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Mécanismes hormonaux impliqués dans l'induction de l'abandon du nid chez un oiseau marin longévif : le manchot Adélie

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Articles et Communications

Articles inclus dans ce mémoire

1. **Spée M.**, Beaulieu M., Dervaux A., Chastel O., Le Maho Y., Raclot T. (2010). Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin. *Hormones and Behavior* 58, 762-768.

2. **Spée M.**, Marchal L., Thierry, A.M., Chastel O., Enstipp M., Le Maho Y., Beaulieu M., Raclot T. Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins (*Pygoscelis adeliae*). (*soumis*)

3. **Spée M.**, Marchal L., Lazin D., Le Maho Y., Chastel O., Beaulieu M., Raclot T. How does corticosterone trigger egg abandonment in free-living Adélie penguins? (*soumis*)

4. **Spée M.**, Galland C., Lazin D., Le Maho Y., Chastel O., Beaulieu M., Raclot T. An experimental decrease in prolactin leads to behavioral changes but does not affect nest desertion in Adélie penguins (*en préparation*)

Articles non-inclus dans ce mémoire

1. Beaulieu M., **Spée M.**, Lazin D., Ropert-Coudert Y., Le Maho Y., Ancel A., Raclot T. (2010). Ecophysiological response of Adélie penguin parents facing an experimental increase in breeding constraints. *Journal of Experimental Biology*. 213, 33-39.

2. Beaulieu M., Dervaux A., Thierry A.M., 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, 93-102.

3. Beaulieu M., **Spée M.**, Chastel O., Le Maho Y., Lacroix A., Ancel A., Raclot T. Examination of prolactin as a regulator of foraging trip duration in the Adélie penguin. *General and Comparative Endocrinology* (en révision).

4. Rey B., **Spée M.**, Belouze M., Girard A., Prost J., Roussel D., Duchamp C. (2010) Oxygen recovery up-regulates avian UCP and ANT in newly hatched ducklings. *Journal of Comparative Physiology B.* 180, 239-246.

Communications orales

1. **Spée M.**, Beaulieu M., Dervaux, A., Chastel O., Le Maho Y., Raclot T. Contrôle hormonal de l'abandon du nid chez le manchot Adélie. Comité National Français des Recherches Arctique et Antarctique; 5èmes Journées Scientifiques, Octobre 2008, Paris, France.

2. **Spée M.**, Beaulieu M., Marchal L., Lazin D., Thierry A.M., Chastel O., Le Maho Y., Raclot T. Corticosterone is involved in the control of nest abandonment in breeding Adélie penguins (*Pygoscelis adeliae*). 7th European Ornithologists' Union Conference, Août 2009, Zurich, Suisse.

3. Beaulieu M., **Spée M.**, Lazin D., Le Maho Y., Ancel A, Raclot T. Ecophysiological response of Adélie penguins facing an experimental increase in breeding constraints. 7th European Ornithologists' Union Conference, Août 2009, Zurich, Suisse.

4. Beaulieu M., Dervaux A., Thierry A.M., Lazin D., Le Maho Y, Ropert-Coudert Y., **Spée M.**, Raclot T, Ancel A. Ecophysiological response of Adélie penguin parents facing contrasting timing of sea-ice retreat. Xth SCAR (Scientific Committee on Antarctic Research) International Biology Symposium, Juillet 2009, Sapporo, Japon.

Communications affichées

1. **Spée M.**, Beaulieu M., Dervaux A., Chastel O., Le Maho Y., Raclot T. Hormonal control of nest abandonment in breeding Adélie penguins. Society of Experimental Biology. Juillet 2008, Marseille, France.

2. **Spée M.**, Marchal L., Thierry A.M., Lazin D., Beaulieu M., Le Maho Y., Raclot T. Implication de la corticostérone dans l'induction du signal de réalimentation: approche expérimentale chez le manchot Adélie. Comité National Français des Recherches Arctique et Antarctique; 5èmes Journées Scientifiques, Octobre 2008, Paris, France.

3. **Spée M.**, Beaulieu M., Marchal L., Dervaux A., Lazin D., Thierry A.M, Le Maho Y., Raclot T. Hormonal control of the refeeding signal: effect of exogenous corticosterone in Adélie penguins. Xth SCAR International Biology Symposium, Juillet 2009, Sapporo, Japan.

4. **Spée M.**, Beaulieu M., Thierry A.M., Dervaux A., Lazin D., Le Maho Y., Raclot, T. Both corticosterone and prolactin affect locomotor activity but corticosterone is more relevant to mimic a late stage of fasting in captive Adélie penguins (*Pygoscelis adeliae*). 7th European Ornithologists' Union Conference. Août 2009, Zurich, Suisse.

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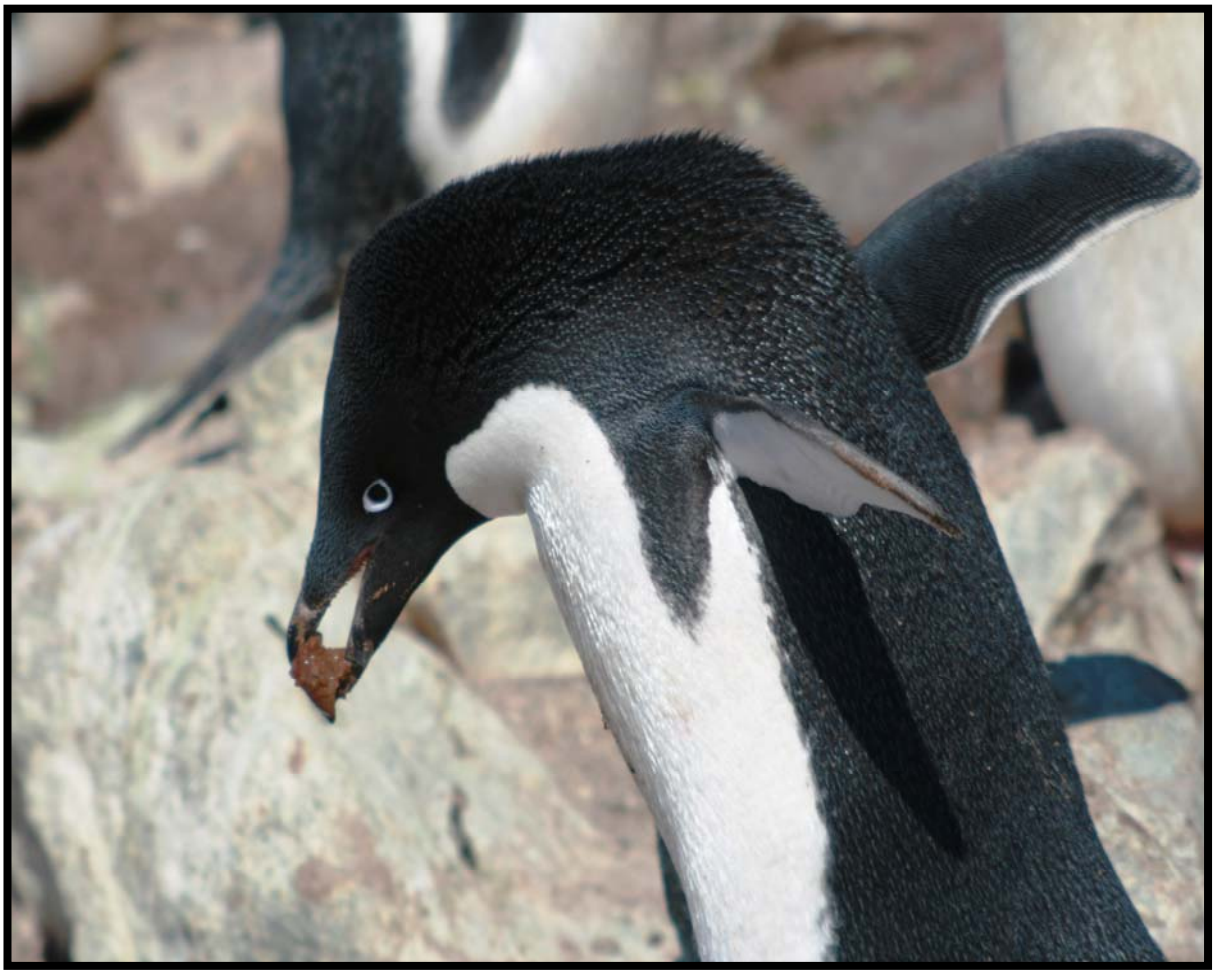
Sommaire

Chapitre 1

Introduction



1) Contexte général

Dans chaque écosystème, la quantité d'énergie dont les êtres vivants disposent est limitée et doit être allouée aux différentes fonctions biologiques telles que la croissance, la reproduction et la survie. L'acquisition de quantités suffisantes d'énergie pour subvenir aux exigences énergétiques des individus et la gestion de cette énergie comptent parmi les principaux facteurs qui détermineront les décisions d'allocation et ainsi le degré de pérennité des espèces et des populations. A l'échelle de l'organisme, le contrôle de la quantité d'énergie assimilée et dépensée et ainsi le maintien de la balance énergétique est un élément crucial permettant de s'adapter à son milieu. Les organismes sont en effet contraints tout au long de leur vie de faire des compromis pour allouer l'énergie dont ils disposent aux différentes fonctions. Ainsi, l'augmentation de l'énergie allouée à l'une des fonctions résultera en une diminution de l'énergie allouée à l'autre (Stearns 1992 ; Zera et Harshman 2001). La théorie des traits d'histoire de vie prédit que les décisions adoptées par les individus doivent leur permettre d'optimiser la transmission de leur patrimoine génétique aux générations futures, *i.e.* leur valeur sélective (Williams 1966 ; Stearns 1992). Par conséquent, pendant la reproduction chez les espèces itéropares (*i.e.* espèces chez lesquelles les individus peuvent tenter plusieurs événements reproducteurs au cours de leur vie), un compromis peut exister entre l'investissement dans la reproduction actuelle d'une part et la capacité à survivre qui permettra de tenter d'autres événements reproducteurs d'autre part. L'investissement reproducteur correspond ainsi au temps et à l'énergie allouée à la reproduction (Trivers 1972). Il comprend l'investissement sexuel (temps et énergie consacrés à la recherche et l'obtention d'un partenaire) et l'investissement parental (temps et énergie consacrés aux soins des jeunes).

L'impact négatif de l'investissement parental sur les chances de survie et de reproductions futures représente le coût de la reproduction.

Les études sur les oiseaux ont été à la pointe de l'élaboration d'une compréhension mécanistique de la diversification de l'histoire de vie dans un contexte naturel (Stearns, 1989 ; Ricklefs 2000 ; Ricklefs et Wikelski 2002). De plus, les oiseaux présentent un large spectre quant à l'importance de leur investissement reproducteur et notamment dans les soins apportés aux jeunes. L'ampleur de l'attention parentale envers les œufs et les poussins dépend du degré de maturité à l'éclosion et de l'état de développement des poussins, et varie le long du spectre « nidifuge – nidicole ». Le comportement parental est essentiellement destiné à la distribution des soins aux jeunes et comprend la présence au nid et la recherche alimentaire. Chez les espèces nidifuges (poussins mobiles et émancipés thermiquement dès l'éclosion), la phase au nid se limite à l'incubation et l'élevage correspond à un simple gardiennage. Chez les espèces nidicoles (poussins thermiquement dépendants des parents), la phase au nid comprend l'incubation et l'élevage des poussins, au moins jusqu'à ce que ces derniers deviennent indépendants thermiquement. Chez ces espèces, la période d'élevage est plus élaborée et comprend l'apport de chaleur et le nourrissage des poussins. Chez 90 % des espèces d'oiseaux, les deux parents prennent part à l'incubation et/ou l'élevage des poussins (Kokko et Jennions 2008). Cela implique une présence constante au nid d'au moins un des parents pendant une durée plus ou moins longue (4-5 jours chez le manchot pygmée *Eudyptula minor* , Chiaradia et Kerry 1999; jusqu'à 4 mois chez le manchot empereur *Aptenodytes forsteri*, Robin et al. 1998). Du fait de la distance séparant les zones de recherche alimentaire et le site de reproduction, ces périodes au nid sont en général synonymes de jeûne. Pour pallier cela, deux stratégies sont possibles chez les espèces biparentales : 1) un des deux parents effectue des voyages alimentaires et approvisionne son partenaire (*ex.* les rapaces, Donozar et al. 1992) ou les deux parents alternent présence à terre et recherche de nourriture (*ex.* les oiseaux marins, Tveraa et al. 1997).

2) Décisions d'allocation

Les stratégies d'histoire de vie et les décisions de reproduction peuvent être orientées par les conditions climatiques, la disponibilité alimentaire (Drent et Dann 1980 ; Weimerskirch et al. 2001 ; Reid et al. 2003), mais aussi par « l'état » de l'individu, qui regroupe entre autres paramètres son âge, la qualité de son territoire, sa capacité à acquérir de la nourriture et ses réserves énergétiques (McNamara et Houston 1996 ; Fig. 1).

2.1. Longévité, âge et expérience

Le choix d'un individu d'allouer plus d'énergie à la reproduction actuelle ou à la survie, et donc à la reproduction future, va dépendre de l'importance relative de ces deux composantes en termes de maximisation de la valeur sélective. Par exemple, à l'échelle de l'espèce, les individus d'espèces longévives devraient se comporter en «parents prudents» et ne pas mettre en péril leur propre survie lors de la reproduction (Drent et Daan 1980; Stearns 1992), puisque le succès reproducteur cumulé sur leur durée de vie est avant tout fonction de la survie des adultes plutôt que de la fécondité saisonnière (Williams 1966). Chez ces espèces, les individus devraient donc maintenir leur survie aux dépens de la reproduction en cours, si celle-ci devient trop coûteuse en énergie (Stearns 1992 ; Fig. 1). Au contraire, les individus des espèces à durée de vie courte devraient maximiser la reproduction en cours, au risque de mettre en péril leur chance de survie. Ainsi, Chastel et collaborateurs (1995a) mettent en évidence chez trois Procellariiformes à durée de vie plus ou moins longue deux stratégies différentes dans les décisions de reproduction lorsque les conditions environnementales avant la saison de reproduction sont défavorables et affectent la condition corporelle des oiseaux. En effet, les auteurs mettent en évidence qu'un grand nombre d'individus ne s'engagent pas dans la reproduction ou s'engagent mais désertent leur œuf chez le pétrel bleu *Halobaena caerulea* (oiseau longévif). En revanche, chez deux autres espèces à durée de vie plus courte (le prion de Belcher *Pachyptila belcheri* et le

pétrel plongeur *Pelecanoides urinatrix*), les individus maintiennent leur engagement dans la reproduction, probablement en s'investissant davantage durant l'épisode reproducteur.

A l'échelle intra-individuelle, les individus peuvent avoir des intérêts divergents à s'investir dans un épisode de reproduction donné en fonction de la valeur de la reproduction actuelle comparée à celle de reproductions futures. Ainsi, en fonction de leur âge et de leur expérience, les individus adoptent des stratégies de reproduction qui diffèrent (Fig. 1). Plus précisément, un individu âgé a tout intérêt à s'investir au maximum dans la reproduction en cours puisque les chances de tenter des événements reproducteurs futurs sont faibles (Wingfield 1995 ; Wingfield et Sapolsky 2003 ; Heidinger et al. 2006). Réciproquement, un individu jeune ou inexpérimenté à intérêt à maximiser ses chances de survie et tenter d'autres événements reproducteurs si la reproduction en cours est trop contraignante.

2.2. Disponibilité alimentaire et acquisition d'énergie

L'acquisition d'énergie d'un individu va dépendre de la disponibilité des ressources dans l'environnement mais aussi de sa capacité à trouver les proies (Fig. 1). Celles-ci doivent être accessibles et abondantes « au bon moment ». Ainsi, la phénologie de la reproduction (*i.e.* l'entrée en période de reproduction) va dépendre de la disponibilité des proies, elle-même sous la dépendance des conditions climatiques (Durant et al. 2007), et peut être décalée en fonction de ces paramètres (Monaghan et al. 1992 ; Meijer et Drent 1999 ; Barbraud et Weimerskirch 2006). A titre d'exemple, la date de ponte chez des hirondelles bicolores *Tachycineta bicolor* vivant en Amérique du nord a été avancée d'une dizaine de jours entre les années 60 et 90 (Dunn et Winkler 1999). Les auteurs proposent que cet avancement dans la phénologie de la reproduction de ces oiseaux soit dû au décalage de l'émergence des insectes aériens (proies des hirondelles bicolore), leur abondance étant directement reliée à la température de l'air. Ceci peut ainsi expliquer que la période de reproduction coïncide généralement avec une forte disponibilité des ressources dans l'environnement. Les parents ayant à nourrir leurs poussins

pourront ainsi trouver un maximum de ressources disponibles pour assurer leur propre maintenance et celle de leur progéniture.

L'acquisition d'énergie via l'approvisionnement alimentaire est susceptible d'agir comme un facteur proximal influençant les décisions de reproduction des individus. La reproduction étant une période du cycle de vie particulièrement coûteuse en énergie, l'acquisition d'énergie avant la saison de reproduction est une étape cruciale qui permettra à l'organisme de subvenir à ses exigences énergétiques. Ceci est d'autant plus important chez les espèces qui utilisent leurs réserves endogènes accumulées avant la saison de reproduction pour assurer le succès de celle-ci (« capital breeder »). Par exemple, face à une pénurie alimentaire au début de la saison de reproduction, les parents peuvent décider de ne pas se reproduire et attendre la prochaine saison (Drent et Dann 1980). L'acquisition d'énergie pendant la saison de reproduction est également une étape importante 1) chez les reproducteurs sur approvisionnement, et 2) chez les espèces qui effectuent des voyages alimentaires pour se réapprovisionner et assurer l'élevage des poussins au cours de leur cycle de reproduction (ex : les oiseaux marins).

Au final, la disponibilité des ressources dans l'environnement influe sur le niveau de réserves énergétiques que l'organisme pourra acquérir et par ce biais participe à l'orientation des décisions de reproduction (Fig. 1). Par exemple, une diminution de la disponibilité en lançons *Ammodytes marinus* (proies des sternes arctiques *Sterna paradisaea*) dans les îles Shetland a permis de mettre en évidence une relation étroite entre pénurie alimentaire, condition corporelle des adultes et investissement dans la reproduction (Monaghan et al. 1989, 1992).

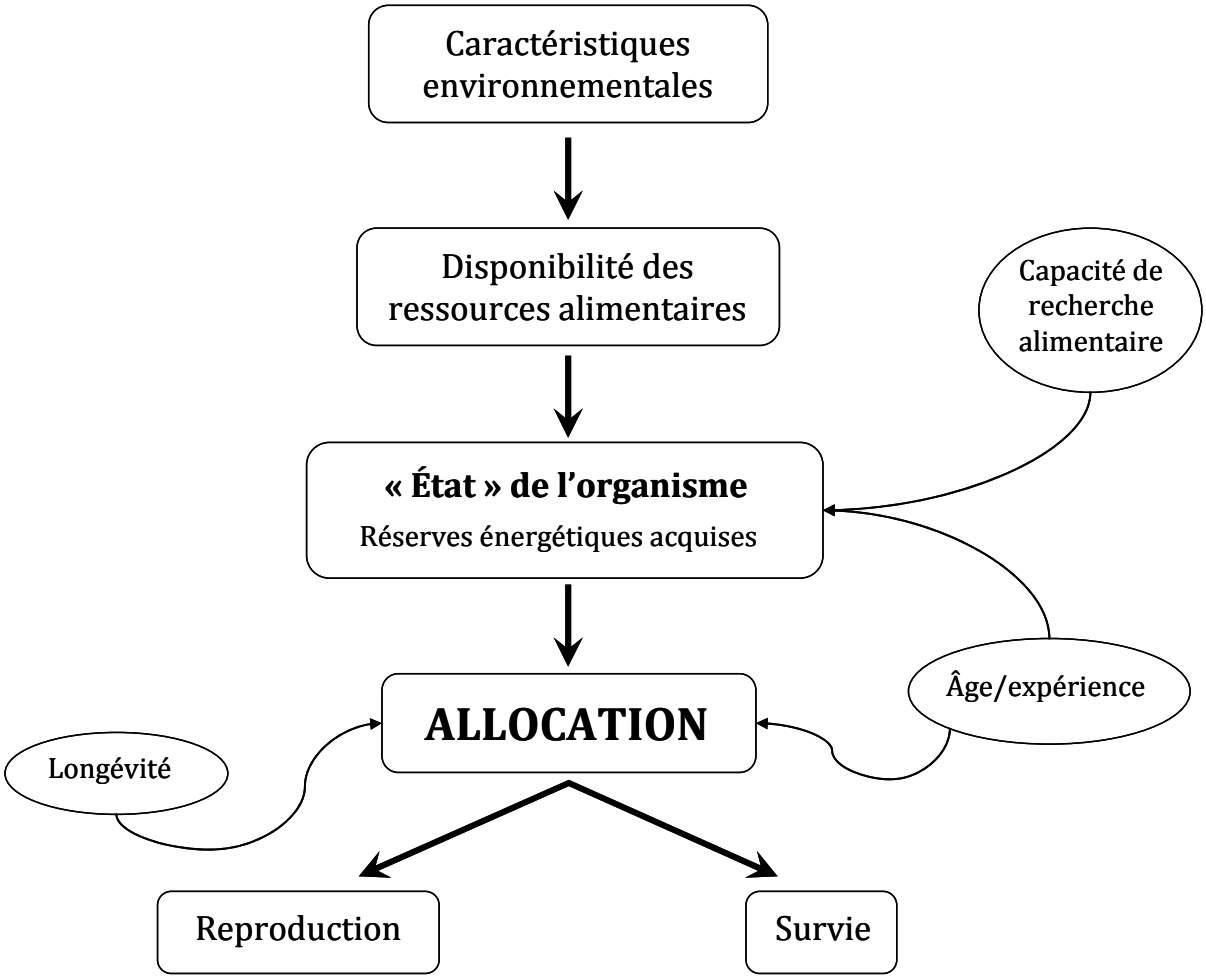


Figure 1. Lors de la reproduction, un compromis peut exister entre l'investissement de l'énergie disponible dans la reproduction actuelle d'une part et la capacité à survivre qui permettra de tenter d'autres événements reproducteurs d'autre part. Cette allocation d'énergie est influencée par les caractéristiques environnementales, la disponibilité des ressources alimentaire et « l'état » de l'organisme. L'état de l'organisme est notamment fonction des ressources énergétiques dont il dispose, de son âge et peut être affecté par sa capacité à rechercher de la nourriture. A l'échelle de l'espèce, la durée de vie (longévité) des individus influe également sur les décisions d'allocation.

2.3. Réserves énergétiques et condition corporelle

Les exigences énergétiques de la reproduction affectent la condition corporelle des individus (Monaghan et al. 1989) et celle-ci a en retour un effet sur l'investissement reproducteur (Fig. 1). D'après Drent et Dann (1980), l'état des réserves énergétiques est un facteur crucial pouvant influer sur la décision de l'individu à 1) entreprendre, ou 2) mener à terme ou non une période de reproduction. Dans cette optique, Chastel et collaborateurs (1995b) observent que la condition corporelle avant la reproduction du mâle ou de la femelle pétrel bleu influence la décision de se reproduire. De plus, Vleck et Vleck (2002) montrent que des manchots Adélie femelles qui viennent sur le site de reproduction mais sans se reproduire ont une masse corporelle inférieure de 10-12 % par rapport à celles qui s'engagent dans la reproduction. Cependant, certains oiseaux « décident » de se reproduire quand même, au risque de ne pas mener à bien leur reproduction. Ainsi, Barbraud et Chastel (1999) ont mis en évidence que des femelles pétrels des neiges *Pagodroma nivea* en mauvaise condition corporelle avaient un faible succès à l'éclosion. De plus, des manchots royaux *Aptenodytes patagonicus* qui abandonnent leur œuf avaient une masse corporelle plus faible en début de reproduction que ceux qui ont mené à terme l'incubation (Gautier-Clerc et al. 2001). Une condition corporelle faible en début de saison de reproduction pourrait être attribuée à l'âge et/ou à l'expérience de l'individu (Chastel et al. 1995b; Limmer et Becker 2007) et pourrait résulter d'une capacité plus ou moins importante à rechercher de la nourriture.

Au cours de la période d'incubation ou d'élevage des poussins, la condition corporelle influe positivement sur la taille des repas donnés aux poussins (Tveraa et al. 1998), leur condition corporelle (O'Dwyer et al. 2006) ou leur vitesse de croissance (Takahashi et al. 2003). Réciproquement, une mauvaise condition corporelle est corrélée à une augmentation du temps passé à rechercher de la nourriture aux dépens du temps passé au nid (Angelier et al. 2007a). De plus, l'atteinte d'un niveau critique de réserves énergétiques pendant l'incubation ou pendant la période d'élevage des poussins peut entraîner l'abandon de la reproduction (Cherel et al. 1994 ; Chaurand et Weimerskirch 1994; Ancel et al. 1998 ; Groscolas et al. 2008).

L'acquisition de quantités suffisantes de réserves énergétiques, le stockage et l'utilisation de ces réserves, ainsi que l'énergie qui en découle sont des facteurs essentiels modulant les décisions de reproduction. Ceci revêt une importance particulière chez les oiseaux marins qui se nourrissent en mer et sont donc sujets aux variations des ressources alimentaires dues à l'hétérogénéité et au dynamisme spatio-temporels des écosystèmes marins. De plus, ils doivent pour se reproduire effectuer de longues périodes de jeûne à terre pendant lesquelles leur survie repose sur les réserves énergétiques accumulées avant la saison de reproduction. Les réserves énergétiques se présentent ainsi sous deux formes : 1) les glucides, stockés principalement sous formes de glycogène dans le foie et les muscles, et qui sont rapidement mobilisables, et 2) les lipides, stockés sous formes de triacylglycérols dans le tissu adipeux blanc, qui représentent une forme de réserves énergétiques mobilisable sur le long terme et notamment lors d'un déficit énergétique (*ex.* jeûne ou exercice prolongé). Les protéines représentent une forme alternative de substrat énergétique en cas de déplétion sévère. D'autre part, les oiseaux marins sont longévifs et se reproduisent tous les ans ou tous les deux ans (ex : le grand albatros *Diomedea exulans*, Weimerskirch et al. 1992 ; Angelier et al. 2006a). Ils ont donc de nombreuses occasions de se reproduire au cours de leur vie et sont ainsi particulièrement amenés à faire des compromis pour allouer l'énergie dont ils disposent dans la reproduction actuelle ou dans leur capacité à survivre et à tenter d'autres événements reproducteurs futurs.

3) Un modèle d'étude du compromis survie / reproduction : le jeûne prolongé pendant l'incubation

De nombreux compromis n'interviennent que lorsque l'énergie est limitée. Le compromis entre reproduction et survie n'apparaît qu'en situation de stress nutritionnel (Stearns 1992). Les oiseaux qui font face à une période de jeûne prolongé au cours de leur

reproduction sont enclins à atteindre un stade de stress nutritionnel et peuvent ainsi avoir à choisir entre poursuivre leur épisode reproducteur ou assurer leur survie.

3.1. Les phases du jeûne prolongé

Le jeûne prolongé se divise en 3 phases, définies par l'évolution de la perte de masse spécifique (ou vitesse d'amaigrissement) et caractérisées par une mobilisation séquentielle des substrats énergétiques (Le Maho et al. 1981 ; Cherel et al. 1988a ; Robin 1998 ; Fig. 2).

- La phase I (PI), dite d'adaptation, est caractérisée par un amincissement rapide, un épuisement des réserves de glycogène (Cherel et al. 1992) et une mobilisation progressive des réserves lipidiques (Le Maho et al. 1981, Cherel et al. 1988a).
- La phase II (PII), dite d'économie, est la plus longue du jeûne prolongé. La diminution de la masse y est lente et régulière, les lipides constituant la principale source d'énergie (Cherel et al. 1988a ; Robin et al. 1998). Les lipides possèdent en effet une teneur énergétique plus élevée que celle des glucides et des protéines (Cherel et al. 1988b). Ceci se traduit par une augmentation du niveau plasmatique d'acides gras libres issus de la lipolyse du tissu adipeux et de corps cétoniques (β-hydroxybutyrate, βOHB) provenant de l'oxydation des lipides. La production de glucose est assurée par la néoglucogenèse hépatique. La principale adaptation au jeûne prolongé est la mise en place d'une épargne protéique liée à un faible catabolisme protéique, comme en témoigne le niveau faible d'acide urique, produit final du métabolisme azoté chez les oiseaux (Cherel et al. 1988a ; Robin et al. 1998). On note tout de même une utilisation incompressible des protéines liée aux besoins énergétiques des tissus gluco-dépendants (principalement le cerveau) qui nécessitent une formation continue de glucose à partir de précurseurs endogènes incluant les acides aminés. L'adiposité en début de jeûne déterminera l'efficacité de cette épargne protéique et la durée de la phase II (Le Maho et al. 1988 ; Cherel et al. 1992). Cependant une réserve trop importante de graisse apparaît comme néfaste. En effet, il a été mis en évidence que lors d'une longue privation de nourriture, la perte

faible mais cumulée de protéines pouvait entraîner la mort soudaine de certains patients obèses alors que leurs réserves lipidiques n'étaient pas épuisées (Le Maho et al. 1988). Dans ce cas, la perte cumulée des protéines apparaît comme le facteur limitant pour la survie.

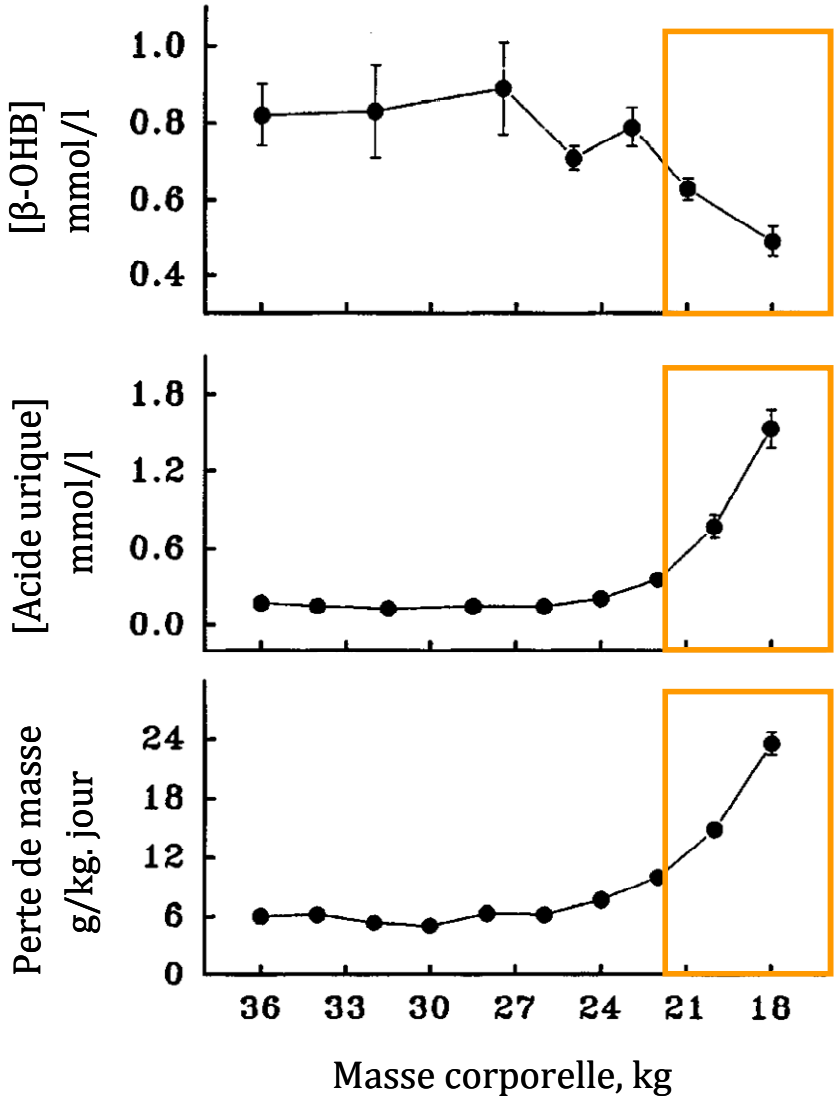


Figure 2. Evolution de la perte de masse spécifique et des taux plasmatiques d'acide urique et de β-hydroxybutyrate (β-OHB) en fonction de la masse corporelle chez des manchots empereurs au cours du jeûne prolongé. Les parties blanche et encadrée en orange représentent respectivement la phase II et la phase III de jeûne. *Modifié de Robin et al. 1998*.

- La phase III (PIII), dite critique, est caractérisée par une levée de l'épargne protéique comme l'atteste l'augmentation du niveau plasmatique d'acide urique (Cherel et al. 1988a). Cette augmentation serait le reflet de l'augmentation de la protéolyse mais aussi de la diminution de la protéosynthèse (Cherel et al. 1991). Parallèlement, on note une diminution de la contribution des lipides à la dépense énergétique (Cherel et al. 1988a). A ce stade, les lipides ne sont pas totalement épuisés (Robin et al. 1998). L'entrée en PIII n'est donc pas la simple conséquence d'un épuisement des réserves lipidiques. Par ailleurs, l'augmentation du catabolisme protéique anticipe l'épuisement total des réserves adipeuses, ce qui suggère que la disponibilité d'une certaine quantité de réserves lipidiques apparaît importante pour la survie (Groscolas et Robin 2001).

3.2. Modifications hormonales

L'entrée en période de jeûne s'accompagne également de modifications hormonales qui permettent une mobilisation séquentielle des réserves énergétiques, en modulant les métabolismes lipidiques et protéiques. Au cours de la majeure partie du jeûne (PII), les niveaux plasmatiques d'hormones thyroïdiennes, de corticostérone (CORT) et d'insuline sont maintenus à un niveau bas tandis que les niveaux plasmatiques de glucagon augmentent progressivement, participant ainsi à la diminution du métabolisme de base, à la mise en place de l'épargne protéique et à la mobilisation des réserves lipidiques (Cherel et al. 1988a). Lors de l'entrée en PIII, les niveaux de CORT et de glucagon augmentent et participent respectivement à l'augmentation de l'utilisation des protéines et au maintien de la néoglucogenèse à partir des acides aminés (Le Ninan et al. 1988; Cherel et al. 1988a).

3.3. Le signal de réalimentation

Malgré de longues périodes de jeûne (jusqu'à 50 jours chez le manchot Adélie *Pygoscelis adeliae*, Vleck et Vleck 2002 ; jusqu'à 4 mois chez le manchot empereur, Robin et al. 1998), aucun animal n'a été retrouvé mort suite à l'épuisement de ses réserves énergétiques. Ces observations ont conduit à émettre l'hypothèse de l'existence d'un signal de réalimentation qui inciterait l'animal à entreprendre une activité de recherche alimentaire pour assurer sa survie, aux dépens de l'activité en cours (Le Maho et al. 1988 ; Robin et al. 1998 ; Groscolas et Robin 2001). D'un point de vue évolutif, le concept du signal de réalimentation n'est valide que s'il permet à l'animal de survivre et d'être capable de restaurer ses réserves en mer. Le fait que les individus qui ont abandonné leur nid suite à l'atteinte de la PIII survivent ultérieurement (manchot empereur, Robin et al. 1998) et reviennent sur la colonie après s'être nourris en mer avec une masse corporelle similaire à celle des oiseaux ayant été relevés par la femelle (manchot royal, Robin et al. 2001 ; Gautier-Clerc et al. 2001) illustre l'efficacité du signal de réalimentation en termes de mécanisme adaptatif dans le compromis entre la survie et la reproduction.

L'hypothèse du signal de réalimentation a été testée chez des manchots empereurs en période de reproduction mais non incubant et gardés en captivité. Leur masse corporelle ainsi que leur activité locomotrice (nombre de pas par jour) ont été suivies tout au long du jeûne (Robin et al. 1998). Les manchots sont très peu mobiles pendant la plus grande partie du jeûne. Cependant, l'activité locomotrice augmente lorsqu'un niveau critique de réserves énergétiques est atteint. Cette modification comportementale est interprétée comme une motivation accrue à rechercher de la nourriture. Les variations des concentrations de βOHB et d'acide urique (marqueurs respectifs de l'utilisation des lipides et des protéines comme substrats énergétiques) attestent que ces modifications comportementales surviennent lors de l'entrée en PIII (Robin et al. 1998). La question concernant le comportement d'un oiseau en cours d'incubation lorsqu'il atteint un niveau critique de déplétion de ses réserves énergétiques s'est ensuite posée. En suivant le comportement d'incubation et l'évolution de la température de l'œuf chez le manchot royal, Groscolas et collaborateurs (2000) ont mis en évidence des abandons

transitoires de plus en plus fréquents et de plus en plus longs avant l'abandon définitif de l'œuf, reflétant ainsi une augmentation de la motivation à rechercher de la nourriture et une diminution de la motivation à incuber.

Ainsi, lors de l'atteinte d'un niveau critique de déplétion des réserves énergétiques, un signal de réalimentation se met en place et incite l'oiseau à partir en mer se réalimenter. Ce signal permet ainsi à l'organisme d'allouer ses ressources encore disponible vers la survie, au dépens de la reproduction en cours.

3.4. Hypothèses quant à l'origine et la nature du signal de réalimentation

Plusieurs hypothèses ont été avancées pour préciser l'origine et la nature de ce signal de réalimentation.

Robin et collaborateurs (1998) suggèrent que la stimulation de la motivation à se réalimenter est associée à l'atteinte d'un niveau seuil d'adiposité. Une diminution de la contribution de l'oxydation des acides gras à la production énergétique pourrait en effet résulter d'une diminution de la libération des acides gras par le tissu adipeux et serait à l'origine des changements métaboliques qui mènent de façon ultime à la stimulation de la recherche alimentaire (Robin et al. 1998 ; Groscolas et Robin 2001). Cette hypothèse est toutefois infirmée par les travaux de Bernard et al. (2002a) qui montrent que ni la lipolyse ni la disponibilité en acides gras libres ne sont réduites chez des manchots royaux en PIII, mais au contraire augmentées. Dans la continuité, une autre étude menée chez le manchot royal a montré que l'utilisation d'un bloqueur de l'oxydation des acides gras entrainait notamment une augmentation du catabolisme protéique et de la corticostéronémie, mimant ainsi les transitions métaboliques et endocriniennes caractéristiques de l'entrée en PIII (Bernard et al. 2002b). Basés sur ces résultats les auteurs ont proposé que la corticostérone (CORT) pourrait être le lien entre l'oxydation des acides gras et le catabolisme protéique.

3.5. Hormones : médiateurs du compromis survie / reproduction

Groscolas et Robin (2001) proposent que les hormones pourraient agir comme médiateurs entre les modifications métaboliques et comportementales qui se produisent lors de l'entrée en phase critique de jeûne (PIII) et ainsi être des acteurs clés dans l'induction du signal de réalimentation. En effet, parmi les facteurs potentiels qui peuvent jouer un rôle dans la réorientation du comportement reproducteur vers un comportement visant à assurer la survie, les hormones apparaissent comme les mécanismes fonctionnels gouvernant les décisions de reproduction (Sinervo et Svennson 1998; Ricklefs et Wikelski 2002 ; Bokony et al. 2009). Chez les oiseaux, le comportement parental est ainsi sous le contrôle de deux hormones principales : la corticostérone qui favorise le comportement de recherche alimentaire et la prolactine qui tient un rôle opposé en stimulant la promotion des soins parentaux.

3.5.1. La corticostérone

La corticostérone (CORT) est le principal glucocorticoïde chez les oiseaux. Elle est sous le contrôle de l'ACTH hypophysaire (*Adrenocorticotropic Hormone*), elle-même sous la dépendance de la CRH hypothalamique (*Corticotropin-Releasing Hormone*), de l'AVT (*Arginine Vasotocin*) et de la mésotocine (Romero et al. 1998a). Elle est sécrétée par les glandes corticosurrénales en réponse à l'activation de l'axe hypothalamo-hypophyso-surrénalien. L'arrêt de la libération de CORT est sous son propre contrôle, *via* un mécanisme de rétrocontrôle négatif au niveau de l'hypothalamus (Romero 2004). Les événements stressants comme des conditions climatiques contraignantes (Rogers et al. 1993 ; Astheimer et al. 1995 ; Romero et al. 2000) ou l'attaque d'un prédateur (Scheuerlein et al. 2001 ; Wingfield 2003) sont définis comme des stimuli néfastes et imprévisibles pour l'individu (Romero 2004). Ils activent l'axe hypothalamo-hypophyso-surrénalien et l'augmentation importante des niveaux de CORT qui en résulte redirigent le comportement et la physiologie des individus vers la promotion de leur survie (Wingfield et al. 1998 ; Sapolsky 2000 ; Wingfield 2003). Des niveaux élevés de CORT stimulent notamment le

catabolisme protéique et lipidique (Cherel et al. 1988 ; Gray et al. 1990 ; Landys et al. 2004 ; Sapolsky 2000), fournissant ainsi l'énergie nécessaire au comportement de fuite. La CORT apparaît ainsi comme étant un facteur endocrinien essentiel dans la médiation de la « réponse au stress », ce terme faisant référence aux mécanismes physiologiques et comportementaux qui suivent l'événement stressant (Romero 2004).

n dehors de ces événements stressants, les taux de CORT varient en fonction du ratio « énergie disponible/demande énergétique » et sont d'autant plus élevés que ce rapport est faible (Landys et al. 2006). Ainsi la sécrétion de CORT augmente lorsque la quantité de ressources disponibles diminue (Kitaysky et al. 1999, 2007 ; Buck et al. 2007 ; Doody et al. 2008), lorsque la demande énergétique est accrue, comme par exemple pendant la période d'élevage des poussins (Chastel et al. 2005 ; Love et al. 2004), ou bien lorsque les réserves énergétiques de l'individu sont faibles (Kitaysky et al. 2001a ; Lynn et al. 2003).

On admet communément que les situations prévisibles d'augmentation de la demande énergétique, telle que la période de jeûne que certaines espèces endurent pendant leur cycle de reproduction, ne constituent pas un stress dans la mesure où les organismes agissent de manière anticipatrice sur leur physiologie et leur comportement (Landys et al. 2006), en emmagasinant par exemple une importante quantité de réserves. Le concept d'allostasie, défini comme l'ensemble des mécanismes physiologiques et comportementaux qui permettent à un individu confronté à des perturbations d'atteindre un état interne stable (*i.e.* un état d'homéostasie), est basé sur la balance entre l'énergie disponible et la demande énergétique et est valable pour les événements prévisibles et imprévisibles de l'environnement (McEwen et Wingfield 2003, Wingfield 2005). Ainsi, lorsque l'augmentation (bien que prévisible) de la demande énergétique se prolonge, elle peut surpasser les capacités de réponse de l'organisme, la quantité d'énergie dont il dispose étant limitée. Ceci provoque une « surcharge allostatique » et l'individu entre alors dans un « état d'urgence ». Les réponses physiologiques et comportementales qui s'en suivent incitent l'organisme à réorienter son activité en cours vers la survie (Wingfield et al. 1998). La CORT a été associée à l'initiation et l'orchestration de cet « état

d'urgence » et provoque des modifications du comportement et de la physiologie des individus ayant pour but d'assurer leur survie (Wingfield et al. 1998 ; Wingfield et Kitaysky 2002 ; McEwen et Wingfield 2003 ; Wingfield 2003, 2005). La PIII correspond ainsi à « l'état» évoqué précédemment. Elle peut également être définie comme un état de « stress nutritionnel », puisque l'atteinte d'un niveau critique de déplétion des réserves corporelles (PIII) entraîne une augmentation importante des niveaux de CORT (Cherel et al. 1988a ; Robin et al. 1998 ; Groscolas et al. 2008 ; Fig. 3). Cette définition est en accord avec celle de Cockrem (2007) qui décrit le stress comme étant un stade où l'axe hypothalamo-hypophyso-surrénalien est activé et où la sécrétion de CORT augmente en réponse à une perturbation.

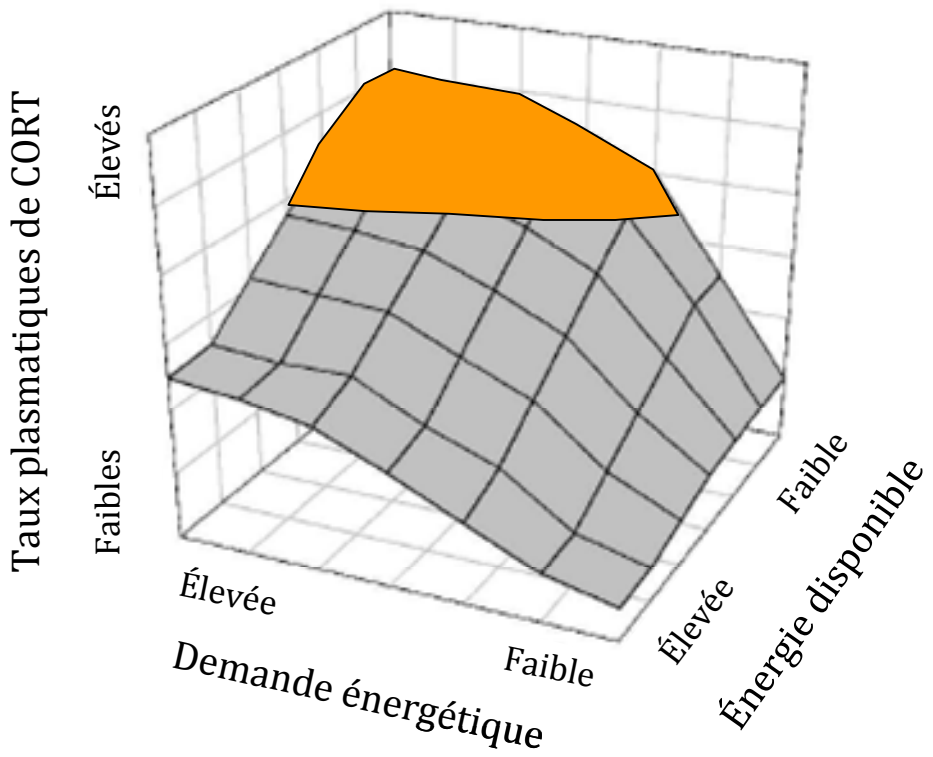


Figure 3. Les taux plasmatiques de CORT sont influencés par le ratio « énergie disponible / demande énergétique ». Les niveaux élevés de CORT (partie orangée) font référence à l'entrée dans un « stade d'urgence ». *Modifié de Landys et al. 2006*.

Ainsi, les niveaux de CORT intègrent les stimulations exogènes (ex : l'occurrence d'événements stressants ; Wingfield et al. 1998) et endogènes (bilan énergétique de l'organisme ; Landys et al. 2006) que supporte l'organisme. Cette hormone apparaît donc comme un médiateur physiologique important capable de moduler les décisions de reproduction en fonction du capital énergétique limité et de ce fait de balancer l'allocation des réserves énergétiques entre la reproduction en cours et la survie de l'animal.

L'action de la CORT sur la physiologie et le comportement va cependant dépendre de sa concentration plasmatique (Romero 2004 ; Landys et al. 2006). La CORT peut notamment se fixer à deux types de récepteurs génomiques à affinités différentielles. Le premier possède une forte affinité pour la CORT et est activé par des niveaux faibles de l'hormone, alors que le second possède une faible affinité et est activé par des taux élevés de CORT. Ainsi, des niveaux bas ou élevés de CORT vont induire des effets différents sur la physiologie et le comportement des organismes en fonction de l'affinité des récepteurs exprimés et fixant l'hormone (Romero 2004 ; Landys et al. 2006). Tandis que des concentrations élevées de CORT plasmatique sont corrélées à une augmentation de la recherche alimentaire (Angelier et al. 2007b), et stimulent l'activité locomotrice (Astheimer et al. 1992 ; Challet et al. 1995 ; Breuner et al. 1998) ; celles-ci provoquent en parallèle une réduction voire une suppression de l'effort parental (Wingfield 2003 ; Wingfield et Sapolsky 2003). En effet, une augmentation expérimentale des niveaux de CORT entraîne une augmentation du temps passé en mer aux dépens du temps passé à élever les poussins de mouette tridactyle *Rissa tridactyla* (Kitaysky et al. 2001b) et des niveaux élevés de CORT ont été associés à l'abandon de la reproduction chez le manchot royal (Groscolas et al. 2008).

Dans certaines situations écologiques, les individus peuvent cependant atténuer leur « réponse au stress », en modulant l'augmentation du niveau de CORT plasmatique sécrétée. Cette élévation des niveaux de CORT est couramment mesurée lors d'un stress de contention qui consiste à capturer et maintenir captif l'animal pendant une durée de 30 minutes (Wingfield 1994), mais peut également faire référence à l'augmentation des niveaux de CORT suite à un

stress nutritionnel. Une diminution de la sécrétion de CORT en réponse au stress peut être observée chez des oiseaux se reproduisant dans des régions où l'environnement est extrême et où la saison de reproduction est courte (Silverin et al. 1997; Silverin et Wingfield 1998 ; O'Reilly et Wingfield 2001). Ceci pourrait s'avérer être une réponse adaptative dans le sens où l'abandon de la reproduction suite à un stress ponctuel (condition climatique défavorable, attaque d'un prédateur) s'accompagne de l'impossibilité temporelle d'en tenter une autre. La sécrétion de CORT en réponse au stress est également atténuée pendant les phases de reproduction où les parents sont le plus investis, notamment pendant les périodes d'incubation et d'élevage des poussins comparées à la période précédant la ponte (Reneerkens et al. 2002 ; Meddle et al. 2003 ; Holberton et Wingfield 2003 ; Pereyra et Wingfield 2003). L'âge et l'expérience des individus est aussi un facteur intervenant dans l'amplitude de la réponse corticale au stress, avec notamment les individus âgés qui montrent une diminution de leur sensibilité au stress (Heidinger et al. 2006).

La sécrétion de CORT semble inversement associée à celle de la prolactine (Chastel et al. 2005 ; Groscolas et al. 2008 ; Angelier et al. 2009a ; Angelier et Chastel 2009). Il apparaît donc essentiel d'examiner en parallèle les niveaux de CORT et de prolactine pour étudier les mécanismes endocriniens qui, lorsque les réserves énergétiques de l'organisme atteignent un niveau de déplétion critique, pourraient stimuler le départ en mer pour rechercher de la nourriture et ainsi provoquer l'abandon de la reproduction.

3.5.2. La prolactine

La prolactine est une hormone protéique sécrétée par les cellules lactotrophes de l'adénohypophyse. Sa libération est sous le contrôle inhibiteur de la dopamine et stimulateur du peptide intestinal vasoactif (*Vasoactive Intestinal Peptide* ou VIP), qui est le facteur hypothalamique de libération de la prolactine chez les oiseaux. Alors que chez les mammifères, les cellules lactotrophes ont spontanément un fort taux de sécrétion de prolactine et sont sous le

contrôle inhibiteur de la dopamine, la sécrétion de prolactine chez les oiseaux n'est pas spontanément élevée et est principalement sous le contrôle stimulateur du VIP (Sharp et al. 1998 ; Sockman et al. 2006).

La prolactine est décrite comme la principale hormone impliquée dans l'expression du comportement parental chez les oiseaux (Buntin 1996 ; Vleck 2002). Elle conditionne l'initiation et le maintien des soins parentaux (Buntin 1996) et alloparentaux (Vleck et al. 1991 ; Schoech et al. 1996). Elle stimule le comportement d'incubation (Youngren et al. 1991 ; Sockman et al. 2000) et le nourrissage des poussins (Duckworth et al. 2003). Elle semble être également impliquée dans la transition de l'activité sexuelle vers l'activité parentale (Sockman et al. 2006). En effet, pendant la période de formation des couples, le niveau de prolactine est bas alors que le niveau des hormones sexuelles stéroïdiennes est élevé (testostérone chez les mâles et œstrogènes et progestérone chez les femelles) (Vleck et al. 1999). Ce profil s'inverse après l'accouplement et la ponte ; les concentrations d'hormones stéroïdiennes diminuent et restent à un niveau bas pendant l'incubation et l'élevage des poussins alors que la concentration de prolactine augmente (Vleck et al. 1999). L'induction de la sécrétion de prolactine pourrait ainsi être liée à l'engagement de l'oiseau dans la reproduction, et être amorcée par des stéroïdes sexuels. En effet, chez des manchots empereurs capturés avant l'accouplement et mis en parc, la concentration de prolactine n'augmente pas (Lormée et al. 1999). De plus, chez des dindes *Meleagris gallopavo* ayant subi une ovariectomie, l'administration de prolactine n'induit l'adoption d'un comportement d'incubation que lorsque les oiseaux sont prétraités avec des stéroïdes sexuels (œstrogène et progestérone ; El Halawani et al. 1986).

La sécrétion de prolactine est initiée au moment de la ponte et est maintenue pendant la période d'incubation chez la majorité des oiseaux (Vleck 2002). Son mode de sécrétion diffère lors de la suite du cycle reproducteur en fonction du type de soins parentaux prodigués (Vleck 1998). Chez les espèces à poussins précoces (poussins mobiles et émancipés thermiquement dès l'éclosion), la concentration de prolactine décroît rapidement après la ponte et atteint de nouveau son niveau initial. En revanche, chez les espèces dont les poussins sont thermiquement

dépendants des parents, elle reste élevée pendant la phase d'élevage du (des) poussins, au moins jusqu'à ce que ce(s) dernier(s) atteignent l'indépendance thermique (Vleck 2002). Chez toutes ces espèces, la présence de stimuli tactiles, visuels ou auditifs émanant des œufs ou des poussins est nécessaire au maintien de la sécrétion de prolactine (Hall 1987; Richard-Yris et al. 1998). Le mode de sécrétion de prolactine diffère également selon la participation relative des deux sexes aux soins parentaux. Les concentrations de l'hormone sont plus élevées chez les canes colvert *Anas platyrhynchos* que chez les mâles, ces derniers ne participant ni à l'incubation ni à l'élevage des poussins (Goldsmith et Williams 1980). Chez les espèces biparentales, les concentrations de prolactine sont généralement plus élevées chez les femelles mais suivent le même profil chez les deux sexes au cours de la reproduction (Vleck et al. 1999).

Chez les oiseaux marins, et en particulier chez les manchots, les niveaux de prolactine restent quasi inchangés pendant toute la période d'élevage (Garcia et al. 1996 ; Lormée et al. 1999 ; Vleck et al. 1999 ; Fig. 4). La sécrétion de prolactine chez ces espèces semble être programmée de façon endogène, indépendamment de stimuli externes et 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 (Lormée et al. 1999). Cette hypothèse est soutenue par le fait que la prolactinémie reste relativement élevée même après un échec de la reproduction (Lormée et al. 1999 ; Vleck et al. 2000a). Ce mode de sécrétion pourrait aussi expliquer des comportements atypiques tels que l'adoption ou le rapt de poussins par des parents en échec de reproduction (Angelier et al. 2006b).

Cependant, la sécrétion de prolactine peut être influencée par des paramètres externes. Par exemple, une diminution de la prolactine est observée lors d'événements stressants (Chastel et al. 2005). Delehanty et collaborateurs (1997) ont montré chez des phalaropes de Wilson *Phalaropus tricolor* en période de reproduction que les niveaux de prolactine sont diminués lorsque les conditions environnementales sont défavorables. Enfin, chez les manchots royaux, des niveaux faibles de prolactine ont été reliés à un niveau de déplétion critique des réserves énergétiques (PIII) et à l'abandon de la reproduction (Cherel et al. 1994 ; Groscolas et al. 2008).

Ainsi, puisque la concentration de prolactine est associée au comportement parental et diminue lorsque la survie de l'individu est menacée, cette hormone pourrait être impliquée dans l'activation de « l'état d'urgence » et influer sur l'issue du compromis entre la reproduction et la survie.

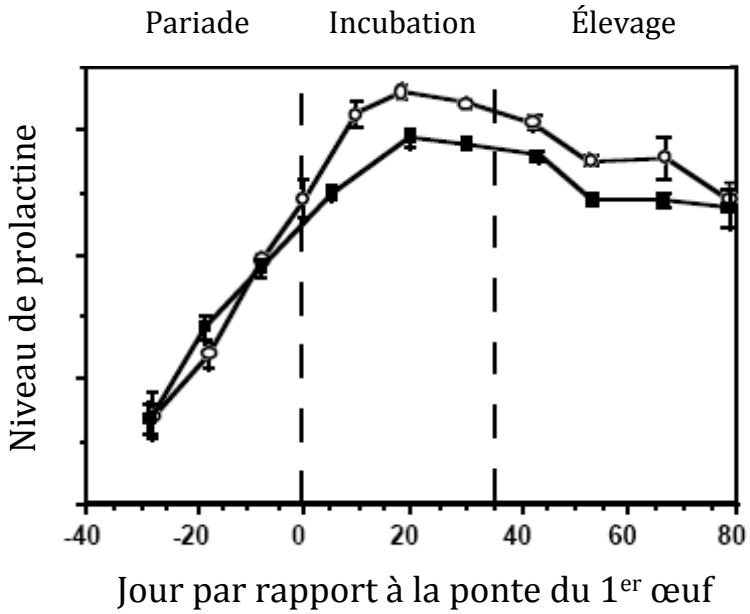


Figure 4. Pattern de sécrétion de prolactine chez les manchots Adélie mâles (carrés noirs) et les femelles (ronds blancs) en fonction du jour par rapport à la ponte du 1er œuf. *Repris de Vleck et al. 1999*.

4) Objectifs et modèle d'étude

4.1. Objectifs

Le signal de réalimentation qui incite les oiseaux à adopter un comportement de recherche alimentaire afin d'assurer leur survie lorsque leurs réserves énergétiques atteignent un niveau critique peut être défini comme l'activation d'un « état d'urgence ». Chez les oiseaux en cours de reproduction, il semble être le résultat d'un système complexe intégratif mettant en jeu des hormones impliquées dans le contrôle du comportement parental. En effet, chez les

oiseaux en cours d'incubation, (contrairement à un animal non engagé dans un cycle de reproduction) une composante supplémentaire s'ajoute : la motivation à incuber. La décision à prendre chez eux est donc plus « complexe » puisqu'il faut que l'individu abandonne la reproduction en cours pour partir se réalimenter. Il y a chez eux un « frein » supplémentaire, une étape de plus à passer. La question est donc : dans quelle mesure la motivation à partir se réalimenter dépasse-t-elle celle à incuber ? Ces deux motivations contraires semblent être orchestrées, respectivement, par les niveaux de CORT et de prolactine. En effet, les études à ce jour indiquent que l'abandon du nid a lieu en PIII et est associé à des niveaux élevés de CORT et faibles de prolactine chez les oiseaux en cours de reproduction (incubation ou élevage des poussins).

Le but de cette thèse est d'examiner les mécanismes sous-jacents à l'abandon du nid en précisant le rôle respectif de la CORT et de la prolactine chez un oiseau marin longévif, le manchot Adélie. Il s'agira notamment de déterminer dans quelles mesures ces deux hormones interagissent pour influencer l'issu du compromis entre la reproduction en cours et la survie.

Pour ce faire, nous avons utilisé des approches corrélatives et expérimentales, chez des oiseaux sauvages en cours de reproduction et captifs en échec de reproduction. Ces derniers ont perdu leur(s) œuf(s) pour des raisons non liées à leur état nutritionnel (prédation, inondation…). Ils nous ont permis d'effectuer des mesures intra-individuelles répétées et de déterminer la cinétique de paramètres métaboliques (β-hydroxybutyrate et acide urique, témoignant respectivement de l'utilisation des lipides et des protéines comme substrat énergétique), endocriniens (prolactine, CORT) et comportementaux (activité locomotrice) au cours du jeûne prolongé (approche corrélative) ou suite à une augmentation de CORT ou une diminution de prolactine (approches expérimentales).

En premier lieu, nous avons cherché à répondre aux questions suivantes (approche corrélative sur oiseaux sauvages):

1) **L'entrée en PIII est-elle systématiquement associée à l'abandon de la reproduction** ?
2) **La CORT et la prolactine sont-elles concomitamment affectées par la contrainte énergétique prolongée, *i.e.* l'entrée en PIII ?**

Pour ce faire, nous avons déterminé le statut nutritionnel et hormonal de manchots Adélie mâles et l'avons relié au succès d'incubation (relève de la femelle ou abandon du nid).

L'étude de Criscuolo et al. (2005) a mis en évidence par une approche expérimentale que l'augmentation seule de le CORT ne suffisait pas à provoquer l'abandon du nid chez des femelles Eiders à duvet *Somateria mollissima*. Les auteurs proposent que cette absence d'effet pourrait être due à 1) une faible durée du traitement, puisque les niveaux de CORT retrouvent un niveau basal 4 jours après le traitement, 2) à une dose trop forte. Sachant que l'action de la CORT sur l'activité locomotrice est dépendante de sa concentration (Breuner et al. 1998 ; Breuner et Wingfield 2000), nous avons implanté en sous-cutané de la CORT à différentes doses et diffusant pendant une longue période afin de répondre aux questions suivantes :

1) **La CORT exogène est-elle susceptible de mimer les modifications métaboliques, hormonales et comportementales caractéristiques de l'entrée en PIII ? Si oui, à quelle dose ?** (approche expérimentale sur oiseaux captifs)
2) **Le maintien de taux élevés de CORT affecte-t-il l'occurrence des abandons du nid ? Les niveaux de prolactine en sont-ils affectés ?** (approche expérimentale sur oiseaux sauvages)

Sachant que la CORT peut modifier le succès reproducteur subséquent (Criscuolo et al. 2005), nous avons également déterminé l'effet du traitement sur la durée des voyages alimentaires, le nombre d'œufs et de poussins et la masse de la couvée.

Enfin, sachant que la prolactine tient un rôle clé dans l'expression du comportement parental chez les oiseaux (Buntin 1996 ; Vleck 2002), nous avons implanté de la bromocriptine

(inhibiteur de la sécrétion de prolactine) à différentes doses et diffusant pendant une longue période pour répondre aux questions suivantes :

1) **Une diminution expérimentale du niveau de prolactine plasmatique provoque-t-elle les modifications comportementales caractéristiques de l'entrée en PIII, affecte-t-elle le niveau plasmatique de CORT et induit-elle un shift dans le type de substrat énergétique utilisé?** (approche expérimentale sur oiseaux captifs)
2) **Une diminution seule du niveau de prolactine plasmatique entraîne-t-elle une diminution de l'attention parentale, au point de stimuler la désertion du nid ? Affecte-t-elle le succès reproducteur subséquent (durée des voyages alimentaires, nombre d'œufs et de poussins, masse de la couvée) ?** (approche expérimentale sur oiseaux sauvages).

4.2. Le manchot Adélie, modèle d'étude

Les manchots Adélie sont des oiseaux marins. A ce titre, ils présentent des stratégies d'histoire de vie extrêmes caractérisées par une grande longévité (âge maximum estimé à 20 ans), une maturité sexuelle retardée (âge de première reproduction : 5 ans) et une faible fécondité (2 œufs par reproduction) (Ainley 2002). Ils sont monogames (*i.e.* n'a qu'un seul partenaire par saison de reproduction) et prodiguent des soins biparentaux.

Ils font partie avec les manchots empereurs des deux espèces de manchot se reproduisant uniquement en bordure du continent Antarctique. Ils se répartissent de 54° à 77° de latitude Sud (Williams 1995) et viennent se reproduire pendant l'été austral (d'octobre à mars, entre la débâcle estivale de la banquise et le début de l'embâcle hivernale). Ils se regroupent à terre et forment de larges colonies pouvant atteindre des dizaines de milliers de couples.

Leur cycle de reproduction comprend cinq stades : la pariade, l'incubation, le stade de garde, le stade de crèche et la mue (Fig. 5).

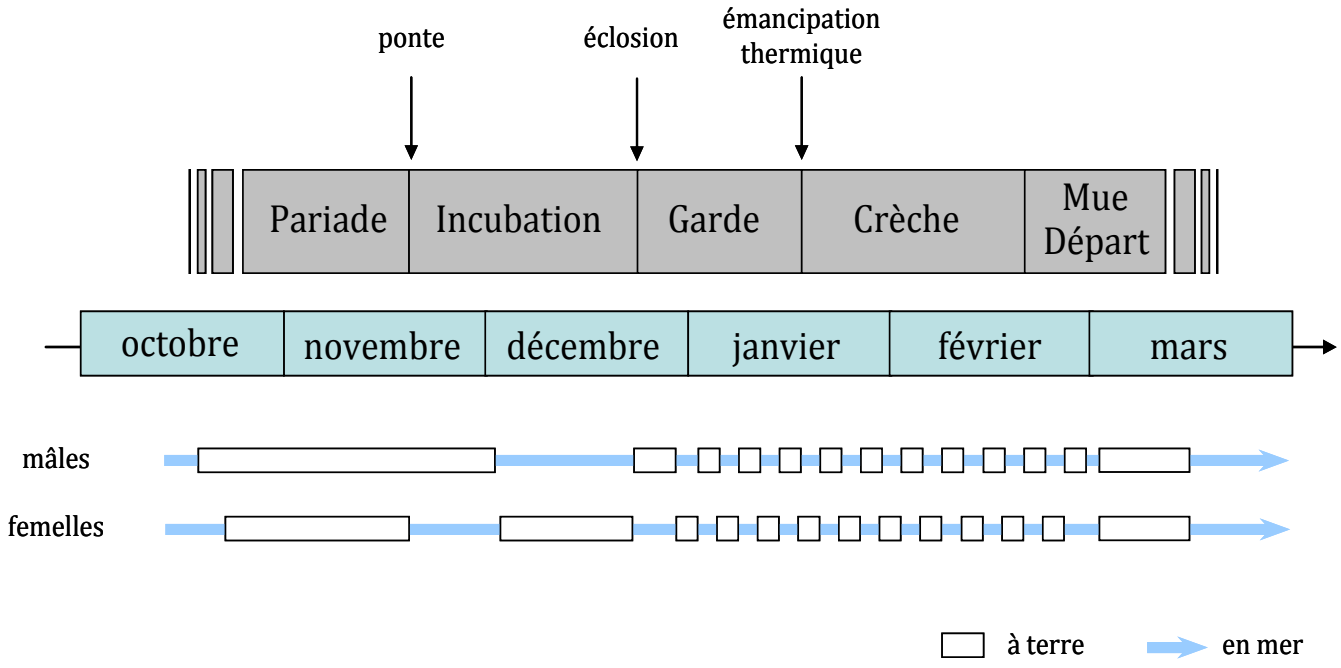


Figure 5. Le cycle de reproduction du manchot Adélie s'étend d'octobre à mars, entre la débâcle estivale de la banquise et le début de l'embâcle hivernale. Les deux membres du couple alternent présence à terre pour couver les œufs puis nourrir les poussins, et recherche alimentaire en mer.

1) Pariade. Les mâles arrivent sur le site de reproduction, suivis quelques jours après des femelles qui choisissent leur partenaire. Les deux membres du couple construisent un nid, le défendent, paradent et s'accouplent.

2) Incubation. La ponte de 1 à 2 œufs (1,9 en moyenne ; Ainley 2002) met fin à la période de pariade. Trois jours en moyenne séparent la ponte des deux œufs (Ainley 2002). La femelle part en mer se réalimenter et le mâle assure la première partie de l'incubation (*i.e.* le premier « shift » d'incubation), poursuivant ainsi son jeûne. Mâles et femelles alternent un ou deux voyages en mer jusqu'à la fin de l'incubation, au moment de l'éclosion des œufs. La durée de l'incubation est en moyenne de 34 jours.

3) Stade de garde. Cette phase dure en moyenne 3 semaines. Elle requiert la présence d'au moins un des deux parents pour protéger les poussins contre les prédateurs potentiels (skua

antarctique *Catharacta maccormicki*) et leur assurer un apport de chaleur. Ils alternent donc présence au nid et recherche alimentaire en mer, tous les 1 à 3 jours.

4) Stade de crèche. Les poussins ayant atteint leur indépendance thermique peuvent rester seuls et les parents peuvent partir simultanément en mer. Les poussins se regroupent et forment des « crèches » (définies par le regroupement d'au moins trois poussins).

5) Mue. Les manchots muent chaque année pour renouveler leur plumage. Les parents partent en mer emmagasiner suffisamment d'énergie pour la mue à venir. Les poussins sont les premiers à muer, ils perdent leur duvet qui est remplacé par des plumes. Les parents reviennent ensuite à terre et jeûnent pendant environ deux semaines où leur plumes sont renouvelées. Une fois la mue terminée, ils partent pour leur migration hivernale.

Les manchots Adélie représentent un modèle pertinent pour l'étude du compris entre la reproduction et la survie. En effet, ils se nourrissent en mer et se reproduisent à terre, ces périodes à terre étant synonymes de jeûne du fait de la distance séparant les zones d'alimentation des sites de reproductions. La durée de jeûne peut aller jusqu'à 50 jours chez les mâles au cours de la pariade et de l'incubation (Vleck et Vleck 2002). Ce sont des reproducteurs sur capital (« capital breeder »), qui ont donc à puiser dans leurs réserves endogènes accumulées avant la saison de reproduction pour subvenir aux exigences énergétiques de celle-ci. Les manchots Adélie ont ainsi pu mettre en place des adaptations métaboliques, hormonales et comportementales au jeûne prolongé. Si leurs réserves énergétiques atteignent un niveau critique de déplétion, ils peuvent être amenés à abandonner leur nid pour assurer leur survie. Du fait de leur longue durée de vie, la stratégie optimale pour un oiseau incubant et entrant en PIII de jeûne serait d'abandonner la reproduction en cours plutôt que de risquer sa propre survie. Dans le cadre du compromis entre la reproduction et la survie, l'abandon du nid correspond à une situation extrême où la survie est favorisée aux dépens de la reproduction en cours.

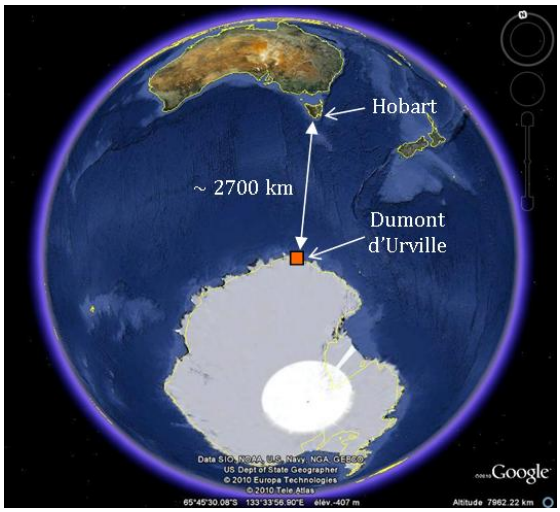
Chapitre 2

Méthodologie

1) Site d'étude

Au cours de ces trois années de thèse, j'ai effectué une campagne d'été (3 mois et demi) sur la base scientifique française de Dumont d'Urville, 66°40'S, 140°01'E (du nom de l'explorateur qui découvrit la Terre Adélie en 1840), en Terre Adélie. La Terre Adélie se trouve sur le continent Antarctique et constitue, avec les îles sub-antarctiques (Kerguelen, Crozet et Amsterdam), les Terres Australes et Antarctiques Françaises (TAAF). Elle représente la partie française de l'Antarctique et couvre environ 3% de l'ensemble du continent. La base scientifique française de Dumont d'Urville (Figure 6) se trouve à la pointe de la Terre Adélie, sur l'île des Pétrels, dans l'archipel de Pointe Géologie.

A



B

Figure 6. Situation géographique (A) et vue du ciel (B) de la base scientifique française de Dumont d'Urville, au bord du continent Antarctique à 2700 km environ de Hobart, Tasmanie, Australie.

2) Procédure

Pour étudier les mécanismes hormonaux impliqués dans l'induction de l'abandon du nid lors du jeûne d'incubation chez les manchots Adélie, nous avons utilisé des approches corrélatives et expérimentales sur oiseaux sauvages en cours de reproduction et captifs en échec de reproduction. Nous nous sommes particulièrement intéressés aux mâles. En effet, ils endurent la période de jeûne la plus longue qui peut durer jusqu'à 50 jours (Vleck et Vleck 2002) pendant la pariade et l'incubation. Si leurs réserves énergétiques atteignent un niveau critique de déplétion, ils peuvent être amenés à abandonner leur nid pour assurer leur survie.

Les données relatives à la réalisation de ce mémoire sont issues de 3 campagnes d'été (2004-05, 2005-06, 2007-08). Les travaux effectués ont été validés par le comité d'éthique de l'Institut polaire français Paul Emile Victor (IPEV).

La procédure générale a été la même lors de ces différentes campagnes d'été, ce qui a permis de suivre les oiseaux de façon identique.

2.1. Suivi des oiseaux sauvages en cours de reproduction

Dans le but de déterminer le statut nutritionnel et hormonal des oiseaux aux différents moments de leur cycle reproducteur, les manchots Adélie sauvages ont été capturées à deux occasions (Figure 7) : quelques jours avant la ponte et lors du départ en mer du mâle, consécutif au retour de la femelle ou à l'abandon du nid. Les manchots ont été suivis tout au long de la période de reproduction. Les nids ont été observés toutes les 2-3 heures pour déterminer quel membre du couple était présent, la relève de la femelle ou l'abandon du nid. Concernant les oiseaux non traités et en prenant en compte 1) la masse corporelle du mâle lors de la première capture et 2) la perte de masse journalière moyenne (0,052 kg/jour, Chappell et al. 1993; 0,042 kg/jour, Vleck and Vleck, 2002), il a été possible d'identifier au jour le jour les oiseaux d'intérêt, *i.e.* susceptibles d'atteindre une masse corporelle faible marquant l'entrée en PIII (voisine de 3,5 kg chez le manchot Adélie mâle, Cockrem et al. 2006). La pression d'observation a été

particulièrement importante pour ces quelques manchots. La durée des voyages alimentaires des deux membres du couple, les dates de ponte et d'éclosion ainsi que les nombres d'œufs et de poussins ont également été déterminés.

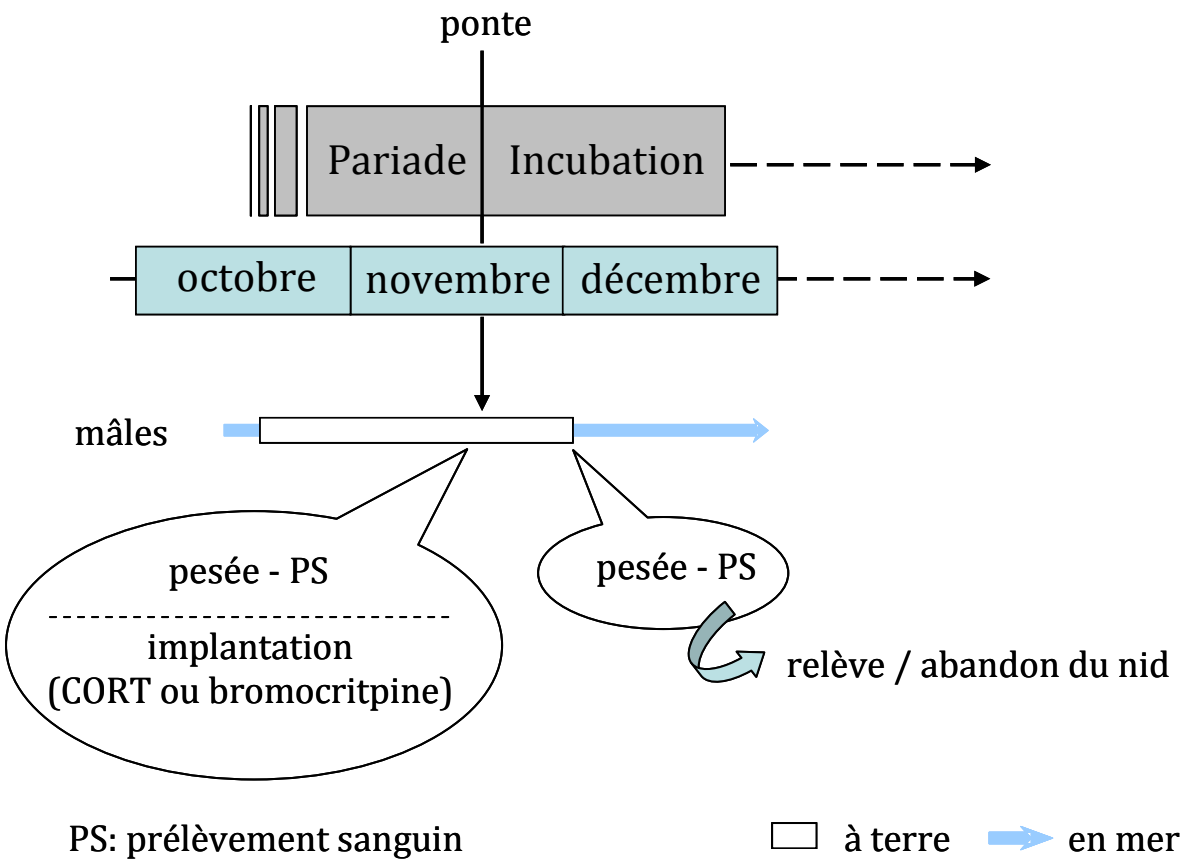


Figure 7. Les manchots Adélie mâles ont été capturés, pesés et un prélèvement sanguin à été effectué à deux occasions : quelques jours avant la ponte et lors du départ en mer du mâle (consécutif au retour de la femelle ou à l'abandon du nid). Concernant les approches expérimentales, les manchots ont été implantés avec de la CORT (corticostérone) ou de la bromocriptine quelques jours avant la ponte.

2.1.1. Marquage des oiseaux

Les deux membres du couple ont été capturés entre la formation des couples et la ponte. Cette échelle de temps est importante à respecter pour la capture des oiseaux, de façon à ne marquer que les couples bien établis et ainsi limiter le dérangement et la probabilité de divorce. Sachant que le dimorphisme sexuel est très peu marqué chez cette espèce, chaque manchot a été identifié grâce au marquage d'un numéro sur leur thorax à l'aide d'un colorant (Nyanzol ®). Ainsi, au sein d'un même couple, l'un des deux partenaires porte un numéro x et l'autre est marqué du même numéro additionné d'une barre (Figure 8).

Figure 8. Manchot numéro 59, marqué avec du Nyanzol ®.

2.1.2. Détermination du sexe

La détermination des sexes a été effectuée par une combinaison de paramètres incluant l'inspection cloacale avant la ponte (Figure 9), la routine de l'incubation (les mâles assurent dans la plupart des cas la première partie de l'incubation (Ainley 2002) et la mesure de lipémie plasmatique. En effet, lors de la période de ponte, les femelles ont à produire une couvée d'œufs

qui exigent le dépôt de grande quantité de lipides. De ce fait, elles présentent avant la ponte une lipémie plasmatique plus élevée que celle des mâles (Kern et al. 2005 ; Beaulieu et al. 2010).

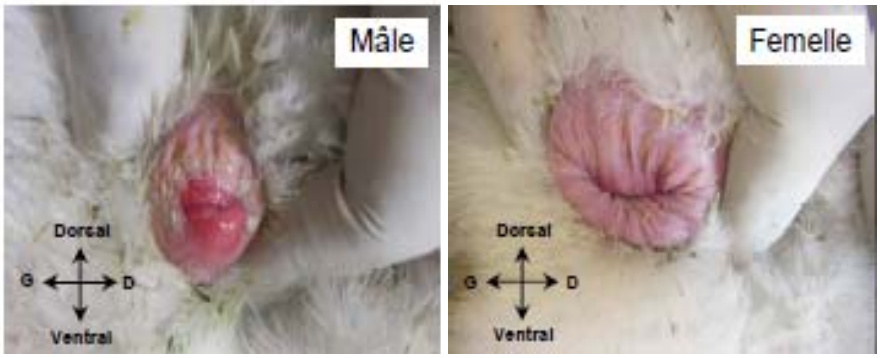


Figure 9. L'inspection cloacale quelques jours avant la ponte est un des paramètres utilisés pour la détermination des sexes des manchots Adélie.

2.2. Suivi des oiseaux captifs en échec de reproduction

Sachant que le nombre de capture possible est limité chez les oiseaux sauvages en cours de reproduction (afin d'éviter l'induction d'un stress trop important ou répété pouvant perturber le décours de l'incubation), nous avons également suivi un groupe d'oiseaux captifs. Ces derniers sont en échec de reproduction, c'est-à-dire qu'ils ont perdu leur(s) œuf(s) pour des raisons non liées à leurs états nutritionnels (prédation, inondation…). Ils nous permettent d'effectuer des mesures intra-individuelles répétées et de déterminer la cinétique de paramètres métaboliques, endocriniens et comportementaux (activité locomotrice) au cours du jeûne prolongé (approche corrélative) ou suite à une augmentation de corticostérone (CORT) ou une diminution de prolactine (approches expérimentales).

Les manchots captifs sont placés dans un enclos grillagé (5,20 m * 2,40 m) et sont soumis à un jeûne total, comme c'est le cas pour leur congénères toujours en cours de reproduction. Le nombre de manchots présents en même temps dans l'enclos ne dépasse pas 8 individus. Ils sont pesées tous les deux jours et des prélèvements sanguins sont régulièrement effectués (voir le détail dans la partie « Matériels et méthodes » des études 2 et 4). Un à deux jours après leur mise

en parc, les oiseaux sont équipés de deux podomètres, afin de mesurer leur activité locomotrice. Ces derniers ont été recouverts de mastic pour assurer leur étanchéité et collés avec de la colle forte (loctite) sur le plumage au niveau de la ceinture pelvienne en haut de chacune des pattes. Sur chaque oiseau, les deux podomètres donnent des valeurs proches et ceci nous permet de n'utiliser qu'un des deux podomètres si l'un d'eux vient à se casser (Figure 10). Ces derniers n'ayant pas été calibrés sur l'animal, les valeurs lues sont considérées comme des unités arbitraires. Ce sont les variations d'un podomètre donné au cours du temps pour un individu qui sont prises en compte. Les podomètres ont été relevés quotidiennement.

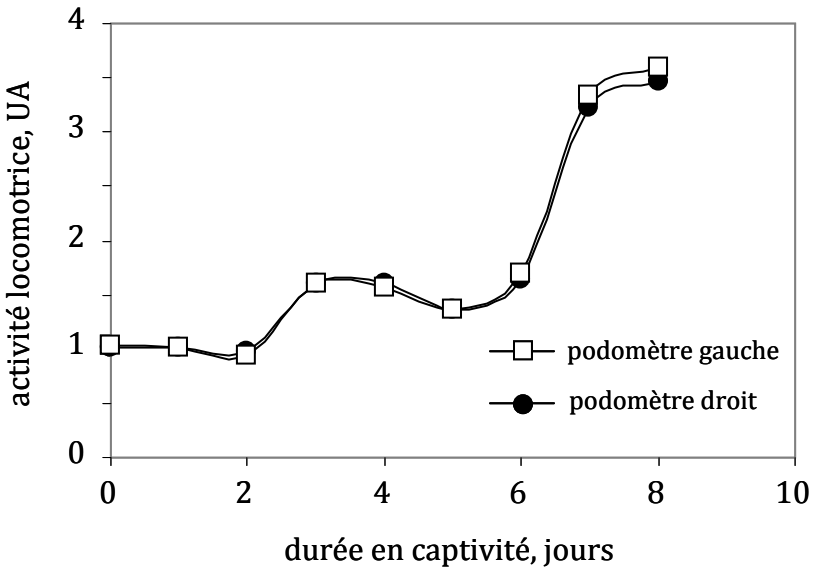


Figure 10. Evolution journalière des données enregistrées par des podomètres placés en haut des pattes gauche et droite chez un manchot Adélie captif.

2.3. Pesée, mesure morphométrique et prise de sang

Les manchots ont été pesés à l'aide d'une balance électronique (Ohaus, ± 2g) et la longueur de l'aileron gauche a été mesurée (± 1 mm).

Les prélèvements sanguins ont été effectués au niveau de la veine alaire ou de la veine de la patte, dans les cinq minutes suivant la capture de l'oiseau puisqu'il a été montré que les

manipulations d'une durée inférieure à 5 minutes n'avaient pas d'effet sur les niveaux basaux de CORT chez le manchot Adélie (Vleck et al. 2000b). Le sang recueilli est transvasé dans un tube Eppendorf contenant de l'héparine ou de l'EDTA (ethylenediamine tetraacetic acid). Deux types d'anticoagulant ont été utilisé puisque l'héparine a une activité lipolytique et pourrait ainsi altérer les mesures d'acides gras libres plasmatiques (rat, Patten et Hollenberg 1969 ; poulet, Cupp et al. 1987 ; humain, Millot et al. 1987). Les échantillons de sang sont centrifugés (5000 rpm, 10 minutes, 4°C), le plasma obtenu est aliquoté et conservé à -80°C jusqu'à ce qu'il soit analysé.

2.4. Approche expérimentale : implantation de CORT ou de bromocriptine

Dans le cas des approches expérimentales, les oiseaux ont été implantés avec différentes doses de CORT ou de bromocriptine (Innovative Research of America, Sarasota, FL, USA), de façon à augmenter leur niveau de CORT et à diminuer la concentration de prolactine plasmatique, respectivement. La bromocriptine est un agoniste de la dopamine (récepteur D2) qui inhibe la sécrétion de prolactine chez les mammifères (Roberts et al. 2001) et les oiseaux (Jouventin et Mauget 1996 ; Angelier et al. 2006 ; Reddy et al. 2007). Les implants ont une durée de diffusion de 21 jours. Une petite incision égale à la taille de l'implant a été faite et l'implant a été inséré sous la peau au niveau de la nuque. L'incision a été fermée avec un point stérile, nettoyée avec de la Bétadine puis aspergée de poudre d'aluminium.

2.5. Dosages des paramètres plasmatiques

Les dosages de métabolites et d'hormones relatifs à ce mémoire ont permis de déterminer le statut nutritionnel et endocrinien des oiseaux sauvages et captifs.

2.5.1. Métabolites

Les niveaux de métabolites plasmatiques sont connus pour varier au cours du jeûne prolongé et sont considérés comme étant de bons indicateurs du statut nutritionnel chez les oiseaux (Cherel et al. 1988a ; Jenni-Eiermann et Jenni 1998). Ils nous ont permis, avec la masse corporelle des individus, de déterminer dans quelle phase de jeûne les manchots de trouvaient lors de l'incubation.

Les concentrations plasmatiques en acide urique (kit de dosage Sigma Diagnostics) β-hydroxybutyrate (β-OHB), glucose (kits de dosage Randox) et en acides gras non estérifiés (kit de dosage Wako Oxoid) ont été déterminées à partir de volumes de 25 ; 20 ; 10 et 12,5µL de plasma, respectivement, selon les recommandations décrites par les fournisseurs. Les mesures de densité optique ont été réalisées à 520nm (pour l'acide urique), à 340nm (pour le β-OHB), à 505nm (pour le glucose) et à 550nm (pour les acides gras non estérifiés) en utilisant un spectrophotomètre.

Le principe du dosage plasmatique de ces métabolites repose sur la formation de produits colorés en proportions stoechiométriques connues. Ces produits sont issus de réactions enzymatiques mettant en jeu spécifiquement les métabolites à doser.

Les mesures de densité optique (DO) sont effectuées dans les maximums d'absorbance des différents produits colorés. En se référant à un ou plusieurs étalons de référence de concentration connue, une détermination de la concentration des métabolites d'intérêt est obtenue.

2.5.2. Hormones

La concentration plasmatique de corticostérone a été déterminée par méthode immuno-enzymatique (dosage EIA = dosage Elisa) en utilisant un kit issu du commerce (AssayMax Corticosterone ELISA Kit, EC3001-1, AssayPro). Le principe de ce dosage consiste à mettre en compétition un antigène « froid » (la corticostérone libre que l'on veut doser) et un antigène

conjugué (corticostérone biotinylée) pour les sites de liaison d'un anticorps. Les deux antigènes peuvent former des complexes avec les anticorps et ont la même affinité pour ces derniers. La compétition réside dans le fait que la quantité d'anticorps présente dans le milieu est insuffisante pour permettre la fixation de tous les antigènes. La quantité d'antigène conjugué introduite est connue, seule la quantité de l'antigène « froid » que l'on veut doser varie. Ainsi, plus la concentration de ce dernier est élevée, plus le nombre de complexes antigène « froid » - anticorps formés sera important, au détriment du nombre de complexes antigène conjugué – anticorps. Après avoir éliminé les antigènes libres qui n'ont pas formé de complexes, l'addition d'une enzyme qui réagira avec l'antigène conjugué permettra d'obtenir un produit coloré. L'intensité de cette coloration est déterminée à l'aide d'un lecteur de plaque Elisa et est proportionnelle à la quantité d'antigène « froid » présente dans le milieu. Par comparaison avec les concentrations des différents points de la gamme étalon définie par le fournisseur, on peut alors déterminer la concentration de l'hormone dans l'échantillon biologique.

La concentration plasmatique de prolactine a été déterminée en collaboration avec le Centre d'Etude Biologiques de Chizé (A. Lacroix et O. Chastel) selon le protocole décrit par Lormée *et al.* (2000). Le principe de ce dosage (radio-immunologique) repose, comme celui de la corticostérone, sur une compétition antigène – anticorps. La prolactine étant une hormone protéique, sa nature diffère en fonction des espèces. Son dosage nécessiterait donc la purification de prolactine standard pour l'espèce étudiée (ici le manchot Adélie) ainsi que la préparation d'anticorps spécifiques, ce qui n'est pas raisonnablement envisageable puisque cela nécessiterait le sacrifice d'un grand nombre d'animaux. Il n'existe pas de kit dans le commerce pour l'oiseau, en général et le manchot en particulier. La technique employée pour ce dosage utilise donc de la prolactine et des anticorps spécifiques issus d'espèces domestiques proches de celle que l'on étudie (prolactine de poulet et anticorps anti-prolactine de poulet) ; on parle de dosage hétérologue (Figure 11). Pour valider ce dosage, il faut effectuer une courbe dose réponse en réalisant des dilutions de plasma contenant l'hormone à doser et vérifier qu'elle est proche de celle issue de poulet. Comme pour la corticostérone, la concentration de l'hormone

dans l'échantillon biologique est déterminée par comparaison avec différents points d'une gamme étalon obtenue à partir de la dilution d'une solution standard de concentration connue.

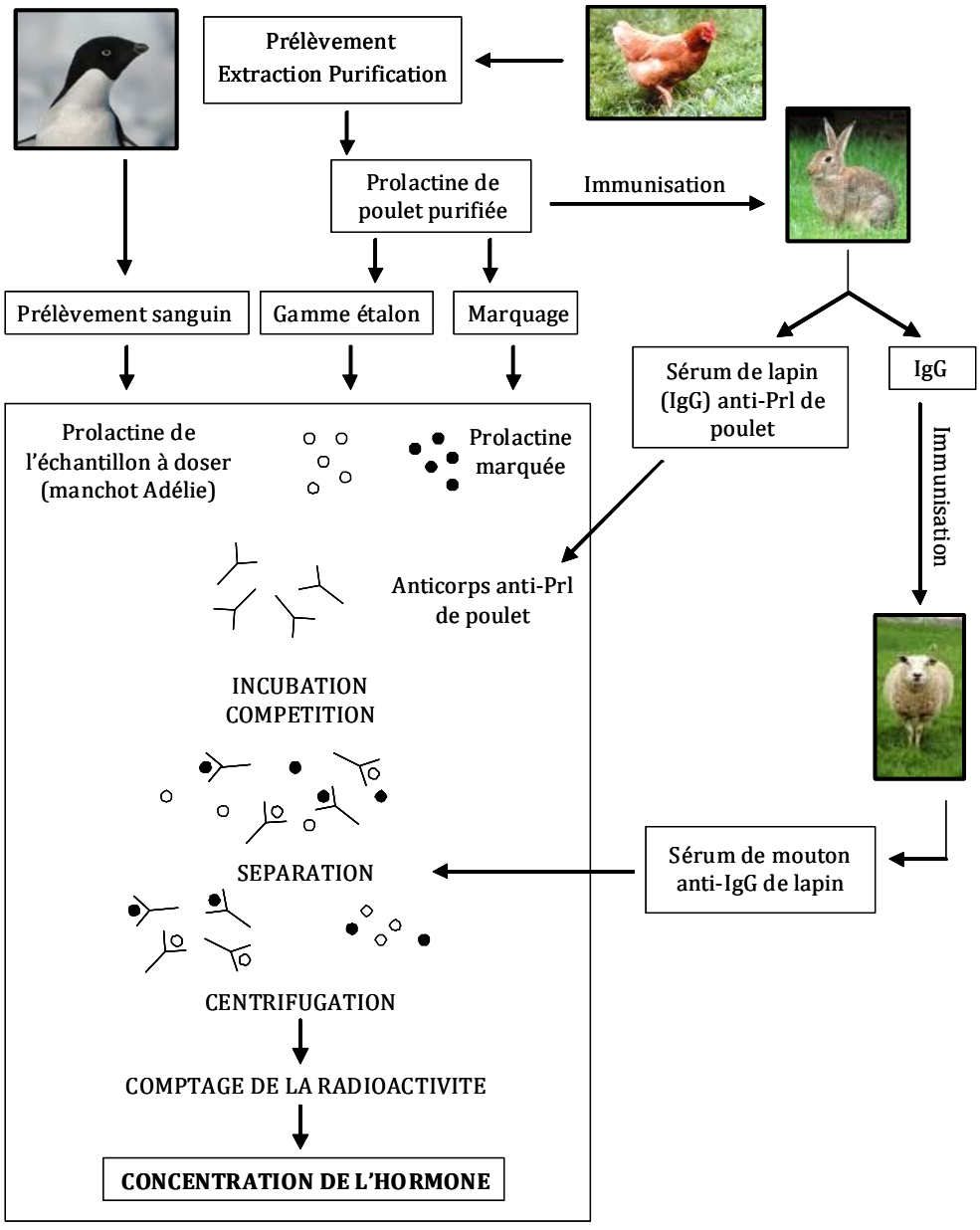
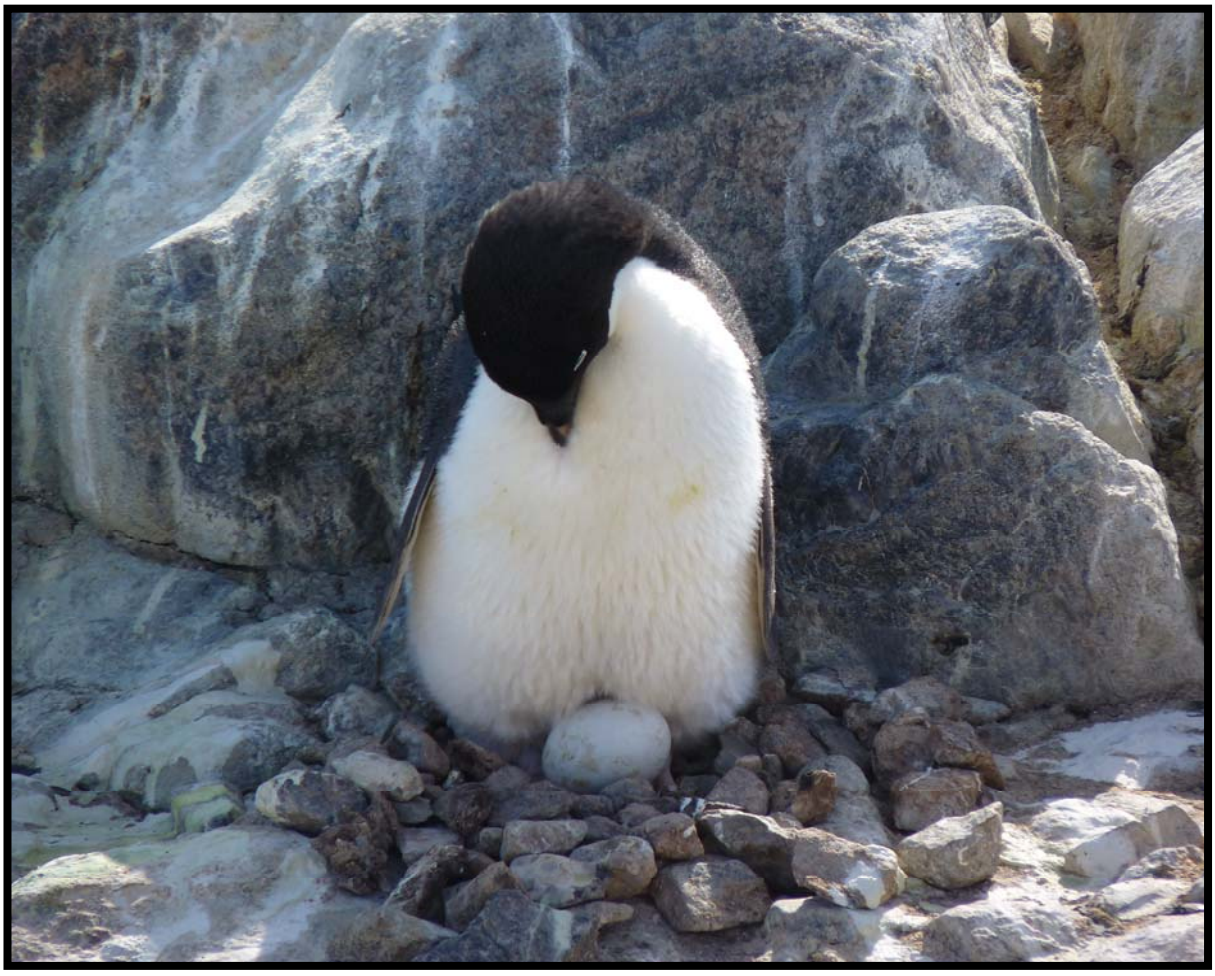


Figure 11. Principe du dosage hétérologue de la prolactine.

Etudes



Résumé - Etude 1

Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin

Introduction

La théorie des traits d'histoire de vie prédit que les oiseaux longévifs devraient favoriser leur propre survie lorsque la reproduction devient trop coûteuse. Chez certaines espèces d'oiseaux marins, les individus effectuent un jeûne prolongé lors de l'incubation. En dessous d'un seuil de déplétion critique des réserves énergétiques, les oiseaux entrent en phase III (PIII). A ce stade, des changements métaboliques caractérisés par une augmentation de l'utilisation des protéines et une diminution des lipides en tant que substrat énergétique principal surviennent. Ces modifications métaboliques sont associées à des changements comportementaux, tels que l'abandon du nid et la stimulation d'un comportement de recherche alimentaire, et à des changements hormonaux. Le comportement parental est en effet sous le contrôle de deux hormones ayant des effets opposés : la CORT et la prolactine, stimulant, respectivement, le comportement de recherche alimentaire et le comportement d'incubation. Les études à ce jour indiquent que chez les oiseaux en cours de reproduction (incubation ou élevage des poussins), le signal de réalimentation qui incite les oiseaux à abandonner leur nid a lieu 1) en PIII et 2) est associé à des niveaux élevés de CORT et faibles de prolactine. Il semble dans ce cas que la motivation à se réalimenter, reflétée par des niveaux hauts de CORT, dépasse celle à incuber, reflétée par des niveaux bas de prolactine.

Chez les manchots Adélie, le mâle et la femelle prennent part à l'incubation des œufs et à l'élevage des poussins, alternant présence à terre et recherche de nourriture en mer. Après la ponte, la femelle part en mer se réalimenter et c'est le mâle qui assure la première partie de l'incubation. Il est ainsi soumis à un jeûne prolongé et peut être susceptible d'atteindre la PIII et

d'abandonner son nid. Le but de cette étude est de déterminer 1) si l'entrée en PIII est systématiquement associée à l'abandon de la reproduction et 2) si la CORT et la prolactine sont concomitamment affectées par la contrainte énergétique prolongée (*i.e.* l'entrée en PIII).

Matériels et méthodes

Nous avons déterminé le statut nutritionnel (masse corporelle, niveaux plasmatiques de β-OHB et d'acide urique, témoins respectifs de l'utilisation des lipides et des protéines en tant que substrat énergétique) et hormonal (niveaux plasmatiques de CORT et de prolactine) de manchots Adélie mâles 1) quelques jours avant la ponte et 2) au moment du départ en mer du mâle (consécutif au retour de la femelle ou à l'abandon du nid). Parmi 27 mâles capturés en début d'incubation, 23 ont été relevés par la femelle alors que 4 ont abandonné leur nid.

Résultats et discussion

Les manchots en PIII qui abandonnent leur nid présentent des taux élevés de CORT et des niveaux faibles de prolactine. De façon intéressante, nous avons mis en évidence des oiseaux dans le même état nutritionnel (PIII) qui n'abandonnent pas le nid mais qui sont relevés par leur partenaire. Ces oiseaux présentent des taux élevés de CORT mais également des taux important de prolactine. Ainsi, dans certains cas, il y a un découplage entre le statut nutritionnel des oiseaux (PIII) et leur réponse comportementale. Ainsi, la PIII n'est pas systématiquement associée à l'abandon de la reproduction. De plus, les changements hormonaux à ce stade sont dynamiques, de telles sortes que la CORT et la prolactine sont successivement affectées par la contrainte énergétique prolongée (*i.e.* l'entrée en PIII). Ainsi, la CORT pourrait agir en premier mais ne semble pas suffisante à elle seule pour provoquer l'abandon du nid. Une approche expérimentale visant à augmenter la CORT permettrait de valider cette hypothèse.

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Should I stay or should I go? Hormonal control of nest abandonment in a long-lived bird, the Adélie penguin

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ABSTRACT

According to life-history theory, long-lived birds should favor their survival over the current reproductive attempt, when breeding becomes too costly. In seabirds, incubation is often associated with spontaneous long-term fasting. Below a threshold in body reserves, hormonal and metabolic shift characteristics of a switch from lipid to protein utilization (phase III, PIII) occur. These metabolic changes are paralleled by nest abandonment and stimulation of refeeding behavior. Parental behavior is then under control of two hormones with opposite effects: corticosterone (CORT) and prolactin which stimulate foraging and incubation behavior, respectively.

The aim of this study was to determine the respective role of these two hormones in nest abandonment by Adélie penguins. To this end, plasma hormone levels were measured before egg-laying and at departure from the colony (i.e. when birds were relieved by their partner or abandoned their nest), and related to nutritional state and incubation success.

We found that males abandoning their nest in PIII presented high CORT levels and low prolactin levels. Interestingly, males which presented high plasma levels of prolactin in PIII did not abandon. We show that although CORT is the first hormone to be affected by prolonged energy constraints, the combined effects of high CORT and low prolactin levels are necessary for parents to favor self-maintenance and abandon the nest. We provide insights into time-course changes of the endocrine profile as PIII proceeds and report that reaching proteolytic late fasting is not sufficient to induce nest abandonment in a long-lived bird.

Introduction

Life-history theory predicts that during the breeding season long-lived birds should favor their own survival over the current reproductive attempt when energetic constraints become too serious (Stearns, 1992). In several bird species, breeding is associated with fasting because foraging might not be possible during incubation. For instance, long fasting periods are common in seabirds, since they feed exclusively at sea whereas breeding occurs on land. To cope with such reproductive patterns (i.e. breeding associated with sustained fasting bouts), biparental species may adopt one of two strategies: either (1) one parent may remain constantly on the nest being provisioned by its partner (e.g. raptors, Donozar et al., 1992) or (2) both parents may alternate between nest attendance and foraging at sea (e.g. seabirds, Tveraa et al., 1997). The parent assuming incubation duty must have previously accumulated sufficient body reserves to sustain long-term fasting. However, birds are likely to discontinue incubation when energy reserves are reaching a critical point of exhaustion. Indeed, below a threshold in body reserves, birds enter phase III of fasting (PIII). At this stage, a metabolic shift occurs: uric acid levels increase (indicating protein catabolism), while plasma levels of β-hydroxybutyrate (βOHB) decrease, indicating a reduction in the utilization of lipids as the main energy substrate (Cherel et al., 1988; Robin et al., 1998). Moreover, behavioral changes, such as an increase in locomotor activity (Robin et al., 1998) and nest abandonment (Groscolas et al., 2008) have been reported from birds in PIII, indicating that when body reserves are close to exhaustion, the promotion of behaviors related to self-maintenance is favored.

Among the potential factors that may play a role in redirecting breeding behavior (e.g. incubation) to behavior ensuring survival (e.g. foraging activity), hormones can offer great insights into the mechanisms that mediate some life-history trade-offs (Sinervo and Svensson, 1998). Corticosterone (CORT), the major avian glucocorticoid, has been associated with the promotion of an emergency life-history stage, inducing behavior that ensures survival (Wingfield et al., 1998). This suggestion is supported by the finding that CORT levels increase in PIII (Cherel et al., 1988; Robin et al., 1998), and stimulates protein catabolism (Challet et al., 1995). Moreover, experimental administration of CORT mimics the fasting-induced rise in locomotor activity in laboratory rats (Challet et al., 1995) and in Adélie penguins (Spée et al.,

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unpublished data). In addition, CORT levels have been shown to be markedly increased in PIII king penguins *Aptenodytes patagonicus* abandoning their egg, suggesting that this hormone plays an important role in the decision to give up a breeding attempt by stimulating the refeeding drive (Groscolas et al., 2008).

The pituitary hormone prolactin, on the other hand, has the opposite effect to CORT with respect to parental behavior and is known to stimulate incubation and brooding behavior in birds (Buntin, 1996; Youngren et al., 1991). Consequently, it is important to investigate the levels of both hormones (CORT and prolactin), when studying the physiological mechanisms that underlie parental behavior in birds. In penguins, prolactin secretion seems to be endogenously programmed on a long-term basis, being poorly influenced by external stimuli (Garcia et al., 1996; Lormée et al., 1999; Vleck et al., 2000a). Prolactin levels increase during courtship, peak in mid-incubation, and remain elevated until the end of chick brooding (Vleck et al., 1999). However, a decrease in prolactin levels has been reported in king penguins that abandoned their nest during PIII (Cherel et al., 1994; Groscolas et al., 2008), suggesting that prolactin secretion could be modulated by energy constraints on a short term basis. Such a drop in prolactin levels might decrease the incubation drive and favor nest abandonment.

Studies to date indicate that the induction of a refeeding signal leading to nest abandonment is related to PIII of fasting (Groscolas et al., 2008). At this stage, the increase in plasma CORT levels and the decrease in prolactin levels seem to play a role in motivating the parent's decision to abandon the nest, by stimulating the drive to refeed and by diminishing the drive to incubate, respectively (Groscolas et al., 2008). However, the respective role of these two hormones in stimulating nest desertion remains unclear. Moreover, whether prolonged energy constraints affect CORT and prolactin levels concomitantly or successively remains to be determined. In other words, whether reaching proteolytic PIII during late fasting is sufficient to induce nest abandonment alone in long-lived birds requires further consideration.

In penguins, both males and females take part in incubation and brooding duties, alternating fasting on land and foraging at sea. After egg-laying, females return to sea in order to forage and replenish their energy reserves while males take on the first incubation shift. In the present study, we investigated the nutritional and hormonal patterns of Adélie penguins (1) at the beginning of the incubation fast and (2) when males departed from the colony to forage at sea. Male penguins left the colony either because they were relieved by their partner or because they abandoned the nest. The nutritional state of birds was determined from their body mass and from plasma metabolites, which are known to be good indicators of the nutritional state in free-living animals (Jenni-Eiermann and Jenni, 1998). Recording these nutritional parameters in parallel with plasma CORT and prolactin levels, we determined whether the entrance into PIII was consistently associated with nest abandonment and refeeding. We also assessed the respective role of CORT and prolactin in the induction of this behavioral shift. Moreover, we examined whether the entrance into PIII was the consequence of a lower body mass at the beginning of the fast or if these males had fasted for a longer period. Indeed, the body condition of male penguins at the beginning of the fast seems to be an important predictor of incubation success (Vleck and Vleck, 2002).

Methods

The study was conducted in Dumont d'Urville Station (66°40′S, 140°01'E), Adélie land, Antarctica, during the 2005–2006 austral summer and was supported by the Ethics Committee of the French Polar Institute Paul Emile Victor (IPEV).

Sampling state

During the study period, we followed 92 pairs of Adélie penguins. Birds were captured on two occasions (see Fig. 1).

First, both members of a pair were captured between pair formation and egg-laying (pre-laying stage). Blood was collected from the wing vein within 5 min of initial capture since it has been shown that handling durations <5 min have no effect on baseline CORT levels in Adélie penguins (Vleck et al., 2000b). Samples were subsequently transferred into pre-treated tubes (using heparin or EDTA) and centrifuged at 4 °C (5000 rpm for 10 min). The plasma was then collected and kept frozen in aliquots at −20 °C until subsequent analyses. All birds were weighed to the nearest 2 g using an Ohaus electronic precision balance and individually marked with a number

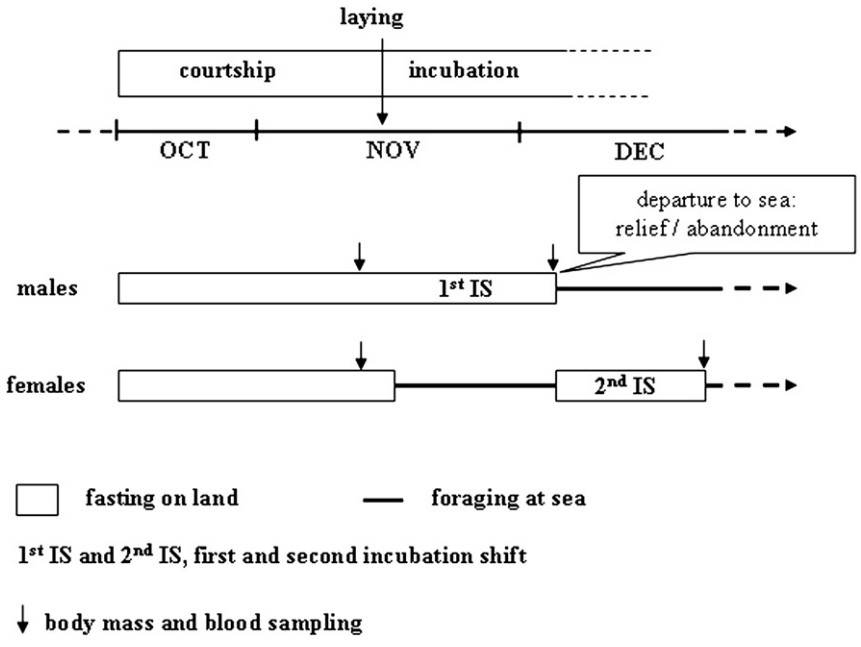

Fig. 1. Study protocol. Birds were weighed and sampled for blood on two occasions: at the pre-laying stage (males and females) and when departing to sea for refeeding (at the end of the first and second incubation shift for males and females, respectively). See Methods section for details.

painted on their chest using Nyanzol D. Sex was determined by observing nest attendance patterns (males usually take on the first incubation shift; Ainley et al., 1983) and by measuring plasma lipemia (females exhibit higher plasma lipemia before egg-laying than males, Beaulieu et al., 2010; Kern et al., 2005). The assigning of sex by these methods has already been used by Vleck and colleagues (2000a).

Nests were observed in 3–4 h intervals to determine bird attendance, egg-laying dates, number of eggs in the nest, and to note relief by the partner or nest abandonment. This study focused on the overall relatively small proportion of male penguins which were likely to reach PIII of fasting and were therefore prone to abandon their nest towards the end of the first incubation shift. Knowing the body mass of these birds at the pre-laying stage, the rate of daily body mass loss (0.052 kg/day, Chappell et al., 1993; 0.042 kg/day, Vleck and Vleck, 2002), and their critical body mass (3.5 kg, Cockrem et al., 2006), it was possible to estimate the approximate date at which birds should reach PIII, and were thus likely to desert their nest. Nest observations were especially important for the males at this point in time and were carried out on an hourly basis.

Second, males were recaptured at the end of the first incubation shift, when they left the colony to forage at sea (when their partner had returned or when they abandoned the nest). Birds were weighed and blood samples were taken. We also recaptured females when they left the colony to refeed at sea at the end of the second incubation shift.

We captured and weighed most males when they left the colony but did not systematically sample blood in all of these males 1) to avoid excessive manipulation and subsequent disturbance; 2) because the procedure was time-consuming; and 3) because many birds with a similar body mass and, thus, a similar nutritional state (PII of fasting) had already been sampled.

In total, during the first capture, 92 pairs were marked, weighed and measured (bill and flipper) but only 48 males and 48 females were sampled for blood. We were able to recapture and weigh most of the marked males when they left the colony at the end of the first incubation shift. At this stage, only 44 males were sampled for blood, of which 27 had already been sampled at the first capture. Of these 27 birds, four males abandoned their nest. In addition, 53 females were recaptured and weighed at the end of the second incubation shift and 27 of them were sampled for blood.

Plasma analysis

Metabolites

Concentrations of uric acid and β-hydroxybutyrate (βOHB) were measured by the enzymatic colorimetric method using commercial kits (uric acid: Sigma Diagnostics; βOHB: Randox). The measurement was conducted on undiluted plasma (uric acid: 25 µl; βOHB: 20 µl).

Hormones

CORT concentrations were determined by a quantitative competitive sandwich enzyme immunoassay technique (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etudes Biologiques de Chizé (CEBC; France). Pooled plasma samples of Adélie penguins produced a dose response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance). For prolactin measurements, intra and inter-assay variations were 6% and 9%, respectively. For corticosterone determinations, these variations were 5% and 7%, respectively.

Determination of fasting phases and statistics

To determine whether birds were either in PII or PIII at the end of their first incubation shift, we used a Principal Component Analysis (PCA) specifying body mass, uric acid levels (reflecting protein catabolism) and βOHB concentrations (reflecting lipid utilization) as variables in the analysis. These parameters are indeed known to be good indicators of the nutritional state of free-living birds (Jenni-Eiermann and Jenni, 1998). PCA was conducted with R (2.8.1) using the FactorMineR package. The number of dimension selected for analysis was reduced to one axis following the Kaiser criteria, i.e. only considering axes with an Eigenvalue >1.

Birds were divided into three groups: ePII (males at the end of PII which were relieved by their partner, $n=18$), PIII + re (males in PIII relieved by their partner, $n=5$) and PIII + ab (males in PIII which abandoned their nest before having been relieved by their partner, $n=4$). General linear mixed models (GLMMs) were used to compare plasma parameters between groups. We included individuals as a random factor and "sampling period" (at the pre-laying stage and at departure to sea), "group" (ePII, PIII + re and PIII + ab) and their interaction as fixed factors, the "sampling period" being the repeated measure. Normality was assessed using a Shapiro–Wilk test. When normality was not met, a generalized estimated equation (GEE) was used. To compare body mass at the pre-laying stage and daily body mass loss, we used a general linear model (GLM). The clutch size and the duration of fasting were compared using a generalized linear mixed model (GzLM) with a Poisson distribution. Post-hoc comparisons were made using Bonferroni tests. We used Student's *t*-test to compare PC1-scores between birds in PIII + re and birds in PIII + ab.

Analyses were conducted using Minitab 15 software and SPSS 16.02 (SPSS Inc., Chicago, Ill., USA). Results are expressed as means ± S.E. and significance level was set at $\alpha=0.05$.

Results

Males during the first incubation shift

Mean body mass of the 27 males captured at the pre-laying stage was 5.05 ± 0.11 kg (range: 4.13 to 6.61 kg). During incubation, 23 of these males were relieved by females and only four abandoned their nest.

The first dimension of the PCA (with body mass, uric acid and βOHB as variables) explained 68.9% of the total variation. However, when we corrected body mass for morphological size, the PCA only explained 67.1% of the total variation. Consequently, we only consider here the PCA which included body mass alone and did not correct for morphological size. According to the individuals' factor map (and taking into account only the first dimension), we were able to distinguish two groups having distinct fasting status among birds that left the colony to refeed at sea: penguins in PII and penguins in PIII (PIII + re and PIII + ab). The frequency distribution of the PC1-scores of these males is shown in Fig. 2. We found no significant difference between PC1 scores of birds in PIII + re and those of birds in PIII + ab (PIII + re = −1.36 ± 0.16 and PIII + ab = −2.13 ± 0.70; $t=1.07$, $df=3$, $p=0.36$).

Finally, of the 23 males relieved by females, 18 were in ePII, while five were in PIII (PIII + re). However, all the birds that abandoned their nest were in PIII (PIII + ab).

Body mass at the pre-laying stage, clutch size, body mass loss and fasting duration

Male body mass at the pre-laying stage (i.e. at first capture) differed significantly between groups ($F_{2,\ 22}=11.71$, $p<0.001$; Table 1). Penguins that abandoned their nest ($p=0.001$) and birds that were relieved in PIII ($p=0.009$) had significantly lower body mass during the pre-laying period than birds that left the colony in ePII (16% and 12% lower, respectively). Hence, penguins in PIII had similar body masses at this stage, regardless of the outcome of the first incubation shift ($p>0.99$).

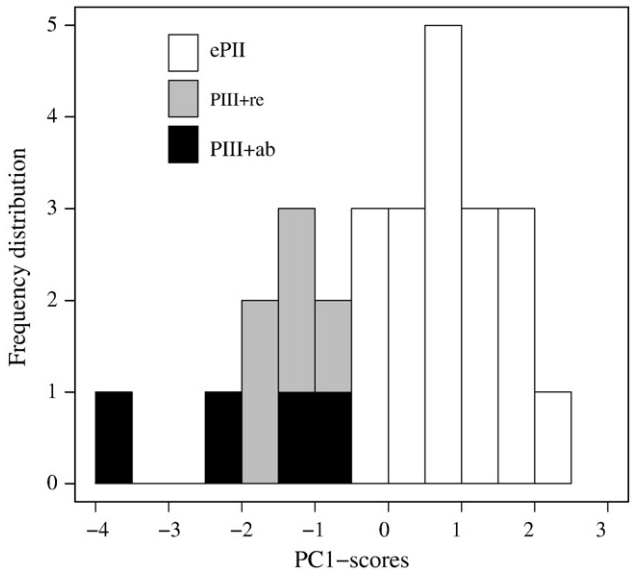

Fig. 2. Frequency distribution of PC1-scores of male Adélie penguins that fall into ePII (males at the end of phase II relieved by their partner, white bars, $n=18$), PIII + re (males in phase III relieved by their partner, grey bars, $n=5$) or PIII + ab (males in phase III that abandoned their nest, black bars, $n=4$) at the end of the first incubation shift.

Penguins had similar clutch size, regardless of whether they fell into ePII (1.88 ± 0.08), PIII + re (1.80 ± 0.20) or PIII + ab (2.00 ± 0.00) at the end of the first incubation shift (Wald$\chi^2=0.05$, df = 2, $p=0.98$).

Fasting duration for males between the first and the second capture was similar for all groups (Wald$\chi^2=1.64$, df = 2, $p=0.44$; Table 1). Daily body mass loss of males was not significantly different between groups ($F_{2,\ 22}=1.37$, $p=0.28$; Table 1).

Plasma levels of metabolites

Uric acid levels of birds in PIII (PIII + re and PIII + ab) were affected by the sampling period ($F_{1,\ 3}=13.4$, $p=0.04$; Fig. 3A). Indeed, birds in PIII + re and PIII + ab exhibited higher uric acid levels when departing to sea than during the pre-laying stage. However, uric acid concentrations were not affected by the group (PIII + re and PIII + ab; $F_{1,\ 3}=1.26$, $p=0.35$) and by the interaction group* sampling period ($F_{1,\ 3}=1.26$, $p=0.35$).

In addition, βOHB levels of birds in PIII were influenced by the period of sampling ($F_{1,\ 6}=11.2$, $p=0.02$), with birds in PIII + re and PIII + ab showing lower βOHB levels when departing to sea than during the pre-laying stage. However, βOHB concentrations were not affected by the group (PIII + re and PIII + ab; $F_{1,\ 7}=0.003$, $p=0.96$) and by the interaction between group*period of sampling ($F_{1,\ 6}=0.49$, $p=0.51$).

Table 1
Profile of breeding male Adélie penguins according to reproductive performance and nutritional status at the end of the first incubation shift.

	Relief by female		Abandonment
Nutritional state	ePII	PIII + re	PIII + ab
Body mass at the pre-laying stage (kg)	5.28 ± 0.08 [a]	4.67 ± 0.16 [b]	4.44 ± 0.27 [b]
Fasting duration between first and second capture (days)	20.6 ± 0.9	21.0 ± 1.7	17.7 ± 2.8
Body mass loss (g/day)	55.8 ± 1.7	55.8 ± 3.2	62.0 ± 3.4

ePII, males at the end of phase II relieved by females, $n=18$; PIII + re, males in phase III relieved by females, $n=5$; PIII + ab, males in phase III that abandoned their nest, $n=4$. Results are means ± S.E. For the body mass at the pre-laying stage, values that do not share the same superscript letter are significantly different. For the other parameters, no significant differences were detected between groups.

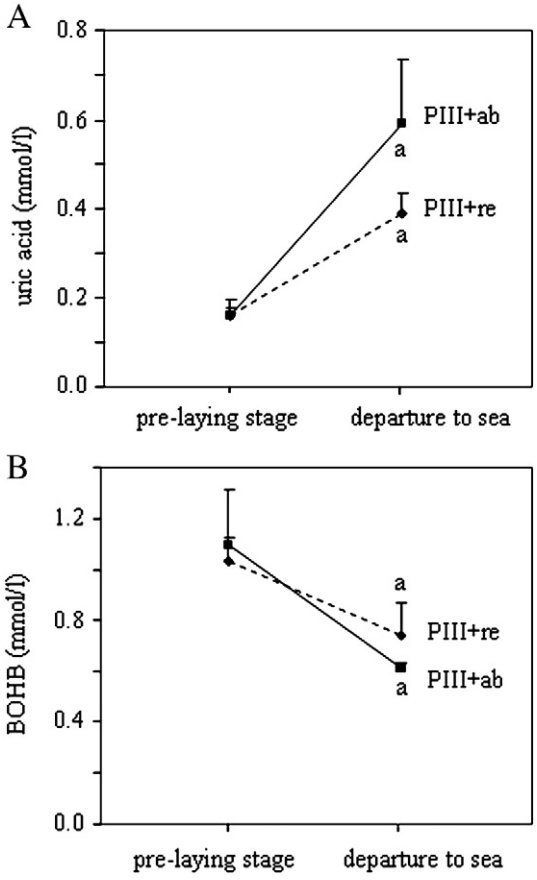

Fig. 3. Plasma levels of uric acid (A) and βOHB (B) of breeding male Adélie penguins in PIII at the pre-laying stage and when departing to sea (relief by their partner or nest abandonment). PIII + re: males in phase III relieved by females, $n=5$; PIII + ab: males in phase III that abandoned their nest, $n=4$. Results are means ± S.E. Bars not sharing the same superscript letter are significantly different.

Plasma levels of hormones

CORT levels were affected by the sampling period (Wald$\chi^2=28.5$, df = 1, $p<0.001$; Fig. 4A), the group (Wald$\chi^2=18.5$, df = 2, $p<0.001$) and their interaction (Wald$\chi^2=13.4$, df = 2, $p=0.001$; Fig. 4A). CORT levels at the pre-laying stage were similar for all penguins ($p>0.99$ for each comparison). At departure to sea, birds in PIII + re and PIII + ab had significantly higher CORT levels than at the pre-laying stage ($p=0.004$ and $p=0.01$, respectively), while we found no difference for birds in ePII ($p=0.19$). Moreover, plasma levels of CORT were not significantly different for birds in PIII + re and PIII + ab ($p>0.99$).

Prolactin levels were influenced by the group ($F_{2,\ 24}=6.59$, $p=0.005$; Fig. 4B) and by the interaction between group*sampling period ($F_{1,\ 23}=20.5$, $p<0.001$) but were not affected by the sampling period ($F_{1,\ 24}=0.27$, $p=0.61$). Prolactin levels at the pre-laying stage were similar for all birds ($p>0.99$ for each comparison). At departure to sea, prolactin levels of penguins in PIII + ab were 62% lower than at the pre-laying stage ($p<0.001$). Interestingly, penguins in PIII + ab had 70% lower prolactin concentrations than birds in PIII + re ($p<0.001$), while they were in a similar nutritional state (enhanced proteolysis).

Females at the pre-laying stage and at the end of the second incubation shift

At the pre-laying stage, mean body mass of females was 4.4 ± 0.1 kg (ranging from 3.67 to 6.11 kg). Their plasma levels of uric acid, CORT, and prolactin were 0.11 ± 0.01 mmol/l, 4.2 ± 0.7 ng/ml, and 91 ± 11 ng/ml, respectively.

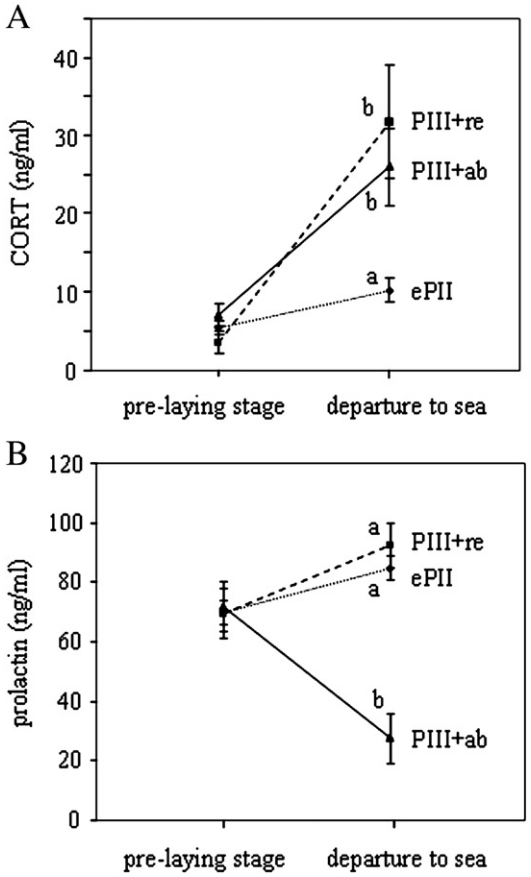

Fig. 4. Plasma levels of CORT (A) and prolactin (B) of breeding male Adélie penguins at the pre-laying stage and when departing to sea (relief by their partner or nest abandonment). ePII: males at the end of phase II relieved by their partner, $n = 18$; PIII + re: males in phase III relieved by their partner, $n = 5$; PIII + ab: males in phase III that abandoned their nest, $n = 4$. Results are means ± S.E.

At the end of the second incubation shift, all females that departed to sea were relieved by their respective mate. Of the 27 females captured at this time, 25 were in ePII, while two were in PIII (PIII + re).

Given the small sample size of birds in PIII ($n = 2$), statistical comparison between females in ePII and females in PIII + re was not performed. However, the trend observed for each parameter measured is indicated in Table 2. We found that females in PIII + re tended to have lower body masses, higher plasma uric acid and CORT levels than females in ePII. By contrast, prolactin levels tended to be similar for all females.

Discussion

Our study shows that reaching proteolytic PIII during late fasting is not sufficient to induce nest abandonment in a long-lived seabird, the Adélie penguin. This finding indicates a decoupling between the metabolic status of a bird and its behavioral response. We found that whereas all breeding males presented elevated CORT levels in PIII, only those which also presented high prolactin levels did not abandon incubation. Conversely all males presenting low prolactin levels in PIII abandoned the nest and left to forage at sea. In addition, penguins which entered PIII did so because their body mass at the beginning of the incubation fast was low, suggesting that these birds might have been less experienced breeders or that they might have presented poor foraging abilities at the time.

Table 2
Profile of breeding female Adélie penguins according to nutritional status at the end of the second incubation shift.

Nutritional state	ePII	PIII + re	Trend (PIII + re *vs.* ePII)
Fasting duration (2nd shift) (days)	12.71 ± 0.38	14.50 ± 0.50 (14 and 12)	→
Body mass (kg)	4.04 ± 0.07	3.08 ± 0.05 (3.03 and 3.13)	↘
Uric acid (mmol/l)	0.18 ± 0.02	0.33 ± 0.03 (0.31 and 0.36)	↗
CORT (ng/ml)	10.03 ± 1.67	20.41 ± 3.65 (16.75 and 24.06)	↗
Prolactin (ng/ml)	96.99 ± 2.01	95.15 ± 6.45 (88.71 and 101.6)	→

ePII, females at the end of phase II relieved by males, $n = 25$; PIII + re, females in phase III relieved by males, $n = 2$. CORT, corticosterone. Results are means ± S.E.

Nutritional state and hormonal status at relief/abandonment

According to the result of the PCA, we found that all abandoning penguins were in PIII (PIII + ab). Birds showed increased uric acid levels and decreased βOHB concentrations (Fig. 3), indicating a metabolic shift from lipid towards protein utilization. Moreover, their body mass was lower than the 3.5 kg critical body mass threshold that typically signals the entrance of male Adélie penguins into PIII (Cockrem et al., 2006). Our results for Adélie penguins are similar to the situation found in king penguins, where nest abandonment occurs during the late stage of fasting (Groscolas et al., 2008). Given their long life span, the optimal strategy for an incubating penguin entering PIII may be to abandon the current reproductive effort in favor of its own survival and thereby ensure future reproductive attempts. Surprisingly however, we found that some birds in a similar nutritional state (i.e. in PIII) did not abandon their nest but were relieved by their partner (PIII + re). This result suggests a decoupling between the metabolic status and the behavioral response.

As expected, abandoning birds in the current study had high levels of CORT and low concentrations of prolactin (Fig. 4), indicating a stimulation of the refeeding drive, while the incubation drive was depressed. Such hormonal patterns seem to be the characteristic for the process of nest abandonment in penguins, as deserting king penguins show comparable changes in CORT and prolactin levels (Groscolas et al., 2008). We found that prolactin levels were sharply decreased in abandoning birds, while they remained high in birds in PIII that did not desert their nest. Hence, in addition to increased levels of CORT, decreased prolactin levels are also required to induce nest abandonment in Adélie penguins. We also found some females in PIII which did not desert their nest at the end of the second incubation shift (Table 2). These females presented high CORT and prolactin levels, indicating that such a situation is not restricted to males at the end of the first incubation shift.

Our findings suggest that the hormonal changes occurring in PIII would be dynamic. The increase in the refeeding drive orchestrated by increased CORT levels seems to precede the decrease in the incubating drive, which is modulated through a decline in prolactin concentrations.

Why do only some penguins in PIII abandon their nest?

Three main reasons can be proposed to explain why prolactin levels only declined in abandoning penguins.

First, assuming that baseline prolactin concentrations are indicative of the amount and quality of parental care provided (Angelier et al., 2007a,b; Angelier and Chastel, 2009), we can hypothesize that the parental care provided by abandoning penguins was already reduced at the start of incubation, when compared to that of non-

abandoning birds. Hence, prolactin levels would already be low at the beginning of the incubation fast in abandoning birds. However, we show that the three groups of penguins had similar prolactin levels at the pre-laying stage (Fig. 4B), supporting the view that all birds were similarly motivated to incubate.

Second, the differences observed in prolactin patterns between abandoning birds and birds in PIII which were relieved by their mates may be explained by a differential regulation of prolactin secretion in response to the stress caused by prolonged fasting. Factors that modulate the prolactin response to stress are usually examined using a standardized stress protocol (Angelier et al., 2007a, 2009; Chastel et al., 2005). Although it could be argued that fasting during incubation is predictable and should therefore not be associated with stress, the prolonged energy constraint that induces birds to enter PIII most likely exposes birds to physiological stress. This view is supported by increased CORT levels at this stage of fasting (Fig. 4A). Moreover, because parental effort has been shown to modulate the prolactin response to stress (chick-rearing *vs.* failed black-legged kittiwakes *Rissa tridactyla*; Chastel et al., 2005; Angelier and Chastel, 2009 for a review), we can hypothesize that penguins should be more reluctant to abandon two eggs than one. However, clutch size of penguins that left the colony to forage at sea in ePII, PIII + re, and PIII + ab was similar.

On the other hand, there is evidence that the prolactin response to stress is modulated by age (Angelier et al., 2007a). In fact, young breeding Adélie penguins are reportedly more likely to desert their nest than older ones (Davis and McCaffrey, 1986). In this respect, we can hypothesize that penguins in PIII + re were older breeders (which were more resistant to stressful situations) and that their prolactin levels remained elevated when CORT increased. Future studies performed on birds of known age or that provide information about the estimated relative age (Haussmann et al., 2003a,b) of abandoning penguins will allow testing this hypothesis.

Third, we can hypothesize that birds in PIII + ab were in more advanced in PIII than penguins in PIII + re, i.e. they could have been in PIII for a longer period. Results from abandoning king penguins are in line with this hypothesis. Groscolas and colleagues found that body mass in abandoning king penguins was about 1 kg below the critical body mass value, with the total duration for the egg abandonment process ranging from 20 h to 5 days (Groscolas et al., 2000). Thus, entrance into an emergency life-history stage driven by elevated CORT levels that redirect behavior towards survival (Wingfield et al., 1998) can take a long time to develop. We found no significant differences in body mass loss and PC1-scores between birds in PIII that abandoned the nest and birds in PIII that were relieved by females, despite a tendency for abandoning birds. Due to our low sample size ($n = 5$ for birds in PIII + re and $n = 4$ for birds in PIII + ab), we cannot provide any statement based on this non-statistical significance. Thus, we are only able to suggest that abandoning birds could have been more advanced in PIII than birds which were relieved.

Do prolactin levels have to reach a threshold concentration in abandoning penguins?

In the context of nest abandonment as an adaptive behavior promoting survival, CORT could affect endocrine mechanisms involved in parental activities, such as prolactin. Indeed, CORT and prolactin are mechanistically linked. For birds it has been shown that an experimentally induced rise in CORT levels leads to a decrease in prolactin levels (Angelier et al., 2009; Criscuolo et al., 2005) and it has been suggested that the effects of CORT on the expression of parental behavior may be mediated through modulation of prolactin levels (Angelier et al., 2009). However, the inhibitory action of CORT on plasma prolactin seems to be complex, since prolactin levels have been shown to decrease only slowly and progressively in response to CORT manipulation (Angelier et al., 2009). For instance, an experimental increase in CORT levels in black-legged kittiwakes was accompanied by a reduction in plasma prolactin concentrations and subsequently (from day 3 after treatment) by a reduction in nest attendance (Angelier et al., 2009), suggesting that prolactin levels probably need to reach a low threshold value to affect parental behavior. Interestingly, observed prolactin levels in abandoning penguins seem to be fairly similar (king penguins, Cherel et al., 1994; Groscolas et al., 2008; Adélie penguins, this study), reinforcing the idea that prolactin has to reach a threshold concentration, below which the drive to incubate is inhibited. Accordingly, we propose that abandoning penguins (PIII + ab) would have been in PIII for a longer period than birds which were relieved (PIII + re). Such a difference in timing is most likely required for CORT to decrease prolactin to a sufficiently low level. However, we cannot reject the hypothesis that birds in PIII + ab could be younger breeders that are less resistant to stressful situations and that their prolactin levels decreased as CORT increased.

The decline in prolactin levels could also be a consequence of the progressive decrease of attentiveness, driven by CORT, which precedes definitive nest desertion. Transitory abandonments, when birds leave the nest for progressively increasing durations and wander further and further away from their egg, have been reported in king penguins (Groscolas et al., 2000) and Red-footed booby *Sula sula* (Chastel and Lormée, 2002). Whether transitory abandonments occur in Adélie penguins, and whether the decline in prolactin levels precedes the decrease in attentiveness or merely follows, remains however unknown. A study conducted in meerkats *Suricata suricatta* supports the former course of events as high prolactin levels preceded the decision to engage in parental care (Carlson et al., 2006).

Conclusion — perspectives

In conclusion, we show that the PIII of fasting is not necessarily associated with nest abandonment in a long-lived seabird, the Adélie penguin. In some cases, we found a decoupling between the metabolic status (PIII) and the behavioral response of abandonment. Whereas CORT and prolactin are both involved in the induction of the refeeding signal that ultimately leads to nest abandonment, we propose that hormonal changes occurring in PIII would be dynamic, so that CORT and prolactin would be successively affected by prolonged energy constraints. In that respect, we hypothesized that CORT would act first and is not sufficient by itself to induce nest abandonment. To test this hypothesis, it would be interesting to examine the extent to which exogenous CORT mimics a PII–PIII transition and provokes nest abandonment, potentially mediated through a decline in prolactin concentrations. Similarly, to gain further insight into the role of prolactin in the control of incubation behavior, it would be of great interest to examine the effects of an experimental decrease in prolactin levels that is not accompanied by an increase in CORT levels. A better understanding of the interrelationships between marine resources, food availability, body condition, and breeding success in long-lived seabirds will require future studies to consider the proportion of birds reaching late fasting, the extent to which they desert their nest and the effect of environmental conditions on such parameters, in years to come.

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Résumé - Etude 2

Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins (*Pygoscelis adeliae*)

Introduction

Nous avons vu dans l'étude 1 (Spée et al. 2010) qu'un niveau élevé de CORT est nécessaire mais non suffisant pour provoquer l'abandon du nid. En accord avec cette hypothèse, l'étude de Criscuolo et ses collaborateurs (2005) montre que l'administration de CORT exogène chez des femelles eider à duvet n'entraîne pas d'abandon du nid lors du jeûne associé à l'incubation mais affecte le succès reproducteur subséquent (reflété par une augmentation de la prédation sur les œufs). Les auteurs proposent que cette absence d'effet pourrait être due à 1) une faible durée du traitement, puisque les niveaux de CORT retrouvent un niveau basal 4 jours après le traitement, 2) une dose trop forte de CORT. Des études ont en effet mis en évidence que l'action de la CORT sur l'activité locomotrice (qui est interprétée comme une motivation à rechercher de la nourriture) est dépendante de sa concentration (Breuner et al. 1998 ; Breuner et Wingfield 2000).

Le but de cette étude est d'examiner dans quelle mesure la CORT exogène peut avoir un rôle actif dans l'induction du signal de réalimentation. Pour ce faire, nous avons implanté de la CORT à différentes doses chez des manchots Adélie captifs afin 1) de déterminer si cette hormone était susceptible de mimer les modifications métaboliques, hormonales et comportementales survenant en PIII, et 2) quelle était la dose efficace.

Matériels et méthodes

Des manchots Adélie mâles captifs, en échec de reproduction, ont été implantés avec 0 (témoins, n=7), 10 (C10 ; n=5), 50 (C50 ; n=4), 100 (C100 ; n=8) ou 200 mg (C200 ; n=4) de CORT, la durée de diffusion de l'implant étant de 21 jours. Les oiseaux ont été pesés tous les deux jours et leur activité locomotrice a été mesurée quotidiennement grâce à la pose de podomètres. Des prélèvements sanguins ont été effectués 1) le jour de l'implantation (jour 0), 2) trois jours après le traitement et 3) le dernier jour passé en captivité (jour 5 à 16, en fonction des individus). Les prélèvements sanguins chez les oiseaux du groupe témoin n'ont eu lieu qu'à deux reprises : 1) le jour de l'implantation et 2) le jour où ils ont été relâchés (après avoir atteint la masse critique d'entrée en PIII, jour 6 à 9 en fonction des individus).

Résultats et discussion

Cette approche expérimentale a permis de mettre en évidence qu'une dose forte mais physiologique de CORT (implant de 100 mg) entraîne 1) une modification du type de substrat énergétique utilisé (augmentation du niveau d'acide urique d'un facteur 3 et diminution du niveau de β-OHB d'un facteur 1.5, dès trois jours après le traitement) , 2) une diminution de 30 % des niveaux de prolactine 8 à11 jours après l'implantation, et 3) une augmentation de l'activité locomotrice d'un facteur 2.5, deux à quatre jours après l'implantation. Ainsi, cette étude montre que la CORT joue un rôle clé dans l'induction du signal de réalimentation survenant en fin de jeûne. De plus, cette hormone semble capable d'entrainer une diminution des niveaux de prolactine et pourrait, par ce biais, provoquer l'abandon de la reproduction. Cependant, une approche expérimentale sur animal sauvage (en cours de reproduction) apparaît nécessaire pour déterminer le rôle de cette hormone dans la promotion de l'abandon du nid.

Exogenous corticosterone mimics a late fasting stage in captive Adélie penguins (*Pygoscelis adeliae*)

Soumis

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Running head: Corticosterone and refeeding signal in Adélie penguins

ABSTRACT

Fasting is part of penguin's breeding constraints. During prolonged fasting three metabolic phases occur successively. Below a threshold in body reserves, birds enter phase III (PIII), which is characterized by hormonal and metabolic shifts. These changes are concomitant with egg abandonment in the wild and increased locomotor activity in captivity; a refeeding signal being triggered. Because CORT enhances foraging activity, we investigated the variations of endogenous CORT, and the effects of exogenous CORT on the behavioral, hormonal, and metabolic responses of failed breeder Adélie penguins.

Untreated and treated captive male were regularly weighed and sampled for blood while fasting, and locomotor activity was recorded daily. Treated birds were implanted with various doses of CORT during phase II.

Untreated penguins entering PIII had increased CORT and uric acid (reflecting protein catabolism) levels, concomitantly with a rise in locomotor activity, while prolactin (involved in parental care in birds) levels declined. In CORT-treated birds, an inverted-U relationship was obtained between CORT levels and locomotor activity. The greatest increase in locomotor activity was observed in birds implanted with a high dose of CORT (C100), locomotor activity being increased four days after implantation to a level similar to that of birds in PIII. Moreover, uric acid levels increased three-fold in C100-birds, while prolactin levels declined by 30 %.

The experimentally-induced rise in CORT levels mimicked metabolic, hormonal, and behavioral changes characterizing late fasting. This study strongly supports a key role for CORT in the induction of a refeeding signal that occurs in fasting birds.

Key words: locomotor activity, long-lived seabird, long-term fasting, glucocorticoid

INTRODUCTION

During their life cycle, several animal species alternate periods of fasting and feeding. For instance, long fasting periods are common in seabird species, especially penguins, since they feed exclusively at sea, while they have to spend time ashore to breed and moult (38). This natural situation of spontaneous prolonged fasting can lead to energy reserve exhaustion and, consequently, to the abandonment of current reproduction.

Prolonged fasting is characterized by three distinct phases, based on patterns of daily body mass loss and substrates used to support cellular metabolism (17). Phase I (PI) is a brief period of adaptation while phase II (PII) is a long period of economy characterized by a constant rate of body mass loss and by protein sparing. The later is indicated by low levels of uric acid, the production of which is the result of protein catabolism (16, 31). At this stage of fasting, plasma levels of β-hydroxybutyrate (βOHB) are elevated, indicating that lipids are the main energy substrate (16, 31). If a minimum body reserve threshold is reached, animals enter phase III (PIII). At this stage hormonal and metabolic shifts occur, which are characteristic for a switch from lipid to protein utilization. In addition to these physiological adjustments, marked behavioural changes have been reported for birds entering PIII. For example, incubating king penguins *Aptenodytes patagonicus* (21) and Adélie penguins (33) were observed to abandon their egg(s), while non-breeding fasting emperor penguins *Aptenodytes forsteri* increased locomotor activity (31). These behavioural changes have been interpreted to reflect an increase in the drive to refeed and are the visible manifestations of endogenous metabolic and endocrine shifts that trigger refeeding (21).

There are different ideas about how such refeeding signal is mechanistically triggered. At the central levels, the expression of several hypothalamic neuropeptides that are involved in the control of feeding behaviour has been reported for rodents during periods of prolonged fasting (7). It was found that the hypothalamic response to long-term fasting (in PIII) is

mediated by the orexigenic system, rather than by the anorexigenic system. The regulation of neuropeptide Y (NPY) and agouti-related peptide (AGRP) expression seems to be primarily involved in the response to prolonged fasting and could mediate the late enhanced drive for refeeding (7). This supports the view that the central effects of long-term fasting are mediated mainly by neuropeptides synthesized in the arcuate nucleus of the hypothalamus (7). Nevertheless, the nature and exact mechanisms of this refeeding signal, which is associated with the late fasting stage, remain partly unknown at the peripheral level. Hormones are known to play an important role in the control of feeding behaviour, and, in penguins, they might act as mediators between metabolic shifts and behavioural changes (23). It can therefore be validly proposed that refeeding is triggered by the hormonal adjustments that characterize the entrance into PIII.

In birds, parental behaviour is controlled by two hormones with opposite effects: (i) prolactin, the major hormone that stimulates incubation and parental care (13, 41), and (ii) corticosterone (CORT), the main avian glucocorticoid, which plays a role in a wide variety of physiological and behavioural processes (28). A rise in plasma levels of CORT was linked to the entrance into PIII in king and emperor penguins (17, 31) and occurs concomitantly with an increase in locomotor activity (31).

Foraging activity was shown to increase in response to CORT treatment in several species of mammals (14) and birds (3, 12, 27). Experimental studies suggest that CORT is involved in the initiation of food-intake during short fasting periods. For example, CORT was shown to stimulate locomotor activity and food searching in white-crowned sparrows *Zonotrichia leucophrys* (3), while it enhances foraging in breeding black-legged kittiwakes *Rissa tridactyla* at the mid-chick-rearing stage (27). Hence, it is tempting to propose that CORT initiates foraging behaviour during late fasting, once critical reserve exhaustion is reached. It would thereby redirect bird behaviour from a costly activity (e.g. reproduction) to

a behaviour promoting survival (e.g. departure to refeed) (40). However, implantation of exogenous CORT in female common eiders *Somateria mollissima* did not lead to nest abandonment (19). This absence of behavioural changes could be due to (i) a short treatment duration, since CORT concentrations returned to basal levels within four days of implantation and/or (ii) a too high dose of CORT, which was possibly not biologically relevant.

In the present study, we investigated the role of CORT in the induction of the refeeding signal that occurs during fasting in Adélie penguins. We mainly focused on male penguins because they undertake the longest fasting period during courtship/incubation (up to 50 days; 37) and, thus, might be prone to reserve exhaustion that can lead to nest desertion. We firstly validated the fasting phases of captive birds, by examining the relationship between uric acid levels and body mass of untreated birds and by defining the body mass threshold, below which they enter PIII. Plasma levels of metabolites are known to be reliable indicators of the nutritional state and fasting phases in birds (17, 24, 31). We used a correlative approach to examine the natural time-course changes of hormones (plasma levels of CORT and prolactin), metabolites (plasma levels of uric acid, βOHB, non-esterified fatty acid and glucose), and behaviour (locomotor activity) in fasting captive birds. Secondly, we investigated whether exogenous CORT might induce (i) a switch in the substrate used to support cellular metabolism (as revealed by plasma metabolites), (ii) a decrease in prolactin levels, and (iii) an increase in locomotor activity. Since the effect of CORT on locomotor activity depends on its concentration (12), we used various doses of CORT.

MATERIALS AND METHODS

Study area and birds

The study was conducted in Dumont d'Urville Station (66°40'S, 140°01E), Adélie Land, Antarctica, during three austral summers (2004-05, 2006-07, and 2007-08). The captive birds in the current study were failed breeders, i.e. they started a reproductive cycle but discontinued incubation prematurely. This was unrelated to parental body condition but explained by extrinsic factors, such as bad weather and flooding.

Study 1: Correlative approach

During the austral summer of 2004-05, a total of 12 male and five female Adélie penguins were captured over the course of the breeding season. They were kept in a pen (5.2 m * 2.4 m), where they fasted until entering PIII (when their body mass fell below ~ 3.5 kg; Cockrem et al. (18) suggested such a body mass threshold to mark the entrance into PIII in male Adélie penguins). The number of birds present at any time in the pen never exceeded 8 individuals. After one or two days of captivity, penguins were equipped with two pedometers each (Dista F100, Decathlon, France) to record their locomotor activity. Pedometers were coated with mastic to waterproof them and attached to the feathers at hip level (one to the left and one to the right) using cyanoacrylate glue (Loctite). Preliminary analysis showed that the two pedometers deployed with each bird gave comparable readings. This enabled us to use recordings from one instrument only, if the other was lost. Locomotor activity was measured daily. Birds were weighed every other day using an electronic balance (Ohaus, ± 2g) and blood samples were taken frequently during the fasting period (between 3 and 10 times, depending on the initial body mass and daily body mass loss of individuals). Blood samples were collected from the alar vein within 5 minutes of capture, a time recommended by Vleck et al. (36), to assess baseline CORT levels in Adélie penguins. No more than 2-3 birds were

sampled per day to avoid biases in CORT levels. Samples were transferred in tubes pre-treated with heparin or EDTA and centrifuged (5000 rpm for 10 minutes at 4°C). Plasma was then collected and kept frozen in aliquots at –20°C until analysed.

Study 2: Experimental approach

During the austral summers of 2006-07 and 2007-08, a total of 28 males was captured and kept in a pen as described above. After a few days in captivity, they were implanted with CORT pellets of either 10 (n=5; C10), 50 (n=4; C50), 100 (n=8; C100) or 200 mg (n=4; C200). CORT-implanted birds with different doses were held together with the total number of birds never exceeding 8 individuals. CORT pellets (21 days release, G-111) were obtained from Innovative Research of America (Sarasota, FL, USA) and implanted in the nape of the neck. For this, a small patch of skin was disinfected with alcohol and betadine (iodine solution) and a small incision, equal to the size of the pellet, was made. The implant was inserted subcutaneously and the incision was closed, cleaned with betadine, and, finally, sprayed with aluminium powder. Control penguins (n=7) underwent the same protocol but either no pellet was inserted or a placebo pellet was used. All birds were weighed every other day and their locomotor activity was recorded daily as described above. Blood samples were taken as follows: treated penguins were sampled at the time of implantation (day 0), three days after implantation (day 3), and on the last day they spent inside the pen (day 7-11, depending on initial body mass and daily body mass loss of individuals). Control penguins were sampled at day 0 and when they entered PIII.

Plasma analysis: metabolites and hormones

Concentrations of uric acid, βOHB, non-esterified fatty acid (NEFA), and glucose were measured by the enzymatic colorimetric method using commercial kits (uric acid: Sigma

Diagnostics, St Louis, MO, USA; βOHB, NEFA, glucose: Randox Laboratories Ltd., UK). The determination was performed on undiluted plasma (uric acid: 25µl; βOHB: 20µl; NEFA: 12.5µl; glucose: 10µl).

CORT concentrations were determined by a quantitative competitive sandwich enzyme immunoassay technique, according to guidelines provided by the manufacturer (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etude Biologiques de Chizé (CEBC, France). Pooled plasma samples of Adélie penguins produced a dose response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, N.H.P.P. Harbor-UCLA Medical Center, Torrance, USA). Intra and inter-assay coefficients of variation for CORT were 5% and 7%, respectively. The corresponding values for prolactin were 6 % and 9 %, respectively.

Determination of fasting phases and statistical analysis

Plasma levels of uric acid are a reliable indicator of fasting phases in birds (17, 24, 31). The entrance into late fasting (PIII) results from reaching a threshold of low body reserves and is not controlled by fasting duration (31). In the current study, we used one dynamic segmented regression analysis (segmented package from R) for all untreated male birds to identify breakpoints in the relationship between uric acid levels and body mass (study 1). Knowing that captive birds are failed breeders and thus have already started to fast when they were captured and kept in the pen, this allowed us to distinguish three stages during the fasting period (PII; PII-PIII, which corresponds to a transition from PII to PIII; and PIII) and also to identify the corresponding body mass (Fig. 1). These three stages were taken as reference points for the subsequent analysis. We also performed a dynamic regression

analysis for untreated female birds (study 1). However, due to the small sample size, we were able to identify only two fasting stages (PII and PIII).

Comparisons of body mass and plasma parameters in relation to fasting stages in untreated males and females (study 1) were analyzed with a general linear mixed model (GLMM). By including a random and a repeated factor, we accounted for pseudoreplication. In our model we included individuals as random factor, the sampling stage (birds were sampled for blood between 3 and 10 times, depending on individuals) as repeated factor, and fasting stages (PII, PII-PIII, PIII for males and PII, PIII for females) as fixed factor. Normality of residuals was assessed using a Shapiro-Wilk test. When normality was not met and the distribution of data was skewed to the right, a generalized estimating equation (GEE) with a gamma distribution was used. The relationships between locomotor activity and plasma levels of CORT and prolactin were tested using Spearman's rank correlation.

In treated birds (study 2), the effects of CORT implants on body mass, plasma metabolites, and locomotor activity were analysed using GLMM or GEE, when normality was not met. We included "treatment", "sampling stage", and their interaction as a fixed factor, with "sampling stage" being a repeated measure. The year during which the experiment was conducted was also added as a fixed factor. The percentage decrease in prolactin levels in response to prolonged fasting in control birds (between PII and PIII) and in CORT-implanted birds (between the time of implantation and days 7-11) was compared using a one-way ANOVA. In C100-penguins, the effect of CORT implants on prolactin levels was analysed using a GLMM with "treatment", "day relative to implantation" (repeated measure), and their interaction as a fixed factor. The relationship between locomotor activity and prolactin in C100-birds was tested using Spearman's rank correlation.

For multiple comparisons, we used Bonferroni post hoc tests. Analyses were performed using SPSS 16.02 (SPSS Inc., Chicago, Ill., USA). Results are expressed as means ± SE and differences were considered as statistically significant when $p<0.05$.

RESULTS

Study 1: Correlative approach

Validation of fasting phases in untreated male Adélie penguins: Body mass, body mass loss, plasma parameters, and locomotor activity

Untreated male Adélie penguins entered into PII-PIII and PIII at a mean body mass of 3.90 ± 0.16 kg and 3.47 ± 0.15 kg, respectively (dynamic regression of analysis). During the various fasting stages their mean body mass was as follows: 4.16 ± 0.12 kg in PII, 3.77 ± 0.11kg during the PII-PIII transition, and 3.39 ± 0.11 kg in PIII. Penguins spent on average 10.75 ± 2.25 days in PII, 4.25 ± 0.30 days in PII-PIII, and 2.92 ± 0.26 days in PIII. The daily body mass loss depended on the nutritional state (Wald χ^2=57.1, df=2, p<0.001; Table 1), with penguins in PII-PIII and PIII losing 25 % and 71 % more body mass per kilogram per day, respectively than penguins in PII (p<0.001 for both). Moreover, nutritional state influenced plasma levels of βOHB ($F_{2, 24}$=6.60, p=0.005; Table 1). Concentrations of βOHB were 34 % lower during PIII, when compared with PII (p=0.01). In contrast, plasma levels of NEFA ($F_{2, 28}$=4.26, p=0.27; Table 1) and glucose ($F_{2, 28}$=0.50, p=0.61; Table 1) did not differ significantly between the different nutritional states of male penguins.

In untreated males, the nutritional state affected CORT levels ($F_{2, 15}$=8.63, p=0.003; Fig. 2A), prolactin concentrations (Wald χ^2=8.63, df=2, p=0.01; Fig. 2B), and locomotor activity (Wald χ^2=36.4, df=2, p<0.001; Fig. 2C). CORT concentrations and locomotor activity in PIII were 253 % and 91 % higher, respectively, than in PII (p<0.01 for both). In contrast, prolactin concentrations were 33 % lower in PIII, when compared with PII (p=0.03). Locomotor activity was positively correlated with plasma CORT levels (r_s=0.69, p<0.001; Fig. 2D) and negatively correlated with prolactin concentrations (r_s= -0.48, p=0.01; Fig. 2E).

Validation of fasting phases in untreated female Adélie penguins: Body mass, plasma parameters and locomotor activity

Untreated female Adélie penguins entered into PIII at a mean body mass of 3.24 ± 0.07 kg (dynamic regression of analysis). Their mean body mass was 3.80 ± 0.03 kg in PII and 3.11 ± 0.03 kg in PIII. The nutritional state of females affected plasma levels of CORT ($F_{1, 3}$=19.7, p=0.02), βOHB ($F_{1, 5}$=8.00, p=0.03), and prolactin (Wald χ^2=66.3, df=1, p<0.001; Table 2). CORT levels were 130 % higher in PIII than in PII, while plasma levels of βOHB and prolactin were 23 % and 53 % lower, respectively, in PIII when compared with PII. NEFA ($F_{1, 6}$=2.39, p=0.17; Table 2) and glucose levels ($F_{1, 8}$=0.004, p=0.95; Table 2) were not influenced by the nutritional state of birds. Locomotor activity was also affected by the fasting stage of females (Wald χ^2=21.0, df=1, p<0.001; Table 2) and was 77 % higher during PIII than during PII.

Study 2: Experimental approach

Effects of CORT implants on CORT levels and locomotor activity

As expected, plasma levels of CORT were selectively affected by the CORT treatment (Wald χ^2=62.2, df=4, p<0.001) and by the interaction treatment*day relative to implantation (Wald χ^2=137.2, df=7, p<0.001; Fig. 3A). At the time of implantation (day 0), CORT concentrations in C10, C50, C100, and C200-penguins were similar to control birds in PII (all p>0.05). In all CORT implanted penguins, with the exception of C10-penguins (p=0.78), plasma levels of CORT increased significantly between day 0 and day 3 (Fig. 3A). This increase was 2.9-fold for C50-penguins, 3.3-fold for C100-penguins, and 5.4-fold for C200-penguins (all p<0.001). On day 3, CORT levels of C50 and C100-penguins were not different from those of control birds in PIII (p=1.00 and p=0.07, respectively). However, CORT levels of C200-birds on day 3 were significantly higher than those of control penguins (C0) in PIII

($p<0.001$). At the third sampling stage (day 7-11, depending on individuals), CORT levels in C50 and C100-penguins were not different from those on day 3 ($p>0.99$) and were similar to those of control birds in PIII ($p>0.99$; Fig. 3A).

Locomotor activity was selectively affected by CORT treatment (Wald $\chi^2=21.0$, df=4, $p<0.001$; Fig. 3B'). In C10, C50, and C200-penguins locomotor activity was similar to that of control birds in PII ($p>0.99$ for all). In C100-birds, however, locomotor activity was significantly higher than that of control birds in PII ($p=0.002$) and, in fact, similar to that of control birds in PIII ($p>0.99$). Moreover, we found that locomotor activity was affected by the interaction treatment*day relative to implantation (Wald $\chi^2=6.836*10^{\wedge 12}$, df=24, $p<0.001$; Fig. 3B). In C100-penguins, locomotor activity reached a plateau at day 4, after which it remained stable. During this time it was ~ 2.5 times that of control penguins in PII ($p<0.005$ for each given day) and was similar to that of control birds in PIII ($p>0.05$).

Effects of CORT implants on body mass and plasma metabolites

Body mass was affected by the treatment (Wald $\chi^2=69.7$, df=4, $p<0.001$), the sampling stage (day 0 and day 3 for CORT-implanted birds, PII and PIII for control birds; Wald $\chi^2=201$, df=1, $p<0.001$), and their interaction (Wald $\chi^2=51.4$, df=4, $p<0.001$). At the time of implantation, the body mass of control birds (C0) in PII was similar to that of C10 ($p>0.99$), C50 ($p>0.99$) and C200-birds ($p=0.32$). However, body mass of C100-penguins was significantly higher at this point ($p<0.001$). At the subsequent sampling stage, all CORT-implanted birds had a significantly higher body mass than control birds in PIII ($p<0.001$ for each comparison). Similarly, uric acid levels were affected by the treatment (Wald $\chi^2=54.4$, df=4, $p<0.001$), the sampling stage (Wald $\chi^2=70.3$, df=1, $p<0.001$) and their interaction (Wald $\chi^2=61.0$, df=4, $p<0.001$). At the time of implantation, uric acid levels of all CORT-implanted birds (C10, C50, C100, and C200-penguins) were similar to those of control birds in PII

($p<0.05$ for each comparison). Three days after implantation, plasma uric acid levels remained unchanged in C10-penguins ($p>0.99$). However, in all other CORT-implanted penguins, uric acid concentrations were significantly increased at this point, when compared with day 0 (C50: 2.2-fold, $p=0.01$; C100: 2.8-fold, $p<0.001$; C200: 2.4-fold, $p<0.001$). At the subsequent sampling stage (i.e. day 3), only C100-birds had uric acid levels similar to those of control birds in PIII ($p>0.99$). Furthermore, the treatment ($F_{4, 41}= 2.79$, $p=0.04$) and the sampling stage ($F_{1, 41}= 16.0$, $p<0.001$) also affected βOHB levels but their interaction was not significant ($F_{4, 41}=0.64$, $p=0.63$). βOHB concentrations between C10 and C100-penguins differed significantly ($p=0.04$). Plasma levels of NEFA were affected by the treatment ($F_{4, 21}=6.03$, $p=0.002$), so that C10-penguins had higher NEFA concentrations than control, C50, and C100-penguins. In addition, glucose levels were also affected by the treatment ($F_{4, 41}=6.32$, $p<0.001$), so that glucose concentrations were higher in C100 and C200-penguins, when compared with control and C50-birds. However, NEFA and glucose levels were not affected by the sampling stage ($F_{1, 41}=3.11$, $p=0.08$ and $F_{1, 20}=0.67$, $p=0.42$ for NEFA and glucose, respectively) and by the interaction between treatment*sampling stage ($F_{4, 20}=0.35$, $p=0.84$ and $F_{4, 41}=1.42$, $p=0.24$ for NEFA and glucose, respectively).

Effects of CORT implants on prolactin levels

Prolactin levels in C100-penguins were significantly affected by sampling stage ($F_{2, 16}=15.18$, $p<0.001$; Fig. 4A). After 8-11 days of treatment, prolactin concentrations in these birds were significantly lower than on day 0 ($p<0.001$). However, while prolactin levels declined in all birds (between PII and PIII in control birds and between the time of implantation and day 7-11 in CORT-implanted birds), the overall decline (expressed in percent) was not significant ($F_{4, 22}=1.81$, $p=0.16$; Fig. 4B). We found a significant negative

relationship between prolactin levels and locomotor activity in C100-penguins (r_s= -0.52, p=0.03; Fig. 4C) but this was not the case for C10-, C50-, and C200-birds (not shown).

DISCUSSION

The present study shows that an experimentally-induced rise in CORT levels of Adélie penguins, which resulted in high but physiologically relevant circulating levels of CORT, mimicked metabolic, hormonal, and behavioural changes characteristic for the phase of late fasting.

Untreated male and female birds: "validation" of the Adélie penguin model

The minimum body mass threshold

We found that untreated male Adélie penguins in Dumont d'Urville entered PIII of fasting at a body mass of ~ 3.5 kg (Fig. 1). This value was obtained from males with different initial body masses (indicating that fasting duration differed) in studies conducted during several consecutive years between 2002 and 2009 (not shown). This indicates that such a minimum threshold might be relatively fixed for a given species, while differences between the sexes have to be accounted for. Our results lend support to the critical body mass value reported by Cockrem et al. (18) for male Adélie penguins on Ross Island. Given our results, this critical body mass value in male Adélie penguins is consistent across studies and the same for both study sites. When below a body mass threshold of ~ 3.2 kg, females Adélie penguins entered PIII and displayed metabolic, hormonal, and behavioural changes, similar to that of males (Table 2). Hence, females also have the capacity to trigger the refeeding signal, promoting their survival in case of critical reserve exhaustion. Mean body mass of male and female Adélie penguins when arriving in the colony was closed to 5.3 – 6.0 kg and 4.7 – 5.2 kg, respectively (34, Spée et al. unpublished data). With critical body masses ~ 3.5 kg and ~ 3.2 kg for males and females, respectively, these birds should be able to support comparable exhaustion of energy reserves (~ 34-42 % and ~ 32-38 % of body mass loss for males and females, respectively).

CORT, prolactin, and locomotor activity

As expected, untreated Adélie penguins in PIII had high levels of CORT (Fig. 2A). This is in agreement with studies conducted in king penguins (17) and emperor penguins (31). Moreover, the fact that PIII is reached when CORT secretion is strongly stimulated supports the idea that this hormone reflects food stress in birds (25). CORT has numerous biological effects, notably by regulating carbohydrate, lipid, and protein metabolism and is thus expected to play a major role during periods of nutritional limitation (32). Prolactin has the opposite effect of CORT in the control of parental behaviour, stimulating incubation in birds (13). In the present study, captive birds were failed breeders, i.e. they started a reproductive cycle but prematurely discontinued incubation, regardless of parental body condition. In previous studies, prolactin levels remained nearly unchanged after nest failure in Adélie penguins (35) and in emperor penguins (29). However, in our study, prolactin levels at the point of capture were low in some birds, indicating that they might have lost their eggs several days ago (35). As was previously reported for emaciated king penguins (15, 22), we also found that prolactin concentration sharply decreased in PIII (Fig. 2B), suggesting that this hormone can be modulated by marked energy constraints and/or stressful situations. Entrance into PIII of fasting is also associated with behavioural changes and Adélie penguins in our study showed an increased locomotor activity at this stage (Fig. 2C). In untreated penguins, locomotor activity was positively related with CORT levels (Fig. 2D), while there was a negative relationship between locomotor activity and prolactin concentrations (Fig. 2E). These results support the idea of a potential involvement of both CORT and prolactin in the induction of the refeeding signal that occurs in birds during late fasting. Altogether, our results from untreated birds illustrate that Adélie penguins entering into PIII show metabolic, hormonal, and behavioural changes that are similar to those of other penguin species (17, 31). This emphasizes the usefulness of the Adélie penguin model for further investigation.

CORT-implanted penguins: experimental study

Effects of CORT implants on CORT levels and locomotor activity

As expected, CORT levels increased with the implanted dosage (Fig. 3A), attesting that our experimental approach was operative. We found that exogenous CORT did not exert a proportional dose-response effect on locomotor activity but the response rather had the shape of an inverted-U curve (mean value of locomotor activity over the entire treatment duration; Fig. 3B). While high levels of CORT (C100) increased locomotor activity of birds, low (C10), intermediate (C50), and very high (C200) CORT levels had little (C50) or no effect (C10 and C200; Fig. 3B). An inverted-U relationship between CORT levels and locomotor activity was also reported for Gambel's white-crowned sparrows (*Zonotrichia leucophrys gambelii*). In these birds, only intermediate levels of CORT activated behavior, while high levels (but still within the range of CORT levels measured in free-living sparrows in response to capture and handling) had no effect (12). In the present study, circulating levels of CORT measured in C100-birds (63 ng/ml at day 3; 52 ng/ml at day 8-11) fell within the range of CORT levels obtained from untreated male Adélie penguins in PIII (from 23 to 67 ng/ml) and were therefore physiologically relevant.

Lack of a behavioral shift in penguins treated with the highest CORT dosage

Circulating CORT levels measured in C200-birds reached 94 ng/ml at day 3 of treatment and decreased slightly thereafter (Fig. 3A). Such extremely high CORT concentrations have been measured in Adélie penguins before. Cockrem and colleagues (18) reported CORT levels for Adélie penguins that left the colony to refeed at sea that ranged between 10.7 and 110.4 ng/ml, after birds had been captured and handled for ~ 30 min. However, it is possible that the highest CORT dose used in our study (C200) was outside the range of physiological relevance for birds at this fasting stage. Accordingly, this dosage might

have been unable to mimic the transition between PII and PIII, leading to the lack of a behavioral response. Furthermore, it should be beneficial to an animal to initiate behavioral responses in a way that is appropriate for the severity of the stressor. Hence, very high circulating levels of CORT could reflect severe environmental perturbations that may be incompatible with increased activity and food-searching behavior, as suggested by Breuner and Wingfield (12). On the other hand, there are many factors downstream of CORT secretion that can affect the behavioral and physiological outcome of a CORT increase. For instance, the binding of CORT to corticosteroid binding globulin (CBG) may regulate its action by altering the amount of CORT reaching target tissues (11). It is possible that the lack of a response in C200-penguins was related to a strong buffering action induced by an increase in CBG capacity. However, in raptor nestlings, the implantation of CORT pellets induced an increase in CBG capacity that resulted only in an attenuated increase of free CORT levels, attesting that total CORT levels were buffered only to a small degree (30). Future studies examining the time course of CBG capacity and free CORT levels in response to a stressor of different severity (or to several doses of CORT), should provide further insights into the mechanisms that regulate CORT actions in fasting seabirds.

Lag time between CORT application and increased locomotor activity

The time-course of the response following CORT treatment in our study on Adélie penguins (2-4 days) is markedly different from that observed in Gambel's white-crowned sparrows by Breuner & Wingfield (12). These authors reported that the increase in locomotor activity in their birds occurred within 15 minutes of hormone application. This led them to suggest that in white-crowned sparrows, CORT probably acts through a membrane-mediated mechanism (a membrane receptor), to rapidly increase locomotor activity. In the present study, the effect of a high dosage of CORT (C100) on behavior was slower and probably

required the recruitment of intracellular receptors. It can also be proposed that CORT exerts its action through indirect effects on specific targets (see below).

Another explanation for the slower and/or dose-effect behavioral responses in penguins following CORT application might be related to their life history strategy at this breeding stage. In contrast to white-crowned sparrows, penguins spontaneously fast for a long period during incubation and they are prepared to cope with this nutritional constraint (17, 31, 37). This energy demanding period is predictable and, therefore, not considered as stressful (39), based on the observation of constant and low levels of CORT during the major part of their prolonged fast i.e. PII (17, 31; this study). At these baseline concentrations, CORT may regulate behavior and physiology to keep internal systems operational, without inducing a facultative emergency response (28). It is thus conceivable that the birds' response to stressors is reduced at this stage of fasting. The finding that CORT responsiveness to stressors in incubating Adélie penguins (PII) is lower than in birds that leave the colony to refeed at sea (PIII) is consistent with this view (18). Moreover, a reduced sensitivity to intermediate levels of CORT during the incubation period might be beneficial to penguins. Incubating birds are likely to suffer attacks from predators or get into conflict with congeners; situations that certainly could lead to increased CORT levels. However, it is of great importance at this time that birds complete incubation and do not desert the nest. This, on the other hand, does not preclude the entrance into an emergency life history stage, initiated by high levels of CORT (40), in situations like those encountered during late fasting.

Effects of CORT implants on plasma metabolites

In C100-penguins, we observed a shift from lipid to protein utilization, as reflected by the increase in uric acid levels and the decrease in βOHB concentrations (Table 3). This

observation is in agreement with the role of CORT in the fasting-induced rise in protein utilization that was reported in rats (14). Interestingly, the highest dose of CORT in our study (C200), which did not affect locomotor activity, also provoked a less pronounced effect on uric acid levels, when compared with C100-penguins (Table 3). Similar to the dose-dependent effect of CORT on locomotor activity, it seems that the action of CORT on protein breakdown also depends on its concentration. Criscuolo et al. (19) found that protein catabolism after CORT implantation of incubating female common eider ducks was not as high as that of control females in PIII (0.21 mmol/l vs 0.75 mmol/l). Because none of the treated females abandoned their nest, the authors suggested that an increase in proteolysis could be an important factor in triggering refeeding behavior. In this context, it was recently shown that proteolytic systems in the skeletal muscles of rodents are only slightly and selectively induced during PII of fasting, while they are strongly upregulated, in a coordinated fashion, during late fasting (6). Hence, one could hypothesize that the effect of CORT on the escape behavior may depend on a synergistic action, with its catabolic peripheral action taking place in the muscle.

Effects of C100 implants on prolactin levels

Some studies have emphasized that the secretion of CORT and prolactin might be mechanistically linked (2, 19). In our study, C100-pellet was clearly the appropriate dose that mimicked metabolic and behavioral changes, characteristic of PIII in Adélie penguins. In our analysis we therefore focused on C100-penguins to examine how exogenous CORT affected prolactin levels. We found a strong decrease in prolactin concentration 8-11 days after treatment, at which point it was 30 % lower than on day 0 (Fig. 4B). This decline in prolactin levels could be indirect and due to the arrival at a minimum body mass (15, 22). However, 8-11 days after treatment, the average body mass of C100-penguins was 3.74 ± 0.13 kg, and,

therefore, above the critical body mass. This suggests that CORT levels affect circulating prolactin concentrations before birds reach the critical body mass threshold. We found a negative relationship between prolactin levels and locomotor activity in C100-penguins (Fig. 4C). Hence, it could be that the effect of CORT implants on behavioral changes of penguins may be reinforced through an effect on prolactin levels. Support for this idea comes from a study in black-legged kittiwakes, where a short-term increase in CORT levels was accompanied by a 30 % decrease in prolactin and a subsequent reduction in nest attendance (2).

PIII of fasting is a stressful situation

We do not know how animals perceive food deprivation during PIII as stressful, nor do we know how such perception can lead to an increase in CORT levels. It has been suggested that the perception of food deprivation as a stressor is dependent upon an individual's assessment of its energy reserves, probably in the form of depot fat (3). In this context, Kitaysky et al. (26) showed that in black-legged kittiwake chicks, CORT levels were negatively correlated with endogenous lipid reserves. A limitation of the contribution from fatty acids to the energy fluxes could be the key metabolic signal. Low fat mobilization during late fasting could be, at least in part, explained by down-regulation of adipose lipases and/or other lipolytic-related factors, as was recently reported for rodents (9). However, triggering of the refeeding signal that redirects the behavior of fasting penguins from incubation towards food searching after entrance into phase III, cannot be solely attributed to a reduction in lipolytic fluxes and NEFA availability (4). Interestingly, a study in king penguins revealed that a three hour blockade of fatty acid oxidation during PII was sufficient, to increase protein breakdown and CORT levels (5). The authors of this study suggested that the entrance into PIII might be related to a depression in fatty acid oxidation, emphasizing that metabolic stress

induces an increase in CORT levels. It is not known, however, whether the rise in CORT observed in PIII is a trigger for this late fasting stage or a consequence of metabolic changes occurring at this stage. It is also possible that adipose tissue, directly or indirectly, informs the central nervous system of its metabolic status or its level of lipid depletion, inducing CORT secretion and, ultimately, initiating feeding behavior. An essential function in regulating energy homeostasis has recently been attributed to adipocytes, through their considerable capacity to release endocrine, paracrine, and autocrine signals, such as leptin (20). Plasma leptin levels are highly correlated with chronic changes in body adiposity, supporting the view that leptin could constitute a sensor for long-term modifications in energy reserves (1). In rodents, leptin (and particularly low levels of plasma leptin) has been proposed as a constituent of a signal that triggers the fasting-induced enhanced drive for refeeding in PIII (8). It has also been reported for rodents that the expression of adipose secreted factors is differentially down-regulated in response to prolonged food deprivation, suggesting a specific role for some of them in the adaptation to prolonged fasting (10). Future studies should examine to what extent adipose tissue of spontaneously fasting birds selectively secretes adipokines.

Conclusions and perspectives

This study shows that an experimentally-induced rise in CORT levels of Adélie penguins, within the physiologically relevant range, mimicked metabolic, hormonal, and behavioral changes, characteristic of birds in PIII. This was illustrated by the observed shift in fuel catabolism (from lipid towards protein utilization), the decline in prolactin levels, and the increase in locomotor activity. Our study emphasizes the role of CORT in the refeeding decision that occurs in late fasting. One behavioral consequence of PIII is nest abandonment in free-ranging birds. To examine the role of hormones in the orchestration of nest

abandonment, it would be of great interest to investigate whether implantation of CORT pellets induces nest desertion in breeding birds, and to what extent this abandonment is facilitated by a decline in prolactin levels. In fact, we recently showed that high levels of CORT concomitant with low levels of prolactin are needed to trigger nest desertion in Adélie penguins (33). Moreover, because prolactin has opposite effect compared to CORT in the control of parental behavior and decline in PIII, it would be interesting to examine whether and to what extent, an experimentally-induced decrease in prolactin concentrations induces behavioral changes characteristic of PIII, independent of a rise in CORT levels.

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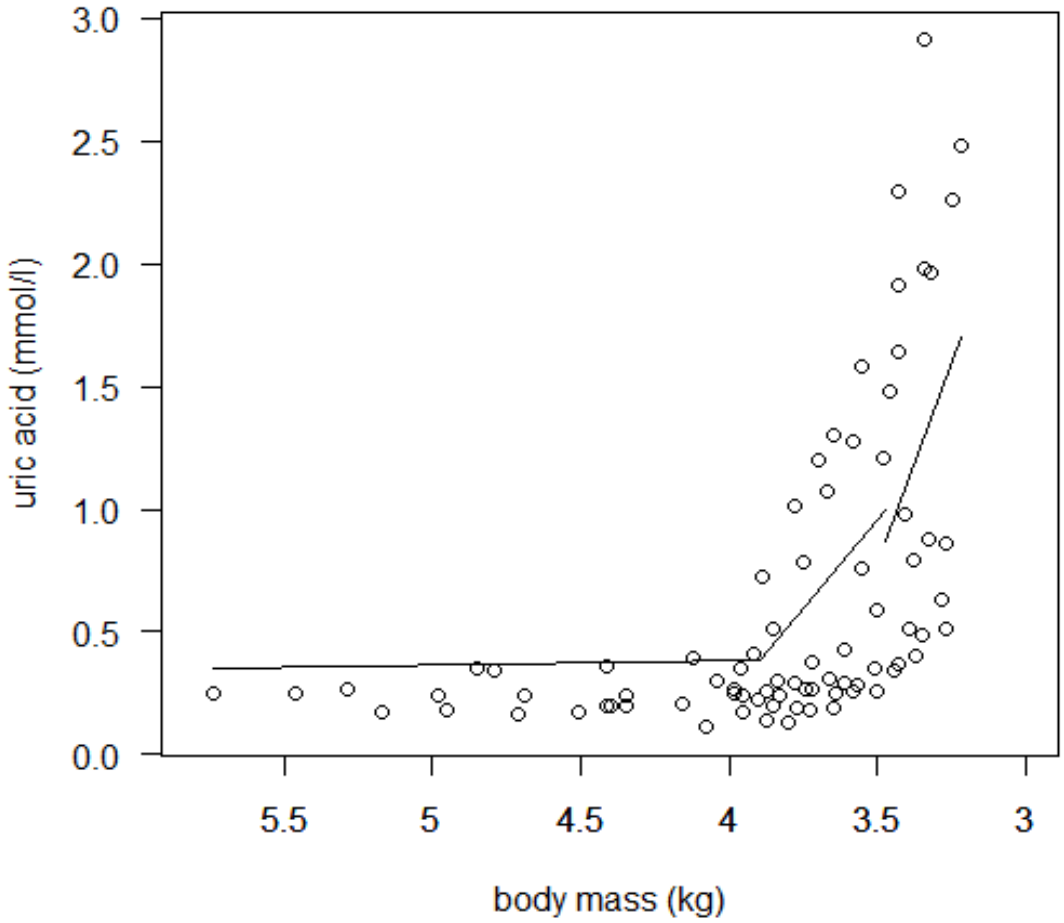
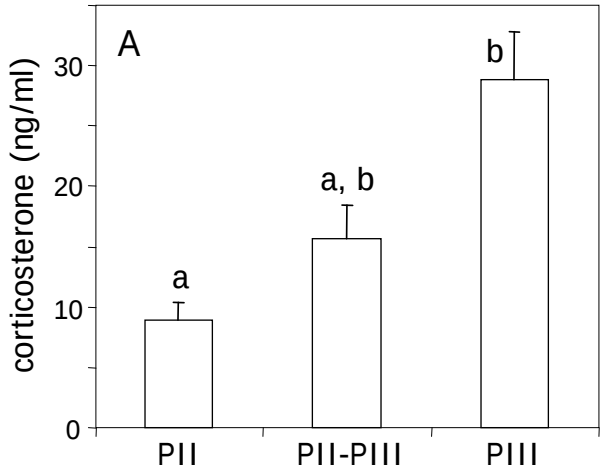
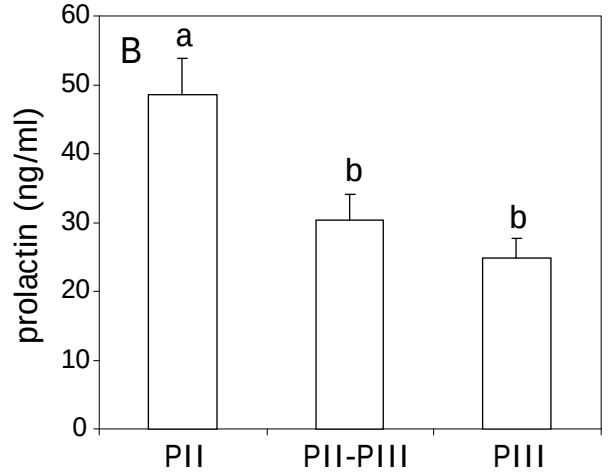
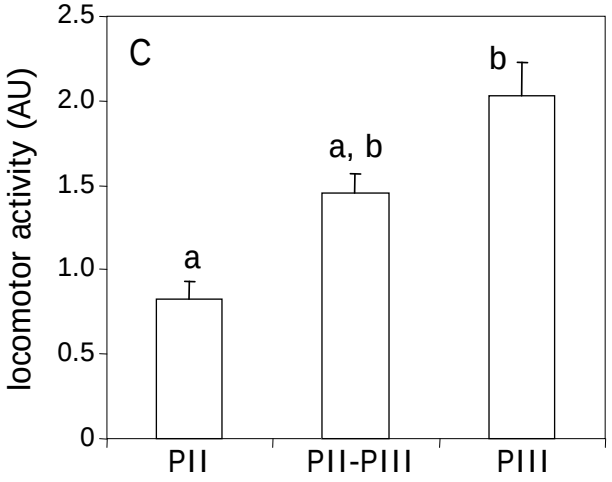
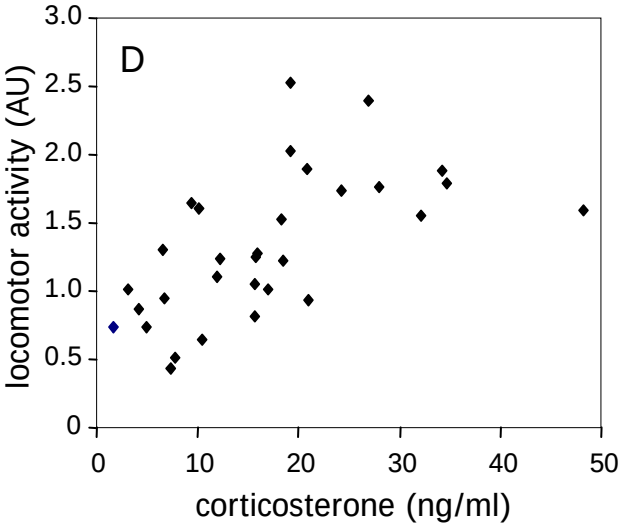


Fig. 1


A
corticosterone (ng/ml)
0
10
20
30
a
a, b
b
PII
PII-PIII
PIII


B
prolactin (ng/ml)
0
10
20
30
40
50
60
a
b
b
PII
PII-PIII
PIII


C
locomotor activity (AU)
0
0.5
1.0
1.5
2.0
2.5
a
a, b
b
PII
PII-PIII
PIII


D
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0.5
1.0
1.5
2.0
2.5
3.0
0
10
20
30
40
50
corticosterone (ng/ml)

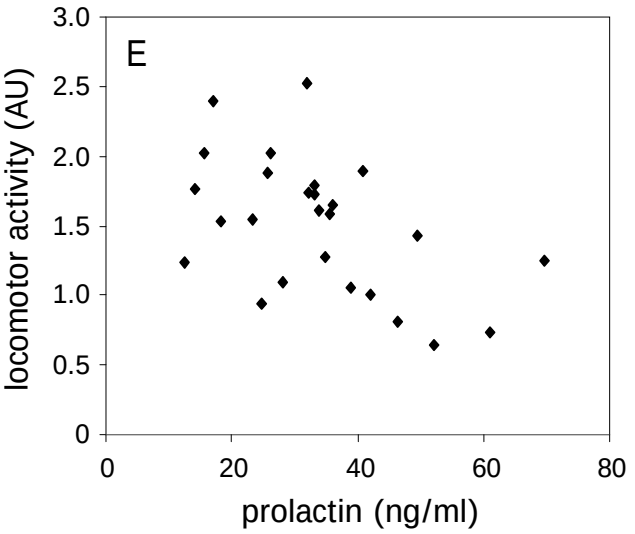

E
locomotor activity (AU)
0
0.5
1.0
1.5
2.0
2.5
3.0
0
20
40
60
80
prolactin (ng/ml)

Fig. 2

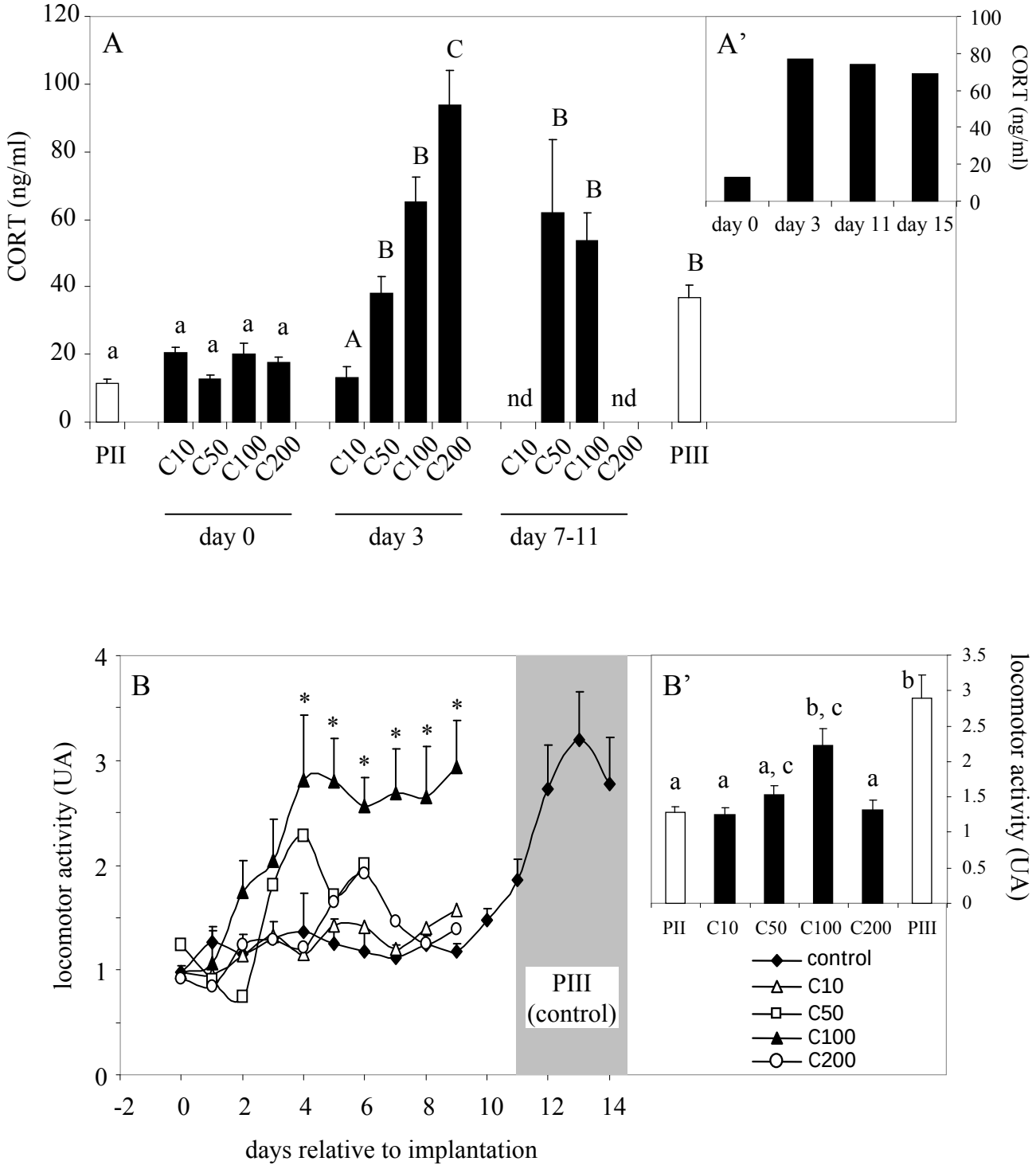
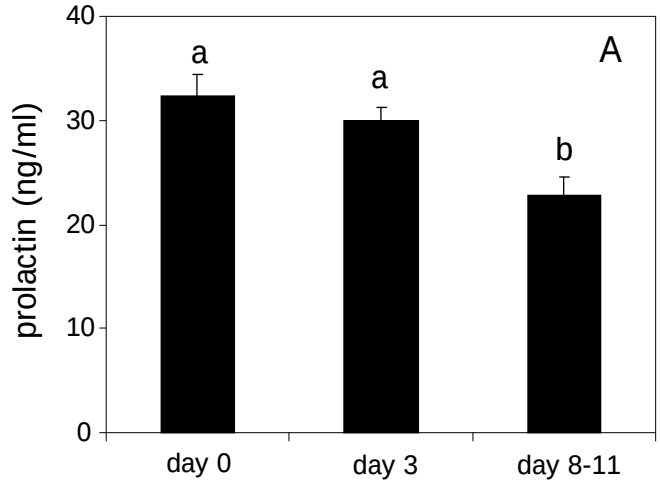
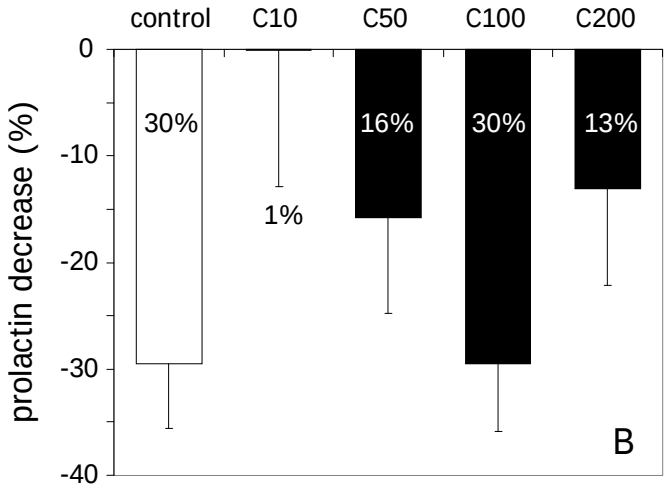

A
A'
CORT (ng/ml)
day 0
day 3
day 11
day 15
PII
PIII
C10
C50
C100
C200
day 0
day 3
day 7-11
nd
B
B'
locomotor activity (UA)
days relative to implantation
PIII
(control)
control
C10
C50
C100
C200

Fig. 3


A
a
a
b
prolactin (ng/ml)
40
30
20
10
0
day 0
day 3
day 8-11


control
C10
C50
C100
C200
0
-10
-20
-30
-40
30%
1%
16%
30%
13%
prolactin decrease (%)
B

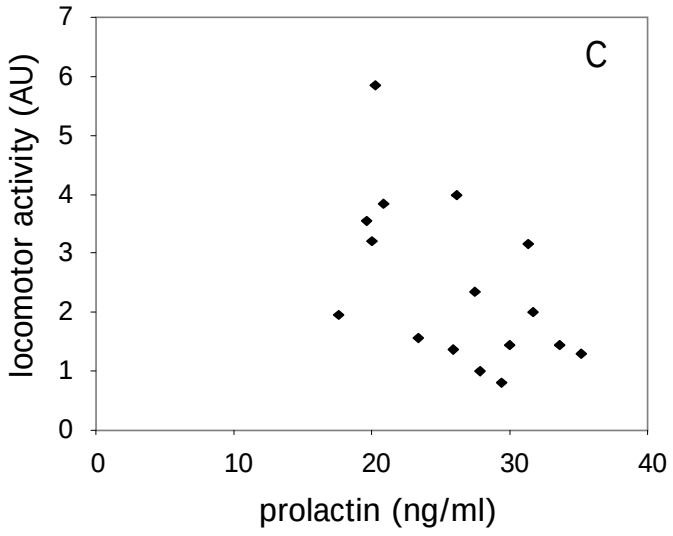

C
locomotor activity (AU)
7
6
5
4
3
2
1
0
0
10
20
30
40
prolactin (ng/ml)

Fig. 4

Fig. 1. Plasma levels of uric acid in relation to body mass changes in captive male Adélie penguins (untreated birds; n=12). The lines within the figure were determined using one dynamic segmented regression analysis for all untreated male birds. Two breakpoints were detected. This allowed us to distinguish three stages during the fasting period and also to identify the corresponding body mass.

Fig. 2. Plasma levels of CORT (**A**; n=12), prolactin (**B**; n=8), and locomotor activity (**C**; n=12) in relation to fasting stage in captive male Adélie penguins (untreated birds). Values are means ± S.E. Bars not sharing the same superscript letter are significantly different ($p<0.05$). Relationship between plasma levels of CORT and locomotor activity (**D**; n=12), and between prolactin levels and locomotor activity (**E**; n=8) in male captive Adélie penguins (untreated birds).

Fig. 3. (**A**) Plasma levels of CORT in control captive male Adélie penguins in PII and PIII of fasting (open bars, n=7) and in CORT-implanted birds (black bars, n=4-8), in relation to sampling stage. CORT concentrations of CORT-implanted birds at day 0 were compared with those of control birds in PII, while CORT levels at day 3 and day 7-11 were compared with those of control birds in PIII. nd, not determined. (Insert **A'**) Characteristic profile of CORT levels in one C100-treated bird weighing 5.8 kg and kept 17 days in the pen. (**B**) Changes in locomotor activity relative to fasting phase in control captive male Adélie penguins (n=7), and relative to implantation day in CORT-treated penguins (n=4-8). * $p<0.05$ in C100 vs control. (Insert **B'**) Effects of treatment on locomotor activity (mean value of locomotor activity over the entire treatment duration). Penguins were implanted with 10 (C10), 50 (C50), 100 (C100) and 200 (C200) mg of CORT. Values are means ± S.E. Bars not sharing the same superscript letter are significantly different ($p<0.05$).

Fig. 4. (**A**) Effect of C100-CORT implants on prolactin levels in captive male Adélie penguins (n=8). Values are means ± S.E. Bars not sharing the same superscript letter are significantly different ($p<0.05$). (**B**) Decrease in prolactin levels (%) in response to prolonged fasting in control birds (between PII and PIII) and in CORT-implanted birds (between the time of implantation and day 7-11). Penguins were implanted with 10 (C10), 50 (C50), 100 (C100) and 200 (C200) mg of CORT. (**C**) Relationship between plasma levels of prolactin and locomotor activity in C100-implanted penguins.

Table 1

Profile of untreated captive male Adélie penguins according to fasting stage.

Nutritional state	PII	PII - PIII	PIII
Body mass loss, g/kg.day^{-1}	17.45 ± 0.52 ^{a}	21.88 ± 0.77 ^{b}	29.88 ± 1.56 ^{c}
βOHB, mmol/l	1.29 ± 0.14 ^{a}	1.10 ± 0.13 $^{a, b}$	0.85 ± 0.12 ^{b}
NEFA, mmol/l	0.84 ± 0.09 ^{a}	0.82 ± 0.08 ^{a}	0.75 ± 0.06 ^{a}
Glucose, mmol/l	12.24 ± 0.61 ^{a}	12.53 ± 0.55 ^{a}	13. 10 ± 0.45 ^{a}

PII, phase II of fasting; PII-PIII, transition from phase II to phase III of fasting; PIII, phase III of fasting; βOHB, β-hydroxybutyrate; NEFA, non esterified fatty acids. Results are means ± S.E. (n = 10-12). Within each line, values that do not share the same superscript letter are significantly different ($p<0.05$).

Table 2

Profile of untreated captive female Adélie penguins according to fasting stage.

Nutritional state	PII	PIII
βOHB, mmol/l	1.11 ± 0.20 [a]	0.86 ± 0.17 [b]
NEFA, mmol/l	0.92 ± 0.08 [a]	0.81 ± 0.09 [a]
Glucose, mmol/l	12.36 ± 0.43 [a]	12.75 ± 0.48 [a]
CORT, ng/ml	11.74 ± 3.17 [a]	27.01 ± 3.33 [b]
Prolactin, ng/ml	56.49 ± 0.99 [a]	26.53 ± 1.55 [b]
Locomotor activity, AU	1.21 ± 0.03 [a]	2.14 ± 0.14 [b]

PII and PIII, phase II and III of fasting; βOHB, β-hydroxybutyrate; NEFA, non esterified fatty acids; AU, Arbitrary Unity. Results are means ± S.E. (n = 4-5). Within each line, values that do not share the same superscript letter are significantly different ($p<0.05$).

Table 3

Body mass and plasma levels of metabolites in control and CORT-implanted captive male Adélie penguins in relation to treatment and sampling stage (day relative to implantation in treated birds and fasting phase in control birds).

	Day relative to implantation	C10 (n=5)	C50 (n=4)	C100 (n=8)	C200 (n=4)	Fasting phase (Control birds)	Control (n=7)
Body mass, kg	0	4.31 ± 0.22 [a, b]	4.37 ± 0.23 [a, b]	4.89 ± 0.16 [a]	4.46 ± 0.09 [a, b]	PII	4.13 ± 0.03 [b]
	3	4.10 ± 0.29 [a]	4.07 ± 0.20 [a]	4.51 ± 0.17 [a]	4.11 ± 0.12 [a]	PIII	3.39 ± 0.05 [b]
Uric acid, mmol/l	0	0.19 ± 0.03 [a]	0.17 ± 0.01 [a]	0.25 ± 0.03 [a]	0.19 ± 0.03 [a]	PII	0.17 ± 0.01 [a]
	3	0.26 ± 0.01 [a]	0.36 ± 0.06 [a, b]	0.70 ± 0.06 [c]	0.46 ± 0.05 [b]	PIII	0.88 ± 0.21 [b, c]
βOHB, mmol/l	0	1.83 ± 0.42	1.74 ± 0.31	1.07 ± 0.12	1.44 ± 0.08	PII	1.79 ± 0.27
	3	1.44 ± 0.41	0.98 ± 0.13	0.72 ± 0.12	0.87 ± 0.15	PIII	0.88 ± 0.21
NEFA, mmol/l	0	1.42 ± 0.27	0.88 ± 0.11	0.68 ± 0.08	0.99 ± 0.06	PII	0.84 ± 0.14
	3	1.41 ± 0.39	0.68 ± 0.11	0.60 ± 0.07	0.99 ± 0.13	PIII	0.71 ± 0.12
Glucose, mmol/l	0	14.34 ± 0.37	13.27 ± 1.89	14.98 ± 0.27	18.22 ± 0.72	PII	13.05 ± 0.78
	3	14.84 ± 1.38	10.75 ± 0.29	15.23 ± 0.23	14.32 ± 1.81	PIII	12.63 ± 0.54

Penguins were implanted with 10 (C10), 50 (C50), 100 (C100), and 200 (C200) mg of CORT, respectively; βOHB, β-hydroxybutyrate; NEFA, non esterified fatty acids. PII and PIII, phase II and III of fasting, respectively. Results are means ± S.E. Superscript letters were not added when the interaction between the treatment and the sampling stage was not significant. Within each line, values that do not share the same superscript letter are significantly different ($p<0.05$). See the Results section for further details.

Résumé - Etude 3

How does corticosterone trigger egg abandonment in free-living Adélie penguins?

Introduction

L'étude 1 (Spée et al. 2010) a permis de mettre en évidence, par une approche corrélative chez des manchots Adélie en cours de reproduction, que la CORT est nécessaire mais non suffisante pour provoquer l'abandon du nid. En effet, une diminution des niveaux de prolactine apparaît cruciale pour stimuler le départ de l'oiseau. L'étude 2 a montré, par une approche expérimentale chez des manchots Adélie captifs, qu'une dose forte mais physiologique de CORT exogène a un rôle actif dans l'induction du signal de réalimentation et entraîne notamment une diminution de la prolactine. Une approche expérimentale chez des oiseaux sauvages en cours de reproduction est cependant nécessaire pour étudier le rôle de la CORT dans la promotion de l'abandon du nid.

Le but de cette étude est de déterminer dans quelle mesure la CORT exogène 1) entraine l'abandon du nid, 2) affecte les niveaux de prolactine et 3) a des effets subséquents sur la durée des voyages alimentaires et sur le succès reproducteur.

Matériels et méthodes

Les manchots Adélie mâles ont été capturés à deux occasions : 1) quelques jours avant la ponte où ils ont été implantés avec 0 (témoins ; n=29), 10 (C10 ; n=15) ou 100 mg (C100 ; n=15) de CORT et 2) au moment du départ en mer du mâle, consécutif au retour de la femelle ou à l'abandon du nid. A ces deux périodes, les oiseaux ont été pesés et un prélèvement sanguin a été effectué.

Nous avons également déterminé les dates de ponte et d'éclosion ainsi que le nombre d'œufs. La durée des voyages alimentaires a été déterminée, ainsi que le succès reproducteur

final (estimé par le nombre de poussins atteignant le stade de crèche, la mortalité étant très rare à cette période, Clarke et al. 2002; Beaulieu et al. 2009, 2010).

Résultats et discussion

L'augmentation expérimentale de CORT a provoqué 60 % d'abandon du nid, ~ 14 jours après le traitement (contre 7 % chez les oiseaux témoins). Une diminution concomitante de prolactine a également été observée. La CORT est donc clairement impliquée dans la promotion de l'abandon du nid lorsque l'incubation est associée à un jeûne prolongé. Il apparaît également que la CORT agit à travers la diminution de la prolactine pour provoquer cette désertion. De façon intéressante, les manchots traités qui n'abandonnent pas leur nid mais sont relevés par la femelle présentent des niveaux de prolactine initiaux (quelques jours avant la ponte) et finaux (lors du départ en mer) plus élevés que ceux des manchots traités qui abandonnent leur nid. Cela suggère fortement que l'atteinte d'un seuil bas de prolactine est nécessaire pour provoquer la désertion du nid. De plus, nous pouvons émettre l'hypothèse que les manchots présentant des niveaux initiaux élevés de prolactine pourraient être de « meilleurs parents », comme en témoigne l'absence d'effet du traitement sur leur succès reproducteur.

Afin de mieux comprendre le rôle de la prolactine dans l'induction de l'abandon du nid, il serait également intéressant d'examiner l'effet d'une diminution expérimentale des niveaux de cette hormone (indépendamment d'une augmentation des niveaux de CORT).

How does corticosterone trigger egg abandonment in free-living Adélie penguin?

Soumis

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ABSTRACT

1. During the breeding season, individuals enter an emergency life-history stage when their body reserves reach a minimum threshold. Consequently, they redirect current activity towards survival. One example of such behaviour is egg abandonment in birds.

2. Corticosterone (CORT), the major avian glucocorticoid, is known to promote this emergency-life-history stage. How and to what extent CORT triggers egg abandonment when breeding is associated with a naturally prolonged fasting period, however, requires further investigation. It has been proposed that CORT could affect breeding success by acting through other endocrine factors regulating parental care (like prolactin) or by affecting foraging performance.

3. We tested these hypotheses by implanting free-living male Adélie penguins with CORT pellets before their laying period. We examined (1) their behavioural response to increased CORT levels with respect to nest abandonment in parallel with (2) their prolactin levels, and (3) the subsequent effects of the treatment on foraging trip duration and breeding success.

4. Exogenous CORT induced egg abandonment in 60 % of the treated penguins ~ 14 days after initiation of the treatment. We also observed a concomitant decline in prolactin levels. Interestingly, treated penguins that did not abandon their nest but were relieved by females, experienced a similar decline in prolactin levels as penguins that abandoned their nest. However, prolactin concentrations in the former birds were higher at the point of implantation and also after being relieved by females, when compared with penguins that abandoned their nest.

5. Our results suggest that CORT alone is not sufficient to trigger nest abandonment in Adélie penguins. Instead, CORT seems to act through a decline in prolactin levels, and this latter hormone has to reach a low threshold value to trigger nest desertion. Furthermore, when prolactin levels are high before the incubation fast, penguins are more likely to maintain

parental care following CORT treatment and, consequently, avoid nest desertion. We propose that penguins with higher prolactin levels in our study could be "better parents", as their breeding success was similar to that of control birds.

Key words: breeding success, glucocorticoid, incubation, long-term fasting, long-lived bird, parental care, prolactin, *Pygoscelis adeliae*

INTRODUCTION

During the demanding breeding period, life-history theory predicts that individuals should favour their own survival over that of their offspring, when breeding becomes too costly in terms of body maintenance (Stearns 1992). Once the parents' energetic state reaches a low threshold, they enter an emergency life-history stage triggering physiological and behavioural changes that enhance survival (Wingfield *et al.* 1998). One example of such behaviour is egg abandonment in birds.

Several studies examining the mechanisms that mediate life-history trade-offs in vertebrates, have emphasised the importance of glucocorticoids. Corticosterone (CORT), the primary glucocorticoid hormone in birds, has been linked to the promotion of an emergency life-history stage (Wingfield *et al.* 1998) and has also been associated with the reduction or suppression of parental care (Wingfield 2003, Wingfield & Sapolsky 2003). For instance, exogenous CORT increases foraging activity at the expense of chick brooding/guarding in black-legged kittiwakes (Kitaysky, Wingfield & Piatt 2001) and elevated CORT levels have been linked to egg abandonment in incubating fasting penguins (Groscolas, Lacroix & Robin 2008; Spée *et al.* 2010). However, Criscuolo and colleagues (2005) showed that an experimentally-induced rise in CORT concentration alone was not sufficient to trigger nest desertion in incubating female common eider ducks *Somateria mollissima*. The authors suggested that the lack of effect could be due to 1) a too short duration of treatment since CORT concentration returned to its baseline level within four days of treatment, 2) a too high dose and/or 3) another mechanism involving other endocrine factors.

Recent studies strongly suggest that CORT could affect other endocrine factors promoting the expression of parental care (Angelier *et al.* 2009, Criscuolo *et al.* 2005). In this context, the pituitary hormone prolactin is of particular interest, since it is involved in the initiation and the maintenance of avian incubation behaviour (Buntin 1996). Correlative

studies revealed that an increase in CORT levels resulting from acute stress or prolonged energy constraints is associated with a decrease in prolactin levels (Angelier *et al.* 2007a, Chastel *et al.* 2005, Groscolas *et al.* 2008). In agreement with these observations, experimental studies have showed that exogenous administration of CORT leads to a reduction in plasma prolactin concentrations (Angelier *et al.* 2009, Criscuolo *et al.* 2005). These studies suggest that the secretion of CORT and prolactin might be mechanically linked. We therefore suggest that increased CORT levels, induced by prolonged energy constraints like fasting, could act through prolactin concentrations and thereby stimulate egg abandonment. The finding that Adélie penguins with high CORT concentrations and low prolactin levels desert their nest, whereas birds with both high CORT and prolactin levels do not (Spée *et al.* 2010), strongly supports this hypothesis.

Long-lived seabirds are good models to study the underlying endocrine mechanisms that mediate the decision to promote self-maintenance at the expense of current reproduction. In seabirds and especially in penguins, reproduction is associated with long fasting periods (up to four months in the emperor penguin *Aptenodytes forsteri*) (Groscolas & Robin 2001), since foraging and breeding areas are generally far apart. In Adélie penguins *Pygoscelis adeliae*, males and females both migrate to the breeding colony, where females fast until the clutch is complete. As they return to sea to feed, males usually take on the first incubation shift (Ainley 2002). Thereafter both parents alternate in caring for the egg(s) and chick(s). However, birds are likely to abandon incubation, once energy reserves are nearing a critical point of exhaustion. In this case, they enter a proteolytic stage, as revealed by increased uric acid levels, (Cherel *et al.* 1988, Robin *et al.* 1998), the so-called phase III of fasting (PIII), and are prone to abandon their nest. Given their long life span, the optimal strategy for an incubating penguin entering PIII is to abandon the current reproductive effort in favour of its own survival, thereby ensuring future reproductive attempts.

In this study, we firstly examined whether the maintenance of high CORT levels can induce abandonment of reproduction in free-living Adélie penguins. We experimentally increased CORT levels to mirror the activation of an emergency life-history stage and examined to what extent this affected the completion of incubation (whether penguins abandoned their nest in response to the treatment or not). Secondly, knowing that prolactin plays a key role in the control of parental behaviour in birds and that this hormone might also be linked with CORT, we examined whether CORT administration affected prolactin levels. Finally, because CORT can modify foraging behaviour (Angelier *et al.* 2008, Angelier *et al.* 2007b) and subsequent reproductive success (Criscuolo *et al.* 2005), we also monitored the effects of the treatment on foraging trip duration in treated males and their partners and the number of surviving chicks.

MATERIALS AND METHODS

Field procedure

The study was conducted in Dumont d'Urville Station (66°40'S; 140°01'E), Adélie land, Antarctica, during the 2007-2008 austral summer and was approved by the ethics committee of the French Polar Institute (IPEV) and the Terres Australes et Antarctiques Françaises (TAAF).

Free-living male Adélie penguins were captured on two occasions (Fig. 1). Firstly, penguins were captured between the periods of pair formation and egg-laying. To determine baseline levels of CORT, a blood sample was collected from the wing vein within less than 5 minutes of capture, as already reported in Adélie penguins (Vleck *et al.* 2000). Samples were transferred into tubes pre-treated with heparin and centrifuged (5000 rpm for 10 minutes at 4°C). Plasma was then collected and kept frozen in aliquots at – 20°C until laboratory analysis. Each penguin was then implanted with CORT pellets, placed subcutaneously in the nape of the neck. Because the action of CORT on escape behaviour is known to be dependant on its concentration (Breuner & Wingfield 2000, Spée, Marchal, Thierry, Chastel, Enstipp, Le Maho, Beaulieu & Raclot, unpublished data), birds were implanted with 0 (control; n=29), 10 (C10; n=15) or 100 (C100; n=15) mg of CORT. CORT pellets (21 days release, G-111) were obtained from Innovative Research of America (Sarasota, FL, USA). All birds were weighed with an electronic balance (Ohaus, ± 2 g) and marked with a Nyanzol-D mark, painted on the breast feathers. Sex was determined by a combination of parameters including cloacal inspection before egg laying, incubation routine (males usually take on the first incubation shift; Ainley 2002), and measure of plasma lipemia (females exhibit higher lipemia before egg-laying than males; Beaulieu *et al.* 2010a, Kern *et al.* 2005).

Secondly, males were recaptured at the end of the first incubation shift, when they left the colony to refeed at sea, either because (1) their partner had returned to relieve them or (2)

because they abandoned their nest. Among the control birds, two males abandoned the nest while 27 were relieved by females. In C10-penguins only one bird abandoned the nest, while 14 were relieved by females. In C100-birds nine abandoned the nest and six were relieved by females. During this second capture, birds were weighed and another blood sample was taken.

Nests were observed in 2-3 hours intervals to determine (1) which birds were present on the nest and potential nest abandonments, (2) the date of egg laying, (3) the number of eggs per pair, and (4) the date of hatching. Nest observations were especially important for males that were close to their critical body mass (~ 3.5 kg,Cockrem, Potter & Candy 2006, Spée, Marchal, Thierry, Chastel, Enstipp, Le Maho, Beaulieu & Raclot, unpublished data) or that were less attentive during incubation, and were therefore carried out on an hourly basis. We also monitored foraging trip duration (during incubation and the chick-rearing stage, until the seventh foraging trip in succession) in males and their partners. The number of chicks was determined one week after hatching date and during the crèche stage, when they reached their peak body mass (~ 42-45 days after hatching, Ainley 2002). At this latter stage, chicks were weighed with an electronic balance (Ohaus, ± 2 g). Reproductive success was estimated from the number of chicks surviving to this latter stage, since after this point chick mortality is usually very rare (Beaulieu *et al.* 2010b, Beaulieu *et al.* 2009, Clarke *et al.* 2002).

Laboratory analyses

Plasma CORT concentrations were determined by immunoassay according to guidelines provided by the manufacturer (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Plasma concentrations of prolactin were determined by a heterologous radioimmunoassay (RIA) at the Centre d'Etude Biologiques de Chizé (CEBC; France). Pooled plasma samples of Adélie penguins produced a dose response curve that paralleled chicken prolactin standard curves (bAFP 4444BQ, source: Dr. Parlow, N.H.P.P. Harbor-

UCLA Medical Center, Torrance). Intra and inter assay variations for prolactin were 6 % and 9 %, respectively, while they were 5 % and 7 %, respectively for CORT.

Data analyses and statistics

We used a generalized linear mixed model (GzLM) with a binomial distribution family to examine the effect of treatment on the percentage of nest abandonment.

To analyse the effect of "treatment" (control, C10, and C100) and "completion of the first incubation shift" (relieved by females or nest abandonment) on most of the variables investigated (date of implantation and departure to sea; body mass and body condition index at these stages; fasting duration between the time of implantation and departure to sea; daily body mass loss; dates of egg-laying and hatching; number of egg(s), chick(s), and brood mass) and their interactions, we did not use a general linear model because the resulting groups were unbalanced. In fact, due to the small number of abandoning birds within the control and C10-groups (n=2 and n=1, respectively) and the large number of abandoning C100-penguins (n=9), the statistical results would automatically be biased. Instead, we considered (1) control, C10, and C100-penguins relieved by females and (2) control and C100-penguins that abandoned their nest. We were not able to conduct statistical analyses in C10-penguins because only one bird abandoned the nest in this group. General linear models (GLM) were used to compare body mass, fasting duration, and daily body mass loss between groups. A generalized linear mixed model (GzLM) with a gamma distribution was used to compare the brood mass because data were not distributed normally but were skewed to the right. GzLM's with a Poisson distribution were used for count data (date of implantation, departure to sea, egg-laying and hatching, number of eggs and chicks).

The aim of our study was to examine whether exogenous CORT might induce nest abandonment and to shed light into the associated endocrine changes in free-living male

Adélie penguins. For this reason we compared CORT and prolactin levels in treated birds (C100-penguins) with those of control birds that abandoned their nest (high CORT and low prolactin levels;Groscolas *et al.* 2008, Spée *et al.* 2010), and not with control birds that were relieved by females (low CORT and high prolactin levels;Groscolas *et al.* 2008, Spée *et al.* 2010). Accordingly, we divided birds into three groups: C100 Re (penguins implanted with 100 mg of CORT that were relieved by females), C100 Ab (birds implanted with 100 mg of CORT that abandoned their nest), and control Ab (control birds that abandoned their nest). The effects of CORT implants on circulating prolactin and CORT levels were examined using a general linear mixed model (GLMM) and a generalized estimating equation (GEE) with a gamma distribution, respectively, since CORT data were not distributed normally. We included individuals as a random factor and "group" (C100 Re, C100 Ab, and control Ab), "sampling period" (the day of implantation and the day of departure to sea) and their interactions as fixed factors, with "sampling period" being a repeated measure.

As the duration of the first foraging trip, which takes place during the incubation stage, and the duration of subsequent trips differ in Adélie penguins, comparisons between groups (control and C100) were carried out separately. To compare the duration of the first foraging trip between control and C100 penguins in males and their partners, we used a Student's *t*-test or a Mann-Whitney test, when normality was not met (for females). A GEE with a gamma distribution was used to compare the duration of subsequent foraging trips in males and their partners. We included individuals as a random factor and "treatment" (control and C100), "trip number" (from the second to the seventh foraging trip in succession) and their interactions as fixed factors, with "trip number" being a repeated measure.

For multiple comparisons we used Bonferroni post hoc tests. Analyses were performed using SPSS (Vers. 16.02; SPSS Inc., Chicago, Ill., USA). Results are expressed as means ± SE and differences were considered as statistically significant when $p<0.05$.

RESULTS

Nest abandonment

We found a strong effect of treatment on nest abandonment in free-living male Adélie penguins (6.9 %, 6.7 %, and 60 % in control, C10, and C100-penguins, respectively; Wald χ^2=14.4, df=2, p<0.001; Fig. 2). C100-birds were seven times more likely to abandon their nest than control (p<0.001) and C10-penguins (p=0.001).

Abandoning birds (control, C10, and C100-penguins)

At the time of implantation, control and C100-penguins that abandoned their nest had similar body masses ($F_{1, 9}$=3.72, p=0.09; Table 1) and were implanted at the same time (Wald χ^2=0.55, df=1, p=0.46; Table 1). Abandoning C100-penguins spent 40 % less time ashore between the time of implantation and their departure to sea ($F_{1, 9}$=10.7, p=0.01; Fig. 3A), and they lost ~ 32 % more body mass per day than control birds that deserted their nest ($F_{1, 9}$=8.10, p=0.02; Fig. 3C). At the time of departure to sea, abandoning C100-penguins left the colony earlier (Wald χ^2=67.5, df=1, p<0.001; Table 1) and their body mass was 18 % higher than that of control birds that deserted their nest ($F_{1, 9}$=8.74, p=0.02; Fig. 3B).

We found no significant effect of the treatment on laying date (Wald χ^2=0.03, df=1, p=0.87; Table 1) and the number of eggs (Wald χ^2=0.04, df=1, p=0.84; Table 1) in birds that deserted their nest.

Birds relieved by females (control, C10, and C100-penguins)

At the time of implantation (implantation date was similar between groups, Wald χ^2=1.29, df=2, p=0.53; Table 1), control, C10, and C100-birds that were relieved by females had similar body masses ($F_{2, 44}$=2.81, p=0.07; Table 1). Fasting duration for males between the time of implantation and their departure to sea was also similar between groups ($F_{1,}$

$_9$=0.46, p=0.63; Fig. 3A). In contrast, daily body mass loss was significantly different between groups ($F_{2, 35}$=11.5, p<0.001; Fig. 3C). C100-penguins had a daily body mass loss that was 15 % and 20 % greater than that of C10 (p=0.007) and control birds (p<0.001), respectively. The date of departure to sea differed between control, C10, and C100-penguins that were relieved by females (Wald χ^2=8.58, df=2, p=0.01; Table 1) but body masses were similar at this stage ($F_{2, 35}$=1.71, p=0.20, Fig. 3B).

We found no significant effect of treatment on laying date (Wald χ^2=0.16, df=2, p=0.92; Table 1) and the number of eggs (Wald χ^2=0.19, df=2, p=0.91; Table 1) in birds relieved by females. In contrast, the treatment significantly affected hatching date (Wald χ^2=39.7, df=2, p<0.001; Table 1). Eggs of control birds hatched ~ 2 and 3 days earlier than that of C10 and C100-penguins, respectively (p<0.001 for both). The treatment did not affect the number of chicks, neither one week after the hatching date (Wald χ^2=0.42, df=2, p=0.81; Table 1), nor when chicks reached their peak body mass (Wald χ^2=0.25, df=2, p=0.88; Table 1). Moreover, the brood mass at this latter stage was not affected by the treatment (Wald χ^2=0.57, df=2, p=0.75; Table 1).

CORT and prolactin levels in abandoning control and C100-penguins

CORT levels changed significantly in all penguins during the study period (Wald χ^2=17.2, df=1, p<0.001; Fig. 4A). At the time of implantation (initial levels), circulating levels of CORT were 70 % lower than when they left the colony to refeed at sea (final levels) (p<0.001). However, plasma levels of CORT during that period did not differ between groups (Wald χ^2=1.47, df=2, p=0.48) and were not influenced by the interaction between group and sampling period (Wald χ^2=1.02, df=2, p=0.60).

By contrast, there was a significant effect of group ($F_{2, 12}$=4.84, p=0.03; Fig. 4B) and sampling period ($F_{1, 12}$=49.4, p<0.001) on prolactin concentrations. C100-penguins that were

relieved by females (C100 Re) had higher initial and final prolactin levels than abandoning C100-penguins (C100 Ab; p=0.049) and abandoning control-birds (control Ab; p=0.048). Initial and final prolactin levels were similar in abandoning C100-penguins (C100 Ab) and abandoning control-birds (control Ab) (p=1.00). Moreover, prolactin decline between the initial and final sampling stage was similar in all groups, as indicated by a lack of interaction between group and sampling period with respect to the effect on plasma prolactin levels ($F_{2, 12}$=0.22, p=0.80).

Foraging trip duration in control and C100-penguins

Duration of the first foraging trip in males, which takes place during the incubation stage, was affected by the treatment (t=2.32, df=30, p=0.03; Fig. 5A). C100-penguins spent 13 % less time at sea, when compared with control birds. Subsequent foraging trip duration was also affected by the treatment (Wald χ^2=29.7, df=1, p<0.001), trip number (Wald χ^2=295, df=5, p<0.001), and their interaction (Wald χ^2=249, df=5, p<0.001; Fig. 5A). C100-penguins spent 75 % more time at sea during their second foraging trip, when compared with control birds (p<0.001). However, during subsequent foraging trips there were no significant differences between groups.

Treatment of male penguins had no effect on foraging trip duration of their female partners, neither during incubation (U=50, p=0.36; Fig. 5B), nor during the chick-rearing period (Wald χ^2=1.71, df=1, p=0.19; Fig. 5B), indicating that females did not compensate.

DISCUSSION

In our study, exogenous CORT (C100) induced a high proportion of egg abandonment in free-living Adélie penguins, which was paralleled by a decline in prolactin levels. We suggest that 1) CORT alone is not sufficient to trigger nest abandonment in Adélie penguins, and 2) prolactin levels may need to reach a low threshold value to stimulate nest desertion.

CORT treatment and egg abandonment

Our experimentally-induced rise in CORT levels mimicked the activation of an emergency life-history stage, as indicated by a significant rate of nest abandonment in the group treated with the high dose of CORT (C100). Nest abandonment in this latter group was 60 %, while it was around 7 % in control and C10-penguins. In contrast to C100-penguins, birds in these two other groups had body masses at the time of abandonment that were lower than the critical body mass threshold which typically characterizes the entrance into PIII in male Adélie penguins (Cockrem *et al.* 2006, Spée, Marchal, Thierry, Chastel, Enstipp, Le Maho, Beaulieu & Raclot, unpublished data). Moreover, the time that C10-penguins spent fasting before deserting their nest was similar to that of abandoning control birds. Thus, the most likely explanation for the only abandoned nest in C10-penguins that we observed is reach of a low threshold in body reserves rather than a direct response to low dose of CORT.

In addition, we found that C100-penguins abandoned their nest ~ 14 days (range: from 9 to 19 days) after the start of the treatment, while control penguins deserted ~ 23 days after manipulation. This suggests that the entrance into an emergency life-history stage, driven by elevated CORT levels, that redirects behaviour towards survival (Wingfield *et al.* 1998) might need a sufficient amount of time to develop. Moreover, it suggests that the effect of CORT on the triggering of nest abandonment is indirect and probably depends on the sensitivity of individuals to CORT. The progressive effect of CORT on prolactin levels might also play a

role (Angelier *et al.* 2009). In fact, the inhibitory action of CORT on plasma prolactin seems to be complex, since prolactin levels decrease only slowly and progressively in response to an increase in CORT levels (Angelier *et al.* 2009).

Why some treated birds did not abandon their nest

Egg abandonment occurs when CORT secretion is strongly stimulated (Groscolas *et al.* 2008, Spée *et al.* 2010). We found that abandoning control birds had similar CORT levels than those experienced by C100-birds in response to the treatment, suggesting that the three groups (control Ab, C100 Ab and C100 Re) underwent similar stress levels, as a result of critical exhaustion of energy reserves or in response to the treatment. There are many downstream factors of CORT secretion, which can affect the behavioural and physiological outcome of a CORT increase. For instance, the binding of CORT to corticosteroid binding globulin (CBG) may regulate the action of CORT by altering its amount reaching target tissues (Breuner & Orchinik 2002). According to the free hormone hypothesis, the unbound hormone is the biologically active fraction, able to enter cells and activate receptors. In this framework, the primary role of CBG would be to regulate the bioavailability and clearance rate of CORT (Ekins 1990). It has been reported that free baseline CORT levels of nest-abandoning female European starlings *Sturnus vulgaris* are significantly higher than those of non-abandoning birds (Love *et al.* 2004). Future studies examining CBG capacity could test the hypothesis that C100-penguins that did not abandon their nest have a higher CBG capacity, which would make them less sensitive to exogenous CORT. In addition, CORT interacts with receptors to induce behavioural and physiological actions. Certain hormone levels can decrease the number of receptors in an attempt to compensate for elevated CORT concentrations (Landys et al. 2006; Romero 2004). In the present study, we suggest that abandoning C100-birds did not markedly reduce receptor production, which still resulted in a

high sensitivity to CORT treatment. Furthermore, a number of studies in birds highlighted a deactivation of the hypothalamic-pituitary-adrenal-system after CORT administration, which leads to a down-regulation of the endogenous CORT secretion (Busch *et al.* 2008, Müller *et al.* 2009). However, because the exogenous dose of CORT we implanted was very high in comparison to endogenous baseline levels of CORT in penguins, such down-regulation might not explain the selective behavioural response we observed.

Interestingly, we found that the main difference between C100-birds relieved by their partner (C100 Re) and abandoning C100-penguins (C100 Ab) was their prolactin profile. The magnitude of the decrease in prolactin levels between the time of implantation and departure to sea was similar in both groups (41 % and 46 % for C100 Re and C100 Ab, respectively), but they had different initial (at the time of implantation) and final prolactin concentrations (when relieved by their partner or when they abandoned their nest). Prolactin levels were significantly higher in C100-birds relieved by their partner when compared with abandoning C100-penguins. If we consider the rate of decrease in prolactin levels between the two groups (expressed as the percentage of prolactin decline per day), we found that prolactin levels decreased more rapidly in abandoning birds, when compared with C100-birds relieved by their partners (3.5 % and 1.8 % per day, respectively; Fig. 6). Angelier and Chastel (2009) proposed that the rate of decrease in prolactin levels in response to a standardized stress protocol (capture followed by 30 min restraint) is directly related to the motivation of the individual to maintain parental care. The same hypothesis could apply to the present results.

The discrepancies in incubation behaviour among C100-treated birds emphasises the need of prolactin levels to reach a minimum threshold value before nest desertion is triggered. There is growing evidence in the literature that the relationship between parental behaviour and prolactin levels is non-linear and depends on a threshold value of prolactin levels (Angelier & Chastel, 2009). It has been proposed that after reaching a minimum threshold

level of prolactin, post-hatching parental care can no longer be provided or maintained (Boos *et al.* 2007, Criscuolo *et al.* 2002).

Why did initial prolactin levels differ between birds?

In penguins, prolactin levels increase gradually throughout courtship and peak during the middle of the incubation phase (Vleck *et al.* 1999). We could simply assume that C100-penguins that were relieved by females had higher prolactin levels at the time of implantation because they were sampled at an earlier point of their breeding cycle. However, this was not the case, as control birds, C100-penguins relieved by females, and C100-penguins that abandoned their nest were sampled at the same point in time. Moreover, their laying date was similar, indicating that their reproductive cycle was synchronized.

In addition, there is evidence that breeding experience can affect baseline prolactin levels. Prolactin levels are generally lower in inexperienced parents than in experienced ones (Angelier *et al.* 2007a, Angelier *et al.* 2006, Christensen & Vleck 2008). Age also affects baseline prolactin levels (Angelier *et al.* 2007a, Deviche, Wingfield & Sharp 2000, Preault *et al.* 2005), with young birds having lower baseline prolactin levels than older breeders. Future studies carried out with birds either of known age or for which information about the biological age is measurable (by determining telomere length for example; (Monaghan & Haussmann 2006) will allow to test the hypothesis that C100-penguins relieved by females were older breeders, while C100-birds that abandoned their nest belonged to inexperienced parents.

Effect of treatment on foraging trip duration and reproductive success

C100-penguins that were relieved by females spent less time at sea during their first foraging trip when compared with control birds. They also had high CORT levels when they

left the colony to undertake their first foraging trip, at which point they were still within the delivery period of the implant. It has been reported earlier that Adélie penguins with high CORT levels before a foraging trip spend less time at sea and engage in a greater foraging effort, while foraging success is lower (Angelier *et al.* 2008). We can thus assume that treated birds were not as efficient as usual during their first foraging trip and needed to replenish their body reserves when undertaking their second foraging trip. Accordingly, the second foraging trip in these birds was longer than in control birds. Foraging trip duration of the partner of CORT treated males was not different from that of partners of control birds. In accordance with our result, Beaulieu and colleagues found that females of handicapped male Adélie penguins (which spent more time to forage at sea than control birds) did not change their foraging trip duration (Beaulieu *et al.* 2010c). In further studies, it would be of interest to examine the effect of CORT treatment on foraging performance as reflected in diving behaviour and spatial distribution; this could be done by monitoring the foraging behaviour with miniaturized dive recorders and GPS loggers. Moreover, determination of parental body mass before and after a foraging trip would permit to quantify the amount of food stored in the stomach and subsequently delivered to the chick(s), thus allowing to determine parental provisioning rates.

We found that reproductive success (as determined by the number of chicks that survived until they reached peak body mass) and brood mass were similar in control and C100-birds relieved by their partners. Accordingly, this suggests that their adjustment in foraging behaviour was sufficient to ensure self-maintenance and survival of their chicks.

Conclusions and perspectives

This study clearly illustrates the involvement of CORT in the decision-making process regarding egg abandonment in penguins, when incubation is associated with a natural long

fast. However, it is also clear that CORT alone is not sufficient to trigger nest abandonment but that a marked decline in prolactin levels to a low threshold value is also required (Fig. 6). Interestingly, C100-penguins that were relieved by females had higher initial and final prolactin levels than abandoning C100-birds. This suggests that high prolactin levels prior to the incubation fast prompts penguins to maintain parental care in stressful situations, such as induced by prolonged energy constraints. We suggest that penguins with higher initial prolactin levels could be "better parents" (see Preault *et al.* 2005). The finding that reproductive success in these penguins was similar to that of control birds (similar number of chicks that survived until they reached their peak body mass) is consistent with this suggestion but this remains to be tested experimentally in further studies. Future investigations should also examine whether CORT-treated birds remain able to adopt an emergency coping response when facing life-threatening situations (Wingfield *et al.* 1998) and whether their survival rate is impaired, as has been suggested recently (Goutte *et al.* 2010). Moreover, to shed more light into the hormonal control of egg abandonment, it would be of great interest to examine the effect of an experimental decrease in prolactin levels - not accompanied by an increase in CORT concentrations - in incubating birds.

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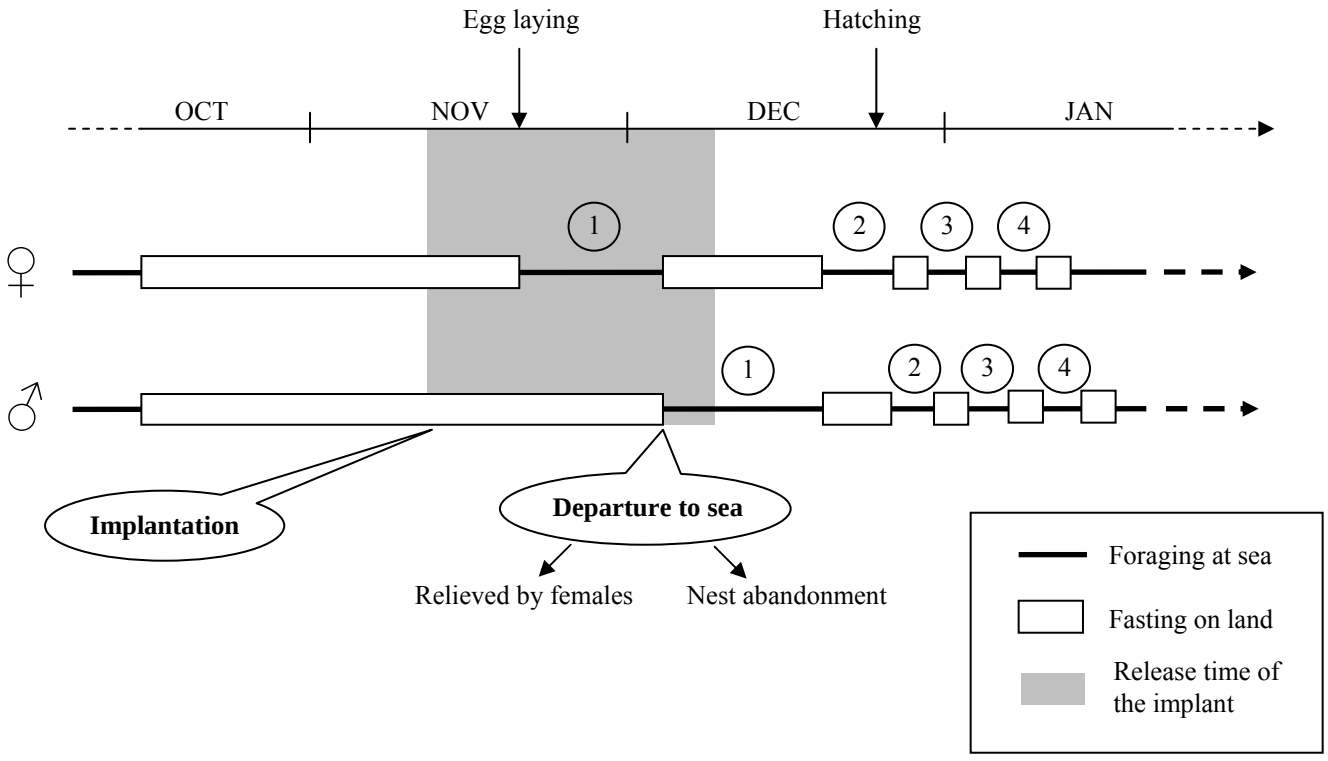

Egg laying
Hatching
OCT
NOV
DEC
JAN
1
2
3
4
♀
♂
Implantation
Departure to sea
Relieved by females
Nest abandonment
Foraging at sea
Fasting on land
Release time of the implant

Fig. 1

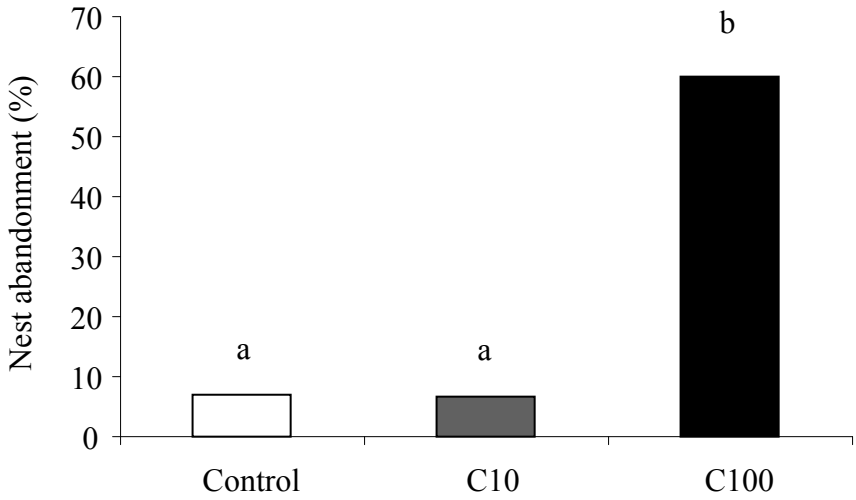


Fig. 2

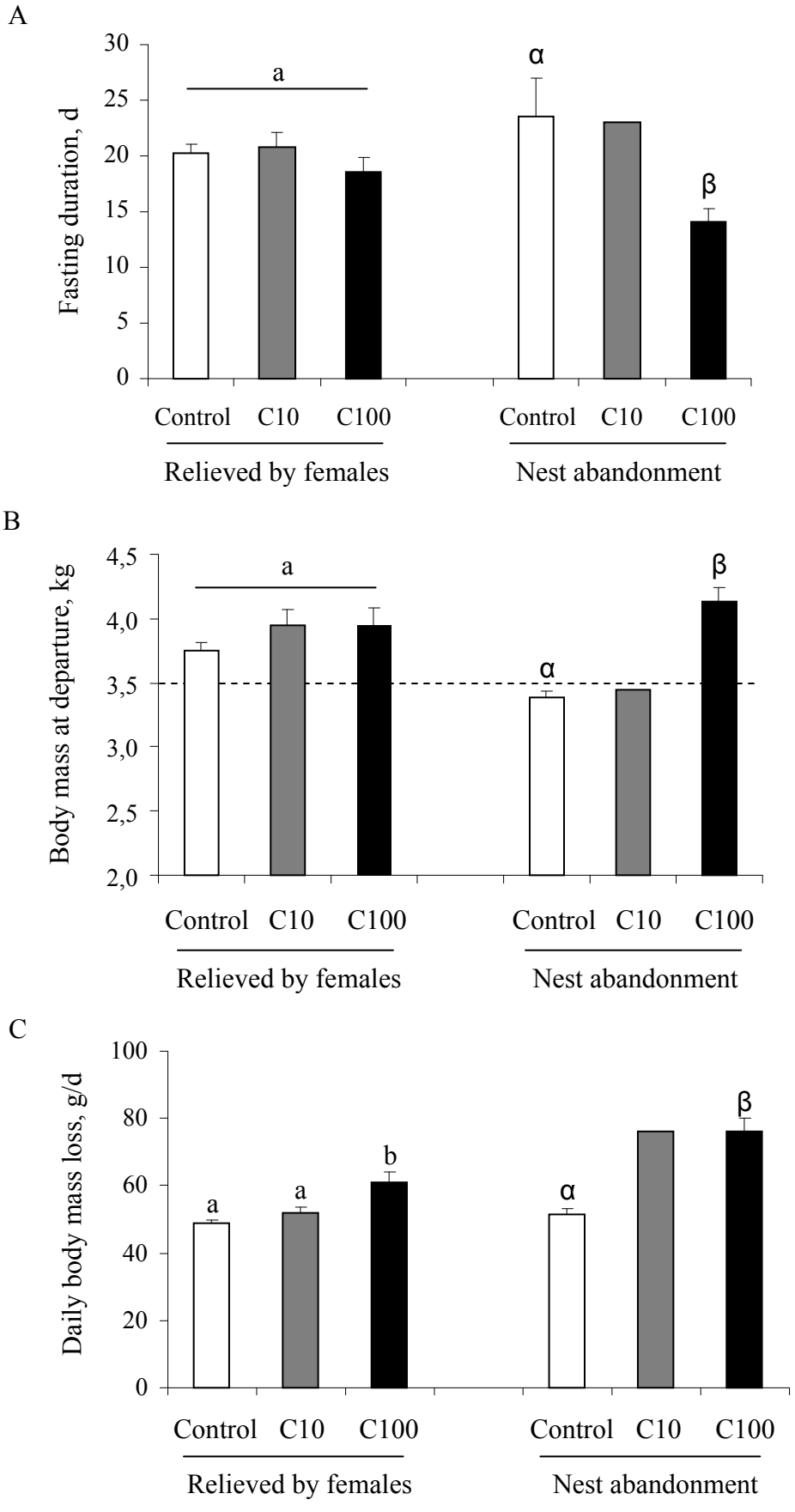
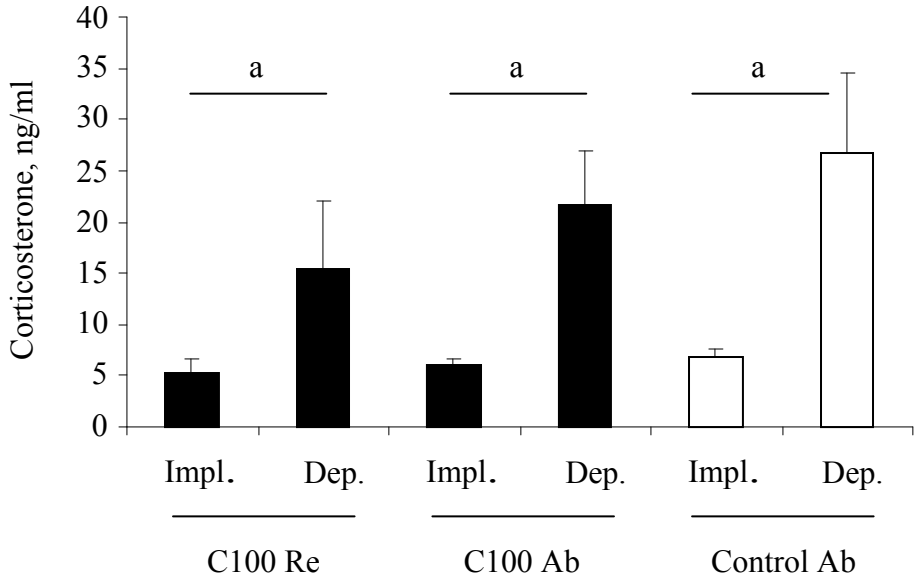

A
Fasting duration, d
30
25
20
15
10
5
0
a
α
β
Control
C10
C100
Control
C10
C100
Relieved by females
Nest abandonment
B
Body mass at departure, kg
4,5
4,0
3,5
3,0
2,5
2,0
a
α
β
Control
C10
C100
Control
C10
C100
Relieved by females
Nest abandonment
C
Daily body mass loss, g/d
100
80
60
40
20
0
a
a
b
α
β
Control
C10
C100
Control
C10
C100
Relieved by females
Nest abandonment

Fig. 3

A


Corticosterone, ng/ml
40
35
30
25
20
15
10
5
0
a
a
a
Impl.
Dep.
Impl.
Dep.
Impl.
Dep.
C100 Re
C100 Ab
Control Ab

B

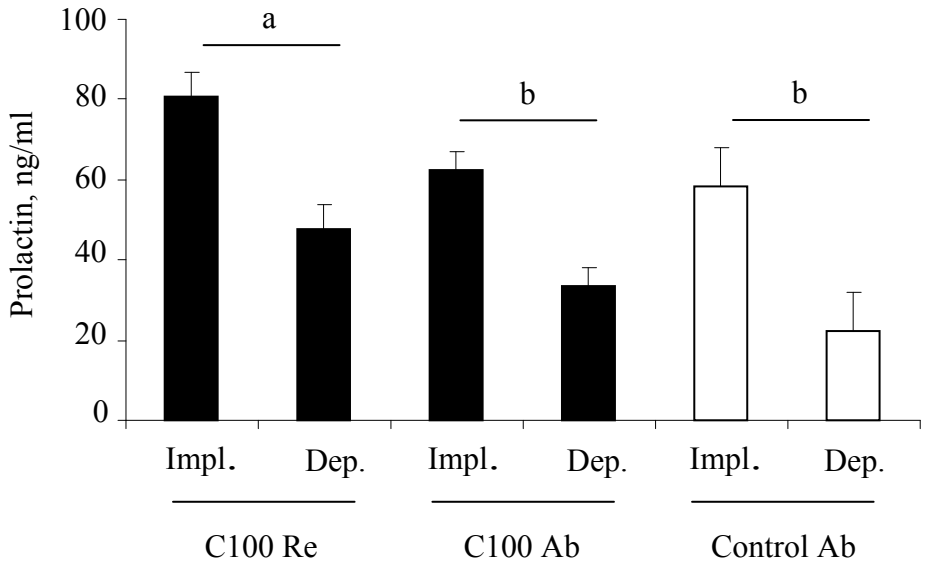
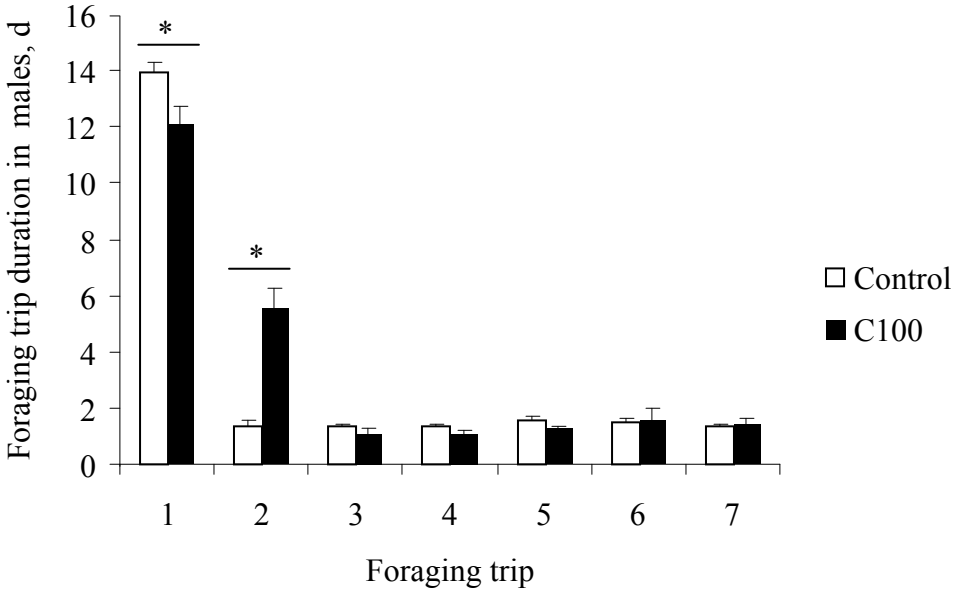

Prolactin, ng/ml
100
80
60
40
20
0
a
b
b
Impl.
Dep.
Impl.
Dep.
Impl.
Dep.
C100 Re
C100 Ab
Control Ab

Fig. 4

A


Foraging trip duration in males, d
16
14
12
10
8
6
4
2
0
*
*
1
2
3
4
5
6
7
Foraging trip
Control
C100

B

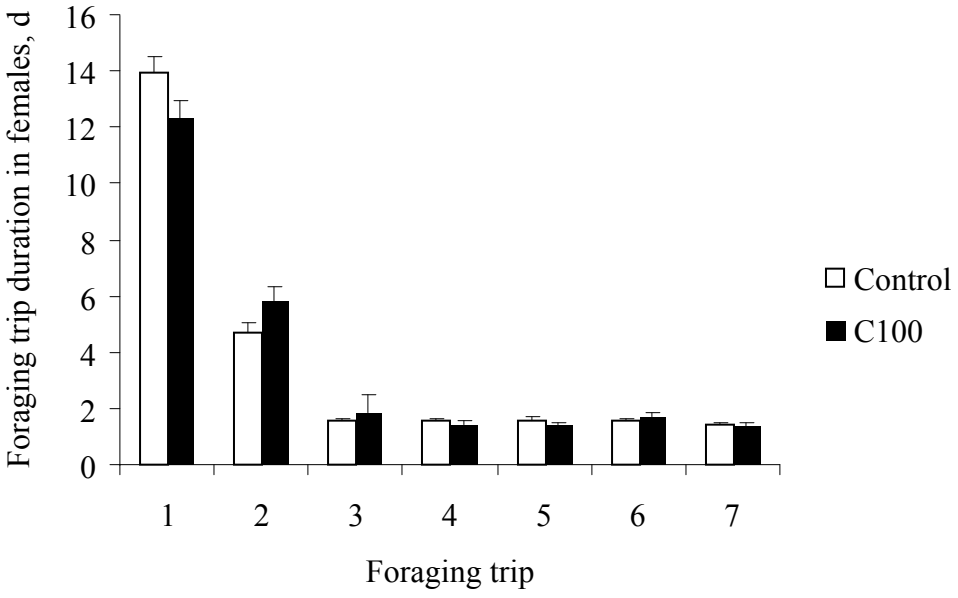

Foraging trip duration in females, d
16
14
12
10
8
6
4
2
0
1
2
3
4
5
6
7
Foraging trip
Control
C100

Fig. 5

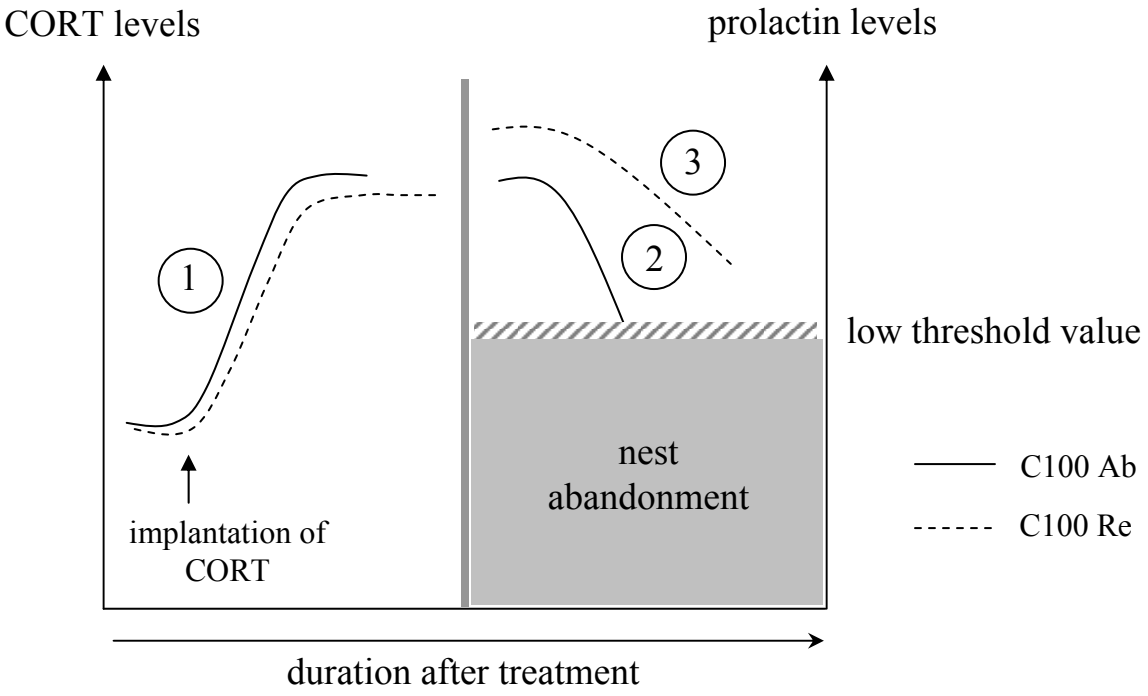

CORT levels
prolactin levels
1
2
3
low threshold value
nest
abandonment
implantation of
CORT
C100 Ab
C100 Re
duration after treatment

Fig. 6

Fig. 1. Study protocol. Male Adélie penguins were weighed and blood samples were taken at the time of implantation and when they departed to sea (when their partner had returned or when they abandoned their nest). Numbers indicate successive foraging trips of males and their female partners. The grey area represents the period during which CORT was released from the implant. See Materials and methods section for details.

Fig. 2. Effect of treatment on nest abandonment in free-living male Adélie penguins. C10 and C100 represent penguins implanted with 10 and 100 mg of CORT, respectively.

Fig. 3. Effect of CORT implants on fasting duration (A), body mass at the time of departure to sea (B), and daily body mass loss (C) in free-living male Adélie penguins, according to incubation success (relieved by females or nest abandonment). C10 and C100 represent penguins implanted with 10 and 100 mg of CORT, respectively. The dotted line (B) represents the body mass threshold below which control male Adélie penguins enter phase III of fasting and spontaneously abandon their nest (Cockrem *et al,* 2006; Spée *et al.* 2010). Results are means ± S.E. For all graphs, among birds relieved by females and abandoning penguins bars with different superscript letters are significantly different from each other.

Fig. 4. Effect of CORT implants on corticosterone (A) and prolactin (B) levels in free-living male Adélie penguins. Impl., the time of implantation; Dep., the time of departure to sea (after relieved by females or after nest abandonment); C100 Re, birds implanted with 100 mg of CORT that were relieved by females; C100 Ab, birds implanted with 100 mg of CORT that abandoned their nest; Control Ab, control penguins that abandoned their nest. Results are means ± S.E. Bars with different superscript letters are significantly different from each other.

Fig. 5. Effect of CORT implants on foraging trip duration in free-living male Adélie penguins relieved by females (A) and in their female partners (B). C100 represents males implanted with 100 mg of CORT. Results are means ± S.E. Bars with different superscript letters are significantly different from each other.

Fig. 6. Schematic representation of how exogenous CORT acts on prolactin levels, leading to nest abandonment (or not) in free-living male Adélie penguins. (1) The implantation of CORT induced a similar rise in CORT levels in both groups (C100 Ab, birds implanted with 100 mg of CORT that deserted their nest ~ 14 days after the start of the treatment, solid line; and C100 Re, birds implanted with 100 mg of CORT that were relieved by females ~ 19 days after the start of the treatment; dotted line). (2) Initial prolactin levels were lower in treated birds that abandoned their nest when compared with birds relieved by females. The rate of decline in prolactin levels in response to the CORT treatment was also greater in the former group, so that their prolactin levels reached a low threshold value, triggering egg abandonment. (3) By contrast, prolactin levels in treated birds relieved by females declined at a slower rate and did not reach the low threshold value until males were successfully relieved by females.

Table 1

Profile of control and CORT treated-penguins (C10 and C100) according to their incubation success.

Completion of the 1st incubation shift	Relieved by females			Nest abandonment		
Treatment	Control (n=27)	C10 (n=14)	C100 (n=6)	Control (n=2)	C10 (n=1)	C100 (n=9)
Date of implantation	16/11 ± 0.64 ^{a}	16/11 ± 1.06 ^{a}	17/11 ± 0.98 ^{a}	16/11 ± 0.00 $^{\alpha}$	16/11	16/11 ± 0.80 $^{\alpha}$
Body mass at implantation (kg)	4.75 ± 0.06 ^{a}	5.10 ± 0.15 ^{a}	4.94 ± 0.21 ^{a}	4.59 ± 0.10 $^{\alpha}$	4.89	5.18 ± 0.14 $^{\alpha}$
Departure date	6/12 ± 0.62 $^{a, b}$	7/12 ± 0.15 ^{a}	5/12 ± 0.47 ^{b}	9/12 ± 3.50 $^{\alpha}$	9/12	30/11 ± 1.02 $^{\beta}$
Laying date	20/11 ± 0.47 ^{a}	20/11 ± 0.62 ^{a}	20/11 ± 0.95 ^{a}	19/11 ± 1.50 $^{\alpha}$	24/11	19/11 ± 0.62 $^{\alpha}$
No. of eggs per pair	1.78 ± 0.08 ^{a}	1.93 ± 0.07 ^{a}	2.0 ± 0.0 ^{a}	2.0 ± 0.0 $^{\alpha}$	1	1.78 ± 0.15 $^{\alpha}$
Hatching date	23/12 ± 0.48 ^{a}	25/12 ± 0.62 ^{b}	26/12 ± 0.68 ^{b}	-	-	-
No. of chicks per pair *	1.23 ± 0.13 ^{a}	1.36 ± 0.20 ^{a}	1.60 ± 0.24 ^{a}	-	-	-
No. of chicks per pair $	1.13 ± 0.11 ^{a}	1.31 ± 0.20 ^{a}	1.40 ± 0.24 ^{a}	-	-	-
Brood mass $	4.29 ± 0.26 ^{a}	5.73 ± 0.58 ^{a}	5.26 ± 0.93 ^{a}	-	-	-

C10 and C100: male Adélie penguins implanted with 10 and 100 mg of CORT, respectively. Results are expressed as means ± SE. Among birds that were relieved by females and abandoning birds, values with different superscript letters are significantly different from each other. * one week after the hatching date; $ when chicks reached their peak body mass (42 to 45 days after hatching; Ainley, 2002).

Etude 4

An experimental decrease in prolactin leads to behavioral changes but does not affect nest desertion in Adélie penguins

En préparation.

Marion Spée, Claire Galland, David Lazin, Yvon Le Maho, Olivier Chastel, Michaël Beaulieu &Thierry Raclot.

OBJECTIFS

Nous avons vu lors des chapitres précédents que la CORT est fortement impliquée dans le processus d'abandon du nid lorsque l'incubation est associée à un jeûne prolongé. Toutefois, il apparait que l'augmentation seule de cette hormone n'est pas suffisante pour stimuler l'abandon. Nous avons en effet mis en évidence que des oiseaux en PIII de jeûne n'abandonnant pas leur nid présentaient un taux élevé de CORT et une concentration de prolactine également haute. Au contraire les oiseaux en PIII qui abandonnent leur nid présentent un niveau élevé de CORT et faible de prolactine (étude 1: Spée et al. 2010). Il ressort ainsi que la CORT pourrait agir en premier dans le processus de désertion spontanée, potentiellement via l'altération de la prolactine, hormone fortement impliquée dans la stimulation et le maintien du comportement parental chez l'oiseau Dans cette optique, nous avons mis en évidence qu'une augmentation expérimentale des niveaux de CORT pouvait induire une diminution de prolactine de 40-45 % (étude 3). De façon intéressante, les oiseaux traités qui n'ont pas abandonné leur nid présentaient des niveaux de prolactine en début d'incubation et après la relève de la femelle plus élevés que les oiseaux ayant déserté leur nid. Ceci souligne fortement l'existence d'un seuil de prolactine en dessous duquel la motivation à incuber est inhibée.

Puisque la prolactine tient un rôle clé dans l'expression du comportement parental (Buntin, 1996; Vleck, 2002), il est intéressant d'examiner dans quelle mesure une diminution expérimentale de la prolactine, sans modifier les taux de CORT, pourraient sensiblement altérer la motivation des oiseaux à incuber, au point de provoquer leur départ.

Nous avons également suivi un groupe de manchots captifs (oiseaux en échec de reproduction pour des raisons non liées à leur état nutritionnel mais à des perturbations externes du type prédation ou inondation du nid). Ils nous permettent, une fois placés en captivité, d'effectuer des mesures intra-individuelles répétées et de déterminer la cinétique de paramètres métaboliques (β-hydroxybutyrate ou β-OHB et acide urique, témoignant respectivement de l'utilisation préférentielle des lipides et des protéines) endocriniens (prolactine, CORT) et comportementaux (activité locomotrice) suite au traitement.

Afin de diminuer expérimentalement les niveaux de prolactine, nous avons utilisé de la bromocriptine, un agoniste de la dopamine (récepteur D2) qui inhibe la sécrétion de prolactine chez les mammifères (Roberts et al., 2001) et les oiseaux (Angelier et al., 2006; Jouventin and Mauget, 1996; Reddy et al., 2007). Plusieurs doses de bromocriptine ont été utilisées. Cette étude s'attachera à examiner les points suivants :

1) La diminution expérimentale de la concentration de prolactine provoque-t-elle les modifications comportementales caractéristiques de l'entrée en PIII chez des oiseaux captifs (*i.e.* une augmentation d'activité locomotrice) ?

2) Le traitement à la bromocriptine a-t-il un effet sur le niveau de CORT plasmatique? Induit-il une transition dans le type de substrat énergétique utilisé ?

3) La diminution expérimentale de concentration de prolactine modifie-t-elle l'attention parentale, au point de stimuler la désertion du nid ? Affecte-t-elle le succès reproducteur subséquent (durée des voyages alimentaires, nombre d'œufs et de poussins, masse de la couvée) ?

MATERIELS ET METHODES

Cette étude a été réalisée sur la base scientifique française de Dumont d'Urville sur l'île des Pétrels (66°40'S, 140°01'E) en Terre Adélie (Antarctique).

Oiseaux captifs

Lors des campagnes d'été 2006-07 et 2007-08, 25 mâles ont été placés en parc (enclos grillagé ; 5,2*2,4 m) et ont été amenés à jeûner jusqu'à ce qu'ils atteignent un niveau seuil de réserve corporelle (quand leur masse corporelle était inferieure à ~ 3,5 kg, un seuil de masse corporelle qui marquent l'entrée en PIII ; Cockrem et al. 2006 ; étude 2). Après un ou deux jours de captivité, les manchots ont été équipés de deux podomètres (Dista F100, Décathlon, France), afin de mesurer leur activité locomotrice. Ces podomètres ont été recouverts de mastic pour assurer leur étanchéité et collés avec du cyanoacrylate (Loctite) sur le plumage au niveau de la ceinture pelvienne en haut de chacune des pattes. Sur chaque oiseau, les deux podomètres donnent des valeurs proches et ceci nous permet de n'utiliser qu'un des deux podomètres si l'un d'eux vient à ne plus fonctionner. Ces derniers n'ont pas été calibrés sur l'animal, les valeurs lues sont considérées comme des unités arbitraires. Ce sont les variations d'un podomètre donné au cours du temps pour un individu qui sont prises en compte. Les podomètres ont été relevés quotidiennement. Les oiseaux ont été pesés tous les deux jours avec une balance électronique (Ohaus, ± 2 g).

Quelques jours après leur mise en captivité (2 à 5 jours), les oiseaux ont été implantés avec 0,5 (B0,5, n=6), 5 (B5, n=4) ou 10 mg (B10, n=7) de bromocriptine. La bromocriptine est un agoniste de la dopamine (récepteur D2) qui inhibe la sécrétion de prolactine chez les mammifères (Roberts et al., 2001) et les oiseaux (Angelier et al., 2006; Jouventin and Mauget, 1996; Reddy et al., 2007). Les implants ont une durée théorique de diffusion de 21 jours (bromocritpine mesylate, Innovative Research of America, Sarasota, FL, USA). Une petite

incision égale à la taille de l'implant a été faite et l'implant a été inséré sous la peau. L'incision a été fermée avec un point stérile, nettoyée avec de la Bétadine puis aspergée de poudre d'aluminium. Les oiseaux témoins ont subi le même protocole mais sans insertion d'implant (témoins, n=8).

Les prélèvements sanguins ont été effectués dans la veine alaire ou dans la veine de la patte (1) le jour de l'implantation (jour 0), (2) trois jours après (jour 3) et (3) lorsque les manchots ont été relâchés (jour 7 à 12, en fonction des individus). Les animaux témoins n'ont été prélevés que deux fois, le jour de l'implantation et le jour où ils ont été relâchés (après avoir atteint la masse critique d'entrée en phase III). Tous les prélèvements sanguins ont été réalisés dans un délai inférieur à 5 minutes, pour évaluer les niveaux basaux d'hormone de stress (CORT) chez le manchot Adélie (Vleck et al., 2000b). Les échantillons de sang ont été transférés dans des tubes contenant de l'héparine et centrifugés (5000 rpm, 10 minutes, 4°C). Le plasma a été aliquoté et les échantillons ont été conservés à - 80°C jusqu'aux analyses réalisées au laboratoire de Strasbourg.

Lors du dernier échantillonnage, certains oiseaux traités avaient une masse corporelle proche ou inférieure à la masse d'entrée en phase III (~ 3,5 kg chez le manchot Adélie mâle, Cockrem et al., 2006 ; étude 2). Dans ces cas, les paramètres plasmatiques (CORT et prolactine) et comportementaux (activité locomotrice) n'ont pas été pris en compte, pour éviter un éventuel effet cumulé de l'atteinte d'un état critique d'origine nutritionnel et du traitement hormonal.

Pendant la période de mue, 8 manchots ont été capturés, mis en parc et amenés à jeûner. Après quelques jours de captivité, ils ont été équipés de podomètres pour mesurer leur activité locomotrice, comme décrit précédemment. 4 d'entres eux ont été implantés avec 10 mg de bromocriptine (B10), alors que les 4 autres ont subi le même protocole sans

insertion d'implant. Les manchots ont été pesés tous les deux jours, comme décrit précédemment.

Oiseaux sauvages en cours de reproduction

Lors de la campagne d'été 2007-08, 60 couples de manchots Adélie sauvages ont été suivis. Les oiseaux ont été capturés à deux occasions (Fig. 1).

Premièrement, les mâles ont été capturés entre la pariade (formation des couples) et la ponte. Ils ont alors été implantés avec 0,5 (B0,5, n=15), 5 (B5, n=15) ou 10 mg (B10, n=10) de bromocriptine, un prélèvement sanguin a été effectué et ils ont été pesés, comme décrit précédemment. Le même protocole a été suivi concernant les oiseaux témoins (n=20), mais sans insertion d'implant. Chaque individu a été marqué d'un numéro à l'aide de Nyanzol D. Ce mélange est composé de gomme arabique, de sulfate de sodium anhydre, de p-Phenylènediamine et d'éthanol. Il est activé en ajoutant du peroxyde d'hydrogène. Le sexe a été déterminé par une combinaison de paramètres incluant l'inspection du cloaque avant la ponte, la routine de l'incubation (les mâles assurent dans la plupart des cas la première partie de l'incubation, Ainley, 2002) et la mesure de lipémie plasmatique (les femelles ont une lipémie plus élevé que les mâles avant la ponte, Kern et al., 2005).

Deuxièmement, les mâles ont été recapturés à la fin de la première partie de l'incubation, lors du départ en mer (1) après qu'ils aient été relevés par la femelle ou (2) après qu'ils aient abandonné leur nid.

Les nids ont été observés toutes les 3-4 heures pour déterminer quel membre du couple était présent sur le nid, la relève de la femelle ou l'abandon du nid, les dates de ponte et d'éclosion, les nombres d'œufs et de poussins par couple. Le nombre de poussins par couple a été déterminé à quatre reprises, lorsque les poussins étaient âgés d'environ (1) 10 jours, (2) 20 jours, (3) 25 jours et (4) lorsqu'ils ont atteint leur masse maximale (42 à 45 jours après

l'éclosion ; Ainley, 2002). A ces deux derniers stades, les poussins ont été pesés. La durée des voyages alimentaires a également été déterminée.

Analyses en laboratoire

La concentration plasmatique de prolactine a été déterminée par méthode radio-immunologique (RIA) en collaboration avec le Centre d'Etude Biologiques de Chizé (A. Lacroix et O. Chastel, CEBC, France). La concentration plasmatique de CORT a été déterminée par méthode immuno-enzymatique (dosage EIA = dosage Elisa), au laboratoire de Strasbourg, en utilisant un kit issu du commerce (AssayPro, AssayMax Corticosterone ELISA Kit, EC3001-1). Les variations inter et intra essais sont de 6 % et 9 %, respectivement, pour les mesures de prolactine et de 5 % et 7 %, respectivement, pour celles de CORT.

Les concentrations plasmatiques en acide urique et β-hydroxybutyrate (β-OHB) ont été déterminées par une méthode enzymatique colorimétrique en utilisant des kits de dosages (acide urique : Sigma Diagnostics ; β-OHB : Randox).

Analyse statistique

Oiseaux captifs

Pour comparer la masse corporelle des individus au début du jeûne en captivité, nous avons utilisé un modèle linéaire général (GLM). Pour tester l'effet du traitement à la bromocriptine sur les concentrations de prolactine et de CORT, les niveaux d'acide urique et de βOHB, la perte de masse spécifique (dm/m.dt ; pendant le jeûne de reproduction et le jeûne de mue) et l'activité locomotrice (pendant le jeûne de reproduction et le jeûne de mue), nous avons utilisé des modèles linéaires généralisés mixtes (GLMM) ou des modèles d'équations d'estimations généralisées (GEE) lorsque la distribution des résidus ne suivait pas une loi normale (test de Shapiro-Wilk). L'individu a été inclus comme facteur aléatoire et le

"traitement", le "jour par rapport à l'implantation" et leur interaction comme facteurs fixes, le "jour par rapport à l'implantation" étant le facteur répété. L'année (2006-07 et 2007-08) a été ajoutée en covariable.

Oiseaux sauvages

Les relations entre la concentration de prolactine à l'implantation et (1) la durée séparant l'implantation et la ponte et (2) la masse corporelle des individus ont été testées à l'aide de corrélations de Spearman. Pour tester l'effet du traitement à la bromocriptine sur les concentrations de prolactine, nous avons utilisé un GLMM, en incluant l'individu comme facteur aléatoire et le "traitement", la "période d'échantillonnage" et leur interaction comme facteurs fixes, la "période d'échantillonnage" étant le facteur répété. La durée séparant l'implantation et la ponte a été ajoutée en covariable. L'effet du traitement à la bromocriptine sur le nombre moyen d'œufs et de poussins par couples a été analysé à l'aide d'un GEE (distribution de Poisson puisqu'il s'agit de données discrètes).

Nous avons utilisé un GLM pour tester l'effet du traitement à la bromocriptine sur la durée du 1er voyage alimentaire (pendant l'incubation). Nous avons utilisé une GEE (distribution gamma) pour tester l'effet du traitement à la bromocriptine sur la durée des voyages alimentaires suivant (pendant le stade d'élevage de(s) poussin(s), du 2^{e} au 8^{e} voyage, après quoi nous n'avons plus assez de données). Le "traitement", le "numéro du voyage" et leur interaction ont été inclus comme facteurs fixes, le "numéro du voyage" étant le facteur répété.

Pour comparer les dates d'implantation, de départ en mer, de ponte et d'éclosion entre les groupes, nous avons utilisé des modèles linéaires généralisés (GzLM) avec une loi de Poisson, les données étant discrètes. Pour la comparaison des masses corporelles à l'implantation et au départ en mer, la perte de masse journalière, la durée entre l'implantation,

la durée d'incubation et le départ en mer et la masse de la couvée, nous avons utilisé des GLM ou des GzLM lorsque la distribution des résidus ne suivait pas une loi normale.

Les comparaisons multiples ont été effectuées à l'aide de tests de Bonferroni. Les analyses statistiques ont été effectuées sur le logiciel SPSS 16.02 (SPSS Inc., Chicago, Ill., USA). Les résultats sont exprimés en moyenne ± ESM. Le seuil de significativité a été fixé à α=0.05.

RESULTATS

Oiseaux captifs

La masse corporelle des individus à l'implantation (témoins : 4,06 ± 1,63, B0,5 : 4,42 ± 2,13, B5 : 4,22 ± 2,40, B10 : 4,52 ± 1,85) ne diffèrent pas significativement entre les groupes (Wald χ^2=0.05, df=3, p=0.99). La perte de masse spécifique (témoins en PII : 19,91 ± 1,58, B0,5 : 21,12 ± 2,24, B5 : 22,89 ± 2,45, B10 : 28,90 ± 1,81, témoins en PIII : 28,76 ± 1,95) est affectée par le traitement ($F_{3, 20}$=5,56, p=0,006). En effet, elle tend à augmenter d'autant plus que la dose de bromocriptine appliquée est forte. Cependant, seuls les individus du groupe B10 montrent une perte de masse qui diffèrent significativement de celle des individus témoins en PII (p=0,007) et qui est similaire à celles des individus témoins en PIII (p>0.99). Les individus des groupes B0,5 et B5 ont une perte de masse spécifique qui ne diffèrent ni des individus témoins en PII (p>0,99 pour chacune des comparaisons) ni de celles des oiseaux témoins en PIII (p=0,22 et p=0,85, respectivement).

Effet du traitement à la bromocriptine sur les concentrations de prolactine et de CORT

La concentration plasmatique de prolactine n'est pas influencée par le traitement ($F_{3, 22}$= 0,87, p=0,47) mais est affectée par le jour par rapport à l'implantation ($F_{2, 31}$= 20,9, p<0,001) et l'interaction jour par rapport à l'implantation*traitement ($F_{5, 30}$=6,13, p<0,001 ; Fig. 2A). Au moment de l'implantation, les concentrations de prolactine sont similaires entre les groupes (p>0,15 pour chacune des comparaisons). Seul le traitement par une dose de 10 mg de bromocriptine induit une diminution significative de prolactine de 64 % et 40 %, respectivement au jour 3 (p<0,001) et au jour 7-12 (p<0,001) par rapport au jour 0.

La concentration de CORT n'est pas affectée par le traitement (Wald χ^2=5,54, df=3, p=0,14) ni par le jour par rapport à l'implantation (Wald χ^2=5,64, df=2, p=0,06). En revanche, nous trouvons un effet de leur interaction (Wald χ^2=17,4, df=5, p=0,004 ; Fig. 2B), les

individus témoins en PIII ayant un niveau de CORT 2,6 fois plus importante qu'en PII (p=0,013).

Effet du traitement à la bromocriptine sur les concentrations d'acide urique et de βOHB

La concentration d'acide urique est affectée par le traitement (Wald χ^2=12,7, df=3, p=0,005), le jour par rapport à l'implantation (Wald χ^2=56,6, df=2, p<0,001) et leur interaction (Wald χ^2=15,3, df=5, p=0,009). Le jour de l'implantation, tous les groupes d'oiseaux (témoins, B0,5, B5 et B10) présentent des niveaux similaires d'acide urique (témoins : 0,17 ± 0,02 mmol/l, B0,5 : 0,17 ± 0,02 mmol/l, B5 : 0,19 ± 0,04 mmol/l, B10 : 0,17 ± 0,02 mmol/l ; p>0,99 pour chacune des comparaisons). Les individus du groupe B0,5 ne montrent aucune différence de concentration d'acide urique au jour 3 et jour 7-12 comparé au jour 0 (p=0,064 et p>0,99). Chez les oiseaux du groupe B5, la concentration d'acide urique augmente significativement d'un facteur 1.8 au jour 3 par rapport au jour 0 (p<0,001). Aucune différence significative n'est observé au jour 7-12 par rapport au jour 0 (p>0,99). Chez les oiseaux du groupe B10, la concentration d'acide urique augmente significativement d'un facteur 1,7 et 1,6 au jour 3 et 7-12, respectivement, par rapport au jour 0 (p<0,001 pour chacune des comparaisons). Chez les individus témoins en PIII, le niveau d'acide urique augmente significativement d'un facteur 4 par rapport à celui des oiseaux en PII (p=0,003).

La concentration de βOHB n'est pas affectée par le traitement (Wald χ^2=0,89, df=3, p=0,83), ni par le jour par rapport à l'implantation (Wald χ^2=1,48, df=2, p=0,48). Par contre, le niveau de βOHB est influencé par leur interaction (Wald χ^2=18,6, df=5, p=0,002). Le jour de l'implantation, tous les groupes d'oiseaux (témoins, B0,5, B5 et B10) présentent des niveaux similaires de βOHB (témoins : 2,08 ± 0,26 mmol/l, B0.5 : 1,61 ± 0,34 mmol/l, B5 : 1,42 ± 0,42 mmol/l, B10 : 1,54 ± 0,10 mmol/l ; p>0,99 pour chacune des comparaisons). Les oiseaux témoins montrent une diminution significative d'un facteur 2 en PIII par rapport à la

PII (p=0,006). En revanche, chez les oiseaux traités, nous n'observons pas de différences significatives dans les concentrations de βOHB au jour 3 et 7-12 par rapport au jour 0 (p>0,99 pour chacune des comparaisons).

Effet du traitement à la bromocriptine sur l'activité locomotrice

L'activité locomotrice (lors du jeûne de reproduction) est affectée par le traitement (Wald χ^2=25,9, df=3, p<0,001 ; Fig. 3B). En effet, elle tend à augmenter d'autant plus que la dose de bromocriptine appliquée est forte. Cependant, seuls les individus du groupe B10 montrent une activité locomotrice qui diffèrent significativement de celle des individus témoins en PII (p<0,001) et similaires et celles des oiseaux témoins en PIII (p>0,99). L'activité locomotrice est également affectée par le jour par rapport à l'implantation (Wald χ^2=245, df=8, p<0,001) et par l'interaction traitement*jour par rapport à l'implantation (Wald χ^2=1003, df=17, p<0,001 ; Fig. 3A). Chez les individus B10, l'activité locomotrice augmente significativement dès 2 jours après l'implantation et reste 4 à 4,5 fois plus élevée que celles des individus témoins jusqu'au jour 4.

Effet du traitement à la bromocriptine sur l'activité locomotrice lors de la mue

Lors de la mue, la concentration de prolactine est pratiquement indétectable (0,29 ± 0,13 ng/ml) et l'effet du traitement à la bromocriptine n'est donc pas visible. A cette même période, l'activité locomotrice n'est pas affectée par le traitement (témoins *vs* B10 ; $F_{1, 5}$=1,15, p=0,33), le jour par rapport à l'implantation ($F_{8, 42}$=0,84, p=0,57) ou leur interaction ($F_{8, 42}$=0,74, p=0,65 ; Fig. 4). La perte de masse spécifique n'est pas affectée par le traitement ($F_{1, 40}$=0,623, p=0,43), le jour par rapport à l'implantation ($F_{7, 40}$=0,90, p=0,51) ou leur interaction ($F_{7, 40}$=0,18, p=0,99).

Oiseaux sauvages

Chez les individus témoins, 2 oiseaux ont abandonnés leur nid. Chez les individus du groupe B0,5, un seul abandon a eu lieu, 21 jours après l'implantation. Cet individu n'a pas été recapturé. Un seul individu du groupe B5 et un seul oiseau de groupe B10 ont abandonné leur nid, respectivement 12 et 9 jours après l'implantation. Leurs masses corporelles lors de l'abandon sont de 4,06 et 4,14 kg, respectivement.

Les résultats qui suivent ne concernent que les individus ayant été relevés par la femelle.

Profil des oiseaux témoins et traités à la bromocriptine

La date d'implantation (Wald χ^2=1,10, df=3, p=0,78 ; Table 1) et la masse corporelle des oiseaux à cette période (Wald χ^2=0,02, df=3, p>0,99 ; Table 1) ne diffèrent pas selon les groupes. Le traitement à la bromocriptine a une influence sur la date de départ en mer (Wald χ^2=29,5, df=3, p<0,001; Table 1), les individus du groupe B10 quittant la colonie pour se réalimenter en mer plus tôt que les oiseaux des groupes témoins, B0,5 et B5 (p<0,001 pour chacune des comparaisons). Par contre, la masse corporelle des oiseaux au départ en mer est similaire (Wald χ^2=0,03, df=3, p=0,99; Table 1). La perte de masse journalière est influencée par le traitement ($F_{3, 49}$=3,69, p=0,02; Table 1). En effet, elle tend à augmenter d'autant plus que la dose de bromocriptine appliquée est forte. Cependant, seuls les individus du groupe B10 montrent une perte de masse qui diffèrent significativement de celle des individus témoins (p=0,03). La durée séparant l'implantation et le départ en mer n'est pas affectée par le traitement ($F_{3, 50}$=1,07, p=0,37; Table 1).

Effet du traitement à la bromocriptine sur la concentration de prolactine

Les niveaux de prolactine lors de l'implantation sont positivement corrélés au temps qui sépare l'implantation et la ponte (r_s=0,32, p=0,01 ; Fig. 5A). En d'autres termes, plus la date de ponte est proche, plus la concentration de prolactine est élevée. En revanche, les niveaux de prolactine à l'implantation ne sont pas influencé par la masse corporelle (r_s=-0,19, p=0,13 ; Fig. 5B) à cette même période. La durée séparant l'implantation et la ponte ayant été ajoutée en covariable, il apparaît que les niveaux de prolactine ne sont pas influencés par le traitement ($F_{3, 51}$=0,91, p=0,44), la période d'échantillonnage (implantation et départ en mer ; $F_{1, 95}$=0,02, p=0,89) ou leur interaction ($F_{3, 50}$=2,12, p=0,11 ; Fig. 6). La diminution de prolactine tend cependant à être plus marquée chez les individus B10 (21 %) que chez les autres groupes (témoins : 6 %, B0,5 : 5 %, B5 : 13 %).

Effet du traitement à la bromocriptine sur le succès reproducteur : de l'œuf au poussin

Le traitement n'a pas d'effet sur la date de ponte (Wald χ^2=6,45, df=3, p=0,09 ; Table 1), la date d'éclosion (Wald χ^2=4,57, df=3, p=0,06 ; Table 1) ou la durée d'incubation n'est pas influencée par le traitement ($F_{3, 29}$=1,56, p=0,22).

Les nombres moyens d'œufs et de poussins par couple sont affectés par le traitement (Wald χ^2=8,02, df=3, p=0,046), les individus du groupe B10 ayant un nombre d'œufs / poussins significativement plus faible que les individus B5 (p=0,03) mais similaires à ceux des groupes B0.5 et témoins (p=0,47 et p=0,60, respectivement). La période d'échantillonnage a également un effet sur le nombre moyen d'œufs et de poussins par couple (Wald χ^2=109, df=4, p<0,001 ; Fig. 7). En effet, tous les groupes montrent une diminution du nombre de poussins (~ 10 jours après l'éclosion) par rapport au nombre d'œufs pondus (p<0,001). Une nouvelle diminution du nombre moyen de poussins est observée lorsque ces

derniers sont âgés d'environ 25 jours et 42-45 jours après l'éclosion, lorsqu'ils ont atteint leur masse maximale (p=0,04 et p=0,02).

La masse de la couvée lorsque les poussins sont âgés d'environ 25 jours ($F_{3, 39}$=2,62, p=0,06; Table 1) et lorsqu'ils atteignent leur masse corporelle maximale (42 à 45 jours après l'éclosion) (Wald χ^2=0,77, df=3, p=0,86 ; Table 1) n'est pas affectée par le traitement.

Effet du traitement à la bromocriptine sur la durée des voyages alimentaires

La durée du 1er voyage alimentaire des mâles (pendant l'incubation, témoins : 14,0 ± 0,48, B0,5 : 14,5 ± 0,56, B5 : 14,2 ± 0,58, B10 : 14,8 ± 0,67) n'est pas affectée par le traitement ($F_{3, 48}$=0,36, p=0,78). La durée des voyages suivants (du 2^{e} au 8^{e}) est affectée par le traitement (Wald χ^2=14,1, df=3, p=0,003), les individus du groupe B0.5 effectuant en général des voyages plus longs que ceux des oiseaux du groupe B5. La durée des voyages n'est pas influencée par le numéro du voyage (Wald χ^2=5,78, df=6, p=0,45) mais est affectée par l'interaction traitement*voyage (Wald χ^2=81,3, df=18, p<0,001). En revanche, les comparaisons multiples (tests de Bonferroni) ne mettent en évidence aucune différence de durée chez les différents groupes au sein d'un même voyage.

DISCUSSION

Niveau de prolactine et modification comportementale chez les manchots captifs

Contrairement à d'autres oiseaux marins (Chastel and Lormée, 2002), la perte accidentelle de l'œuf chez les manchots n'a que très peu d'effet sur la concentration de prolactine (Lormée et al., 1999; Vleck et al., 2000a). Les oiseaux captifs dans cette étude présentent en effet des niveaux de prolactine plus faible que ceux des individus sauvages mais bien supérieurs aux taux basaux (pendant la mue). Chez ces oiseaux captifs, la diminution de prolactine suite au traitement tend à être d'autant plus marquée que la dose est forte. Cependant, seuls les individus du groupe B10 montrent une diminution significative de la concentration de prolactine de 64 % au jour 3 et de 40 % au jour 7-12. Le traitement a donc été efficace.

L'augmentation de l'activité locomotrice est d'autant plus importante que la dose implantée est élevée. Chez les individus du groupe B10, l'activité locomotrice est significativement plus importante que celle des témoins dès 2 jours après l'implantation. Cette augmentation d'activité ne semble pas liée à un effet de la CORT puisque le traitement à la bromocriptine n'a pas eu d'influence significative sur les niveaux de CORT plasmatique. De plus, cette modification comportementale ne semble pas relever d'un effet intrinsèque de la bromocriptine, éventuellement autre que son action inhibitrice sur la concentration de prolactine. En effet, l'activité locomotrice des oiseaux pendant la mue, période à laquelle les niveaux de prolactine sont pratiquement indétectables et donc non susceptibles d'être baissée par la bromocriptine, n'est pas affectée par le traitement. Ainsi, l'augmentation d'activité observée lors du jeûne de reproduction semble donc être due à une diminution de la concentration de prolactine.

Les individus du groupe B10 montrent une perte de masse spécifique plus importante que les oiseaux témoins en PII. Cette augmentation ne semble pas due à un shift dans le type

de substrat énergétique utilisé (passage de l'utilisation des lipides à une utilisation préférentielle des protéines, caractéristique de l'entrée en PIII). En effet, bien qu'on observe une augmentation significative des niveaux d'acide urique chez les oiseaux traités avec la dose la plus forte de bromocriptine, elle est bien moindre que l'augmentation obtenue chez les oiseaux témoins passant de la PII à la PIII (facteur 1,7 et 4, respectivement). De plus les niveaux de βOHB ne varient pas chez ces mêmes oiseaux. Par contre, on peut attribuer l'élévation de la vitesse d'amaigrissement à une augmentation importante de l'activité locomotrice chez les oiseaux du groupe B10.

Ainsi, à la vue des résultats obtenus chez les manchots en captivité traités à la bromocriptine, il apparaît qu'une diminution de la prolactine seule est susceptible d'induire des modifications comportementales caractéristiques de l'entrée en phase critique de jeûne (PIII). On s'attend ainsi à observer un effet du traitement (B10) sur l'occurrence de désertion spontanée chez les oiseaux sauvages en cours de reproduction.

Niveau de prolactine et abandon du nid chez les manchots sauvages

La diminution expérimentale de prolactine n'a provoqué qu'un seul abandon du nid dans chacun des groupes d'oiseaux traités. L'abandon de l'oiseau du groupe B0,5 a eu lieu 21 jours après l'implantation mais celui-ci n'a pas été recapturé. Sachant que les individus traités avec 0,5 mg de bromocriptine ont une perte de masse journalière de 53,6 g/jour, l'oiseau ayant abandonné son nid aurait atteint une masse corporelle d'environ 3,3 kg. Il semble donc que son abandon soit dû à l'atteinte d'un niveau seuil de réserves corporelles (masse corporelle d'entrée en PIII ~ 3,5 kg chez les oiseaux témoins, Cockrem et al., 2006; Spée et al.,2010; étude 2) et non pas au traitement. Chez les individus des groupes B5 et B10, la cause du départ ne semble pas être due à l'atteinte d'un stade critique, au vu de leur masse corporelle à l'abandon. Quoi qu'il en soit, un seul abandon par groupe, soit 6.7 % chez les

oiseaux des groupes B0,5 et B5 et 10 % chez les individus du groupe B10 contre 5 % chez les manchots témoins, ne permet pas de conclure sur un éventuel rôle du traitement sur l'induction de l'abandon du nid.

Chez les oiseaux sauvages à l'implantation, les niveaux de prolactine ne sont pas corrélés avec la masse corporelle des individus. Ainsi, mise à part l'atteinte d'un niveau seuil de réserves corporelles (PIII, Cherel et al., 1994; Groscolas et al., 2008; Spée et al., 2010), la concentration de prolactine n'est pas influencée par l'état des réserves chez le manchot Adélie en début d'incubation. Chez les manchots, la concentration de prolactine est connue pour augmenter graduellement au cours de la pariade et atteint son niveau le plus élevé en milieu d'incubation (Vleck et al., 1999). Ainsi, comme attendu, nous trouvons que les niveaux de prolactine sont d'autant plus élevés que la date de ponte est proche.

Malgré une tendance chez les oiseaux du groupe B10, le traitement n'a pas induit de diminution significative des niveaux de prolactine. L'administration d'une dose de bromocriptine plus forte et induisant une diminution de prolactine plus marquée (au-delà du seuil relatif en dessous duquel la motivation à incuber est inhibée) permettrait de déterminer plus précisément son effet sur l'induction de l'abandon du nid.

Pourquoi n'observe-t-on pas d'effet du traitement sur la concentration de prolactine et sur l'occurrence d'abandon du nid chez les oiseaux sauvages ?

La durée théorique de diffusion de l'implant est de 21 jours. Il est possible cependant qu'il ne diffuse pas aussi longtemps et que la prise de sang que nous avons effectuée au moment du départ en mer (~ 17-19 jours après l'implantation) ait eu lieu alors que l'implant ne diffusait plus. Chez les oiseaux, la sécrétion de prolactine est de façon prédominante sous le contrôle stimulateur du peptide intestinal vasoactif (*Vasoactive Intestinal Peptide* ou VIP ; Sockman et al., 2006). La bromocriptine agit en tant qu'agoniste des récepteurs

dopaminergiques D2, présents sur les neurones hypothalamiques qui libèrent le VIP. C'est donc en empêchant la stimulation des cellules sécrétrices de prolactine que la bromocriptine induit une baisse de la concentration de prolactine. On peut émettre l'hypothèse que l'arrêt de diffusion de l'implant ait levé cette inhibition et que le VIP ait repris son action stimulatrice sur la sécrétion de prolactine. Il est ainsi possible que la concentration de prolactine que nous avons mesurée lors du départ en mer des oiseaux soit revenue à un niveau plus élevé que lorsque l'implant diffusait. Müller et ses collaborateurs (2009) ont montré que des implants de CORT, provenant du même fabricant que les implants de bromocriptine utilisés dans cette étude et conçus pour diffuser pendant 7 jours n'avaient entraîné une augmentation de CORT que pendant 2-3 jours. Ce résultat va dans le sens de notre hypothèse selon laquelle la durée réelle de diffusion de l'implant est moins longue que sa durée théorique.

D'autre part, des études ont mis en exergue que la relation liant le comportement parental et les niveaux de prolactine est non linéaire et dépend d'une valeur seuil de prolactine. En effet, l'expression du comportement parental pourrait être maximisée au dessus d'un niveau seuil de prolactine (Boos et al., 2007) et inhibée en dessous du seuil (Angelier et al., 2006; Angelier and Chastel, 2009; Spée et al., 2010; étude 3). Il est possible que la dose B10 utilisée dans cette étude n'ait pas été assez forte pour induire une diminution de prolactine en dessous de laquelle la motivation à incuber est inhibée. Une question se pose alors : l'ampleur de la diminution de prolactine est-elle la même chez les oiseaux captifs et chez les oiseaux sauvages ? Ou plutôt a-t-elle été la même si l'on émet l'hypothèse que la concentration de prolactine chez les oiseaux sauvages ait diminué mais soit revenue à un niveau plus haut lors de la recapture des manchots (au moment de leur départ en mer). Les oiseaux sauvages ont une concentration de prolactine lors de l'implantation supérieure à celle des individus captifs. En appliquant aux individus sauvages le même pourcentage de diminution de prolactine que celui observé chez les oiseaux captifs (*i.e.* 64 % au jour 3), on

trouve une valeur proche de 25-30 ng/ml. Cette valeur avoisine celle obtenue chez des manchots sauvages abandonnant leur nid (Spée et al., 2010). Si une diminution de concentration de prolactine aussi importante que celle obtenue chez les oiseaux captifs a effectivement été induite chez les manchots sauvages, on aurait pu s'attendre à ce que les oiseaux abandonnent leur nid, une valeur seuil ayant été atteinte. Ce scénario pourrait néanmoins s'appliquer aux oiseaux des groupes B5 et B10 ayant déserté leurs œufs. Il est fortement probable cependant que la régulation de la prolactine diffèrent entre les oiseaux ayant perdu leur œufs (captifs) et les individus incubant (sauvages). Bien que programmé de façon endogène, la sécrétion de prolactine pourrait en effet être soutenue par la présence de l'œuf. Par exemple, la concentration de prolactine augmente pendant un séjour d'incubation à terre chez les manchots royaux (Garcia et al., 1996). Par ailleurs, Chastel et ses collaborateurs (2005) ont mis en évidence chez la mouette tridactyle qu'en réponse à un stress la baisse de concentration de prolactine était plus forte chez des oiseaux ayant perdu leurs œufs que chez des oiseaux toujours impliqués dans l'effort parental (élevage de poussins).

Incubation… Poussins et voyages alimentaires

Bien que le traitement n'ait pas provoqué l'abandon du nid, il est possible qu'il ait altéré la vigilance du mâle lors de l'incubation, induisant ainsi une diminution de son attention au nid. Chez des femelles eiders, une diminution de la concentration de prolactine de 14 % en réponse à une augmentation expérimentale de CORT (diminution de prolactine non suffisante pour les faire abandonner leur nid) a en effet induit une diminution de leur attention au nid, reflétée par une augmentation de la prédation (Criscuolo et al., 2005). Au cours de l'incubation, une baisse d'attention pourrait se traduire par une diminution du temps de contact entre l'adulte et l'œuf. Chez les individus traités à la bromocriptine, la perte de masse est d'autant plus importante que la dose administrée est élevée, avec une différence

significative chez les individus du groupe B10 par rapport aux oiseaux témoins. Cette perte de masse plus élevée pourrait être le reflet d'un comportement plus actif (agitation de l'animal sur son nid, passage de la position couchée à debout plus fréquente) des oiseaux B10 par rapport aux manchots témoins. Il est important de noter que cette augmentation de la perte de masse ne semble pas due à un effet intrinsèque de la bromocriptine mais plutôt à son action inhibitrice sur la prolactine, puisqu'il n'y a pas d'effet du traitement sur la perte de masse lors de la mue. Ainsi, une diminution de l'attention du parent pourrait entraîner une diminution de la température d'incubation ou provoquer des variations de température de plus grande ampleur. De telles modifications de la température d'incubation peuvent induire une augmentation de la durée de celle-ci et affecter le succès à l'éclosion, la croissance ou la condition corporelle du poussin (Ardia et al., 2010; Durant et al., 2010; Hepp et al., 2006). Dans notre étude, ni les dates de pontes et d'éclosion, ni la durée d'incubation n'ont été altérés par le traitement, malgré une tendance non significative chez les B10 à incuber plus longtemps (37,2 jours chez les B10 contre 35,5 jours chez les témoins). Le nombre moyen de poussins environ une semaine après l'éclosion tend cependant à être plus faible chez les oiseaux du groupe B10, malgré une absence de significativité. De plus, le succès reproducteur estimé par le nombre de poussins atteignant leur pic de masse corporelle (au stade de crèche où la mortalité est très rare ; Beaulieu et al., 2010; Beaulieu et al., 2009; Clarke et al., 2002) et la masse de la couvée à cette période tendent à être plus faible chez les individus du groupe B10. Il serait intéressant de suivre précisément le comportement d'incubation des oiseaux traités avec de la bromocriptine (en utilisant une dose plus forte) afin de déterminer si le traitement affecte leur attention au nid et induit une diminution de la température d'incubation. Ceci serait rendu possible par l'utilisation de capteurs de température placés dans le nid.

Il est également possible que la stratégie des oiseaux du groupe B10 lors des voyages alimentaires ait été affectée par le traitement. En effet, les voyages alimentaires courts sont plutôt orientés vers un approvisionnement du poussin alors que les voyages longs sont plutôt dévolus à la reconstitution des réserves énergétiques du parent (Ropert-Coudert et al., 2004). Sachant que les individus du groupe B10 ont une perte de masse journalière plus importante que celle des oiseaux témoins, on pourrait ainsi s'attendre à ce qu'ils aient effectué des voyages alimentaires plus longs que ceux des oiseaux témoins, « négligeant » ainsi plus leur(s) poussin(s). Ceci pourrait expliquer en partie le nombre plus faible de poussins chez les oiseaux du groupe B10. Cependant, aucune différence notable dans la durée des voyages alimentaires n'a été mise en évidence suite au traitement dans cette étude. Il est néanmoins possible que le comportement de plongée ou l'efficacité de recherche alimentaire des oiseaux du groupe B10 aient été affectés par le traitement. L'utilisation de capteurs de pression et d'accéléromètres permettrait de tester cette hypothèse.

Conclusion et perspectives

Lors de cette étude, nous avons mis en évidence qu'une diminution expérimentale des niveaux de prolactine provoquait, chez des manchots Adélie captifs, une réponse comportementale similaire à celle observée lors de l'entrée en PIII de jeûne. De plus, cette modification du comportement ne semble pas être expliquée par un effet de la CORT ou du type de substrat énergétique utilisé (modifications endocrinienne et métaboliques caractéristiques de la PIII). En revanche, le traitement n'a pas induit de diminution notable de la concentration de prolactine chez les oiseaux sauvages, empêchant ainsi de conclure sur le rôle d'une baisse de la prolactine sur l'occurrence d'abandon du nid. Nous avons cependant pu mettre en évidence une tendance chez les oiseaux du groupe B10 à avoir un succès reproducteur moins bon que celui des manchots témoins. De prochaines études utilisant des

doses plus fortes de bromocriptine permettraient d'induire une diminution plus marquée de prolactine, notamment en dépassant le seuil en dessous duquel la motivation à incuber est inhibée, et d'examiner l'effet sur l'occurrence d'abandon du nid. Par ailleurs le suivi précis du comportement d'incubation (utilisation d'œufs factices mesurant la température) et de recherche alimentaire (utilisation de capteur de pression et d'accéléromètre) suite au traitement pourrait nous donner des informations sur les perturbations éventuelles de l'investissement du parent consécutives au traitement.

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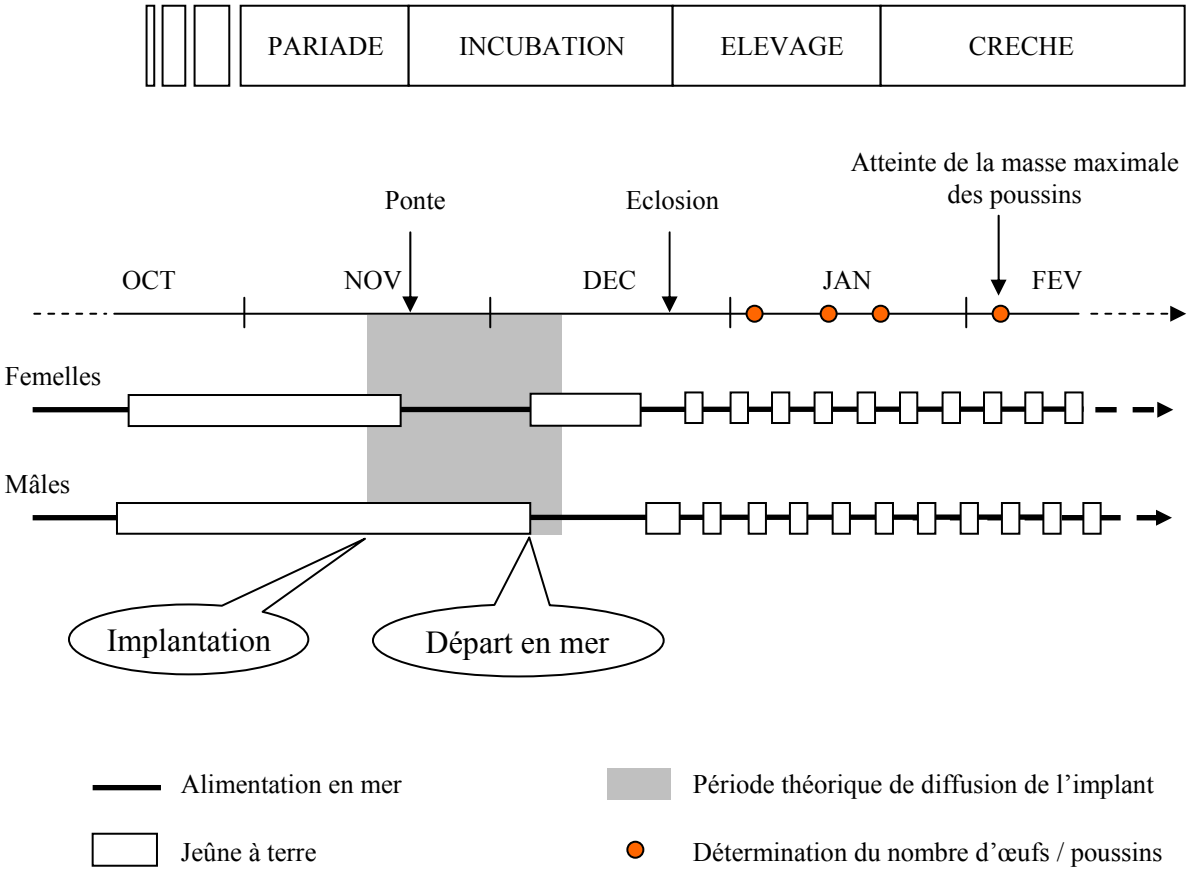

PARIADE
INCUBATION
ELEVAGE
CRECHE
Atteinte de la masse maximale des poussins
Ponte
Eclosion
OCT
NOV
DEC
JAN
FEV
Femelles
Mâles
Implantation
Départ en mer
Alimentation en mer
Jeûne à terre
Période théorique de diffusion de l'implant
Détermination du nombre d'œufs / poussins

Fig. 1

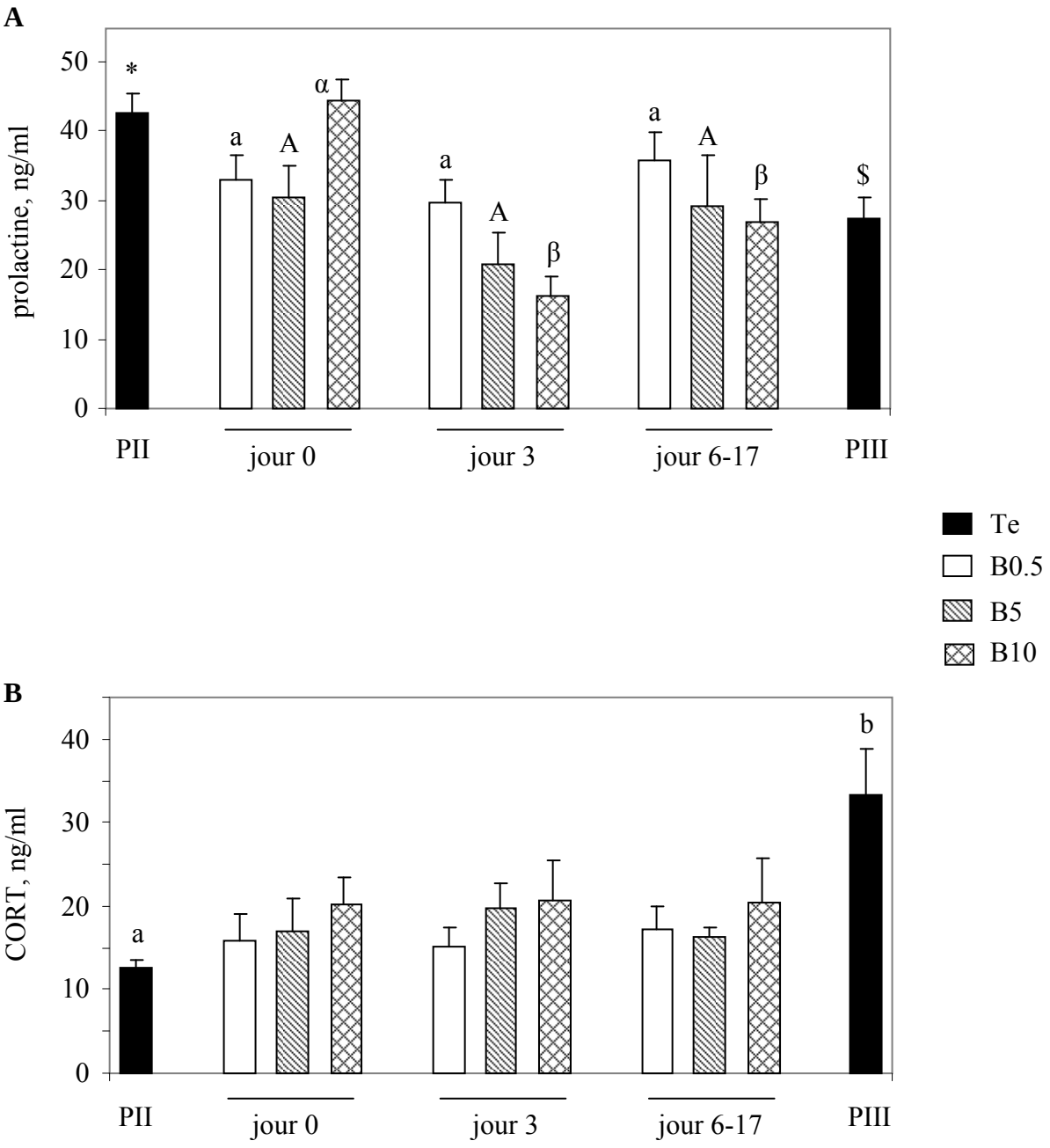
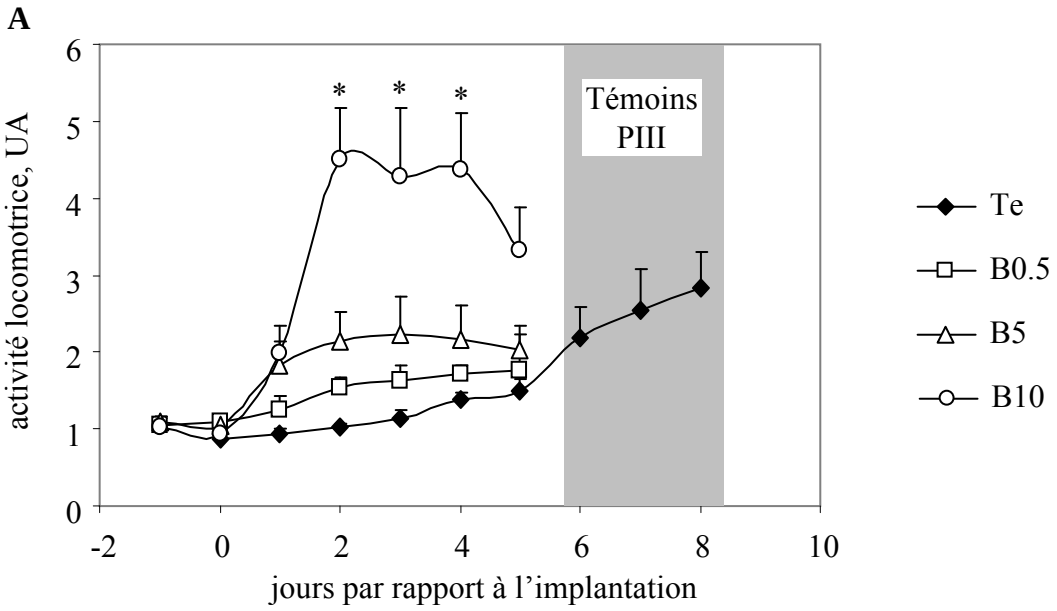

A
prolactine, ng/ml
50
40
30
20
10
0
*
a
A
α
a
A
β
a
A
β
$
PII
jour 0
jour 3
jour 6-17
PIII
Te
B0.5
B5
B10
B
CORT, ng/ml
40
30
20
10
0
a
b
PII
jour 0
jour 3
jour 6-17
PIII

Fig. 2


A
activité locomotrice, UA
jours par rapport à l'implantation
Témoins
PIII
Te
B0.5
B5
B10
*
*
*

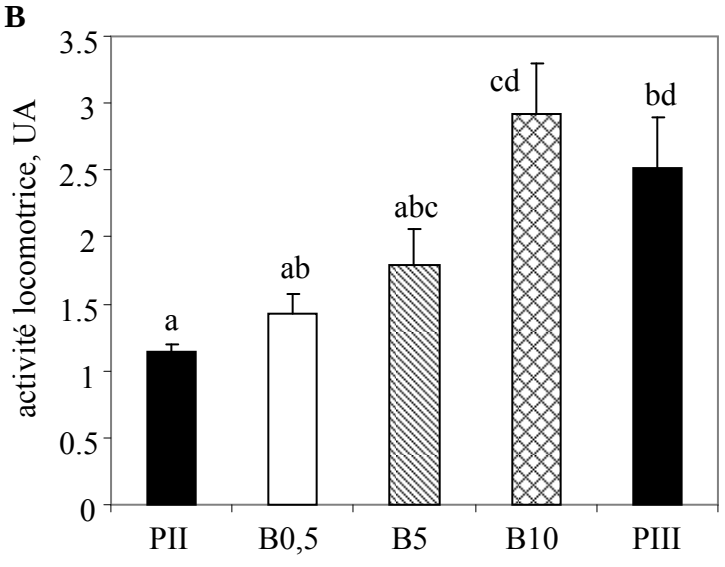

B
activité locomotrice, UA
a
ab
abc
cd
bd
PII
B0,5
B5
B10
PIII

Fig. 3

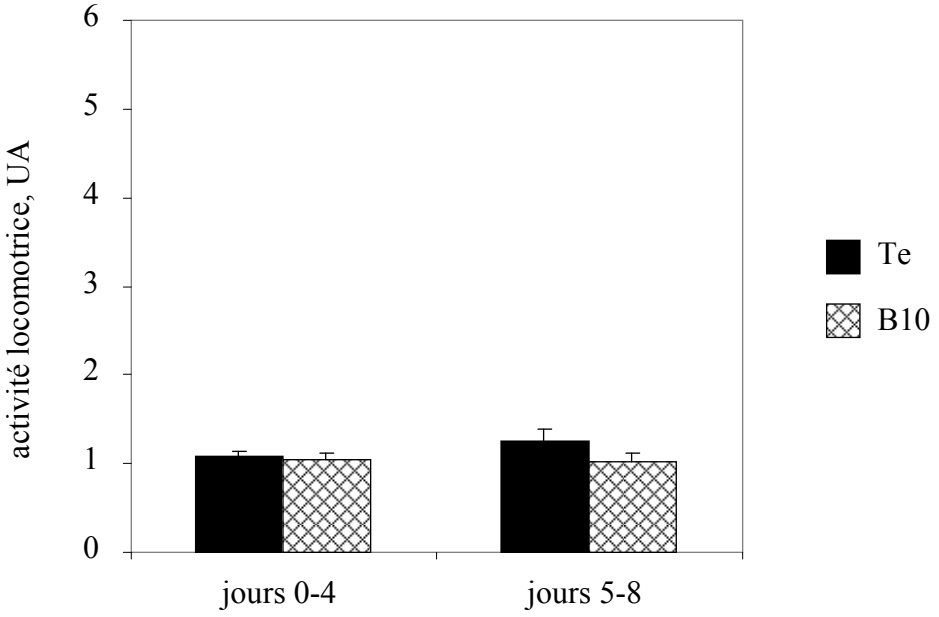


Fig. 4

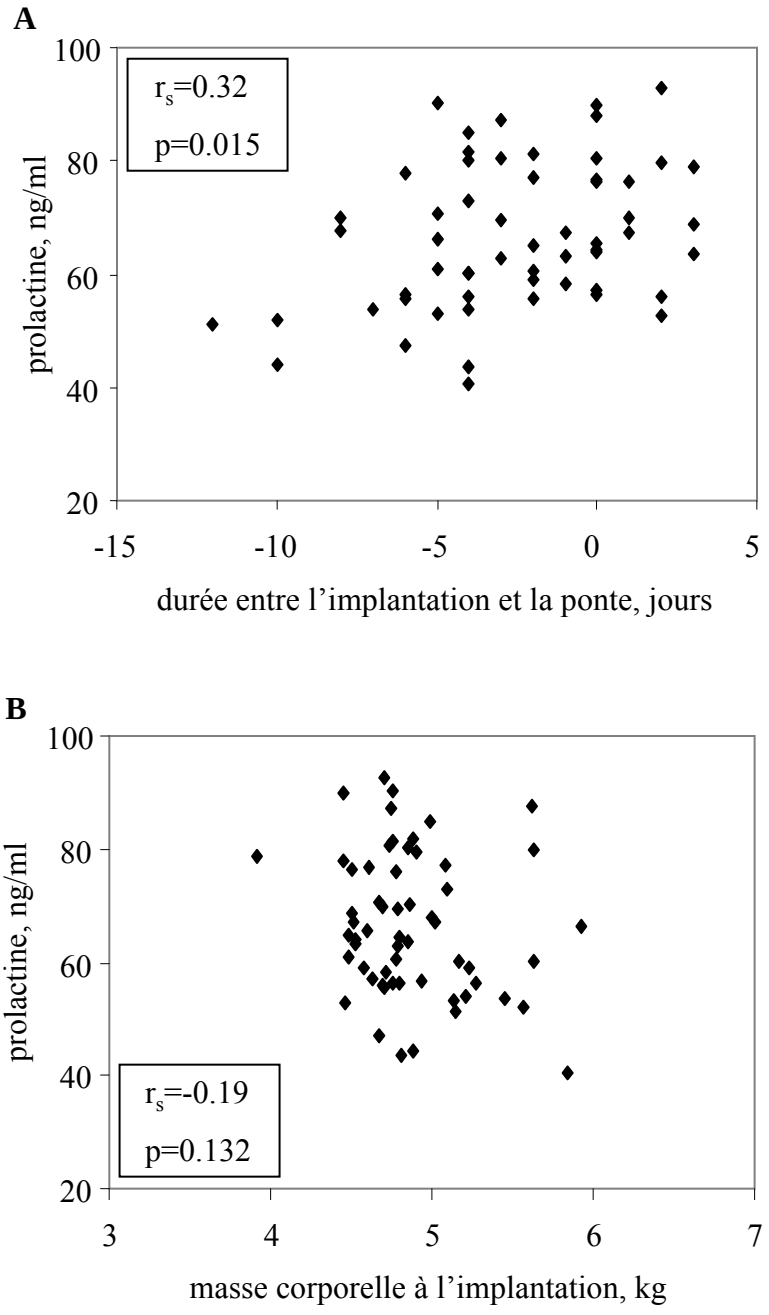

A
100
80
60
40
20
prolactine, ng/ml
r_s=0.32
p=0.015
-15
-10
-5
0
5
durée entre l'implantation et la ponte, jours
B
100
80
60
40
20
prolactine, ng/ml
r_s=-0.19
p=0.132
3
4
5
6
7
masse corporelle à l'implantation, kg

Fig. 5

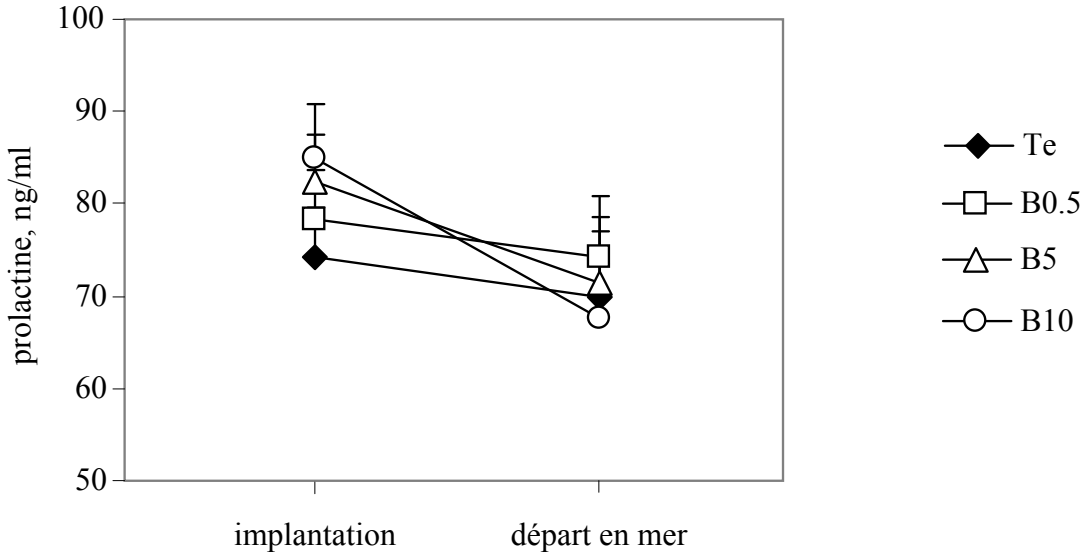

100
90
80
70
60
50
prolactine, ng/ml
implantation
départ en mer
Te
B0.5
B5
B10

Fig. 6

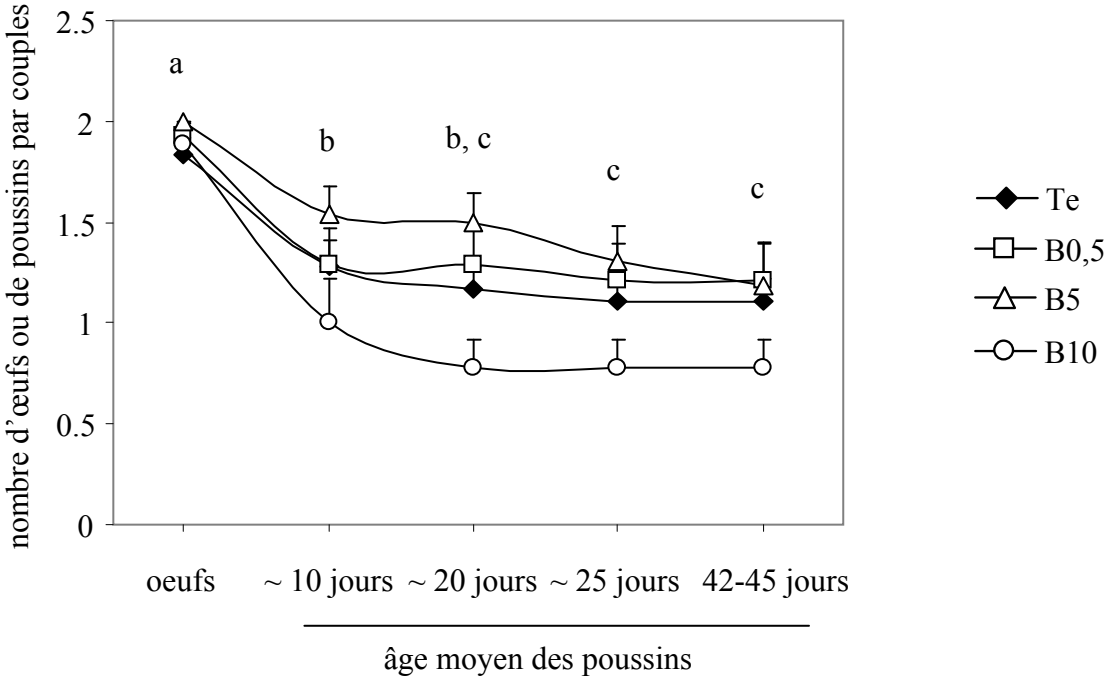

nombre d'œufs ou de poussins par couples
2.5
2
1.5
1
0.5
0
a
b
b, c
c
c
oeufs
~ 10 jours
~ 20 jours
~ 25 jours
42-45 jours
âge moyen des poussins
Te
B0,5
B5
B10

Fig. 7

Fig. 1. Schéma explicatif du protocole d'étude. Les manchots Adélie mâles ont été pesés et un prélèvement sanguin a été effectué au moment de l'implantation (0, 0,5, 5 ou 10 mg de bromocriptine) et du départ en mer (après la relève de la femelle ou l'abandon du nid). Le nombre d'œufs / poussins a été évalué lorsque les poussins étaient âgés d'environ 10 jours, 20 jours, 25 jours et lorsqu'ils ont atteint leur masse maximale (42 à 45 jours après l'éclosion ; Ainley, 2002). A ce dernier stade, les poussins ont été pesés. La durée des voyages alimentaires des parents a également été déterminée. La partie grisée représente la période théorique de diffusion de l'implant.

Fig. 2. Effet du traitement à la bromocriptine sur les concentrations de prolactine (A) et de CORT (B) chez des manchots Adélie mâles captifs. Te, individus témoins ; B0,5, B5, B10, individus implantés avec 0,5, 5, 10 mg de bromocriptine, respectivement. PII et PIII, oiseaux témoins en phase II et III, respectivement. Les résultats sont exprimés en moyenne ± ESM. Pour un même traitement, les barres d'histogrammes dont l'exposant diffère sont significativement différentes.

Fig. 3. (A) Evolution de l'activité locomotrice chez des manchots Adélie mâles captifs en fonction du traitement et du jour par rapport à l'implantation. *, indique une différence significative entre les individus du groupe B10 et les témoins pour un jour donné. (B) Effet du traitement à la bromocriptine sur l'activité locomotrice. Te, individus témoins ; B0,5, B5, B10, individus implantés avec 0,5, 5, 10 mg de bromocriptine, respectivement. PII, oiseaux témoins en phase II au jour 0 ; PIII, oiseaux témoins en phase III au jour 6-9. Les résultats sont exprimés en moyenne ± ESM. Les barres d'histogrammes dont l'exposant diffère sont significativement différentes.

Fig. 4. Effet d'un traitement avec 10 mg de bromocriptine sur l'activité locomotrice chez des manchots Adélie captifs en mue. Les résultats sont exprimés en moyenne ± ESM.

Fig. 5. Relation entre la concentration de prolactine à l'implantation et (A) la durée séparant l'implantation et la ponte, (B) la masse corporelle à l'implantation, chez des manchots Adélie mâles sauvages.

Fig. 6. Effet du traitement à la bromocriptine sur la concentration en prolactine à l'implantation et au départ en mer, chez des manchots Adélie mâles sauvages. Te, individus témoins ; B0,5, B5, B10, individus implantés avec 0,5, 5, 10 mg de bromocriptine, respectivement. Les résultats sont exprimés en moyenne ± ESM.

Fig. 7. Effet du traitement à la bromocriptine sur le nombre moyen d'œufs / poussins par couples. Te, individus témoins ; B0,5, B5, B10, individus implantés avec 0,5, 5, 10 mg de bromocriptine, respectivement. Les résultats sont exprimés en moyenne ± ESM, les lettres font références à l'effet de la période d'échantillonnage (quelque soit le traitement). Des lettres différentes indiquent une différence significative.

Table 1

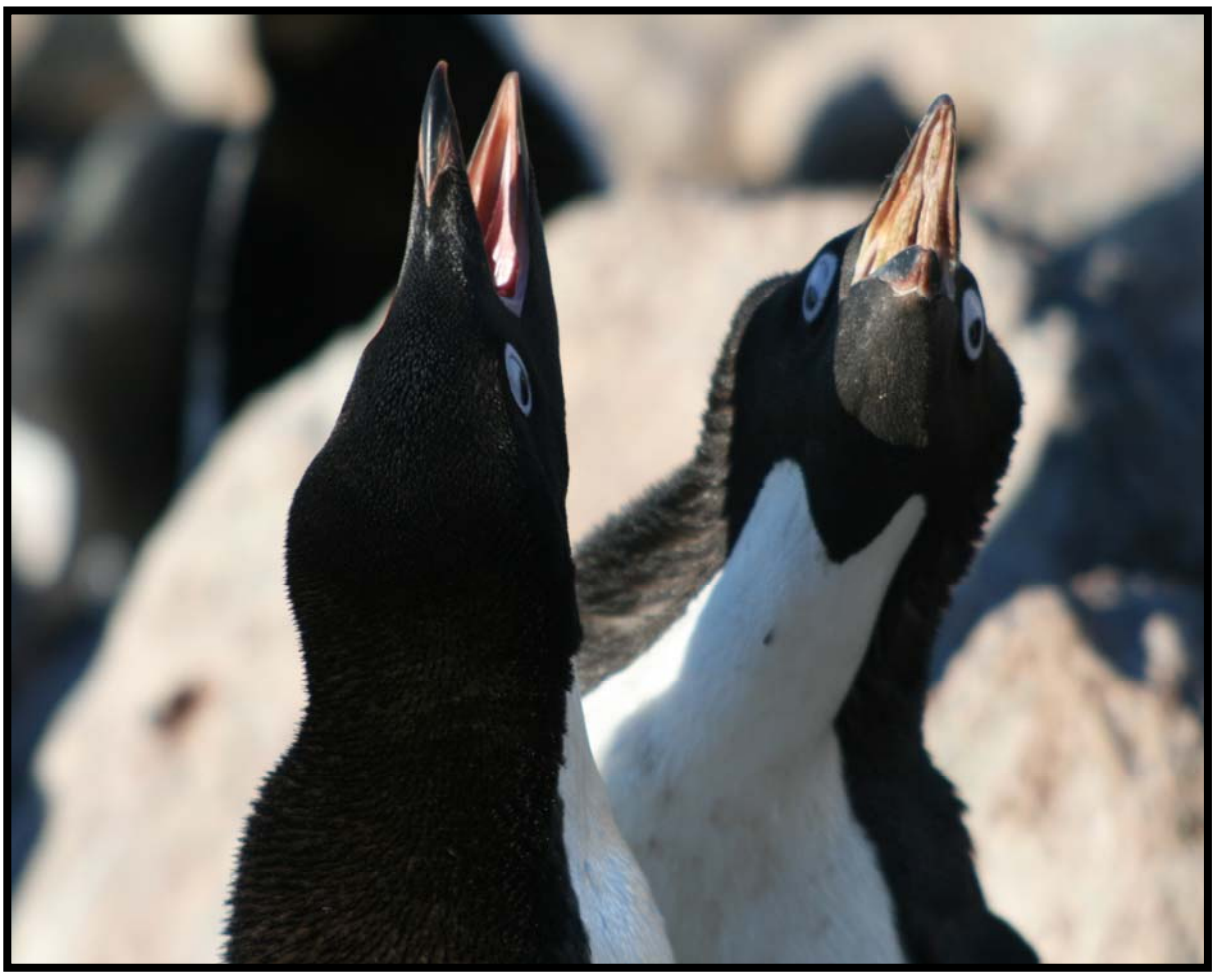
Profil des manchots Adélie mâles témoins et traités avec de la bromocriptine ayant été relevés par la femelle.

Traitement	Témoins (n=18)	B0,5 (n=14)	B5 (n=13)	B10 (n=9)
Date d'implantation	16/11 ± 0,84 [a]	16/11 ± 0,74 [a]	16/11 ± 1,06 [a]	17/11 ± 0,71 [a]
Masse corporelle à l'implantation, kg	4,77 ± 0,09 [a]	4,94 ± 0,07 [a]	4,98 ± 0,12 [a]	5,04 ± 0,16 [a]
Date de départ en mer	06/12 ± 0,62 [a]	05/12 ± 0,75 [a]	05/12 ± 0,81 [a]	02/12 ± 0,39 [b]
Masse corporelle au départ en mer, kg	3,79 ± 0,06 [a]	3,92 ± 0,06 [a]	3,97 ± 0,10 [a]	4,12 ± 0,14 [a]
Perte de masse journalière, g/jour	49,13 ± 1,02 [a]	53,59 ± 1,37 [a, b]	54,80 ± 2,16 [a, b]	56,11 ± 2,75 [b]
Durée entre l'implantation et le départ en mer, jour	19,83 ± 1,03 [a]	19,07 ± 0,85 [a]	18,83 ± 0,78 [a]	16,56 ± 0,78 [a]
Date de ponte	19/11 ± 0,59 [a]	19/11 ± 0,53 [a]	18/11 ± 0,76 [a]	18/11 ± 0,53 [a]
Date d'éclosion	24/12 ± 0,54 [a]	25/12 ± 0,85 [a]	23/12 ± 0,65 [a]	25/12 ± 0,86 [a]
Durée d'incubation	35,54 ± 0,43 [a]	36,33 ± 0,67 [a]	35,17 ± 0,60 [a]	37,20 ± 1,02 [a]
Masse de la couvée $	2,69 ± 0,14 [a]	3,43 ± 0,30 [a]	3,53 ± 0,35 [a]	2,83 ± 0,13 [a]
Masse de la couvée *	4,21 ± 0,29 [a]	5,38 ± 0,59 [a]	5,39 ± 0,56 [a]	3,92 ± 0,24 [a]

B0,5, B5 and B10, manchots implantés avec 0,5, 5 and 10 mg de bromocriptine, respectivement. Les valeurs représentent les moyennes ± ESM. Les valeurs d'une même ligne dont l'exposant diffèrent sont significativement différentes ($p<0,05$). $ Lorsque les poussins sont âgés d'environ 25 jours et * lorsqu'ils atteignent leur masse maximale (42 à 45 jours après l'éclosion; Ainley, 2002).

Chapitre 4

Synthèse, discussion et perspectives



Synthèse, discussion et perspectives

L'objectif de ce doctorat était d'examiner l'induction de l'abandon du nid lors du jeûne d'incubation chez le manchot Adélie au travers de mécanismes hormonaux impliqués dans l'expression du comportement parental : la CORT qui stimule le comportement de recherche alimentaire et la prolactine qui tient un rôle opposé en stimulant la promotion des soins parentaux.

1) Rappel des principaux résultats

Les résultats obtenus montrent que la CORT et la prolactine sont successivement affectées par l'entrée en phase critique du jeûne prolongé associé à l'incubation (étude 1 : Spée et al. 2010). La concentration de CORT augmente en premier et les niveaux élevés ainsi atteints entraînent une diminution de la concentration de prolactine. Les résultats obtenus mettent en évidence le fait que la prolactine doit atteindre un niveau seuil bas pour finalement provoquer l'abandon de la reproduction (études 1 : Spée et al. 2010 et 3). Ainsi, l'effet de la CORT sur la concentration de prolactine apparaît comme étant un mécanisme hormonal crucial impliqué dans la décision d'interrompre un épisode reproducteur lorsque la survie du parent est menacée. De plus, si l'atteinte de cet état hormonal (niveaux hauts de CORT et bas de prolactine) déclenche l'abandon du nid, il semble que la modification seule de l'une ou l'autre de ces hormones impliquées dans le contrôle du comportement parental ne soit pas suffisante pour provoquer la désertion du nid (études 1 : Spée et al. 2010, 3 et 4).

Les résultats obtenus dans le cadre de ce doctorat mettent également en évidence l'existence d'un délai plus ou moins long entre l'atteinte de niveaux hauts de CORT (suite à une implantation de CORT) et l'apparition des modifications comportementales (l'activité

locomotrice augmente 2 à 4 jours après le traitement chez les oiseaux captifs et les oiseaux sauvages abandonnent leur nid ~ 14 jours après l'implantation de CORT ; études 2 et 3).

Dans la présente discussion, nous allons tout d'abord évoquer la notion de seuil *i.e* les niveaux que les hormones (CORT et prolactine) ont à atteindre, suite à l'entrée en phase critique de jeûne ou à la suite de modifications expérimentales de leurs concentrations, pour influer sur la physiologie et le comportement. Nous discuterons ensuite de la modulation des niveaux de CORT et de prolactine en réponse à un stress nutritionnel ou consécutive à une manipulation expérimentale (implantation de CORT) afin d'examiner les raisons pour lesquelles 1) certains oiseaux n'abandonnent pas leur nid et 2) le délai entre l'atteinte d'un niveau adéquat de CORT et de prolactine et l'apparition des modifications comportementales varie selon nos études. Nous discuterons subséquemment des premiers résultats d'études en cours non présentées dans le manuscrit (réalisées lors de la dernière campagne d'été - 2009-10 - à Dumont d'Urville) et de leur implication dans la compréhension de l'induction du signal de réalimentation. Nous discuterons également de l'implication possible d'autres mécanismes dans la stimulation du comportement de recherche alimentaire. Enfin, nous évoquerons le rôle éventuel des femelles dans le processus d'abandon du nid par les mâles.

2) La notion de seuil

Romero (2004) souligne l'importance de considérer la valeur atteinte par une hormone en réponse à un stress (stress de contention / stress nutritionnel) plutôt que d'examiner sa variation entre un niveau initial et un niveau final. Les hormones doivent, en effet, se fixer sur des récepteurs pour provoquer des modifications de la physiologie et/ou du comportement, les niveaux d'hormones étant en outre susceptibles d'agir sur le nombre de récepteurs (Landys et al. 2006). Par exemple, le nombre de récepteurs aux glucocorticoïdes peut diminuer pour

compenser l'élévation à long terme des niveaux CORT. Dans ce cas, puisqu'il y a moins de récepteurs, une concentration donnée de CORT n'aura pas les mêmes effets biologiques.

L'ampleur de la réponse au stress (qu'il soit de contention ou nutritionnel) et par conséquent les niveaux atteints par les hormones vont influencer l'issue du compromis entre la reproduction en cours et la survie. Lorsque la valeur de la reproduction en cours est plus importante que les perspectives de reproductions futures, la réponse au stress devrait être atténuée (Lendvai et al. 2007 ; Bokony et al. 2009 ; Angelier et al. 2007c). Ainsi, l'induction des modifications comportementales relatives à l'arrêt de la reproduction et à la stimulation de la recherche alimentaire peut dépendre de paramètres individuels intrinsèques induisant une modulation de la réponse au stress ; c'est-à-dire, dans le cadre de notre étude, de l'amplitude de l'augmentation de CORT et de la diminution de prolactine et par conséquent les niveaux atteints par ses hormones suite à un stress nutritionnel ou à la manipulation expérimentale des concentrations de CORT. Dans cette optique, l'hypothèse d'un niveau seuil bas de prolactine en dessous duquel la motivation de l'oiseau à incuber est dépassée par sa motivation à rechercher de la nourriture, elle-même contrôlée par des niveaux élevés de CORT (études 2 et 3) est particulièrement intéressante à considérer.

3) CORT et modulation de la réponse au stress

3.1. Niveaux de CORT

Chez l'animal sauvage, l'augmentation de la CORT en réponse à un stress nutritionnel permet de restaurer l'équilibre énergétique de l'organisme en provoquant des changements de sa physiologie et de son comportement (Wingfield et al. 1998 ; McEwen et Wingfield 2003). Dans les études 1 et 3, nous avons mis en évidence que les niveaux de CORT (endogènes et exogènes) étaient similaires chez les oiseaux qui ont abandonné leur nid et ceux qui ne l'ont pas abandonné

mais ont été relevés par leur partenaire. Sachant que les niveaux élevés de CORT reflètent un stress nutritionnel (Kitaysky et al. 2007), ces quatre groupes d'oiseaux (manchots en PIII 1) abandonnant leur nid et 2) relevés par la femelle ; manchots traités avec de la CORT 3) abandonnant leur nid et 4) relevés par la femelle) présentent le même niveau de stress, alors que leur réponse comportementale diffère. Cependant, les niveaux de CORT présentés dans ce mémoire concernent les concentrations de CORT totale et ne nous renseigne que partiellement sur l'action biologique de l'hormone. Dans la circulation sanguine, la CORT se trouve majoritairement sous une forme liée à une protéine de transport (*Corticosteroid Binding Globulin*, CBG), la fraction restante étant sous forme libre. Il est admis que la CORT libre représente la fraction biologiquement active (Ekins 1990 ; Breuner et Orchinik 2002), capable de se fixer aux récepteurs et d'influer sur la physiologie et le comportement des individus. En effet, deux individus ayant le même niveau de CORT totale peuvent « en réalité » être dans deux « stades d'activation » de CORT distincts.

3.2. Modulation de la CORT en réponse à un stress

La production, l'action et la bio-disponibilité de la CORT peuvent être modulées par de nombreux facteurs (Wingfield et Sapolsky 2003 ; Romero 2004 ; Landys et al. 2006). La sensibilité de la CORT au stress peut notamment être minimisée 1) au niveau central par une modulation de l'axe hypothalamo-hypophyso-surrénalien (Romero et al. 1998b ; Romero et Wingfield 2001 ; Heidinger et al. 2008) et 2) aux niveaux plasmatique et cellulaire par des facteurs en aval de cet axe (Breuner et Orchinik 2001 ; Breuner et Orchinik 2002). Ces facteurs étant cruciaux dans la compréhension de la modulation de la réponse au stress, ces deux derniers points sont développés ci-dessous.

Dans la circulation sanguine, un niveau de CBG plasmatique plus important permettrait de minimiser la sensibilité de la CORT en réponse à un stress en diminuant la fraction d'hormone libre (Breuner et al. 2006). Breuner et collaborateurs (2003) ont souligné

l'importance de considérer le niveau de CBG et donc de CORT libre pour examiner les questions de modulation de la réponse au stress et de stratégies d'histoire de vie. Ainsi, chez des individus de trois populations de bruants à couronne blanche *Zonotrichia leucophrys* se reproduisant à des latitudes différentes et possédant des fenêtres temporelles variables pour se reproduire, les niveaux de CORT totale suite à un stress de contention sont similaires. Il était pourtant prédit que les individus de la population se reproduisant le plus au nord présenteraient une augmentation plus modérée de la CORT face à un stress. De façon intéressante, les auteurs ont montré que les niveaux de CBG et de CORT libre différaient entre les populations : les individus de la population la plus au nord présentaient 1) des niveaux plus hauts de CBG et 2) des concentrations de CORT libre plus faibles en réponse au stress. Dans le contexte de notre étude, l'hétérogénéité comportementale (abandon/non abandon) et les niveaux différents de prolactine observés chez des individus étant dans le même « état de stress apparent » (*cf.* niveaux de CORT totale similaires) pourraient être expliqués par des niveaux de CBG et donc de CORT libre différents. La mesure des niveaux de CBG permettrait ainsi de déterminer si les individus qui désertent leur nid présentent des niveaux de CBG plus faibles et donc de CORT libre plus élevés. A titre d'exemple, le niveau de CORT libre chez les femelles étourneau sansonnet *Sturnus vulgaris* qui abandonnent la reproduction est plus important que chez celles qui n'abandonnent pas (Love et al. 2004). La question des facteurs individuels pouvant moduler le niveau de CBG se pose alors. Il est envisageable que les oiseaux qui entament une période prévisible de besoins métaboliques accrus telle que la reproduction présentent des niveaux de CBG élevés, leur permettant ainsi de disposer d'un « réservoir » d'hormone couramment inactive mais qui peut être rapidement mobilisée en fonction des besoins (Malisch et Breuner 2010). Sachant que le jeûne entraîne une diminution de la capacité de liaison des CBG (Lynn et al. 2003, 2010), on peut supposer que les individus présentant les plus hauts niveaux de CBG en début du jeûne de reproduction auront également les niveaux les plus élevés en fin de jeûne. Ceux-ci seraient donc plus aptes à modérer leur réponse physiologique et comportementale suite à un stress nutritionnel (ex : atteinte de la PIII). Ainsi dans l'étude 1 (Spée et al. 2010), les oiseaux qui

sont entrés en PIII sans abandonner leur nid pourraient présenter des niveaux de CBG plus hauts en début de reproduction comparés aux oiseaux qui sont entrés en PIII et qui ont déserté leurs œufs. Au niveau interindividuel, les niveaux de CBG peuvent être modulés de différentes manières :

1) La condition corporelle en début de jeûne peut avoir une influence sur les niveaux de CBG. Il existe en effet une corrélation positive entre la condition corporelle et le niveau de CBG chez le macareux huppé *Fratercula cirrhata* (Williams et al. 2008).

2) Deviche et ses collabrateurs (2001) ont montré chez des juncos ardoisés *Junco hyemalis* que les niveaux de CBG étaient plus importants chez les oiseaux ayant une concentration de testostérone naturellement élevée (ex : en début de reproduction) ou expérimentalement haute. En effet, chez les oiseaux, contrairement aux mammifères, les hormones sexuelles n'ont pas de protéines de transport plasmatique spécifiques mais se fixent aux CBG (Wingfield et al. 1984; Breuner et Orchinik 2002). Dans le cadre de notre étude, on peut émettre l'hypothèse que les manchots présentant les plus hauts niveaux de testostérone en début de reproduction pourraient également avoir les niveaux les plus hauts de CBG. Sachant que le niveau de testostérone chute en début d'incubation (Vleck et al. 1999), les CBG seraient alors disponibles pour fixer la CORT.

3) Une étude menée chez une espèce longévive (mouette tridactyle, Shultz et Kitaysky 2008) propose que les variations des niveaux de CBG pourraient être fonction de la dynamique de la disponibilité alimentaire ou de la composition du régime. En effet, des coquelets *Gallus domesticus* nourris avec un régime alimentaire pauvre en protéines présentent des niveaux de CBG diminués par rapport aux oiseaux nourris avec un régime plus riche en protéines (Carsia et al. 1988). Dans le cadre de notre étude, les manchots pourraient présenter des niveaux variables de CBG en fonction de la disponibilité et du type de proies qu'ils ont pu ingérer avant d'arriver sur le site de reproduction.

Au niveau cellulaire, la CORT peut se fixer à plusieurs types de récepteurs à affinité et vitesse de réponse différentielles (deux récepteurs génomiques à réponse lente et un récepteur

membranaire à réponse rapide ; Breuner et Orchinik 2001 ; Breuner et Orchinik 2009). Nos résultats indiquent que les délais entre l'atteinte d'un niveau haut de CORT et l'apparition des modifications comportementales sont longs (augmentation de l'activité locomotrice 2-4 jours après le traitement, étude 2 et abandon du nid ~ 14 jours après l'implantation de CORT, étude 3) par rapport à ce qui a été obtenu chez des bruants à couronne blanche (augmentation d'activité locomotrice 15 minutes après l'ingestion d'un repas de ver contenant de la CORT, Breuner et Wingfield 2000). Ces auteurs ont proposé que l'effet rapide de la CORT sur l'activité locomotrice chez ces oiseaux pouvait passer par des récepteurs membranaires. Dans notre étude cependant, il est probable que les effets de la CORT sur le comportement locomoteur aient comme médiateurs les récepteurs génomiques. Le nombre de ces récepteurs exprimés peut influer sur la réponse induite par la CORT et réciproquement, des niveaux élevés de CORT peuvent diminuer le nombre de récepteurs capables de fixer l'hormone, permettant d'éviter les effets délétères d'un niveau élevé de CORT et ainsi de moduler la sensibilité des tissus à l'action de l'hormone (Breuner et Orchinik 2001 ; Romero 2004 ; Landys et al. 2006). Dans les études 1 (Spée et al. 2010) et 3, il est possible que les oiseaux qui abandonnent leur nid présentent un nombre de récepteurs plus important que les oiseaux qui ne désertent pas, étant ainsi plus sensibles à l'action de la CORT.

Ainsi, les niveaux de CBG, le nombre et la nature des récepteurs sont parmi les facteurs permettant une modulation de la réponse de la CORT au stress, en influençant l'action de l'hormone sur la physiologie et le comportement et par conséquent l'issue du compromis entre la survie et la reproduction en cours. Cependant, parce que la CORT semble nécessaire mais non suffisante à elle seule pour provoquer l'abandon du nid (étude 1 : Spée et al. 2010, étude 3), nous nous intéresserons dans la partie suivante à une autre hormone ayant un rôle opposé à celle de la CORT dans le contrôle du comportement parental : la prolactine. En effet, son niveau ainsi que sa sensibilité au stress peuvent influer sur la décision d'abandonner un épisode reproducteur.

4) Prolactine et modulation de la réponse au stress

4.1. Niveau seuil de prolactine

La relation liant le comportement parental et les niveaux de prolactine est non linéaire et dépend d'un seuil (Angelier et Chastel 2009). Par exemple, il a été proposé que les soins parentaux post-éclosion ne pouvaient être mis en place et/ou soutenus que si la concentration de prolactine après l'éclosion se maintenait au dessus d'un seuil (Criscuolo et al. 2002 ; Boos et al. 2007). A l'inverse, la réduction voire la suppression des soins parentaux semble dépendre de l'atteinte d'un niveau seuil bas de prolactine (Angelier et al. 2009a ; Spée et al. 2010). A titre indicatif, il semble au vu des résultats obtenus chez les manchots Adélie mâles abandonnant leur nid, que le niveau seuil de prolactine en dessous duquel la motivation à incuber est inhibée se situe autour de 30 ng/ml.

Les résultats obtenus dans les études 2 et 3 ainsi que les données de la littérature indiquent que l'action de la CORT sur la diminution des niveaux de prolactine est complexe, puisque la concentration de prolactine décroît lentement et progressivement suite à une augmentation expérimentale de la concentration de CORT (Angelier et al. 2009a). Dans notre étude, il semble que le délai entre l'atteinte d'un niveau élevé de CORT et l'apparition des modifications comportementales dépende de la vitesse de diminution de la concentration de prolactine jusqu'à l'atteinte d'un niveau seuil bas. En effet, chez les manchots captifs traités à la CORT, le niveau de prolactine est aux environs de 30 ng/ml 3 jours après l'implantation (première prise de sang effectuée après l'implantation) et l'activité locomotrice augmente 2 à 4 jours après le traitement. Chez les manchots sauvages traités à la CORT, la prolactine n'atteint ce niveau que chez les individus qui abandonnent, et ce, environ 14 jours après le traitement. Enfin, des données préliminaires indiquent que chez des oiseaux sauvages traités à la fois avec de la CORT et de la bromocriptine (25 mg ; dose plus forte que celles utilisées dans l'étude 4), le traitement a induit 70 % d'abandon du nid, et ce au bout de 9 jours environ (de 6 à 12 jours).

Dans ce dernier cas, il est fortement probable que la concentration de prolactine ait atteint un seuil bas plus rapidement que chez les oiseaux traités avec de la CORT seule.

4.2. Modulation de la prolactine en réponse à un stress

Angelier et Chastel (2009) proposent que l'ampleur de la diminution de la concentration de prolactine suite à un stress de contention et donc la vitesse d'atteinte d'un certain niveau dépendent de la motivation de l'oiseau à s'investir dans la reproduction en cours (Angelier et Chastel 2009). Par exemple, les niveaux de prolactine diminuent plus rapidement suite à un stress de contention chez des oiseaux impliqués dans l'élevage de leur poussin (mouettes tridactyles, Chastel et al. 2005) ou incubant (pétrels des neiges, Angelier et al. 2009b) par rapport aux oiseaux ayant perdu leur œuf, et ce, indépendamment de leur condition corporelle. Malgré le fait que les individus captifs présentent un niveau de prolactine plus faible que celui des oiseaux sauvages au moment de l'implantation de CORT, ceci pourrait expliquer le délai plus court d'apparition des changements comportementaux chez les manchots captifs *i.e.* ayant perdu leurs œufs (étude 2) et sauvages traités avec de la CORT (étude 3). De plus, les résultats obtenus dans l'étude 4 indiquent que la même dose de bromocriptine induit une augmentation de l'activité locomotrice chez les manchots captifs mais n'entraîne pas d'abandon du nid chez les oiseaux sauvages. Il est donc fortement probable que la régulation de la prolactine diffère entre les oiseaux ayant perdu leur œufs (captifs) et les individus incubant (sauvages). Setiawan et collaborateurs (2007) proposent que le contexte social, *i.e.* la présence d'autres oiseaux reproducteurs, pourrait participer à la stimulation de la sécrétion de prolactine. En effet, les auteurs ont mis en évidence que des manchots antipodes *Megadyptes antipodes* exposés à des stimuli auditifs (reflétant un contexte social) durant la période de parade et de construction du nid présentaient des niveaux de prolactine plus hauts au moment de la ponte que des oiseaux non soumis à ces stimuli. Ceci est cependant peu probable dans notre étude étant donné que les oiseaux captifs sont placés à proximité des oiseaux incubant et peuvent les entendre. Bien que

programmée de façon endogène (Lormée et al. 1999), il est possible que la sécrétion de prolactine soit soutenue par la présence de l'œuf, la concentration de l'hormone augmentant pendant un séjour à terre chez le manchot royal (Garcia et al. 1996).

La sensibilité de la prolactine suite à un stress peut également être modulée en fonction de l'âge des individus. Chez les espèces longévives, les individus âgés ont en effet plus intérêt à maximiser leur reproduction en cours et donc réprimer leur sensibilité à un stress puisque les chances de survie et donc de reproductions futures diminuent avec l'âge (Wingfield et Sapolsky 2003). Par exemple, les niveaux de prolactine en réponse à un stress de contention diminuent de façon moins importante chez des pétrels des neiges âgés comparés aux oiseaux plus jeunes (Angelier et al. 2007c). Il est ainsi possible que les manchots des études 1 et 3 qui présentent des niveaux bas de prolactine et abandonnent leur nid soient plus jeunes ou moins expérimentés que les oiseaux dans le même « état de stress » (jeûne prolongé et atteinte de la PIII ou implantés avec de la CORT) mais qui présentent des niveaux de prolactine plus hauts et qui n'abandonnent pas la reproduction. Le fait que les manchots traités à la CORT qui abandonnent leur nid présentent une vitesse de diminution des niveaux de prolactine 2 fois plus élevée que ceux qui n'abandonnent pas (étude 3) va dans le sens de cette hypothèse. De futures études menées chez des individus d'âge connu permettraient de tester cette théorie. Par ailleurs, la détermination de l'âge « biologique » des individus serait extrêmement intéressante dans le contexte de notre étude et serait rendue possible par la détermination de la longueur des télomères (Monaghan et Haussmann 2006). Les télomères sont des séquences nucléiques non-codantes qui chapeautent les extrémités des chromosomes des cellules eucaryotes et stabilisent le génome. Ils se raccourcissent classiquement à chaque division cellulaire. Lorsque la longueur des télomères a atteint un certain seuil, la cellule rentre en apoptose *i.e.* mort cellulaire programmée (Blackburn 2000). Bize et collaborateurs (2010) ont mis en évidence que la longueur des télomères dans les cellules sanguines et leur vitesse de raccourcissement étaient reliées au taux de survie chez le martinet alpin *Apus melba*. De plus, il a été montré que des hirondelles bicolores âgées de 1 an et possédant des télomères relativement courts avaient une chance de survie (estimée par le taux

de retour des oiseaux sur leur site de reproduction les années suivantes) plus faible que des oiseaux du même âge ayant des télomères plus longs (Haussmann et al. 2005). Si des individus possédant des télomères courts sont moins enclins à survivre, ils devraient privilégier leur maintenance aux dépens de la reproduction en cours si celle-ci devient trop coûteuse. Ainsi, une atténuation de la réponse de la prolactine au stress dépendante de l'âge « chronologique » ou « biologique » pourrait être un mécanisme adaptatif permettant de maintenir les soins parentaux lors de situations stressantes.

5) Premiers résultats issus d'études en cours

Nous avons vu que plusieurs facteurs intrinsèques pouvaient influer sur la modulation de la CORT et de la prolactine en réponse à un stress, influençant ainsi l'issue du compromis entre la reproduction en cours et la survie. Il est clair que l'atteinte d'un certain état hormonal (niveaux hauts de CORT et bas de prolactine) déclenche l'abandon du nid, mais il semble cependant que la modification seule de l'une ou l'autre de ces hormones ne soit pas suffisante pour provoquer la désertion du nid (études 1, 3 et 4). L'utilisation d'une dose plus forte de bromocriptine chez les animaux sauvages fait l'objet d'une étude en cours dont les premiers résultats indiquent qu'un seul abandon du nid a été observé sur 10 individus traités (soit 10 % de désertion du nid contre 7 % chez les oiseaux témoins). Ceci renforce le fait qu'une diminution seule de prolactine n'est pas suffisante pour déclencher la désertion du nid. Le dosage de la prolactine chez ces oiseaux reste néanmoins à effectuer pour déterminer si le traitement a induit une diminution significative du niveau de prolactine, éventuellement jusqu'à une valeur basse seuil chez l'oiseau ayant abandonné son nid. Chez les oiseaux du groupe B25, comme chez ceux du groupe B10 (étude 4), la perte de masse est plus importante que celle des manchots témoins (11 et 14 % plus importante chez les B10 et B25, respectivement). Ainsi, on peut penser que le traitement, à défaut de provoquer l'abandon du nid, a altéré l'attention au nid du parent, le

rendant plus actif (passage de la position couchée à debout plus fréquente). L'utilisation d'accéléromètres posés sur les animaux incubant traités avec de la bromocriptine permettrait d'obtenir des informations sur la position de l'animal (debout/couché) et sur son activité sur le nid (agité/immobile). Par ailleurs, l'utilisation d'œufs loggers mesurant la température permettrait d'avoir une estimation de l'attention du parent en postulant que si la température est plus basse, le parent a passé plus de temps dans la position debout.

Cette même dose de bromocriptine (B25) a été implantée chez des oiseaux captifs et les premiers résultats indiquent que le profil d'activité locomotrice induit par le traitement (augmentation de facteurs 2 et 5, respectivement un jour et deux jours après le traitement; Figure 12) est similaire à celui obtenu chez les oiseaux traités avec 10 mg de bromocriptine (B10, étude 4). On peut s'attendre à ce que la diminution de prolactine soit plus importante que celle observée chez les oiseaux du groupe B10. L'ampleur des modifications comportementales ne paraît donc pas dépendre de l'amplitude de la diminution du niveau de prolactine. Ces changements comportementaux semblent plutôt mis en place de façon similaire chez les oiseaux des groupes B10 et B25 en dessous d'un niveau seuil minimum de prolactine. Ceci s'oppose à l'effet de la CORT sur l'induction de l'activité locomotrice qui suit une courbe en « U renversé » (étude 2).

D'autre part, sachant que les niveaux de prolactine plasmatique tendent à baisser chez les individus en échec de reproduction (oiseaux captifs), il est possible que l'effet de la CORT exogène sur l'induction des modifications comportementales soit amplifié par la baisse de prolactine. Ceci pourrait ainsi expliquer le délai plus court d'apparition des modifications comportementales observé chez les oiseaux captifs (l'activité locomotrice augmente 2 à 4 jours après le traitement, étude 2) par rapport aux oiseaux sauvages (abandon du nid ~ 14 jours après l'implantation de CORT, étude 3). Pour tester cette hypothèse, nous avons administré en sous cutané, chez des oiseaux captifs, de la prolactine *via* l'implantation d'une pompe osmotique afin de maintenir un niveau comparable à celui des oiseaux sauvages avant de leur implanter de la CORT (C100). Les résultats préliminaires indiquent que l'activité locomotrice augmente un jour

plus tard que chez les oiseaux traités seulement avec de la CORT et que son amplitude est moindre (C100 : augmentation d'un facteur 2 trois jours après l'implantation ; C100+prolactine : augmentation d'un facteur 1,5 quatre jours après le traitement ; Figure 12). Au contraire, des oiseaux captifs implantés à la fois avec de la CORT et de la bromocriptine (B25+C100) montrent une activité locomotrice plus importante (augmentation de facteurs 2, 5 et 7-8, respectivement un, deux et trois jours après le traitement; Figure 12) que celle des oiseaux traités avec de la CORT seule (augmentation d'un facteur 2 trois jours après le traitement; Figure 12) ou de la bromocriptine seule (augmentation de facteurs 2 et 5, respectivement un jour et deux jours après le traitement; Figure 12). Il semble ainsi que le maintien d'un niveau haut de prolactine s'oppose à l'effet de la CORT sur les modifications comportementales alors qu'une diminution concomitante de la concentration de prolactine renforce au contraire l'effet de la CORT.

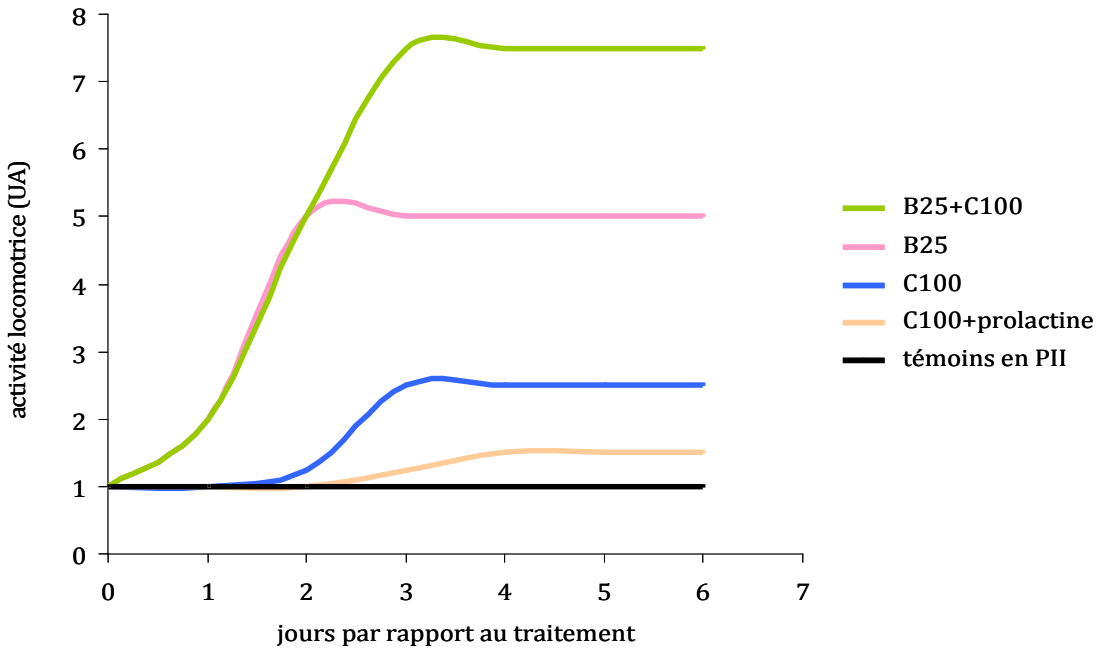


Figure 12. Représentation schématique du profil d'activité locomotrice de manchots Adélie captifs traités avec 100 mg de CORT (C100), 25 mg de bromocriptine (B25), 100 mg de CORT et 25 mg de bromocriptine (B25+100), 100 mg de CORT et de la prolactine (C100+prolactine) et témoins en PII. Données préliminaires issues de travaux effectués lors de la compagne d'été 2009-10 à Dumont d'Urville.

Au final, la mise en place du signal de réalimentation qui incite l'individu à adopter un comportement de recherche alimentaire est plus complexe chez les oiseaux sauvages incubant que chez les oiseaux captifs en échec de reproduction. Chez les oiseaux en cours de reproduction, « partir se réalimenter » implique d'« abandonner son nid » et donc la reproduction en cours. Chez eux, le maintien d'un niveau élevé de prolactine semble contrecarrer l'action initiatrice de la CORT dans la stimulation d'un comportement de recherche alimentaire.

6) Autres mécanismes potentiellement impliqués dans l'induction du signal de réalimentation

Dans la présente discussion, nous avons mis en évidence que la modulation de la sécrétion de CORT et de celle de la prolactine en réponse à un stress nutritionnel ou à une modification expérimentale des niveaux d'hormones pouvait influer sur la décision d'abandonner le nid ou affecter le délai d'apparition de ce comportement de désertion. Cependant, chez les oiseaux témoins en PIII qui abandonnent leurs œufs, le délai séparant l'atteinte d'un niveau haut de CORT et la désertion du nid est nettement plus rapide que ce que rapportent les résultats de l'étude 3 (environ 14 jours) et les résultats préliminaires obtenus chez des oiseaux traités à la fois avec de la CORT et de la bromocriptine (environ 9 jours). Il est donc probable que d'autres facteurs soient impliqués dans la décision de déserter les œufs.

Criscuolo et collaborateurs (2005) ont mis en évidence que l'induction du catabolisme protéique après implantation de CORT chez des femelles eiders lors du jeûne d'incubation n'était pas aussi importante que celle de femelles en PIII. Sachant qu'aucune des femelles traitées n'a abandonné son nid, les auteurs ont proposé que l'augmentation de la protéolyse pourrait être un facteur impliqué dans la stimulation de la réalimentation. Ainsi, l'action de la CORT sur le comportement de désertion spontanée pourrait dépendre d'un effet synergique avec son action

périphérique sur le catabolisme protéique. Chez le rat de laboratoire soumis à un jeûne prolongé, les niveaux d'ARNm de trois systèmes protéolytiques dans le muscle squelettique (système ubiquitine-protéasome, cathepsines, calpaïnes) sont sélectivement induits en PII et sont régulés à la hausse de façon coordonnée en PIII (Bertile et al. 2003c). Ainsi, la stimulation du comportement de recherche alimentaire pourrait dépendre de l'activation coordonnée de différents systèmes protéolytiques. De façon intéressante, des résultats préliminaires montrent que les oiseaux traités à la fois avec de la CORT et de la bromocriptine qui désertent leurs œufs présentent une perte de masse quotidienne 20% plus élevée que celle des individus traités avec de la CORT seule qui abandonnent leur nid (calculée entre le moment de l'implantation et le départ en mer). Cette perte de masse plus élevée pourrait témoigner d'un comportement plus actif, mais pourrait également être le reflet d'un catabolisme protéique plus important puisque celui-ci augmente en miroir avec la perte de masse quotidienne lors de l'entrée en PIII chez des individus témoins (Cherel et al. 1988a ; Robin et al. 1998). Une protéolyse importante pourrait être une composante du signal de réalimentation qui incite les oiseaux à abandonner leur reproduction en cours pour assurer leur survie.

Par ailleurs, l'étude d'hormones issues des adipocytes et capables d'agir au niveau central et de stimuler des voies orexigènes pourrait mettre en évidence d'autres acteurs impliqués dans la stimulation du comportement de recherche alimentaire. Ainsi, le clonage du gène *ob* (Zhang et al. 1994), la mise en évidence de l'activité endocrine de l'adipocyte et la sécrétion de la protéine *ob* ou leptine par le tissu adipeux (Halaas et al. 1995) suggèrent un lien permettant de renseigner le système nerveux central sur l'état des réserves adipeuses. L'administration de leptine recombinante chez des souris obèses entraîne en effet une diminution de la prise alimentaire et une augmentation de la dépense énergétique (Pelleymounter et al. 1995). Basée sur l'observation que le niveau de leptine est corrélé à l'adiposité corporelle (Maffei et al. 1995), la leptine pourrait constituer un senseur des changements à long-terme des réserves énergétiques. Elle semble également représenter un médiateur des changements à court-terme étant donné que le niveau de leptine chute

brutalement en début de jeûne (Ahima et al. 1996; Ahima et Flier 2000). La leptine module l'activité neuroendocrine de noyaux hypothalamiques exprimant des neuropeptides orexigènes (agouti-related peptide, AgRP et neuropeptide Y, NPY) et anorexigènes (pro-opiomélanocortine, POMC et cocaine and amphetamine regulated transcrit, CART) (Ahima et Lazar 2008). En PIII, il a été montré que l'expression génique des neuropeptides AgRP et NPY était fortement augmentée au niveau de l'hypothalamus, constituant ainsi une importante réponse hypothalamique de la PIII (Bertile et al. 2003a). Une concentration faible de leptine plasmatique à cette période pourrait permettre l'élaboration d'une réponse hypothalamique qui entraînerait la stimulation d'un comportement de recherche alimentaire. Cependant, l'administration de leptine recombinante chez des rats soumis à un jeûne prolongé a induit une atténuation mais pas une suppression de l'augmentation de l'expression des gènes codant l'AgRP et NPY (Bertile et al. 2003b). Il est toutefois important de noter que l'activité locomotrice, reflet d'un comportement de recherche alimentaire, n'a pas été déterminée dans cette étude. Chez les oiseaux, le clonage du gène codant la leptine (poulet, Taouis et al. 1998) et l'identification du gène codant pour son récepteur (Horev et al. 2000) suggèrent fortement la présence de leptine chez les oiseaux. De plus, Denbow et collaborateurs (2000) ont mis en évidence qu'une injection intracérébroventriculaire de leptine recombinante humaine entraînait une diminution de la prise alimentaire chez le poulet, suggérant ainsi un rôle similaire de la leptine à celui décrit chez les mammifères. Cependant, le clonage du gène codant la leptine a été remis en question (Friedman-Einat et al. 1999). Récemment, Friedman-Einat et collaborateurs (in press) ont mis en évidence, à l'aide d'un test basé sur l'activité du récepteur à la leptine de poulet, une absence de leptine dans des échantillons de sang d'oiseaux sauvages présentant des variations saisonnières extrêmes dans l'apport de nourriture et dans leurs réserves adipeuses (manchot Adélie et barge rousse *Limosa lapponica*). Les auteurs suggèrent que chez les oiseaux, un mécanisme alternatif à celui des mammifères pourrait intervenir dans la communication entre l'état des réserves adipeuses et le contrôle central de l'homéostasie énergétique.

7) Rôle des femelles dans le processus d'abandon du nid par les mâles

Dans ce travail de doctorat, nous nous sommes focalisés sur les manchots Adélie mâles. En effet, ils effectuent naturellement le jeûne le plus long pendant la pariade et la première partie de l'incubation et peuvent de ce fait être amenés à abandonner leurs œufs si l'état de leurs réserves énergétiques atteint un niveau critique. Leur capacité à effectuer un jeûne prolongé est fonction 1) du temps passé à jeûner et 2) de la quantité de réserves énergétiques dont ils disposent en début de jeûne. A titre d'exemple, des manchots Adélie arrivant sur le site de reproduction avec une masse corporelle faible en raison de conditions environnementales défavorables atteignent un niveau de déplétion critique de leurs réserves corporelles à une date précoce et abandonnent leur nid (Cockrem et al. 2006). Nous rapportons également dans l'étude 1 (Spée et al. 2010) que les manchots Adélie mâles qui entrent en PIII avaient une masse corporelle en début du jeûne d'incubation plus faible que ceux qui quittent le site en PII après avoir été relevés par leur partenaire (Figure 13). Les manchots qui abandonnent ont passé entre 9 et 15 jours à jeûner sur le nid depuis le départ en mer des femelles. On peut donc supposer que si leurs partenaires femelles avaient passé aussi peu de temps en mer que les femelles des couples en succès (6-8 jours, la durée des voyages des femelles des couples en succès oscillant entre 6 et 18 jours), certains mâles auraient pu être relevés à temps. Chez le pétrel antarctique *Thalassoica antarctica*, il existe une corrélation positive entre le temps passé en mer par les femelles et la condition corporelle de leurs partenaires (Tveraa et al. 1997). Dans notre cas, il semble que les manchots Adélie femelles ne prennent pas en compte l'état des réserves énergétiques de leurs partenaires mâles avant de partir en mer pour effectuer leur voyage alimentaire.

Chez les espèces où les individus prodiguent des soins biparentaux et où les deux parents prennent part à l'incubation, le temps que l'un des parents passe à rechercher de la nourriture peut en effet contraindre le temps que l'autre parent passe à jeûner (Tveraa et al. 1997). Ainsi, l'atteinte d'un niveau critique de réserves corporelles conduisant à l'abandon de la reproduction peut être due à un voyage en mer trop long du partenaire (Davis 1982 ; Watanuki 1993). A titre

d'exemple, l'allongement expérimental de la durée des voyages alimentaires chez des femelles pétrels antarctique est associé à un taux de désertion de leur partenaire 4.5 fois plus important que chez les couples témoins (Tveraa et al. 1997). Sur le site d'étude de Dumont d'Urville, nos résultats indiquent un taux d'abandon du nid chez les manchots Adélie mâles d'environ 5-7% (étude 1 : Spée et al. 2010 et étude 3). Ce pourcentage de désertion est faible par rapport à ceux rapportés chez les individus de la même espèce à des sites d'études différents (21 % à Cape Bird, Davis 1982 ; 16 % à Lützow-Holm Bay, Watanuki 1993). De façon intéressante, la durée moyenne des voyages alimentaires des femelles est plus importante dans ces sites (16.6 et 18.3 jours, respectivement à Cape Bird et à Lützow-Holm Bay) que celle des femelles qui se reproduisent à Dumont d'Urville (12-13 jours), pouvant ainsi expliquer le taux d'abandon plus important. La masse des mâles en début d'incubation n'a pas été déterminée dans ces études. Sachant que les conditions environnementales peuvent affecter la durée des voyages alimentaires (Beaulieu et al. 2010), on peut émettre l'hypothèse selon laquelle les femelles passeraient plus de temps en mer à rechercher de la nourriture lors des années où la disponibilité alimentaire est faible. Par conséquent, leurs partenaires devraient attendre leur retour pendant plus longtemps et pourraient voir leurs réserves énergétiques atteindre un niveau critique et être ainsi amenés à abandonner leur nid.

Une meilleure compréhension des relations liant la disponibilité des ressources marines, l'acquisition de réserves énergétiques, l'état des réserves corporelles des mâles et des femelles et le succès reproducteur subséquent chez les oiseaux marins longévifs permettrait de déterminer dans quelles mesures les conditions environnementales, et notamment leurs variations face aux changements climatiques, affectent la proportion des oiseaux atteignant la phase critique du jeûne et abandonnant leur nid.

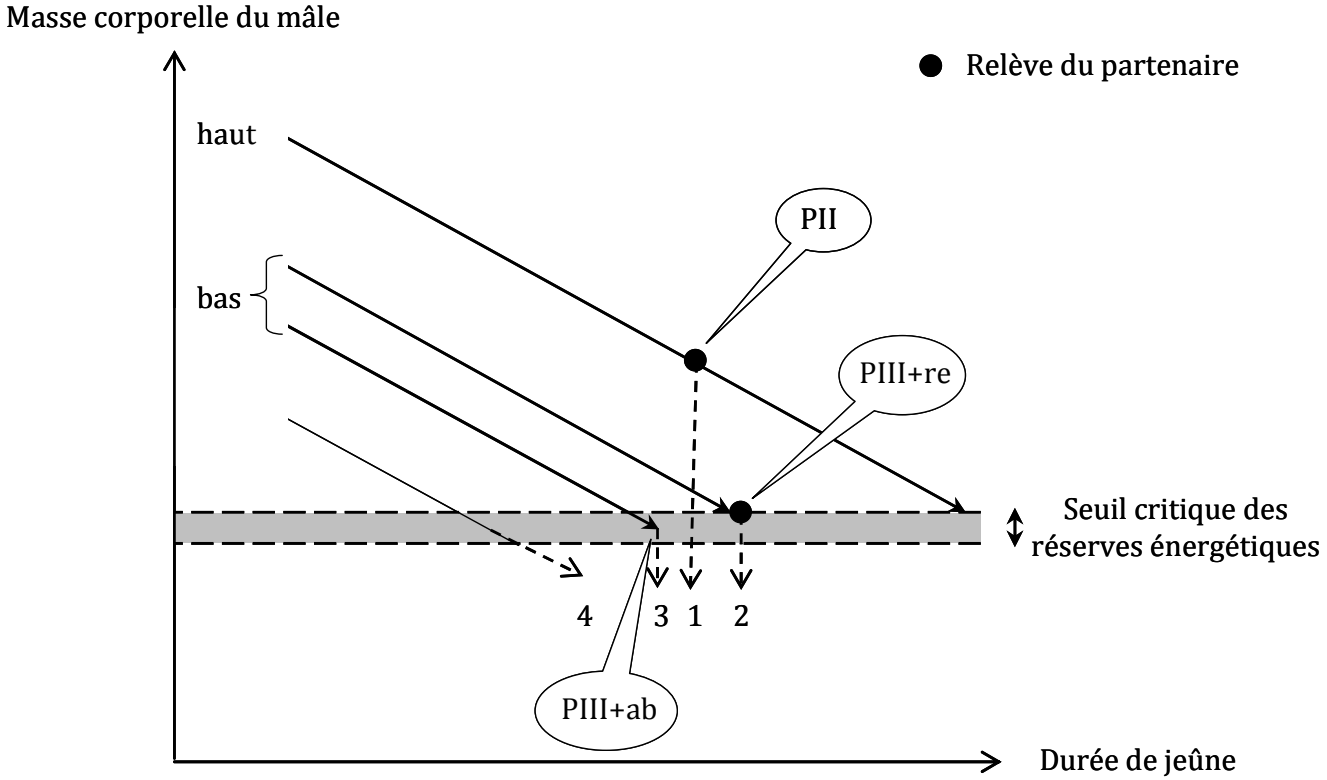


Figure 13. Représentation schématique de la durée de jeûne d'incubation que les mâles peuvent effectuer avant d'atteindre un niveau critique d'épuisement de leurs réserves énergétiques qui les inciterait à abandonner leurs œufs. Les variables les plus importantes sont la masse corporelle du mâle en début de jeûne et la durée du jeûne effectué. Concernant les mâles présentant une masse corporelle initiale élevée, leurs partenaires femelles reviennent sur le site de reproduction alors qu'ils ont une marge de sécurité encore importante avant d'atteindre le niveau seuil (1). Les femelles partenaires des mâles ayant une masse faible en début de jeûne (PIII+re) reviennent « juste à temps » pour relever leurs partenaires qui ont atteint un niveau critique de réserves corporelles (2). Les manchots abandonnent leur nid (PIII+ab) avant le retour de la femelle à cause d'une masse faible en début d'incubation et non pas à cause d'une durée de jeûne trop importante (3). Le quatrième stade est purement théorique puisqu'aucun manchot n'a jamais été retrouvé mort sur œuf (Le Maho et al. 1988). *Modifié à partir de Tveraa et al. 1997.*

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Annexes

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Ecophysiological response of Adélie penguins facing an experimental increase in breeding constraints

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SUMMARY

Foraging strategies play a key role in breeding effort. Little is known, however, about their connection with hormonal and nutritional states, especially when breeding constraints vary. Here, we experimentally increased foraging costs and thus breeding constraints by handicapping Adélie penguins (*Pygoscelis adeliae*) with dummy devices representing 3–4% of the penguins' cross-sectional area. We examined food-related stress (*via* plasma corticosterone concentration) and nutritional state (*via* metabolite levels). Concurrently, we investigated the use of ecological niches *via* the isotopic signature of red blood cells indicating the trophic position ($\delta^{15}N$) and the spatial distribution ($\delta^{13}C$) of penguins. Handicapped birds performed ~70% longer foraging trips and lost ~60% more body mass than controls and their partners. However, corticosterone levels and the nutritional state were unchanged. The isotopic signature revealed that males and females differed in their foraging behaviour: upper trophic levels contributed more in the males' diet, who foraged in more pelagic areas. Handicapped and partner birds adopted the same strategy at sea: a shift towards higher $\delta^{13}C$ values suggested that they foraged in more coastal areas than controls. This change in foraging decisions may optimize feeding time by decreasing travelling time. This may partly compensate for the presumed lower foraging efficiency of handicapped birds and for the energetic debt of their partners who had to fast ~70% longer on the nest. We propose that this flexible use of ecological niches may allow birds facing increased breeding constraints to avoid chronic stress and to minimize the impact on their body condition.

Key words: corticosterone, foraging, handicap, isotopic signature, metabolite, stress.

INTRODUCTION

In an unpredictable environment, breeding constraints may vary between years or within one single reproductive season. To cope with these fluctuating breeding constraints, animals have to be able to adapt and change their behaviour accordingly. One major component of reproductive effort is foraging activity. Several studies have examined whether animals are able to modify their foraging behaviour according to different breeding constraints, in different foraging locations (Wienecke et al., 2000; Tremblay and Cherel, 2003; Lescroël and Bost, 2005), under different environmental conditions (Green et al., 2005; Yoda and Ropert-Coudert, 2007) or at different stages of the breeding cycle (Clarke et al., 1998; Clarke, 2001).

Though changes in foraging behaviour provide worthwhile information on the response of parents when facing variable breeding constraints, understanding of the regulation of animal behaviour can be further enhanced by the examination of a combination of physiological parameters. These may provide useful information on: (1) food-related stress, (2) nutritional condition and (3) the use of ecological niches by experimental animals (Kern et al., 2007; Navarro and González-Solís, 2007; Navarro et al., 2008).

Glucocorticoids play an important role in the regulation of feeding, locomotor activity and energy metabolism (see Landys et al., 2006). For instance, in Adélie penguins (*Pygoscelis adeliae*, Hombron and Jacquinot 1841), baseline corticosterone levels have been correlated with foraging behaviour (Angelier et al., 2008). Moreover, corticosterone is a stress hormone that increases when parents have to work harder (Storey et al., 2006) or when they have to face an unpredictable situation (Pravosudov et al., 2001; Reneerkens et al., 2002). Finally, corticosterone levels have been proposed as a reliable measure of food-related stress and, as a consequence, a direct measure of food availability in free-living birds (Kitaysky et al., 2007).

Changes in foraging decisions may also affect the nutritional state of parents. For this purpose, metabolites can be used as indicators of the nutritional state in free-living animals (Jenni-Eiermann and Jenni, 1998). For example, plasma triglyceride concentration is an indicator of fattening because it increases with the amount of food absorbed and it decreases during heavy endurance exercise. An increase in uric acid levels characterizes the rise in protein breakdown which occurs once a critical threshold has been reached in the depletion of body fuel reserves (see Lindström and Piersma, 1993) or may result from higher muscle activity and from a higher dietary protein fraction. It is also useful to investigate metabolites and hormone levels in parallel as glucocorticoids may increase protein breakdown (Jenni et al., 2000) and decrease plasma triglyceride levels (Remage-Healey and Romero, 2001; Kern et al., 2007).

The measurement of stable isotope ratios is a valuable tool for examining the use of ecological niches by animals (Kelly, 2000; Inger and Bearhop, 2008). The concept of the isotopic method is that animals are constituted by what they consume. For example, as trophic level increases, the quantity of ^{15}N increases, so the ratio $^{15}N/^{14}N$ (expressed as $\delta^{15}N$) indicates the trophic position of the

consumer (Bearhop et al., 2002). The ratio $^{13}C/^{12}C$ (expressed as $\delta^{13}C$) is more stable in marine foodwebs and its variation instead reflects the spatial distribution of consumers (Inger and Bearhop, 2008), with high values being found in coastal foragers and low values in pelagic foragers (Hobson et al., 1994; Cherel and Hobson, 2007). The isotopic signature of the consumer thus reflects the isotopic signature of the consumed prey species.

The main goal in the present study was to enhance the understanding of foraging decisions in Adélie penguins when they face an increase in their breeding constraints. We increased the foraging cost of breeding males and females by equipping them with large dummy devices known to affect the drag of these streamlined animals (Culik and Wilson, 1991; Culik and Wilson, 1992; Watanuki et al., 1992; Miller and Davis, 1993). We thus examined the consequences of this experimental increase in foraging cost on foraging trip duration, body mass loss and the profile of physiological parameters. Handicapped birds were expected to be exposed to a chronic stress due to the presence of the instrument and the difficulty it causes in catching prey efficiently, as well as to an extra foraging cost. In addition, if handicapped birds performed longer foraging trips, their partners were expected to endure longer fasting periods on the nest and consequently to face an additional energetic debt when returning to the sea to feed. For these reasons, we expected corticosterone levels to increase in handicapped and partner birds. Moreover, we expected a decrease in triglyceride levels and an increase in uric acid concentrations in handicapped birds because they would have to make a greater effort (Culik and Wilson, 1991) while being less efficient at catching prey (Ropert-Coudert et al., 2007).

MATERIALS AND METHODS

Study species and area

Fieldwork was carried out during the austral summer 2006–2007 in Dumont d'Urville (66°40′S; 140°00′E), Adélie Land, Antarctica. The Adélie penguin breeding cycle comprises four phases: courtship, incubation [males are in charge of the first incubation shift (~12 days) while females re-feed at sea], guard stage (when the two parents alternate foraging at sea and chick attendance at the nest) and crèche stage (when the two parents forage at the same time leaving the young alone on the colony). This study focused on the incubation and the guard stage, because during the crèche stage it was impossible to precisely monitor the birds.

Thanks to the method of stomach flushing (Ridoux and Offredo, 1989; Kent et al., 1998; Wienecke et al., 2000; Ropert-Coudert et al., 2002; Libertelli et al., 2003), Adélie penguins are known to prey upon two trophic levels: krill (mainly *Euphausia superba* and *Euphausia cristallorophias*) and fish (mainly *Pleuragramma antarcticum*). These prey species have been segregated by their overall isotopic signature in Adélie Land, Antarctica: *Euphausia superba* constitutes a lower trophic level than fish and lives in more oceanic areas (Cherel, 2008). In addition, diet determined by stable-isotope analysis closely mirrors that determined from stomach content (Tierney et al., 2008) and there is a positive relationship between the proportion of fish consumed by Adélie penguins and their $\delta^{15}N$ values (Ainley et al., 2003).

Study protocol

This study was approved by the ethics committee of the French Polar Institute Paul Emile Victor.

Eighty individuals belonging to 40 pairs were followed. A few days before egg laying, the birds were weighed (electronic balance, ±2 g; Ohaus, Pine Brook, NJ, USA) and individually marked for identification with a subcutaneous transponder and a letter painted on their chest with Nyanzol-D, and some of them were handicapped (see below). Sex was determined *a posteriori* by using a combination of parameters including cloacal inspection before egg laying, copulatory position and incubation routine (Taylor, 1962; Kerry et al., 1993).

From the beginning of the incubation period to the crèche stage, we increased the cost of foraging by equipping one bird per pair (N=25 birds) with a large dummy Plexiglas device (25 mm×35 mm×60 mm, 60 g) attached with mastic, cyanoacrylate glue, Tesa tape and cable ties to the middle back feathers (Wilson et al., 1997). When considering the deleterious effects of instrumentation in diving animals, three main parameters have to be taken into account (Bannasch et al., 1994): (1) the shape of the device, (2) the attachment position and (3) the cross-sectional area (CSA) of the device relative to the animal's CSA. The consensus recommends attaching hydrodynamic instruments on the lower back, with a CSA of less than 1% of that of the animals, so as to prevent the generation of extra drag and extra foraging cost (Culik and Wilson, 1991; Bannasch et al., 1994). In the present study, the dummy devices were parallelepipeds (not hydrodynamic), attached to the middle back and their CSA represented 3–4% of the penguins' CSA. An instrument with a CSA 3.5% that of the penguin is likely to produce a drag similar to that of the bird (Bannasch et al., 1994). In addition, Culik and Wilson (Culik and Wilson, 1991) reported that the cost of transport was increased by 25% in penguins equipped with instruments representing ~2% of their CSA. As a result, we can confidently assume that our dummy devices increased the foraging cost of handicapped penguins. However, the size of the dummy device was chosen so as not to be too deleterious for the birds, according to previous studies which used devices of comparable size on Adélie penguins (Culik and Wilson, 1991; Culik and Wilson, 1992; Watanuki et al., 1992; Miller and Davis, 1993).

In total, 15 pairs were assigned to the control group (where neither mate in a pair was handicapped), 12 pairs to the handicapped-female group (where only the females were equipped with the device) and 13 pairs to the handicapped-male group (where only the males were equipped with the device). We distinguished three treatments at the pair level (control, handicapped-female and handicapped-male pairs), therefore resulting in six treatments at the parent level (Table 1).

Foraging trip duration was determined by visual nest observation ranging from every 2 h to continuous. The birds were captured and weighed a second time during the guard stage (40–45 days after egg laying), after a nest relief and just before leaving the colony to forage at sea. Body mass loss was defined as the difference between the first and the second weighing. Blood was collected from the wing vein with a heparinized syringe and centrifuged. Plasma and red blood cells were then quickly stored at –20°C. Because the capture and restraint constitute an acute stress which may influence baseline blood parameters (Jenni-Eiermann and Jenni, 1998; Cockrem et al., 2008), great attention was paid to minimizing the stress for the birds. The pengiun's head was covered by a hood (Cockrem et al., 2008) and handling duration was minimized and measured from the approach of the experimenter towards the nest until the end of blood sampling. A 5 min threshold was chosen as it has been shown that handling durations of less than 5 min had no effect on corticosterone levels in Adélie penguins (Vleck et al., 2000). Blood sampling depended on the bird departure and therefore occurred at any time of the day. Note that in Adélie penguins, no daily rhythm of corticosterone secretion has been reported (Vleck and Van Hook, 2002; Angelier et al., 2008).

Table 1. Foraging trip duration, body mass loss and physiological parameters of Adélie penguins according to their sex and their status (control, handicapped or partner birds)

	Control pairs (*N*=15)		Handicapped-female pairs (*N*=12)		Handicapped-male pairs (*N*=13)	
	Control males	Control females	Partner males	Handicapped females	Handicapped males	Partner females
Foraging trip duration (days)	0.97±0.28	1.02±0.28	1.01±0.31	1.84±0.72	1.62±1.08	1.05±0.29
Body mass loss (g)	504±288	386±260	487±320	696±198	752±274	378±234
[Corticosterone] (ng ml^{-1})	2.49±2.55	1.56±1.34	3.35±2.57	2.14±1.64	2.92±3.24	1.59±1.56
[Triglycerides] (mmol l^{-1})[a]	1.21±0.53	1.14±0.51	1.50±0.71	1.28±0.43	1.60±0.50	1.41±0.49
[Uric acid] (mmol l^{-1})	0.30±0.11	0.34±0.16	0.29±0.15	0.36±0.18	0.31±0.16	0.35±0.16

Data are means ± s.d.
Partner birds formed pairs with handicapped birds.
[a][Triglycerides] corresponds to estimated marginal means obtained by a general linear model with handling time as a covariate.

Laboratory analyses

Analyses of the plasma concentrations of corticosterone, triglycerides and uric acid were carried out at the IPHC-DEPE, France. Corticosterone levels were determined by immunoassay (Assay Pro, AssayMax Corticosterone ELISA Kit, St Charles, MO, USA) and concentrations of triglycerides and uric acid were measured using enzymatic colorimetric tests (Sigma Diagnostic, St Louis, MO, USA). Intra-assay and inter-assay coefficients of variation were between 1% and 3% for metabolite measurements and were 5% and 7%, respectively, for corticosterone measurements.

Tissue isotopic signature mirrors the diet throughout the period of tissue synthesis (Bearhop et al., 2002). For the birds of this study, the period between the first time they fed at sea and blood sampling was 37.6±2.0 days for females and 25.1±3.2 days for males (means ± s.d.). This time corresponds to the turnover of red blood cells (Hobson and Clark, 1993; Haramis et al., 2001; Bearhop et al., 2002). For these reasons, we chose to investigate isotopic signature in red blood cells because it reflects the diet of birds over the whole study period. Before isotopic analyses, red blood cells were lyophilized (48 h) and powdered (Hobson et al., 1997) but were not delipidated (Cherel et al., 2005). Stable carbon and nitrogen isotope assays were carried out at the Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés (CRELA), L'Houmeau, France. Intra-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0.88% and 0.63%, respectively. Inter-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0.42% and 0.24%, respectively. Results are expressed in the standard δ notation (‰) relative to PDB (Peedee belemnite) for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

Data analyses

Body mass and body mass changes were compared between groups with general linear models. Comparisons of foraging trip duration were performed using a generalized linear model with a gamma distribution. For these comparisons, we considered only the guard stage period because corticosterone levels and nutritional state measured 40–45 days after egg-laying should better reflect this period.

For plasma parameters, we first checked whether handling duration was correlated with the plasma concentrations of corticosterone, triglycerides and uric acid, using Spearman correlations. Then, to compare these plasma concentrations between the different groups of birds, we used general linear models. If handling duration was correlated with the considered parameter, it was added as a covariate in the model. Normality of residuals was assessed using a Shapiro–Wilk test. When this condition was not fulfilled (corticosterone), we used a generalized linear model with a gamma distribution.

Analyses were conducted using SPSS 16.02 (SPSS, Chicago, IL, USA). Results are expressed as means ± s.d. and significance level was set at α=0.05.

RESULTS

Foraging trip duration

During the guard stage, bird treatment affected foraging trip duration (Wald χ^2=70.33, d.f.=2, P<0.001) with handicapped birds performing longer foraging trips (1.73±0.91 days) than control birds (1.00±0.28 days, P<0.001) and partner birds (1.03±0.30 days, P<0.001). In contrast, foraging trip duration was not influenced by the sex of the bird (Wald χ^2=2.78, d.f.=1, P=0.10) or by the interaction between the sex and the treatment of the bird (Wald χ^2=0.99, d.f.=2, P=0.61, Table 1).

Body mass

Bird body mass was similar during the courtship period between the three treatment groups ($F_{2,66}$=0.42, P=0.66) and between the treatment groups within males and females (interaction sex×treatment, $F_{2,66}$=1.21, P=0.31). However, during the courtship period, males were heavier than females (5.17±0.45 and 4.60±0.27 kg, respectively; $F_{1,66}$=39.13, P<0.001).

Forty to forty-five days after egg laying, handicapped birds had lost ~60% more mass (724±233 g, $F_{2,64}$=8.25, P=0.001) than control birds (445±276 g, P=0.002) and partner birds (432±285 g, P=0.003). Male and female birds lost body mass at the same rate ($F_{1,64}$=2.10, P=0.15) and the interaction sex×treatment was not significant ($F_{2,64}$=0.09, P=0.92, Table 1).

Effects of handling duration

Corticosterone levels and plasma concentrations of uric acid were not correlated with handling duration (Spearman correlations: R=–0.13, P=0.26; R=–0.05, P=0.63, respectively). However, plasma concentrations of triglycerides were correlated with handling duration (Spearman correlation, R=–0.42, P<0.001).

Corticosterone

Corticosterone levels were higher in males than in females (2.92±2.81 and 1.77±1.50 ng ml^{-1}, respectively; Wald χ^2=4.71, d.f.=1, P=0.03) but were not affected by the treatment or the interaction sex×treatment (Wald χ^2=0.86, d.f.=2, P=0.65 and Wald χ^2=0.57, d.f.=2, P=0.75, respectively; Table 1).

Triglycerides

Males and females exhibited similar plasma concentrations of triglycerides (1.43±0.62 and 1.27±0.49 mmol l^{-1}, $F_{1,69}$=1.83, P=0.18). This concentration did not significantly differ between the different treatment groups ($F_{2,69}$=2.52, P=0.09). In addition, the

interaction sex×treatment was not significant ($F_{2,69}$=0.45, P=0.64; Table 1).

Uric acid

Plasma concentrations of uric acid did not differ between males and females (0.30±0.14 and 0.35±0.16 mmol l^{-1}; respectively, $F_{1,79}$=2.37, P=0.13) and between the different treatment groups ($F_{2,79}$=0.01, P=0.91). The interaction sex×treatment was also not significant ($F_{2,79}$=0.05, P=0.95; Table 1).

Isotopic signature

Males had higher values of δ^{15}N (8.93±0.34‰, $F_{1,71}$=11.03, P=0.001) and lower values of δ^{13}C (–24.60±0.24‰, $F_{1,71}$=17.07, P<0.001) than females (δ^{15}N=8.67±0.36‰, δ^{13}C=–24.31±0.38‰). The value of δ^{15}N was not significantly affected by the treatment ($F_{2,71}$=2.36, P=0.10) whereas the value of δ^{13}C differed according to the treatment ($F_{2,71}$=5.22, P=0.008), with control birds presenting lower values than partner birds (P=0.02) and handicapped birds (P=0.01). In contrast, handicapped and partner birds presented similar δ^{13}C values (P=0.84). The interaction sex×treatment was not significant for δ^{15}N ($F_{2,71}$=1.67, P=0.20) or for δ^{13}C ($F_{2,71}$=0.44, P=0.65; Fig. 1).

DISCUSSION

Sex-specific foraging strategies

Our results highlight variable ecophysiological trends according to the sex or the treatment of birds. First, we confirmed the sex-specific foraging behaviour of Adélie penguins (Clarke et al., 1998). Males had higher values of δ^{15}N and lower values of δ^{13}C than females, suggesting that higher trophic levels contributed more to the diet of males than to that of females and that males tended to forage in more pelagic areas than females. Using the model proposed by Tierney and colleagues (Tierney et al., 2008) and the isotopic signature of prey (*E. superba* and *P. antarcticum*) given by Cherel (Cherel, 2008), krill contribution for females' diet was 42% while it was 37% for males' diet. The difference in δ^{15}N values between males and females was small but Ainley and colleagues (Ainley et al., 2003) found a positive relationship between the proportion of fish consumed by Adélie penguins and δ^{15}N values over a relatively small range of δ^{15}N values. δ^{15}N measurement therefore appears to be a sensitive tool able to detect small differences in the diet of animals. Moreover, our results on trophic levels *via* δ^{15}N values confirmed what is known about the diet of Adélie penguins: males and females both feed on krill (lower trophic level) but males feed more extensively on fish (higher trophic level) than females (Clarke et al., 1998; Tierney et al., 2009). This trophic difference between males and females is not exceptional in animals, particularly amongst penguins (Volkman et al., 1980; Forero et al., 2002; Forero et al., 2005; Norris et al., 2005; Bearhop et al., 2006; Awkerman et al., 2007). This may be due to different feeding requirements and/or foraging capacities between males and females and may serve to reduce the intra-specific competition on feeding grounds. Moreover this difference in the use of the habitat between males and females may be modulated by corticosterone levels, which are known to affect feeding behaviour and locomotor activity. The 60% higher corticosterone levels in males may drive them to forage in more pelagic areas. To confirm this hypothesis, further studies should experimentally modulate corticosterone levels and examine simultaneously the consequences in terms of the use of the habitat by males and females.

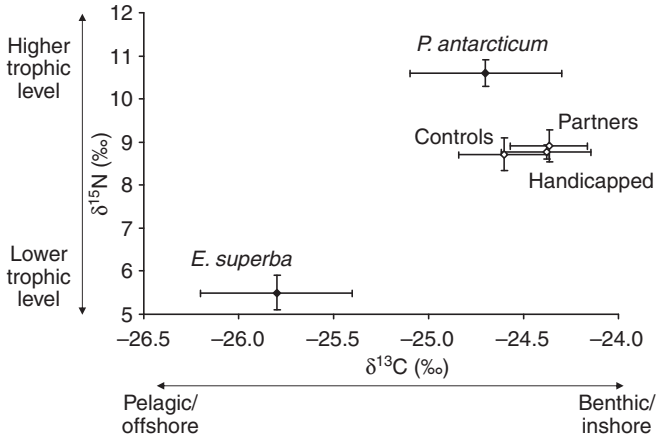

Fig. 1. δ^{15}N and δ^{13}C values of Adélie penguins (open symbols) and their prey (filled symbols). Values for prey come from Cherel (Cherel, 2008). Results are presented as means ± s.d.

Foraging strategies of handicapped penguins and their partners

As shown by prolonged foraging trips in handicapped birds, the handicap affected foraging behaviour. Consequently, we first hypothesized that handicapped and partner birds were exposed to a situation of stress (longer foraging trips for handicapped birds, suggesting a lower foraging efficiency, and prolonged fasting periods for their partners) so corticosterone levels should have increased and nutritional state should have been altered. However, our results show that handicapped and partner birds maintained their corticosterone levels and their nutritional state in a range comparable to that of control birds. Even in handicapped birds, in which body mass loss was increased (Table 1), corticosterone levels and nutritional state remained unchanged. In our study, corticosterone levels were low but comparable to those measured in Adélie penguins by Cockrem and colleagues (Cockrem et al., 2008), just after bird capture, so we can be confident that these values reflect baseline corticosterone levels. This consistency in corticosterone levels was also found in handicapped pied flycatchers *Ficedula hypoleuca* (Kern et al., 2007) and Cory's shearwaters *Calonectris diomedea* (Navarro et al., 2008).

At the beginning of the experiment, the handicap may have elevated corticosterone levels but this increase may have been only temporary (Suedkamp Wells et al., 2003). Several non-exclusive hypotheses may explain why corticosterone levels were not increased several weeks after the beginning of the experiment: (1) the handicap did not represent a significant chronic stress, (2) birds may have habituated to the stressor, (3) birds may have changed their foraging decisions to avoid a chronic stress. Indeed, as the stressor was always the same throughout the experiment, after some time its effects would no longer be unpredictable and thus the handicap probably would not represent a stressor anymore (hypothesis 1). Moreover, animals are expected to avoid situations of chronic stress to remain healthy because chronic stress is associated with physiologically deleterious effects (Sapolsky et al., 2000). To this end, after a repeated or a chronic exposure to a stressor, an animal is expected to habituate (Fig. 2) and to reduce its glucocorticoid response through an acceptation of the stressor and/or physiological feedback (hypothesis 2) (Romero, 2004).

In our study, birds even seem to have coped with the stressor, as they changed their foraging strategies (hypothesis 3, Fig. 2): they still fed on the same trophic levels but foraged in more coastal areas as suggested by the small but significant shift towards higher δ^{13}C

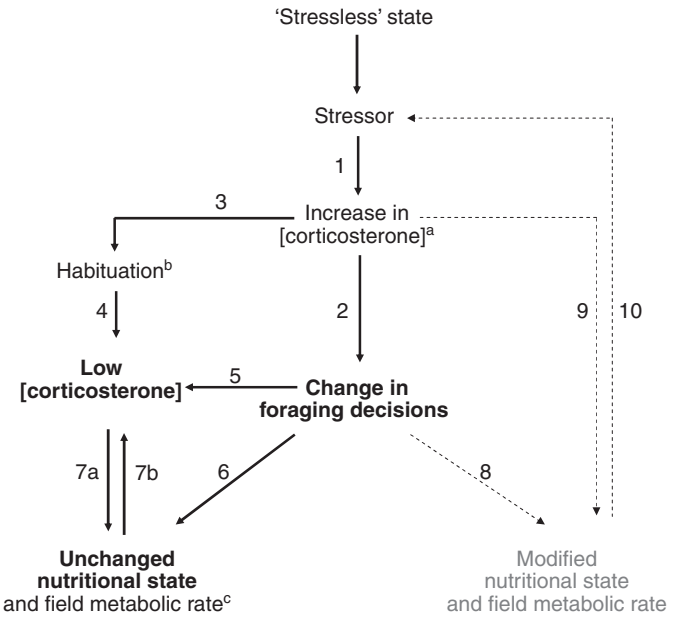

Fig. 2. Schematic view showing the potential ecophysiological responses of Adélie penguins to increased breeding constraints. Superscript letters refer to data from other studies, potentially transposable to our study: [a](Suedkamp Wells et al., 2003); [b](Romero, 2004); [c](Culik and Wilson, 1992). Data in bold were obtained in this study and bold arrows indicate the most probable linkage between these data. In our study, the stressors we considered were the low foraging efficiency due to the large dummy device attached to handicapped penguins and the prolonged periods of fasting for their mates. This may have led to a temporary increase in corticosterone levels (arrow 1) but the birds may have solved this stressful situation by foraging in more costal areas thus optimizing feeding time at the expense of travelling time (arrow 2). This behavioural change, associated with habituation to the stressor, for instance through an acceptation of the stressor and/or physiological feedback (arrow 3), may explain the low corticosterone levels (arrows 4 and 5) and unchanged nutritional state and field metabolic rate (arrows 6 and 7a). Conversely, unchanged nutritional state and metabolic rate may also allow corticosterone levels to remain low (arrow 7b). Though not observed in our study, other responses could have occurred: changes in foraging behaviour could have been insufficient to cope with the stressor (arrow 8) or could have not occurred at all (arrow 9), thus resulting in modified nutritional state and field metabolic rate. Finally, the modified nutritional state and field metabolic rate could be perceived as a potential stressor (arrow 10), thus resulting in a vicious circle, which does not seem viable over a long time scale.

values in handicapped penguins. Interestingly, Navarro and Gonzáles-Solís (Navarro and Gonzáles-Solís, 2007) found that handicapped Cory's shearwaters also modified their spatial distribution in the Atlantic Ocean (although $\delta^{13}C$ values remain constant) but did not change their diet (as suggested by constant $\delta^{15}N$ values). In our study, the difference in $\delta^{13}C$ values might be due to a difference in metabolic rate between handicapped and control penguins, but Carleton and Martínez del Rio (Carleton and Martínez del Rio, 2005) found in birds that an increased metabolism had no effect on the rate of ^{15}N incorporation into red blood cells and had a very small effect on the rate of ^{13}C incorporation. In addition, in our study, partner and control birds that were supposed to present similar metabolic rates, nevertheless exhibited different isotopic signatures. This suggests that the different $\delta^{13}C$ values observed between the experimental groups cannot be (fully) explained by different isotopic incorporation rates. In our study, the difference in $\delta^{13}C$ values between groups was small presumably because our $\delta^{13}C$ values encompassed several consecutive foraging trips, which were not necessarily all coastal. Yet, the resulting average still indicates that the overall $\delta^{13}C$ values were significantly smaller in controls than in other groups. The strategy of foraging in more coastal waters may allow birds to optimize feeding time by reducing travelling phases (back and forth) between the colony and the feeding grounds. The presumably lower efficiency of handicapped birds while travelling, diving and catching prey is likely to explain this change in their foraging behaviour. Culik and Wilson (Culik and Wilson, 1991) reported that instrumented Adélie penguins, swimming in a canal, had a 25% higher swimming metabolic rate than controls but they paradoxically found that the field metabolic rate during one foraging trip at sea was similar to that of controls (Culik and Wilson, 1992). They suggested that this discrepancy was possible if the foraging range was reduced. Our present data may confirm this hypothesis as instrumented penguins foraged in more coastal areas, presumably less distant than offshore areas.

Surprisingly, the partners of handicapped penguins adopted the same strategy as their mates. Because of the prolonged trips of handicapped birds, partners had to fast ~70% longer at the nest than control birds and therefore had an energetic debt when returning at sea to feed. If we consider that penguins lose 50 g per day when fasting (Chappell et al., 1993), the partners of handicapped penguins should have weighed approximately 250 g less than controls when they were weighed during the guard stage. Moreover, considering that the energy cost of fat and protein deposition is 53 kJ g^{-1} and that krill has a metabolizable energy content of 3.5 kJ g^{-1} (Chappell et al., 1993), partner birds would need an additional 3.8 kg of krill to compensate for their prolonged fasting periods. To maintain their body mass constant (Table 1), the alternatives for them could be: (1) to lengthen the duration of their foraging trips to catch more prey items, (2) to reduce the quantity of food given to the chicks or (3) to increase the rate of prey capture per foraging trip. The first strategy was not observed in our study while the other two seem possible. In addition, the data from our study give some support to the third hypothesis: the $\delta^{13}C$ signature showed that partner birds foraged in more coastal waters than control birds thus probably reducing travelling phases and optimizing feeding time per foraging trip. It would be worthwhile examining whether the same foraging strategy is adopted by penguins in natural conditions (i.e. poor food conditions) obliging the penguins to forage for longer and then forcing their partners to fast longer on the nest.

However these results raise a new question: why did control penguins not optimize their foraging trips similarly? One reason may be that they avoided a higher feeding competition in coastal areas by foraging offshore. Another hypothesis explaining the difference between control and handicapped penguins is that coastal waters are more predictable (Weimerskirch, 2007) but less productive than oceanic areas. Handicapped penguins were unable to forage and/or could not 'take the risk' of foraging in unpredictable oceanic waters even though these may be more productive. Partners of handicapped birds adopted a similar cautionary strategy and chose to forage in coastal waters in response to the long trips of their handicapped mates. In contrast, control birds with better foraging ability may be more flexible in exploring their environment and thus may be better able to cope with resource unpredictability and may find oceanic waters to be more productive grounds.

In our study, handicapped penguins opted for changing their foraging behaviour and not abandoning their breeding attempt, while sacrificing their body condition. This suggests that Adélie penguins can tolerate a lower body condition when breeding constraints

increase. However, such a strategy is not expected in long-lived animals that should prioritize their body maintenance. This may be because the body condition of handicapped penguins was not drastically altered. Indeed, Adélie penguins are able to support severe body mass losses (more important than that experienced by handicapped individuals) during their breeding cycle and particularly when they incubate (Cockrem et al., 2006). Even though handicapped individuals lost more body mass than control birds, this mass loss was in the physiological range for this species. In addition, abandoning their breeding attempt would obviously have allowed handicapped penguins to forage only for themselves but it would also have implied negative effects: (1) no breeding success the year they were handicapped and (2) a potentially diminished breeding success the subsequent year. Indeed, information on breeding performance can affect the probability of divorce (Dubois and Cézilly, 2002), potentially altering breeding success in Adélie penguins (Ainley et al., 1983).

Finally, our study could have been extended to the physiological and behavioural responses of handicapped-pair young as they represent the final level of parental investment. Because handicapped parents performed longer foraging trips, handicapped-pair chicks were less frequently provisioned during the guard stage (provisioning rate=1/foraging trip duration). Moreover, handicapped parents may have reallocated energy for their own maintenance and transferred the extra cost induced by the handicap to their offspring. To what extent handicapped-pair young present a lower body mass, an altered nutritional state and higher levels of corticosterone (known to affect begging behaviour) should be considered in future studies.

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When sea-ice clock is ahead of Adélie penguins' clock

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Abstract

1. In Polar Regions, the extent and dynamics of sea-ice are changing. This affects the ocean productivity which consecutively impacts plankton communities and polar top predators like penguins. Yet, the underlying behavioural and physiological mechanisms remain poorly understood.

2. Here we monitored the ecophysiological responses of Adélie penguin (*Pygoscelis adeliae*) pairs during two seasons of contrasting timing of sea-ice retreat. Beside classical breeding parameters like foraging trip duration, body mass and reproductive success, we also investigated food-related stress (*via* plasma corticosterone concentration), nutritional state (*via* metabolite levels) and the use of penguins' habitat (*via* blood isotopic values).

3. Body mass and reproductive success remained unchanged but foraging trips were shorter when sea-ice retreated earlier. Constant plasma corticosterone concentrations indicated that none of the feeding conditions resulted in a food-related stress. However metabolite levels were lower when sea-ice retreated early, suggesting that the foraging performance and the quality/quantity of food differed. Indeed isotopic ratios indicated that coastal prey like fish contributed more to the penguins' diet when sea-ice retreated prematurely.

4. The early sea-ice retreat was related to higher chlorophyll concentrations, known to favour krill recruitment. Paradoxically, this was not associated to a higher krill contribution in the penguins' diet. We propose that a shift in the phytoplankton quality (rather than quantity), affecting krill recruitment, forced penguins to switch to more available prey like coastal fish.

5. In some Antarctic regions, sea-ice is retreating earlier and earlier. In the present study, even though the timing of sea-ice retreat and the consecutive ocean productivity differed drastically between the 2 years, Adélie penguins were not severely affected because they were able to adjust their at-sea behaviour and thus maintained their body condition and reproductive success unchanged.

6. This suggests that the timing of sea-ice retreat does not represent an important threat to populations of Adélie penguins at least as long as alternative resources are still available and other environmental parameters like winter sea-ice extent are not dramatically altered.

Key-words: food availability, krill, phytoplankton, seabird, sea-ice retreat

Introduction

From a predator's perspective, the quality of a particular habitat can be considered as the matching between its requirements and the food available in terms of timing, abundance and accessibility (Durant *et al.* 2007). When the breeding season is conditioned by resource availability, several alternatives are possible for potential breeders when a decrease in food resources happens: animals may (1) skip one breeding season (Drent & Daan 1980), (2) breed but shift their breeding timing according to food availability (Barbraud & Weimerskirch 2006), (3) breed without altering their breeding phenology but mismatch the peak of food availability. In this latter case, animals may change their foraging behaviour and shift to other preys to cope with the lower availability of their usual food resource (Croxall, Reid & Prince 1999; Miller & Trivelpiece 2008; Nicol *et al.* 2008).

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Fig. 1. Adélie penguins on sea-ice edge. Photo by Michaël Beaulieu©.

Because the quality of the habitat is likely to induce effects on the animal physiology and behaviour, the examination of the adequacy between food availability and the animal requirements can be carried out by the investigation of its manifestations on the animal itself. For instance, corticosterone levels have been proposed as a reliable measure of food-related stress and as a consequence a direct measure of food availability in free-living birds (Kitaysky, Piatt & Wingfield 2007). This stress hormone plays an important role in the regulation of feeding, locomotor activity and energy metabolism (see Landys, Ramenofsky & Wingfield 2006) and may affect foraging decisions (Angelier *et al.* 2008). Similarly, plasma metabolite levels highly depend on food intake and can be used as indicators of the nutritional state of free-living animals (Jenni-Eiermann & Jenni 1998). For instance, triglyceride levels reflect the amount of food absorbed and the time since when it was ingested, while uric acid levels characterize protein breakdown which occurs once a critical threshold has been reached in the depletion of body fuel reserves or may result of high muscular activity. Moreover, metabolite levels may also depend on the seasonal requirements of animals; for instance in female birds, before egg laying, triglyceride and uric acid levels reflect the increased liver activity involved in lipid and protein production for oogenesis (Vézina & Williams 2003; Kern *et al.* 2005). Finally a shift in the use of the habitat can be examined by the measurement in animal tissues of stable isotope ratios which indicate simultaneously the trophic position, through $^{15}N : {}^{14}N$ ratio (further expressed as $\delta^{15}N$), and the spatial distribution, through $^{13}C : {}^{12}C$ ratio (further expressed as $\delta^{13}C$), of the consumer. Indeed, in marine food-webs, low values of $\delta^{15}N$ reflect a diet based on preys found at the bottom of the food-web and high values of $\delta^{13}C$ are found in coastal foragers (for the principle of this measurement, see Inger & Bearhop 2008).

In the Southern Ocean, interactions between algae and krill (mainly *Euphausia superba*) represent the basis for energy flux to higher trophic levels like fish, seabirds and mammals. Krill is essentially herbivorous and grazes on phytoplankton. The intensity of its reproduction is therefore highly correlated with annual primary production in the water column, which in turn depends on sea-ice extent and the timing of its retreat (Quetin & Ross 2001). As a result, the summer krill density and quality (reproductive krill are more energetic) correlate positively with chlorophyll concentrations (Cripps *et al.* 1999; Atkinson *et al.* 2004). In the Arctic, it has been shown that the timing of sea-ice retreat affects the timing of the phytoplankton bloom (Hunt & Stabeno 2002). Indeed sea-ice acts as a physical barrier between the atmosphere and the ocean, preventing daylight from penetrating into the water column. The timing of sea-ice retreat may be variable since sea-ice is relatively thin and is therefore vulnerable to perturbations from the ocean and the atmosphere. In certain regions of Antarctica, on top of a decrease in its extent (Moline *et al.* 2008), sea-ice is also forming ~55 days later and retreating ~30 days earlier than 40 years ago (Stammerjohn *et al.* 2008). As a result, these premature retreats are likely to modify the timing and the intensity of the spring phytoplankton bloom (Moline *et al.* 2008) which by a cascade effect may affect the whole food-web structure.

In this study, we focused on two successive austral summers in Adélie Land which presented contrasting conditions in sea-ice retreat and therefore followed the general trend observed in some Antarctic regions. In 2006–2007, fast sea-ice retreated in late September consecutively to a strong windstorm while in 2007–2008, it retreated, more typically, 3 months later in late December (Fig. 2). This difference of timing was likely to induce consequences on the subsequent phytoplankton bloom, krill and krill-eating predators. Here we examined the link between habitat quality in terms of timing and abundance of phytoplankton and the reproductive and ecophysiological responses of Adélie penguins (*Pygoscelis adeliae*, Fig. 1). During the breeding season in Adélie Land, Adélie penguins principally feed upon krill *Euphausia superba*. However they are not krill-specialists since they can also feed upon Antarctic fish (*Pleuragramma antarcticum*, Ridoux & Offredo 1989; Clarke *et al.* 1998; Wienecke *et al.* 2000). Adélie penguins present a high degree of seasonality: in Adélie Land, they arrive on the breeding grounds in mid-October, lay 1–2 egg(s) in mid-November that hatch in mid-December, chicks are then guarded until mid-January and left in crèches until mid-February (Fig. 3). Food requirement peaks during the chick-rearing period (guard and crèche stages, Chappell *et al.* 1993) when parents have to feed their growing chick(s) and insure their own maintenance. In addition, Adélie pen-

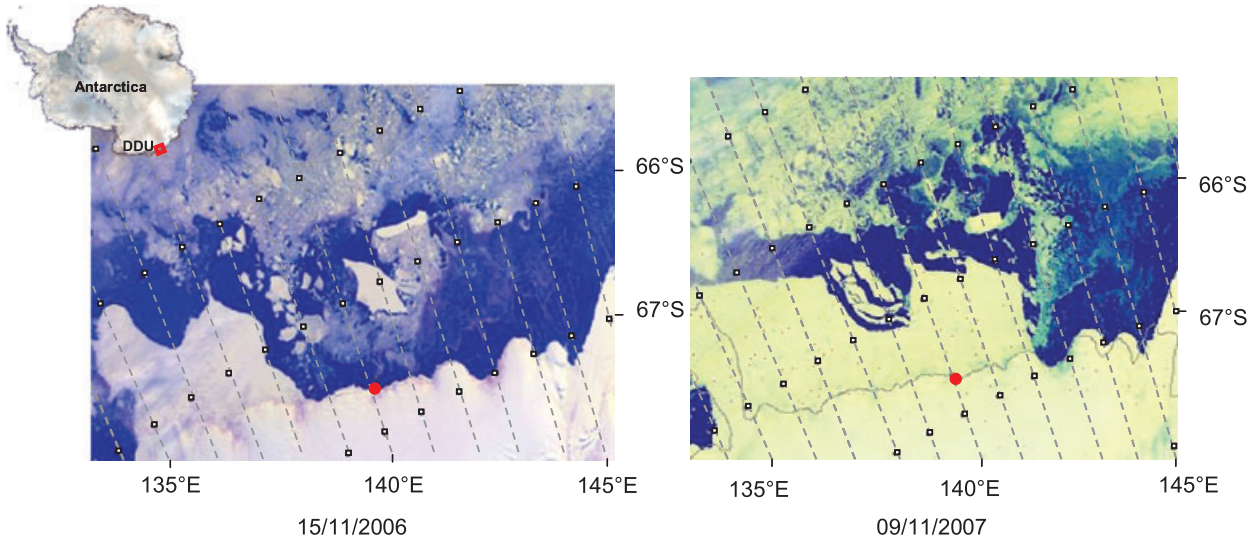

Fig. 2. Satellite images showing sea-ice conditions in Adélie Land in November 2006 and November 2007. Open water areas are represented in blue while ice is represented in white (2006) or yellow (2007). Dumont d'Urville Station (DDU), located on the coast, is symbolized by a red circle. These images were provided by Météo France.

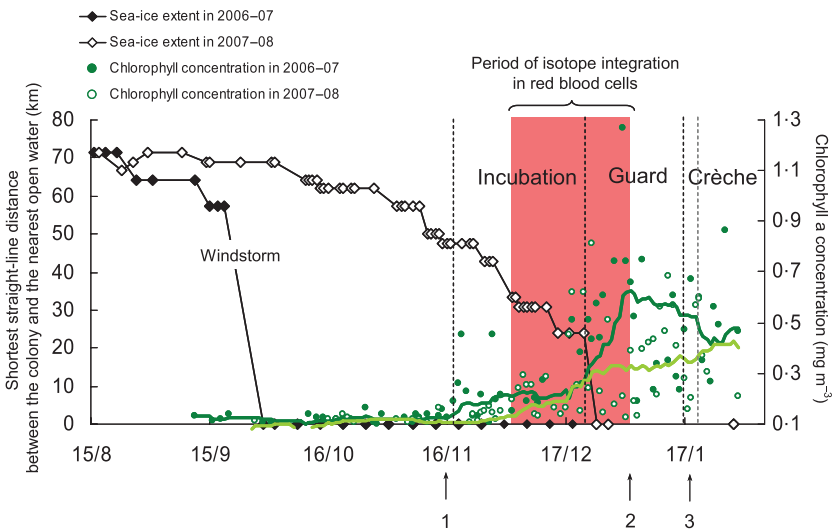

Fig. 3. Evolution of the shortest distance from the colony to the nearest open water due to fast-ice retreat (2006–2007: black symbols, 2007–2008: white symbols) and the consecutive chlorophyll concentration in open water (2006–2007: green-filled symbols, 2007–2008: green-unfilled symbols) from 15 August to 31 January. The moving averages of chlorophyll concentration are represented to facilitate the visualization of its evolution (2006–2007: dark-green line, 2007–2008: light-green line). The breeding phenology of Adélie penguins is superimposed: the limits between two stages were the same in 2006–2007 and 2007–2008 except the limit between the guard and the crèche stages which tended to differ – although non significantly –between the 2 years (2006–2007: black-dashed line, 2007–2008: grey-dashed line). Penguins were weighed and bled twice (arrows 1 and 2) and chicks were weighed at the end of the guard stage (arrow 3). The red area represents the period of isotope integration in red blood cells for the second blood sample.

guins are forced to forage in a limited area (30–110 km from the colony, Angelier *et al*. 2008) because of the constraint to feed chicks regularly. Since sea-ice retreat was premature in 2006–2007, a mismatch between phytoplankton bloom (and presumably krill recruitment) and the requirements of Adélie penguins was likely to happen. In our study, we examined whether differences in the timing of sea-ice retreat affected the timing and/or the abundance of phytoplankton and to what extent it may have affected Adélie penguins' body condition, metabolisms of lipids and proteins, foraging decisions, diet quality and ultimately breeding success.

Materials and methods

FIELD PROCEDURE

In 2006–2007, at the end of the courtship period, 32 birds from 16 different pairs from Dumont d'Urville, Pétrel Island (66°40′S, 140°01′E;

Fig. 2), were captured on their nest and weighed with an electronic balance (Ohaus, ±2 g). The birds were then identified with a Nyanzol-D mark painted on the breast feathers and with a subcutaneous passive transponder (Renner & Davis 2000). To minimize disturbance due to consecutive captures on the same nest, the partner underwent a similar treatment 2 days later.

Until the end of the guard stage, the 16 nests were observed from a blind overhanging the nests, about 20 m apart, every 2 h at worst and continuously at best to monitor copulation behaviours, laying, foraging trip duration and reproductive success. Laying date was defined as the laying date of the first egg. Provisioning rate was defined from the chick perspective and was calculated as the number of parent returns (male + female) from the sea during the guard stage. The number of chicks in crèche was used to estimate the final reproductive success of the focal pairs since at this stage, chick mortality is low in Adélie penguins (Davis & McCaffrey 1986; Clarke *et al.* 2002).

The birds were captured and weighed a second time during the guard stage (40–45 days after laying), after a nest relief and just before leaving the colony to forage at sea (Fig. 3). Body mass loss was defined as the difference between the first and the second weighing.

Blood was collected during the two captures (courtship and guard stage, Fig. 3) from the wing vein with a heparinized syringe. After centrifugation, plasma and red blood cells were quickly stored at −20 °C. Because the capture and the restraint constitute an acute stress that may influence baseline parameters in blood (Jenni-Eiermann & Jenni 1998; Cockrem *et al.* 2008), most attention was paid to minimize the stress for birds. The head of the penguin was covered by a hood (Cockrem *et al.* 2008) and handling duration was minimized and timed from the approach of the experimenter towards the nest until the end of blood sampling. A 5-min threshold was chosen since it has been shown that handling durations < 5 min had no effect on corticosterone levels in Adélie penguins (Vleck *et al.* 2000). Timing of blood sampling depended on the bird departure and therefore occurred at any time of the day. However corticosterone concentration is not affected by daytime in Adélie penguins (Vleck & Van Hook 2002, Angelier *et al.* 2008).

At the end of the guard stage, the chicks were weighed on their nest with a spring balance (Salter, ±20 g) when they were left unguarded for the first time.

In 2007–2008, at the end of the courtship period and twice during the incubation period, all the nests occupied by pairs in 2006–2007 were checked with a hand held antenna to search for penguins identified with transponders. For the rest of the breeding cycle, the procedure was strictly the same as that described in 2006–2007.

In both years, adults were sexed by a combination of parameters including cloacal inspection before egg laying, copulatory position and incubation routine (Taylor 1962; Kerry, Clarke & Else 1993). Sex determination carried out in 2007–2008 totally confirmed the sexing of all birds achieved 1 year before.

LABORATORY ANALYSES

Analyses of the plasma concentrations of corticosterone, triglycerides and uric acid were conducted at the IPHC-DEPE, France. Corticosterone levels were determined by immunoassay (AssayMax Corticosterone ELISA Kit; AssayPro, St. Charles, Missouri, US) and concentrations of triglycerides and uric acid were measured using enzymatic colorimetric tests (Sigma Diagnostic, St. Louis, Missouri, US). Intra- and inter- assay variations were 5% and 7%, respectively for corticosterone measurements and were comprised between 1% and 3% for metabolite measurements. In addition, the cross-reactivity of the corticosterone antibody with other steroids is low (comprised between 0 and 2%, Assay Pro). No relationship between handling time and corticosterone levels was found (courtship 2006: $r_s = 0{\cdot}04$, $P = 0{\cdot}86$; courtship 2007: $r_s = -0{\cdot}26$, $P = 0{\cdot}29$; guard stage 2006–2007: $r_s = -0{\cdot}03$ $P = 0{\cdot}91$; guard stage 2007–2008: $r_s = -0{\cdot}20$ $P = 0{\cdot}41$), so that we considered these corticosterone levels reflected baseline values.

Tissue isotopic values mirror the diet throughout the period of tissue synthesis (Bearhop *et al.* 2002). We chose to investigate isotopic values of red blood cells, which require 3–4 weeks to turn-over (Hobson & Clark 1993; Haramis *et al.* 2001; Bearhop *et al.* 2002) and thus integrated the diet of the bird from the end of the incubation period to the early guard stage (second blood sample, Fig. 3). Before isotopic analyses, red blood cells were lyophilized (48 h) and powdered (Hobson, Gibbs & Gloutney 1997). Lipids were not extracted as this is not necessary when using red blood cells (Cherel *et al.* 2005). Stable-carbon and nitrogen isotope assays were carried out at the Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés (CRELA), L'Houmeau, France. Results are expressed in the standard δ notation (‰) relative to PDB belemnite for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$. Intra-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0·88% and 0·63%, respectively. Inter-assay coefficients of variation for $\delta^{13}C$ and $\delta^{15}N$ values of standard acetanilide were 0·42% and 0·24%, respectively.

ENVIRONMENTAL ANALYSIS

In both years, we measured the shortest straight-line distance between the colony and the nearest open water on cloud-free satellite images (resolution: 1 km) provided by Météo France. To measure chlorophyll concentration, we used SEAWIFS satellite data (NASA-OrbImage, resolution: 1 km) that provided mean chlorophyll *a* concentration in open-water areas in a region that extended latitudinally from 65°00′S to 66°40′S and longitudinally from 135°00′E to 145°00′E (Fig. 2). Since chlorophyll concentration varies as a function of daytime (McMinn *et al.* 2007), it was measured only between 13:00 and 15:00 local time. Environmental data were analysed from 15 August to 31 January.

DATA ANALYSIS

A population is characterized by diversity and variance as it is composed of animals of different age, experience, quality etc. In order to perform inter-annual comparisons and avoid confounding factors due to inter-individual variability, two alternate sampling protocols are conceivable: (1) sample each year a large number of individuals equally sampling all the categories found in the population (or even better in the species) or (2) repeatedly sample each year the same number of animals so that the experimenter is sure that the confounding factors due to sampling are the same year after year. As the first alternative is logistically too difficult, we chose the second option thus assuming that the potential inter-individual confounding factors were the same each year. Since newly-established pairs have a lower breeding success in Adélie penguins (Ainley, Leresche & Sladen 1983) and that a change of mate may increase corticosterone levels (Angelier *et al.* 2007), we removed the potential bias due to pair stability by considering only stable pairs from 2006–2007 to 2007–2008. This resulted in the exclusion of five pairs that divorced or in which one mate was absent in 2007–2008. Moreover, because metabolic levels do not lead to the same interpretation during incubation and chick-rearing periods (see introduction) and corticosterone levels may also

change within a breeding season (Lanctot *et al.* 2003), we conducted comparisons between the 2 years during the incubation period and during the guard stage independently.

The concentrations of chlorophyll were compared between the 2 years after the chlorophyll bloom (15 November) with a Mann–Whitney test. Using the date of laying as the reference date, we assessed with general linear models whether the dates of manipulation and sampling differed between the 2 years. Almost all other comparisons were carried out using general linear mixed models to avoid the problem of pseudoreplication since our statistical analyses involved repeated observations of the same subjects. Individuals were considered as a random factor while the year, the sex and their interaction were used as fixed factors. Normality of residuals was assessed by Shapiro-Wilk tests. When this condition was not fulfilled, we used generalized linear models with a normal distribution (duration of the first foraging trip during incubation) or a gamma distribution of dependent data (plasmatic parameters, foraging trip duration during the guard stage). Generalized linear models with a Poisson distribution were also used in the case of count data (reproductive success) considering the stage (egg laying, hatching, guard stage and crèche stage), the year and the interaction of these two factors as fixed factors. Multiple comparisons were undertaken using the post hoc Bonferroni test.

All analyses were conducted using SPSS 16.02 (SPSS Inc., Chicago, IL, USA). Results are expressed as means ± SE and significance level was set at $\alpha = 0{\cdot}05$.

Results

ENVIRONMENTAL PARAMETERS

The distance from the colony to the nearest open water was similar (70 km) in mid-August 2006 and 2007. In 2006–2007, open water reached the coast of the colony 3 months before it did in 2007–2008 (Fig. 3). Chlorophyll concentrations began to increase at the same time in both years (15 November). After this date, chlorophyll mean concentration was 30% lower in 2007–2008 ($0{\cdot}287 \pm 0{\cdot}024$ mg m^{-3}) than in 2006–2007 ($0{\cdot}404 \pm 0{\cdot}035$ mg m^{-3}, $U = 880$, $P = 0{\cdot}006$, Fig. 3).

PRE-LAYING PERIOD

There was no difference in the handling date between the 2 years ($F_{1,\ 39} = 1{\cdot}05$, $P = 0{\cdot}31$) and females and males were manipulated at the same time ($F_{1,\ 39} = 3{\cdot}29$, $P = 0{\cdot}08$). The interaction year × sex was not significant ($F_{1,\ 39} = 2{\cdot}28$, $P = 0{\cdot}14$, Table 1).

Before egg laying, males were heavier than females ($5{\cdot}12 \pm 0{\cdot}10$ and $4{\cdot}53 \pm 0{\cdot}10$ kg, respectively; $F_{1,\ 38} = 18{\cdot}12$, $P < 0{\cdot}001$) but neither the year ($4{\cdot}83 \pm 0{\cdot}10$ kg in 2006 and $4{\cdot}82 \pm 0{\cdot}10$ kg in 2007, $F_{1,\ 38} = 0{\cdot}01$, $P = 0{\cdot}94$) nor the interaction sex × year ($F_{1,\ 38} = 0{\cdot}74$, $P = 0{\cdot}40$) had an effect on body mass (Fig. 4).

Concentrations of corticosterone were similar between males and females ($2{\cdot}32 \pm 0{\cdot}51$ and $1{\cdot}86 \pm 0{\cdot}53$ ng mL^{-1}, respectively; Wald $\chi^2 = 0{\cdot}39$, d.f. = 1, $P = 0{\cdot}53$) and between years ($1{\cdot}92 \pm 0{\cdot}46$ in 2006 and $2{\cdot}26 \pm 0{\cdot}45$ ng mL^{-1} in 2007, Wald $\chi^2 = 0{\cdot}40$, d.f. = 1, $P = 0{\cdot}53$). The interaction sex × year was also not significant (Wald $\chi^2 = 1{\cdot}83$, d.f. = 1, $P = 0{\cdot}18$, Fig. 4).

Plasma concentrations of uric acid and triglycerides followed the same trends: metabolite concentrations were lower in males than in females (uric acid: $0{\cdot}21 \pm 0{\cdot}01$ and $1{\cdot}16 \pm 0{\cdot}08$ mmol L^{-1}, respectively; Wald $\chi^2 = 9{\cdot}88$, d.f. = 1, $P = 0{\cdot}002$, triglycerides: $1{\cdot}33 \pm 0{\cdot}12$ and $10{\cdot}96 \pm 1{\cdot}26$ mmol L^{-1}, respectively; Wald $\chi^2 = 58{\cdot}21$, d.f. = 1, $P < 0{\cdot}001$) and there were differences between years (uric acid: $0{\cdot}60 \pm 0{\cdot}04$ in 2006–2007 and $0{\cdot}77 \pm 0{\cdot}05$ mmol L^{-1} in 2007–2008, Wald $\chi^2 = 15{\cdot}46$, d.f. = 1, $P < 0{\cdot}001$, triglycerides: $3{\cdot}09 \pm 0{\cdot}73$ in 2006–2007 and $9{\cdot}20 \pm 0{\cdot}78$ mmol L^{-1} in 2007–2008, Wald $\chi^2 = 54{\cdot}51$, d.f. = 1, $P < 0{\cdot}001$). The interaction sex × year (uric acid: Wald $\chi^2 = 9{\cdot}88$, d.f. = 1, $P = 0{\cdot}002$, triglycerides: Wald $\chi^2 = 48{\cdot}74$, d.f. = 1, $P < 0{\cdot}001$) indicated that metabolite concentrations remained stable in males (uric acid: $P = 0{\cdot}79$, triglycerides: $P = 0{\cdot}13$) while they increased between 2006–2007 and 2007–2008 in females (uric acid: $P = 0{\cdot}001$, triglycerides: $P < 0{\cdot}001$, Fig. 4).

INCUBATION PERIOD

There was no difference in the laying date between both years ($F_{1,\ 9} = 0{\cdot}02$, $P = 0{\cdot}90$, Table 1). The first foraging trip was longer in 2007–2008 than in 2006–2007 ($13{\cdot}47 \pm 0{\cdot}32$ and $10{\cdot}83 \pm 0{\cdot}26$ days, respectively; Wald $\chi^2 = 45{\cdot}68$, d.f. = 1, $P < 0{\cdot}001$) but neither the sex (Wald $\chi^2 = 1{\cdot}64$, d.f. = 1, $P = 0{\cdot}20$) nor the interaction year × sex affected its duration (Wald $\chi^2 = 0{\cdot}15$, d.f. = 1, $P = 0{\cdot}70$, Fig. 4). Egg hatching

Table 1. Principal dates of the breeding cycle of the studied Adélie penguin pairs and dates of handling

	2006–2007		2007–2008	
	Females ($n = 11$)	Males ($n = 11$)	Females ($n = 11$)	Males ($n = 11$)
Duration between the first handling and egg laying (days)	−5·8 ± 0·9	−2·8 ± 0·9	−5·4 ± 0·9	−5·09 ± 0·9
Date of laying	17/11 ± 0·6 days	–	17/11 ± 1·1 days	–
Date of hatching	23/12 ± 0·8 days		23/12 ± 0·8 days	
Duration between egg laying and the second handling (days)	41·2 ± 0·6	41·3 ± 0·6	40·6 ± 0·6	40·2 ± 0·6
Duration of the guard stage (days)	25·2 ± 1·5		28·7 ± 1·6	

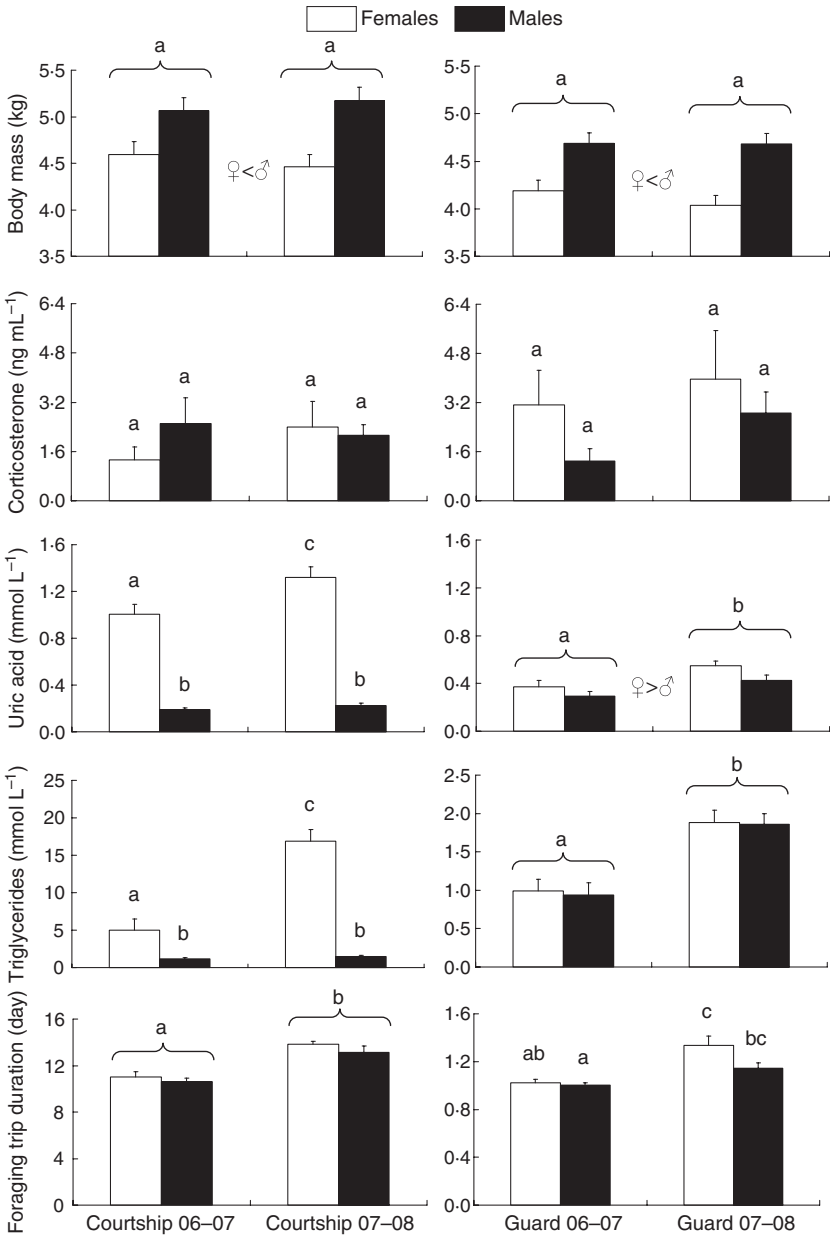

Fig. 4. Body mass, plasmatic parameters (corticosterone, uric acid and triglyceride) and foraging trip duration during the courtship and the incubation periods (left column) and during the guard stage (right column) in 2006–2007 and 2007–2008. White histograms refer to females and black histograms refer to males. Results are presented as means ± SE. Comparisons were carried out during the courtship and the incubation periods and during the guard stage independently. Different letters correspond to significant differences between two groups for a considered parameter and the brackets indicate the results of the comparison between the 2 years whatever the sex of the individuals.

happened at the same date ($F_{1,\ 9} = 0{\cdot}03$, $P = 0{\cdot}88$) in 2006–2007 and in 2007–2008 (Table 1).

GUARD STAGE

There was no difference in the handling date during the guard stage between the 2 years ($F_{1,\ 36} = 2{\cdot}13$, $P = 0{\cdot}15$), between sexes ($F_{1,\ 36} = 0{\cdot}04$, $P = 0{\cdot}84$) and the interaction year × sex was not significant ($F_{1,\ 36} = 0{\cdot}16$, $P = 0{\cdot}69$, Table 1).

Body mass was higher in males than in females (4·69 ± 0·10 and 4·11 ± 0·10 kg, respectively; $F_{1,\ 18} = 16{\cdot}08$, $P = 0{\cdot}001$) but neither the year (4·44 ± 0·08 kg in 2006–2007 and 4·36 ± 0·08 kg in 2007–2008, $F_{1,\ 17} = 1{\cdot}73$, $P = 0{\cdot}21$) nor the interaction sex × year ($F_{1,\ 17} = 1{\cdot}57$, $P = 0{\cdot}23$) influenced body mass (Fig. 4). Body mass changed similarly between the pre-laying period and the guard stage (mean body mass loss: 393 ± 59 g) in both years ($F_{1,\ 16} = 0{\cdot}41$, $P = 0{\cdot}53$) and in males and females ($F_{1,\ 17} = 1{\cdot}74$, $P = 0{\cdot}21$). The interaction sex × year was not significant ($F_{1,\ 17} = 0{\cdot}78$, $P = 0{\cdot}79$).

Corticosterone remained constant between males and females (2·07 ± 0·47 and 3·54 ± 0·82 ng mL^{-1}, respectively; Wald $\chi^2 = 2{\cdot}41$, d.f. = 1, $P = 0{\cdot}12$) and between years (2·21 ± 0·59 in 2006–2007 and 3·40 ± 0·87 ng mL^{-1} in 2007–2008, Wald $\chi^2 = 1{\cdot}07$, d.f. = 1, $P = 0{\cdot}30$). The interaction sex × year was also not significant (Wald $\chi^2 = 0{\cdot}10$, d.f. = 1, $P = 0{\cdot}75$). Metabolite concentrations were higher in 2007–2008 than in 2006–2007 (uric acid: 0·49 ± 0·03 and 0·33 ± 0·03 mmol L^{-1}, respectively, Wald $\chi^2 = 16{\cdot}74$, d.f. = 1, $P < 0{\cdot}001$, triglycerides: 1·87 ± 0·10 and 0·96 ± 0·11 mmol L^{-1}, respectively; Wald $\chi^2 = 54{\cdot}42$, d.f. = 1, $P < 0{\cdot}001$) and sex had only an effect on uric acid concentrations (uric acid: 0·36 ± 0·03 mmol L^{-1} in males and 0·46 ± 0·04 mmol L^{-1} in females, Wald $\chi^2 = 1{\cdot}11$, d.f. = 1, $P = 0{\cdot}04$, triglycerides: 1·40 ± 0·13 mmol L^{-1} in

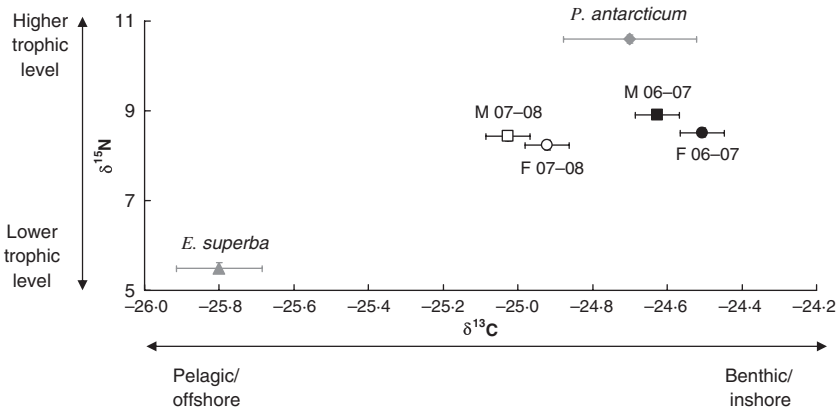

Fig. 5. Mean $\delta^{15}N$ and $\delta^{13}C$ (± SE) values of adult Adélie penguins, males (squares) and females (circles) in 2006–2007 (filled symbols) and 2007–2008 (empty symbols). Values for *Euphausia superba* and *Pleuragramma antarcticum* are also represented (from Cherel 2008).

males and 1·44 ± 0·12 mmol L^{-1} in females, Wald χ^2 = 0·04, d.f. = 1, P = 0·84). The interaction sex × year was not significant neither for uric acid (Wald χ^2 = 0·29, d.f. = 1, P = 0·59) nor for triglycerides (Wald χ^2 = 0·01, d.f. = 1, P = 0·91, Fig. 4).

During the guard stage, foraging trip duration was affected by the sex (1·07 ± 0·03 days in males and 1·17 ± 0·04 days in females, Wald χ^2 = 4·12, d.f. = 1, P = 0·04), the year (1·01 ± 0·02 days in 2006–2007 and 1·24 ± 0·04 days in 2007–2008, Wald χ^2 = 28·03, d.f. = 1, P < 0·001) and the interaction sex × year (Wald χ^2 = 3·98, d.f. = 1, P = 0·05) with males performing 15% longer foraging trips in 2007–2008 than in 2006–2007 (P = 0·001) and females performing 30% longer foraging trips in 2007–2008 than in 2006–2007 (P < 0·001, Fig. 4). Over the guard stage, the provisioning rate was similar in 2006–2007 and in 2007–2008 (2006–2007: 22·70 ± 1·54 parent returns, 2007–2008: 20·44 ± 1·63 parent returns, $F_{1, 17}$ = 1·01, P = 0·33). This was due to the nearly significantly longer guard stage (~3·5 days) in 2007–2008 than in 2006–2007 ($F_{1, 9}$ = 4·14, P = 0·07, Table 1), since provisioning rate was significantly different between the 2 years after controlling for the duration of the guard stage (2006–2007: 24·06 ± 0·98 visits, 2007–2008: 18·93 ± 1·04 visits; $F_{1, 16}$ = 12·14, P = 0·003).

Isotopic values differed according to the sex and the year: males had higher levels of $\delta^{15}N$ than females (8·7 ± 0·1 ‰ and 8·4 ± 0·1 ‰, respectively, $F_{1, 18}$ = 6·29, P = 0·02) but $\delta^{13}C$ values were similar in both sexes (males: −24·8 ± 0·1 ‰, females: −24·7 ± 0·1 ‰, $F_{1, 18}$ = 2·67, P = 0·12). Both $\delta^{15}N$ and $\delta^{13}C$ values were different between the 2 years with higher $\delta^{15}N$ values in 2006–2007 than in 2007–2008 (8·7 ± 0·1 ‰ and 8·3 ± 0·1 ‰, respectively, $F_{1, 18}$ = 23·20, P < 0·001), associated with higher $\delta^{13}C$ values (2006–2007: −24·6 ± 0·1 ‰, 2007–2008: −25·0 ± 0·1 ‰, $F_{1, 18}$ = 74·35, P < 0·001). The interaction sex × year was not significant neither for $\delta^{15}N$ values ($F_{1, 18}$ = 1·67, P = 0·21) nor for $\delta^{13}C$ values ($F_{1, 18}$ = 0·03, P = 0·87, Fig. 5).

REPRODUCTIVE SUCCESS AND BROOD MASS

Even though the breeding stage had an effect on reproductive success (Wald χ^2 = 35·56, d.f. = 3, P < 0·001, Fig. 6), there was no difference between the 2 years (Wald χ^2 = 0·30, d.f. = 1, P = 0·59) and the interaction stage × year was not significant (Wald χ^2 = 1·64, d.f. = 3, P = 0·65). At the end of the guard stage, brood mass was similar in 2006–2007 and in 2007–2008 (3·01 ± 0·34 kg and 3·02 ± 0·35 kg, respectively, $F_{1, 9}$ = 0·00, P = 0·98).

Discussion

Few studies have tried to establish a link between ecological, behavioural, dietary and physiological parameters in wild animals. Here, we showed that a 3-months earlier fast-ice retreat was associated to a 30% higher chlorophyll production in open water and it affected Adélie penguins who performed shorter foraging trips, and fed on a diet with higher $\delta^{15}N$ and $\delta^{13}C$ values. They presented lower plasma metabo-

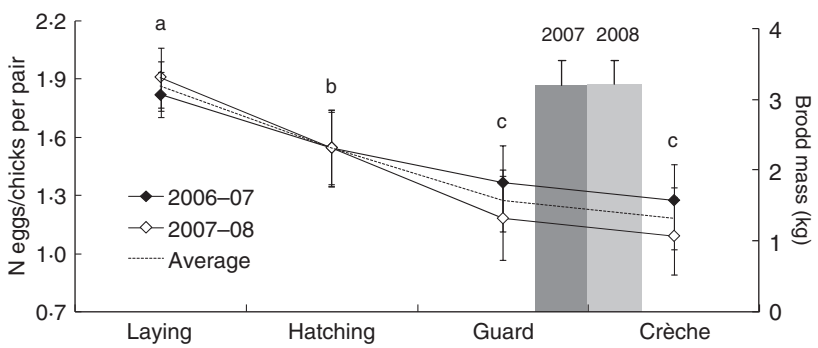

Fig. 6. Reproductive success of Adélie penguin pairs as a function of the number of eggs laid, hatched, the number of chicks during the guard and the crèche stages in 2006–2007 and 2007–2008. Histograms represent brood mass at the end of the guard stage. Results are presented as mean ± SE. Different letters correspond to significant differences between two consecutive stages for the mean reproductive success.

lite levels but corticosterone levels, body mass and reproductive success were not altered.

Interestingly, the early fast sea-ice retreat did not result in a premature phytoplankton bloom as previously described in the Arctic (Hunt & Stabeno 2002). As a result, Adélie penguins, who did not modify their breeding phenology, did not mismatch the peak of primary productivity. This match in timing between phytoplankton bloom and Adélie penguin breeding season occurred probably because both rely on the same environmental cues (i.e. daylight duration and intensity). However the early fast-ice retreat was followed by a 30% higher peak of primary production. In 2006–2007, krill stocks were thus expected to be more abundant and presumably more energetic since the recruitment must have been higher (Cripps *et al*. 1999; Quetin & Ross 2001; Atkinson *et al*. 2004). However, looking at isotopic values, higher values of $\delta^{15}N$ indicated that penguins did not feed more at lower trophic levels (krill) than at higher trophic levels (fish) in 2006–2007. This discrepancy between expected krill availability (estimated through chlorophyll concentration) and krill exploitation by Adélie penguins may come from a mismatch between phytoplankton bloom and krill recruitment in 2006–2007. Adult krill is known to feed on the phytoplankton bloom at the ice edge (Nicol 2006). In 2006–2007, as the ice edge had totally disappeared, krill recruitment may have been altered. Indeed it has been shown that an early sea-ice retreat was associated with the dominance of 'small' pico-nanophytoplankton (< 20 μm, cryptophytes) on 'large' microphytoplankton (> 420 μm, diatoms; Montes-Hugo *et al*. 2008). As a result, even if the overall chlorophyll concentration was higher in 2006–2007 than in 2007–2008, a shift in the size distribution of the phytoplankton community is likely to have occurred, with small phytoplankton prevailing in 2006–2007. As the grazing efficiency of *Euphausia superba* decreases significantly with particles < 20 μm (Moline *et al*. 2004), these specific conditions could have led to a lower krill recruitment in 2006–2007 than in 2007–2008. As a result, a high chlorophyll production is not necessarily associated with high krill recruitment. This may explain why, in 2006–2007, Adélie penguins were forced to partially shift to a diet with a higher contribution of fish resulting in higher $\delta^{15}N$ plasma values. Ainley *et al*. (2003) also found that Adélie penguins eat more fish in years with less sea-ice in the Ross Sea, suggesting that a low sea-ice cover may increase the relative abundance and/or the accessibility of fish.

As suggested by isotopic values, *Pleuragramma antarcticum* inhabits more coastal areas (higher $\delta^{13}C$ values) than *Euphausia superba* (lower $\delta^{13}C$ values, Cherel 2008). In agreement with this, isotopic measures showed that penguins fed more on higher trophic levels in coastal areas (higher $\delta^{15}N$ and $\delta^{13}C$ values) while they fed more on lower trophic levels in pelagic areas (lower $\delta^{15}N$ and $\delta^{13}C$ values, Fig. 5). Two non-exclusive reasons can explain why penguins fed more predominantly on krill in more pelagic areas in 2007–2008: (1) the presence of the fast-ice forced the penguins to reach the limit ice-edge/open water, 20–30 km away from the colony (Fig. 3), where krill is present (Nicol 2006), (2) Adélie penguins are known to feed preferentially on gravid energetic female krill (Clarke *et al*. 2002; Nicol *et al*. 2008) that live and spawn offshore (Nicol 2006). This may also explain why penguins performed longer foraging trips in 2007–2008, even when fast-ice disappeared during the guard stage.

The absence of changes in body mass and corticosterone levels between the 2 years suggested that none of the environmental conditions in the 2 years induced a food-related stress for birds. The fact that the diet differed between both years does not necessarily mean that 1 year was inferior to the other in terms of food availability. Overall food availability (krill and/or fish) may not have been drastically different and because Adélie penguins are generalist feeders, they were able to adjust their diet to the contrasting environmental conditions. Nevertheless, our results may have been biased by our selective sampling method since only birds remaining in a stable pair and present each year in the colony were included in the analysis; we may have thus selected only the most competitive birds able to respond to different levels of food availability without experiencing any food-related stress.

Metabolite levels were higher in 2007–2008 than in 2006–2007. The diet richer in krill (2007–2008) could be therefore considered as more profitable for birds than the diet richer in fish (2006–2007): as triglyceride and uric acid levels were higher in 2007–2008, one can hypothesize that birds consumed larger amount of food, stored more fat and had a higher-protein diet in 2007–2008 than in 2006–2007. However, body mass was not higher in 2007–2008 than in 2006–2007. Moreover protein content is similar in krill and fish (*Euphausia superba*: 10% of wet weight, *Pleuragramma antarcticum*: 11% of wet weight, Reinhardt & Van Vleet 1986) and lipid content is lower in krill than in fish (*Euphausia superba*: 5% of wet weight, *Pleuragramma antarcticum*: 10% of wet weight; Clarke 1980; Friedrich & Hagen 1994). One alternative explanation could be that krill lipids and proteins may be better assimilated than those of fish. Another way to interpret higher observed uric acid levels in 2007–2008, is that penguins increased protein breakdown by providing a higher muscular effort (Jenni-Eiermann & Jenni 1998) when they foraged on krill in more pelagic areas and during longer foraging trips. In the same line of thought, since foraging trip duration was longer in females than in males, females may have to provide a greater effort than males to obtain the same diet, which may explain why their uric acid levels were higher than those of males.

Comparing our results to other studies dealing with Adélie penguins facing different environmental conditions, it is interesting to notice that all parameters are not similarly modified in all studies (Table 2). The laying date, the laying success or the hatching date did not vary in any study. In contrast, some parameters changed in all studies (foraging trip duration, decreased meal size, duration of the guard stage) and can therefore be considered as sensitive indicators of a modification in the environment of Adélie penguins. Arrival body mass, diet quality, breeding success and fledgling mass varied in some studies but not in others. This is likely to be due to variable differences in food availability between the two

Table 2. Comparison of four studies dealing with consequences of inter-annual environmental conditions and breeding in Adélie penguins

	This study	Clarke *et al.* 2002*	Nicol *et al.* 2008;	Olmastroni *et al.* 2004;	Clarke *et al.* 2002†
Location	Dumont d'Urville	Béchervaise Island	Béchervaise Island	Edmonson Point	Béchervaise Island
Difference in primary production	30%	NI	NI	NI	NI
Difference in krill availability	NI	NI	70%	NI	NI
Decreased arrival body mass	No	No	No	Yes	No
Decreased laying success	No	NI	No	No	NI
Delayed laying	No	No‡	No	No‡	No‡
Delayed hatching	No	NI	No	No	NI
Increased foraging trip duration	F (30%) M (15%)	F (30%) M (20%)	F (50%) M (40%)	NI	F (150%) M (30%)
Change in diet quality	Yes	No	Yes	NI	No
Increased corticosterone levels	No	NI	NI	NI	NI
Different metabolic state	Yes	NI	NI	NI	NI
Decreased meal size	NI	Yes	Yes	NI	Yes
Delayed crèching date	(Yes)	NI	Yes	Yes	NI
Decreased breeding success	No	Yes	Yes	Yes	Yes
Decreased fledgling mass	No	Yes	No	NI	All chicks died

Studies are presented from the left to the right according to the percentage of increased foraging trip duration. Data from Clarke *et al.* (2002) are presented for years 1993–1994 (†) and 1997–1998 (*) and are completed with data provided by Emmerson *et al.* (2003). For some studies, the laying date was approximated by the female departure after egg laying (‡). The increase in foraging trip duration is presented in percentage for females (F) and males (M) during the guard stage. 'Yes' indicates a difference and 'No' indicates no differences between the two considered years (the reference year being that when foraging trips were the shortest). NI indicates that the parameter was not investigated.

considered years. It is also important to note that most studies, like ours, usually consider a limited number of years with different level of food availability and thus do not cover the full spectrum of feeding conditions. Some parameters vary with a small difference between two environmental situations while others vary only if this difference is important. For instance, we did not detect any effect of environmental variability on breeding success while other studies did. It is likely because the birds of our study experienced the smallest modification of their environment compared to other studies. This is in agreement with foraging trip duration which increase was one of the smallest of all studies.

In Béchervaise Island, between 1997 and 1998, foraging trip duration increased similarly as in our study (Clarke *et al.* 2002). However, in contrast to our data, their breeding success decreased the year when penguins foraged for a longer time. This discrepancy may come from the possibility that our penguins had to change the quality of their diet so that they could feed their chicks properly. This shift in diet was not observed in Béchervaise Island between 1997 and 1998, and may explain why breeding success was affected. This hypothesis is reinforced by Nicol *et al.* (2008): in their study, even though foraging trip duration increased more importantly than in our study, breeding success remained unchanged. Birds may have been able to maintain their breeding success because, like in our study, they were able to modify their diet.

Our study is a first attempt to relate environmental conditions to behavioural, dietary, breeding and physiological parameters in Adélie penguins. This approach is promising but proved to be an uneasy task because it integrates many levels (sea-ice, phytoplankton, krill, fish, penguins' behaviour and physiology). Consequently results are sometimes difficult to interpret. Unfortunately we were not able to compare our physiological data to other data since no other studies, examining the effects of contrasting environmental conditions on Antarctic top predators, investigated the animals' physiology (Table 2). In the other studies dealing with Adélie penguins, birds appeared to have experienced more severe modifications of their environment so that their physiological response is likely to have been different to that in our study. In addition, we have considered the isotopic values of prey as constant over time while they may also have fluctuated between years. To better understand the underlying mechanisms between environmental constraints and ecophysiological responses of Antarctic top predators, further studies should integrate data on animals' behaviour, prey, endocrinology and physiological state over a large spectrum of environmental conditions.

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ORIGINAL PAPER

Oxygen recovery up-regulates avian UCP and ANT in newly hatched ducklings

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Abstract At hatching, breaking eggshell induces a surge in oxygen availability that is likely to generate oxidative stress in newborn chicks. To investigate the involvement of potential adaptive antioxidant mechanisms, we explored some markers of oxidative stress and the regulation of muscle avian uncoupling protein (avUCP) and adenine nucleotide translocase (ANT) in ducklings in the peri-hatching period. When compared with pre-hatching levels, the amount of peroxidized lipids were increased 24 h after external pipping in gastrocnemius muscle (+37%) and heart (+39%) as well as the muscle avUCP mRNA expression (+60%) but the susceptibility of red blood cells to free radicals (a functional test of oxidative status) was not affected. In order to relate these changes to the oxidative transition of hatching, an imposed hypoxia/re-oxygenation protocol was used. Hatched chicks that had spent the last 24 h of incubation in artificial severe hypoxia showed a rise in muscle (+50%) and heart (+69%) lipid peroxidation, an increased susceptibility of red blood cells to free radicals, a marked over-expression of avUCP mRNA (+105%) and a rise in mitochondrial ANT content (+54%). These results suggest that avian UCP and ANT may contribute to prepare incubating eggs to the oxidative stress generated by the hypoxia/re-oxygenation transition naturally occurring at hatching.

Keywords Birds · Uncoupling · Reactive oxygen species · Hypoxia

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Introduction

In eucaryotes, aerobic processes of oxidative metabolism are accompanied by continuous intracellular generation of reactive oxygen species (ROS). These reactive molecules are generated as a by-product of several metabolic pathways but predominantly because of the natural functioning of mitochondrial respiratory chain (Boveris and Chance 1973; Nohl 1994). Within mitochondria, ROS are produced by the one electron reduction of oxygen during the reactions of ATP synthesis, which couples the proton pumping-related electrochemical gradient to oxidative phosphorylation (Cadenas and Davies 2000). At high concentration, ROS have some effects on cellular components and lead to oxidative stress causing deleterious damages to nuclear and mitochondrial DNA, membrane lipids, and proteins (Finkel and Holbrook 2000). To prevent oxidative damages, animals develop a number of molecular antioxidant mechanisms that include accumulation of vitamin A, C, E, glutathione, and expression of antioxidant enzymes protecting cells from superoxide injuries (Storey 1996).

The rate of mitochondrial superoxide production is highly affected by the oxygen tension surrounding mitochondria (Turrens et al. 1982), which is clearly influenced by environmental, behavioral, and/or physiological factors. These include variations in oxygen availability, prolonged

apnoeic breathing, and changes in organ temperature or in metabolic activity (Hermes Lima and Zenteno Savin 2002; Storey 1996). Birds naturally experience such stress during the hatching period. Indeed, during the last stages of incubation, the embryo must cope with low-oxygen pressure in the egg internal environment because of low oxygen transfer through eggshell and increased tissue metabolic needs. By contrast, hatching leads to rapid increase in tissue oxygen tension when the newborn chick has a direct access to air. This transition between hypoxia and tissue re-oxygenation is functionally analogous to ischemia/reperfusion that is associated with a dramatic increase in mitochondrial oxyradical generation (Storey 1996). So far, most of the attention in this field has focused on the accumulation of antioxidant defences in newly hatched chicks, which include enzymes involved in superoxide detoxification, such as superoxide dismutase, glutathione peroxidase and catalase, and chain-breaking antioxidant molecules, such as vitamin E, ascorbic acid, carotenoids, and glutathione (Surai 1999; Surai and Sparks 2001). By contrast, little is known on the preventive mechanisms that would contribute to limit mitochondrial ROS generation during chick embryogenesis and bird postnatal development.

Mitochondrial ROS production is highly sensitive to mitochondrial membrane potential (Skulachev 1996) and may thus be regulated by internal factors that directly or indirectly influence the mitochondrial inner membrane conductance to proton. Avian uncoupling protein (avUCP) is known to catalyze a nucleotide-sensitive inducible proton leak when activated by fatty acid and superoxides (Talbot et al. 2003, 2004). Although there is still some intense debate in the literature on the putative role of UCP in birds as either playing a thermogenic role, favoring lipid substrates oxidation or being involved in antioxidant system (Collin et al. 2003a, b, 2005; Criscuolo et al. 2005; Raimbault et al. 2001; Rey et al. 2008a; Talbot et al. 2004), the UCP-dependent reduction in membrane potential may prevent ROS generation in muscle mitochondria (Abe et al. 2006; Mujahid et al. 2006, 2007b). Adenine nucleotide translocase (ANT) may also be implicated in the modulation of ROS generation by virtue of its significant contribution to basal proton leak (Brand et al. 2005; Talbot et al. 2004). Evidences for such control of ROS production by ANT in mammals were also demonstrated by gene knockout mice lacking adenine nucleotide carrier (Esposito et al. 1999).

Presuming that UCP and/or ANT are directly or indirectly involved in the prevention of deleterious mitochondrial ROS production in birds, we predicted that their expression might be stimulated in situation of acute oxidative stress naturally occurring at hatching. To test this hypothesis, we compared natural situation of hatching to a forced hypoxia/re-oxygenation experience in pre-hatched ducklings. Hence, the first objective of this study was to determine in both experiments, the extent of hatching-generated oxidative stress by a functional test based on the red blood cells susceptibility to ROS and by measuring lipid peroxidation in gastrocnemius muscle, heart, and liver tissues. In order to connect our results with variation in tissue oxygen availability, we determine the muscle expression of the hypoxia-inducible factor 1 alpha subunit (HIF-1α), which is known to play a key role in cellular responses to hypoxia and might be a potential candidate for transcription factor modulating mitochondrial proteins in response to variation in ambient oxygen availability (Semenza 2007). Finally, we investigate whether the expression of avian UCP and ANT was affected by oxygen recovery naturally or artificially occurring at hatching time.

Materials and methods

Egg care

Incubating eggs of muscovy ducks (*Cairina moschata,* Linnaeus; pedigree R31, Institut National de la Recherche Agronomique) were provided from a commercial stock breeder (Grimaud, Roussay, France) 72 h before predicted time of hatching. Eggs were placed in an incubator at 37°C and were split into experimental groups as described below.

Experiment A

A first set of 8 ducklings was euthanized 24 h before estimated time of external pipping (group A "−24 h"). At that time, birds were deemed to be in a natural hypoxic condition (Surai 1999). A second set of birds was euthanized 24 or 48 h (6 birds per group) after they had naturally perforated the egg membranes and shell (natural pipping) and thus returned to normoxia (group A "+24 h", "+48 h").

Experiment B

Twenty-four hours before estimated time of pipping, the eggshell of 12 eggs was perforated at the level of the gas chamber under controlled normobaric hypoxia (10 ± 0.2% oxygen; Biospherix, Redfield, NJ, USA).

At their natural time of pipping, a set of six ducklings was euthanized (group B "24 h hypoxia") while a second set of six birds was placed under normoxic conditions for another 24 h before being euthanized (group B "24 h hypoxia + 24 h reox").

For each bird, blood sample was taken under heparin and conserved in KRL medium (see below), gastrocnemius muscles, heart and liver were dissected, frozen in liquid nitrogen, and kept at −80°C prior to biochemical and molecular analysis.

Red blood cells susceptibility to free radicals

A blood aliquot was diluted 1/25 in KRL buffer (340 mosm L^{-1}) and kept until analysis. Susceptibility of blood cells to free radicals aggression was tested as the capacity of erythrocytes to withstand free radical-induced hemolysis. KRL test (Brevet Spiral V02023, Couternon France) was performed according to Blache and Prost (1992) and adapted to birds physiological parameters (Alonso-Alvarez et al. 2004) by monitoring the rate of free radical-induced hemolysis with a microplate titrator (Blache and Prost 1992). The kinetics of erythrocyte resistance to hemolysis was determined at 40°C by continuous monitoring of changes in absorbance at 540 nm. The time to reach 50% of total hemolysis, an index of susceptibility of blood cells to free radical insults (Blache and Prost 1992), was retained for group comparisons.

Muscle lipid peroxidation

The amount of lipid peroxides was estimated in gastrocnemius muscle, heart, and liver using spectrophotometric method, measuring malondialdehyde (MDA) as thiobarbituric acid-reactive substance (TBARS) following the method described by Ohkawa et al. (1979). Results are expressed as nmol MDA/g muscle.

Relative abundance of HIF-1α and avian UCP transcripts in gastrocnemius muscle

HIF-1α, avian UCP mRNA expression, and β-actin as standard gene were assessed by reverse transcription-polymerase chain reaction (RT–PCR). Total RNA was isolated from 0.1 g of frozen tissue using Trizol reagent (Eurome-dex, France). Reverse transcription (RT) reactions consisted of 1 µg total RNA, 200 IU M-MLV-RT (Promega, France), 2 µg mL^{-1} Poly T primers (Invitrogen, France), 1 mM deoxyribonucleotides and 25 IU RNase inhibitor (RNAsin, Promega, France). Polymerase chain reaction (PCR) was then performed in 50 µL reaction containing: 2.5 µL of the RT reaction, 0.2 mM dNTPs (Eurobio, France), 1 µM forward and 1 µM reverse specific primers of HIF-1α (5′-CCC ATC CAT GTG ACC ATG AGG-3′; 3′-TCA GCA CCA AGC ACG TCA TAG G-5′), avian UCP or β-actin (for primers sequences see Talbot et al. 2004), 2.5 U of Taq DNA polymerase (Eurobio, France), reaction buffer and 1.5 mM $MgCl_2$ in a Hybaid thermocycler (Ashford). Thermal cycling parameters where an initial denaturation at 94°C for 2 min followed by 28, 27, and 22 cycles (for HIF-1α, avian UCP and β-actin, respectively) of denaturation at 94°C for 45 s, annealing at 65°C for 60 s, and extension at 72°C for 60 s. A final extension was performed at 72°C for 10 min. The amplified products were separated according to their size on 1.5% agarose gel containing 0.5 mg/mL ethidium bromide (Sigma, France). Numeric photographs of the gels were acquired using Kodak Digital Science 1D Image Analysis Software.

Determination of mitochondrial ANT content

Mitochondrial adenine nucleotide translocase (ANT) content was determined by western blot analysis. Mitochondria were extracted by differential centrifugation in extraction buffer containing 100 mM sucrose, 50 mM Tris base, 50 mM KCl, 5 mM EDTA (pH adjusted to 7.4 with HCl at 4°C). Mitochondrial protein (30 µg) was separated by SDS-PAGE on 13% acrylamide gels, and was transferred to polyvinylidene fluoride membranes. Immunological detection was performed using a mouse anti-serum against bovine heart ANT1 (generous gift of B. Notario, T. Mampel, and O. Vinas, Department of Biochemistry and Molecular Biology, Barcelona; 1:20,000) as previously used for birds (Talbot et al. 2004). The binding of antibody was detected with horseradish peroxidase-coupled anti-mouse (Bio-Rad; 1:5,000) secondary antibody and an enhanced chemiluminescence (ECL) detection kit (Amersham, UK). Protein size was determined using molecular-mass standard (Fermentas, France) and ANT positive control. Quantification of autoradiography was performed by scanning densitometry.

Statistical analysis

The statistical analysis was performed separately for each experiment (A and B) using the Statview computer statistical package. Data from experiment A were analyzed using one-way ANOVA followed by a post hoc Tukey HSD test. Data from experiment B were compared using a *t* test. All the results are presented as mean ± SEM with significance considered at $P < 0.05$.

Results

Susceptibility of red blood cells to free radical aggressions (KRL test)

Table 1 shows that the time to reach 50% hemolysis was not significantly reduced after hatching. This indicates that erythrocytes were poorly affected by oxygen recovery during natural hatching period suggesting either that the oxidative insult was minimal or that erythrocytes were well protected against oxidative stress.

By contrast, blood from birds that experienced 24 h normoxia following 24 h of severe hypoxia after breaking eggshell presented a significant reduction in the time to reach 50% hemolysis (−15%; $p < 0.05$, Table 1). This result

Table 1 Oxidative stress markers measured in different tissues of duckling in two independent experiments

	−24 h	+24 h	+48 h
a			
KRL test (min)	57.2 ± 1.7 (8)	54.0 ± 1.5 (6)	52.5 ± 2.3 (6)
TBARS content (nmol MDA/g WW)			
Muscle	17.0 ± 1.0 (5)	23.4 ± 2.2 (5)[§]	23.3 ± 1.8 (5)[§]
Heart	30.7 ± 4.8 (5)	42.8 ± 2.6 (5)[§]	34.2 ± 3.6 (5)
Liver	23.6 ± 0.6 (6)	25.8 ± 2.1 (7)	24.0 ± 1.3 (5)
	Hypoxia 24 h		Hypoxia + Reox
b			
KRL test (min)	61.7 ± 2.8 (6)		52.3 ± 0.9 (6)*
TBARS content (nmol MDA/g WW)			
Muscle	21.6 ± 1.6 (5)		32.4 ± 5.1 (5)*
Heart	31.9 ± 4.5 (7)		53.9 ± 9.0 (5)*
Liver	22.8 ± 1.5 (6)		21.8 ± 0.3 (6)

The susceptibility of duckling red blood cells to free radical aggressions (KRL test) is expressed in time (min) to reach 50% hemolysis. The thiobarbituric acid reactive substance (*TBARS*) content was determined in gastrocnemius muscle, heart, and liver tissues

Numbers of independent values for each experimental set of data is given in *parentheses*

[a] Blood sample and tissues were obtained from ducklings euthanized 24 h before hatching (−24 h; natural hypoxia), or 24 and 48 h after external pipping ("+24 h", "+48 h"; natural reoxygenation)

[b] Blood sample and tissues were obtained from ducklings placed at 10% oxygen with the eggshell manually pierced 24 h before the expected time of hatching ("hypoxia 24 h") or after an additional 24 h period of re oxygenation under normoxic conditions ("hypoxia 24 h + reox 24 h")

Means are presented ±SEM

[§] Significant difference ($p < 0.05$) with birds euthanized 24 h before hatching (−24 h, group A)

* Significant difference between groups in experiment B

suggested that erythrocytes were weakened by ROS generated during the oxygen recovery following artificial hypoxia.

Gastrocnemius muscle, heart, and liver lipid peroxidation content

As presented in Table 1, the level of lipid peroxidation was significantly increased 24 h after external pipping in gastrocnemius muscle (+37%, $p < 0.05$) and in heart (+39%, $p < 0.05$) when compared with pre-hatching levels. No further modification in TBARS content was detected 48 h after hatching suggesting that the oxidative stress and tissue damages were occurring at the time of hatching. The TBARS content remains unchanged in liver 24 and 48 h after hatching compared to pre-hatching ducklings ($p = 0.56$).

Table 1 shows that 24 h of normoxia following a severe forced hypoxia during the last 24 h of incubation significantly increased TBARS content in gastrocnemius muscle (+50%, $p < 0.05$) and in heart (+69%, $p < 0.05$). In liver, TBARS content was not modified by oxygen recovery following forced hypoxia experiment ($p = 0.50$).

HIF-1α mRNA expression in gastrocnemius muscle

As noticeable in Fig. 1a, the relative abundance of mRNA encoding HIF-1α was significantly reduced 24 h (−30%, $p < 0.05$) and 48 h (−56%, $p < 0.01$) following natural shell pipping as compared with pre-hatching levels. HIF-1α expression tend to be lower in group "+48 h" when compared to group "+24 h" but the differences did not reach sig nificance ($p = 0.062$). As predicted, newly hatched ducklings recovering from 24 h under 10% hypoxia shows a clear reduction in HIF-1α expression in gastrocnemius muscle (−55%, $p < 0.05$, Fig. 1b).

Avian UCP mRNA expression in gastrocnemius muscle

Figure 2a shows that the relative abundance of mRNA encoding avian UCP was increased in gastrocnemius muscle from newly hatched ducklings 24 h (+60%, $p < 0.05$) and 48 h (+85%, $p < 0.05$) after natural shell pipping as compared with pre-hatching levels. No significant difference was noticeable between groups "+24 h" and "+48 h" ($p = 0.19$). Figure 2b shows that in newly hatched ducklings recovering from 24 h under 10% hypoxia the UCP expression pattern in gastrocnemius muscle was the same that observed in naturally hatched ducklings except that the increase was more pronounced (+105%, $p < 0.01$).

Mitochondrial ANT content in gastrocnemius muscle

ANT protein content was measured on mitochondria extracted from gastrocnemius muscle. Figure 3a shows no variation in ANT content during natural hatching when comparing pre-hatchling and hatchling groups (24 and 48 h) (one-way ANOVA, $p = 0.56$), whereas ducklings that had recovered from 24 h under artificial 10% hypoxia showed a significant increase in mitochondrial ANT content (+54%; $p < 0.05$; Fig. 3b).

Discussion

The present study provides the first evidences that (1) in ducklings, hatching is associated with cellular oxidative stress occurring in muscle (gastrocnemius and heart) but not in liver, (2) muscle avian UCP mRNA is increased in parallel with a drastic reduction in HIF-1α expression during natural hatching following the oxidative stress generated by the transition from hypoxia to re-oxygenation and (3) ANT protein abundance is also increased at the time

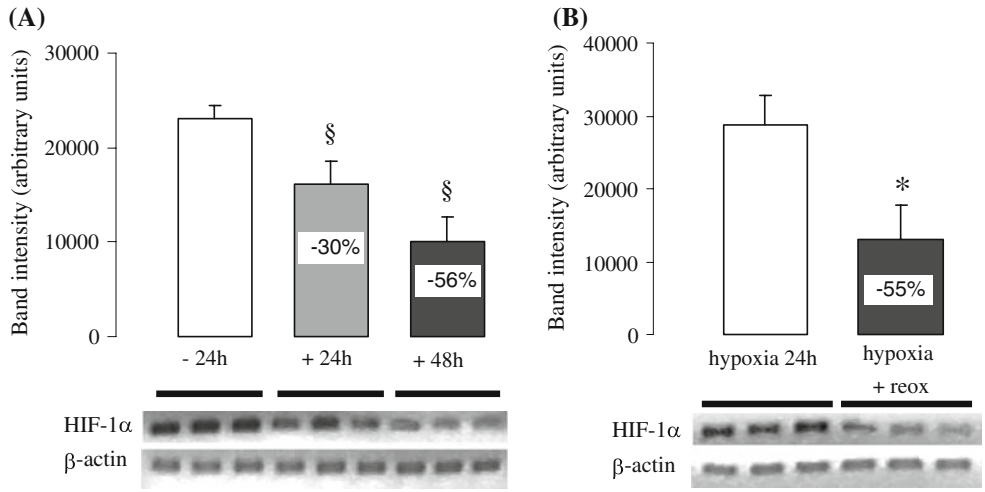

Fig. 1 Determination of HIF-1α gene expression in gastrocnemius muscle of ducklings. **a** Gastrocnemius muscle was obtained from ducklings euthanized 24 h before hatching (−24 h; natural hypoxia), or 24 and 48 h after external pipping ("+24 h"; "+48 h"; natural reoxygenation). **b** Gastrocnemius muscle was obtained from ducklings placed at 10% oxygen with the eggshell manually pierced 24 h before the expected time of hatching ("hypoxia 24 h") or after an additional 24 h period of re oxygenation under normoxic conditions ("hypoxia 24 h + reox 24 h"). Means are presented ±SEM, $n = 5$ in each group, *section symbol* indicates a significant difference ($p < 0.05$) with birds euthanized 24 h before hatching (−24 h, group A), *asterisk* indicates a significant difference between groups in experiment B

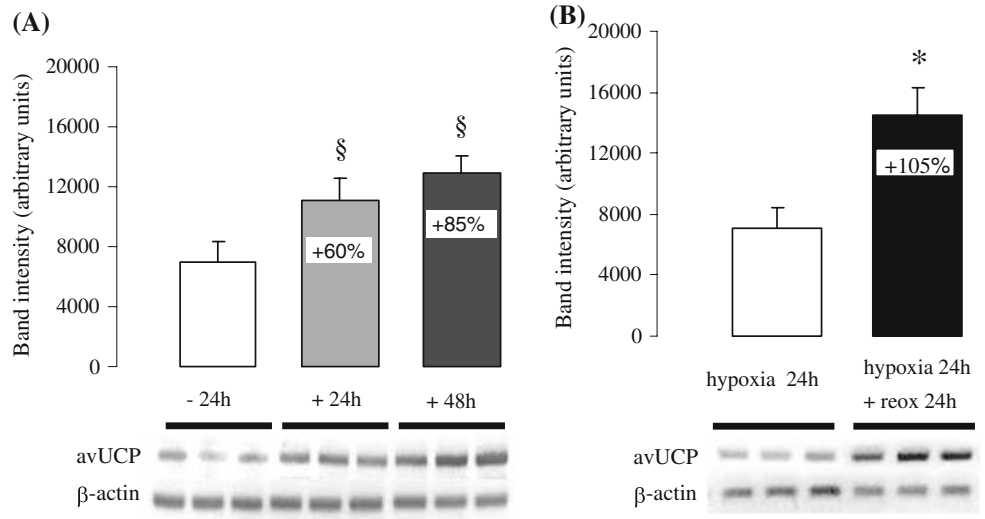

Fig. 2 Determination of avian UCP (avUCP) gene expression in gastrocnemius muscle of ducklings. **a** Gastrocnemius muscle was obtained from ducklings euthanized 24 h before hatching (−24 h; natural hypoxia), or 24 and 48 h after external pipping ("+24 h"; "+48 h"; natural re oxygenation). **b** Gastrocnemius muscle was obtained from ducklings placed at 10% oxygen with the eggshell manually pierced 24 h before the expected time of hatching ("hypoxia 24 h") or after an additional 24 h period of re oxygenation under normoxic conditions ("hypoxia 24 h + reox 24 h"). Means are presented ±SEM, $n = 5$ in each group, *section symbol* indicates a significant difference ($p < 0.05$) with birds euthanized 24 h before hatching (−24 h, group A), *asterisk* indicates a significant difference between groups in experiment B

of re-oxygenation when the magnitude of the hypoxia to re-oxygenation transition is enlarged.

Oxidative stress and oxygen availability in hatching ducklings

In all bird species, high production of reactive oxygen species may be suspected at the hatching time because of the marked increase in partial oxygen pressure surrounding the embryo (Ar and Mover 1994) concomitant with the rise in metabolic activity (Moriya et al. 2000). Blood is one of the first tissues that undergo oxidative effects resulting from large variations in environment oxygen availability. Indeed, the susceptibility of erythrocytes was shown to be enhanced after acute oxidative stress (Lesgards et al. 2002). Thus, the time to reach 50% hemolysis gives information on the

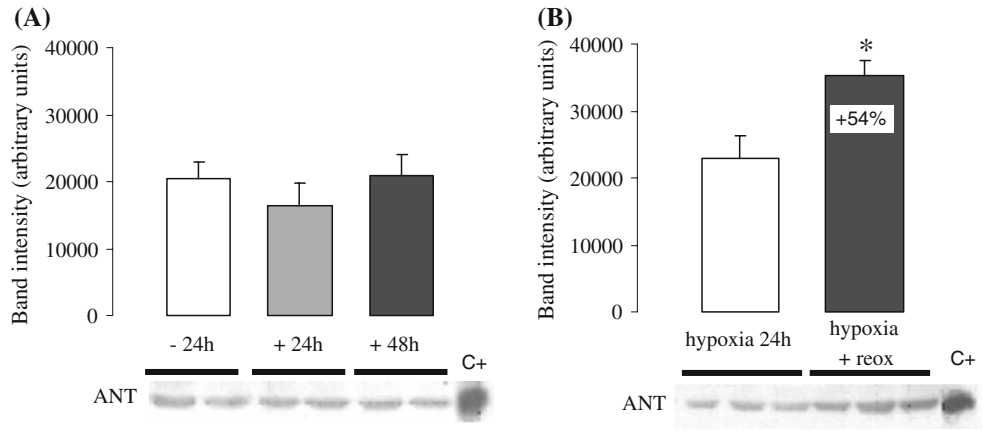

Fig. 3 ANT content (Western Blot, arbitrary unit) in mitochondria isolated from gastrocnemius muscle of ducklings. **a** Gastrocnemius muscle was obtained from ducklings euthanized 24 h before hatching (−24 h; natural hypoxia), or 24 and 48 h after external pipping ("+24 h"; "+48 h"; natural re oxygenation). **b** Gastrocnemius muscle was obtained from ducklings placed at 10% oxygen with the eggshell manually pierced 24 h before the expected time of hatching ("hypoxia 24 h") or after an additional 24 h period of reoxygenation under normoxic conditions ("hypoxia 24 h + reox 24 h"). Means are presented ±SEM, $n = 5$ in each group, *asterisk* indicates a significant difference ($p < 0.05$) between groups in experiment B

balance between the intensity of radical aggression and blood antioxidant capacities. Present results showed no differences in red blood cells susceptibility to ROS before and after natural pipping while TBARS levels in skeletal muscle and heart were increased in post-hatching ducklings (Table 1). Surprisingly, TBARS content was unchanged in liver. Because the intensity of oxidative stress is related to the balance between pro- and antioxidant molecules, it may be postulated that plasma, red blood cells and liver of newly hatched ducklings might dispose of appropriate antioxidant defences to prevent excessive oxidative damages naturally occurring at hatching (Speake et al. 1996; Surai et al. 1996). However, because of large tissue differences in antioxidant levels (Surai et al. 1996), the constitutive antioxidant defences that accumulate during chick embryogenesis might not be fully effective to prevent oxidative damages to lipids during oxygen recovery in all tissues and particularly in heart and skeletal muscles (Table 1).

In the present study, we also show that embryos that hatched 24 h after severe hypoxia were exposed to more pronounced oxidative stress than birds that naturally hatched, as indicated by changes in both red blood cell susceptibility to ROS, gastrocnemius, and heart TBARS content (Table 1). This suggests that the susceptibility of tissues to oxidative stress depends on the magnitude of the changes in partial oxygen pressure occurring at hatching. Among different birds species, such a magnitude might be critically influenced by physical and biological parameters, such as egg size (shell surface/embryo ratio), shell properties (gas conductance, Leon-Velarde and Monge 2004) or altitude of incubation (from sea level up to 6,500 m, Carey et al. 1982), and might therefore clearly influence the intensity of oxidative stress encountered by newborn chicks.

Changes in expression of HIF-1α, avUCP and ANT in hatching ducklings

Next, we explore the pattern of expression of several transcripts in gastrocnemius muscle. We first show that the relative abundance of HIF-1α mRNA is drastically down-regulated after oxygen recovery in both experiments (Fig. 1a, b). HIF-1α is a critical mediator of cellular response to hypoxia that is rapidly affected by variation in oxygen availability and that control many biological processes influencing both oxygen delivery and cell adaptation to oxygen deprivation. Therefore, our results confirm that, at a cellular level, muscles were rapidly recovered from natural (Fig. 1a) or experimental (Fig. 1b) period of oxygen deprivation occurring at the end of the incubation period.

Our results also show that the relative abundance of avUCP mRNA is significantly increased in skeletal muscle after external pipping (Fig. 2a). Since it was first reported in 2001, the possible role of avUCP as antioxidant has rapidly propagated throughout literature (Criscuolo et al. 2005; Mujahid et al. 2006, 2007a, b, 2009; Rey et al. 2008a; Talbot et al. 2004). Although direct evidences of such role are not yet provided; recent study from Toyomizu group presents interesting correlative data showing that heat stress-induced reduction in avian UCP expression is associated with both an increase of membrane potential and a concomitant rise of mitochondrial ROS production in chickens (Mujahid et al. 2009). These effects that were reversed by controlled increase in avUCP expression using olive oil-supplemented diet, suggest that avUCP mainly alleviates mitochondrial ROS production and oxidative damages at least in the acute heat stress protocol. Considering the role of avUCP as a potential modulator of

mitochondrial ROS production, the upregulation observed in hatching ducklings might be interpreted as an additional protective mechanism developed by embryos to counteract the effect of tissue re-oxygenation during hatching period. Interestingly, same pattern of variation in UCP expression was also found in muscle from ectotherms following anoxia/normoxia or rewarming induced oxygen recovery experiment (Issartel et al. 2009; Rey et al. 2008b). These data also support an important antioxidant role played by UCPs in crucial periods of oxygen recovery.

Finally, our results show that the mitochondrial ANT content was not modified during natural hatching period, but its level was significantly increased after the transition from severe hypoxia to normoxia (Fig. 3a, b). By virtue of its involvement in basal proton conductance (Brand et al. 2005) and ROS production (Esposito et al. 1999), this may also suggest a role of ANT as an additional antioxidant mechanism, which would take place following a severe oxidative stress. Overall, it appears from the present study that adaptive responses to hatching-related overproduction of ROS would primarily lead to the upregulation of avian UCP in order to reinforce mitochondrial antioxidant defences in skeletal muscle, while ANT would be secondarily involved when the magnitude of oxidative stress increases. Although we only provide mRNA and protein level measurements, there is clear evidences that an up-regulation of UCP and ANT genes in birds is associated with an increased activity of these proteins in mitochondria, especially their capacity to lower the mitochondrial membrane potential when appropriately stimulated (Talbot et al. 2004).

However, following this interpretation and considering the implication of avUCP and ANT as antioxidant defences, one would expect that oxidative stress markers might be unchanged in our experiments. These apparent equivocal data suggest at least two different interpretations, which will warrant specific experiments to be done. First interpretation concerns the increase in lipids susceptibility to ROS occurring at the end of incubation. Indeed, embryo development is associated with large changes in fatty acid profile with enhancement of polyunsaturated fatty acid of several tissues (Speake et al. 1998) rendering the embryonic tissues highly susceptible to free radicals. We can also hypothesized that increase in oxidative stress markers would reflects the dynamic balance between ROS generation and the concordant activation of avUCP and ANT expression in myocytes.

In our study, the expression of avUCP and ANT was positively related with the amplitude of the changes in oxygen availability, being larger in chicks after exposure to severe hypoxia than after natural hatching. These results favor a link between the magnitude of oxygen availability, oxidative stress and a functional role of uncoupling proteins as antioxidant defence. Among potential activating mechanisms, it was shown in mice mitochondria that more generation of ROS induces peroxidation of membrane phospholipids and leads to the production of reactive aldehydes such as 4-hydroxy-2-nonenal (4HNE) that induce UCP1, 2, 3 and ANT-dependent decreases in mitochondrial membrane potential and drop in ROS production (Echtay et al. 2003). Because lipid peroxidation increases in hatching ducklings, such peroxidation of membrane phospholipids may occur and would provide a feedback control over ROS production by activation of avian UCP and ANT transcription and activity. This implies that activation of a signaling pathway, followed by the triggering of specialized transcription factors that initiate an increase in the expression of avUCP, and possibly avian ANT. At this early point, although the molecular basis of such signaling pathway in newborn ducklings is currently not known, we show that avUCP is inversely correlated to HIF-1α expression, suggesting a possible signaling link between UCP expression and HIF-1α in birds, but this hypothesis clearly deserves further investigations.

Altogether, present data suggest that avian UCP and ANT may contribute preparing to incubate eggs to the stress of reestablishment of efficient oxygen delivery by air breathing at the time of hatching following relative oxygen limitation by the end of incubation. It is anticipated that these proteins may play antioxidant roles by contributing to adaptive responses of birds subjected to large environmental variations in oxygen availability.

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