ; p=0,007). La masse corporelle des manchots traités CORT 90j était significativement plus faible lors du retour de mer à la fin de l'incubation (Fig. 2B, $F_{1,27}$=5,72 ; p=0,024). Lors de l'élevage, les niveaux de corticostérone et la masse corporelle des manchots qui n'étaient pas en échec de reproduction n'étaient pas différents entre les oiseaux témoins et traités (corticostérone : $F_{1,13}$=1,33 ; p=0,269, masse : $F_{1,13}$=1,30 ; p=0,275).

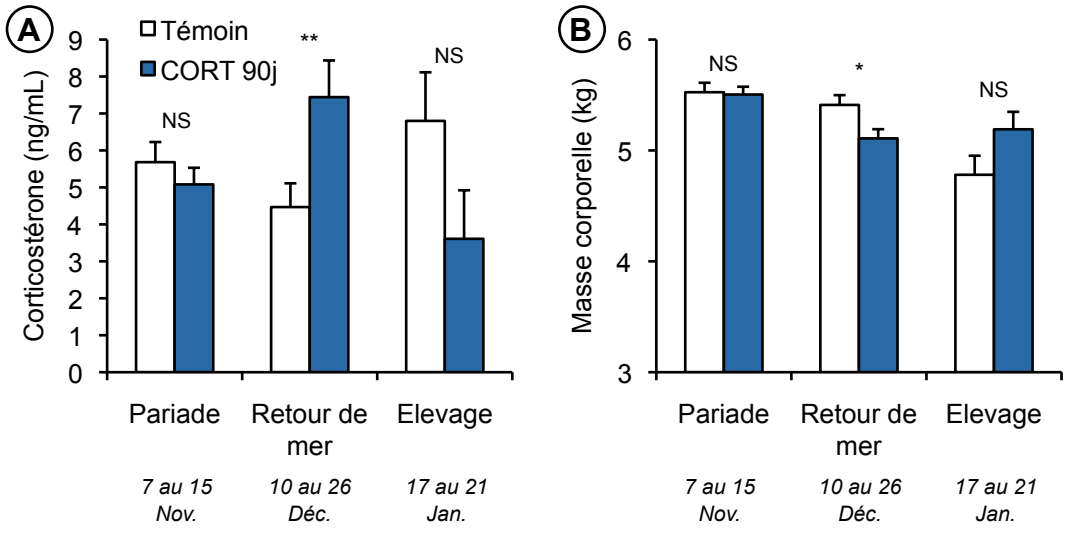


**Figure 2 :** Niveaux de corticostérone (**A**) et masse corporelle (**B**) de manchots Adélie mâles témoins et traités avec un implant de corticostérone diffusant l'hormone pendant 90 jours

*EFFETS DU TRAITEMENT SUR L'INVESTISSEMENT PARENTAL PENDANT L'INCUBATION*

Pendant l'incubation, un manchot témoin et trois manchots traités CORT 90j ont abandonné la reproduction. Le taux d'abandon était similaire entre les manchots témoins et traités (test exact de Fisher : p=0,601). Les effectifs ne permettent pas de comparer statistiquement les dates d'abandon du nid, les masses et niveaux de corticostérone de ces manchots lors de cet abandon et lors de leur retour de mer après l'abandon (Fig. 3) mais les résultats suggèrent que l'abandon a eu lieu alors que tous ces manchots avaient atteint un stade de déplétion critique de leurs réserves énergétiques (masse inférieure à la masse critique d'entrée en phase critique du jeûne prolongé de 3,5kg chez le manchot Adélie mâle, Spée *et al.* 2010).

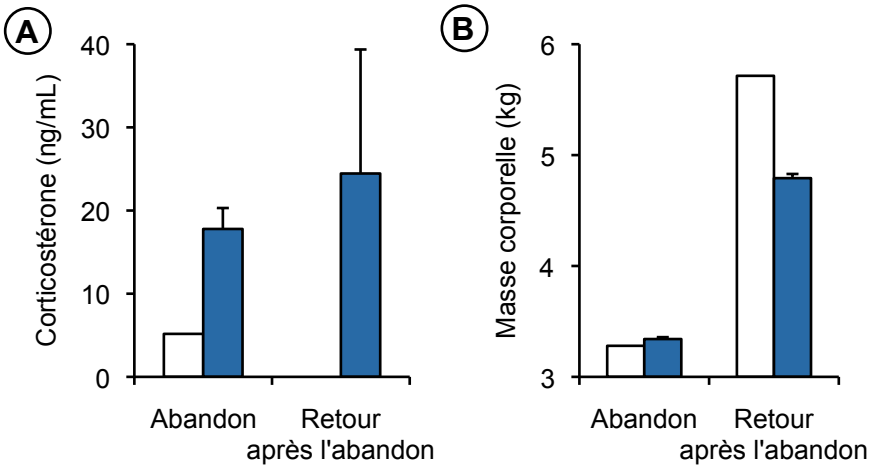


**Figure 3 :** Niveaux de corticostérone (**A**) et masse corporelle (**B**) lors de l'abandon du nid au cours de l'incubation et lors du retour de mer après l'abandon

A l'approche de l'abandon, le pourcentage de temps passé debout sur le nid a tendance à augmenter, bien que ce ne soit pas systématiquement le cas (Fig. 4).

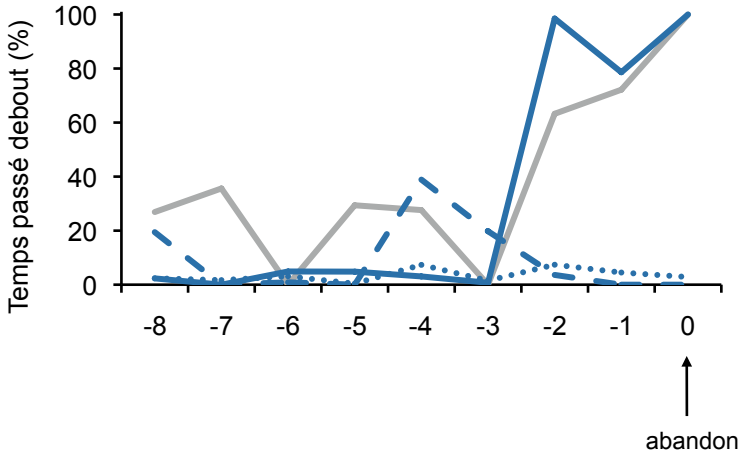


**Figure 4 :** Modifications du pourcentage de temps passé en position debout sur le nid à l'approche de l'abandon

Les observations correspondent à un manchot Adélie témoin (ligne grise) et trois manchots traités CORT (lignes bleues).

De façon générale, le budget temps comportemental des manchots Adélie mâles pendant l'incubation a été peu affecté par le traitement (Fig. 5, Tab. 2). Cependant, le temps alloué aux interactions sociales positives (ecstatic display et bill-to-axilla display) a augmenté au fil du temps chez les manchots traités alors qu'il restait stable chez les manchots témoins (moins de 1% des observations, Fig. 5C)

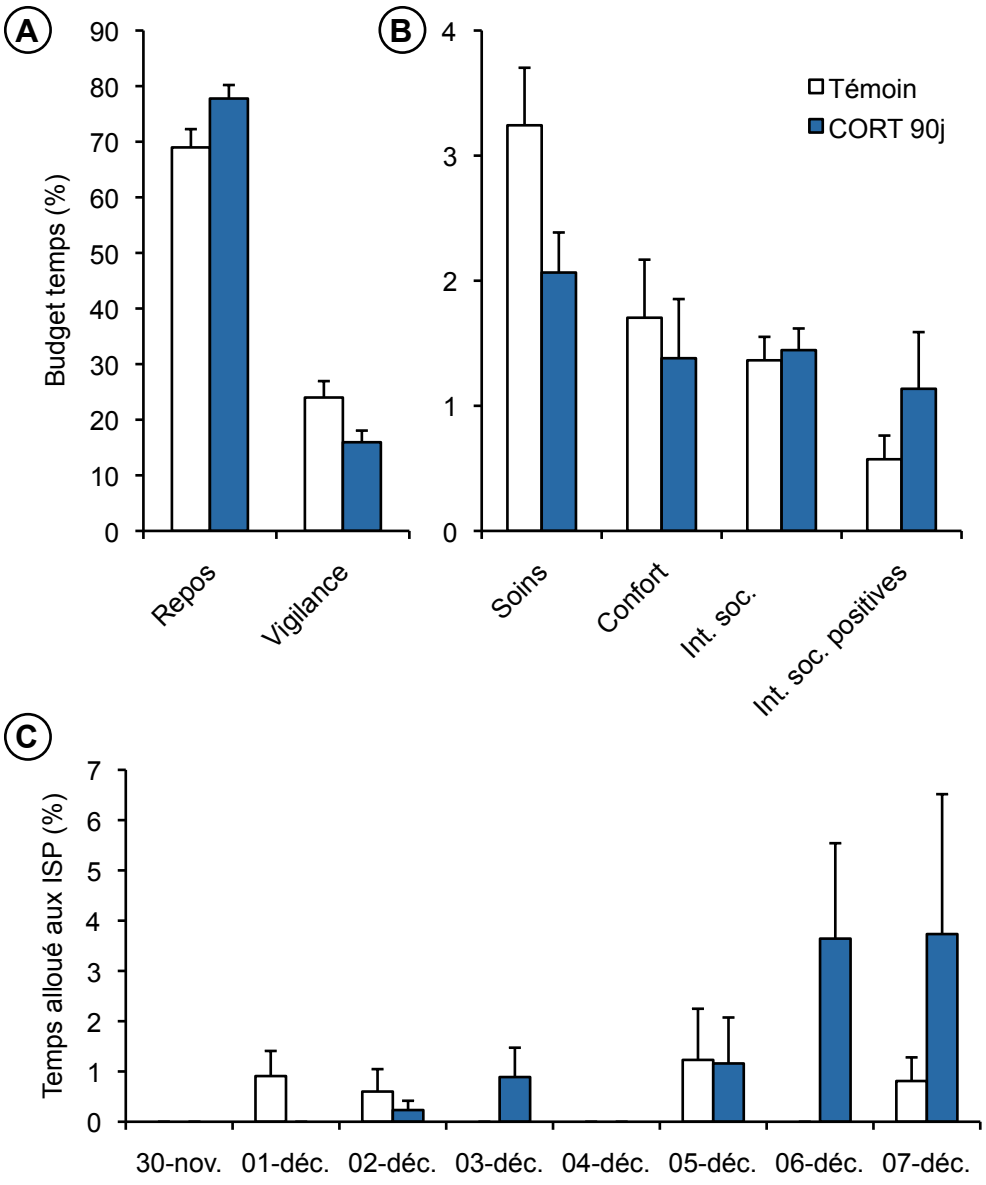


**Figure 5 :** Budget temps comportemental de 8 manchots Adélie témoins et 8 traités avec un implant de corticostérone pendant l'incubation (**A, B**) et évolution du budget temps alloué aux interactions sociales positives (ISP) au cours du temps (**C**)

**Tableau 2 :** Résultats des GEE (distribution binomiale) mesurant l'effet du traitement, de la date et de l'interaction entre traitement et date sur le budget temps comportemental de manchots Adélie pendant l'incubation

| | Traitement | Date | Traitement x Date |
|---|---|---|---|
| Repos | NS | - (0.028) | NS |
| Vigilance | NS | NS | NS |
| Attention au nid | - (0.020) | NS | NS |
| Confort | NS | + (<0.001) | NS |
| Interactions agonistiques | NS | NS | NS |
| Interactions sociales positives | - (<0.001) | NS | + (<0.001) |

Après ces quatre abandons pendant l'incubation, d'autres couples ont échoué pendant la période d'incubation de la femelle et au moment de l'éclosion. Au total, 56% des couples pour lesquels le mâle était traité CORT 90j ont échoué entre la ponte et l'éclosion contre 17% pour les témoins. La durée d'incubation et le nombre de poussins à l'éclosion ne différaient pas significativement entre témoins et traités CORT 90j encore en reproduction (durée d'incubation : 37,2 ± 0,59 jours pour 15 témoins et 36,6 ± 0,93 pour 5 traités CORT 90j, GLM avec une distribution de Poisson : z=-0,19 ; p=0,849 ; nombre de poussins à l'éclosion : 1,60 ± 0,13 pour les témoins et 1,40 ± 0,24 pour les traités, z=-0,31 ; p=0,756).

*EFFETS DU TRAITEMENT SUR L'INVESTISSEMENT PARENTAL PENDANT L'ÉLEVAGE DES POUSSINS*

Le nombre d'œufs ou de poussins par nid a diminué avec le temps (Fig. 6A, GEE avec une distribution de Poisson : Wald $\chi^2$=33,40 ; p<0,001), et ce plus particulièrement pour les manchots traités CORT 90j (interaction traitement x période : Wald $\chi^2$=11,83 ; p<0,001). La masse des poussins n'était pas affectée par le traitement (Fig. 6B, GEE : Wald $\chi^2$=0,27 ; p=0,606) ni par l'interaction entre âge du poussin et traitement (Wald $\chi^2$=0,30 ; p=0,584), et augmentait significativement avec l'âge (Wald $\chi^2$=304,79 ; p<0,001). La masse de la couvée était également uniquement affectée par l'âge des poussins (Wald $\chi^2$=61,58 ; p<0,001), mais pas par le traitement ni l'interaction entre âge et traitement (p>0.96).

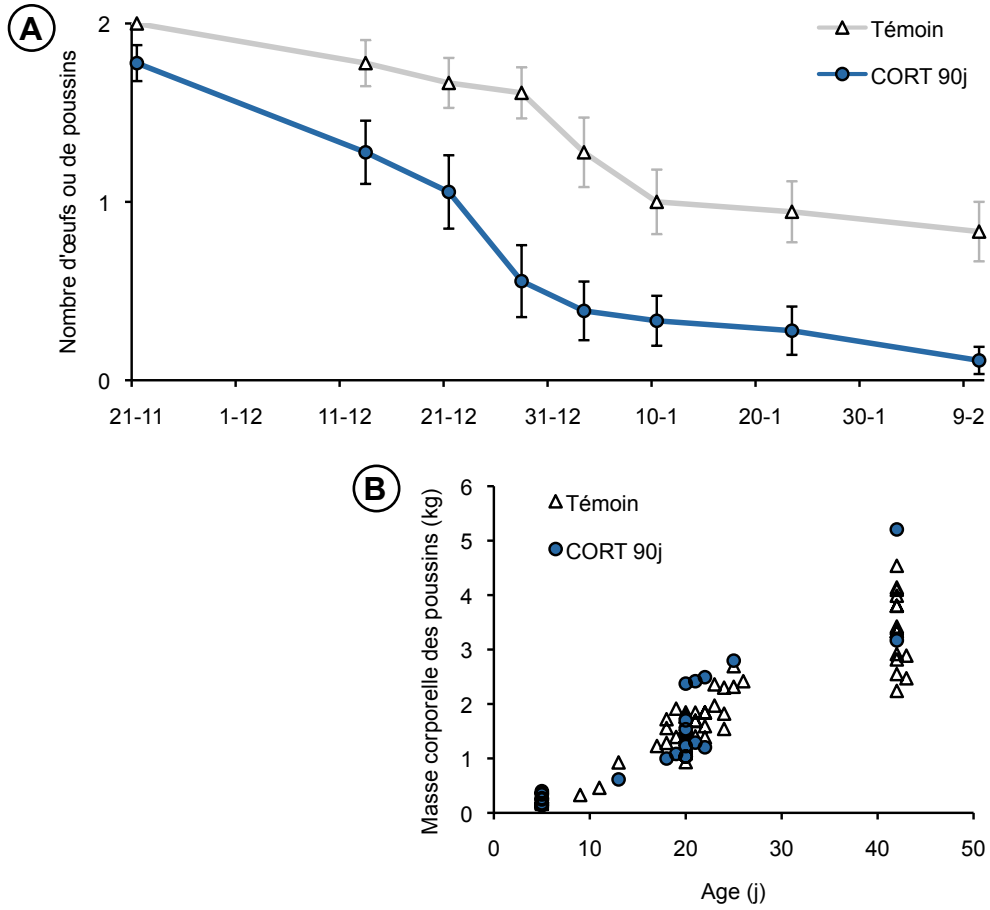


**Figure 6 :** Evolution du nombre d'œufs ou de poussins (**A**) et de la masse des poussins (**B**) pendant la saison de reproduction

La durée de la période de garde était similaire entre les nids de manchots témoins et traités (25,6 ± 1,0 jours pour 13 nids témoins et 22,4 ± 1,4 pour 4 nids CORT 90j, GLM avec une distribution de Poisson : z=-1,51 ; p=0,131). La durée des voyages en mer n'a pas été affectée par le traitement pendant l'élevage des poussins (données non présentées). Pour les nids en succès, le nombre de poussins à la fin de la saison de reproduction était de 1,25 ± 0,13 pour 12 nids témoins et de 1,00 ± 0,00 pour 2 nids CORT 90j.

*EFFETS DU TRAITEMENT À PLUS LONG TERME*

12 manchots témoins et 13 traités CORT 90j ont été revus dans la colonie d'étude l'année suivant la manipulation expérimentale, soit un taux moyen de retour de 69% similaire entre les groupes (test exact de Fisher, p=1). Ces manchots avaient des niveaux de corticostérone et une masse corporelle similaires (ANOVA, corticostérone : $F_{1,19}$=0,76 ; p=0,395 ; masse : $F_{1,16}$=1.87, p=0.190).

## Discussion

*VALIDATION DU TRAITEMENT*

L'utilisation d'implants diffusant 100mg de corticostérone pendant une période de 21 jours a été validée dans le cadre d'études précédentes (Spée *et al.* 2011a, 2011b ; Etudes 1 et 6), avec une augmentation effective des niveaux de l'hormone jusqu'à 18 jours après l'implantation pendant l'incubation (Spée *et al.* 2011a ; Etude 1), et un retour à des niveaux comparables aux manchots témoins 17 jours après l'implantation pendant l'élevage des poussins (Etude 6). Les implants diffusant la même quantité d'hormone pendant une période de 90 jours utilisés dans cette étude ont légèrement augmenté les niveaux de corticostérone jusqu'au premier retour de mer des mâles, en moyenne 27 jours après l'implantation (Fig. 2A). Des études en conditions naturelles ne permettent pas un suivi précis de la cinétique de diffusion des implants, des captures trop fréquentes pouvant affecter le comportement des oiseaux voire entraîner un échec précoce de la reproduction. De plus, l'implant diffuse sur une période trop longue pour mesurer sa cinétique de diffusion chez des manchots en échec de reproduction maintenus en captivité, comme pour les études avec des implants diffusant plus rapidement (Spée *et al.* 2011b ; Etudes 2 et 3). Le retour à des niveaux d'hormone comparables aux manchots témoins lors de l'élevage des poussins (environ 56 jours après l'implant) confirme que les implants d'Innovative Research of America diffusent la molécule d'intérêt sur une période plus courte que celle décrite par le fournisseur (voir aussi Müller *et al.* 2009).

*CORTICOSTÉRONE ET SUCCÈS REPRODUCTEUR*

Malgré une augmentation des niveaux de corticostérone sur une période sensiblement plus courte que celle décrite par le fournisseur, cette étude a permis de s'intéresser aux effets d'une faible élévation des niveaux de corticostérone sur l'effort reproducteur pendant l'incubation et le début de l'élevage des poussins. Le suivi régulier des nids d'étude a permis de montrer une diminution importante du succès

reproducteur pour les nids où le mâle était traité CORT 90j, avec des échecs pendant l'incubation (mâles et femelles) et au moment de l'éclosion. Ces résultats ne sont pas en accord avec l'idée que les niveaux basaux de corticostérone peuvent être corrélés positivement au succès reproducteur (Bonier *et al.* 2009b ; Ouyang *et al.* 2011). 70% des manchots traités CORT 21j ont abandonné leur nid environ 14 jours après l'implantation (données cumulées provenant de Spée *et al.* 2011a et de l'Etude 1). De façon intéressante, un même pourcentage de manchots traités CORT 90j a échoué à se reproduire (Fig. 7A), mais pas nécessairement au cours de la période d'incubation du mâle (Fig. 7B).

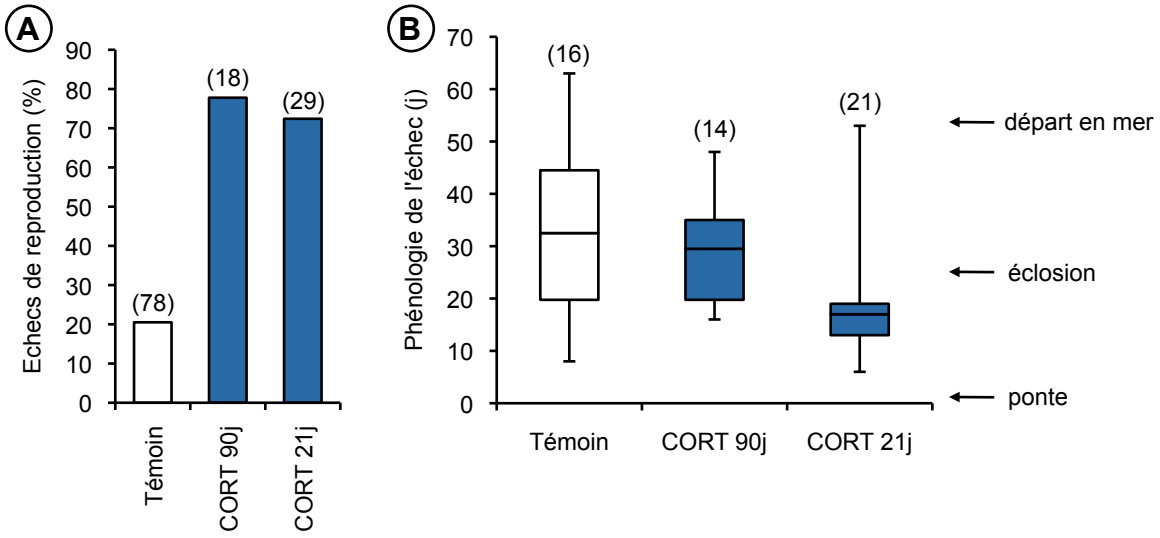


**Figure 7 :** Pourcentage d'échecs pendant l'incubation (**A**) et timing de l'échec (**B**) pour des manchots Adélie témoins et traités avec un implant de corticostérone diffusant une même quantité d'hormone pendant 21 ou 90 jours.

Les effectifs sont indiqués entre parenthèses.

*CORTICOSTÉRONE ET ABANDON DE LA REPRODUCTION*

Seuls un manchot témoin et trois manchots traités CORT 90j ont quitté définitivement leur nid au cours de la première partie de l'incubation. Cet abandon a eu lieu alors que ces manchots avaient atteint un stade critique de déplétion de leurs réserves énergétiques (phase III du jeûne prolongé), correspondant à une masse corporelle inférieure à 3.5kg chez les mâles (Cockrem *et al.* 2006 ; Spée *et al.* 2010). Cette observation et le fait que les manchots traités CORT 21j ont abandonné leur nid pour une masse d'environ 4kg (Spée *et al.* 2011a) confirment que des niveaux élevés de corticostérone, supérieurs à un seuil qui reste à définir, participent à l'induction de l'abandon du nid. Cependant, les mécanismes impliqués dans l'induction de l'abandon sont susceptibles d'être différents entre les manchots traités CORT 21j et CORT 90j vu les masses corporelles différentes à l'abandon. La détermination méthodique du pourcentage de temps passé debout sur le nid à l'approche de l'abandon a permis de confirmer une hypothèse émise au cours des missions précédentes en Antarctique concernant les variations interindividuelles du comportement des manchots Adélie avant l'abandon. En effet, certains manchots passent plus de temps debout sur le nid à l'approche de l'abandon, mais pas nécessairement, et ce a priori indépendamment des niveaux de corticostérone (Fig. 4). L'observation d'un plus grand nombre de manchot au cours des jours précédant

l'abandon, associée à des mesures de masse corporelle et de différents paramètres physiologiques, restent nécessaires pour déterminer précisément les facteurs physiologiques et environnementaux contrôlant la décision d'abandonner définitivement la reproduction en cours.

*CORTICOSTÉRONE ET "ECSTATIC DISPLAY" PENDANT L'INCUBATION*

La faible augmentation des niveaux de corticostérone n'était pas associée à une modification importante du budget temps comportemental. Il faut cependant noter l'augmentation du temps alloué aux interactions sociales positives ("ecstatic display" et "bill-to-axilla display") chez les oiseaux traités CORT 90j (Fig. 5C). L'ecstatic display est décrit comme un signal territorial utilisé par les mâles et les femelles dans le cadre de la défense du nid. Il pourrait également servir d'indice de la qualité du mâle lors de la pariade, et donc jouer un rôle dans le choix du partenaire par les femelles (Marks *et al.* 2010). En effet, les vocalisations émises lors de l'ecstatic display varient en fonction de la condition corporelle du mâle. Les oiseaux traités CORT 90j pourraient ainsi informer leur partenaire sur leur état nutritionnel. Il pourrait donc s'avérer intéressant de considérer à la fois le budget temps alloué à ce comportement et le spectre sonore des vocalisations associées pour les manchots témoins et traités CORT 90j dans le cadre d'analyses complémentaires à ce travail.

*PERSPECTIVES*

Les résultats présentés ici sont les données préliminaires issues d'une étude plus complète qui s'intéresse non seulement aux effets d'une faible augmentation des niveaux de corticostérone sur les performances de reproduction mais également sur différents marqueurs physiologiques caractérisant l'état des oiseaux. Des mesures en cours de stress oxydatif (capacité anti-oxydante et dégâts oxydatifs) et de longueur de télomères pourraient permettre de préciser les relations entre corticostérone et vieillissement chez les oiseaux, et de façon plus générale entre conditions environnementales et traits d'histoire de vie (Haussmann & Marchetto, 2010)

**Remerciements**

Nous remercions Sophie Brajon pour la mise au point de l'éthogramme des comportements du manchot Adélie pendant l'incubation (Etude 2).

# Discussion & Perspectives

# 5. Discussion et Perspectives

Dans cette partie, les principaux résultats sont résumés et discutés à la lumière de la littérature récente sur le sujet. Une discussion spécifique à chaque étude est par ailleurs détaillée dans les différents articles. La discussion générale se poursuit par la description de différentes perspectives s'offrant à ce travail de thèse.

## 5.1 Synthèse des principaux résultats

L'objectif général de cette thèse était d'étudier les relations entre statut endocrinien et effort reproducteur d'un animal dans son milieu naturel, à l'aide d'approches principalement expérimentales. Nous avons pu mettre en évidence un rôle clé de la corticostérone et de la prolactine dans la régulation de l'effort reproducteur au cours de l'incubation, ainsi que des effets dose, état, et contexte dépendants d'une modification des niveaux de corticostérone sur l'effort reproducteur.

Les tableaux suivants récapitulent les résultats obtenus dans le cadre des études concernant la période d'incubation (Tab. 5-1) et des études au sujet de la corticostérone (Tab. 5-2).

**Tableau 5-1 :** Récapitulatif des principaux résultats concernant la période d'incubation

| **Etude** | **1** | **2** | **3** | **4** |
|---|---|---|---|---|
| Facteur étudié | **++ corticostérone** | **- prolactine** | **+ testostérone** | **Conditions environnementales** |
| Durée d'incubation | ↗ | ↗ | = | ↗ *(neige)* |
| Températures d'incubation | ↘ | ↘↘ | ↘↘ | ↗ *(temp. extérieure)* ↘ *(vent)* |
| Taux de rotation des œufs | = | ↗ | = | ↗ *(temp. extérieure)* ↘ *(vent, humidité)* |
| Temps passé debout sur le nid | NE | ↗ | ↗ | NE |
| Succès à l'éclosion | ↘ | ↘ | = | ↘ *(neige)* |

Pour les études 1 à 3, le sens des variations (=, ↗, ↘) est donné par rapport au groupe de manchots témoins. NE : Non Evalué.

**Tableau 5-2 :** Récapitulatif des principaux résultats concernant la modification des niveaux de corticostérone à différentes étapes du cycle de reproduction et/ou à différentes doses

| **Etude** | **1** | **5 et 6** | **7** |
|---|---|---|---|
| Traitement<br>Durée de diffusion de l'implant | **++ corticostérone**<br>(21 j) | **++ corticostérone**<br>(21 j) | **+ corticostérone**<br>(90 j) |
| Période | Incubation | Elevage | Incubation et Elevage |
| Succès reproducteur | ↘↘ | ↘ | ↘↘ |
| Abandon de la reproduction ? | ↗ (60%) | = | = |
| Décours temporel de la diminution du succès reproducteur | ↘<br>(~14 jours post-implant) | A la fin (Etude 5) ou après le traitement (Etude 6) | = |
| Effets environnementaux | Effets ↗ avec blizzard | Différences interannuelles | NE |
| Taux de retour l'année suivant la manipulation | = | NE | = |

Le sens des variations (=, ↗, ↘) est donné par rapport au groupe de manchots témoins. NE : Non Evalué.

## 5.2 Discussion générale : effets dose, état, et contexte dépendants d'une modification du statut endocrinien

### 5.2.1 Nature de l'hormone

Les hormones étudiées étant impliquées dans la régulation de différentes fonctions biologiques, nous ne nous attendions pas à des résultats comparables en manipulant les niveaux de corticostérone, de prolactine ou de testostérone pendant l'incubation. Cependant, une augmentation expérimentale des niveaux de corticostérone est associée à une diminution des niveaux de prolactine (Criscuolo *et al.* 2005 ; Angelier *et al.* 2009 ; Spée *et al.* 2011a). Des niveaux élevés de corticostérone et faibles de prolactine sont également mesurés lors de l'abandon du nid en cas de déplétion critique des réserves énergétique (Groscolas *et al.* 2008 ; Spée *et al.* 2010). Ces observations pouvaient laisser penser qu'une diminution seule des niveaux de prolactine serait susceptible d'induire l'abandon du nid. Certaines études suggèrent d'ailleurs qu'il existe un seuil de prolactine en dessous duquel la propension à couver est inhibée (Boos *et al.* 2007 ; Spée *et al.* 2010 ;Spée *et al.* 2011a). Une immunisation active contre la prolactine chez des dindes a diminué les niveaux de prolactine et empêché l'expression du comportement d'incubation chez la dinde (Crisóstomo *et al.* 1998). Pourtant, des études corrélatives n'ont pas mis en évidence de relations entre les niveaux de prolactine et un déclin de la motivation à incuber chez le bécasseau semipalmé *Calidris pusilla* (Gratto-Trevor *et al.* 1990) et le gobemouche noir *Ficedula hypoleuca* (Silverin & Goldsmith, 1990).

Distinguer les effets d'une diminution des niveaux de prolactine associée à une augmentation de corticostérone d'une diminution seule des niveaux de prolactine sur le comportement d'incubation du manchot Adélie apparaissait donc nécessaire. L'étude 2 montre qu'une diminution seule des niveaux de prolactine n'entraîne pas l'abandon du nid, bien que le traitement ait induit une diminution de l'investissement parental pendant l'incubation. Les résultats de cette étude confirment le rôle de la prolactine dans la régulation des soins parentaux chez les oiseaux, rôle parfois discuté (Williams, 2012), en montrant notamment que les températures d'incubation sont corrélées aux niveaux de prolactine chez le manchot Adélie. Pourtant, les manchots présentant des niveaux diminués de prolactine n'ont pas alloué plus de temps à réaménager leur nid que les oiseaux témoins. Plusieurs hypothèses peuvent être proposées pour expliquer l'absence de diminution de l'attentivité au nid chez les oiseaux traités :

- La diminution des niveaux de prolactine a pu ne pas être suffisamment importante pour affecter le budget temps comportemental des manchots. Cette hypothèse est peu probable dans la mesure où les niveaux de prolactine atteints en fin de traitement lors de l'étude 2 sont comparables aux niveaux de prolactine de manchots traités avec un implant de corticostérone (30-40 ng/mL, Spée *et al.* 2011a). Ils sont également similaires aux niveaux de prolactine d'oiseaux témoins abandonnant leur nid : environ 30 ng/mL, au lieu de 80 ng/mL pour des oiseaux n'abandonnant pas leur nid (Spée *et al.* 2010).

- Les oiseaux pourraient être peu sensibles à une diminution des niveaux de prolactine à cette période du cycle de reproduction.
- Et/ou d'autres hormones pourraient intervenir dans le contrôle du comportement d'incubation. En effet, une injection de progestérone, mais pas de prolactine, a induit la mise en place de comportements d'incubation chez la tourterelle domestique *Streptopelia risoria* (Lehrman & Brody 1961). Seule l'injection d'un mélange d'œstrogènes et de progestérone suivi par l'injection de prolactine a pu induire l'incubation chez les dindes ovariectomisées, alors que l'injection de prolactine seule a pu maintenir les comportements d'incubation après leur induction (El Halawani *et al.* 1986), justifiant l'étude de la prolactine seule dans cette thèse. Le rôle d'autres hormones devrait donc être considéré pour mieux comprendre l'induction et le contrôle des comportements d'incubation chez le manchot.

### 5.2.2 Dose de l'hormone

Si les hormones peuvent agir à de très faibles doses, leurs rôles biologiques sont susceptibles de varier en fonction de leur concentration. En particulier, l'action de la corticostérone sur l'activité locomotrice est connue pour être dépendante de sa concentration, avec des effets comportementaux maximaux pour des niveaux intermédiaires de l'hormone (Breuner *et al.* 1998 ; Breuner & Wingfield 2000 ; Spée *et al.* 2011b). Ces effets dose de la corticostérone sont en partie expliqués par le fait que l'hormone peut se fixer à deux types de récepteurs : des récepteurs possédant une grande affinité pour la corticostérone, activés par de faibles taux de l'hormone, et d'autres avec une faible affinité et activés par des taux élevés (Reul *et al.* 1987 ; de Kloet *et al.* 1993 ; Kalman & Spencer 2002).

D'autre part, les effets de la corticostérone sont susceptibles d'être différents selon que l'on considère les niveaux basaux *(baseline)* des niveaux aigus *(acute, stress-induced)* de l'hormone. Il est clair qu'une augmentation rapide des niveaux de corticostérone est mesurée en réponse à une perturbation brève et intense telle qu'une capture suivie d'une période de contention. Mais lorsque le début d'une perturbation n'est pas rapide (comme une période de jeûne prolongé dont la fin n'est pas définie chez le manchot, ou bien comme une tempête, au cours de laquelle la couverture neigeuse réduit drastiquement la disponibilité alimentaire de passereaux pendant plusieurs heures et non quelques minutes), doit-on considérer la lente augmentation des niveaux de corticostérone associée comme une réponse au stress ? Un stress chronique est-il uniquement défini lorsqu'une perturbation est suffisamment longue ? Enfin, lorsqu'un animal est stressé de façon chronique, par exemple du fait d'une subordination sociale ou lors d'une diminution chronique de la disponibilité alimentaire, comment faut-il évaluer la réponse à autre type de dérangement (Dallman *et al.* 1994 ; Romero & Wikelski 2001 ;Boonstra 2013) ? Une revue récente suggère qu'il faudrait peut être considérer des variations continues des niveaux endogènes plasmatiques de glucocorticoïdes plutôt que de distinguer les niveaux basaux et aigus (Crespi *et al.* 2013).

Les études 1 et 7 montrent des conséquences différentes d'une augmentation des niveaux de corticostérone sur les performances de reproduction des oiseaux en fonction des niveaux de corticostérone des oiseaux traités, les implants utilisés dans ces études ayant diffusé une même dose de l'hormone pendant une période différente. Il est intéressant de noter que l'augmentation des niveaux de corticostérone a entraîné une diminution similaire du succès reproducteur avec un décours temporel des échecs de reproduction différent entre ces deux études (Fig. 7 de l'étude 7). Des analyses complémentaires sont nécessaires pour comprendre cette différence. L'administration de corticostérone peut induire un rétrocontrôle négatif sur la sécrétion endogène de l'hormone pendant une durée prolongée (Romero *et al.* 2005 ; Vandenborne *et al.* 2005 ; Busch *et al.* 2008 ; Müller *et al.* 2009). Par exemple, une étude réalisée chez des mouettes tridactyles montre que l'injection d'une faible dose de corticostérone a affecté l'axe corticotrope et réduit la sécrétion de corticostérone en réponse à une capture (Goutte *et al.* 2011). Ainsi, la capacité des oiseaux à répondre à une perturbation (c'est-à-dire la sécrétion endogène de corticostérone en réponse à un événement stressant) a pu être affectée différemment lors des études 1 et 7.

De façon plus générale, ces résultats soutiennent l'idée d'une relation non-linéaire entre glucocorticoïdes et performances de reproduction (Breuner *et al.* 2008 ; Bonier *et al.* 2009a ; Busch & Hayward 2009). Ils confirment également l'importance de considérer des modifications expérimentales du statut endocrinien à différents niveaux avant de pouvoir généraliser les relations entre hormones, performances de reproduction et valeur sélective (Crespi *et al.* 2013). Par ailleurs, les relations entre paramètres physiologiques et variations individuelles de l'effort reproducteur peuvent être de type *tout-ou-rien* (succès ou échec de la reproduction) ou bien *continus* (différences de succès reproducteur entre les individus, en termes de nombre, taille et/ou masse des poussins). Dans la mesure du possible, il est donc important de corréler les niveaux des différentes hormones d'intérêt avec les paramètres comportementaux mesurés.

### 5.2.3 Hormones & période du cycle de reproduction

L'utilisation d'implants diffusant la même dose de corticostérone pendant une même durée a eu des effets différents sur le succès reproducteur selon la période du cycle de reproduction où le statut endocrinien était manipulé (Etudes 1, 5 et 6). Si l'augmentation expérimentale des niveaux de corticostérone a induit une diminution du succès reproducteur dans ces différentes études, la magnitude de cette diminution n'a pas été la même entre l'incubation et l'élevage des poussins. Plusieurs hypothèses peuvent être proposées pour expliquer cette différence :

- Il a été montré qu'une élévation expérimentale des niveaux de corticostérone augmente le temps passé à se déplacer et à rechercher de la nourriture pour des mouettes tridactyles ayant une bonne condition corporelle, mais pas pour celles présentant une mauvaise condition corporelle (Angelier *et al.* 2007a). Le statut nutritionnel et la condition corporelle des manchots diffèrent entre l'incubation (oiseaux

soumis à un jeûne prolongé de plusieurs semaines) et l'élevage des poussins (oiseaux régulièrement nourris). Ceci pourrait expliquer les différences observées.

- L'augmentation des niveaux de corticostérone en réponse à un événement stressant, et donc la réponse comportementale et physiologique à une perturbation, dépend de la valeur de la nichée (Lendvai & Chastel, 2008 ; Bokony *et al.* 2009). Les effets différents d'une même augmentation des niveaux de corticostérone sur l'effort reproducteur du manchot Adélie, et plus particulièrement le fait que le traitement n'ait pas induit d'abandon de la reproduction pendant l'élevage des poussins, pourraient donc s'expliquer par une valeur de nichée plus importante pendant cette période.
- De plus, seule la corticostérone libre, c'est-à-dire la portion de corticostérone non liée à des protéines de transport *(Corticosteroid Binding Globulin, CBG)* semble être biologiquement active (Ekins, 1990 ; Rosner, 1990 ; Hammond, 1995 ; Breuner & Orchinik, 2002), c'est-à-dire capable de se fixer aux récepteurs et d'induire des modifications physiologiques et comportementales. Les niveaux de CBG varient en fonction de la saison (Deviche *et al.* 2001) et aussi du statut nutritionnel des oiseaux. Par exemple, les niveaux totaux de corticostérone ne sont pas affectés par une courte période de jeûne chez des bruants à couronne blanche, mais les niveaux de CBG diminuent entraînant une augmentation des niveaux de corticostérone libre (Lynn *et al.* 2003). Une manipulation apparemment similaire des niveaux de corticostérone pourrait donc affecter différemment les niveaux de corticostérone libre de manchots à jeun et nourris, et donc avoir des effets différents sur les performances de reproduction.
- Enfin, une combinaison de ces différents facteurs pourrait également être à l'origine des différences entre les effets d'un même traitement sur les performances de reproduction des manchots Adélie mâles.

Concernant la prolactine, une diminution expérimentale des niveaux de l'hormone non pas pendant l'incubation (Etude 2) mais au cours de l'élevage des poussins n'a pas eu d'effets sur la condition corporelle des manchots ni sur le succès reproducteur en termes de nombre et masse des poussins, bien que des effets négatifs du traitement sur l'effort de plongée à court terme aient été mesurés (Cottin, 2012 ; Cottin *et al.* in prep). Cette diminution de l'effort de plongée chez les oiseaux traités peut être interprétée comme une moindre motivation à rechercher de la nourriture. La détermination du budget temps comportemental au nid de ces oiseaux, comme pour l'étude 6, permettrait d'avoir un indicateur de la fréquence de nourrissage des poussins. Elle permettrait également de tester l'hypothèse d'une implication de la prolactine dans le contrôle hormonal de la phénologie de la reproduction proposée dans l'étude 2. Chez les oiseaux, les niveaux de prolactine sont susceptibles de diminuer en réponse à un événement stressant tel qu'une capture et une période de contention (Chastel *et al.* 2005 ; Angelier & Chastel ,2009). Il est intéressant de noter que cette modification des niveaux de prolactine en réponse au stress n'est pas constante au cours de la reproduction comme le montre une étude récente chez le puffin des Anglais *Puffinus puffinus* (Riou *et al.* 2010). La relation entre niveaux de prolactine et effort reproducteur pourrait donc également différer en fonction de la période du cycle de reproduction.

### 5.2.4 Hormones & conditions environnementales

Les conditions météorologiques sont connues pour influencer certains paramètres biologiques chez de nombreuses espèces d'oiseaux, dont les paramètres et rythmes d'incubation (Hébert, 2002). Ceci est particulièrement vrai pour les espèces se reproduisant dans l'environnement contraignant que présentent les régions polaires. Qui plus est, de mauvaises conditions météorologiques peuvent également modifier le statut endocrinien des oiseaux. Par exemple, des juncos ardoisés *Junco hyemalis* présentaient des niveaux plus élevés de corticostérone pendant une tempête de neige qu'avant ou après cette tempête (Rogers *et al.* 1993). De même, les niveaux de corticostérone de bruants à couronne blanche étaient particulièrement élevés pendant une tempête qui entraîna l'abandon du nid et du territoire. Lors du retour sur le nid, les oiseaux présentaient des niveaux comparables à ceux d'avant la tempête (Wingfield *et al.* 1983). Une diminution des niveaux de testostérone suite à une tempête au cours de l'hiver ou au début du printemps a également été mesurée chez le bruant chanteur *Melospiza melodia*, mais pas à la fin du printemps lorsque les oiseaux nourrissaient leurs poussins (Wingfield, 1985). Enfin, s'il est connu que les niveaux de prolactine diminuent en réponse à un événement stressant chez les oiseaux (Chastel *et al.* 2005 ; Angelier & Chastel, 2009), aucune étude ne semble s'être intéressée à la modification des niveaux de prolactine en réponse à de mauvaises conditions météorologiques.

Peu d'études ont, à notre connaissance, considéré les effets comportementaux de mauvaises conditions météorologiques en interaction avec une modification du statut endocrinien (Breuner & Hahn, 2003). L'étude 1 a permis de mettre en évidence que des conditions météorologiques sévères, telles qu'un épisode de blizzard suivi de températures élevées entraînant la fonte de la neige, affectaient l'incubation des manchots Adélie plus particulièrement lorsque ceux-ci présentaient des niveaux élevés de corticostérone, justifiant l'importance de l'étude des interactions organismes-environnement dans un environnement changeant (Wingfield *et al.* 2011). L'absence de conditions météorologiques sévères au cours de l'étude 2 n'a pas permis de mesurer des effets potentiellement cumulés de telles conditions avec une diminution des niveaux de prolactine. Enfin, des épisodes neigeux n'ont pas modifié la sensibilité réduite des manchots Adélie à une augmentation des niveaux de testostérone pendant l'incubation (Etude 3). Des études complémentaires sont nécessaires pour déterminer dans quelle mesure de mauvaises conditions météorologiques peuvent affecter différemment le statut endocrinien des oiseaux en fonction de la période du cycle de reproduction et de la valeur de la nichée. Elles permettraient ainsi de préciser les relations entre statut endocrinien et effort reproducteur dans un environnement changeant.

Si les conditions météorologiques peuvent affecter les performances de reproduction du manchot Adélie (Emmerson *et al.* 2011 ; Etude 4), d'autres paramètres sont également à considérer dans l'étude des relations entre hormones, effort reproducteur et conditions environnementales. Par exemple, l'étendue et la concentration de la glace de mer peut affecter le succès reproducteur (Emmerson & Southwell, 2008 ; Nicol *et al.* 2008 ; Emmerson *et al.* 2011), à travers des effets sur la disponibilité alimentaire (Beaulieu *et al.* 2010a), le comportement de recherche alimentaire (Watanuki *et al.* 1993, 1997) et le type de

proies consommées (Ainley *et al.* 2003). Les conditions de glace de mer peuvent également affecter le statut endocrinien des manchots Adélie (Cockrem *et al.* 2006 ; Ninnes *et al.* 2011). Les études 5 et 6 ont mis en évidence une diminution du succès reproducteur consécutive à l'augmentation expérimentale des niveaux de corticostérone, mais dont le décours temporel diffère entre les deux études, malgré le fait qu'un même protocole ait été suivi. Ces études ont été réalisées au cours de deux étés austraux consécutifs, pendant lesquels des conditions environnementales différentes, en termes de glace de mer par exemple, ont pu affecter la disponibilité alimentaire en mer et donc le nourrissage des poussins. Les données récoltées n'ont pas permis de déterminer les raisons précises de ce décalage dans le décours temporel de la diminution du succès reproducteur consécutive à l'augmentation expérimentale des niveaux de corticostérone. L'utilisation de la signature isotopique en carbone ($\delta^{13}C$) et en azote ($\delta^{15}N$) d'échantillons sanguins peut renseigner sur le régime alimentaire des oiseaux, tels que le type de proies ingérées et la zone prospectée (McCutchan *et al.* 2003 ; Cherel & Hobson, 2007). Des différences de signature isotopiques pourraient ainsi mettre en évidence des différences de performances de recherche alimentaire entre les deux années. Des études complémentaires devront également prendre en compte la condition corporelle et le statut nutritionnel des oiseaux, ainsi que les conditions de glace de mer et la disponibilité alimentaire. Cependant, ces résultats soulignent l'importance de considérer les interactions entre une modification expérimentale du statut endocrinien et les conditions environnementales.

### 5.2.5 Des limites à une modification expérimentale du statut endocrinien

Des études expérimentales se sont avérées nécessaires pour préciser les relations entre statut endocrinien et performances de reproduction. Plusieurs techniques sont disponibles pour modifier le niveau d'une hormone d'intérêt, présentant chacune des avantages et des inconvénients. Augmenter le niveau de corticostérone peut par exemple se faire à l'aide d'injections, uniques ou répétées (Loiseau *et al.* 2008), en ajoutant l'hormone dans la nourriture ou l'eau de boisson (Breuner *et al.* 1998), en implantant un tube silastique rempli d'hormone cristallisée (par exemple : Angelier *et al.* 2007a ; Goutte *et al.* 2010a), une pompe osmotique (Donker & Beuving, 1989) ou encore un implant dégradable (Bourgeon & Raclot, 2006 ; Almasi *et al.* 2008 ;Müller *et al.* 2009 ; Spée *et al.* 2010, 2011a, 2011b). Les injections d'hormone nécessitent des manipulations répétées qui peuvent activer l'axe hypothalamo-hypophyso-surrénalien. L'ingestion de glucocorticoïdes avec la nourriture ne permet pas de contrôler facilement l'ampleur du traitement puisque les niveaux dépendront de la quantité de nourriture ingérée, et peut donc varier en fonction des individus. De plus, ces deux techniques ne sont pas adaptées aux animaux sauvages dans leur milieu naturel. Au contraire, le recours aux implants permet de modifier de façon comparable le statut endocrinien d'un groupe d'animaux à l'aide d'un nombre limité d'intervention, une seule pour les implants utilisés dans nos études. De tels implants sont censés délivrer une dose constante de l'hormone par dégradation de la matrice de l'implant. Pourtant, plusieurs études ont montré qu'ils ne diffusent pas strictement comme le prévoit le fournisseur, avec un possible pic dans l'élévation des niveaux de

l'hormone et une modification du statut endocrinien pendant une période qui peut être plus courte que la période théorique (Müller *et al.* 2009).

S'il est difficile de contrôler tous les effets secondaires d'une modification du statut endocrinien à l'aide d'implants diffusant l'hormone dans le cadre d'une étude en milieu naturel, il est important de vérifier la pertinence physiologique de la modification expérimentale du statut endocrinien. Ceci est particulièrement important dans la mesure où un implant peut également affecter la sécrétion endogène d'une hormone (Goutte *et al.* 2011). Mesurer régulièrement le statut endocrinien d'animaux sauvages peut être source de dérangement en cas de captures et prélèvements sanguins répétés. D'autres méthodes de prélèvements pourraient permettre de suivre l'évolution du statut endocrinien consécutivement à l'implantation tout en minimisant le dérangement lié aux captures répétées. Par exemple, une méthode non-conventionnelle de prélèvements sanguins consiste à utiliser des insectes hématophages (des punaises ou Hétéroptères de la sous-famille des Triatominae) contenus dans des œufs factices (Arnold *et al.* 2008). Les niveaux de corticostérone mesurés dans le sang prélevés par ces insectes sont comparables à ceux mesurés dans le sang prélevé de façon classique. L'introduction d'espèces exotiques en Antarctique n'est ni recommandée ni autorisée, mais l'utilisation de ce type de prélèvement pourrait permettre de limiter les captures répétées pour de nombreuses espèces. Une autre possibilité consisterait à prélever régulièrement le guano des manchots témoins et traités en vue de la mesure des glucocorticoïdes fécaux (Harper & Austad, 2000, Keay *et al.* 2006), tout en prenant en compte le fait que cette mesure est susceptible de varier en fonction de prise alimentaire, notamment pour des animaux soumis à de longues périodes de jeûne (Touma & Palme, 2005).

## 5.3 Perspectives

A l'aide d'une approche pluridisciplinaire combinant des techniques issues notamment de la physiologie (manipulation du statut endocrinien et détermination des niveaux hormonaux), de l'écologie et de l'écologie comportementale (suivi de la phénologie et du succès reproducteur, détermination du budget temps comportemental au nid) et du bio-logging (utilisation d'œufs factices enregistreurs des paramètres physiques de l'incubation), les travaux présentés dans cette thèse soulignent une relation complexe entre statut endocrinien et effort reproducteur chez un oiseau marin longévif, le manchot Adélie. De nombreuses perspectives se dessinent dans la continuité ou en complément de ce travail de thèse et seront présentées succinctement, afin d'éviter des redondances avec les paragraphes précédentes.

Ces perspectives permettraient notamment d'enrichir nos travaux par des mesures de paramètres nouveaux *(perspectives 1, 2, 3 et 6)* et de les élargir en ne considérant pas uniquement le statut endocrinien des mâles mais aussi celui des femelles, les interactions entre mâles et femelles, et entre femelles et œufs *(perspective 4)*. Enfin, s'il est évidemment essentiel de préciser les relations entre différents paramètres environnementaux et les performances de reproduction en termes de succès reproducteur à l'envol, il serait également intéressant de considérer la phénologie *(perspective 5)* ou encore la qualité des poussins à la fin de la saison de reproduction *(perspective 7),* en fonction des conditions environnementales et/ou du statut endocrinien des parents. De telles perspectives pourraient être développées dans le cadre du protocole général déjà mis en place sur le manchot Adélie à Dumont d'Urville depuis plusieurs années et/ou élargies à d'autres sites de reproduction et d'autres espèces.

### 5.3.1 Différentes mesures du fonctionnement de l'axe corticotrope

La relation entre corticostérone et performances de reproduction ne dépend pas seulement des conditions environnementales (Breuner & Hahn, 2003 ;Etude 1), mais également des espèces (Bonier *et al.* 2009a), du sexe (Bonier *et al.* 2007 ; Angelier *et al.* 2010), des stratégies de reproduction (Lancaster *et al.* 2008), de la condition corporelle (Angelier *et al.* 2007a ; Loiseau *et al.* 2008) et aussi des individus (Koolhaas *et al.* 1999 ; Dingemanse *et al.* 2010). De plus, la production et l'action de la corticostérone sont régulées par de nombreux facteurs (Wingfield & Sapolsky, 2003 ; Crespi *et al.* 2013), parmi lesquels figurent la nature et le nombre de récepteurs, ainsi que plusieurs neurohormones peptidiques interagissant pour réguler la sécrétion des glucocorticoïdes par les glandes surrénales : le CRH *(Corticotropin-Releasing Hormone)* et l'ACTH *(Adrenocorticotropic Hormone)*. Les facteurs susceptibles d'affecter la variabilité des niveaux de glucocorticoïdes sont résumés dans le tableau suivant.

**Tableau 5-3 :** Facteurs affectant la variabilité des niveaux de glucocorticoïdes, d'après Crespi *et al.* 2013

| | |
|---|---|
| **Facteurs intrinsèques** | Phylogénie |
| | Age |
| | Etape du cycle de reproduction |
| | Sexe |
| | Statut de reproduction |
| | Système d'appariement |
| | Contraintes énergétiques |
| | Espérance de vie |
| **Facteurs extrinsèques** | Conditions météorologiques |
| | Type de climat |
| | Disponibilité alimentaire |
| | Compétition |
| | Pression de prédation |
| | Perturbations humaines |
| | Polluants chimiques |
| **Axe hypothalamo-hypophyso-surrénalien (ou axe corticotrope)** | CRH et récepteurs |
| | ACTH et récepteurs |
| | Glucocorticoïdes et récepteurs |
| | CBGs |
| **Conceptualisation du stress** | Niveaux basaux |
| | Niveaux aigus |
| | Niveaux aigus répétés |
| | Stress chronique |

L'axe hypothalamo-hypophyso-surrénalien est influencé par de nombreux facteurs (Tab. 5-3, Crespi *et al.* 2013). Par exemple, même si le rôle biologique des hormones stéroïdiennes sous leur forme libre et non liée à des CBG est encore débattu (Malisch & Breuner, 2010), certaines études montrent des relations différentes entre corticostérone et performances de reproduction selon que l'on considère les niveaux totaux ou libres de l'hormone (Breuner & Orchinik, 2001 ; Love *et al.* 2004), justifiant la mesure de la fraction libre et des niveaux totaux de corticostérone (Breuner *et al.* 2013). Des mesures complémentaires aux niveaux totaux de corticostérone, réalisées dans le cadre d'études similaires aux études 1, 5, 6 et 7 permettraient de décrire comment le fonctionnement de l'axe corticotrope a pu être affecté par le traitement, ainsi que de possibles différences en fonction du type d'implant utilisé et de la période du cycle de reproduction.

**Perspective 1 : Axe corticotrope et performances de reproduction**

- Mesurer des paramètres de l'axe corticotrope autres que les niveaux totaux de corticostérone, tels que les CBGs et donc les niveaux de corticostérone libre.
- Mesurer la réponse au stress des manchots, c'est-à-dire la sécrétion de corticostérone endogène en

réponse à un protocole standardisé de capture et contention. Ce type de mesure augmenterait le dérangement causé par l'étude, et ne doit donc pas être systématique.
- Répéter ces mesures dans différentes situations telles que de mauvaises conditions environnementales susceptibles d'affecter la disponibilité alimentaire et donc l'état des réserves énergétiques, une manipulation expérimentale des niveaux de corticostérone et/ou en fonction du statut nutritionnel des oiseaux.
- Relier ces mesures aux performances de reproduction des oiseaux.

### 5.3.2 Caractéristiques individuelles affectant le statut endocrinien

Parmi différentes caractéristiques individuelles (Tab. 5-3), l'âge et l'expérience de reproduction semblent d'un intérêt particulier puisque susceptibles d'affecter le statut endocrinien des oiseaux (Angelier *et al.* 2006b ; Heidinger *et al.* 2006 ; Angelier *et al.* 2007d), bien que cette relation ne soit pas systématique (Goutte *et al.* 2010b). Par exemple, les niveaux de corticostérone et prolactine augmentent avec l'expérience de reproduction chez l'albatros hurleur *(Diomedea exulans)*, avec un maximum lors de la sixième tentative de reproduction pour la corticostérone (Angelier *et al.* 2006b). Cette augmentation pourrait refléter un investissement parental plus important et/ou une régulation du système endocrinien plus efficace pour faire face aux contraintes énergétiques de la reproduction, et ce notamment pour les individus expérimentés. La diminution des niveaux de corticostérone, mais pas de prolactine, chez les oiseaux âgés pourrait être la cause ou la conséquence d'une diminution de l'investissement parental chez ces oiseaux ou bien de capacités réduites à sécréter et maintenir des niveaux élevés de corticostérone pendant une longue période.

La gêne causée par la pose de bagues alaires sur les manchots Adélie (Jackson & Wilson, 2002 ; Dugger *et al.* 2006) ne permet pas raisonnablement de les marquer pour connaître leur âge. L'utilisation de transpondeurs passifs, associant un numéro d'identification unique à chaque manchot, et d'antennes de détection situées sur les principales voies d'accès aux colonies apparait comme une alternative intéressante au bagage des manchots (Saraux *et al.* 2011). Pourtant, des effets potentiellement néfastes de l'injection sous-cutanée de transpondeurs (Clarke & Kerry, 1998) justifient une utilisation non systématique de cet outil. D'autre part, l'utilisation d'indices de l'âge biologique des animaux tels que la longueur des télomères pourrait également être une alternative intéressante au baguage des manchots. Les télomères sont des séquences répétitives et non codantes d'ADN protégeant l'extrémité des chromosomes, qui raccourcissent avec les divisions cellulaires (Blackburn, 1991). Des études, principalement conduites chez l'homme, montrent que la dynamique d'érosion des télomères peut être affectée par différents facteurs environnementaux (Lin *et al.* 2012), tels qu'un stress psychologique (Epel *et al.* 2004). Si une tendance générale à une diminution de la longueur des télomères avec l'âge existe (Hall *et al.* 2004 ; Haussmann *et al.* 2005), la longueur des télomères doit être utilisée comme un marqueur d'un âge biologique plutôt que chronologique (Monaghan & Haussmann, 2006 ; Monaghan, 2010).

**Perspective 2 : Age biologique, réponse au stress et performances de reproduction**

- Mesurer la longueur des télomères, validée comme indicateur de l'âge biologique du manchot Adélie (Haussmann *et al.* 2003). Cette mesure peut être réalisée à l'IPHC à partir de cellules sanguines d'oiseaux (Criscuolo *et al.* 2009).
- Relier cette mesure à la capacité des oiseaux à faire face à une perturbation (réponse au stress), dans le cadre d'une augmentation expérimentale des niveaux de corticostérone ou sans manipulation du statut endocrinien.

### 5.3.3 La corticostérone, indicateur de la santé des populations ?

Une idée récurrente dans la littérature récente est que les hormones, et notamment les glucocorticoïdes, peuvent être considérés comme des indicateurs de la santé des individus et des populations (Busch & Hayward, 2009) et de la capacité des populations à s'adapter à des changements de leur environnement (Meylan *et al.* 2012). Cette idée est intéressante dans la mesure où les glucocorticoïdes peuvent être mesurés de façon peu invasive à l'aide de prélèvements sanguins (Angelier *et al.* 2011), voire non invasive en dosant les métabolites des glucocorticoïdes dans les fèces (Harper & Austad, 2000 ; Keay *et al.* 2006). Cependant, pour pouvoir effectivement relier glucocorticoïdes et santé des populations, il est nécessaire de bien comprendre les relations entre hormones, performances de reproduction et survie. Ceci est d'autant plus vrai que les effets physiologiques et comportementaux d'une modification du statut endocrinien sont dose, état et condition dépendants, comme le confirment les études présentées dans cette thèse.

La théorie des traits d'histoire de vie prédit l'existence de compromis entre différentes fonctions biologiques, comme celui entre la reproduction et la survie (Stearns, 1992). Nos résultats montrent qu'une augmentation des niveaux de corticostérone est associée à une diminution des performances de reproduction (Etudes 1, 5, 6 et 7), qui peut être interprétée comme une diminution de l'investissement dans la reproduction au profit de la survie. Déterminer les conséquences de variations des niveaux de corticostérone sur la valeur sélective nécessite donc de s'intéresser à la fois à la reproduction et à la survie, et non pas à l'un ou l'autre. Pourtant, les relations entre corticostérone et survie ont été peu étudiées chez les oiseaux en comparaison aux relations entre corticostérone et performances de reproduction. Nous avons évalué le taux de retour des oiseaux sur la colonie un an après la manipulation et n'avons trouvé aucune différence de survie apparente entre les oiseaux témoins et traités avec un implant de corticostérone (Etudes 1 et 7). Chez la mouette tridactyle, une diminution du taux apparent de survie pendant les deux années qui ont suivi l'expérimentation a été mesurée chez des oiseaux traités avec un implant de corticostérone par rapport aux témoins (Goutte *et al.* 2010a). Les auteurs suggèrent que le traitement a pu affecter la sécrétion de corticostérone endogène (voir aussi Goutte *et al.* 2011). Cette diminution de la survie pourrait être liée au fait que les oiseaux traités n'aient pas pu répondre à un stress de façon appropriée, et donc n'aient pas ou

peu été capables d'ajuster leur comportement et leur physiologie face aux contraintes environnementales. Ces observations posent la question de la gestion d'un stress chronique à long terme.

Une façon de mieux comprendre les relations entre glucocorticoïdes et survie serait de suivre les oiseaux pendant plusieurs années après le traitement, tout en dissociant dispersion et survie. Cependant, de telles études peuvent être délicates à mettre en place en conditions naturelles. Une alternative pourrait être de s'intéresser aux relations entre la sécrétion d'hormones de stress et de possibles variations de l'érosion des télomères à travers une approche longitudinale (Haussmann & Marchetto, 2010).

**Perspective 3 : Stress, corticostérone et survie**

- Mesurer les conséquences d'une modification expérimentale du statut endocrinien sur la survie apparente d'oiseaux manipulés en comparaison à un groupe d'oiseaux témoins et identifiés à l'aide de transpondeurs (en déterminant le taux de retour dans la colonie pendant au moins deux années après le traitement).
- Tester l'existence de corrélations entre les niveaux de corticostérone (totaux et libres) et l'érosion des télomères (nécessitant un suivi longitudinal des oiseaux et des mesures répétées), pour des oiseaux témoins et/ou dans de bonnes conditions environnementales, en comparaison avec des oiseaux traités avec un implant et corticostérone et/ou dans de mauvaises conditions environnementales.

### 5.3.4 Femelles et effets maternels

Les différentes études présentées ici font partie d'un projet plus large qui s'est initialement intéressé au jeûne prolongé et aux mécanismes physiologiques sous-jacents à l'induction de l'abandon du nid chez le manchot Adélie mâle (thèse de Marion Spée). Le projet s'est ensuite intéressé aux mécanismes hormonaux impliqués dans l'acquisition de l'énergie à travers l'étude des performances de recherche alimentaire en mer, toujours sur les mâles (thèse de Manuelle Cottin). Pendant cette thèse, nous avons uniquement considéré les effets physiologiques et comportementaux d'une modification du statut endocrinien de manchots Adélie mâles pour des raisons en partie « historiques », dans l'idée de comparer nos résultats à ceux obtenus dans le cadre des études précédentes. Pourtant, l'étude conjointe des mâles et des femelles est recommandée dans la mesure où les deux partenaires participent généralement à l'élevage des jeunes chez les oiseaux, et des conclusions différentes peuvent être formulées selon que l'on s'intéresse à l'un ou l'autre sexe (Caro, 2012).

Lorsque le phénotype d'une femelle affecte directement celui de sa descendance, et donc la valeur sélective de celle-ci, on parle d'effet maternel. Les femelles peuvent notamment transmettre à leur progéniture des informations concernant les conditions environnementales qu'elles rencontrent à travers les niveaux de

certaines hormones dans les œufs, les préparant ainsi à leur futur environnement. Une augmentation des niveaux plasmatiques de corticostérone chez les femelles est suivie par une augmentation de l'hormone dans les œufs, avec des conséquences négatives sur le développement de l'embryon et le phénotype des poussins après l'éclosion (Groothuis *et al.* 2005 ; Hayward & Wingfield, 2006). Une telle augmentation a notamment été associée à un moindre succès à l'éclosion (Saino *et al.* 2005) et à une diminution de la masse corporelle et de la taille des poussins (Saino *et al.* 2005 ; Love *et al.* 2008 ; Spencer & Verhulst, 2008). Cependant, des niveaux élevés de corticostérone dans l'œuf peuvent être bénéfiques pour les poussins lorsque les conditions environnementales pendant la croissance ne sont pas favorables, agissant comme un lien adaptatif entre la qualité maternelle, l'investissement dans la progéniture et la fitness des femelles (Love *et al.* 2005 ; Love & Williams, 2008). Si l'étude des mâles permettait de s'affranchir d'effets maternels dans cette thèse, l'étude des femelles s'avère nécessaire pour compléter la compréhension des relations entre glucocorticoïdes et fitness au sein du couple.

**Perspective 4 : Hormones et effets maternels**

- Mesurer les effets d'une augmentation expérimentale des niveaux de corticostérone ou d'œstrogènes chez les femelles et/ou dans les œufs sur le succès reproducteur, sur la croissance et la qualité des poussins
- Déterminer dans quelle mesure les effets d'une telle augmentation peuvent varier en fonction des conditions environnementales et en fonction de la période du traitement (pendant la pariade avant la ponte, pendant l'incubation ou pendant l'élevage des poussins)
- Comparer les résultats d'une augmentation des niveaux de corticostérone pendant l'élevage des poussins aux études réalisées sur les mâles (Etudes 5 et 6).

### 5.3.5 Hormones et phénologie

La phénologie est l'étude de l'apparition d'événements périodiques au cours de la vie des organismes et est déterminée par les variations saisonnières du climat. Les variations phénologiques sont un des indicateurs privilégiés des effets des changements climatiques sur les populations et les écosystèmes dans la mesure où elles sont particulièrement faciles à détecter (Walther *et al.* 2002). Par exemple, l'arrivée des oiseaux en Terre Adélie a lieu en moyenne 9,1 jours plus tard par rapport aux années 1950, et la reproduction 2,1 jours plus tard (Barbraud & Weimerskirch, 2006). Qui plus est, se reproduire au bon moment est considéré comme un trait d'histoire de vie déterminant le succès reproducteur (Lack, 1968 ; Nager & Van Noordwijk 1995). Déterminer les relations entre hormones et phénologie à une période donnée du cycle de reproduction permettrait de mieux comprendre les mécanismes physiologiques sous-jacents aux capacités d'adaptation des animaux aux changements de leur environnement (Goutte *et al.* 2010b).

Les résultats des études 2 et 3 permettent de suggérer une implication de la prolactine et de la testostérone dans le contrôle hormonal de la phénologie de la reproduction. Le suivi détaillé de la reproduction de manchots Adélie pendant plusieurs saisons de reproduction consécutives, associé à des prélèvements sanguins et la détermination des niveaux de certaines hormones, pourrait aider à préciser les relations entre phénologie – et plus particulièrement date de ponte – et statut endocrinien dans un environnement changeant. Une telle étude permettrait de mieux comprendre pourquoi l'arrivée des oiseaux sur la colonie est plus retardée que la date de ponte (Barbraud & Weimerskirch, 2006). Il conviendrait de mesurer les niveaux de corticostérone comme indicateurs de la disponibilité alimentaire et de l'état des réserves énergétiques des oiseaux en début de reproduction d'une part, et d'autre part les niveaux d'hormones sexuelles (testostérone chez les mâles, œstradiol chez les femelles) comme indicateurs de la phénologie du début de la reproduction (Williams, 1992 ; Davis *et al.* 1995). Ces mesures pourraient également être associées à des mesures de performances et du succès de reproduction, et mises en relation avec les conditions environnementales telle que l'étendue de la glace de mer au début de la saison de reproduction. Ce sont les objectifs d'une étude préliminaire commencée dans le cadre de la thèse (Etude 8) et présentée ci-dessous.

## Perspective 5 : Hormones et phénologie dans un environnement changeant

### Hormonal correlates of phenology in Adélie penguins breeding in a changing environment

Anne-Mathilde Thierry, Yan Ropert-Coudert, Thierry Raclot

Au cours de six saisons de reproduction consécutives, nous avons suivi la reproduction de couples de manchots Adélie pour lesquels une prise de sang a également été réalisée. La figure 1 montre les variations de la date de départ en mer des femelles après la ponte, utilisée comme un indice de la date de ponte (Ainley, 2002) pour ces différentes années. La date précise d'arrivée de ces femelles sur le site de reproduction n'est pas connue, mais il est possible d'estimer une date moyenne d'arrivée d'après les dates d'arrivée des premiers manchots et d'après le pic d'arrivée sur les colonies.

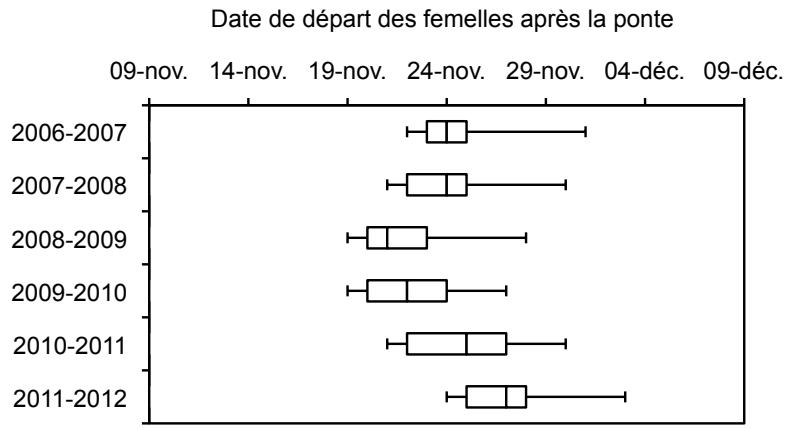


**Figure 5-1 :** Date de départ des femelles après la ponte pendant six saisons de reproduction consécutives.

La détermination des niveaux de différentes hormones, telles que la corticostérone et les hormones sexuelles stéroïdiennes, permettra de tester l'existence de corrélations entre phénologie et statut endocrinien. Différents paramètres environnementaux pourront être considérés, dont l'étendue de la glace de mer en début du cycle de reproduction. Les résultats préliminaires de cette étude sont présentés dans le tableau suivant. La littérature et les résultats des études précédentes permettent de prédire une corrélation positive entre date de ponte et niveaux d'hormones sexuelles stéroïdiennes.

**Tableau 5-4 :** Comparaisons de différentes études s'intéressant aux conséquences de variations interannuelles des conditions environnementales sur la phénologie et le statut endocrinien de manchots Adélie mâles et femelles

| **Etude** | Thierry *et al.* (in prep.) Etude 8 | Ninnes *et al.* 2011 | Beaulieu *et al.* 2010a |
|---|---|---|---|
| Site d'étude | Dumont d'Urville | Ross Island* | Dumont d'Urville |
| Masse corporelle en début de saison de reproduction | ↘ (pour une même date) | NE | = |
| Nombre d'œufs pondus | = | NE | = |
| Date de ponte | ↗ | ↗ | = |
| Date d'éclosion | ↗ | NE | = |
| Niveaux de **corticostérone** (mâles et femelles) | *A déterminer* | ↗ | = |
| Niveaux de **testostérone** (mâles) | *A déterminer* | ↘ | NE |
| Niveaux d'œstradiol (femelles) | *A déterminer* | ↘ | NE |

= : Pas de différence entre les deux étés étudiés. NE : Non Evalué.
*Deux colonies distantes de 80 km

- Cette étude pourra être complétée par la comparaison du statut endocrinien des oiseaux avec les performances de reproduction, à travers la mesure de différents paramètres tels que la durée d'incubation, le nombre de poussins à l'éclosion et à l'envol.
- Il conviendra également de préciser les paramètres environnementaux à considérer, tels que l'étendue et la concentration de la glace de mer à différentes périodes ou encore les conditions météorologiques sur le site de reproduction.

### 5.3.6 D'autres marqueurs physiologiques des effets de conditions environnementales changeantes sur les organismes ?

Les relations dose, état et contexte dépendants entre niveaux de corticostérone, performances et succès reproducteur limitent la généralisation de l'utilisation des niveaux de glucocorticoïdes comme marqueur unique des effets de changements des conditions environnementales. Ceci est d'autant plus vrai qu'une lacune majeure dans le domaine de la physiologie du stress est le fait que l'on utilise seulement les niveaux

totaux de glucocorticoïdes comme un indicateur d'événements stressants et/ou de stress chronique (voir perspective 1).

Des études, initialement conduites chez l'homme (McIntosh & Sapolsky, 1996), ont permis de mettre en évidence un lien entre la sécrétion de glucocorticoïdes et le stress oxydant (Costantini *et al.* 2011). Le stress oxydant est défini comme un état de déséquilibre entre la production de radicaux chimiques dérivés de l'oxygène *(Reactive Oxydative Species, ROS)* qui peuvent causer des dommages sur les molécules biologiques et la capacité antioxydante de l'organisme (Finkel & Holbrook, 2000). Une possibilité pourrait donc être de mesurer la capacité antioxydante et les dégâts oxydatifs d'un organisme en fonction des conditions environnementales auxquelles il fait face. Par exemple, une étude récente propose d'utiliser ce type de mesure en biologie de la conservation puisque les niveaux de stress oxydant sont corrélés avec des marqueurs de la dynamique de populations de manchots Adélie et papous (Beaulieu *et al.* 2013, présenté en Annexe 2) :

**Perspective 6 : Dynamique de population et stress oxydant**

**Integrating oxidative ecology into conservation physiology**

Michaël Beaulieu, Anne-Mathilde Thierry, Daniel González-Acuña, Michael J. Polito

**Abstract.** Ecologists have recently shown great interest in using physiological markers as indicators of the health of animal populations. In this context, the measurement of markers of oxidative balance, such as antioxidant defences and oxidative damage, may be a valuable tool. Indeed, at the individual level, antioxidant defences are positively associated with fertility and survival probability, while elevated oxidative damage during reproduction or growth may negatively affect recruitment and survival. Therefore, variation in oxidative balance is likely to influence demographic processes. This suggests that conservationists may be able to use oxidative markers to monitor population health. Yet, the connection between these markers and demographic parameters first needs to be established. We present here preliminary results obtained in colonies of breeding Gentoo *(Pygoscelis papua)* and Adélie penguins *(Pygoscelis adeliae)*, showing that antioxidant defences strongly reflect population trends. However, population trend was not related to oxidative damage. This suggests that in the context of the emerging field of conservation physiology, antioxidant defences may represent a key parameter to monitor population health. We therefore exhort other research teams to assess the generality of this finding in other biological models, especially in species of conservation concern.

Il serait intéressant de confirmer les résultats de cette étude en s'intéressant à d'autres sites de reproduction, d'autres espèces et/ou d'autres étapes de la vie des animaux, en mesurant les niveaux de corticostérone, la capacité antioxydante et les dégâts oxydatifs pour de mêmes individus. En particulier, les poussins sont très

sensibles à une diminution de l'approvisionnement alimentaire par les parents au cours de la croissance. Il paraît donc intéressant de mesurer les effets de mauvaises conditions environnementales sur la physiologie de poussins au cours de la croissance, comme un indice de l'effort reproducteur des adultes. Une autre étude réalisée pendant la thèse s'est ainsi intéressée aux effets d'une étendue inhabituelle de glace de mer sur la survie, la croissance et la physiologie de poussins de manchots Adélie (Etude 9, présentée en Annexe 3).

**Perspective 7 : Physiologie, croissance et survie de poussins dans un environnement changeant**

**Harsh environmental conditions stress the importance of good physiological parameters to survive in Adélie penguin chicks**

Anne-Mathilde Thierry, Françoise Amélineau, Mathieu Boureau,
Barbara Class, François Criscuolo, Thierry Raclot

**Abstract.** Early-life environmental conditions can have detrimental effects on chick growth and survival, as well as long-term consequences on future key life-history components. But the mechanisms by which compensatory growth may affect adult phenotype are only starting to emerge, suggesting a role for biological markers of ageing such as oxidative stress and telomere erosion. The objectives of this study were to test the effects of contrasting environmental conditions on the early-life of Adélie penguin chicks. To do this, survival, body condition, plasmatic parameters, oxidative damage, and telomere length were determined. We also assessed whether a particular phenotype was associated with a higher survival rate. Our study highlights marked differences in Adélie penguin chick growth depending on environmental conditions, with chick mass gain and growth accelerated in 2012 when compared to 2011. This compensatory growth was associated with changes in physiological parameters, including increased DNA oxidative damage. Interestingly, there were no differences either in corticosterone levels or in telomere erosion rates, indicating differential effects of environmental changes on physiological parameters.

# Références

## Références


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# Annexes

## Annexe 1 : Liste complète des articles publiés

1. Beaulieu M, **Thierry AM**, Le Maho Y, Ropert-Coudert Y, Ancel A. 2009. Alloparental feeding in Adélie penguins: why is it uncommon? Journal of Ornithology. 150: 637-643

2. Beaulieu M, **Thierry AM**, Raclot T, Le Maho Y, Ropert-Coudert Y, Gachot-Neveu H, Ancel A. 2009. Sex-specific parental strategies according to the sex of offspring in the Adélie penguin. Behavioral Ecology. 20: 878-883

3. Beaulieu M, Dervaux A, **Thierry AM**, Lazin D, Le Maho Y, Ropert-Coudert Y, Spée M, Raclot T, Ancel A. 2010. When sea-ice clock is ahead of Adélie penguins' clock. Functional Ecology. 24(1): 93-102

4. Beaulieu M, **Thierry AM**, Handrich Y, Le Maho Y, Massemin S, Ancel A. 2010. Adverse effects of instrumentation in incubating Adélie penguins. Polar Biology. 33(4): 485-492

5. Spée M, Marchal L, **Thierry AM**, Chastel O, Enstipp M, Le Maho Y, Beaulieu M, Raclot T. 2011. Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins *(Pygoscelis adeliae)*. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 300(5): R1241-1249

6. Cottin M, Kato A, **Thierry AM**, Le Maho Y, Raclot T, Ropert-Coudert Y. 2011. How does corticosterone affect diving behaviour of male Adélie Penguins? A preliminary experimental study. Ornithological Science 10(1): 3-11

7. Legrand M, Gros V, Preunkert S, Sarda-Estève R, **Thierry AM**, Pépy G, Jourdain B. 2012. A reassessment of the budget of formic and acetic acids in the boundary layer at Dumont d'Urville (coastal Antarctica): The role of penguin emissions on the budget of several oxygenated volatile organic compounds. Journal of Geophysical Research 117(D6)

8. **Thierry AM**, Massemin S, Handrich Y, Raclot T. 2013. Elevated corticosterone levels and severe weather conditions decrease parental investment of incubating Adélie penguins. Hormones and Behavior 63(3): 475-483

9. McCafferty DJ, Gilbert C, **Thierry AM**, Currie JI, Le Maho Y, Ancel A. 2013. Temperature variation across Emperor penguin body surfaces. Biology Letters 9(3) DOI: 10.1098/rsbl.2012.1192

10. Beaulieu M, **Thierry AM**, González-Acuña D, Polito MJ. 2013. Integrating oxidative ecology into conservation physiology. Conservation Physiology 1(1) DOI: 10.1093/conphys/cot004

11. **Thierry AM**, Ropert-Coudert Y, Raclot T. 2013. Elevated corticosterone levels decrease reproductive output but do not affect chick mass at fledging in chick-rearing Adélie penguins. Conservation Physiology, 1(1) DOI: 10.1093/conphys/cot007

12. **Thierry AM**, Brajon S, Massemin S, Handrich Y, Chastel O, Raclot T. 2013. Decreased prolactin levels reduce parental commitment, egg temperatures, and breeding success of incubating male Adélie penguins. Hormones and Behavior. In press

Perspective

# Integrating oxidative ecology into conservation physiology

**Michaël Beaulieu[1,*], Anne-Mathilde Thierry[2], Daniel González-Acuña[3] and Michael J. Polito[4]**

[1]Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstraße 1, 79104 Freiburg, Germany
[2]Université de Strasbourg, IPHC, 23 rue Becquerel, 67087 Strasbourg, France
[3]Facultad de Ciencias Veterinarias, Universidad de Concepción, Casilla 537, Chillán, Chile
[4]Woods Hole Oceanographic Institution, 266 Woods Hole Road MS 50, Woods Hole, MA 02543, USA;

***Corresponding author**: Faculty of Biology, Department of Evolutionary Biology and Animal Ecology, University of Freiburg, Hauptstraße 1, 79104 Freiburg, Germany. Email: miklvet@hotmail.fr

**Ecologists have recently shown great interest in using physiological markers as indicators of the health of animal populations. In this context, the measurement of markers of oxidative balance, such as antioxidant defences and oxidative damage, may be a valuable tool. Indeed, at the individual level, antioxidant defences are positively associated with fertility and survival probability, while elevated oxidative damage during reproduction or growth may negatively affect recruitment and survival. Therefore, variation in oxidative balance is likely to influence demographic processes. This suggests that conservationists may be able to use oxidative markers to monitor population health. Yet, the connection between these markers and demographic parameters first needs to be established. We present here preliminary results obtained in colonies of breeding Gentoo (*Pygoscelis papua*) and Adélie penguins (*Pygoscelis adeliae*), showing that antioxidant defences strongly reflect population trends. However, population trend was not related to oxidative damage. This suggests that in the context of the emerging field of conservation physiology, antioxidant defences may represent a key parameter to monitor population health. We therefore exhort other research teams to assess the generality of this finding in other biological models, especially in species of conservation concern.**

**Key words:** Antarctica, demography, oxidative balance, penguins, population decline, seabirds

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## Why may markers of oxidative balance be worth examining in the context of conservation physiology?

Conservationists need accurate tools to evaluate the health of animal populations in their natural habitat. Towards this end, they have recently started to use physiological markers as indicators of population health (Wikelski and Cooke, 2006). So far, most research in conservation physiology has concentrated on endocrine and immunological parameters in response to environmental perturbations (Stevenson *et al.*, 2005). In comparison to endocrine and immunological ecology, oxidative ecology is a relatively recent research field, and has not yet been considered with respect to conservation issues. Yet, the measurement of oxidative markers may be valuable in view of the ubiquity of oxidative processes in biological systems and of their effects on individuals' fitness.

Excesses of reactive oxygen species (ROS) relative to antioxidant defences result in the production of oxidative damage [because reactive nitrogen species are thought not to be as damaging as ROS (Monaghan *et al.*, 2009), we will not consider them in the present manuscript]. For instance, this may happen

when organisms experience acute or prolonged physical activity (Costantini *et al.*, 2008; Fletcher *et al.*, 2012), when they reproduce (Alonso-Álvarez *et al.*, 2010; Christe *et al.*, 2012; Van de Crommenacker *et al.*, 2012), or when they are exposed to anthropogenic pro-oxidative agents (Bonisoli-Alquati *et al.*, 2010; Koivula and Eeva, 2010). High levels of oxidative damage can lead to accelerated cell ageing, thereby decreasing fertility and survival probability (Haenold *et al.*, 2005; Monaghan *et al.*, 2009). Accordingly, individuals with enhanced antioxidant defences or reduced oxidative damage have better fertility and survival (Bize *et al.*, 2008; Freeman-Gallant *et al.*, 2011; Saino *et al.*, 2011; Losdat *et al.*, 2012; Noguera *et al.*, 2012). Moreover, the intrinsic oxidative balance of organisms interacts with the oxidative constraints (e.g. radiation, temperature, and prey availability) imposed by environmental conditions (Beaulieu *et al.*, 2010). Such interaction may lead populations with low antioxidant defences to decrease when faced with pro-oxidative environmental conditions (Møller and Mousseau, 2007). This raises the hypothesis that oxidative balance and demographic processes may be interrelated, and that oxidative markers, reflecting demographic trends, may be used by conservationists as indicators of population health. Yet, the connection between oxidative balance and demographic parameters still needs to be established.

It may be initially assumed that increased levels of physiological markers classically associated with deteriorated fitness parameters at the individual level would reflect poor population health. However, such an assumption does not necessarily hold true. For instance, even if increased corticosterone levels predict decreased reproductive performance in common murres (*Uria lumvia*), corticosterone levels do not differ significantly between increasing and declining colonies, even when population trends differ drastically (Kitaysky *et al.*, 2007). Consequently, even if corticosterone levels may act on fitness components, their measurement appears inappropriate to estimate the health of populations of common murres. This example emphasizes the necessity to assess the relationship between physiological markers and demographic indices directly, before being able to use them as indicators of population health. The examination of oxidative balance in populations of known contrasting trends may therefore be a useful first step to assess the validity of the relationship between oxidative balance and population health.

## Examination of oxidative balance in populations of *Pygoscelis* penguins with contrasting population trends

According to the International Union for Conservation of Nature (IUCN), two of the three currently existing *Pygoscelis* species, namely the Gentoo penguin (*Pygoscelis papua*) and the Adélie penguin (*Pygoscelis adeliae*), are near threatened (IUCN, 2012). However, their status varies greatly between populations, which show contrasting demographic trends depending both on species and region. Indeed, over the few last decades, populations of Gentoo penguins have been stable or increasing around the Antarctic Peninsula, while populations of Adélie penguins have been decreasing (Hinke *et al.*, 2007). Conversely, in other regions of Antarctica, populations of Adélie penguins have been increasing (Table 1). The high dependence of Adélie penguins on decreasing stocks of Antarctic krill (*Euphausia superba*) around the Antarctic Peninsula is likely to be responsible for their decline in this region (Trivelpiece *et al.*, 1987; Hinke *et al.*, 2007). Compared with other populations, Adélie penguins from the Antarctic Peninsula may therefore have to intensify their foraging effort to feed. This may result in lower antioxidant defences and higher oxidative damage, as observed in birds increasing their flying effort (Costantini *et al.*, 2008). Moreover, they may be limited in their ability to invest in antioxidant defences, because their feeding requirements may not be entirely satisfied, and their endogenous resources may be limited. Finally, low consumption of krill, rich in antioxidants (Tou *et al.*, 2007), may result in low antioxidant defences and high oxidative damage (Beaulieu *et al.*, 2010). These potential changes in oxidative balance may contribute to explaining the negative impact of krill depletion on demographic parameters in Adélie penguins (Nicol *et al.*, 2008; Tierney *et al.*, 2009; Trivelpiece *et al.*, 2011).

As Adélie penguins from the Antarctic Peninsula exhibit decreasing population trends, they are expected to have lower antioxidant capacity and higher oxidative damage than (i) Gentoo penguins from the same region, and (ii) Adélie penguins from other Antarctic regions. In order to assess this prediction, we examined the oxidative balance of breeding Gentoo and Adélie penguins from three different regions of the Antarctic Peninsula (Ardley Island, Gabriel González Videla, and Admiralty Bay) and from Adélie Land (Dumont d'Urville). For each colony, the published results of long-term population-monitoring studies provided us with mean annual population changes (Table 1). Oxidative balance was measured in plasma samples collected during the chick-rearing period of the austral summer 2010–2011, by using the OXY-adsorbent tests (Diacron International, Grosseto, Italy), which measures total antioxidant capacity, and the d-ROM test (Diacron International, Grosseto, Italy), which measures hydroperoxide, resulting from the attack of ROS on organic substrates (and therefore reflecting oxidative damage; for details on the procedure, see Beaulieu *et al.*, 2010).

In agreement with our initial hypothesis, penguins from increasing populations had higher antioxidant capacity than penguins from decreasing populations (Fig. 1). This was true when considering each penguin colony independently (Table 2, Model 1; Fig. 2) or irrespective of species, location (Table 2, Model 2), and levels of oxidative damage (Table 2, Model 3). This positive relationship between antioxidant capacity and population trend may be related to the adaptive advantages conferred by higher antioxidant defences, such as enhanced fertility and survival, as already observed in other bird species (Bize *et al.*, 2008; Saino *et al.*, 2011; Losdat *et al.*, 2012). Moreover, penguins from declining populations may be unable to keep antioxidant defences as high as

**Table 1:** Average annual population changes of Gentoo and Adélie penguins from the Antarctic Peninsula (Ardley Island, Gabriel González Videla, and Admiralty Bay) and Adélie Land (Dumont D'Urville)

| Species | Population | Coordinates | Annual population change (%) | No. of samples |
|---|---|---|---|---|
| Gentoo | Ardley Island | 62°13′S, 58°54′W | +4.7 (1973–2005)[a] | 14 |
| Gentoo | Gabriel González Videla | 64°49′S, 62°52′W | +3.8 (1986–1997)[b] | 25 |
| Adélie | Dumont d'Urville | 66°40′S, 140°01′E | +1.8 (1984–2003)[c] | 23 |
| Gentoo | Admiralty Bay | 62°11′S, 58°26′W | 0 (1977–2005)[d] | 20 |
| Adélie | Admiralty Bay | 62°11′S, 58°26′W | −2.3 (1979–2009)[e] | 22 |

The time scale during which population changes were calculated is indicated in parentheses. The population trend of Gentoo penguins from Gabriel González Videla was approximated from that of the 10 km distant colony on Goudier Island (Cobley and Shears, 1999). The co-ordinates and the number of penguins from each population included in our study are also indicated. [a]Antarctic Treaty Consultative Meeting (2010); [b]Cobley and Shears (1999); [c]Jenouvrier *et al.* (2005); [d]Hinke *et al.* (2007); and [e]Trivelpiece *et al.* (2011).

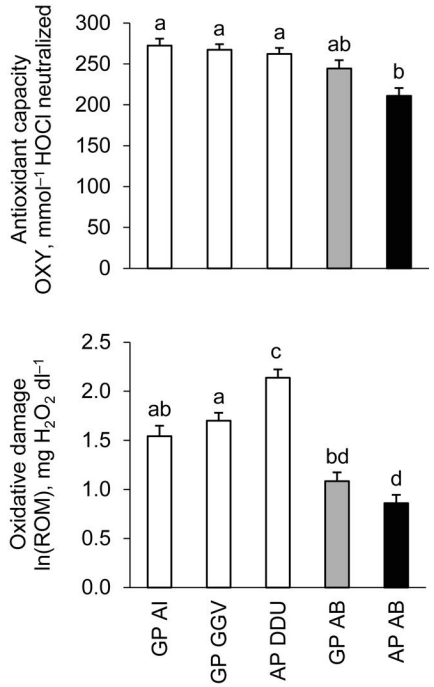


**Figure 1:** Plasma antioxidant capacity and oxidative damage in Gentoo (GP) and Adélie (AP) penguins from Ardley Island (AI), Gabriel González Videla (GGV), Dumont d'Urville (DDU), and Admiralty Bay (AB). Different letters indicate significant differences between colonies, as calculated by Bonferonni tests following Model 1 (Table 2). Increasing colonies are represented in white, stable in grey, and decreasing in black. Results are presented as means ± SEM.

**Table 2:** Results of the statistical models used to assess the relationship between plasma oxidative balance and annual population change in *Pygoscelis* penguins

| Model | OXY | d-ROM |
|---|---|---|
| Model 1: GLM | | |
| Fixed factor: colony | $F_{4,99} = 14.94$, $P < 0.001$ | $F_{4,99} = 35.68$, $P < 0.001$ |
| Model 2: GLMM | | |
| Covariate: annual population change | $F_{1,3} = 26.84$, $P = 0.012$ | $F_{1,3} = 2.24$, $P = 0.23$ |
| Model 3: GLMM | | |
| Covariate: annual population change | $F_{1,3} = 28.92$, $P = 0.009$ | $F_{1,3} = 1.74$, $P = 0.28$ |
| Covariate: d-ROM | $F_{1,17} = 2.82$, $P = 0.11$ | — |
| Covariate: OXY | — | $F_{1,98} = 1.84$, $P = 0.18$ |

Abbreviations: d-ROM, oxidative damage; and OXY, antioxidant capacity. The d-ROM data were ln transformed for normality. We checked for potential effects of the date of sampling and the sex of penguins on oxidative balance but did not find any (all $P > 0.13$). Consequently, we did not include these parameters in final models. In Model 1 (general linear model; GLM), we compared oxidative balance between the five considered colonies (Table 1). in order to assess if, irrespective of location and species, annual oxidative balance was related to the population trend of *Pygoscelis* penguins, we carried out Model 2 (general linear mixed model; GLMM), with OXY or d-ROM as dependent factors and annual population change as covariate. In this model, species was nested in location (as both Gentoo and Adélie penguins were sampled at Admiralty Bay) and used as a random factor. Finally, in Model 3, we repeated Model 2 but with d-ROM or OXY as covariate. Analyses were performed in SPSS 17.00 (SPSS Inc., Chicago, IL, USA).

penguins from increasing colonies, because they are energetically limited and cannot invest effectively in antioxidant defences (Fletcher *et al.*, in press).

Given that low oxidative damage may confer the same advantages in terms of fertility and survival as high antioxidant capacity (Helfenstein *et al.*, 2010; Freeman-Gallant *et al.*, 2011; Noguera *et al.*, 2012), lower levels of oxidative damage were also expected in increasing populations. However, even though oxidative damage also differed between colonies (Table 2, Model 1; Fig. 1), there was no general trend between oxidative damage and population trend (Fig. 2). This absence of relationship was confirmed when the species, the location, or the antioxidant capacity were taken into account in statistical analyses (Table 2, Models 2 and 3). The absence of relationship between oxidative damage and population trend may come from the fact

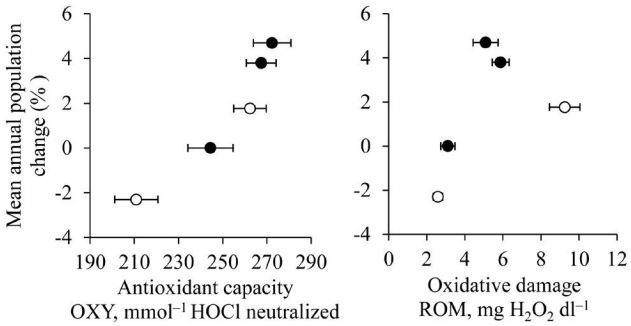


**Figure 2:** Relationships between mean annual population change and antioxidant capacity (means ± SEM, left), and between mean annual population change and oxidative damage (means ± SEM, right) in populations of Gentoo (filled circles) and Adélie penguins (open circles).

that we sampled breeding individuals. Indeed, low levels of oxidative damage, expected in increasing populations, may be counterbalanced by elevated oxidative damage related to high investment in reproduction (Fletcher *et al.*, in press; Stier *et al.*, 2012), which is also expected in increasing populations, hence hiding any visible relationship between oxidative damage and population trend. In this context, it would be theoretically interesting to examine whether a negative relationship between oxidative damage and population trend is observed in penguins outside of the breeding season [which may be practically difficult because (i) birds are generally not present on the colony at that time, and (ii) population trend is generally calculated during breeding].

## Future directions and potential applications to conservation

The aim of this article is to stimulate conservation ecologists to integrate markers of oxidative balance into the array of physiological parameters used to monitor the health of animal populations in their natural habitat (Wikelski and Cooke, 2006). Recently published articles have provided solid evidence that variation in oxidative balance of free-ranging animals affects fitness components (Bize *et al.*, 2008; Freeman-Gallant *et al.*, 2011; Saino *et al.*, 2011; Losdat *et al.*, 2012; Noguera *et al.*, 2012). Here, by measuring the oxidative balance of *Pygoscelis* penguins from colonies with contrasting demographic trends, we show that variation in oxidative balance may be related to demographic processes, because we found a strong association between plasma antioxidant defences and population trends. These results suggest that antioxidant defences could be used by conservationists as an indicator to estimate the health of populations of unknown demographic trend. For instance, Gentoo penguins from O'Higgins Base (Antarctic Peninsula, 69°19′S, 57°53′W) have an antioxidant capacity of 253.1 ± 8.0 mmol$^{-1}$ HOCl neutralized ($n$ = 17, M. Beaulieu, personal data), but their population trend is unknown. Based on the results of our study (Fig. 2), this population is likely to be stable or slightly increasing.

As oxidative balance potentially shapes life-history trade-offs (Monaghan *et al.*, 2009), animal species are likely to modulate their oxidative balance with respect to their own life-history traits. For instance, experimentally increased breeding constraints result in opposite effects on the antioxidant defenses of Adélie penguins and zebra finches (*Taeniopygia guttata*), which show increased and decreased antioxidant capacity, respectively (Alonso-Álvarez *et al.*, 2004; Wiersma *et al.*, 2004; Beaulieu *et al.*, 2011). This discrepancy may be explained by the fact that Adélie penguins are long lived and favour self-maintenance, while zebra finches are short lived and favour current reproduction at the expense of self-maintenance (Stearns, 1989). It is therefore possible that a study examining the relationship between oxidative balance and population trend in a short-lived species would show opposite results to those obtained in long-lived *Pygoscelis* penguins. In that case, increasing populations of zebra finches would have lower antioxidant defences during reproduction than declining populations. This example also emphasizes the importance of considering the life stage when measuring oxidative balance to assess population health. Indeed, because the regulation of oxidative balance must be critical in terms of fitness only during oxidatively challenging conditions, oxidative markers may reflect population health only during life stages associated with higher vulnerability to oxidative damage (e.g. growth, reproduction, and ageing) or in oxidatively challenging environmental conditions (e.g. radioactive, hot environment). It is therefore possible that the relationship between population trend and antioxidant capacity that we observed in breeding *Pygoscelis* penguins would disappear in non-breeding penguins. In contrast, a negative relationship between oxidative damage and population trend may be apparent only outside the breeding season (see above). These examples emphasize the necessity to conduct further studies examining the relationship between oxidative balance and population trends in various conditions, for conservationists to use oxidative markers as indicators of population health. We therefore urge other research teams to explore this relationship further (i) in biological systems with different life-history traits, (ii) during different life stages, and (iii) in variable environmental conditions.

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## Annexe 3 : Physiologie, croissance et survie des poussins de manchot Adélie dans un environnement changeant

### Harsh environmental conditions stress the importance of good physiological parameters to survive in Adélie penguin chicks

Anne-Mathilde Thierry, Françoise Amélineau, Mathieu Boureau,
Barbara Class, François Criscuolo, Thierry Raclot

**Abstract**

Early-life environmental conditions can have detrimental effects on chick growth and survival, as well as long-term consequences on future key life-history components. But the mechanisms by which compensatory growth may affect adult phenotype are only starting to emerge, suggesting a role for biological markers of ageing such as oxidative stress and telomere erosion. The objectives of this study were to test the effects of contrasting environmental conditions on the early-life of Adélie penguin chicks. To do this, survival, body condition, plasmatic parameters, oxidative damage, and telomere length were determined. We also assessed whether a particular phenotype was associated with a higher survival rate. Our study highlights marked differences in Adélie penguin chick growth depending on environmental conditions, with chick mass gain and growth accelerated in 2012 when compared to 2011. This compensatory growth was associated with changes in physiological parameters, including increased DNA oxidative damage. Interestingly, there were no differences either in corticosterone levels or in telomere erosion rates, indicating differential effects of environmental changes on physiological parameters.

**Keywords:** compensatory growth, oxidative stress, *Pygoscelis adeliae*, sea-ice extent, telomere length

## Introduction

Natural selection is expected to maximise fitness, which for iteroparous organisms includes the contributions of both current and expected future reproduction. Long-lived animals should particularly behave as "prudent parents" (Drent & Daan, 1980) and avoid increased risks of mortality during reproduction because their lifetime reproductive success is mainly a factor of adult survival (Williams, 1966). When breeding under conditions of limited food availability, animals may reduce offspring provisioning to avoid abandonment of the breeding effort, with negative consequences on offspring growth rates. Early-life nutritional restrictions are often followed by compensatory growth, involving prolongation of the growth period and/or growth acceleration (Metcalfe & Monaghan, 2001) to attain normal adult size. Such compensatory growth, which is known to occur in the wild (Bize *et al.*, 2006; Bjorndal *et al.*, 2003; Geiger *et al.*, 2012; Johnsson & Bohlin, 2005), is associated with both physiological and life-history costs (Metcalfe & Monaghan, 2001, 2003; Monaghan, 2008). While it is now widely accepted that early life conditions and growth can have long-term consequences, the mechanisms by which compensatory growth may affect adult phenotype are only starting to emerge (Lindström, 1999; Metcalfe & Monaghan, 2001; Tarry-Adkins & Ozanne, 2011). In particular, poor development conditions have been related to accelerated ageing through increased oxidative stress and telomere erosion in laboratory rats (Tarry-Adkins *et al.*, 2013). Telomeres are highly conserved sequences at the end of chromosomes (Blackburn, 1991). Their erosion with time is one of the main mechanisms explaining cell senescence (Monaghan & Haussmann, 2006). Physiological stress, through the activation of the hypothalamic-pituitary-adrenal axis and consequent glucocorticoid release, may also accelerate the ageing process (Haussmann & Marchetto, 2010). While experiments carried out under controlled environmental conditions can help understanding the proximate mechanisms underlying the growth-ageing relationship, studies examining the effects of a bad early-life conditions on physiological parameters during growth in a naturally fluctuating environment remain scarce. To our knowledge, only one study has showed that telomere shortening was related to different growth trajectories in wild king penguin *(Aptenodytes patagonicus)* chicks; small chicks which grew faster displaying increased oxidative damage and accelerated telomere loss (Geiger *et al.*, 2012). Adélie penguin *(Pygoscelis adeliae)* is widely distributed in Antarctica and its obligate association with pack ice makes it an interesting species to monitor the ecological consequences of environmental changes on bird physiology and survival in Polar Regions. The objectives of this study were to describe the effects of contrasting environmental conditions on the early-life of Adélie penguin chicks, in terms of body mass and size, metabolites, stress hormone (corticosterone), antioxidant defences, oxidative damage, and telomere length. We also assessed whether a particular phenotype was associated with a higher survival rate.

## Methods

*FIELD WORK*

This study was carried out during two consecutive austral summers with constrasting sea-ice conditions (Fig. 1) at Dumont d'Urville in Antarctica (66°40'S, 140°01'E), between December and February of 2010-2011 and 2011-2012. The protocol was approved by an independent ethics committee commissioned by the French Polar Institute and authorised by the French Southern and Antarctic Lands (decrees 2010-67,

2010-79, 2011-99, and 2011-107).

20 nests in 2010-2011 and 90 nests in 2011-2012 were monitored several times a day from the courtship period. 24 chicks hatched on 15 nests and 73 on 49 nests in December of 2010 and 2011, respectively. The number of chicks on these nests was monitored once a day from hatching until the end of the chick-rearing period. Chicks were sampled, weighed and measured 5, 20, and 42 days after hatching. In 2011-2012, chicks that died were also measured and sampled up to a few hours after death but before freezing or being eaten by predators. Blood was sampled from a foot vein with Na-heparin capillaries when chicks were 5-days old, and with capillaries or a heparinised 1mL-syringe with a 25G needle when chicks were older. On that occasion, chicks were weighed with an electronic balance (± 2 g, Ohaus, Giessen, Germany). The length of the left flipper was measured to the nearest mm, as well as the length of the head and bill to the nearest 0.2 mm. Blood samples were centrifuged (5000 rpm, 10 min, 4 °C) and frozen at -20 °C until analyses were carried out. Chicks were also weighed with a spring balance (± 50g, Pesola AG, Baar, Switzerland) and measured at the beginning of the crèche stage, when they were 15 to 26 days old. They were individually identified with coloured fishtags during the first capture following that of five days after hatching (TBA standard anchor t-bag tag, Hallprint, Hindmarsh Valley, SA, Australia). Fishtags were removed at the last chick capture.

*LABORATORY ANALYSES*

*Sexing:*

Adélie penguin chicks were sexed using genomic DNA extracted from red blood cells using DNeasy Blood & Tissue kit (Qiagen), following a protocol adapted from Kahn *et al.* (1998) and Ellegren (1996). Genomic DNA quality and concentrations were assessed using a Nanodrop spectrophotometer (Thermo Scientific, France). Polymerase chain reaction (PCR)–based sexing was performed using the 1237L/1272H primer pair (Kahn *et al.*, 1998). PCR amplifications were carried out in 25 µL volumes, containing 100 ng of genomic DNA, 0.9 units of Taq DNA polymerase (MBI Fermentas), 200 µM of each dNTPs, 0.22 µM of each primers, 1.75 mM MgCl2 (MBI Fermentas) and 1X PCR reaction buffer (750 mM Tris-HCl pH 8.8, 200mM $(NH_4)_2SO_4$, 0.1 % Tween 20; MBI Fermentas). Reactions were performed in a Master Cycler Gradient (Eppendorf) under following thermal conditions: 2 min at 94 °C; 10 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 35 s; 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 35 s; and 5 min at 72 °C before holding at 4 °C. After amplification, PCR products were separated in 2 % agarose (agarose DNA, grade-type D5, Euromedex) gels containing 0.5 µg/mL ethidium bromide in 90 mM Tris-borate/1mM EDTA (TBE, Euromedex) at 90V for 10 min and then at 130V for 1 hour. The 100bp DNA Ladder Plus (Euromedex) was used as a molecular size standard.

*Metabolites*

Plasma glucose, triglycerides, and uric acid levels were assayed using enzymatic colorimetric tests according to guidelines provided by the manufacturer (GL 364, TR 210, and UA 1613, respectively, RANDOX Laboratories Ltd., Crumlin, UK). Intra- and inter-assay variations were 3.9 ± 0.6 and 4.4 % for glucose, 5.9 ± 0.4 and 9.3 % for triglycerides, and 6.2 ± 1.3 and 7.4 % for uric acid, respectively.

*Corticosterone*

Plasma corticosterone levels were determined in duplicate by immunoassay (Corticosterone EIA Kit, Cayman Chemical Company, Ann Harbor, MI, USA). Plasma samples were diluted 1:10 into EIA diluent before assay. Intra- and inter-assay variations were 5.6 ± 0.8 and 11.4 %, respectively.

*Oxidative status*

Plasma concentrations of non-enzymatic antioxidant capacity (OXY-Adsorbent) were assayed in duplicates (Diacron International, Italy) as previously described in penguins (Beaulieu *et al.* 2010, see Costantini *et al.* 2006 for a detailed description of the methodology). Intra- and inter-assay variations were 7.1 ± 0.5 and 13.3 %, respectively. Oxidative damage on the DNA was assessed with a competitive ELISA kit for the quantification of 8-oxo-d-Guanosine (DNA damage, 8-OH-dG StressXpress® EIA Kit, SK-120-96, StressMarq Biosciences Inc., Victoria, BC, Canada). 1 µg of DNA was totally digested as described in Quinlivan & Gregory (2008), with 2.5 U Benzonase nuclease (# E1014, Sigma-Adlrich), 2 U Phosphatase alkaline (# P7923, Sigma-Adlrich), and 3mU of Phosphodiesterase I (# P3243, Sigma-Adlrich) using 20 mM Tris-HCl buffer at pH 7.9, and incubated 37 °C during 6 hours. Samples were then stored at -20 °C until analysis. 8-oxo-d-Guanosine ELISA was performed according to the recommendations of the manufacturer. Intra- and inter-assay variations were 9 ± 1.3 and 8.6 %, respectively.

*Telomere length*

Telomere length was measured by qPCR as described by Cawthon *et al.* (2002) and adapted to bird samples by Criscuolo *et al.* (2009). The relative telomere length of Adélie penguins was evaluated by determining the ratio of telomere repeats (T) versus a control (S) gene (Smith *et al.* 2011). Primer sequences for telomere amplification were similar to previous studies (Criscuolo *et al.* 2009). We used the *Pygoscelis adeliae* zinc finger protein (NCBI accession number AF490195) as a single control gene, with forward and reverse primer sequences defined as: (Adel1: 5'-CAACTGCCGTTTAAGTTTTCC-3'; Adel2: 5'-AATATGGCCCTGCAAATTCC-3'). Primer concentrations in the final qPCR mix were 100 mM for telomere length determination and 300 mM for the control gene. Telomere and control gene PCR conditions were 10 min at 95 °C followed by 40 cycles of 15 s at 95 °C, 30 s at 54 °C, 1 min 30 s at 72 °C. Telomere length was measured on a CFX384 real-time PCR detection system (Bio-Rad Laboratories, France). Real-time qPCR amplifications for both telomere and zinc finger protein were performed on the same plate using 5 ng of DNA per reaction. Serial dilutions of a reference sample (8 ng, 1:4 dilutions, 4 points) were repeated on each plate and used to generate a reference curve, in order to control for the amplifying efficiency of the qPCR. Mean amplification efficiency of the qPCR runs were comprised between 99.9 and 100.3 % for telomere and between 99.4 and 100.3 % for the control gene. Intra-plate coefficients of variation for Ct values were 2.2 ± 0.2 % and 1.7 ± 0.2 % for the telomere and control gene assays, respectively. Inter-plate coefficients of variation based on repeated samples were 3.0 and 1.5 %, respectively. To take into account the slight variation of efficiencies between telomere and control gene amplifications, relative telomere lengths were calculated using the method suggested by Pfaffl (2001): $T/S=(1+E_{\text{control gene}})^{\Delta Ct \text{ control gene (golden sample - sample)}}/(1+E_{\text{telomere}})\Delta^{Ct \text{ telomere (golden sample - sample)}}$.

Final telomere values were expressed as the ratio between telomere and the single control gene number of amplification cycles (T/S ratio, Criscuolo *et al.* 2009).

*STATISTICAL ANALYSES*

Statistical analyses were performed with R 2.13.2 (R development core team, 2011).

A Generalised Linear Model (GLM) with a Poisson distribution was used to compare the number of chicks at fledging between years. Generalised Estimating Equations (GEE) were used (*geeglm* function of the *geepack* package) to compare the reproductive output between years with breeding period (laying, before hatching, hatching, guard stage, fledging), and the interaction between year and breeding period as factors, nest as identity, and an AR1 correlation structure. GEEs were also used to compare physiological and growth parameters between chicks surviving and not-surviving, with age, survival status, year, and the interaction between survival status and year as covariate and factors, chick as identity, and an exchangeable correlation structure. GEEs were carried out to evaluate the relationships between physiological or growth parameters in 42 days-old chicks and year, sex, number of chicks per nest, and number of chicks at hatching as factors and covariates, and nest as identity. Models were compared using ANOVA in geepack (Zuur *et al.*, 2009) and QIC (Pan, 2001). Model selection was performed by excluding non-significant interactions first, and then all non-significant factors. The best predictors of chick survival among different variables were defined using a cox regression (*coxph* function of the *survival* package).

**Results**

The reproductive output at fledging was significantly reduced in 2011-2012 (Fig. 1, GLM with a Poisson distribution, $z=-4.07$, $p<0.001$). When considering the whole breeding season, the time and the interaction between time and year significantly predicted the number of eggs or chicks per nest (GEE, time: Wald-$\chi^2=22.83$, $p<0.001$, time x year: Wald-$\chi^2=19.45$, $p<0.001$). Sex ratio was not affected by chick age, year, and the interaction between chick age and year (GLM with a binomial distribution, $p>0.419$). Chick mass, head-bill and flipper length, glucose levels, antioxidant capacity and DNA oxidative damage significantly increased with age (GEE, $p<0.001$ except $p=0.002$ for DNA oxidative damage), while triglycerides and telomere length significantly decreased with age ($p=0.013$ and $p=0.001$, respectively). Chick sex had no effect on morphological and physiological parameters. Morphological and physiological parameters were further compared separately for the different ages (5, 20, and 42 days old, see Fig. 2). The effects of survival, year, and the interaction between survival and year on the different parameters at 5 and 20 days after hatching are presented in Table 1. On the whole, 5 and 20 days old chicks were smaller and lighter during the 2011-2012 summer. This trend was even more marked for chicks, which did not survive.

Chick mass and size did not differ between years 42 days after hatching (mass: $p=0.983$, flipper length: $p=0.173$, head-bill length: $p=0.386$). Amongst surviving chicks, DNA oxidative damage (Wald-$\chi^2=9.98$, $p=0.002$) and uric acid levels (Wald-$\chi^2=4.14$, $p=0.042$) were significantly higher in February 2012. Males had decreased triglycerides (Wald-$\chi^2=8.43$, $p=0.004$) and increased corticosterone levels (Wald-$\chi^2=9.58$, $p=0.002$). All the other measured physiological parameters were not significantly affected by sex, number of chicks per nest, and year.

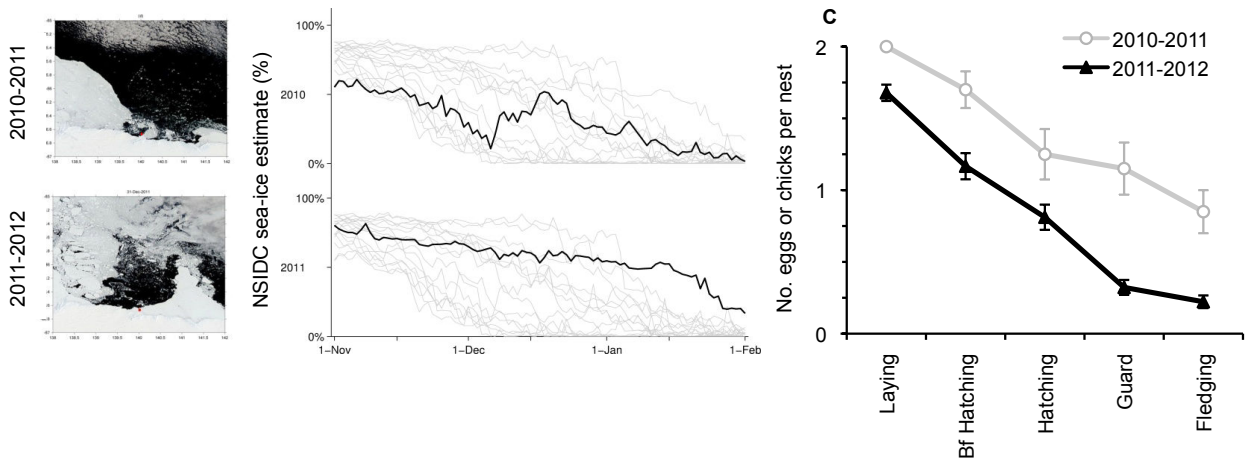


**Figure 1:** Evolution of sea-ice conditions (**A, B**) and reproductive output (**C**) of Adélie penguins over two consecutive austral summers

Chicks growing in January 2012, which died before they were 20 days old, weighed less than the other chicks (p=0.003) and had increased corticosterone levels (p=0.048). During this same year, chicks which did not survive until 42 days after hatching also had increased corticosterone levels (p=0.024) together with shorter telomeres (p<0.001). The best predictors of chick survival between hatching and fledging were chick mass, DNA oxidative damage, and corticosterone levels five days after hatching (Table 2, Cox regression, $R^2$=0.505, p<0.001).

**Table 1:** Effets of year, survival, and the interaction between year and survival on morphological and physiological parameters at 5, 20, and 42 days after hatching using Generalised Estimating Equations

| | Age | Year | Survival | Year x Survival |
|---|---|---|---|---|
| Mass | 5 | 0.664 | 0.647 | - 0.003 |
| | 20 | - <0.001 | - <0.001 | NS |
| | 42 | NS | | |
| Head-bill length | 5 | - 0.019 | - 0.011 | NS |
| | 20 | - <0.001 | - 0.003 | NS |
| | 42 | NS | | |
| Flipper length | 5 | NS | - <0.001 | NS |
| | 20 | - <0.001 | - <0.001 | NS |
| | 42 | NS | | |
| Uric acid | 5 | NS | NS | NS |
| | 20 | NS | NS | NS |
| | 42 | + 0.042 | | |
| Glucose | 5 | + 0.016 | - 0.046 | NS |
| | 20 | NS | NS | NS |
| | 42 | NS | | |
| Triglycerides | 5 | + 0.005 | - 0.020 | NS |
| | 20 | NS | NS | NS |
| | 42 | NS | | |
| Corticosterone | 5 | - 0.010 | 0.493 | + 0.048 |
| | 20 | 0.689 | - <0.001 | + 0.024 |
| | 42 | NS | | |
| Antioxidant capacity | 5 | + 0.006 | NS | NS |
| | 20 | - <0.001 | NS | NS |
| | 42 | NS | | |
| DNA oxidative damage | 5 | NS | + <0.001 | NS |
| | 20 | + 0.039 | NS | NS |
| | 42 | + 0.002 | | |
| Telomere length | 5 | NS | NS | NS |
| | 20 | 0.54 | + <0.001 | - <0.001 |
| | 42 | NS | | |

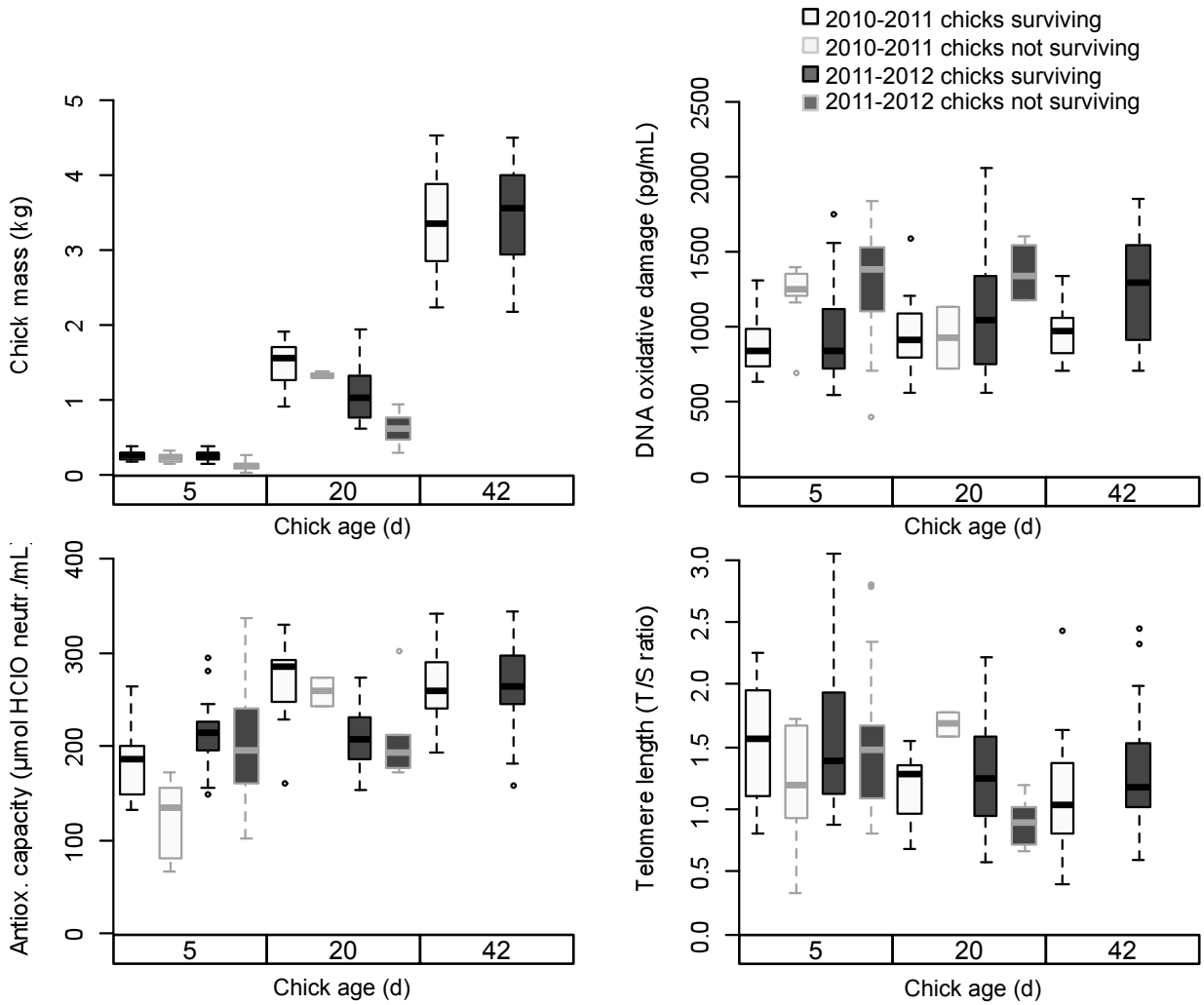


**Figure 2:** Chick body mass, antioxidant capacity, DNA oxidative damage, and telomere length during two consecutive austral summers depending on chick age and survival

**Table 2:** Early physiological and morphological predictors (5 days after hatching) of survival until fledging (Cox regression)

| Variable | Exp(B) | z | p |
|---|---|---|---|
| Chick mass | 0.000 | -3.34 | <0.001 |
| DNA oxidative damage | 1.002 | 2.84 | 0.005 |
| Corticosterone | 1.186 | 2.45 | 0.014 |

## Discussion

Our study highlights marked differences in growth and survival of Adélie penguin chicks depending on environmental conditions.

### *EFFECTS OF CONTRASTING SEA-ICE CONDITIONS ON REPRODUCTIVE OUTPUT*

Given the short time available for breeding in Antarctica, Adélie penguins face particularly strong trade-offs between chick feeding and self-maintenance. The decreased reproductive output associated with extended sea-ice conditions (Fig. 1) is in agreement with previous studies (e.g. Clarke *et al.*, 2002; Emmerson *et al.*, 2003; Olmastroni *et al.*, 2004). Penguins facing extended sea-ice conditions raised only one chick, but surviving chicks had similar body mass and size at the end of the reproductive season than during the summer with usual sea-ice conditions. This trade-off between chick quality and quantity does not necessarily result from direct breeding decisions but may be an indirect effect of sibling competition for limited resources (Williams and Croxall, 1991). Indeed, it has been suggested that there is a threshold chick mass at fledging, below which chicks either die at the colony before fledging or have reduced survival after fledging (Emmerson *et al.*, 2006), supporting the idea that breeding Adélie penguins should favour chick quality over chick quantity.

### *EFFECTS OF CONTRASTING SEA-ICE CONDITIONS ON CHICK GROWTH*

Seabirds raising their offspring under conditions of limited food availability may avoid abandonment of the breeding effort by reducing chick provisioning and chick growth rates (Lack, 1968; Ricklefs *et al.*, 1985). In our study, both body mass and size (flipper and head-bill lengths) during penguin chick growth were affected by severe environmental conditions. Yet, fat accumulation may be excluded as a component of growth rate, with growth rates of feathers, appendages, and various organs described as providing better indexes of growth than body mass. For instance, captive seabird nestlings preferentially allocate resources to bill and wing growth over body components of lower priority, such as tarsus and mass under restricted feeding conditions (Benowitz-Fredericks *et al.*, 2006; Øyan and Anker-Nilssen, 1996). The effects of food restriction on chick growth depend on the timing of this restriction as well as on an organism's ability to change its patterns of allocation in response to variation in resource availability (Schew and Ricklefs, 1998). Interestingly, adults of some species of pelagic seabirds are able to increase food delivery in response to increased demand (Shea and Ricklefs, 1985). Common murres *(Uria aalge)* have the ability to change their foraging strategies to deal with some variability in prey resources, but not under exceptionally poor environmental conditions (Schrimpf *et al.*, 2012). The same probably applies to our study.

### *SHORT- AND LONG-TERM COSTS OF COMPENSATORY GROWTH*

Adélie penguin chicks have been described as particularly vulnerable to a decreased availability of food during the first 3 weeks after hatching (Clarke *et al.*, 2002). The unusual sea-ice conditions penguins faced in our study were associated with compensatory chick growth. Early-life environmental conditions can

have important consequences on juvenile animals in terms of growth, but also on future key life-history components such as survival and lifetime reproductive success (but see Drummond *et al.*, 2011; e.g. Vincenzi *et al.*, 2013), with potentially important consequences on population dynamics (Lindström, 1999). Telomere erosion and oxidative stress have been proposed as potential links between growth and ageing (Monaghan, 2010; Monaghan *et al.*, 2009). Indeed, king penguins chicks, which grew faster, had higher oxidative damage and accelerated telomere loss (Geiger *et al.*, 2012). We show that compensatory growth was also associated with increased oxidative damage in Adélie penguin chicks, but not with accelerated telomere erosion. These results suggest that the earlier costs of compensatory growth may consist of increased oxidative damage, and that ageing through reduced telomere length may occur later. While it would be interesting to measure long-term effects of extended sea-ice conditions and consecutive nutritional restrictions during growth, this would require monitoring hundreds of birds, which do not return on land until 3-5 years after they were born (Ainley, 2002). An alternative explanation would be that Adélie penguin chicks are resistant to deleterious oxidative effects through mechanisms that remain to be elucidated.

**Acknowledgments**

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## Annexe 4 : Nouveau Chapitre de la Thèse

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# Anne-Mathilde Thierry

*Ecole doctorale ED414 Vie et Santé*
*Université de Strasbourg*

*Nom du mentor : Pascale Klein*

# Hormones, reproduction, et conditions environnementales chez le manchot Adélie

Date de présentation orale du NCT : 14 juin 2013

Sujet académique de la thèse : Statut endocrinien et effort reproducteur chez un oiseau marin longévif, le manchot Adélie, dans un environnement changeant

*Nom des directeurs de thèse : Yan Ropert-Coudert et Thierry Raclot*
*Date de soutenance de la thèse : 13 septembre 2013*

# 1. Cadre général, enjeux et présentation du projet de recherche

## 1.1. Présentation succincte

Pour mieux comprendre comment les changements climatiques affectent les écosystèmes, il est nécessaire d'étudier les capacités d'adaptation des organismes face à des changements de leur environnement.

Les milieux polaires présentent une saisonnalité importante et des contraintes environnementales marquées, et les espèces qui y vivent sont particulièrement intéressantes à étudier car très sensibles aux changements de leur environnement. Des études démographiques ont notamment montré l'existence de corrélations entre les conditions environnementales et la dynamique des populations de différentes espèces d'oiseaux marins polaires. Ces études doivent être complétées par des travaux à l'échelle des individus afin de mieux comprendre comment les organismes font face à un environnement changeant.

Au cours de leur vie, les animaux prennent des 'décisions' de reproduction, comme se reproduire ou non, quand se reproduire ou encore combien d'énergie investir dans la reproduction. Les hormones jouent un rôle particulièrement important dans ces décisions, en permettant aux organismes d'ajuster leur physiologie et leur comportement face aux modifications prévisibles ou non de leur environnement.

L'objectif de ce travail de thèse est de préciser les relations entre le taux de certaines hormones –dont les hormones dites de *stress* (glucocorticoïdes) – les conditions environnementales et la capacité des oiseaux à mener à terme la reproduction avec succès chez un oiseau antarctique, le manchot Adélie.

## 1.2. La thèse dans son contexte

***La thèse dans un projet plus large***

Cette thèse s'inscrit dans un projet plus large sur les capacités d'adaptation comportementales et physiologiques des manchots face à la variabilité climatique, projet soutenu et financé par l'Institut polaire français Paul-Emile Victor (IPEV) depuis une dizaine d'années. Ce projet implique différents chercheurs de l'Institut Pluridisciplinaire Hubert Curien (IPHC, UMR 7178, CNRS – Université de Strasbourg).

La thèse a été réalisée à l'IPHC, au sein du Département Ecologie, Physiologie et Ethologie (DEPE), dans l'Equipe Ecologie Fonctionnelle. La thèse était rattachée à l'école doctorale des Sciences de la Vie et de la Santé de l'Université de Strasbourg et a été financée par une allocation de recherche de l'Ecole Normale Supérieure de Cachan.

Les travaux réalisés au cours de cette thèse ont bénéficié du soutien logistique et financier de l'IPEV dans le cadre du programme de recherche 137/ECOPHY. Les données ont été récoltées sur la base française Dumont d'Urville en Terre Adélie, Antarctique (66°40' S, 140°00' E) entre 2007 et 2012.

Les compétences d'un ingénieur d'étude et d'un technicien de laboratoire ont été partiellement mises à la disposition de ce projet. Onze étudiants de l'Université de Strasbourg (L3, M1, M2) ont travaillé sur différents aspects du projet dans le cadre de stages de recherche, pour un total de 22 mois cumulés. Quatre Volontaires Civils à l'Aide Technique (VCAT), travaillant pour le compte de l'IPHC et payés par l'IPEV, ont participé à la récolte des données en Antarctique.

***Ma place dans ce contexte***

Dès mes études secondaires, j'ai montré un intérêt particulier pour les sciences. Mon parcours universitaire, en classes préparatoires d'abord puis à l'Ecole Normale Supérieure de Cachan, m'a permis d'effectuer plusieurs stages dans des laboratoires de recherche en France et à l'étranger, confirmant cet intérêt pour les sciences et la recherche scientifique. C'est donc naturellement que j'ai choisi de préparer un doctorat. Le choix du sujet de thèse s'est fait à la fois par intérêt scientifique et pour une question d'opportunité.

De novembre 2007 à février 2009, j'ai séjourné sur la base Dumont d'Urville en Antarctique en tant que Volontaire Civile à l'Aide Technique pour récolter des données sur les manchots Adélie et empereur pour des chercheurs et doctorants de l'IPHC. J'ai ensuite eu l'opportunité de travailler sur les données recueillies pendant mon séjour en Antarctique lors d'un stage de recherche de Master 2. Ce stage s'est poursuivi par une thèse, toujours sur les manchots Adélie, me permettant d'acquérir des compétences sur l'ensemble du travail de recherche, et non seulement sur le travail de récolte de données sur le terrain.

Cette expérience de terrain acquise en Antarctique m'a permis de participer de façon importante à la définition et à la programmation de mon projet de thèse. Dans un premier temps, plusieurs mois avant le début de la thèse, j'ai rédigé la demande de financement de thèse en collaboration avec mon futur encadrant. Pendant mon stage de Master 2, puis pendant la thèse, j'ai préparé les campagnes de terrain en Antarctique : rédaction des protocoles en accord avec mes encadrants, demandes d'autorisation pour expérimenter auprès du comité d'éthique de l'IPEV et des Terres Australes et Antarctiques Françaises (TAAF), commande du matériel et préparation des colis pour envoi en Antarctique, recrutement et formation des futurs hivernants.

En parallèle de la thèse, j'ai participé au développement et à la mise en place d'un projet éducatif pour vulgariser les sciences polaires en France. Il existe de nombreux projets de communication et vulgarisation des sciences polaires auprès des écoles pour la communauté internationale, proposant de nombreuses activités, mais il n'y a pas d'équivalent en France. Pourtant, les enseignants, notamment de

primaire, sont très ouverts à ce type d'activité. C'est ainsi qu'est née « l'Ecole des pôles » : une semaine pendant laquelle ont été proposées différentes activités en rapport avec les pôles, en parallèle de la semaine polaire internationale organisée deux fois par an par l'association des jeunes chercheurs en sciences polaires (APECS Association of Polar Early Career Scientists) et un groupe d'éducateurs polaires internationaux (PEI Polar Educators International). Pour cette première édition, des scientifiques ont répondu aux questions des élèves, des webinars (présentations en ligne d'une heure environ permettant à plusieurs classes de suivre en même temps) ont été organisés sur des thèmes tels que la vie dans un village au Groenland, la vie sur une base antarctique ou encore les animaux polaires. 15 classes de 10 écoles différentes (9 écoles élémentaires dont 1 école allemande et 1 classe de collège) ont assisté à 1 à 4 webinars (en moyenne 2). En tout 332 élèves ont suivi un ou plusieurs webinars, et les retours positifs ont été nombreux. Cette semaine polaire française a abouti à la création du comité national français de l'APECS, dont je suis une des deux correspondantes. Le but de ce comité national est de mettre en relation les jeunes chercheurs en sciences polaires français ou francophones, de les aider dans le développement de leur carrière et d'améliorer la communication et la vulgarisation des sciences polaires en France. La prochaine semaine polaire, dont l'affiche est présentée sur la page suivante, est actuellement en préparation.

Au cours de ma dernière année de thèse, j'ai également participé à l'organisation des neuvièmes rencontres « Ecology & Behaviour » qui ont eu lieu Strasbourg en avril 2013. Ce sont des rencontres pour les doctorants organisées par des doctorants ; elles ont lieu chaque année en France depuis 2005. Le principe est le suivant : les étudiants qui présentent leurs travaux de recherche ne paient pas de frais d'inscription, d'hébergement et de couvert. Cette démarche permet d'accueillir un grand nombre d'étudiants dans un contexte économique difficile. L'équipe d'organisation de la conférence, dont j'étais membre, a ainsi géré un budget de 18000 € pour accueillir pendant cinq jours 80 participants (dont seulement la moitié étaient français) et 10 chercheurs européens invités. J'étais plus particulièrement chargée de la gestion financière et de l'édition de l'abstract book (livret de 120 pages présentant le programme de la conférence et les résumés de toutes les communications).

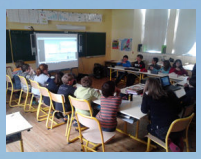
# LA SEMAINE POLAIRE

La semaine polaire est née de la volonté de jeunes scientifiques travaillant dans les régions polaires de partager leurs expériences de l'Arctique et de l'Antarctique auprès des plus jeunes.

En partenariat avec APECS international (Association of Polar Early Career Scientists) et PEI (Polar Educators International), APECS France propose aux écoles primaires, collèges et lycées de participer à plusieurs activités pédagogiques sur les régions polaires, dans le cadre de l'organisation bi-annuelle d'une semaine thématique sur l'Arctique et l'Antarctique.

La prochaine semaine polaire aura lieu du **30 septembre au 4 octobre 2013**.






## DECOUVRIR

Les scientifiques présenteront différents sujets lors de vidéoconférences thématiques d'une heure environ (les webinars). Les élèves pourront poser leurs questions directement à l'intervenant via l'interface informatique (en parlant avec un micro ou bien par chat). Le matériel minimum nécessaire est un ordinateur et une connexion à internet, avec idéalement un vidéo projecteur. Les présentations seront adaptées à différents niveaux (primaire, collège, lycée).

## QUESTIONNER & COMPRENDRE

Vous pourrez échanger par e-mail avec les scientifiques qui ont vécu ou vivent actuellement dans les régions polaires.

## ACTIVITE A DEFINIR

Une activité commune à toutes les classes sera proposée, comme une leçon de science associée à une activité artistique. Le descriptif de cette activité sera communiqué début septembre.

Pour plus d'informations : apecs.france@gmail.com

**Figure 1 :** Affiche de la prochaine semaine polaire

## 2. Déroulement, gestion et coût du projet

### 2.1. Préparation et cadrage du projet

***Facteurs de risque et stratégie de maîtrise des risques***

Les facteurs de risques sont nombreux lorsque l'on réalise une thèse s'intéressant aux milieux polaires. Ils sont principalement liés aux contraintes du travail dans ces régions : isolement, éloignement, difficulté d'acheminement du personnel et du matériel, conditions environnementales difficiles et changeantes, voire extrêmes…
L'existence d'un programme de recherche sur les manchots Adélie à Dumont d'Urville depuis une dizaine d'années a permis d'aborder le projet de thèse dans de bonnes conditions. La principale stratégie de maîtrise des risques a consisté à faire la thèse dans le cadre d'un projet déjà existant, permettant de modifier le sujet de thèse en cas de problèmes lors de la récolte des données en Antarctique. En effet, des données sauvegardées sur support informatique et échantillons stockés au congélateur, récoltés lors des années précédentes, étaient à ma disposition. Concernant le matériel nécessaire à la récolte d'échantillons en Antarctique, nous avons fait en sorte que la majorité du matériel, principalement consommables, soient déjà présents sur la base à la fin de la campagne précédente.
Du fait de différentes contraintes de terrain, j'ai aussi dû adapter une partie des protocoles à la réalité du travail en Antarctique. Bien que les risques soient relativement connus, ils n'en sont pas toujours prévisibles (par exemple, arrivée sur la base avec un mois de retard par rapport au planning).

***Partenaires scientifiques et financiers***

Le partenaire principal pour cette thèse a été l'IPEV qui a assuré la logistique du projet ainsi qu'un soutien financier. Les financements internes à l'équipe étant relativement limités, j'ai effectué des demandes de financements externes. Une bourse de la fondation britannique Antarctic Science a permis de réaliser une partie des analyses en laboratoires (5500€). Des bourses ont également été demandées pour participer à des congrès nationaux et internationaux (journées scientifiques du Comité National Français des Recherches Arctiques et Antarctiques, Brest, mai 2012 - XIth Biology Symposium of the Scientific Committee on Antarctic Research, Barcelone, juillet 2013). J'ai également reçu en octobre 2012 la bourse L'Oréal France – UNESCO – Académie des Sciences Pour les Femmes et la Science qui m'a aidé à valoriser ma dernière année de thèse.
Toutes les espèces se reproduisant en Antarctique sont protégées dans le cadre du traité de l'Antarctique. Les études sur ces espèces sont soumises à autorisation de l'IPEV et des TAAF. Chaque année, des demandes doivent être transmises pour accord auprès de ces organisations.
Le projet éducatif « Ecole des pôles » s'est fait en collaboration avec l'APECS (notamment par la mise à disposition de la plateforme de Webinar) et PEI.

## 2.2. Conduite du projet

***Principales étapes***

Pour mener à bien ce projet, il a été important de planifier à la fois les campagnes de récolte de données en Antarctique et l'analyse des données en laboratoire à Strasbourg dès le début de la thèse. Les principales étapes du projet sont résumées dans la figure 2.

Un fichier décrivant l'avancement du projet ainsi qu'une liste de points à traiter ont été régulièrement mis à jour tout au long de la thèse (environ une fois par mois hors missions en Antarctique). Nous avons fait le point régulièrement sur l'avancement du projet avec mon responsable de thèse (mensuellement ou plus fréquemment en fonction de mes besoins). Lors des missions en Antarctique, nous faisions le point sur les différents aspects de l'avancement du travail de terrain par e-mail au moins une fois par semaine. Deux comités de thèse ont également été organisés au cours de la thèse et ont été l'occasion de présenter le projet et les résultats à deux chercheurs extérieurs au projet. Lors de périodes d'encadrement des stagiaires, nous faisions une réunion hebdomadaire où chaque étudiant présentait l'avancement de ses travaux, nous permettant de recadrer le projet si besoin.

Dans le cadre de la préparation des missions en Antarctique, j'ai communiqué avec différents personnels de l'Institut polaire pour préparer les campagnes de terrain (demandes d'autorisation pour expérimenter, commandes de matériel, acheminement du matériel et des personnels).
Une partie de l'analyse des données a été sous-traitée à une autre équipe du laboratoire, avec laquelle j'ai régulièrement communiqué.

***Problèmes et solutions***

Le principal problème rencontré pendant le projet est lié aux conditions de travail en milieu isolé lors des missions en Antarctique.
Les missions polaires sont fortement tributaires des conditions environnementales, qui peuvent être source de retard dans l'acheminement du personnel et du matériel, et malheureusement également d'accidents. En octobre 2011, un accident d'hélicoptère a coûté la vie à quatre personnes. Nous sommes arrivés avec plusieurs semaines de retard sur la base. Il est difficile de reporter les tâches lorsque le personnel est réduit. Le protocole a donc été adapté et modifié pour qu'il soit réalisable par la seule personne de l'équipe qui était sur place.

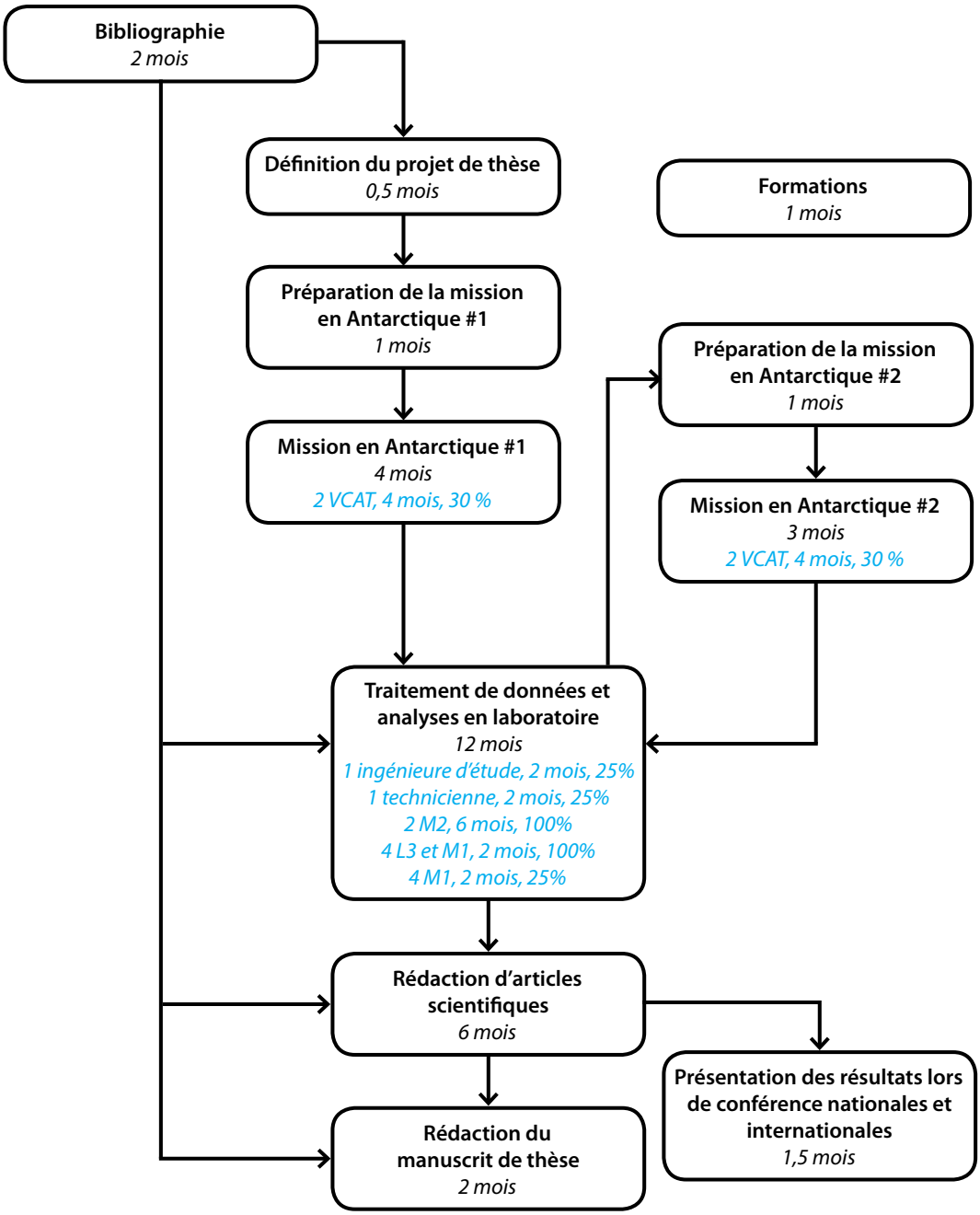



**Figure 2 :** Planification du projet de thèse.

Les durées en italique correspondent aux ressources humaines affectées au projet : ce qui me concerne en noir et différentes personnes ayant participé au projet en bleu, dont des Volontaires Civils à l'Aide Technique (VCAT), des étudiants de troisième année de licence (L3), de première (M1) et deuxième (M2) année de Master.

La base Dumont d'Urville est un lieu exceptionnel pour la facilité d'accès au sujet d'étude de ma thèse que sont les manchots Adélie (les bâtiments sont à quelques mètres des colonies de manchots). Mais j'ai également pu y expérimenter les relations sociales en milieu isolé. En particulier, j'ai dû faire face au manque de motivation d'une partie du personnel à des périodes où le travail ne manquait pas. La récolte des données aurait donc pu pâtir de cette situation. Le projet a pu avancer grâce à l'aide d'un certain nombre de personnes présentes sur la base dont l'étude des manchots n'était pas la préoccupation principale.

La dernière difficulté rencontrée pendant ma thèse est liée au grand nombre de données dont j'ai disposé, données récoltées non seulement pendant la thèse mais aussi pendant plusieurs missions en Antarctique antérieures à la thèse. De ce fait, il aurait pu y avoir plusieurs sujets de thèse possibles selon l'intérêt du doctorant. Mes encadrants m'ont laissé relativement libre dans l'orientation du sujet de thèse, tout en me guidant lorsque cela était nécessaire.

## 2.3. Estimation et prise en charge du coût de votre projet

***Ressources humaines***

Des moyens en personnel ont été mis à ma disposition pour mener à bien ce projet. Le tableau 1 détaille les ressources humaines affectées au projet, ainsi que les dépenses associées.
En France, j'ai bénéficié de l'encadrement de deux chargés de recherche (Thierry Raclot et Yan Ropert-Coudert). Une ingénieure d'étude et une technicienne de laboratoire ainsi que plusieurs stagiaires (deux stagiaires de deuxième année de Master, huit stagiaires de première année de Master et une stagiaire de Licence) ont participé à l'analyse des données (dosages en laboratoire, traitement de données sur informatique, visionnage de vidéos pour déterminer le comportement des manchots à certaines étapes clés du cycle de reproduction).

Une mission en Antarctique ne peut se réaliser sans un certain nombre de personnes. Dans un premier temps, le trajet en bateau entre l'Australie et la station de recherche occupe une quinzaine de personnes (capitaine, second, chef mécanicien, différents membres d'équipage, cuisinier). Un ou deux pilotes d'hélicoptère et un mécanicien sont également présents pour assurer la dernière partie du trajet jusqu'à la station de recherche, trajet en hélicoptère qui peut durer une à deux heures en début d'été austral. Sur la base, un certain nombre de techniciens (mécanicien, électricien, plombier, chauffagiste, soudeur), des logisticiens, un cuisinier, un médecin et un chef de base font en sorte que tout se passe bien sur la base, ce qui permet aux scientifiques de mener à bien leur travail de récolte de données. Trois personnels de Météo France sont également présents sur la base pour le fonctionnement et l'entretien de la station météorologique, et rédigent chaque jour des prévisions très utiles pour prévoir le travail en extérieur du lendemain. Enfin, plusieurs jeunes scientifiques (Volontaires Civils à l'Aide Technique) ont participé de façon intensive au travail de récolte de données en Antarctique.

| **1. Ressources Humaines** | **Salaire mensuel net** | **Coût salarial** *toutes charges comprises* | **Mois** | | **Quote part (%)** | **Total € TTC** |
|---|---|---|---|---|---|---|
| Doctorant | 1 400 | 2 534 | 36 | | 100 | 91 224 |
| Encadrant 1 : directeur de thèse | 3 000 | 5 430 | 36 | | 5 | 9 774 |
| Encadrant 2 : chargé de recherche | 3 000 | 5 430 | 36 | | 20 | 39 096 |
| Ingénieure d'étude | 1 600 | 2 896 | 2 | | 25 | 1 448 |
| Technicienne laboratoire | 1 400 | 2 534 | 2 | | 25 | 1 267 |
| Personnel administratif | 1 500 | 2 715 | 36 | | 1 | 977 |
| Stagiaires | 400 | 400 | 12 | | 100 | 4 800 |
| Volontaire Civil à l'Aide Technique | 900 | 900 | 24 | | 30 | 6 480 |
| Personnels de campagne d'été (10 personnes) | 5 000 | 9 050 | 80 | | 5 | 36 200 |
| **Sous-total** | | | | | | **191 266** |
| **2. Infrastructures** | **Loyer annuel consolidé** *entretien, secrétariat, maintenance, chauffage, électricité, gaz, eau* | **m² utilisés** | **Mois** | | **Quote part (%)** | **Total € TTC** |
| Bâtiment | 80 € / $m^2$ | 40 | 36 | | 90 | 9 600 |
| Mission en Terre Adélie | 2000 € / $m^2$ | 15 | 8 | | 100 | 20 000 |
| **Sous-total** | | | | | | **29 600** |
| **3. Consommables** | **Dépenses globales équipe par an** | **Coût plateforme** *coût consolidé* | **Mois** | **Nombre d'unités** | **Quote part (%)** | **Total € TTC** |
| Consommables de laboratoire | 10 000 | | 36 | | 25 | 7 500 |
| Consommables spéciaux | 5 500 | | 12 | | 100 | 5 500 |
| Consommables de laboratoire (Antarctique) | 5 000 | | 8 | | 75 | 2 500 |
| Plateforme analyse | | 100 | | 20 | 100 | 2 000 |
| Plateforme PCR quantitative | | 400 | | 5 | 100 | 2 000 |
| **Sous-total** | | | | | | **19 500** |
| **4. Matériel** | **Amortissement (%)** | **Amortissement utilisé (%)** | **Utilisation** *(années)* | **Coût achat € TTC** | **Quote part (%)** | **Total € TTC** |
| Ordinateur | 33,33 | 100 | 3,0 | 800 | 100 | 800 |
| Licenses logiciels | 100 | 100 | 3,0 | 500 | 100 | 500 |
| Machine PCR | 20 | 10 | 0,5 | 10 000 | 20 | 200 |
| Congélateurs -80°C | 10 | 50 | 5,0 | 5 000 | 10 | 250 |
| Congélateurs -20°C | 10 | 50 | 5,0 | 2 000 | 50 | 500 |
| **Sous-total** | | | | | | **2 250** |
| **5. Déplacement** | **Montant** | | **Nombre** | | | **Total € TTC** |
| Mission en Antarctique : Avion (A/R Paris-Hobart) | 2 500 | | 2 | | | 5 000 |
| Trajet Hobart - Dumont d'Urville (bateau) | 2 100 | | 4 | | | 8 400 |
| Trajet Astrolabe - Dumont d'Urville (hélicoptère) | 1 000 | | 3 | | | 3 000 |
| Repas et logement en Antarctique | 1 000 | | 8 | | | 8 000 |
| Congrès en France | 150 | | 3 | | | 450 |
| Congrès à l'étranger | 500 | | 4 | | | 2 000 |
| **Sous-total** | | | | | | **26 850** |
| **Bilan coût total € TTC** | | | | | | **269 466** |

**Tableau 1 :** Estimation du coût de ma thèse

***Evaluation et prise en charge du coût du projet***

Les principaux contributeurs financiers ont été le CNRS, l'Université de Strasbourg et l'IPEV (Fig. 3). Une plus petite partie du budget a été financée par l'ANR, par une bourse de la fondation Antarctic Science, ainsi qu'une bourse L'Oréal – UNESCO – Académie des Sciences « Pour les Femmes et la Science ».

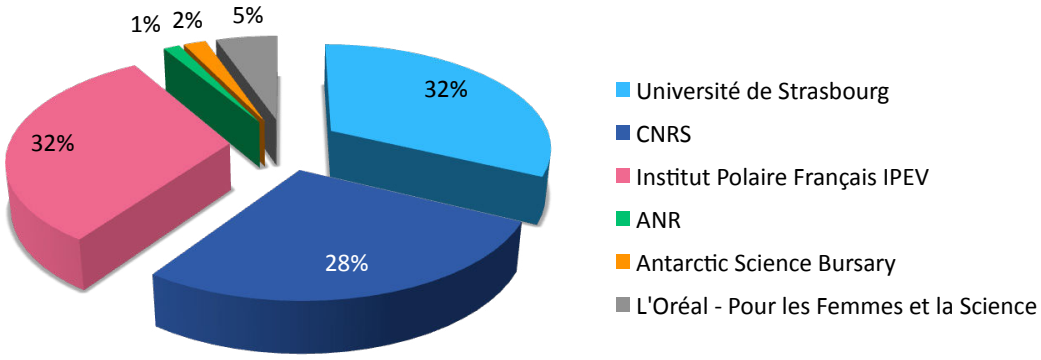



**Figure 3 :** Contributeurs et répartition des ressources financières

## 3. Identification, hiérarchisation et illustration des compétences mises en œuvre

***Compétences scientifiques et techniques***

Mon domaine d'expertise concerne les relations entre hormones et comportements liés à la reproduction chez les manchots. Les connaissances acquises pendant la thèse ne concernent pas uniquement les manchots, mais également les autres espèces d'oiseaux marins, et de façon plus générale les animaux dans leur milieu naturel.
Au cours de ma thèse, j'ai appris à observer un animal sauvage et à collecter des échantillons biologiques en milieu polaire, à analyser les échantillons en laboratoires à l'aide de dosages spécifiques pour mesurer différents paramètres physiologiques et à analyser et modéliser les données récoltées à l'aide de différents outils statistiques requérant de bonnes compétences de programmation.

***Compétences liées à la gestion d'un projet sur trois ans***

J'ai pu acquérir différentes compétences de gestion de projet pendant la thèse. Il s'est agi dans un premier temps de formaliser le projet, puis de planifier son avancement, y compris dans ses aspects

administratifs. Ma thèse a été l'occasion de travailler en équipe, aussi bien lors de la récolte des données en Antarctique que pendant l'analyse des échantillons au laboratoire, en respectant des délais.

***Qualités personnelles***

J'ai développé différentes qualités personnelles au contact de la difficulté de la thèse, et plus particulièrement lors des missions en Antarctique. Il m'a ainsi fallu argumenter certains choix faits en amont de la récolte de données lorsque les conditions sur le terrain demandaient adaptabilité et flexibilité. La conduite de ce projet de thèse sur trois ans a également demandé rigueur et persévérance.
J'ai appris à communiquer et valoriser les résultats obtenus, à l'écrit comme à l'oral. La présentation des résultats s'est faite sous la forme d'articles scientifiques rédigés en anglais et à l'oral devant des publics variés, en français ou en anglais, lors de conférences nationales ou internationales. J'ai également eu l'occasion de communiquer autour de mon projet de thèse auprès du grand public, lors de la Fête de la Science ou au cours d'interviews (radios locales et nationale, chaînes de télévision locales) réalisées dans le cadre de la médiatisation de la bourse L'Oréal France – UNESCO – Académie des Sciences « Pour les Femmes et la Science » reçue pendant ma thèse.

***Construction d'un réseau professionnel personnel***

La rencontre de chercheurs spécialistes des milieux polaires et/ou de l'étude des animaux dans leur milieu naturel, lors de conférences nationales et internationales, lors de missions en Antarctique, ou lors d'échanges par e-mail, m'a permis de développer un réseau informel. Plusieurs collaborations sont d'ailleurs en cours avec des scientifiques de différents pays et/ou domaines. Je participe également à la mise en place d'un réseau de jeunes chercheurs en sciences polaires en France, dans le cadre de la création du comité national français de l'APECS.

## 4. Retombées, notamment en termes de pistes professionnelles identifiées

### 4.1. Résultats, impact des recherches

***Résultats***

Dans le cadre d'études expérimentales, j'ai montré qu'une augmentation des niveaux de corticostérone, la principale hormone dite de stress chez les oiseaux, entraîne une diminution du succès de reproduction chez le manchot Adélie. Pendant l'incubation, cette diminution est due à un fort taux d'abandon du nid associé à des températures d'incubation diminuées, alors que le comportement des manchots était peu affecté par l'augmentation des niveaux de corticostérone pendant la période d'élevage des poussins. L'effet de l'augmentation des niveaux de corticostérone était d'autant plus important que les conditions

météorologiques étaient mauvaises, soulignant l'importance de considérer les conditions environnementales dans les études sur les relations entre hormones et comportement chez une espèce animale sauvage. Deux autres études concernant la prolactine, hormone des soins parentaux, et la testostérone, liée aux comportements sexuels et à l'agressivité, a permis de suggérer que ces deux hormones pourraient jouer un rôle important dans la transmission d'informations concernant le timing de la reproduction, du fait de leurs effets sur le comportement des manchots au cours de l'incubation.

***Pour le laboratoire et pour les partenaires du projet***

Ce travail de thèse a fait l'objet de plusieurs articles scientifiques publiés dans des revues à comités de lectures et d'autres sont en préparation. Les résultats ont été présentés lors de colloques nationaux et internationaux. L'obtention de la bourse L'Oréal France– UNESCO – Académie des Sciences « Pour les Femmes et la Science » a été accompagnée de plusieurs interviews, médiatisant ainsi une partie des travaux réalisés au sein du laboratoire.

***Pour moi-même***

La réalisation d'une thèse est une aventure scientifique mais aussi humaine, surtout lorsque l'on a l'opportunité de travailler dans ce milieu isolé qu'est l'Antarctique.

## 4.2. Identification de pistes professionnelles

Le NCT a été l'occasion de clairement identifier plusieurs pistes professionnelles réalistes, à partir d'une réflexion faite sur mes compétences scientifiques, mes compétences en gestion de projet et mes qualités personnelles.

- Chargée de recherche en écologie animale. Ce projet est une des suites possibles à ma thèse. Dans un premier temps, il me faudra réaliser un ou plusieurs post-docs, de préférence à l'étranger, avant de postuler à un concours du type CNRS ou IRD. Je suis actuellement en contact avec une équipe d'un institut de recherche norvégien pour un post-doc sur les renards polaires à Trondheim et avec des chercheurs de l'Université du Québec à Rimouski qui étudient les écosystèmes arctiques.

- Chargée de projet. Dès le début de la thèse, j'ai vite réalisé que la thèse ne serait pas uniquement un travail de questionnement scientifique, de récolte et d'analyses de données. La réflexion faite au cours du NCT a confirmé mon intérêt pour la gestion de projet et permis de faire le bilan des

compétences acquises dans ce domaine. Un poste de chargée de projet en communication scientifique, en environnement ou en solidarité internationale m'intéresserait énormément.

- Gestionnaire ou responsable d'organisation internationale en sciences polaires. Parmi les nombreuses organisations scientifiques internationales en sciences polaires, l'APECS (Association of Polar Early Career Scientists) recrute actuellement un directeur, poste auquel j'ai posé ma candidature. Dans le cas où ma candidature ne donnerait pas suite, j'écrirai aux autres organisations, telles que le SCAR (Scientific Committee on Antarctic Research, un comité inter-disciplinaire du conseil international pour la science, ICSU) ou le programme Arctique du WWF, pour leur faire part de mon intérêt pour leurs activités et leur proposer mes compétences.

**Résumé :** L'étude des mécanismes endocriniens est particulièrement intéressante du fait du rôle majeur des hormones dans la régulation des interactions entre la physiologie d'un organisme, son comportement, et les modifications prévisibles ou non prévisibles de son environnement. Pendant cette thèse, je me suis intéressée aux relations entre le statut hormonal, les performances de reproduction et le succès reproducteur d'un oiseau marin longévif, le manchot Adélie *Pygoscelis adeliae,* tout en considérant des paramètres environnementaux dans certaines études. Le statut endocrinien de manchots mâles a été manipulé en utilisant des implants dégradables sous-cutanés diffusant l'hormone d'intérêt ou un inhibiteur de sa sécrétion. Les effets d'une modification des niveaux d'hormones sur l'investissement parental pendant l'incubation ont été mesurés à l'aide d'observations directes et d'œufs factices enregistrant les paramètres d'incubation. Les niveaux de corticostérone – hormone dite de stress, de prolactine – hormone des soins parentaux, et de testostérone – hormone liée aux comportements sexuels et à l'agressivité, ont été manipulés. Les effets d'une augmentation des niveaux de corticostérone sur les performances et le succès reproducteur pendant la période de l'élevage des poussins ont également été mesurés. Enfin, les conséquences d'une légère élévation des niveaux de corticostérone pendant l'ensemble de la saison de reproduction en termes de comportement et de succès reproducteur ont été examinées. Une augmentation des niveaux de corticostérone a globalement diminué les performances et le succès de reproduction. D'autre part, une modification des niveaux de prolactine ou de testostérone a affecté la durée et les paramètres d'incubation, suggérant une implication de ces deux hormones dans le contrôle de la phénologie de la reproduction. Les résultats présentés dans cette thèse mettent l'accent sur le fait que la relation entre statut endocrinien et performances de reproduction est dose, état et contexte dépendante. Par exemple, les conséquences d'une augmentation des niveaux de corticostérone pendant l'incubation ont été plus importantes lorsque les manchots étaient soumis à des conditions météorologiques sévères. Nos résultats illustrent le rôle majeur des hormones étudiées dans la régulation de l'effort reproducteur, et soulignent également l'importance de considérer les interactions entre les organismes et leur environnement.

**Mots-clés :** comportement, corticostérone, conditions environnementales, élevage des poussins, hormones, incubation, oiseaux marins, prolactine, régions polaires, soins parentaux, stress, succès reproducteur, testostérone

**Abstract:** Studying endocrine mechanisms is of particular interest because of the major role played by hormones in mediating interactions between an animal's physiology, its behaviour, and both predictable and unpredictable regimes of environmental variation. During this PhD, I have investigated the relationships between endocrine status, reproductive performance, and reproductive output in a long-lived polar seabird, the Adélie penguin *Pygoscelis adeliae,* while integrating environmental parameters for some of the studies. The endocrine status of male penguins was experimentally modified using subcutaneous self-degradable pellets, which released either the hormone or an inhibitor of its secretion. The effects of changes in the levels of several hormones on the parental investment during incubation were assessed, using direct observations and dummy eggs to record incubation parameters. The levels of corticosterone – the so-called stress hormone, prolactin – the parental care hormone, and testosterone – the sexual behaviour and aggressiveness hormone, were manipulated. The effects of increased corticosterone levels on reproductive performance and output were also evaluated during the chick-rearing period. Finally, the behavioural consequences of a moderate elevation of corticosterone levels during the whole breeding cycle were assessed. On the whole, an increase in corticosterone levels decreased reproductive performances and output. Changes in prolactin or testosterone levels affected incubation duration and egg temperature, suggesting a role for these hormones in the control of the timing of breeding. The results prevented in this PhD highlight the fact that the relationship between endocrine status and reproductive performance is dose-, state-, and context-dependant. For example, weather conditions appeared to be particularly important in determining the extent to which corticosterone levels affected the behaviour of incubating penguins. Our results illustrate the major role of the hormones considered in our studies in the regulation of reproductive effort. They also underline the importance of considering the interactions of organisms with their environment in studies of animal behaviour and ecophysiology.

**Keywords:** behaviour, chick-rearing, corticosterone, environmental conditions, hormones, incubation, parental care, Polar Regions, prolactin, reproductive output, seabirds, stress, testosterone.

UNIVERSITÉ DE STRASBOURG

# Anne-Mathilde THIERRY

IPHC Institut Pluridisciplinaire Hubert CURIEN STRASBOURG

## Statut endocrinien et effort de reproduction chez un oiseau marin longévif, le manchot Adélie, dans un environnement changeant

## Résumé

L'étude des mécanismes endocriniens est particulièrement intéressante du fait du rôle majeur des hormones dans la régulation des interactions entre la physiologie d'un organisme, son comportement, et les modifications de son environnement. Cette thèse s'est intéressée aux relations entre le statut hormonal, les performances de reproduction et le succès reproducteur d'un oiseau marin longévif, le manchot Adélie *Pygoscelis adeliae,* dans un contexte environnemental soumis à des changements. Le statut endocrinien de manchots mâles a été manipulé en utilisant des implants dégradables sous-cutanés diffusant l'hormone d'intérêt ou un inhibiteur de sa sécrétion. Les effets d'une modification des niveaux d'hormones sur l'investissement parental pendant l'incubation ont été mesurés à l'aide d'observations directes et d'œufs factices enregistrant les paramètres d'incubation. Les niveaux de corticostérone – hormone dite de stress, de prolactine – hormone des soins parentaux, et de testostérone – hormone liée aux comportements sexuels et à l'agressivité, ont été manipulés. Les effets d'une augmentation des niveaux de corticostérone sur les performances et le succès reproducteur pendant la période de l'élevage des poussins ont également été mesurés. Enfin, les conséquences d'une légère élévation des niveaux de corticostérone pendant l'ensemble de la saison de reproduction en termes de comportement et de succès reproducteur ont été examinées. Une augmentation des niveaux de corticostérone a globalement diminué les performances et le succès de reproduction. D'autre part, une modification des niveaux de prolactine ou de testostérone a affecté la durée et les paramètres d'incubation, suggérant une implication de ces deux hormones dans le contrôle de la phénologie de la reproduction. Les résultats présentés dans cette thèse mettent l'accent sur le fait que la relation entre statut endocrinien et performances de reproduction est dose, état et contexte dépendante. Nos résultats illustrent le rôle majeur des hormones étudiées dans la régulation de l'effort reproducteur, et soulignent également l'importance de considérer les interactions entre les organismes et leur environnement.

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## Abstract

Studying endocrine mechanisms is of particular interest because of the major role played by hormones in mediating interactions between an animal's physiology, its behaviour, and both predictable and unpredictable regimes of environmental variation. During this PhD, I have investigated the relationships between endocrine status, reproductive performance, and reproductive output in a long-lived polar seabird, the Adélie penguin Pygoscelis adeliae, while integrating environmental parameters for some of the studies. The endocrine status of male penguins was experimentally modified using subcutaneous self-degradable pellets, which released either the hormone or an inhibitor of its secretion. The effects of changes in the levels of several hormones on the parental investment during incubation were assessed, using direct observations and dummy eggs to record incubation parameters. The levels of corticosterone – the so-called stress hormone, prolactin – the parental care hormone, and testosterone – the sexual behaviour and aggressiveness hormone, were manipulated. The effects of increased corticosterone levels on reproductive performance and output were also evaluated during the chick-rearing period. Finally, the behavioural consequences of a moderate elevation of corticosterone levels during the whole breeding cycle were assessed. On the whole, an increase in corticosterone levels decreased reproductive performances and output. Changes in prolactin or testosterone levels affected incubation duration and egg temperature, suggesting a role for these hormones in the control of the timing of breeding. The results prevented in this PhD highlight the fact that the relationship between endocrine status and reproductive performance is dose-, state-, and context-dependant. Our results illustrate the major role of the hormones considered in our studies in the regulation of reproductive effort. They also underline the importance of considering the interactions of organisms with their environment in studies of animal behaviour and ecophysiology.

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