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Mécanismes hormonaux impliqués dans la phénologie de la reproduction chez les oiseaux marins polaires

Aurélie Goutte

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THESE

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Présentée par :
Aurélie Goutte

Mécanismes hormonaux impliqués dans la phénologie de la reproduction chez les oiseaux marins polaires



Directeur de Thèse : Olivier Chastel

Soutenue le 22 octobre 2010 devant la Commission d'Examen

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Goutte, A., Angelier, F., Clément-Chastel, C., Trouvé, C., Moe, B., Bech, C., Gabrielsen, G.W., Chastel, O. (2010). Stress and the timing of breeding: glucocorticoids-luteinizing hormone relationships in an arctic seabird. *General and Comparative Endocrinology*, in press

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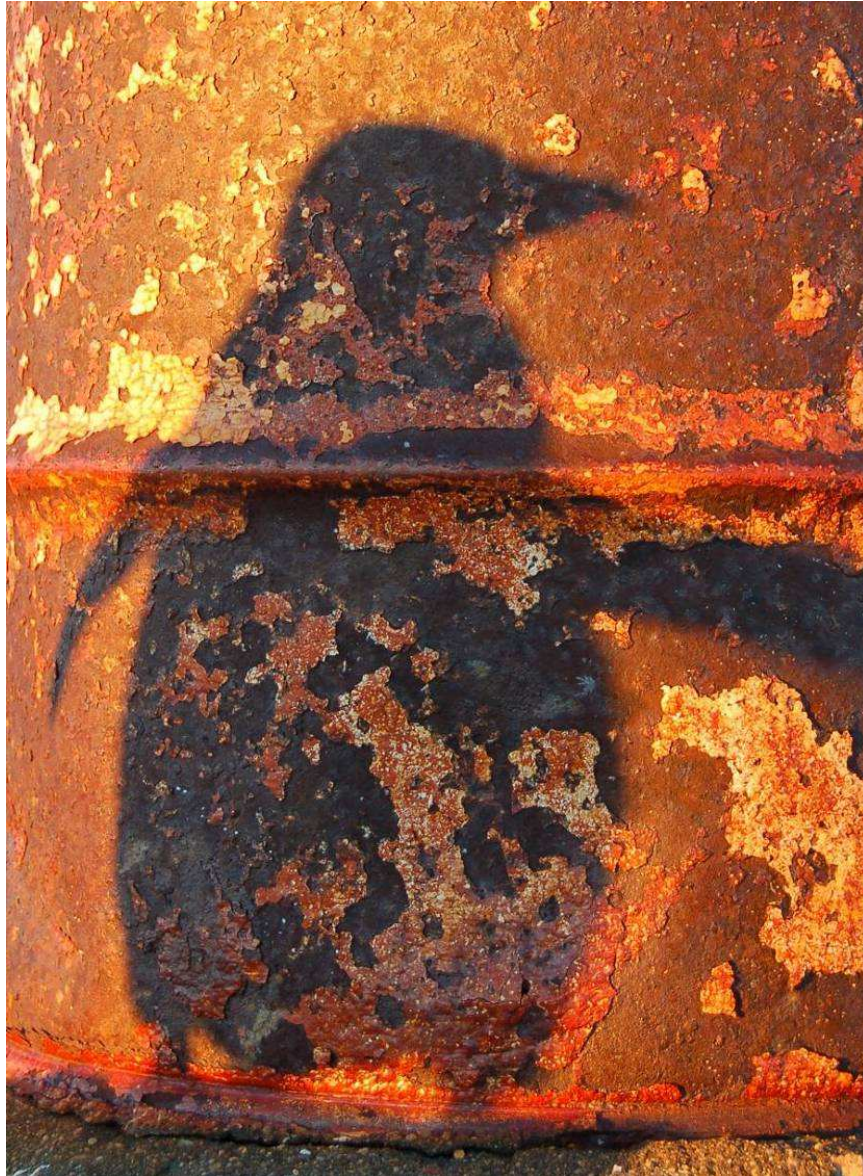
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Introduction



*Il y a une chose dont je suis absolument certain,
c'est que si on se prend au sérieux, on ne peut pas faire de travail sérieux.
Pour faire du travail sérieux, il ne faut pas se prendre au sérieux, car
c'est le seul moyen d'avoir la possibilité de faire une autocritique.
Et si on se prend au sérieux, on ne fait pas d'autocritique.*

Paul Emile Victor

I. Introduction

I.1. Contexte générale

La phénologie est l'étude des évènements majeurs et périodiques des cycles de vie animale et végétale. Parmi ces activités saisonnières, on peut citer les dates de débourrement (ouverture des bourgeons à la sortie de l'hiver), de floraison, de premier vol de papillons, d'arrivée de migrateurs, de ponte, de sevrage et / ou d'hibernation.

I.1.1. La phénologie dans le contexte climatique actuel

i. L'émergence de la phénologie

Le mot 'phénologie', du grec *phainomai* (φαινομαι, pour 'montrer', 'apparaître') est apparu dans le langage scientifique à la fin du 19^{ème} siècle. Cependant, les hommes se sont intéressés aux rythmes naturels saisonniers longtemps auparavant. Au Moyen Age, le suivi des dates de récoltes et de migrations permettaient de programmer les activités agricoles, de cueillette, de pêche et de chasse. Ainsi les dates de vendanges de Bourgogne ont pu être reconstituées jusqu'à 1370 (Chuine *et al.* 2004). Malgré d'importants enregistrements, dont ceux effectués par les stations de Météo-France entre 1880 et 1945, l'engouement scientifique s'est avéré mitigé. Ce n'est qu'au cours des récentes décennies que la compréhension des variations phénologiques a pris son essor, porté par le contexte actuel des changements climatiques.

ii. Une avancée à long terme de la phénologie de la reproduction

Au cours du 20^{ème} siècle, le réchauffement global s'est concrétisé par une élévation des températures de 0,58°C en moyenne (IPCC). Les conséquences d'un tel changement climatique ont pu être appréciées à différentes échelles biologiques : physiologie, phénologie, aire de distributions, processus micro-évolutifs, structure et composition des communautés, déclin des populations (Hughes 2000; Walther *et al.* 2002). Particulièrement faciles à détecter, les variations phénologiques sont devenues l'un des indicateurs privilégiés des changements climatiques (Walther *et al.* 2002).

Dans l'hémisphère Nord, on constate, en effet, une avancée relative de la phénologie printanière des espèces animales et végétales (Parmesan & Yohe 2003), estimée en moyenne à -2.8 ± 0.35 jours par décennie (N = 203 espèces, tous taxons confondus, Parmesan 2007). Pour les oiseaux, l'avancée des dates de ponte est en moyenne de -3.7 ± 0.70 jours par décennies (N = 41 espèces, Parmesan 2007). Cependant, ces études ont été majoritairement menées dans les milieux terrestres. Chez les oiseaux marins, les tendances phénologiques sont moins évidentes et plus contrastées. En mer du Nord, les mouettes tridactyles, *Rissa tridactyla*, et les guillemots de Troïl, *Uria aalge*, présentent un retard de respectivement 5,1 et 2,6 jours par décennies (Frederiksen *et al.* 2004). De même, les fous de Bassan, *Morus bassanus*, ont un retard de 2,1 jours par décennies (Wanless *et al.* 2008). Au contraire, les pontes ont lieu de plus en plus tôt chez les sternes arctiques, *Sterna paradisaea* (Møller *et al.* 2006) et dans d'autres colonies de guillemots de Troïl (Votier *et al.* 2009). Une étude comparative a mis en évidence ces fortes disparités de tendances phénologiques chez les oiseaux marins au Nord-Est de l'Angleterre : trois espèces de sternes (sterne arctique, sterne pierregarin, *Sterna hirundo*, et sterne caugek, *Sterna sandvicensis*) arrivent et se reproduisent plus tôt, tandis que les macareux moines, *Fratercula arctica*, et les mouettes tridactyles se reproduisent de plus en plus tard ou que d'autres espèces comme les pingouins torda, *Alca torda*, et les fulmars boréaux, *Fulmarus glacialis*, ne présentent pas de tendances phénologiques claires (Wanless *et al.* 2009).

Dans les milieux polaires, pourtant très touchés par les changements climatiques, les tendances de phénologie de la reproduction restent rares et peu généralisables. En Terre Adélie, Antarctique, l'arrivée a lieu en moyenne 9,1 jours plus tard et la reproduction, 2,1 plus tard comparées aux années 1950 (Barbraud & Weimerskirch 2006). On constate par contre, un avancement de la date d'arrivée du skua antarctique (*Catharacta macormicki*, Barbraud & Weimerskirch 2006). Au Spitzberg, Arctique norvégien, les mergules nains, *Alle alle*, se reproduisent de plus en plus tôt tandis que les mouettes tridactyles présentent une tendance non-significative à un retard de la reproduction (Moe *et al.* 2009). Au Nunavut, une colonie de guillemots proches du cercle polaire se reproduit de plus en plus tôt, tandis qu'une colonie plus septentrionale ne présente pas de tendance phénologique à long terme (Gaston *et al.* 2005a).

Face à ces fortes différences dans les réponses phénologiques à l'échelle de la population, il apparaît nécessaire d'identifier l'éventail de signaux environnementaux, pouvant affectés la phénologie de la reproduction au niveau individuel afin de comprendre les mécanismes

proximaux, qui font le lien entre ces paramètres environnementaux et l'ajustement de la phénologie de la reproduction au niveau individuel (Fig. 1).

I.1.2. Les signaux environnementaux influençant la phénologie de la reproduction

i. La photopériode

La photopériode est la variation annuelle de la durée de la phase claire du jour. Fixe, fiable et répétable d'une année sur l'autre, c'est le signal primaire qui initie la préparation aux activités de reproduction plusieurs semaines, voire plusieurs mois à l'avance (Fig. 1). Rowan (1925) est le premier à avoir établi un lien entre la lumière et les préparations physiologiques et comportementales aux activités saisonnières de reproduction aviaire. La photopériode a non seulement une action initiatrice sur la reproduction, mais a aussi une fonction terminatrice. Trois phases ont ainsi été définies : les oiseaux sont tout d'abord *photosensibles* à l'allongement de la durée du jour au début du printemps, puis *photostimulés* (développement et maturation des gonades), et enfin *photoréfractaires* (régression des gonades) face à l'exposition à des jours de plus en plus longs (revu dans Dawson *et al.* 2001).

ii. Les signaux secondaires

En plus de ce signal fixe, plusieurs signaux environnementaux sont intégrées et coordonnent des variations phénologiques à plus fine échelle temporelle, de l'ordre de quelques jours ou quelques semaines. Chez les oiseaux, ces informations dites secondaires jouent un rôle inhibiteur ou accélérateur sur la date de ponte (Fig. 1). Ainsi, parmi les facteurs abiotiques, on cite la température ambiante, les précipitations, des événements météorologiques difficiles, l'accessibilité des nids (couverture neigeuse), l'étendue de glace de mer, tandis que parmi les facteurs biotiques, on cite la disponibilité, le timing et l'abondance des proies, la présence et la qualité du partenaire et / ou les stimulations sociales (Ball 1993; Visser *et al.* 1998; Wingfield *et al.* 2003; Schoech *et al.* 2004; Gaston *et al.* 2005a,b; Barbraud & Weimerskirch 2006; Ball & Ketterson 2008; Both *et al.* 2009). De plus, l'activité solaire (tâches solaires) affecte les dates de ponte des mésanges (Visser & Sanz 2009). Visser *et al.* (2009) ont montré un lien direct entre des températures élevées et une ponte précoce. Enfin, une attention

particulière a été dédiée à l'échelle spatiale des variables climatiques (grande échelle : Oscillation Nord Atlantique par rapport aux températures de surface de la mer SST, Frederiksen *et al.* 2004; Moe *et al.* 2009).

En plus de retarder l'initiation de la reproduction, des évènements stressants (raréfaction des ressources environnementales, risque de prédateurs, évènements météorologiques difficiles) tôt dans la saison peuvent influencer la décision de ne pas se reproduire, c'est-à-dire la décision de prendre une année sabbatique (Fig. 1). Ainsi, Chastel *et al.* (1995) ont montré que 50% des pétrels bleus, *Halobaena caerulea*, ne se reproduisent pas lors des années défavorables bien qu'ils soient sexuellement matures.

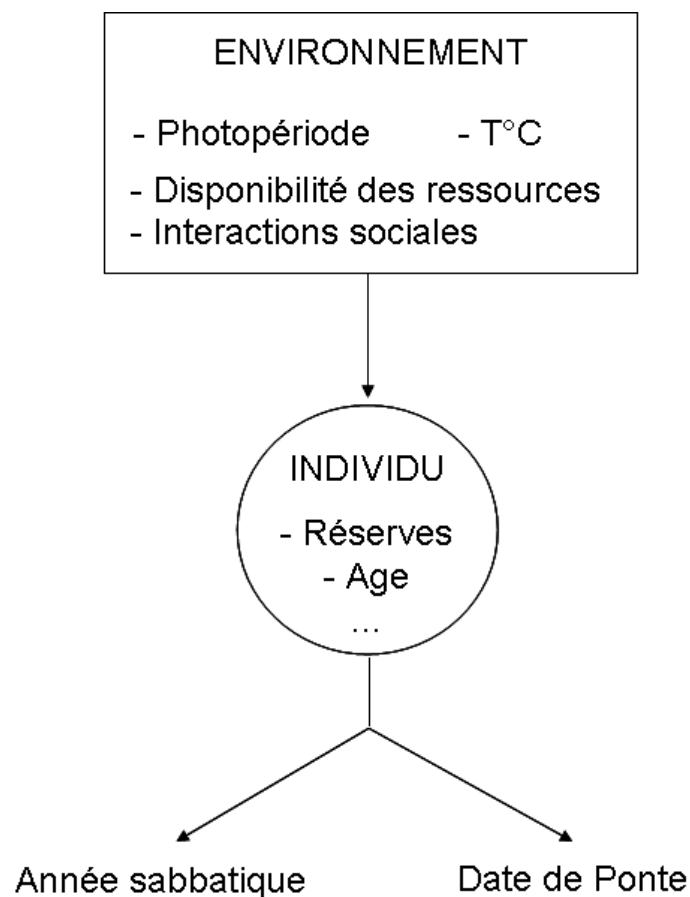


Figure 1 : La date de ponte et la décision de ne pas reproduire sont influencées par les conditions environnementales (signal primaire : la photopériode, signaux secondaires : température, disponibilité des ressources, interactions sociales) et par l'état physiologique de l'individu (réserves énergétiques, âge, etc.)

I.1.3. Les paramètres individuels influençant la phénologie de la reproduction

McNamara & Houston (2008) ont passé en revue les variables individuelles susceptibles d'affecter les traits d'histoire de vie. Nous détaillerons dans ce paragraphe l'importance des réserves énergétiques, de l'âge et les différences entre mâles et femelles, sur la décision de ne pas se reproduire et la date de ponte (Fig. 1). D'autres facteurs interviennent, tels que la capacité à acquérir de la nourriture, la capacité à défendre un territoire de meilleure qualité, des plumes de mauvaise qualité, une blessure et / ou un stress chronique (Nilsson & Svensson 1996; McNamara & Houston 2008). De plus, la date de ponte est un trait déterminé génétiquement et partiellement héritable (e.g. Charmantier *et al.* 2006; Brommer *et al.* 2008). Les tendances phénologiques observés à long terme chez les mésanges résultent cependant de la plasticité phénotypique et non de processus microévolutifs (Charmantier *et al.* 2008).

i. Les réserves énergétiques

La source majeure des variations individuelles des dates de ponte est le niveau de réserves énergétiques nécessaire à la formation des œufs et à l'initiation de la reproduction. On distingue les *income breeders*, pour qui la reproduction est basée sur l'acquisition immédiate de nourriture, des *capital breeders*, qui puisent dans leurs réserves énergétiques, accumulées antérieurement pour préparer la reproduction (Drent & Daan 1980 ; Stearns 1992 ; Jonsson 1997). Certains nutriments sont aussi primordiaux, comme le calcium nécessaire à la formation de la coquille des œufs (Tilgar *et al.* 2002), ou comme certains pigments tels que les caroténoïdes (e.g. Doutrelan *et al.* 2008). Enfin, la capacité à augmenter le taux métabolique de base est associée à une date de ponte précoce (Chastel *et al.* 2003).

Concernant les années sabbatiques, les non-reproducteurs ont une condition corporelle plus faible que les reproducteurs en période pré-nuptiale (Chastel *et al.* 1995).

ii. L'âge

L'âge est un caractère individuel essentiel dans l'étude des traits d'histoire de vie. En règle générale, les jeunes se reproduisent plus tard dans la saison que les oiseaux plus âgés (Martin 1995; DeForest & Gaston 1996; Gonzalez-Solis *et al.* 2004; Ezard *et al.* 2007; McCleery *et al.* 2008; Verhulst & Nilsson 2008; Blas *et al.* 2009). D'un point de vue ultime, ce phénomène

peut être expliqué par (1) une disparition progressive des individus tardifs par sélection naturelle (*selection hypothesis*, Mauck *et al.* 2004), (2) un ajustement individuel des traits d'histoires de vie: les jeunes privilégient leur survie et en vieillissant, favorisent de plus en plus leur succès reproducteur (*restraint hypothesis*, Stearns 1992), (3) une amélioration des performances reproductrices avec l'expérience (*constraint hypothesis*, Curio 1983), ou (4) l'incorporation progressive d'individus précoces dans les classes d'âge (*delayed-breeder hypothesis*, Forslund & Pärt 1995; Blas *et al.* 2009). De plus, il a été montré, mais en de rares occasions, que les individus très vieux se reproduisent plus tard dans la saison, comparés aux oiseaux d'âge moyen (Ezard *et al.* 2007; McCleery *et al.* 2008), ce qui reflète un possible aspect de la sénescence chez certains oiseaux longévifs.

Enfin, la décision de ne pas se reproduire varie avec l'âge : chez les oiseaux longévifs, les très jeunes et / ou les très vieux ont une plus faible probabilité de se reproduire par rapport aux individus d'âge moyen (Cam *et al.* 2002; Berman *et al.* 2009; Goutte *et al.* 2010a).

iii. Les différences entre sexes

Comme suggéré par Ball & Ketterson (2008), mâles et femelles ne présentent pas forcément la même sensibilité aux variables environnementales influençant la phénologie de la reproduction. Les femelles ont, en effet, des coûts énergétiques élevés associés à la formation des œufs, tandis que la compétition entre mâles lors de la période pré-nuptiale pourrait être un critère sélectif privilégiant une rapide préparation des mâles à la reproduction et une certaine insensibilité aux événements stressants (Ball & Ketterson 2008).

Le rôle indirect des mâles sur la date de ponte est controversé. Chez les mésanges bleues (*Cyanistes caeruleus*), les femelles apparaissent comme les seules à décider de la date de ponte (Caro *et al.* 2009), tandis que chez les goélands cendrés (*Larus canus*, Brommer & Rattiste 2008), une espèce aux soins biparentaux, les mâles ont des effets génétiques indirects sur la date de ponte de leur partenaire.

I.2. Fitness¹, décision de reproduction et phénologie de la reproduction

I.2.1 Décision de ne pas se reproduire

Selon la théorie des traits d’histoire de vie (Stearns 1992), un individu alloue de façon différentielle une quantité limitée d’énergie à la reproduction en cours et à sa propre maintenance. Chez de nombreux espèces longévives, des individus matures s’abstiennent de se reproduire lors d’une ou de plusieurs saisons (e.g. Cam *et al.* 1998 ; Chastel *et al.* 1993, 1995; Berman *et al.* 2009; Blas & Hiraldo 2010).

Cette décision de ne pas se reproduire, pourrait être considérée comme une stratégie adaptative consistant à privilégier la survie et les reproductions futures lorsque la valeur de la reproduction immédiate est faible (*prudent parent hypothesis* : Drent & Daan 1980). Ainsi, la prise d’une telle année sabbatique pourrait faciliter le rétablissement, comme cela a été observé chez les fous à pieds bleus, *Sula nebouxii* : les ornements sexuels des individus très âgés sont plus colorés l’année suivant une année sabbatique, que l’année suivant un épisode de reproduction (Velando *et al.* 2010).

La décision de ne pas se reproduire pourrait, non pas être une stratégie adaptative, mais une forme extrême d’échec reproducteur : chez les mouettes tridactyles, les non-reproducteurs ont des taux de survie plus faibles et seraient alors des individus de moindre qualité (Cam *et al.* 1998).

I.2.2 Un ajustement des besoins aux ressources : ‘timing is everything’

Si se reproduire au bon moment est considéré comme un trait d’histoire de vie essentiel au succès reproducteur (Lack 1968; Perrins 1970; Nager & VanNoordwijk 1995), quelles sont les conséquences écologiques qui découlent du lien entre les signaux environnementaux, l’état individuel et la phénologie de la reproduction ?

¹ **Fitness** ou valeur sélective : capacité à transmettre son patrimoine génétique aux générations futures

i. La théorie

La théorie du ‘*match-mismatch*’ a été tout d’abord élaborée à l’échelle des populations et postule que le recrutement augmente lorsque la phase du cycle la plus coûteuse en énergie (généralement, l’élevage des jeunes) coïncide avec le pic de disponibilité des proies dans l’environnement (Visser *et al.* 1998; Stenseth & Mysterud 2002; Durant *et al.* 2007). En effet, l’exploitation optimale des ressources disponibles dans l’environnement permet de subvenir aux besoins énergétiques requis pour la croissance des jeunes (*match*, Fig. 2). Si le pic de nourriture survient plus tôt ou plus tard et si les parents n’ajustent pas leurs activités saisonnières, les besoins énergétiques nécessaires à la reproduction sont décalés par rapport aux ressources disponibles (*mismatch*, Fig. 2), et le recrutement en pâtit.

ii. Le modèle mésange-chenille

L’exemple classique est le modèle mésange-chenille (Visser *et al.* 1998; Durant *et al.* 2007; Both *et al.* 2009). La phénologie de la végétation et des maillons trophiques supérieurs (chenilles, passereaux et rapaces) ont été suivis à long-terme, aux Pays-Bas, au cours des deux dernières décennies. On note un avancement global des dates de débourrement, du pic d’abondance des chenilles, ainsi que des dates d’éclosion de la mésange charbonnière, *Parus major*, de la mésange bleue, *Cyanistes caeruleus*, de la mésange noire, *Periparus ater*, et du gobe-mouche noir, *Ficedula hypoleuca* (Visser *et al.* 1998; Both *et al.* 2009). Pour l’épervier d’Europe, *Accipiter nisus*, en revanche, on ne constate aucune tendance sur long-terme, bien que ses proies se reproduisent plus tôt (Both *et al.* 2009).

A chaque niveau trophique, les réponses phénologiques se font à un degré plus faible que celles de leurs proies, si bien qu’il persiste un décalage entre la date de disponibilité maximale des ressources et la date de ponte (Visser & Both 2005).

iii. Ajustement phénologique et recrutement

Cette limite dans la flexibilité phénologique (*mismatch*) peut avoir de lourdes conséquences démographiques. Chez le gobe-mouche noir, la faible capacité à ajuster la date de ponte aux changements des conditions environnementales (avancée des dates de pic d’abondance des chenilles) mène à un fort déclin de la population (Both *et al.* 2006). Une étude comparative

sur plus de 100 espèces d'oiseaux migrateurs a montré que les populations en déclin sont celles qui ne présentent aucune tendance phénologique à long-terme (Møller *et al.* 2008).

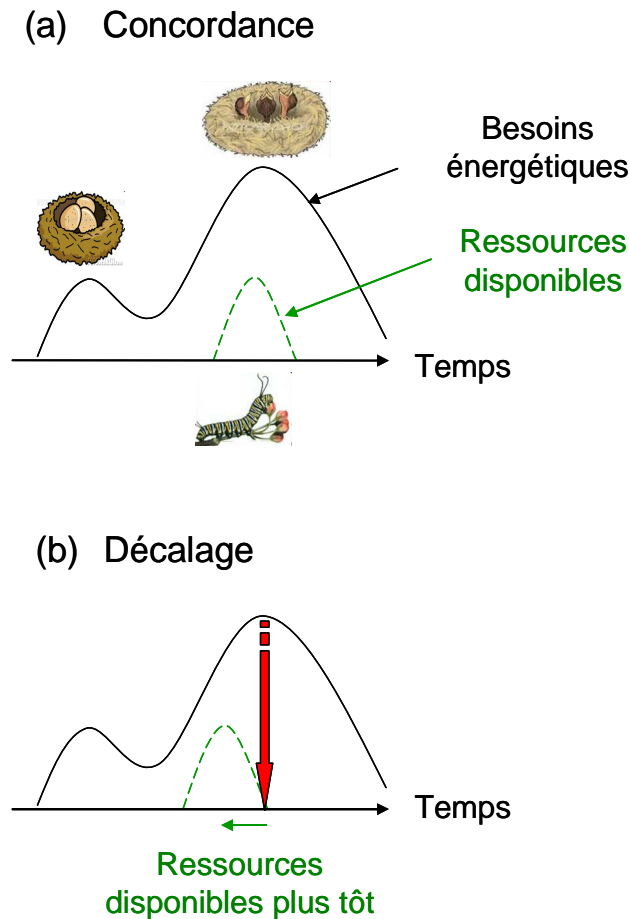


Figure 2 : La théorie du match-mismatch illustrée par le modèle passereaux-chenilles : (a) Concordance entre les besoins énergétiques et le pic d'abondance des chenilles. (b) Décalage en cas d'avancée des ressources D'après Stenseth & Mysterud 2002 et Durant *et al.* 2007

I.2.3. Fitness immédiate et fitness résiduelle

i. Fitness immédiate

Au niveau individuel, les couples reproducteurs précoces dans la saison ont généralement une plus grande couvée, une meilleure réussite à l'éclosion et à l'envol et / ou une plus forte croissance des poussins (Perrins 1970). Ce déclin saisonnier du succès reproducteur a été

attribué à deux phénomènes, non mutuellement exclusifs (revus dans Verhulst & Nilsson 2008) :

(1) La date de reproduction *per se* peut directement affecter le succès reproducteur, via la détérioration de l'environnement au cours de la saison (*date hypothesis*, Brouwer *et al.* 1995; Brinkhof & Cave 1997; Wardrop & Ydenberg 2003; Arnold *et al.* 2004). En effet, des ressources de moins en moins abondantes, des phénomènes croissants de compétition, des pressions de prédation et de parasitisme de plus en plus fortes sont autant de phénomènes saisonniers qui concourent à la réduction des chances de survie des jeunes (Verboven *et al.* 2001; Naef-Daenzer *et al.* 2001; Götmark 2002).

(2) La seconde hypothèse attribue le déclin saisonnier du succès reproducteur à la qualité individuelle (*quality hypothesis*, DeForest *et al.* 1996; Hipfner 1997; Hipfner *et al.* 1999; Daunt *et al.* 1999). Comme détaillé ci-dessus, la qualité est définie par un ensemble de facteurs, tels que l'état énergétique, l'âge, les performances intrinsèques, la qualité du partenaire et / ou la qualité du territoire. Des femelles précoces sont des femelles en meilleure condition et / ou plus âgées (e.g. Dann *et al.* 1990; Daunt *et al.* 1999). Indépendamment de l'environnement et des dates de reproduction *per se*, ces femelles précoces auraient un meilleur succès reproducteur, que des femelles de moindre qualité et tardives.

Afin de mettre à l'épreuve ces deux hypothèses, de nombreuses manipulations expérimentales des dates de reproduction ont été menées via le remplacement de la couvée ou des échanges entre couvées tardives et précoces (Fig. 3, voir Verhulst & Nilsson 2008 pour une revue critique de ces techniques). Les deux hypothèses (date *per se* et qualité du couple) expliquent le déclin saisonnier du succès reproducteur avec un effet plus ou moins prononcé en fonction de l'espèce et de la composante de fitness étudiée (Verhulst & Nilsson 2008).

ii Fitness résiduelle : survie et reproductions futures

Chez les espèces se reproduisant dans des milieux saisonniers, la durée de la reproduction est limitée par des activités post-reproductrices, et plus particulièrement par la mue, c'est-à-dire le renouvellement partiel ou complet du plumage. Ainsi, des couples de mésanges bleues dont la phénologie de reproduction a été retardée expérimentalement ont une dépense énergétique élevée pendant l'hiver -liée à une plus forte thermorégulation- et se reproduisent plus tard l'année suivante par rapport aux couples témoins (Nilsson & Svensson 1996). Ces auteurs

proposent qu'un retard de la reproduction interfère avec la mue, ce qui diminue la qualité des nouvelles plumes, et qui cause de fortes pertes de chaleur pendant l'hiver et des coûts lors des futurs épisodes reproductifs.

A l'opposé, une reproduction précoce peut compromettre la survie. Chez les femelles de foulque macroule (*Fulica atra*, Brinkhof *et al.* 2002), une avancée de la période de reproduction engendre un fort taux de mortalité l'année suivante, dû à un degré d'investissement très élevé dans la reproduction en cours.

I.2.4. L'hypothèse d'optimisation individuelle

i. La théorie

Pour des parents prudents, la date de ponte serait alors un compromis adaptatif entre la valeur de la reproduction en cours et la valeur des reproduction futures (i.e. la survie, Drent & Daan 1980). D'après l'hypothèse d'optimisation individuelle (Drent 2006), la tendance saisonnière de ces deux forces sélectives s'oppose : d'une part, les besoins énergétiques pour la reproduction en cours sont d'autant mieux satisfaits que l'exploitation des ressources disponibles débute tôt dans la saison ; d'autre part la survie des parents, et les possibilités de reproductions futures ne doivent pas être compromises par une reproduction en cours trop précoce et soutenue.

La figure 3 représente les prédictions associées aux différentes hypothèses: selon l'hypothèse de date, un retard de la reproduction induit une baisse saisonnière de fitness dû à une incapacité à subvenir aux besoins des jeunes (*mismatch*), tandis qu'une avancée de la reproduction augmente les chances de subvenir aux besoins des jeunes et optimise donc la fitness. Selon l'hypothèse de qualité, la date *per se* n'est pas la cause du déclin saisonnier et un retard ou une avancée de la reproduction ne modifient donc pas la fitness. Enfin, selon l'hypothèse optimisation individuelle, un retard ou une avancée de la reproduction provoque une perte de fitness dû à une incapacité à subvenir aux besoins des jeunes (*mismatch*) ou à des coûts sur le long terme.

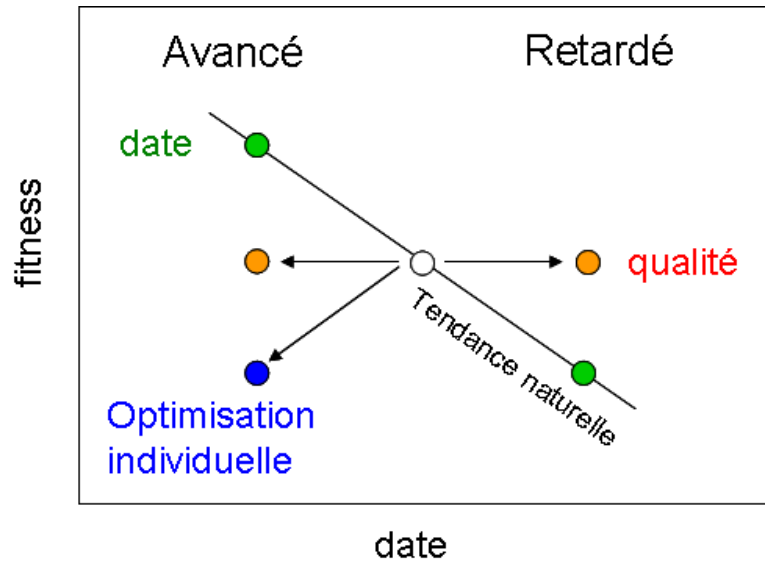


Figure 3 : Prédiction des variations de fitness après une manipulation expérimentale des dates d'éclosion en fonction des trois hypothèses. Inspiré de Drent 2006.

ii. Les conséquences possibles en termes d'investissement parental

Chaque couple, de qualité donnée dans un environnement donné, fait donc face à un compromis entre la reproduction en cours et les opportunités de reproductions futures. Un aspect possible mais peu exploré serait la modification de l'investissement parental suite à une manipulation expérimentale des dates d'éclosion des jeunes. Il est concevable que les parents dévaluent une couvée tardive, puisque la probabilité d'échec augmente avec le décalage des besoins par rapport aux ressources (*mismatch*). En se désinvestissant de leur reproduction tardive, les parents prudents favoriseraient ainsi leur propre survie et leurs reproductions futures (Drent & Daan 1980). Les faibles résultats étayant cette hypothèse sont cependant contrastés : d'une part, le taux métabolique, témoin de l'effort reproducteur, est indépendant de la date de reproduction (Verhulst & Tinbergen 2001) ; d'autre part, le taux de prise alimentaire chez les moineaux domestiques *Passer domesticus*, pourtant nourris *ad libitum*, diminue au cours de la saison, suggérant un plus faible apport alimentaire aux couvées tardives par rapport aux couvées précoces (Moreno-Rueda 2004).

Cette possible diminution de l'investissement parental pourrait être aussi modulée par les opportunités de reproductions futures, c'est-à-dire par l'âge des reproducteurs.

iii. Anticipation par utilisation de signaux précurseurs ou contraintes énergétiques

Puisque la reproduction est initiée bien avant la date de ressources maximales, l'ajustement de la date de ponte se fait en fonction des variables environnementales présentes *avant* l'initiation de la reproduction et en fonction de l'état physiologique des membres du couple. Après la ponte, les seuls ajustements possibles consistent à retarder l'éclosion, en ajustant l'effort d'incubation des œufs, soit en repoussant le début de l'incubation, soit en incubant les œufs par intermittence. Selon certains auteurs, les individus seraient capables de capter des signaux précurseurs qui leur permettent d'anticiper les conditions futures et de prédire la date de disponibilité maximale des ressources (Frederiksen *et al.* 2004; Reed *et al.* 2009). Par exemple, Visser *et al.* (2009) ont montré que des températures élevées sont *directement* liées à une ponte précoce. Ces signaux précurseurs, s'ils sont peu fiables, imprécis, difficilement perceptibles ou mal interprétés peuvent expliquer le décalage entre les besoins énergétiques requis et les ressources disponibles à un temps donné. Dans ce contexte, la prédictibilité de l'environnement a une importance cruciale (Reed *et al.* 2010). Des conditions imprévisibles, telle qu'une raréfaction des ressources, une perturbation météorologique, ou un fort risque de prédation forceraient les individus à se reproduire plus tard que l'optimum.

Cependant, cette hypothèse d'anticipation adaptative n'est pas soutenue par certaines études descriptives et expérimentales, en particulier chez les oiseaux marins migrateurs (Hipfner *et al.* 2008; Shultz *et al.* 2009). Au lieu d'être choisie et anticipée, la date de ponte serait alors imposée par les contraintes environnementales prénuptiales (e.g. Both *et al.* 2009). Pour tester cette hypothèse, il a été proposé de réduire le budget énergétique des femelles et de tester si les dates de pontes sont avancées. Cependant, une avancée de la reproduction n'a pas été mise en évidence suite à la réduction des dépenses énergétiques des mésanges femelles, via le chauffage des niochirs pendant la nuit (Nager & VanNoordwijk 1992). Ainsi il apparaît difficile de discerner l'hypothèse d'une date de ponte choisie et anticipée, d'une date de ponte imposée par les conditions environnementales.

Dans ce contexte controversé, il apparaît donc indispensable d'identifier les mécanismes sous-jacents qui lient les conditions environnementales, l'état physiologique et les décisions de se reproduire et / ou les dates de ponte. Au niveau proximal, nous nous sommes concentrés sur le rôle potentiel des mécanismes hormonaux.

I.3. Mécanismes hormonaux de la décision et de la phénologie de la reproduction

Les hormones sont des messagers chimiques, produits par des cellules ou des glandes sécrétrices en réponse à des stimuli internes ou environnementaux. Principalement transportées dans le système circulatoire (sang et lymphe), elles agissent sur des cellules cibles, en se liant à des récepteurs spécifiques et induisent une réponse physiologique ou comportementale. Leurs actions sont pléiotropiques, c'est-à-dire que plusieurs fonctions différentes sont activées de manière simultanée : la testostérone, par exemple, stimule les parades sexuelles mais inhibe les soins parentaux. Les hormones semblent donc être des médiateurs appropriés entre les stimuli internes, externes et l'ajustement des niveaux physiologiques et comportementaux, ce qui les place au cœur des compromis adaptatifs prédits par la théorie des traits d'histoire de vie (Stearns 1992 ; Ricklefs & Wikelski 2002).

I.3.1 La photopériode et l'axe HPG

L'augmentation de la photopériode (jours de plus en plus longs) est le signal primaire qui active l'axe Hypothalamus-Hypophyse-Gonades (axe HPG, de l'anglais hypothalamic–pituitary–gonadal axis, Fig. 4). Au niveau de l'hypothalamus, une neurohormone, la GnRH (Gonadotropin Releasing Hormone) est exprimée et active à son tour, la sécrétion d'hormones hypophysaires, les gonadotrophines : la FSH (Follicle-Stimulating Hormone) et la LH (Luteinizing Hormone, Dawson *et al.* 2001, Fig. 4). La FSH est impliquée dans l'initiation de la gamétogenèse et la régulation de la croissance des gonades, tandis que la LH régule principalement la maturation des gonades, stimule la production de testostérone par les cellules de Leydig chez les mâles, ou de la production d'œstradiol chez la femelle et provoque l'ovulation. On observe donc un pic marqué de la LH pendant la phase pré-nuptiale chez les individus vivants dans les milieux tempérés et polaires, à la saisonnalité marquée (Fig. 16, partie 'Matériel et Méthodes', exemple des mouettes tridactyles dans l'Arctique norvégien). En zone tropicale, les variations temporelles des taux de LH sont faibles (Lormée *et al.* 2000; mais voir Chastel *et al.* 2005a)

Les gonadotrophines provoquent donc un pic de sécrétion de la testostérone (famille des androgènes) et d'œstradiol, pendant la phase pré-nuptiale (Fig. 4 et 16).

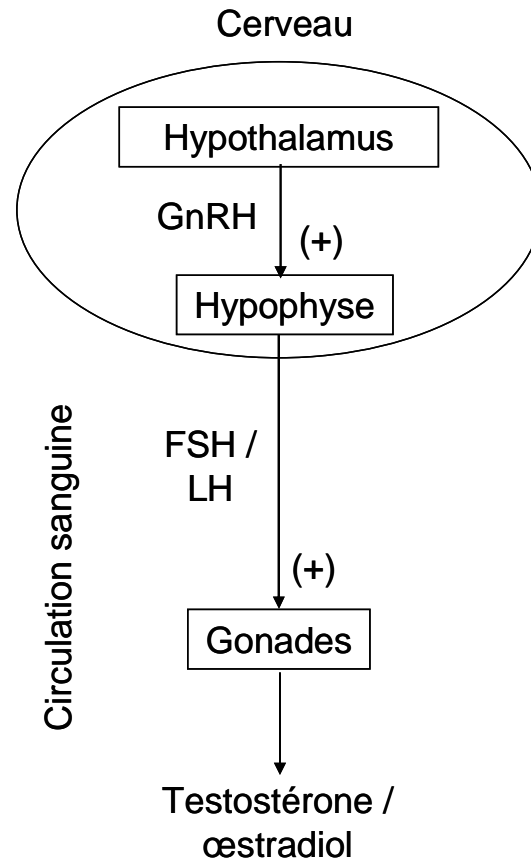


Figure 4 : Axe Hypothalamus-Hypophyse-Gonades et sécrétion de LH, FSH et d'hormones sexuelles (testostérone et œstradiol)

Ces hormones sexuelles concourent à l'expression d'un large éventail de comportements dont la construction du nid, les parades nuptiales et l'accouplement (Ball 1993). Une autre hormone sexuelle, la progestérone a une action moins connue dans la reproduction aviaire : certaines études ne reportent aucune évolution des taux de progestérone au cours de la saison de reproduction (Fivizzani & Oring 1986; Hector & Goldsmith 1985), tandis que d'autres suggèrent une action stimulatrice sur l'incubation (Davis *et al.* 1995; Fivizzani and Oring, 1986) ou sur l'élevage du poussin (Hector *et al.* 1996).

Cependant, la photopériode est un signal constant d'une année sur l'autre, et son action sur l'axe HPG ne suffit pas à expliquer les variabilités des dates de ponte. Quels sont les médiateurs proximaux qui font le lien entre les signaux environnementaux non-photopériodiques et la variation des dates de ponte ?

I.3.2. Les variables environnementales et les hormones de stress

i. Définition

On qualifie de stressant un évènement imprévisible, labile et compromettant potentiellement la survie de l'individu (Wingfield & Kitaysky 2002). A titre d'exemple, on cite la raréfaction des ressources alimentaires, un évènement météorologique particulièrement difficile et / ou un fort risque de prédation. L'allostasie est définie comme l'ensemble des processus physiologiques et comportementaux qui permettent à un organisme confronté à un évènement perturbant d'atteindre un état d'homeostasie, c'est à dire un état interne stable (McEwen 2000, McEwen & Wingfield 2003, Wingfield 2005).

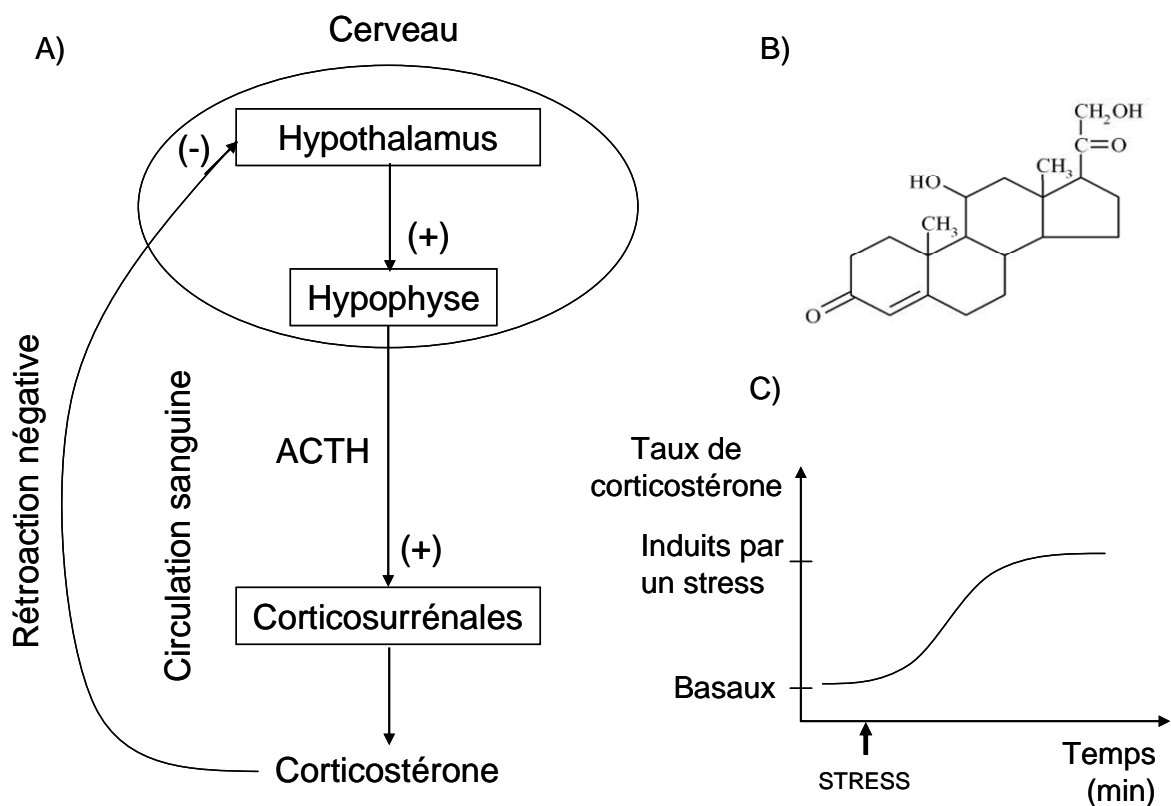
Face à une perturbation stressante, l'individu adopte un état dit d'urgence (Wingfield et al. 1998), qui conduit à différents types de réponses selon le type de perturbations rencontrées. Dans le cas de perturbations instantanées (par exemple, un risque de prédation élevé ou un évènement météorologique particulièrement difficile), une réponse physiologique et comportementale quasi-instantanée se met en place afin d'éviter, ou bien de combattre, le facteur perturbant (*the fight-or-flight response*). Dans le cas de perturbations progressives (par exemple, la raréfaction des ressources alimentaires), ou si une perturbation instantanée perdure dans le temps, l'état d'urgence active alors différents types de stratégies selon la condition physiologique de l'individu (Wingfield & Kitaysky 2002, Wingfield 2003, 2005): la fuite (*the leave-it strategy*), la recherche d'un refuge (*the take-it strategy*), ou bien la recherche d'un refuge puis la fuite si le facteur stressant persiste (*the take-it, then leave-it strategy*).

L'individu alloue donc ses ressources énergétiques à sa survie immédiate, au détriment d'activités secondaires et non-immédiatement vitales, comme la reproduction en cours. Ainsi, un évènement stressant peut engendrer l'abandon temporaire ou définitif de l'œuf ou du poussin (Wingfield & Sapolsky 2003), via notamment l'augmentation d'hormones de stress (corticostérone) et la baisse momentanée des taux d'hormones parentales (prolactine, Chastel *et al.* 2005b; Angelier *et al.* 2007b, 2009; Angelier & Chastel 2009).

Ce stress de courte durée, qualifié de stress aigu, ne doit pas être confondu avec un stress chronique, c'est-à-dire un stress qui persiste sur le long-terme.

ii. Physiologie du stress

Au niveau physiologique, chez les Vertébrés, des conditions stressantes provoquent la sécrétion accrue d'hormones de stress de la famille des glucocorticoïdes (Wingfield *et al.* 1998, Fig. 5B et C). Les glucocorticoïdes sont sécrétés par les glandes corticosurrénales, suite à l'activation de l'axe hypothalamus-hypophyse-glandes corticosurrénales (HPA, Fig. 5A). La corticostérone est le glucocorticoïde principal chez les oiseaux, et est l'équivalent du cortisol chez l'homme.



Cette réponse hormonale au stress permet aux individus de faire face à l'événement perturbateur via la mobilisation des réserves énergétiques, l'adoption de comportements de recherche et de prise alimentaire, de fuite et / ou d'attente dans un refuge, ainsi que la suppression de toutes fonctions non-immédiatement vitales, telle que la reproduction en cours (Wingfield & Sapolsky 2003; Landys *et al.* 2006; Breuner *et al.* 2008). Cette réponse au stress est considérée comme adaptative puisqu'elle permettrait, lors d'un événement stressant, de

rediriger les réserves énergétiques vers la survie immédiate au détriment de la reproduction en cours (Wingfield & Kitaysky 2002; Wingfield & Sapolsky 2003; Breuner *et al.* 2008; Bokony *et al.* 2009).

Au contraire, un stress chronique et des taux élevés de corticostérone sur le long-terme compromettent les capacités cognitives et d'apprentissage, les fonctions immunes et la guérison et diminuent la masse musculaire (revu dans Sapolsky 2000). A terme, l'exposition à des taux élevés de corticostérone diminue la survie (Romero & Wikelski 2001; Brown *et al.* 2005; Kitaysky *et al.* 2007, 2010; Goutte *et al.* 2010b).

Pour bien comprendre le mode d'action des glucocorticoïdes, il est important de distinguer deux niveaux de corticostérone : les taux basaux et les taux induits par un stress (Fig. 5C). En effet, il existe deux types de récepteurs auxquels se fixe la corticostérone : l'un est activé par de faibles taux de corticostérone (taux basaux), tandis que l'autre possède une grande affinité pour de fort taux de corticostérone (taux induits par un stress, Romero 2004).

iii. Niveau basal de corticostérone

Le niveau basal de corticostérone est un marqueur des activités et de l'état énergétique de l'individu (Kitaysky *et al.* 1999; Love *et al.* 2004; Landys *et al.* 2006) et reflète le ratio entre l'énergie disponible (dans l'environnement et / ou dans les réserves) et la demande énergétique pour la réalisation d'une activité spécifique (Landys *et al.* 2006). Le taux de corticostérone basale est élevé lorsque la disponibilité des ressources diminue (Kitaysky *et al.* 1999; Lynn *et al.* 2003; Kitaysky *et al.* 2007), lorsque le succès de recherche alimentaire est faible (Angelier *et al.* 2007c), lorsque les réserves énergétiques de l'individu sont réduites (Cherel *et al.* 1988; Lynn *et al.* 2003) et / ou lorsque les demandes énergétiques sont accrues, comme par exemple lors de la reproduction (Love *et al.* 2004; Landys *et al.* 2006).

Le taux de corticostérone basale est souvent associé à une mesure physiologique et intégrative de la fitness (*Cort-fitness hypothesis*, revu et critiqué par Bonier *et al.* 2009) et par extension de la qualité d'un individu (Angelier *et al.* 2010). Il a été ainsi montré, chez les mâles d'albatros à sourcils noirs, *Thalassarche melanophrys*, que des individus ayant eu, sur le long-terme, le moins de descendants (indice d'une faible qualité) présentaient des taux élevés de corticostérone basale et que ces taux étaient hautement répétables d'une saison à l'autre (Angelier *et al.* 2010). Cependant, il faut garder à l'esprit que certaines activités, telles

que la reproduction, nécessitent un travail soutenu et sont aussi caractérisés par une augmentation des taux de corticostérone basale (Landys *et al.* 2006; Bonier *et al.* 2009).

iv. Niveau de corticostérone induit par un stress

Le niveau maximal de corticostérone (Fig. 5C) est le taux de corticostérone atteint après un épisode stressant, comme par exemple après une situation climatique contraignante (Wingfield *et al.* 1983; Rogers *et al.* 1993; Smith *et al.* 1994; Astheimer *et al.* 1995), un fort risque de prédation (Silverin & Wingfield 1998; Scheuerlein *et al.* 2001) ou la capture (Wingfield *et al.* 1994). Ce taux maximum de corticostérone correspond, en moyenne et selon les espèces, à une multiplication par 5-10 des taux basaux (Bokony *et al.* 2009). Il reflète le degré de sensibilité au stress de l'individu, et est assimilé, par extension, à la perception individuelle du risque ainsi qu'au degré d'investissement dans la reproduction en cours (Ricklefs & Wikelski 2002; Wingfield & Sapolsky 2003; Lendvai *et al.* 2007; Bokony *et al.* 2009). D'après la théorie des traits d'histoire de vie, la réponse au stress peut être atténuée lorsque la valeur de la reproduction en cours est élevée, relativement à la valeur des opportunités de reproductions futures (Wingfield & Sapolsky 2003). Plusieurs études expérimentales et comparatives convergent vers cette hypothèse (*brood value hypothesis*, Lendvai *et al.* 2007; Lendvai & Chastel 2008; Bokony *et al.* 2009).

Ainsi, les glucocorticoïdes orchestrent l'ajustement des compromis adaptatifs. Avant la période de reproduction, ils pourraient jouer le rôle de médiateurs entre les conditions environnementales, individuelles, les décisions de ne pas se reproduire et / ou les dates de ponte.

I.3.3. Le contrôle hormonal de la phénologie de reproduction

i. Mécanismes hormonaux orchestrant la décision de ne pas se reproduire

Schoech *et al.* (1996) a montré que les non-reproducteurs sont physiologiquement aptes à se reproduire chez le geai à gorge blanche, *Aphelocoma coerulescens*, une espèce utilisant un système de coopération pour la reproduction (un couple est aidé par un ou plusieurs oiseaux

non-reproducteurs dit ‘helpers’). En effet, pendant la phase de construction du nid, les mâles et femelles non-reproducteurs, mais ‘helpers’, ont des taux de LH équivalents aux reproducteurs ; en revanche, les mâles non-reproducteurs ont des taux plus faibles de testostérone, du à un manque de stimulation ; les femelles non-reproductrices ont des follicules ovariens moins développés que les femelles reproductrices (Schoech *et al.* 1996). De plus, Blas & Hiraldo (2010) se sont intéressés aux variations saisonnières des hormones sexuelles chez les milans noirs, *Milvus migrans* : entre les mâles reproducteurs et non reproducteurs, les taux de testostérone ne sont pas significativement différents, tandis que les femelles non reproductrices ont des taux d’œstradiol plus faibles que les femelles reproductrices.

Un rôle inhibiteur de la corticostérone sur l’axe HPG et sur la décision de se reproduire semble être très probable. Chez la femelle de diamant mandarin (*Taeniopygia guttata*), l’élévation expérimentale des taux de corticostérone avant la ponte, provoque une diminution du nombre de femelles reproductrices (Salvante & Williams 2003). De même, chez les iguanes marins *Amblyrhynchus cristatus*, les femelles qui ne se reproduisent pas ont les plus fortes concentrations de corticostérone basale et de corticostérone induite par un stress pendant la période pré-nuptiale comparées aux femelles qui se reproduisent (Vitousek *et al.* 2010).

ii. Mécanismes hormonaux orchestrant la date de ponte

Très peu d’études ont réussi à établir un lien entre les niveaux pré-nuptiaux de corticostérone (basaux et /ou maximum, *i.e.* induits par un stress) et la variation individuelle des dates de ponte chez les animaux sauvages (Schoech *et al.* 2009). Les animaux sauvages, et tout particulièrement les femelles, sont en effet moins accessibles avant la période de reproduction que pendant l’incubation et l’élevage des jeunes. Quelques rares études suggèrent néanmoins un rôle inhibiteur de la corticostérone basale sur les décisions de ponte chez les femelles. Une approche descriptive chez le geai à gorge blanche révèle que les femelles avec de forts taux basaux de corticostérone initient leur ponte plus tard que les femelles avec de faibles taux basaux de corticostérone (Schoech *et al.* 2009). Cependant cette relation n’est observée que lors des années défavorables et est masquée lors des années favorables. Une approche expérimentale chez la femelle de diamant mandarin démontre que l’élévation expérimentale des taux de corticostérone avant la ponte provoque un retard de la ponte (Salvante & Williams

2003). Toutefois, l'administration de corticostérone aux femelles de geai à gorge blanche nourries *ad libitum* ne retarde pas les dates de ponte (Schoech *et al.* 2007). A ce jour et à notre connaissance, aucune étude n'a exploré les taux de corticostérone induit par un stress comme mécanisme potentiel de la perception du stress et de l'investissement dans une reproduction précoce (Goutte *et al.* 2010a). La corticostérone semble faire le lien entre des conditions environnementales stressantes et une date de ponte tardive, mais ces relations apparaissent complexes, dépendantes de l'environnement et méritent une investigation plus poussée.

iii. Relation entre les glucocorticoïdes et l'axe HPG

Quelle est l'action fonctionnelle des glucocorticoïdes sur les dates de ponte et les décisions de se reproduire? Comment agit la corticostérone au niveau physiologique ?

Chez les animaux sauvages, le rôle de la corticostérone sur l'axe HPG, et plus particulièrement sur la sécrétion de LH, de FSH et d'hormones sexuelles a été très peu exploré chez les femelles avant la ponte et semble complexe chez les mâles (Wingfield & Sapolsky 2003; Schoech *et al.* 2009). En effet, en captivité, l'injection de corticostérone directement dans l'hypothalamus des mâles de bruant hudsonien (*Spizella arborea*, Wilson & Follett 1975) diminue les taux basaux de LH et la croissance des testicules. A l'opposé, l'implantation sous-cutanée de corticostérone ne modifie pas les taux de LH et de testostérone chez les mâles de cette même espèce, en milieu naturel (Astheimer *et al.* 2000). En outre, le cortisol inhibe le développement testiculaire et la sécrétion de testostérone mais sans réduire la production de LH chez les mâles de carpes (*Cyprinus carpio L.*, Consten *et al.* 2001; Consten *et al.* 2002) et de cochons d'inde (*Cavia aperea porcellus*, Fenske 1997).

Des études menées sur les animaux domestiques peuvent apporter quelques pistes sur l'action des glucocorticoïdes sur l'axe HPG. En effet, chez des brebis, *Ovis hamon*, ayant subi une ovariectomie, un stress psychosocial et / ou l'augmentation expérimentale de cortisol plasmatique réduit considérablement l'amplitude de sécrétion pulsatile de LH, via la suppression de la réponse de l'hypophyse au GnRH (revue dans Breen & Karsch 2006). De plus, l'augmentation expérimentale de cortisol chez les brebis ayant subi une ovariectomie puis traitées à l'œstradiol et à la progestérone, réduit la fréquence des pulses de GnRH (Oakley *et al.* 2009). Ainsi, les stéroïdes ovariens jouent un rôle dans l'inhibition de la GnRH par le cortisol.

On peut donc raisonnablement s'attendre à un effet inhibiteur des glucocorticoïdes sur l'axe HPG chez les animaux sauvages, mais aussi à des différences entre mâles et femelles dans le rôle fonctionnel de la corticostérone sur l'axe HPG, comme suggéré par Ball & Ketterson (2008) et / ou à des variations interindividuelles.

I.3.4. L'effet de l'âge sur les taux d'hormones

Comme évoqué précédemment, la décision de ne pas se reproduire et la date de ponte sont fortement influencées par l'état physiologique des individus, et notamment par leur âge. Les glucocorticoïdes pourraient jouer le rôle de médiateurs entre l'âge et la décision de ne pas se reproduire et / ou les dates de ponte. Quels sont les effets connus de l'âge sur les taux de corticostérone, de LH et d'hormones sexuelles ?

i. Age et corticostérone

Les études présentées ci-dessous ont été menées pendant l'incubation et / ou l'élevage du poussin. Chez les espèces longévives, les taux basaux de corticostérone ne sont pas corrélés à l'âge (grand albatros, *Diomedea exulans*, Angelier *et al.* 2006; sterne pierregarin *Sterna hirundo*, Heidinger *et al.* 2006; pétrel des neiges, *Pagodroma nivea*, Angelier *et al.* 2007b; albatros à sourcils noirs, *Thalassarche melanophris*, Angelier *et al.* 2007d). Cependant, le nombre d'expérience de reproductions antérieures, souvent lié à l'âge, est très corrélé aux taux basaux de corticostérone (Angelier *et al.* 2006; Angelier *et al.* 2007d). Pour le grand albatros en période d'incubation, les taux de corticostérone augmentent avec l'expérience de reproduction avant de diminuer chez les individus sénescents, tandis que pour l'albatros à sourcils noirs en période d'élevage du poussin, les taux de corticostérone sont au contraire plus élevés chez les individus inexpérimentés et sénescents (Angelier *et al.* 2006; Angelier *et al.* 2007d). Ainsi, les patrons obtenus diffèrent grandement entre les espèces et les stades de reproduction.

Concernant les taux de corticostérone induit par un stress, la sensibilité au stress décline avec l'âge chez la sterne pierregarin (Heidinger *et al.* 2006), ce qui est interprétée comme un investissement croissant dans la reproduction en cours. Des injections d'ACTH (Adrenocorticotropie Hormone, Heidinger *et al.* 2008) ont pu montrer que l'atténuation de la réponse au stress (taux maximum de corticostérone) avec l'âge était due à une baisse des

capacités adrénales. Par ailleurs, on constate une diminution avec l'âge de la sensibilité au stress de certaines hormones parentales, telles que la prolactine (Angelier *et al.* 2007b, Angelier & Chastel 2009; Heidinger *et al.* in press).

ii. Age, LH et hormones sexuelles

L'âge influence aussi les taux de LH et d'hormones sexuelles, avant et après la maturité sexuelle. Les résultats sont cependant contrastés, selon les espèces et le sexe. Avant d'atteindre la maturité sexuelle, les femelles de grands albatros sécrètent de la progestérone mais pas d'œstradiol et leur taux de LH basale augmente avec l'âge (Hector *et al.* 1990). Pour les individus matures sexuellement, il existe aussi des relations entre l'âge et les taux de LH et d'hormones sexuelles. Chez les femelles de sterne pierregarin, entre la ponte des deux premiers œufs, le taux basal de LH augmente avec l'âge, tandis que les taux d'œstradiol et de progestérone ne varient pas avec l'âge (Nisbet *et al.* 1999). Chez les mâles de la même espèce, le taux d'androgène augmente pendant les 5 premières années, puis diminue progressivement, de 5 à 21 ans, et le taux de LH ne varie pas avec l'âge (Nisbet *et al.* 1999). De plus, les taux de testostérone et de progestérone des mâles, et les taux d'œstradiol et de progestérone des femelles reproductrices de milan noir ne sont pas liés à l'âge (1 à 13 ans, Blas & Hiraldo 2010).

I.4. Problématique et objectif du doctorat

Les variations de la phénologie de la reproduction dénotent de la grande flexibilité des individus à répondre aux conditions environnementales en fonction de leur état physiologique. Cependant, certains facteurs proximaux, et principalement les mécanismes hormonaux, sous-jacents restent très peu compris et explorés.

Ce doctorat vise à concourir à l'identification des mécanismes hormonaux orchestrant les décisions de reproduction et des dates de ponte.

Ce doctorat s'est concentré sur les milieux polaires, caractérisés par une saisonnalité très marquée et d'importantes contraintes environnementales. Bien que ces milieux soient particulièrement sensibles aux changements globaux, les données phénologiques y sont rares et diffèrent des tendances observées dans les milieux tempérés (Gaston *et al.* 2005a,b; Barbraud & Weimerskirch 2006; Moe *et al.* 2009).

Nos deux modèles d'études sont :

- les mouettes tridactyles, au Svalbard, qui présentent une grande variabilité de phénologie de la reproduction au cours des dernières décennies, dû en partie, aux conditions océanographiques, comme la température de surface de mer et l'étendue de glace (Moe *et al.* 2009). Les années tardives sont associées à un faible succès reproducteur, soulignant l'importance de la date de ponte en termes de valeur sélective (Moe *et al.* 2009). Facilement manipulable et accessible avant la ponte, cette espèce présente des atouts techniques et scientifiques pour l'étude des mécanismes hormonaux pré-nuptiaux contrôlant la décision de reproduction et date de ponte.

- les pétrels des neiges en Antarctique, dont la phénologie de reproduction varient fortement d'une année sur l'autre et qui présentent une tendance au retard de la reproduction (Barbraud & Weimerskirch 2006). Chez cette espèce, l'âge affecte de façon quadratique la décision de se reproduire et la date de ponte (Berman *et al.* 2009; Goutte *et al.* 2010). Grâce à un suivi à long-terme exceptionnel (depuis 1963-1964) et aux connaissances des mécanismes endocriniens pendant la période de reproduction (Angelier *et al.* 2007b), le pétrel des neiges est idéal pour l'intégration d'un volet sur les effets de l'âge sur les mécanismes proximaux

régissant les décisions de reproduction et de dates de ponte. Enfin, l'accès au nid fait du pétrel des neiges, un modèle privilégié pour la manipulation expérimentale de la phénologie, via un échange entre les œufs précoces et tardifs.

Les objectifs de cette étude sont :

(i) de replacer la phénologie dans un contexte d'acquisition et d'allocation d'énergie, avec une approche éco-physiologique.

Chez les mouettes tridactyles, nous avons comparé les ajustements physiologiques, comportementaux et phénologiques lors de deux années aux conditions environnementales contrastées. Chez le pétrel des neiges, nous avons testé les effets d'une manipulation expérimentale des dates d'éclosion (retard ou avancée) sur l'effort parental.

(ii) de comprendre les mécanismes hormonaux impliqués dans les variations des dates de pontes et des décisions de ponte en fonction de l'âge des pétrels des neiges.

Nous nous sommes concentrés non seulement sur les taux de corticostérone, mais aussi sur la dynamique de la sécrétion de la LH.

(iii) de comprendre l'action fonctionnelle de la corticostérone sur la phénologie et sur la sécrétion d'hormones impliquées dans l'initiation de la reproduction chez la mouette tridactyle.

Par une approche descriptive, puis expérimentale, nous avons examiné les liens entre les taux de corticostérone, la décision de ne pas se reproduire et les dates de ponte, ainsi que les liens entre la corticostérone et l'inhibition de la sécrétion de la LH et / ou des hormones sexuelles (testostérone). Une attention particulière a été portée aux différences et similitudes entre mâles et femelles.

Matériels et méthodes



Entre chaque montagne il s'est formé d'immenses glaciers dont la hauteur augmente chaque année ; cette élévation croissante de glaciers est inévitable : un été de quelques semaines ne peut fondre complètement ces immenses amas de neige que répand sur le Spitzberg un hiver de dix mois, et dans un temps donné les glaciers atteindront presque le sommet des pics de granit.

Léonie d'Aunet (1839)

II. Matériels et méthodes

Les données nécessaires à ce travail de thèse ont été récoltées au cours de quatre campagnes de terrain, dans l'Arctique norvégien aux printemps 2008 et 2009, et en Antarctique lors des étés austraux 2007/2008 et 2008/2009. Cette section est consacrée à la présentation des deux sites d'études, à la biologie des espèces, ainsi qu'à la description détaillée des protocoles et des analyses employés.

II.1. Sites et modèles d'étude

II.1.1. Sites d'étude : les milieux polaires

i. Le Svalbard : territoire Arctique

Bordé par l'océan Arctique, le Spitzberg est la plus grande île de l'archipel du Svalbard, au nord de la Norvège (Fig. 6, 7). La colonie d'étude de Mouette tridactyle se situe à Krikjefjellet, en Baie du Roi, près de la base scientifique de Ny-Ålesund (78°56'N, 11°56'E, Fig. 6, 7). La côte ouest du Spitzberg bénéficie de températures légèrement tempérées dû au courant chaud de l'Atlantique Nord, la branche la plus septentrionale du Gulf Stream. Les températures sont en moyenne de 6.7°C pendant le mois le plus chaud (juillet). De mi avril à mi août, le jour y est permanent.

ii. La Terre Adélie : territoire Antarctique

Sur la côte Antarctique, l'île des Pétrels appartient à l'archipel de Pointe Géologie (66°40'S, 140°01'E), en Terre Adélie (Fig. 8, 9). Les colonies d'étude de pétrels des neiges sont dispersées autour de la station scientifique de Dumont d'Urville, basée sur l'île des Pétrels mais aussi sur les îles alentour (Fig. 8, 9). Les vents y sont très violents et les températures sont basses, y compris pendant l'été : au cours du mois le plus chaud (décembre), les températures moyennes sont de -1°C. En été austral, le soleil passe la ligne d'horizon autour de 23 heures pour se lever vers 1h30.



Figure 6 : Carte de l'Arctique. La flèche rouge indique le Svalbard. Source : IPEV

Figure 7 : Vue aérienne du village scientifique de Ny Ålesund et de la Baie du Roi, Svalbard.



Figure 8 : Carte de l'Antarctique. La station scientifique française Dumont D'Urville (flèche rouge) se situe en Terre Adélie. Source : IPEV

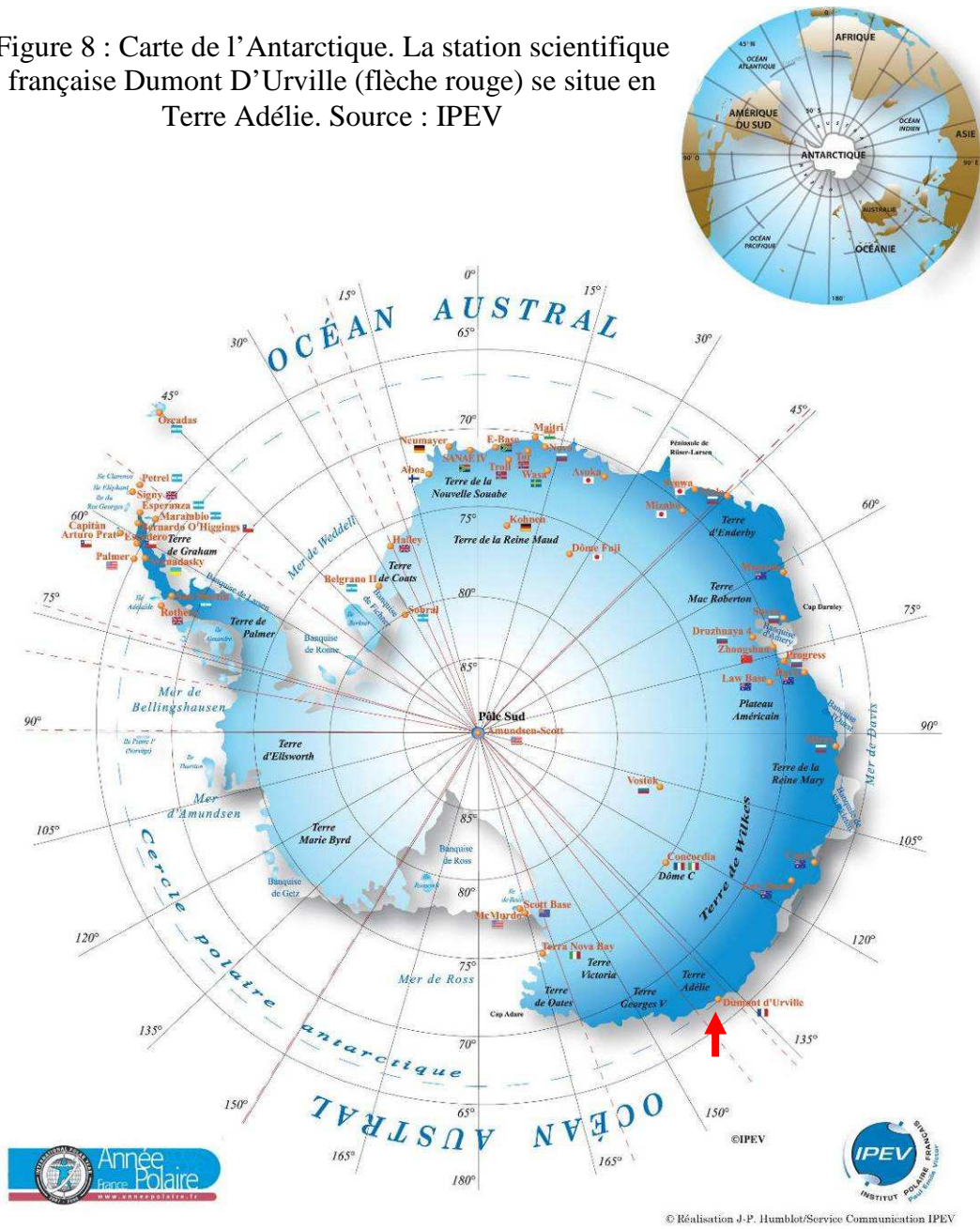


Figure 9 : Vue aérienne de l'archipel de Pointe Géologie, dont la plus grande île est l'île des Pétrels. Source : IPEV



II.1.2. Biologie des espèces étudiées

i. La mouette tridactyle

Oiseau marin pélagique, la mouette tridactyle (*Rissa tridactyla*, Linnaeus 1758) appartient à la famille des *Laridae* et à l'ordre des *Charadriiformes*. D'une longueur de 36 à 40 cm, elle a une envergure de 95 à 120 cm pour un poids de 340 à 500 g. Elle niche en colonies sur les falaises côtières de l'Atlantique Nord, du Pacifique Nord et de l'océan Arctique. Le Spitzberg constitue la limite nord de son aire de répartition (Cramp 1983) et accueille environ 300 000 à 500 000 couples (Mehlum 1990).

En Baie du Roi, les mouettes tridactyles occupent les colonies dès le mois d'avril (Mehlum 1990). La ponte a lieu la deuxième moitié du mois de juin et peut exceptionnellement atteindre trois œufs. Durant la période pré-nuptiale, les mouettes paradent, s'accouplent, défendent et construisent leur nid à partir de débris végétaux. Les œufs, formés en 6-8 jours, sont pondus et incubés pendant approximativement 27 jours, par les deux membres du couple (Coulson & Wooller 1984). La première partie de l'élevage (jeunes poussins) est la plus coûteuse en énergie puisque les parents doivent se relayer au nid pour nourrir et réchauffer leurs poussins (Fyhn *et al.* 2001; Bech *et al.* 2002). Une fois les poussins indépendants thermiquement, les parents partent simultanément en mer acquérir des ressources pour eux-mêmes et pour leurs petits. L'envol a lieu une quarantaine de jours après l'éclosion (Roberts & Hatch 1993), soit en fin août / début septembre (Fig. 10).

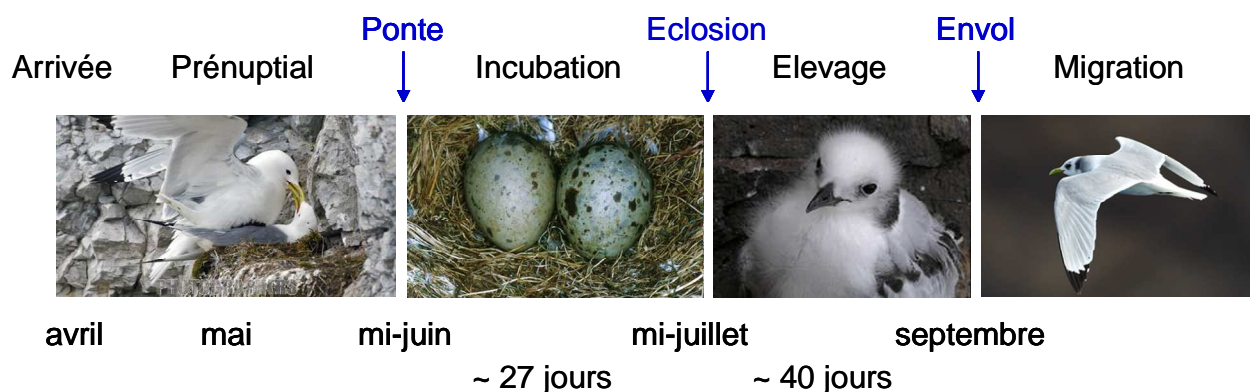


Figure 10 : Cycle de vie de la mouette tridactyle en Baie du Roi, Svalbard

La mouette tridactyle constitue un bon indicateur de la fluctuation de l'environnement. En Baie du Roi, elle fait l'objet d'un suivi démographique à long terme depuis 1970. Les conditions environnementales avant la ponte (avril-mai) déterminent en partie, la phénologie de reproduction : les mouettes sont précoces lors des années de fortes SST (Sea Surface Temperature, Moe *et al.* 2009). De plus, les années tardives sont associées à un fort échec reproducteur (Moe *et al.* 2009). Au Svalbard, le régime alimentaire des mouettes repose essentiellement sur de jeunes morues polaires, *Boreogadus saida*. Cependant, lorsque les ressources se font rares, elle se nourrit de ptéropodes (escargots marins planctoniques) et de crustacés planctoniques (amphipodes).

Accessible sur le nid environ deux mois avant la ponte, cette espèce présente un intérêt considérable pour l'étude des mécanismes hormonaux impliqués dans les traits d'histoire de vie (programme de recherche soutenu par l'IPEV, responsable O. Chastel). Facilement manipulable, elle est appropriée à la pose d'enregistreurs miniaturisés (Angelier *et al.* 2007a) ainsi qu'aux manipulations expérimentales des taux d'hormones (Kitaysky *et al.* 2001; Angelier *et al.* 2007a, 2009; Goutte *et al.* 2010b).

ii. *Le pétrel des neiges*

Le pétrel des neiges (*Pagodroma nivea*, Forster 1777) est un oiseau marin extrêmement pagophile, autrement dit dont l'écologie est étroitement associée à la glace de mer. Il appartient à la famille des *Procellariidae* et à l'ordre des *Procellariiformes*. D'une longueur de 30 à 40 cm, il a une envergure de 75 à 95 cm pour un poids de 315 à 570 g. De distribution circumpolaire, sur la côte antarctique mais aussi à l'intérieur du continent, le pétrel des neiges recherche ses proies au niveau de la banquise et à proximité des icebergs. Fidèle au nid et à son partenaire (Bried *et al.* 2003) il niche en colonies dispersées, dans des crevasses et des trous de rochers, sous les éboulis.

L'arrivée a lieu fin octobre, les parades et les accouplements au cours du mois de novembre (période pré-nuptiale, Fig. 11). Pendant l'exode pré-nuptial (2-3 semaines), la femelle accumule en mer, les réserves énergétiques nécessaires à la formation de l'œuf unique. La taille des femelles explique 24 % de la variation des dates de ponte (Barbraud *et al.* 2000). L'œuf est pondu en décembre, sans remplacement en cas d'échec, et est incubé entre 41 et 49 jours (Fig. 11). Après l'éclosion, en janvier, les parents se relaient au nid pour

garder, nourrir et réchauffer le poussin. Le poussin émancipé (42 - 54 jours) s'envole en mars (Fig. 11).

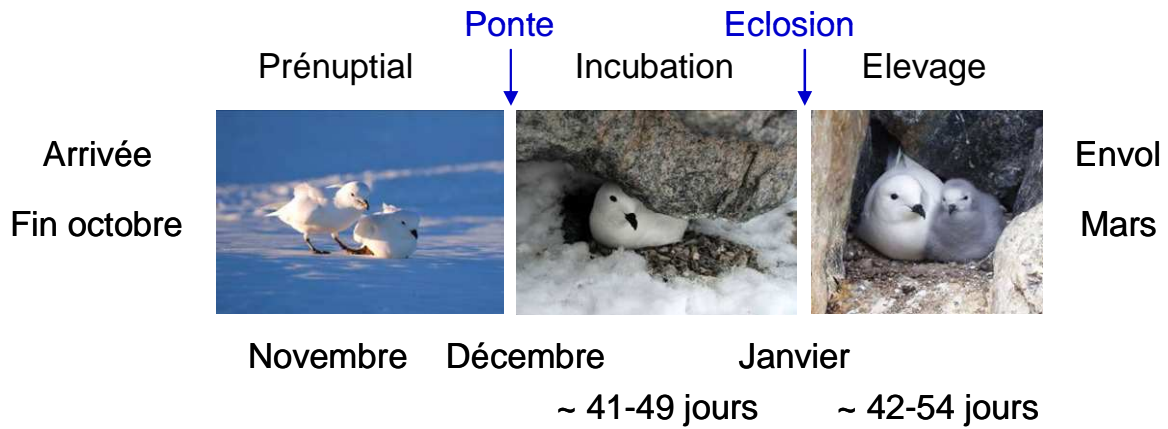


Figure 11 : Cycle de vie du pétrel des neiges en Terre Adélie.

Les dates de ponte affectent le succès reproducteur au niveau individuel chez les pétrels des neiges : en 2007-2008, le succès reproducteur décroît lorsque les dates de ponte sont tardives (Fig. 12).

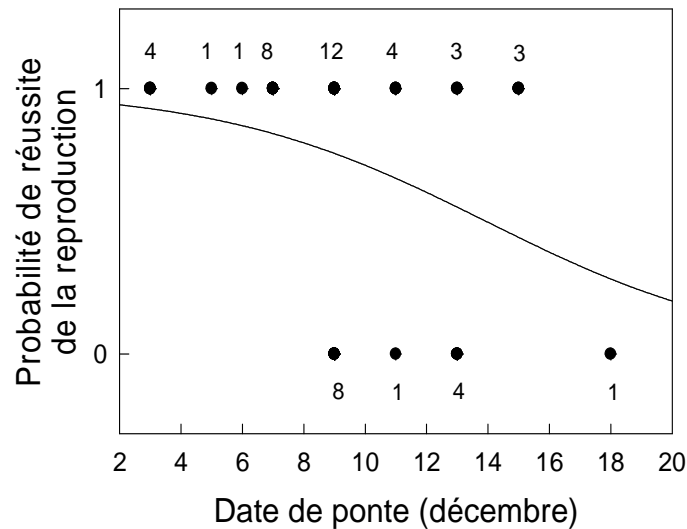


Figure 12 : Probabilité de réussite (poussin à l'envol) en fonction de la date de ponte chez les pétrels des neiges en décembre 2007. Les effectifs sont donnés pour chaque date de ponte.

Les colonies de pétrels des neiges en Terre Adélie font l'objet d'un suivi démographique (programme soutenu par l'IPEV, responsable H. Weimerskirch). Tous les poussins sont bagués avant leur envol, sur l'ensemble des îles de Pointe Géologie. Etant philopatric, un certain nombre d'adultes, bagués poussins, reviennent sur le site d'étude pour s'y reproduire et sont ainsi d'âge connu. Cette espèce a une faible fécondité et est extrêmement longévive (Chastel *et al.* 1993; Jenouvrier *et al.* 2005). Le plus vieux pétrel des neiges manipulé dans le cadre de ce doctorat a été bagué poussin il y a 45 ans. L'âge à la première reproduction est estimé à 9.9 ans en moyenne (Chastel *et al.* 1993), les immatures restant en mer ou visitant occasionnellement les colonies à partir de 5 ans (Weimerskirch *et al.* 1986; Chastel *et al.* 1993).

Afin d'étudier les mécanismes hormonaux impliqués dans la décision de se reproduire et dans la date de ponte, nous avons employé une approche descriptive (covariation naturelle entre les taux d'hormones et la date de ponte) puis une approche expérimentale (manipulation de la date de ponte et manipulation des taux hormonaux).

II.2. Approche descriptive

II.2.1. Captures et baguages

Pendant la période pré-nuptiale, les oiseaux sont attrapés au nid, à l'aide d'une courte gaffe pour les pétrels des neiges, ou d'un nœud coulant à l'extrémité d'une canne à pêche de 5 mètres pour les mouettes tridactyles. Chaque individu reçoit alors une bague métal possédant un numéro unique.



Figure 13 : Identification de mouettes tridactyles au télescope depuis le bas de la colonie grâce à leur bague Darwick. Photos : C. Clément Chastel

Les mouettes reçoivent également une bague en plastique (bague Darwick), comportant un code unique de trois lettres (Fig. 13). L'identification des mouettes ainsi baguées est possible au télescope (Fig. 13) à une certaine distance de la colonie, ce qui limite les perturbations humaines. Pour les deux espèces, une marque au feutre sur la tête permet de plus, d'identifier un oiseau manipulé lorsque sa bague n'est pas directement visible.

II.2.2. Mesures biométriques et indices de condition corporelle

Une fois capturés, les oiseaux sont mesurés afin d'avoir un indice de leur taille corporelle. Le crane (tête + bec) et le tarse sont mesurés à l'aide d'un pied à coulisse (précision de 0.1mm), l'aile pliée grâce à un réglet (précision de 1mm, Fig. 14). De plus, la masse corporelle est enregistrée à l'aide d'une balance Pesola (précision de 1g). Si nécessaire,

l'œuf et / ou le poussin de pétrels des neiges sont aussi mesurés et / ou pesés (Fig. 14). Pour le calcul de l'indice de condition corporelle, on se reportera aux méthodes détaillées dans les articles.



Figure 14 : Mesure biométrique du crâne (tête + bec), du tarse et de l'aile d'un adulte et d'un poussin de pétrel des neiges. Photos : A. Goutte et A.M. Thierry

II.2.3. Prises de sang, réponse au stress, et capacité à sécréter de la LH

i. Prises de sang

Juste après la capture (chronomètre en main), une prise de sang est effectuée par ponction de la veine alaire à l'aide d'une aiguille (25G) fixée à une seringue de 1 mL préalablement héparinée (Fig. 15A). Le chronomètre permet de calculer le temps écoulé entre la capture (début du stress) et la fin de la prise de sang. Cette durée de première prise de sang peut influencer les taux d'hormones mesurés : au-delà de 3 minutes après la capture, la

corticostérone est sécrétée et les taux mesurés ne sont plus basaux (Romero & Reed 2005, Fig 15B). Dans ce cas, un seuil est déterminé, au-delà duquel les taux de corticostérone augmentent significativement avec la durée de première prise de sang et ne sont plus considérées comme basales.

Le sang est recueilli dans un tube Eppendorf, et conservé dans une boîte isotherme à environ 0°C. Moins de 6 heures après la prise de sang, les échantillons sont centrifugés à 7000 tours par minute pendant 10 minutes. Le plasma, où sont diluées les hormones, est pipeté et séparé du culot d'hématie, dont l'ADN servira au sexage. Tous les tubes sont congelés à -20°C, puis rapatriés au CEBC pour les analyses. En plus des taux basaux de corticostérone, cette première prise de sang, immédiatement après la capture, sert à déterminer les taux basaux de LH et de testostérone.

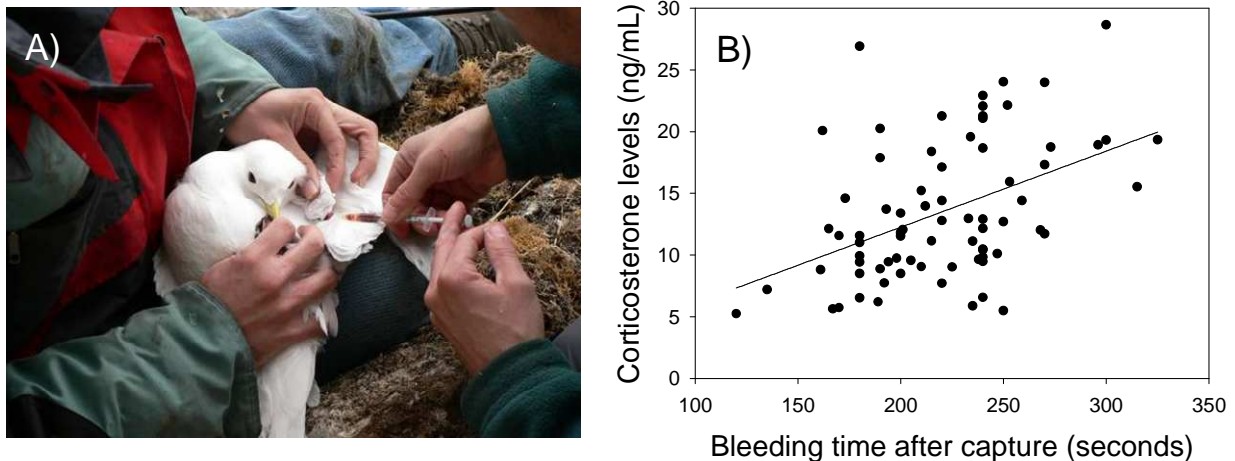


Figure 15 : A) Prise de sang par ponction de la veine alaire d'une mouette tridactyle. (Photo : C. Clément-Chastel), B) Augmentation du taux de corticostérone en fonction du temps de prise de sang. Au-delà des 3 minutes, les taux de corticostérone ne sont plus basaux.

Afin de s'assurer que la phase pré-nuptiale (période de parades et d'accouplements) était la période idéale pour l'étude des mécanismes hormonaux impliqués dans les décisions de ponte, nous avons étudié les variations saisonnières de différentes hormones. Les taux pré-nuptiaux de LH chez les mâles et les femelles, ainsi que les taux de testostérone chez les mâles sont à leurs niveaux maximum, par rapport aux taux à l'arrivée, à l'incubation et / ou pendant l'élevage des poussins (mouettes tridactyles, saison de reproduction 2008, Fig. 16). De plus, les taux de corticostérone sont élevés en phase pré-nuptiale et diminuent après la ponte. Les taux de prolactine, hormone des soins parentaux, augmentent au cours de la saison (Fig. 16).

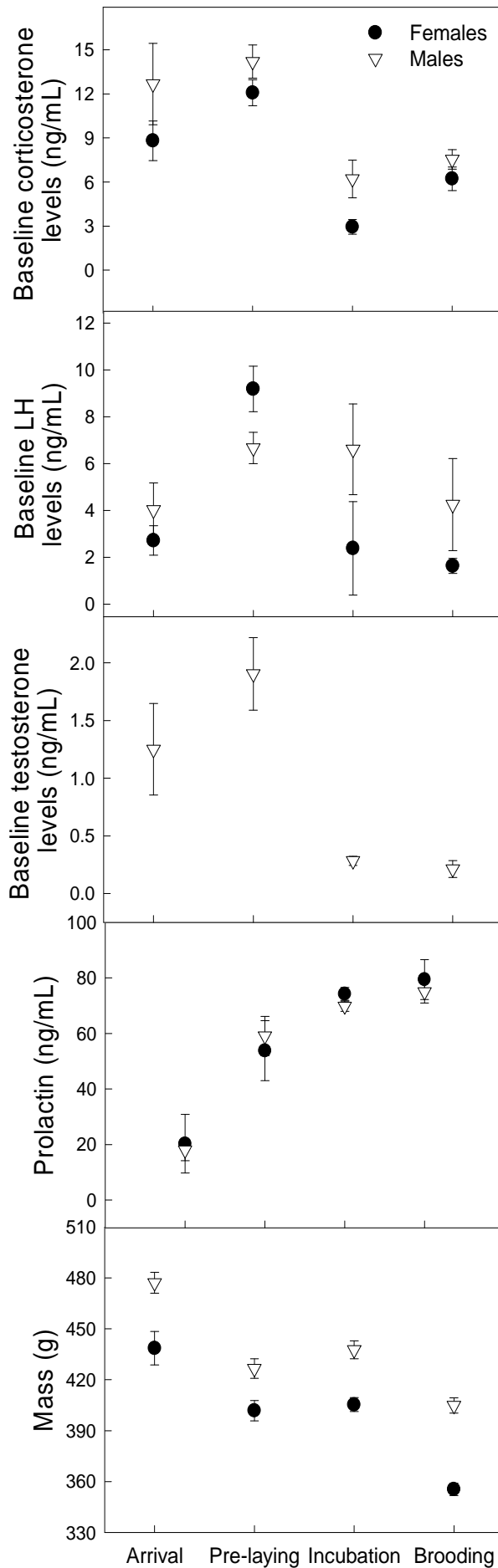


Figure 16: Variation saisonnière des taux basaux de corticostérone, LH, testostérone et prolactine (ng/mL) et de la masse (g) de mouettes tridactyles pendant la saison de reproduction de 2008. Les moyennes et les erreurs types (SE) sont données pour chaque période, à savoir l'arrivée (avril), le pré-nuptial (fin mai / début juin), l'incubation (fin juin) et l'élevage des poussins (fin juillet). Les mâles sont représentés par un triangle blanc et les femelles par un rond noir.

ii. Protocole de réponse au stress

Lors d'un stress, la corticostérone est sécrétée et circule dans le sang, jusqu'à atteindre rapidement une concentration maximale. L'amplitude de cette sécrétion de corticostérone (aussi appelée réponse au stress) témoigne de la perception individuelle du risque et oriente les décisions d'histoire de vie (Wingfield *et al.* 1998). Elaboré par Wingfield *et al.* (1994), un protocole standardisé consiste à prélever du sang immédiatement après la capture, afin de déterminer le taux basal de corticostérone, puis de maintenir l'oiseau captif pendant 30 minutes, dans un sac de toile, et enfin d'effectuer une seconde prise de sang à 30 minutes, afin de déterminer les taux de corticostérone induits par un stress (Fig. 17).

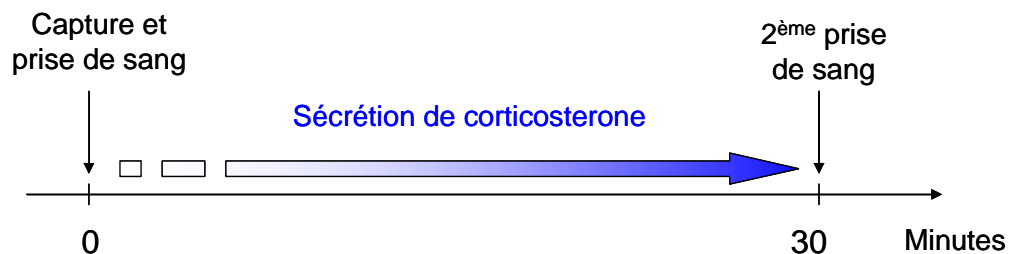


Figure 17 : Protocole de réponse au stress

Les taux de corticostérone augmentent en période prénuptiale, chez les deux espèces étudiées, après un protocole de réponse au stress (Fig. 18 A, B). Ce protocole de stress permet aussi d'étudier la réponse au stress d'autres hormones, comme la LH et la testostérone.

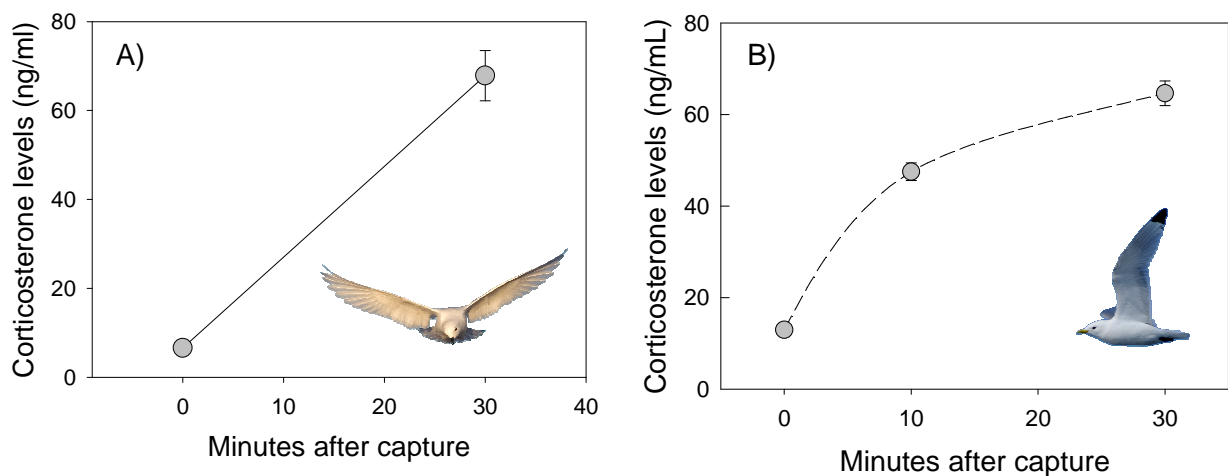


Figure 18 : Evolution des taux de corticostérone avant (0 min) et après (30 min) un protocole de réponse au stress chez les pétrels des neiges (A), et les mouettes tridactyles (B)

iii. Test de réponse à la GnRH

Afin de mesurer la capacité à sécréter de la LH, le test de réponse à la GnRH consiste à injecter une solution de GnRH qui induit un pic rapide et transitoire de LH (Schoech *et al.* 1996). La GnRH est en effet perçue par l'hypophyse comme un signal déclencheur de la production et de la sécrétion de LH. Le protocole de réponse à la GnRH (Fig. 19) consiste à effectuer une première prise de sang, immédiatement après la capture de l'oiseau, puis de lui administrer une solution de GnRH. Dix minutes après l'injection, une seconde prise de sang renseignera sur les taux maximum de LH, induits par cette injection de GnRH. Les taux de LH diminuent progressivement après le pic, si bien qu'une dernière prise de sang à 30 minutes indiquera alors l'amplitude de déclin (Fig. 19).

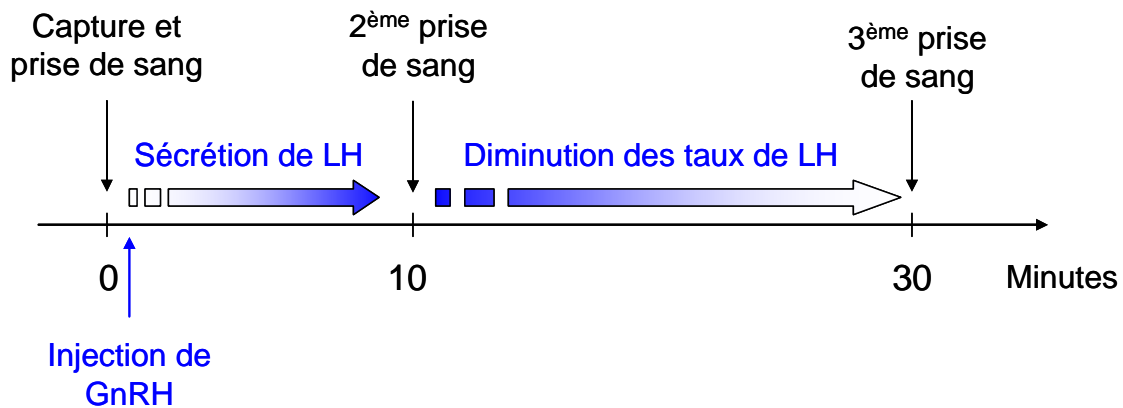


Figure 19 : Protocole du test de réponse à la GnRH. Après une injection de GnRH (ou de LHRH, un équivalent de la GnRH), la sécrétion de LH est stimulée. Des prises à 0, 10 et 30 minutes renseignent sur la dynamique (augmentation et déclin) des taux de LH.

La solution de GnRH a été préparée par dissolution d'un équivalent de la GnRH, la LHRH (Luteinizing Hormone Releasing Hormone, [Gin⁸], Sigma Lot 121H04314) dans une solution physiologique (solution saline à 0,9%), jusqu'à atteindre une concentration finale de 0,6 µg/0,1mL. Chez les oiseaux marins (Jouventin & Mauget 1996), 1,5 µg de LHRH par kilogramme de masse corporelle, suffit à provoquer une sécrétion maximale de LH. Ainsi pour un oiseau de 400g en moyenne (pétrels ou mouettes), le protocole employé a été l'injection de 0,1 mL d'une solution de GnRH à 0,6 µg/0,1mL dans la veine alaire à l'aide d'une seringue à injection de 0,3 mL. Pour les individus témoin, 0,1 mL de solution

physiologique est injectée. Le protocole a été validé chez les mouettes tridactyles (Fig. 20) et les pétrels des neiges.

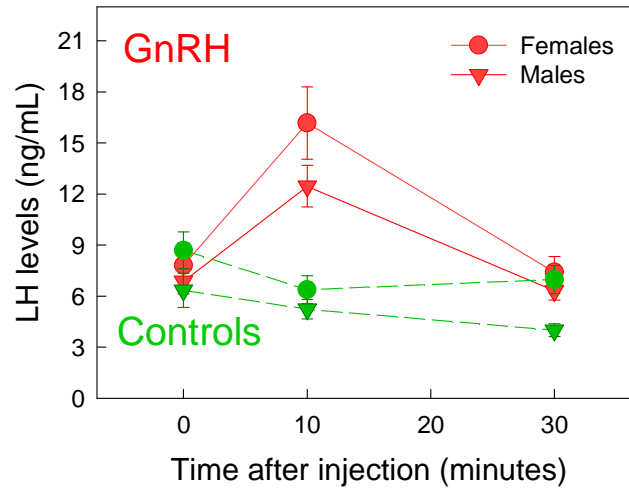


Figure 20 : Evolution des taux de LH avant (0 minutes), 10 et 30 minutes après injection d'une solution de GnRH (points et ligne rouges) ou d'une solution saline (points et ligne verts) chez des mouettes tridactyles mâles (triangles) et femelles (ronds) avant la ponte (année 2008)

En outre, le pic de LH provoqué par l'injection de GnRH va à son tour induire la sécrétion d'hormones sexuelles (œstradiol et testostérone). Contrairement à la LH, les hormones sexuelles augmentent progressivement entre 0 et 30 minutes. La concentration plasmatique des hormones sexuelles (principalement la testostérone) pourra donc être suivie à 0, 10 et 30 minutes après l'injection, via les trois prises de sang réalisées.

L'injection d'une faible dose de GnRH pendant la phase pré-nuptiale ne perturbe pas la décision de ponte et la date de ponte des oiseaux (voir article E).

II.2.4. Suivis phénologiques et comportementaux

i. Suivi des décisions de ponte et de la phénologie

Afin d'enregistrer la présence au nid des deux partenaires du couple, les mouettes tridactyles font l'objet de lectures de bague Darwick à l'aide d'un télescope, une à deux fois par jour. Quant au pétrel des neiges, l'identité des deux partenaires est contrôlée grâce à leur bague métal et / ou à leur marque au feutre.

Lors de la période de ponte du ou des œufs, un passage tous les jours ou tous les 2 jours permet de suivre les dates de ponte. Chaque pétrel est doucement soulevé à l'aide d'une gaffe pour vérifier la présence ou l'absence d'un œuf. Dans la colonie de mouettes, un miroir est hissé à l'extrémité d'une canne à pêche télescopique pour contrôler l'intérieur des nids (Fig. 21). De même, la désertion du ou des œufs, le succès à l'éclosion et le succès final (poussin à l'envol pour le pétrel et nombre de poussin de plus de 12 jours pour les mouettes) sont enregistrés à chaque étape de la saison de reproduction.



Figure 21 : Contrôle d'un nid de mouette (ici, 2 œufs) à l'aide d'un miroir hissé à l'extrémité d'une canne télescopique. Photo : F. Amélineau

ii. Suivi comportemental de présence au nid et de trajets en mer

En 2008 et 2009, le comportement de recherche alimentaire des mouettes a été enregistré à l'aide de GPS miniaturisés de 7 grammes (©TechnoSmart). Imperméabilisés à l'intérieur d'un sac, les GPS sont fixés sur les plumes du dos avec du ruban adhésif ©Tesa. A intervalle régulier, la géo-localisation est précisément enregistrée, ainsi que des paramètres d'activités, comme la vitesse et l'altitude. La durée totale du voyage, la distance totale parcourue et l'éloignement maximal par rapport à la colonie sont donc déduits.

II.3. Manipulation expérimentale

Les approches descriptives, détaillées ci-dessus, sont idéales pour mettre en relation les composantes hormonales, comportementales et phénologiques. Cependant, afin de s'affranchir d'éventuels effets confondants, une approche expérimentale est requise.

II.3.1. Manipulation des dates d'éclosion

L'accessibilité du nid, l'âge des parents et la valeur relative de l'œuf unique font du pétrel des neiges un modèle scientifique et technique privilégié. Les dates de ponte ont été suivies quotidiennement et la variabilité interindividuelle est importante (plus de 2 semaines).

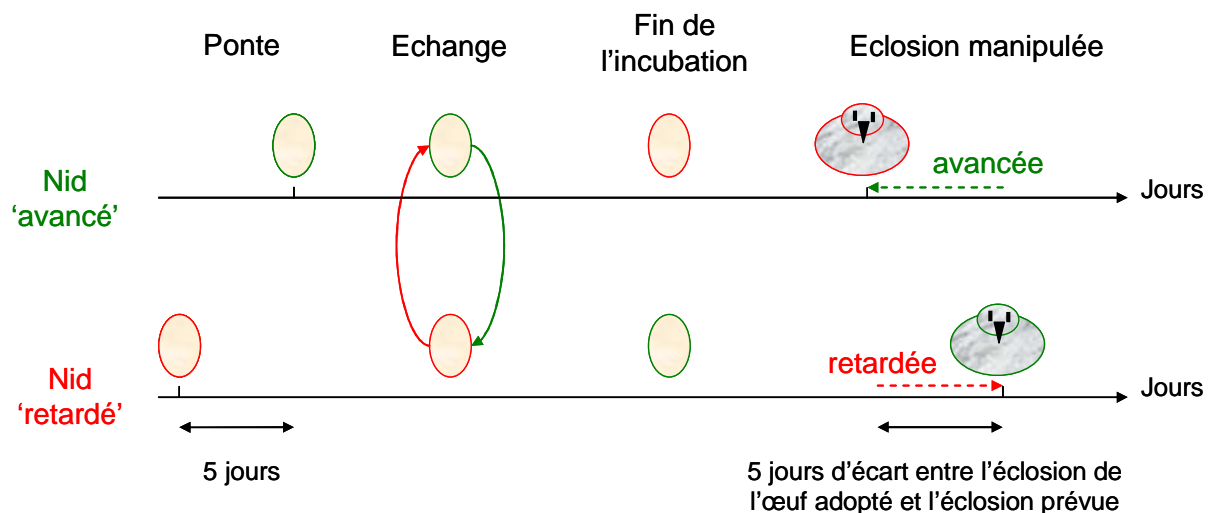


Figure 22 : Schéma de la manipulation expérimentale de la date d'éclosion (la swapette pour les intimes). La ponte est suivie précisément et les nids dont la ponte est espacée de 5 jours sont jumelés. L'échange a lieu pendant l'incubation. Les parents finissent d'incuber l'œuf adopté, dont l'éclosion a lieu plus tôt ou plus tard que prévu.

Les nids ont été jumelés en fonction de la date de ponte : les œufs sont échangés entre deux nids dont la ponte a eu lieu avec 5 jours d'écart (Fig. 22, l'œuf « vert » a été pondu 5 jours plus tard que l'œuf « rouge »). Lors de la deuxième moitié de l'incubation, les œufs jumelés sont transférés d'un nid à l'autre (« Echange », Fig. 22), de sorte que les parents finissent d'incuber l'œuf adopté jusqu'à l'éclosion. Pendant le transfert, un œuf factice est donné au

parent, pour éviter l'abandon. La date d'éclosion est ainsi manipulée par rapport à la date d'éclosion attendue pour leur propre œuf (éclosion environ 5 jours plus tôt pour les nids avancés et environ 5 jours plus tard pour les nids retardés).

Les conséquences sur le succès reproducteur et sur le comportement des parents ont été suivies. La sensibilité au stress des parents pendant l'élevage du poussin, témoin de l'effort parental, a été enregistrée via un protocole de réponse au stress. De plus, une attention particulière a été portée aux effets de l'âge et de la condition corporelle des parents.

II.3.2. Manipulation expérimentale des taux de corticostérone

Les mouettes tridactyles se prêtent facilement aux manipulations expérimentales des taux de corticostérone (Kitaysky *et al.* 2001; Angelier *et al.* 2007a, 2009; Goutte *et al.* 2010b), ce qui permet d'étudier l'influence de la corticostérone sur les ajustements physiologiques et sur les traits d'histoire de vie.

Cette technique repose sur l'implantation sous-cutanée d'un ou de deux tubes de silastic (diamètre interne : 1,47 mm, diamètre externe : 1,96 mm, longueur : 25 mm, Dow Corning, Michigan) remplis avec de la corticostérone cristallisée (C2505, Sigma Chemical Co., St. Louis, USA) ou vides (témoins). Une petite incision cutanée de moins d'un mm de diamètre est effectuée dans le dos des mouettes (entre les deux ailes) afin que l'implant soit glissé sous la peau à l'aide d'une sonde cannelée. Après avoir désinfecté l'incision à l'Alumine, celle-ci est refermée avec de la colle chirurgicale, à usage vétérinaire ©Vetbond 3M. Afin de faciliter la diffusion de la corticostérone, les extrémités du tube ont été coupées juste avant l'implantation.

Au CEBC, les implants ont été testés sur des pigeons en captivité (Fig. 23). Trois se sont vus attribuer un implant de corticostérone (CORT), le quatrième, un implant vide (témoin). Entre le lot CORT et le lot témoin, les taux basaux de corticostérone ont été multipliés par approximativement 3, les deux premiers jours.

Les doses de corticostérone utilisées (un ou deux implants) n'ont pas les mêmes effets. Chez les mouettes tridactyles, il a été largement démontré (Kitaysky *et al.* 2001; Angelier *et al.* 2007a, 2009; Goutte *et al.* 2010b) que l'utilisation de deux implants provoque une augmentation des taux basaux de corticostérone les deux premiers jours (multiplication des taux basaux par 10). Or, la production endogène de corticostérone est inhibée par l'implantation de doses faibles et moyennes de corticostérone exogène chez les rats de

laboratoire (Akana *et al.* 1992). L'administration de corticostérone chez les oiseaux provoque aussi un rétrocontrôle négatif pendant une durée prolongée (Vandenborne *et al.* 2005; Romero *et al.* 2005; Busch *et al.* 2008; Müller *et al.* 2009). Ainsi, il est possible d'altérer la perception du stress (i.e. la sécrétion endogène de corticostérone suite à un stress) via l'administration d'une faible dose de corticostérone exogène.

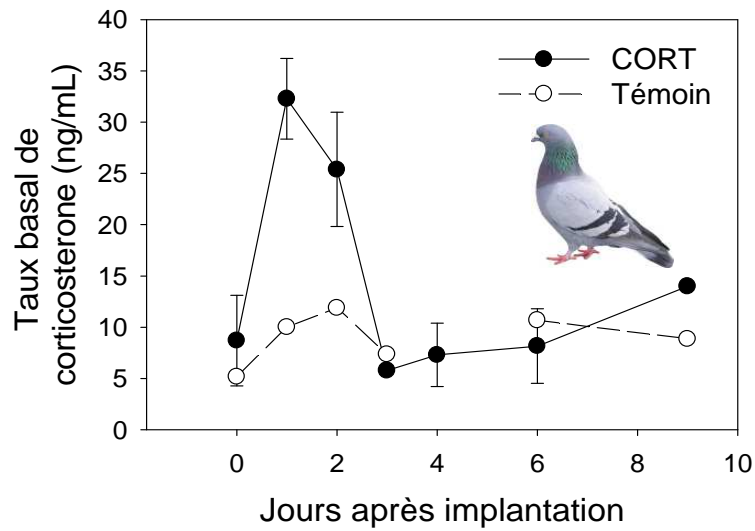


Figure 23 : Validation des implants de corticostérone (CORT) pour des pigeons en captivité.

II.4. Analyses biochimiques

II.4.1. Sexage moléculaire

Le faible dimorphisme sexuel des mouettes et pétrels ne permet pas de distinguer les mâles des femelles à l'œil nu. Le sexe des individus est alors déterminé par la technique de sexage moléculaire (Fridolfsson & Ellegren 1999). L'ADN est extrait au CEBC à partir du culot d'hématies prélevé lors de la prise de sang. Les introns (portions de gènes non codantes) sont isolés à l'aide d'enzymes spécifiques, puis amplifiés par PCR, à partir d'amorces. Les fragments CHD1Z sont présents chez les mâles et les femelles, tandis que les fragments CH1DW sont présents uniquement chez les femelles. De taille et de poids différents, ces fragments sont alors séparés par migration via une électrophorèse sur un gel d'agarose. Un individu présentant deux bandes visibles sur le gel est une femelle, celui n'en présentant qu'un est un mâle.

II.4.2. Dosages hormonaux

La technique de dosages hormonaux utilisés au CEBC repose sur des dosages radio-immunologiques (Berson & Yalow 1968). A partir du plasma récolté sur le terrain ont été déterminés les taux de corticostérone, LH, testostérone, œstradiol et / ou prolactine. Les dosages hormonaux chez les oiseaux sauvages, et notamment les dosages d'hormones hypophysaires, sont très spécifiques et nécessitent la mise au point de système hétérologue de dosage (Fig. 24). Actuellement, de faibles quantités d'hormones de référence purifiées (LH) sont disponibles, et ont été obtenues à partir d'hypophyse d'espèces domestiques (poulet). En France, seul le CEBC réalise ces dosages hétérologues.

i. Principe

Le dosage radio-immunologique est une méthode d'analyse compétitive d'une grande précision basée sur la réaction de deux antigènes identiques (l'un étant marqué) avec leur anticorps. L'hormone à doser est l'antigène froid (non marqué) et est mise en compétition

avec une hormone standard, l'antigène marquée avec un isotope actif. Ces deux antigènes ont une affinité identique pour les sites de liaison d'un anticorps spécifique et forment donc des complexes anticorps-antigènes. La quantité d'anticorps disponible dans le milieu réactionnel étant limitée, tous les antigènes ne pourront pas se lier sous forme de complexes. Ainsi la concentration inconnue de l'antigène froid (hormone à doser) est déterminée en fonction du nombre de complexes anticorps-antigène froid formés, au détriment des complexes anticorps-antigène marqué. Grâce au signal fourni par le marqueur radioactif, la concentration du complexe anticorps-antigène marqué est mesurée pour différentes concentrations de l'antigène froid.

ii. Application

Avant d'être dosées, les hormones stéroïdiennes (la corticostérone, la testostérone, l'œstradiol) nécessitent une extraction préalable à l'aide d'un solvant organique des lipides afin d'éliminer les protéines de transport. On dit que le dosage est total, par opposition au dosage de la fraction d'hormones libres. La LH et la prolactine sont quant à elles, des hormones protéiques, qui peuvent être dosées directement à partir du plasma. Le marquage consiste à fixer un élément radioactif dont le rayonnement constituera un signal mesurable. Le CEBC est spécialisé dans le marquage des hormones hypophysaires (LH, prolactine) à l'iode 125, qui émet un rayonnement X et gamma. Le marquage nécessite une période de pré-incubation et d'incubation dans des conditions spécifiques (temps de réaction, température, etc.). Pour les stéroïdes (corticostérone, testostérone), le marquage est aussi effectué au CEBC, au tritium 3H qui émet un rayonnement bêta (électrons).

Une fois l'incubation terminée, les fractions libres d'hormones marquées et d'hormones "froides" sont séparées des complexes anticorps-hormones par centrifugation après immunoprécipitation (hormones protéiques) ou après adsorption sur charbon recouvert de Dextran (hormones stéroïdiennes). Le milieu ne contenant plus que les complexes anticorps-hormones est analysé sur un compteur à scintillations, soit directement pour l'iode 125 (LH, prolactine), soit avec un liquide scintillant, qui émet des photons de fluorescence, proportionnellement à la radioactivité du tritium (corticostérone, testostérone, œstradiol).

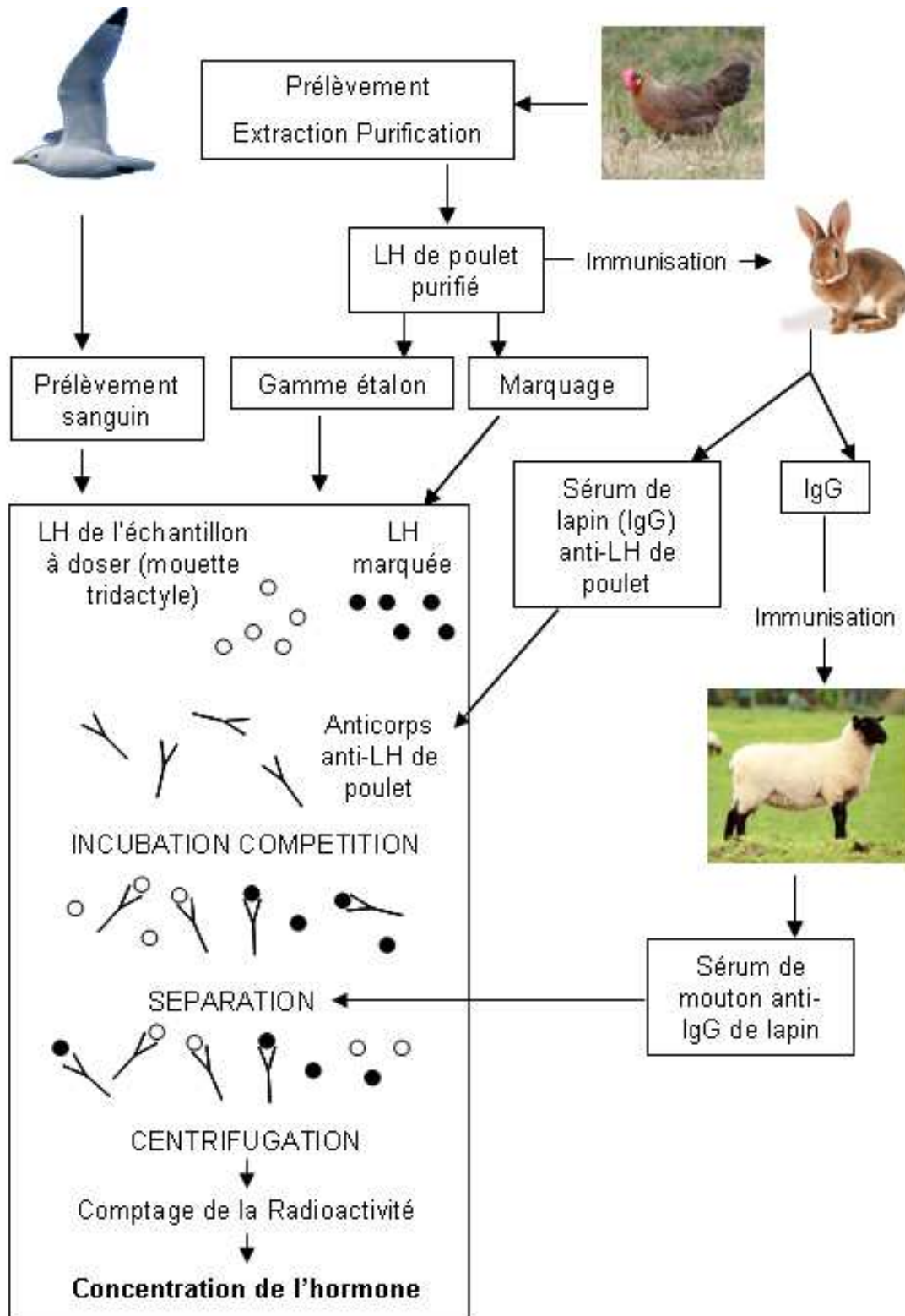


Figure 24: Principe et grandes étapes des dosages hétérologues (exemple du dosage de la LH pour la mouette tridactyle)

iii. Validation

Une courbe d'étalonnage est établie afin de déterminer, par interpolation, les concentrations hormonales de chaque échantillon. Cette courbe est obtenue par dilution d'une solution standard de concentration connue. La mesure du pourcentage de traceur radioactif lié équivaut, par référence à cette courbe, à une mesure de la concentration hormonale de l'échantillon. Dans le cas des dosages hétérologues, la dilution d'un échantillon de plasma riche en hormone permet de s'assurer que l'hormone se comporte de la même manière que l'hormone standard marquée, en termes d'affinité pour l'anticorps. La courbe obtenue à partir de la dilution du plasma riche doit être parallèle à la courbe obtenue avec l'hormone standard (Fig. 25, cas du dosage de la LH chez les mouettes tridactyles). Des échantillons de référence sont dosés plusieurs fois afin de calculer un coefficient de variation intra-dosage, et donc d'estimer l'erreur associée au dosage.

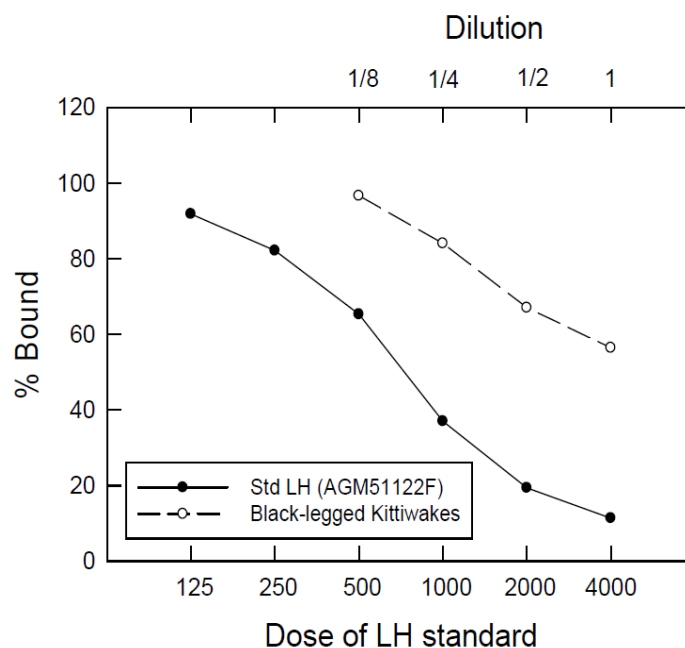


Figure 25 : Validation du dosage de LH par dilution d'un plasma riche en LH de mouette tridactyle. Les doses de LH standards (AGM51122F) sont exprimées en pg par tube.

II.5. Méthodes statistiques

Les analyses statistiques ont été conduites grâce au logiciel R 2.8.0 (R Development Core Team 2008). Les tests utilisés sont détaillés dans chacun des manuscrits.

Résultats



*Mieux vaut aller skier en pensant à Dieu,
Qu'aller à l'église en pensant au sport.*

Fridtjof Nansen

III. Phénologie, acquisition et allocation d'énergie

III.1. Organisation du chapitre

Ce premier chapitre de résultat est consacré à la compréhension des décisions phénologiques dans un contexte d'acquisition et d'allocation des ressources énergétiques.

D'une part, au niveau populationnel, nous avons comparé les ajustements comportementaux (présence au nid et trajets alimentaires pré-nuptiaux via la pose de balises GPS), les ajustements hormonaux (corticostérone, LH, testostérone) et l'adoption de traits d'histoires de vie (décision de se reproduire, date de ponte et taille des pontes) lors de deux années contrastées (2008 & 2009, Article A). En accord avec nos prédictions, une année tardive, avec une faible proportion de couples reproducteurs et peu d'œufs pondus, est caractérisée par des trajets alimentaires longs en période pré-nuptiale, des taux élevés de corticostérone et de faibles taux de LH et de testostérone (Article A).

D'autre part, nous nous sommes intéressés aux conséquences d'un retard ou d'une avancée expérimental(e) des dates d'éclosion sur la valeur de la reproduction en cours et sur les décisions parentales en termes d'allocation énergétiques (Article B). La sensibilité au stress est un moyen efficace et fiable de mesurer le degré d'effort parental dans la reproduction en cours. Nous avons ainsi montré que les pétrels des neiges, dont la reproduction a été retardée, diminuent leur motivation parentale, en abandonnant l'œuf en fin d'incubation et en présentant une robuste réponse au stress (taux élevés de corticostérone induits par un stress) en début d'élevage des poussins. Cette baisse de la motivation parentale n'est pas renforcée par une moindre condition corporelle des poussins et / ou une moindre survie des poussins. Enfin, une avancée de la reproduction n'est pas synonyme d'une motivation parentale accrue (Article B)

III.2. Phénologie, recherche alimentaire et stress: une approche interannuelle

ARTICLE A

Foraging trips and the onset of breeding in an Arctic seabird:
hormonal differences between two contrasted years

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Foraging trips and the onset of breeding in an Arctic seabird: hormonal differences between two contrasted years

Summary

Environmental conditions early in the season greatly influence the inter-annual variations in the number of breeding pairs, the timing of breeding and the clutch size. However, the underlying mechanisms remain poorly understood. In a High Arctic population of Black-legged kittiwakes, we investigated, for the first time, foraging behaviours and endocrine adjustments during the pre-laying period of two contrasted years. Specifically, foraging trips were tracked in male and female kittiwakes, through GPS deployment. At the physiological level, we focussed on corticosterone, a marker of food-related stress and foraging success, and on LH and testosterone, the hormonal drivers of breeding. LH releasing abilities were monitored through GnRH challenges. In 2008 (the “good year”), the number of breeding pairs was higher, egg-laying was earlier and clutch size was bigger than in 2009 (the “bad year”). In parallel, pre-laying foraging trips were further away from the colony and lasted longer during the bad year than during the good year, especially for males. Pre-laying baseline corticosterone levels were higher in 2009 than in 2008, in male but not in female kittiwakes. Baseline LH levels in both sexes, and baseline testosterone in males, were lower in 2009 than in 2008, while the LH releasing ability after a GnRH challenge did not differ between the two years. These results suggest that foraging conditions early in the season may drive breeding decision, egg-laying date and clutch size, through the expression of elevated corticosterone levels and/or low LH and testosterone levels.

Key words: egg-laying date, clutch size, breeding decision, GPS tracking, foraging trips, corticosterone, LH, testosterone, GnRH challenge, *Rissa tridactyla*

Introduction

Long-term phenological trends have provided evidences of the tight link between environmental parameters and the timing of breeding (e.g. Stenseth et al. 2002; Walther et al. 2002; Parmesan & Yohe 2003). In marine top predators, sea surface temperatures, sea ice extent and/or the abundance of the preys have been related to inter-annual phenological variations (Frederiksen et al. 2004; Gaston et al 2005 a,b; Barbraud & Weimerskirch 2006; Moe et al. 2009; Shultz et al. 2009). Indeed, potential breeders adopt various strategies to cope with a depletion of food supply early in the season: they may skip the immediate breeding season, they may decrease their clutch size and/or they may postpone their egg-laying (“prudent parent hypothesis”, Drent & Dann 1980; Stearns 1992). The modulation of these life history traits early in the season has crucial fitness consequences (Lack 1968; Perrins 1970) and requires the adoption of appropriate physiology and behaviour, in response to environmental fluctuations and physiological state (reviewed in Wingfield 2008; McNamara & Houston 2008). For instance, in Adélie penguins, *Pygoscelis adeliae*, foraging trips during the courtship period were shorter when sea-ice retreat was earlier, and pre-breeders switched to other food supply when sea-ice retreat was late (Beaulieu et al. 2009). However, this annual foraging change was not accompanied by a change in the egg-laying date and/or in the reproductive success (Beaulieu et al. 2009). Hence, the proximate mechanisms underlying annual resource variations and life-histories decisions early in the season remain poorly understood and need further investigations.

One potential mediator could be the release of corticosterone in response to stress, which is known to adjust life-history strategies according to environmental and physiological parameters (Ricklefs & Wikelski 2002; Wingfield & Sapolsky 2003). Baseline corticosterone level is a reliable marker of food-related stress and reflects food availability, energetic state and sustained activities (Wingfield et al. 1998; Kitaysky et al. 1999; Wingfield & Kitaysky 2002; Love et al. 2004; Landys et al. 2006; Kitaysky, Piatt & Wingfield 2007; Benowitz-Fredericks et al. 2008). In turn, corticosterone promotes the mobilization of energy reserves, facilitates locomotion and foraging decision (Wingfield & Kitaysky 2002; Wingfield & Sapolsky 2003; Landys et al. 2006; Angelier et al. 2008). High corticosterone levels could interfere with the current reproduction, but the action of corticosterone remains unclear during the pre-laying period. At the individual level, recent studies reported that females bearing elevated baseline corticosterone levels during the pre-laying period did not breed or postponed the onset of breeding (Salvante and Williams 2003 ; Schoech et al. 2009; Vitousek

et al. 2010; Goutte et al. 2010a). It is conceivable that corticosterone interferes with the onset of breeding, through a down-regulation of the hypothalamic–pituitary–gonadal axis (hereafter HPG axis, reviewed in Schoech et al. 2009; Goutte et al. 2010b). Indeed, the HPG axis is activated by photoperiodic changes (increased day length): the expression of the Gonadotropin Releasing Hormone (GnRH) triggers the release of follicle stimulating hormone and luteinizing hormone (LH), the hormonal drivers of reproduction. In turn, LH promotes gonadal development and the secretion of sex-steroids (estradiol and/or testosterone, reviewed in Dawson et al. 2001). This hormonal cascade leads to the adoption of sexual behaviours, such as courtship, mating, and nest building, and promotes the egg-laying (Ball 1993). In female Black-legged kittiwakes, elevated baseline corticosterone levels are associated with low baseline LH levels and with low LH releasing abilities (Goutte et al. 2010b). Hence, it is conceivable that the secretion of corticosterone at the individual level would thus mediate skipped breeding decision, late breeding, and/or low clutch size, through the inhibition of LH release.

This study aims to explore behavioural and physiological causes of annual variations in life history traits, early in the season, and at the population level. We focused on two consecutive and contrasted years (2008 and 2009) in a High Arctic population of Black-legged kittiwake (*Rissa tridactyla*). A long-term study (38 years) has been conducted in this population and has highlighted great inter-annual phenological variations (Moe et al. 2009). In Svalbard, kittiwakes attend the colony during two months before egg-laying, thus offering the opportunity to explore behavioural and physiological parameters during the pre-laying period (*i.e.* copulations and nest building period). In 80 nests, breeding decisions, egg-laying dates and clutch-size were precisely monitored. To track the foraging ranges of male and female kittiwakes, they were equipped with a GPS device during the pre-laying period. The duration of foraging trips were known through nests checking. At the hormonal level, the baseline levels of corticosterone and LH were monitored in pre-laying males and females, and the baseline levels of testosterone, in males only. In Black-legged kittiwakes, baseline LH and testosterone levels reach maximal levels during the pre-laying period, compared to the arrival, incubation and chick-rearing period (Goutte al. unpublished data). Moreover, the readiness of a bird to breed and its ability to release temporary LH can indeed be assessed by administering a small injection of Luteinizing Hormone Releasing Hormone (LHRH, the so-called GnRH challenge, Schoech et al., 1996). We were thus able to compare behavioural, physiological and phenological adjustments between the two years. Hence, we predicted that a year of low breeding decision, late egg-laying and/or small clutch size would be

characterized by longer foraging trips, and by high baseline corticosterone levels, low baseline LH and testosterone levels, compared to a year of high breeding decision, early egg laying and/or big clutch size.

Materials and methods

Study area and birds

Our study was conducted during the 2008 and 2009 pre-laying periods in a colony of Black-legged kittiwakes at Kongsfjorden (78°54'N, 12°13'E), Svalbard, 7 km east of Ny-Ålesund, Norway. Black-legged kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago. We studied kittiwakes in one plot of 80 nests at a height of 5-10 m. These nests were occupied by a pair during the pre-laying period but some of them did not breed. In these nests, pairs were not manipulated during the pre-laying 2008 and 2009, except for the basic endocrine investigations and the GPS equipment, described below.

Life-history traits early in the season

Using a mirror at the end of an 8 m fishing rod, we checked the 80 nests every two days during the egg-laying period. Three life-history traits were thus monitored: the breeding decision, the laying date of the first egg, and the clutch size. Breeding decision is a binomial variable: 0 means no laid eggs, and 1, at least one egg is laid (active nest). Among the active nests, we managed to monitor egg-laying date and clutch size of 71 nests in 2008 and 57 nests in 2009.

Foraging trips

To study the foraging movements of kittiwakes during the pre-laying period, we fitted 14 kittiwakes in 2008 and 13 kittiwakes in 2009 with a miniaturized 7g GPS (©TechnoSmart). The GPS was placed into a waterproof bag and fitted on the bird's back using ©Tesa tape, a method successfully used for activity recorders in kittiwakes (Angelier et al. 2007a; Kotzerka et al. 2010). We are confident that there were no deleterious effects of the GPS loggers on the behavior of kittiwakes since the logger's weight was about 4% of the

mass of the birds, which is far below the limit considered as acceptable for flying birds (Kenward 2001). Due to technical problems with the batteries, we were not always able to track the whole foraging trips, especially for the long foraging trips in 2009.

The majority of kittiwakes in the plot are individually marked with metal rings and plastic rings with a code of three letters allowing identification from a distance without perturbation. We checked the nests and the whole colony and we read the plastic rings from a distance, twice a day. The duration of a foraging trip was estimated as the number of days that a kittiwake was absent from the nest and from the colony. The durations of pre-laying foraging trips were monitored for 128 pre-laying kittiwakes (N = 80 in 2008 and N = 48 in 2009). We excluded uncertain observations, when plastic rings were not clearly visible, for instance.

Blood sampling

We only considered baseline corticosterone, LH and testosterone levels of kittiwakes that did breed in 2008 or in 2009. Indeed, we have previously shown that baseline LH levels were lower and baseline corticosterone levels were higher in kittiwakes that did not breed, compared to breeders (Goutte et al. 2010b, 2008 data set).

Male and female kittiwakes were caught at their nests with a noose on the end of a 5 m fishing rod, during the pre-laying period 2008 (from 19 May to 18 June 2008, N = 48) and 2009 (from 19 May to 18 June 2009, N = 23). Immediately after capture, a first blood sample was collected from the alar vein with a 1 mL heparinised syringe and a 25 gauge needle to assess baseline LH and corticosterone levels in males and females and testosterone in males only. Bleeding time, that is, the time between capture and the end of the blood sampling, was 3 minutes and 36 ± 5 [SE] seconds.

Immediately after this first blood sampling, 32 kittiwakes (22 in 2008 and 10 in 2009) were injected with 0.1 mL of a solution of LHRH ([Gin⁸], Sigma Lot 121H04314) to test the responsiveness of the pituitary gland. The LHRH was dissolved in physiological solution to yield a final dosage of 0.6 µg/0.1mL (1.5 µg/ kg body mass in 1 mL of 0.9% saline solution). This dose of LHRH has been shown to be sufficient to elicit the maximal release of LH in Black-legged kittiwakes (Goutte et al. 2010b). We administered the LHRH solution directly into the alar vein, and collected a second blood sample (*ca.* 0.3 mL) 10 minutes after the injection, to assess LHRH-induced LH levels.

Kittiwakes were weighed to the nearest 2 g using a Pesola spring balance and their skull length (head + bill) was measured to the nearest 0.5 mm with a sliding caliper. Because males are larger than females (Moe et al. 2002), body condition was calculated for males and females separately, from a standardized least-squares linear regression of body mass against head size. Kittiwakes were also marked with spots of dye on the forehead to distinguish them from their partner during observations and were released.

Molecular sexing and hormone assay

Blood samples were centrifuged and plasmas were decanted and stored at -20°C until they were assayed. Red cells were also kept for molecular sexing at the Centre d'Etudes Biologiques de Chizé (CEBC). Sex was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes (Fridolfsson and Ellegren, 1999), as detailed in Weimerskirch et al. (2005).

LH radioimmunoassay was conducted following the methods previously described for other seabirds (Mauget et al., 1994; Chastel et al., 2005b), and especially for Black-legged kittiwake's plasma (Goutte et al. 2010b). The lowest detectable concentration for LH was 0.06 ng/mL and the intra-assay coefficient of variation was 8.7 % (N = 3 duplicates). Plasma concentrations of testosterone were assayed for males only, by radioimmunoassay, at the CEBC as described by Chastel et al. (2003). The lowest detectable concentration for testosterone was 0.05 ng/mL and the intra-assay coefficient of variation was 7 % (N = 3 duplicates). Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC, as described by Lormée et al. (2003). The lowest detectable concentration for corticosterone was 0.5 ng/mL and the intra-assay coefficient of variation was 6.7 % (N = 5 duplicates).

Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team 2008). We used GLM with quasi-Poisson error distribution and a log link function to test an effect for year, sex and year * sex on clutch size and on foraging trip durations. We used generalised linear models (GLM) with a normal error distribution and an identity link function to test an effect for year (2008 and 2009), sex (male and female) and an interaction year * sex on 1) egg-laying dates, 2) body condition, 3) maximum foraging ranges, 4)

baseline corticosterone levels, 5) baseline LH levels, 6) baseline testosterone levels for males, 7), LHRH-induced LH levels. Dependent continuous variables were log-transformed when necessary. Diagnostic plots were assessed to test whether the data sufficiently met the assumptions of the linear model.

Results

Life-history traits early in the season

Among the 80 monitored nest, the breeding decision was ~25 % lower in 2009 than in 2008 (Wald $\text{Chi}^2 = 22.07$, $\text{df} = 1,158$, $p < 0.001$, Fig. 1A). First egg-laying was postponed of about 8 days in 2009 compared to 2008 ($F_{1,126} = 139.30$, $p < 0.001$, Fig. 1B). Clutch size was 14% smaller in 2009 than in 2008 ($F_{1,126} = 9.92$, $p = 0.002$, Fig. 1C). Moreover, we did not record any clutch of 3 eggs in 2009 while 7 % of the active nests had 3 eggs in 2008.

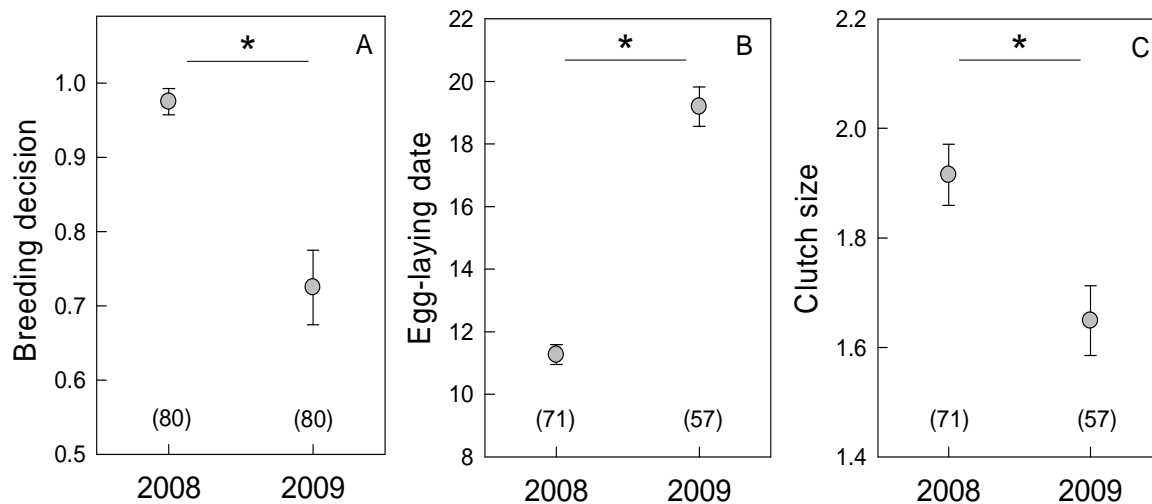


Figure 1: Comparison of (A) breeding decision (0, no egg laid and 1, at least one egg laid), (B) laying date of the first egg in June, and (C) clutch size per active nests in 2008 and 2009. Samples sizes, means and standard errors are given.

Foraging trips

During the pre-laying period, maximum foraging ranges from the colony were higher in 2009, than in 2008 ($F_{1,25} = 6.94$, $p = 0.015$), and higher in males than in females ($F_{1,24} = 28.47$, $p < 0.001$), without an interaction year * sex ($F_{1,23} < 0.01$, $p = 0.969$, Table 1, Fig. 2).

Females did not forage outside the fjord system, while 70 % of males foraged offshore, in deep waters, during the pre-laying period 2008 and 2009 (Fig. 2). Foraging trips durations were around 2 days longer in 2009 (N = 48, 3.75 ± 0.27 days) than in 2008 (N = 80, 1.73 ± 0.13 days, $F_{1,126} = 59.01$, $p < 0.001$), without differences between sexes (sex: $F_{1,125} = 0.03$, $p = 0.859$; year * sex: $F_{1,124} = 0.21$, $p = 0.651$).

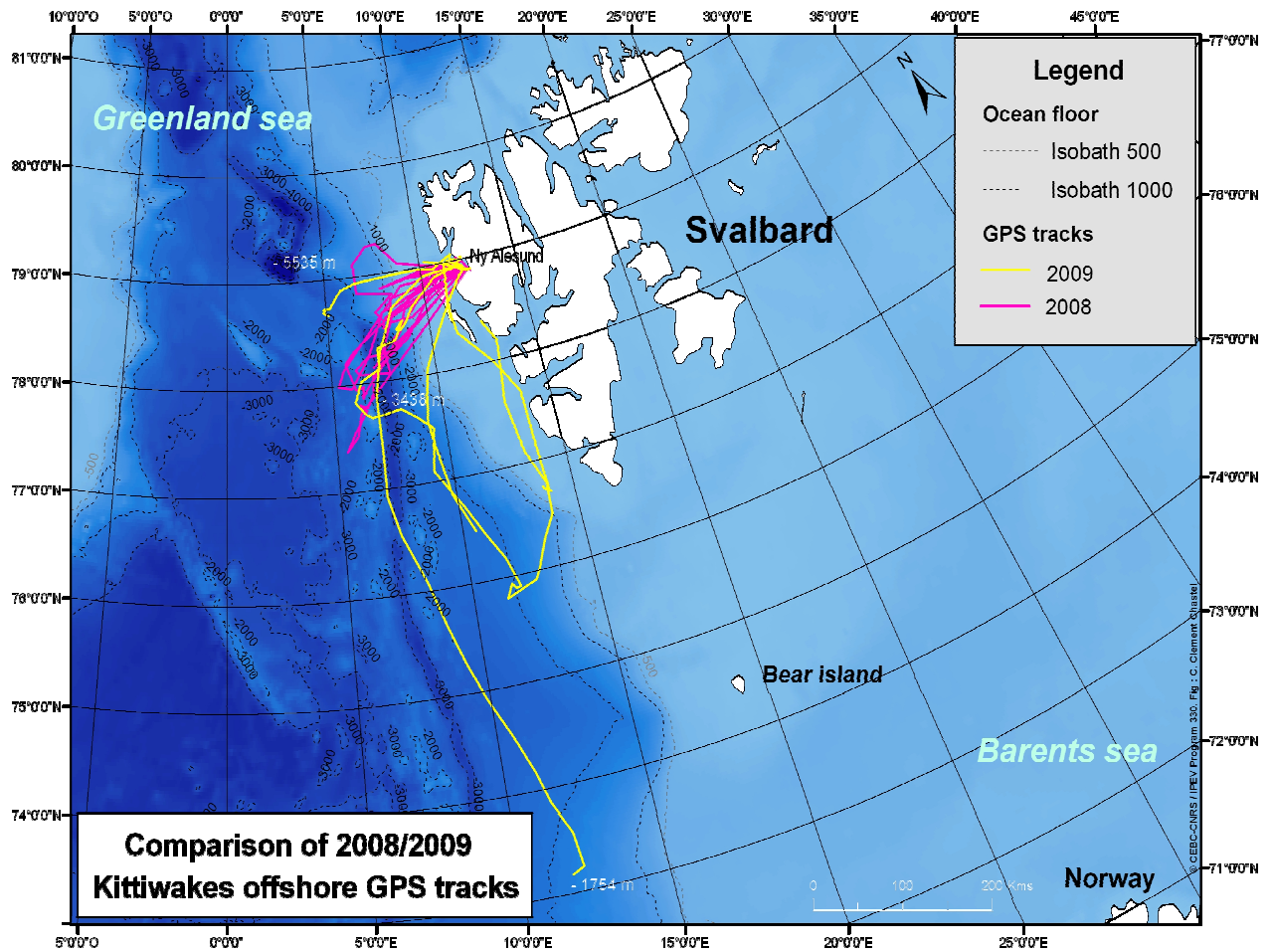


Figure 2: Foraging tracks of pre-laying male kittiwakes during the pre-laying periods in 2008 and in 2009.

	2008	2009	Total
Males	N = 4, 93.3 ± 35.6	N = 9, 188.2 ± 70.5	N = 13, 159.9 ± 50.4
Females	N = 10, 5.2 ± 2.2	N = 4, 4.4 ± 2.0	N = 14, 4.9 ± 1.6
Total	N = 14, 31.2 ± 14.7	N = 13, 131.6 ± 53.8	N = 27, 79.5 ± 28.2

Table 1: Mean and standard error of maximum foraging range (km) of pre-laying kittiwakes.

Comparison of hormones levels

During the pre-laying period, baseline corticosterone levels were significantly higher in 2009 than in 2008, but only in males (year * sex: $F_{1,65} = 6.02$, $p = 0.017$; year: $F_{1,65} = 2.38$, $p = 0.128$; sex: $F_{1,65} = 6.59$, $p = 0.013$; Fig. 3A). Baseline LH levels were lower in 2009 than in 2008 ($F_{1,52} = 6.63$, $p = 0.013$) and were lower in males than in females ($F_{1,52} = 10.01$, $p = 0.003$), without an interaction effect year * sex ($F_{1,51} < 0.001$, $p = 0.998$, Fig. 3B). Baseline testosterone levels in male kittiwakes were higher in 2008 than in 2009 ($F_{1,36} = 7.03$, $p = 0.012$, Fig. 3C). Ten minutes after the LHRH-injection, LH levels did not differ between years ($F_{1,30} = 0.511$, $p = 0.480$), even when considering an effect of sex (sex: $F_{1,29} = 0.314$, $p = 0.580$; year * sex: $F_{1,28} = 0.797$, $p = 0.380$). Body condition during the pre-laying period did not differ between years ($F_{1,69} = 0.13$, $p = 0.724$), even when considering an effect of sex (sex: $F_{1,68} < 0.01$, $p = 0.956$, year * sex: $F_{1,67} = 0.45$, $p = 0.503$).

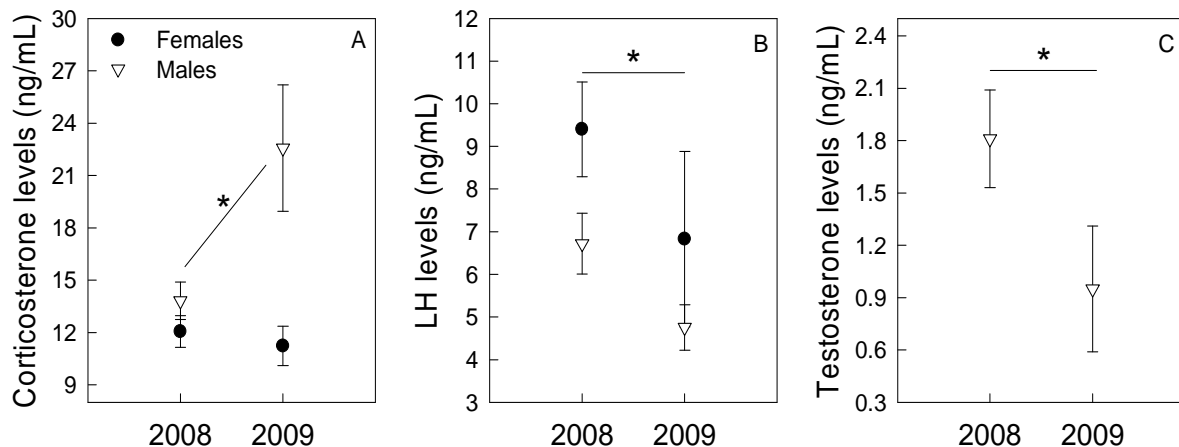


Figure 3: Comparison (means and standard errors) of baseline levels of (A) corticosterone, (B) LH, and (C) testosterone (ng/mL) in 2008 and 2009. Open triangles denote males and filled circles denote females.

Discussion

To our knowledge, this study is the first one to relate foraging and endocrine adjustments, with early life-history decisions (*i.e.* breeding decision, egg-laying date, and clutch size) between two contrasted consecutive years. The results are summarized on table 2.

	2008	2009
Early life-history traits		
Breeding decision	High	Low
Timing of breeding	Early	Late
Clutch size	Big	Small
Foraging trips		
Max. foraging range	Close	Far
Foraging trip duration	Short	Long
Hormonal levels		
Corticosterone (male)	Low	High
Corticosterone (female)	Low	Low
LH (male)	High	Low
LH (female)	High	Low
Testosterone (male)	High	Low

Table 2: Summary of difference in early life-history traits, foraging trips and hormonal levels during the pre-laying period 2008 and 2009.

First of all, the two years greatly differed in the life-history traits early in the season. The 2009 year could be seen as a poor year with low breeding decision, late egg-laying and small clutch size. In contrary, the 2008 year is characterized by high breeding decision, early egg-laying and big clutch size, and could be seen as a good year (Moe et al. 2009). This annual difference have probably emerged from environmental differences between these two consecutive years, such as sea surface temperature, ice extent and/or food supply (Frederiksen et al. 2004; Moe et al. 2009). For instance, a difference in fish abundance would have affected phenology and clutch size, as demonstrated by Reed et al. (2009). Moreover, in the Arctic and in the Antarctic, extended sea-ice cover and late ice break-up are usually related to delayed breeding and small clutch size in seabirds (Gaston et al. 2005a,b; Barbraud & Weimerskirch 2006). Annual variability of sea ice recession affects primary production bloom and imposes temporal asynchronies and spatial separations between energy requirements and food availability, ultimately impacting seabird populations (Moline et al. 2008; Laidre et al. 2008). Although annual variations in environmental parameters were not investigated in this study, we observed a greater sea-ice extent and late sea-ice retreat in 2009, compared to 2008. In turn, it is possible that kittiwakes were negatively affected in 2009, through a reduction of

preys abundance, accessibility and/or distribution. To test this hypothesis, we monitored the foraging ranges and durations during the 2008 and 2009 pre-laying periods.

Pre-laying kittiwakes foraged 4.2 times farther away from the colony in 2009, than in 2008. Moreover, foraging trips lasted 2 days longer in 2009, than in 2008. This lower nest attendance and longer foraging trips in 2009 could have led to the decreased probability to breed, to the delayed breeding and to the small clutch size in 2009 compared to 2008. Specifically, sustained foraging activities in 2009 would have triggered higher energy expenditure and lower nest attendance, compared to 2008, ultimately decreasing the investment in the immediate breeding event. Interestingly, we detected strong sex-difference in foraging range: the majority of males did offshore trips, while all females foraged in the fjord system. As pre-laying kittiwakes displayed courtship feeding (Kempnaers et al. 2007), we suggest strong sex-differences in foraging constraints during the pre-laying period. However, the annual difference in foraging activities and the possible variation in preys' abundance did not influence stored energy reserves, since body condition did not significantly differ between the two years, neither in males, nor in females.

Baseline corticosterone levels were higher in 2009 than in 2008, especially in male kittiwakes, which could reflect an inter-annual variation in activities, energetic state, food availability, and foraging success (Kitaysky et al. 1999; Love et al. 2004; Landys et al. 2006; Angelier et al. 2007b; Benowitz-Fredericks et al. 2008). In addition, corticosterone promotes energy mobilisation and facilitates locomotion (Lynn et al. 2003). Thus, the higher baseline corticosterone observed in males in 2009 are consistent with the higher distance travelled in 2009 and with the sex-difference in maximum foraging range. In females, high baseline corticosterone levels have been previously associated with late breeding at the individual level (Salvantes & Williams 2003; Schoech et al. 2009; Goutte et al. 2010a). Interestingly, baseline corticosterone levels in females did not differ between 2008 and 2009, which suggests that the correlation between corticosterone levels and early life-history traits was not detectable at the population level. Alternatively, as blood sampling was done during the same period in 2008 and 2009, baseline corticosterone levels were monitored around 8 days earlier *relative to* the egg-laying period, in 2008 than in 2009. However, this difference in the breeding schedule should not have strongly affected corticosterone levels, since the date of sampling did not affect hormonal levels during the pre-laying period, within a year and at the individual level (Goutte et al. 2010b). At last, baseline corticosterone levels in males were higher the year of low breeding decision, late breeding and small clutch size. This would suggest an indirect

effect of males on breeding decision, egg-laying date and / or clutch size, through for instance, lower nest attendance and / or courtship feeding.

Concerning the HPG axis, baseline LH levels in males and females were lower in 2009 than in 2008 and baseline testosterone levels in males were lower in 2009 than in 2008. The higher corticosterone levels in 2009 would have inhibited the secretion of LH, as previously highlighted at the individual level (Goutte et al. 2010b). However, the present results are not consistent with the sex-difference in the corticosterone-induced inhibition of LH (Goutte et al. 2010b). Moreover, LHRH-induced LH levels were not lower in 2009 than in 2008, while high corticosterone levels were associated with a low LH releasing ability after an injection of LHRH at the individual level (Goutte et al. 2010b). Alternatively, as kittiwakes were absent from their nest during a more-prolonged period in 2009, compared to 2008, they would have been less stimulated by their mates and by their neighbours. This lack of frequent social interactions could have led to a decrease of LH and testosterone levels and ultimately, to the late breeding and the small clutch size observed in 2009 (e.g. Helm et al. 2006).

To sum up, we argue that phenological variations are influenced by pre-laying foraging distance, which in turn, may trigger the secretion of corticosterone, especially in males. Low nest attendance and/or high baseline corticosterone levels would perturb LH and testosterone release, thereby delaying the onset of breeding and decreasing the clutch size.

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III.3. Manipulation de la phénologie, allocation parentale et sensibilité au stress

ARTICLE B

Experimentally delayed hatching triggers a magnified stress response in a long-lived bird

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Summary

In birds, the timing of breeding is a key life-history trait with crucial fitness consequences. We predicted that parents may value a brood less if it hatched later than expected, thereby decreasing their parental effort. In addition, breeding effort would be further modulated by the age-specific decline of future breeding opportunities. We experimentally investigated whether snow petrels, *Pagodroma nivea*, were less committed to care for a chick that hatched later than expected. The timing of hatching was manipulated by swapping eggs between early and late known-age pairs (7-44 years old), and investigations on hormonal and behavioral adjustments were conducted. As a hormonal gauge of parental commitment to the brood, we measured the corticosterone stress response of guarding adults. Indeed, an acute stress response mediates energy allocation towards survival at the expense of current reproduction and is magnified when the current brood value is low, as it is expected to be in young and/or delayed parents. As predicted, egg desertion and the magnitude of the stress response was stronger in delayed pairs compared to control ones. However, the treatment did not decrease the length of the guarding period, chick condition and chick survival. In addition, old parents resisted stress better (lower stress-induced corticosterone levels) than young ones. Our study provides evidence that snow petrels, as prudent parents, may value a brood less if it hatched later than expected. Thus, in long-lived birds, the responsiveness to stressors appeared to be adjusted according to the individual prospect of future breeding opportunities (age) and to the current brood value (timing of breeding).

Key words: corticosterone, brood value, egg swapping, phenology, parental investment, stress response, age, snow petrel, *Pagodroma nivea*

Introduction

The timing of breeding is a key life-history trait (Lyon et al., 2008; Reed et al., 2009), which greatly influences final reproductive success (Lack, 1950; Perrins, 1970). A brood that hatched later than expected may suffer from higher environmental deterioration, such as resource depletion, competition, and/or predation risk for the offspring ('date hypothesis', Lack, 1968; review in Verhulst and Nilsson, 2008). Moreover, experimental manipulations of the breeding schedule have provided evidence that delayed breeding may not only penalize the brood, but may also induce long-term costs for parents, due to the overlap with post-breeding activities, such as moult (Nilsson and Svensson, 1996; Brinkhof et al., 2002; Verhulst and Nilsson, 2008; McNamara and Houston, 2008). Hence, it is conceivable that long-lived parents would be less committed to care for a brood that hatched later than expected, thereby favouring their own survival and future breeding opportunities ("prudent parent hypothesis", Drent and Daan, 1980). In addition, as future breeding prospects decline with advancing age, old breeders are expected to invest more in current reproduction than young ones (Stearn, 1992; Isaac and Johnson, 2005; but see McNamara et al., 2009). Consequently, the modulation of parental effort should be adjusted according to both the timing of current breeding and the age of the breeder. Such adaptive parental strategies should be regulated in a flexible manner, via the integration of fluctuating environmental conditions and the individual perception of the risks (Wingfield et al., 1998; Ricklefs and Wikelski, 2002).

One potential mechanism underlying the adjustment of parental effort may be the endocrine stress response (Wingfield and Sapolsky, 2003). Vertebrates release glucocorticoid hormones (corticosterone in birds) in response to stressors, such as food scarcity, predator attacks, and harsh weather. This adrenocortical stress response is thought to promote the immediate survival through the mobilization of stored energy and the adoption of emergency behaviors at the expense of current breeding activities (Wingfield and Sapolsky, 2003; but see Breuner et al., 2008). Therefore, the stress response is seen as an adaptive mechanism that reallocates energy toward survival when individual and/or environmental conditions could not sustain the current reproductive effort (Landys et al., 2006). However, when the value of current reproduction is high relative to the value of future breeding opportunities, stress response should be attenuated or even suppressed to ensure that the current breeding success is not threatened (Silverin and Wingfield, 1998; Wingfield and Sapolsky, 2003). This 'brood value hypothesis' is highly supported by recent correlative, comparative and experimental

studies (Wingfield et al. 1995, Heidinger et al. 2006; Lendvai et al., 2007; Lendvai and Chastel, 2008; Bokony et al., 2009). Thus, the magnitude of the stress response appears to be a good proxy for the level of parental commitment to the brood. It is crucial to distinguish the stress response (that is, the stress-induced corticosterone levels) from the baseline corticosterone levels, which are a marker of activities, energetic state and/or food availability (Kitaysky et al., 1999; review in Landys et al., 2006). Increased baseline corticosterone levels would provide information on the levels of sustained reproductive activities / work (Kitaysky et al. 2001; Love et al. 2004). If the brood hatches later than expected and is less valuable, parents are predicted to mount a stronger adrenocortical stress response (stress-induced corticosterone levels) and to be less committed to care for the offspring. In contrary, if they work harder, delayed parents are predicted to exhibit higher baseline corticosterone levels than controls. In addition, older adults should respond less strongly to stressors (lower stress-induced corticosterone levels) than younger ones (Heidinger et al., 2006, 2008; Angelier et al., 2007a). In summary, stress response is likely to reflect the relative value of a brood that hatched earlier / later than expected, for young / old parents.

In this study, we tested whether parental effort declined with delaying breeding through the measure of parental behavior and stress response in a long-lived Antarctic seabird, the snow petrel (*Pagodroma nivea*, Forster 1777). Snow petrels are excellent models for such an investigation because, like all Procellariiformes, they lay only one egg during the extremely short Antarctic breeding season. The breeding schedule is thus of high importance for an optimal allocation of energy to the single chick and thus for reproductive success. Furthermore, snow petrel, as a very long-lived bird (up to 46 years old), should modulate the stress response in relation to the current brood value and to their own age (Angelier et al., 2007a; Bokony et al., 2009).

By swapping eggs between early and late breeding pairs, we manipulated the hatching date. We then measured breeding success, incubating and chick-rearing behavior and the corticosterone stress response of chick-rearing snow petrels through a capture/restraint stress protocol (Wingfield, 1994). Parental behavior and breeding success were expected to be reduced in delayed pairs and enhanced in advanced pairs compared to controls. According to the brood value hypothesis, delayed breeders should exhibit a higher stress-induced corticosterone release and advanced breeders, a lower stress-induced corticosterone release than controls. In addition, stress-induced corticosterone levels are expected to be higher in young than in old parents, as an additional effect of elevated future breeding expectancies.

Methods

Study site and species

The study was carried out on Ile des Pétrels, Adélie Land (66°40'S, 140°01'E), Antarctica. Snow petrels (250–500 g) are long-lived seabirds (> 40 years old) that lay one egg per season with no replacement clutch in case of failure (Warham, 1990). In Adélie Land, the laying period ranges from late November to mid December (Barbraud et al., 2000; Goutte et al., 2010) and incubation lasts 44.2 ± 0.3 days (N = 34). Although males undertake slightly longer incubation shifts than females, males and females provide roughly similar amounts of parental care in this species (Warham, 1990). Nest desertion is a common behavior in snow petrels (Angelier et al., 2007a). At the individual level, reproductive success significantly decreases with increasing egg-laying date during the 2007/2008 season (GLM, N = 50, $\chi^2 = 4.910$, $p = 0.027$). A significant part of the population on Ile des Pétrels is of known age because of intensive yearly banding of chicks since 1963. Some adults were also ringed during their first breeding attempt and a minimum age could be attributed to them by adding the minimum age at first breeding in snow petrels (i.e. 5 years, Chastel et al., 1993) to the time elapsed since ringing.

The treatment: manipulation of hatching date

To manipulate hatching dates, a cross-fostering protocol was applied between paired nests, on the 7th and 8th of January 2008, which was during the second half of the incubating period. In order to avoid egg unviability, we selected nests that were not deserted prior to the treatment and we did not switch eggs with hairlines cracks on their shell. All the eggs were measured (length and breadth) to the nearest 0.5 mm using a calliper, and were swapped with eggs of similar volume to reduce potential egg volume effects (Barbraud et al., 2000). During egg transfer (less than 10 minutes), the egg in the first nest was temporarily replaced with warmed dummy egg that was readily accepted by the incubating parent. Eggs were manipulated with care and transported from one nest to the other in a box covered by cotton. Eggs were swapped between two nests whose egg-laying date differed from 5 days. Egg-laying dates were precisely known by daily nest checking during the egg-laying period (i.e. 3-17 December 2007). A time lag of 5 days seemed to be sufficient as it corresponded to 2.5 ± 0.5 time the standard deviation of the incubation length. Pairs receiving an egg that was laid 5

days earlier than their own were called “advanced pairs” (N = 18 nests), whereas pairs receiving an egg that was laid 5 days later than their own were called “delayed pairs” (N = 20 nests). Pairs with unchanged hatching date constituted the control group (N = 37 nests). Among the 75 studied nests, 63 parents were of known age (banded as chicks) and 12 parents had a minimum estimated age (number of years since banding + 5 years). Groups did not differ in the age of the parents ($F_{2,72} = 0.042$, $p = 0.959$, advanced: 20.0 ± 2.4 [SE], 10-40 (range) years old; control: 20.0 ± 1.3 [SE], 7-44 (range) years old and delayed: 20.7 ± 2.0 [SE], 11-37 (range) years old).

Effect of the treatment on hatching success and brooding effort

The 75 nests were checked every day following the treatment and until the end of the chick-rearing period to monitor parental behavior, hatching success, hatching date, the length of the guarding period and chick survival. The duration of the guarding period (mean \pm SE: 10.2 ± 0.4 days, N = 62 in 2007/2008 and in 2008/2009) is of high importance because young chicks are not thermally independent and require constant attendance by their parents. The opportunities of breeders to forage for themselves are thus constrained (Catry et al., 2006). Male and female snow petrels provide roughly similar amounts of parental care and the shifts during brooding last between 2 to 3 days (Warham, 1990). During early guarding (chick was 4.9 ± 0.1 days old), body mass and skull length (head + bill) of chicks were recorded to the nearest 0.5g using a spring balance and to the nearest 0.5 mm using a calliper. Chick body condition was then calculated from a least-squares linear regression of body mass against skull length ($F_{1,56} = 13.699$, $p < 0.001$).

Measuring the stress response

During the guarding period (chick was 4.9 ± 0.1 days old), one known-age parent of each experimental pair was captured by hand at its nest. An initial blood sample (for baseline corticosterone levels) was collected from the alar vein immediately after capture (mean \pm SE: 119 ± 5 seconds) using a 1-mL heparinized syringe and a 25-gauge needle. For two of them, bleeding time exceeded 3 minutes (i.e. the time required for baseline corticosterone levels, Romero and Reed, 2005), so these two values were not used for the analysis. After the first bleeding, the bird was placed into cloth bags and a second blood sample was collected 30 min later (for stress-induced corticosterone levels) according to the standardized capture/restraint

stress protocol (Wingfield, 1994). Adults were weighted to the nearest 2 g using a spring balance and their skull length (head + bill) was measured to the nearest 0.5 mm using a calliper. In snow petrels, skull length appears to be a reliable measure of the overall size of a bird (see Angelier et al., 2007a). Adult body condition was then calculated from a least-squares linear regression of body mass against skull length ($F_{1,57} = 39.476$, $p < 0.001$). Petrels were marked with spots of dye on the forehead to distinguish them from their partner. During handling of adults, chicks were placed in a box covered with cotton to keep them warm. At the end of the sampling, first chicks then adults were put back onto their nest.

Molecular sexing and hormone assay

Within 6 hours from sampling, blood samples were centrifuged and plasma was decanted and stored at -20°C until assayed. Red cells were also kept frozen for molecular sexing in our laboratory (CEBC). The sex of adults was determined by polymerase chain reaction (PCR) amplification of part of two highly conserved genes (CHD) present on the sex chromosomes (Fridolfsson and Ellegren, 1999), as detailed in Weimerskirch et al. (2005). Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC, as described previously (Lormée et al., 2003). All samples were run in one assay and intra-assay variation was 7.9% ($N = 6$ duplicates).

Data processing and analysis

All analyses were conducted using R 2.8.0 (R Development Core Team 2008). The corticosterone stress response was expressed as the stress-induced levels of corticosterone. Baseline and stress-induced corticosterone levels were not correlated in our sample size ($r = 0.212$, $p = 0.104$, $N = 60$). We first used generalized additive models (GAM) and considered age of adults as a smoothed variable. However, significant effects of age were only linear so we moved to generalized linear model (GLM). GLM with binomial error distribution and a logit link function was used to examine hatching success as a function of treatment (advanced, delayed and control groups) and age of adults. The interaction treatment * age was not included in the analysis, due to the low number of eggs that failed to hatch ($N = 6$). Chick survival was tested as a function of treatment, adult body condition and adult age. The interactions treatment * adult body condition and treatment * age were not included in the analysis, due to the low number of dead chicks ($N = 6$). We used GLM with normal errors and

an identity link function to examine the length of the guarding period and chick body condition as a function of treatment, adult body condition, adult age and the interactions treatment * adult body condition and treatment * adult age. The magnitude of stress response was expressed as the stress-induced levels of corticosterone. We also calculated the amplitude of corticosterone release (i.e. the difference between stress-induced and baseline levels) and the statistical analyses of this variable gave nearly identical results, hence we report only the results for stress-induced corticosterone levels to facilitate the comparison with other published results. Baseline corticosterone and stress-induced corticosterone levels were tested as a function of treatment, sex, adult body condition, adult age and the interactions treatment * adult body condition, treatment * adult age. Dependent continuous variables were log-transformed if necessary. We used a step-down approach starting from the most global models and therefore simplified them by eliminating step by step non-significant independent variables. When treatment significantly affects one of the dependant variables, a post hoc pairwise t-test with Bonferroni correction was used to compare the three groups. The effect of intrinsic breeding schedule was not included in the above models, since the expected hatching date differed between groups (Post hoc Bonferroni correction: delayed-control: $p = 0.012$, advanced-delayed: $p < 0.001$, advanced-control: $p = 0.265$, Fig. 1).

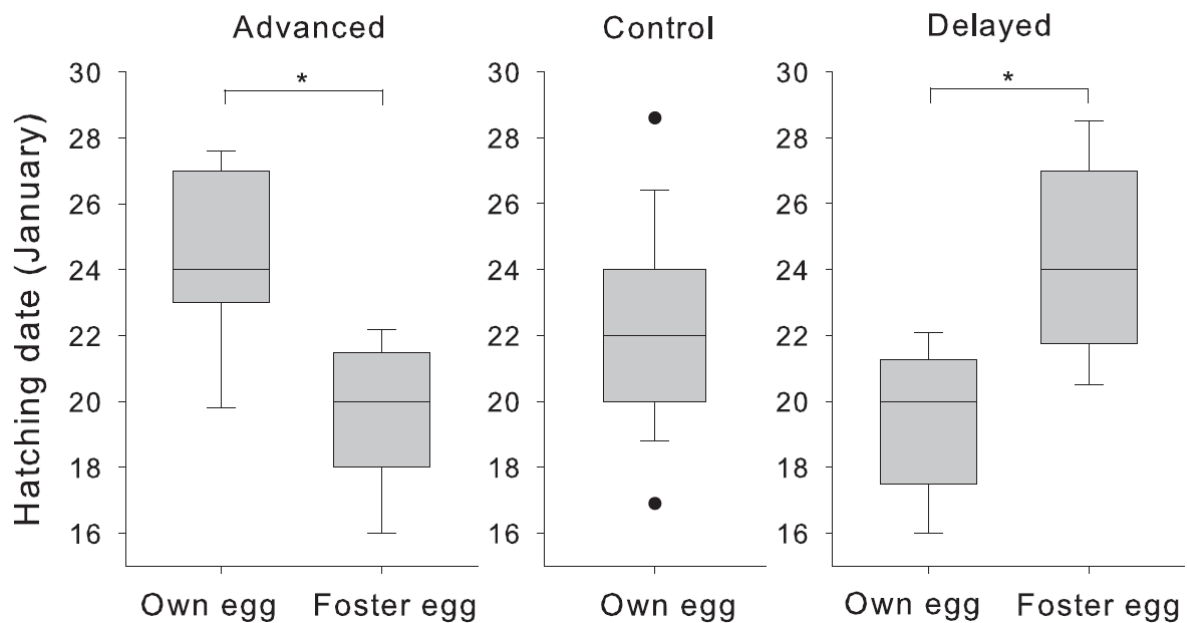


Figure 1: Expected (own egg) and manipulated (foster egg) hatching date in the advanced and the delayed groups, and controls hatching date.

Results

Expected hatching date (own egg) significantly differed from the manipulated hatching date (foster egg) in advanced pairs (paired t-test: $df = 13$, $t = 13.769$, $p < 0.001$, Fig. 1) and in delayed pairs (paired t-test: $df = 12$, $t = -14.506$, $p < 0.001$, Fig. 1). Moreover, the hatching date (January) differed among groups ($F_{2,65} = 9.086$, $p < 0.001$).

Effect of the treatment on reproductive success and brooding effort

Hatching success differed significantly between experimental groups ($N = 75$, $\chi^2 = 12.150$, $p = 0.003$) and was lower in delayed pairs (Post hoc Bonferroni correction: advanced-control: $p = 1$; advanced-delayed: $p = 0.011$, delayed-control: $p = 0.007$, Fig. 2A). Indeed incubation behavior was reduced in the delayed group: five delayed pairs deserted their nest 3.6 ± 2.6 days after the expected hatching date, while only one control pair and no advanced pairs deserted their nest. There was no effect of adult age on hatching success ($N = 75$, $\chi^2 = 1.175$, $p = 0.278$). The treatment and the age of adults did not influence the length of the guarding period and the chick body condition (Table 1a,b). Chick survival was neither influenced by the treatment ($N = 68$, $\chi^2 = 0.398$, $p = 0.820$, Fig. 2B), nor by parents age ($N = 68$, $\chi^2 = 0.053$, $p = 0.819$) and nor by parents body condition ($N = 68$, $\chi^2 = 0.003$, $p = 0.960$).

Table 1: Modelling (a) length of the guarding period and (b) chick body condition at the end of the guarding period according to the treatment, adult body condition and the interactions using GLMs (normal error distribution, identity link function). A step-down approach was used and variables are ordered according to the elimination process.

Dependent variable	Independent variables	df	F-value	p-value
(a) Length of the guarding period (log)	Adult body condition	1,60	2.986	0.089
	Treatment	2,58	1.523	0.227
	Adult age	1,57	0.468	0.497
	Treatment * Body condition	2,55	0.959	0.390
	Treatment * Age	2,53	< 0.001	0.999
(b) Chick body condition	Adult body condition	1,59	2.794	0.099
	Treatment	2,57	0.326	0.723
	Adult age	1,56	0.470	0.496
	Treatment * Age	2,54	1.513	0.229
	Treatment * Body condition	2,52	0.960	0.390

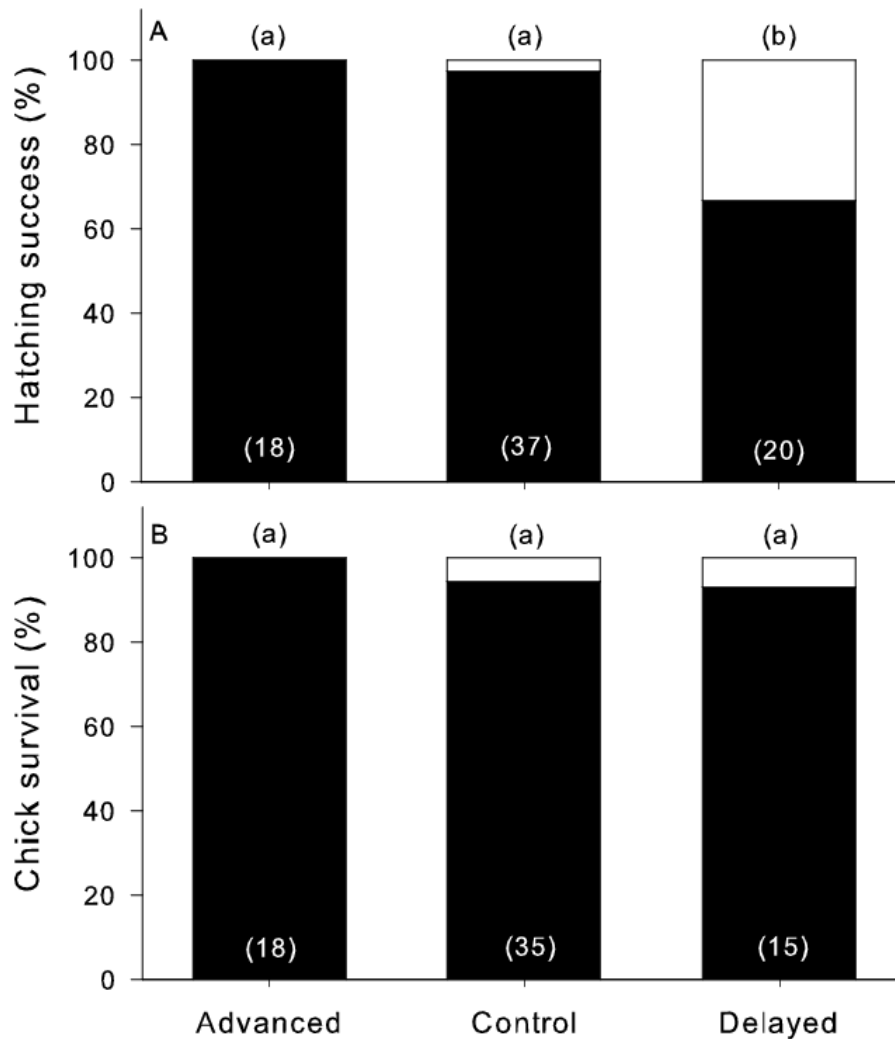


Figure 2: (A) Hatching success in relation to experimental manipulation of hatching dates. (B) Chick survival in relation to experimental manipulation of hatching dates. Sample sizes are given for each group.

Effect of treatment on adult stress response

Adult body condition was not affected by the treatment ($F_{2,56} = 0.584$, $p = 0.561$). Baseline corticosterone levels were not affected by the treatment (Table 2a, Fig. 3A) but decreased significantly with increasing adult age (Table 2a, Fig. 4A). Circulating plasma levels of corticosterone increased significantly following capture and handling ($p < 0.001$ for paired Student's t-tests). There was a significant effect of treatment on stress-induced corticosterone levels (Table 2b, Fig. 3B): delayed birds responded more strongly to the acute stress protocol than control ones (Post hoc Bonferroni correction: delayed-control: $p = 0.015$,

advanced-delayed, $p = 0.143$, advanced-control, $p = 1.000$). In addition, stress-induced corticosterone significantly decreased with increasing adult age (Fig. 4B) and with improving adult body condition (estimate of the slope: -0.080 ± 0.044), without interactions effect treatment * adult age and/or treatment * adult body condition (Table 2b).

Table 2: Modelling baseline (a) and stress-induced (b) corticosterone levels according to the treatment, adult age, sex, adult body condition and the interactions using GLMs (normal error distribution, identity link function). The best models (in bold type) were selected by using a step-down approach starting from the most global model. In this table, variables are ordered according to the elimination process.

Dependent variable	Independent variables	df	F-value	p-value
(a) Baseline corticosterone levels (log)	Adult age	1,56	4.586	0.037
	Treatment	2,54	0.582	0.562
	Treatment * Adult age	2,52	2.726	0.075
	Sex	1,49	0.071	0.791
	Treatment * Sex	2,47	1.684	0.197
	Adult Body condition (BC)	1,45	0.208	0.651
	Treatment * Adult BC	2,43	0.865	0.428
	(b) Stress-induced corticosterone levels	Treatment	1,54	4.733
Adult Body condition (BC)		1,54	4.136	0.047
Adult age		1,54	4.756	0.034
Treatment * Adult BC		2,52	1.945	0.153
Treatment * Adult age		2,50	1.003	0.374
Sex		1,49	0.014	0.905
Treatment * Sex		2,47	0.048	0.954

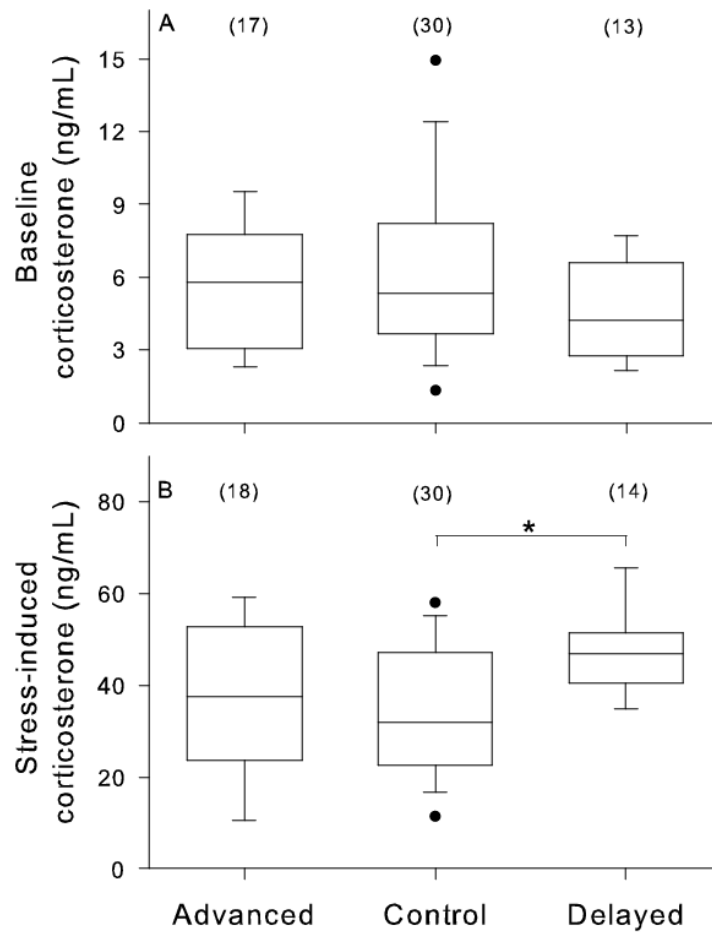


Figure 3: Effect of experimental manipulation of hatching dates on plasma levels of baseline (A) and of stress-induced (B) corticosterone (ng/mL) measured in brooding adults within each experimental group. Sample sizes are given for each group.

Discussion

In this experimental study, we tested whether parental effort is modulated when hatching occurs earlier or later than expected. According to our hypothesis, we found that incubation behavior and hatching success were reduced in delayed snow petrels. Moreover they mounted a stronger stress response during early chick-rearing period compared to control snow petrels. To our knowledge, this is the first study to show that an experimental manipulation of the timing of breeding can influence the magnitude of stress-response and the parental commitment to reproduction. In addition to this treatment effect, the magnitude of the stress response declined with older age and with better body condition.

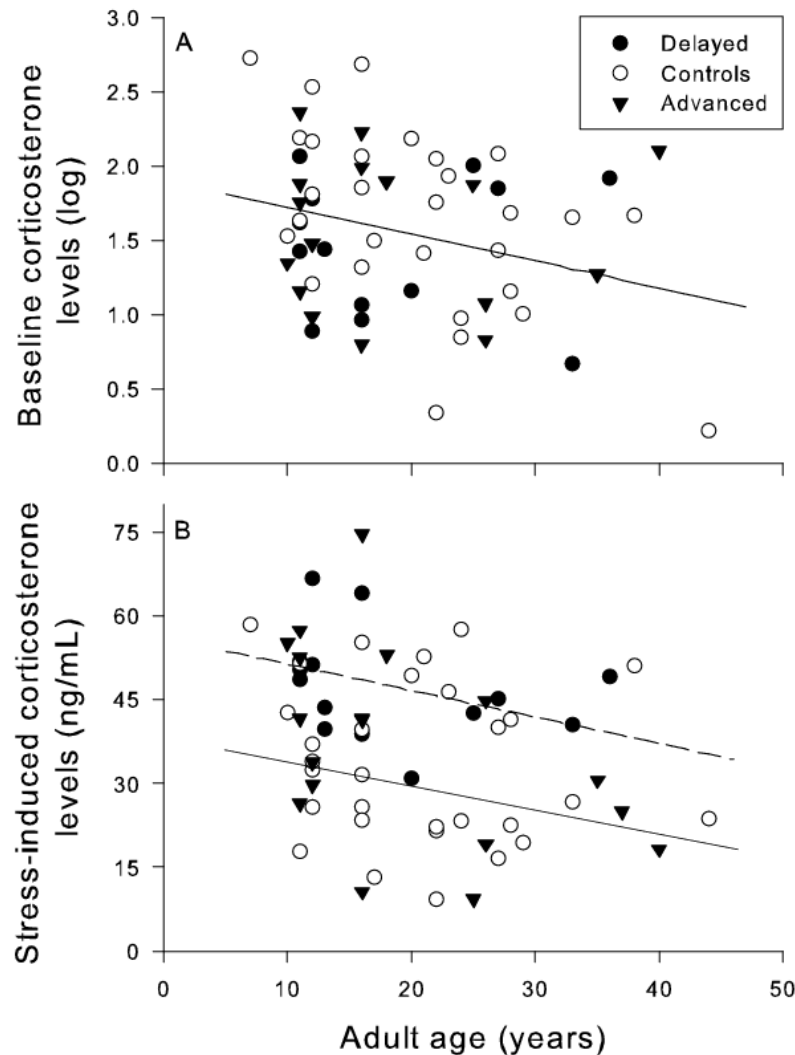


Figure 4: Relationships between age and plasma levels of baseline (A) and (B) stress-induced corticosterone (ng/mL) measured in brooding adults within each experimental group. Open circles denotes controls birds; filled circles, delayed birds; and triangles, advanced birds. The decrease of baseline corticosterone with age (A) did not differ between groups (solid line). The decrease of stress-induced corticosterone levels with age (B) is represented by a solid line for control and advanced birds and by a dashed line for delayed birds.

Fitness consequences of the treatment and parental behavior

Our manipulation had clear fitness consequences, since the overall breeding success of the delayed group was significantly reduced compared to advanced and control pairs. In seabirds, the decline in reproductive success within the season is mainly due to hatching failure, rather than chick mortality (De Forest and Gaston, 1996; Minguéz, 1998). Moreover in petrels, the release of corticosterone in response to stress is higher during incubation than

during brooding period (Adams et al., 2005; Angelier et al., 2009), possibly because of the lower fitness value of an egg relative to chick's one. Our treatment clearly reflected this pattern, since hatching success, but not chick survival, was compromised by the experimentally postponed hatching date. This hatching failure was entirely due to egg desertion, the main source of failure in snow petrels (Angelier et al., 2007a) and this low nest attendance was observed only at the end of the incubation period: delayed snow petrels were more susceptible to desert an egg after the expected hatching date. In addition to this incubation behavior, the hypothesis of reduced parental effort in delayed pairs was supported by a magnified stress response during the early chick-rearing period.

Alternatively, as incubation requires fasting at the nest (Heaney and Monaghan, 1996), delayed birds could have been exhausted by the experimentally prolonged incubation time. However, the adult body condition and/or baseline corticosterone levels (markers of physiological state and activities, Wingfield et al., 1998; Landys et al., 2006) did not differ between delayed petrels and control ones. This suggests that stored energy was not depleted in delayed birds and that the extended incubation effort did not require a significantly harder work, compared to controls. However, we could not exclude the possibility that delayed and failed birds were not able to cope with energetic constraints of extended incubation effort, since body condition and baseline corticosterone levels were not measured during incubation to avoid extra disturbance, and were thus only monitored in birds that did not desert the nest.

In contrast with the incubation period, we did not detect any observable behavioral consequences of the treatment, during the brooding period. Contrary to our expectations, chicks' body condition and survival were not reduced in the delayed groups. However, it is important to notice that the 2007/2008 breeding season was characterized by a high breeding success (68% of successfully fledged chicks, $N = 204$ eggs in the long-term study plots), well above the average 50% recorded for this species in Adélie Land (Chastel et al., 1993). Since adult baseline corticosterone levels and body condition did not differ between groups, delayed birds seemed to have provided the same levels of sustained activities (Landys et al., 2006) and brooding effort compared to control and advanced ones. These results support the ideas that the fitness value of the brood, once hatched, did not differ between delayed, advanced and control pairs. To go further, we investigated the stress response of brooding parents, which are known to be actively modulated according to the brood value (Landvai et al., 2007).

Timing of breeding and parental stress response

According to our prediction, delayed snow petrels responded stronger to acute stress than control ones. An elevated stress response redirects energetic resource away from parental duties and towards survival ('emergency stage', Wingfield et al., 1998). According to the brood value hypothesis, stress response is attenuated or even suppressed, when the value of reproduction is high relative to the value of future breeding opportunities, to ensure that the current breeding success is not threatened (Silverin and Wingfield 1998; Wingfield and Sapolsky, 2003; Lendvai et al., 2007; Lendvai and Chastel, 2008). Hence, our findings support the hypothesis that parents value the brood less if it hatched later than expected. Alternatively, we could not exclude that delayed snow petrels exhibited elevated stress-induced corticosterone levels as a reaction to the delayed hatching date.

Parental behavior during the guarding period was not affected by the treatment. Despite high stress-induced corticosterone levels, the length of guarding period, the chick's body condition, the chick's survival were not reduced in delayed pairs. Thus, how can we interpret the stronger release of corticosterone in response to acute stress in experimentally delayed pairs compared to controls? First, our results are consistent with studies in house sparrows (*Passer domesticus*, Lendvai et al., 2007; Lendvai and Chastel, 2008): an experimentally increased brood value (increased clutch size) did not change adult body condition and baseline corticosterone levels but clearly attenuated the stress-induced corticosterone release. Second, it is important to keep in mind that the stress-induced corticosterone levels reflect the parental motivation in a stressful context (Wingfield and Sapolsky, 2003). In case of unpredictable adverse conditions (e.g. snow storm which cause significant chick mortality in snow petrels, Chastel et al., 1993), delayed pairs would have mounted a higher acute release of corticosterone than controls. In turn, they would likely have provided poorer brooding effort, thereby promoting their own survival in case of a life-threatening perturbation.

Interestingly, advanced pairs did not attenuate their stress response compared to control ones, suggesting that an earlier-hatching chick was not value more than a control one. This contrasts with the results obtained in the short-lived house sparrow (Lendvai et al., 2007): parents with experimentally enhanced brood value (increased clutch size) were able to actively attenuate their adrenocortical response to stress. Our result suggest that an advanced brood was not valued more than the expected one. However, snow petrels, as very long-lived organisms (up to 46 years old) show a stronger and probably less flexible response to stress

than short-lived species (Bokony et al., 2009). In this study, the very long-lived snow petrels tended to behave as prudent parents (Drent and Dann, 1980), and would have not jeopardized their own survival by suppressing their stress response, even for a highly valuable chick. This also highlights possible survival costs of early breeding (Brinkhof et al., 2002; reviewed in Drent, 2006) and the need for long-lived species to adjust parental care for future breeding prospects. It would be interesting to conduct a similar study in short-lived birds to compare the flexibility of the adult stress response in regard to an advanced / delayed brood.

Regarding the age of adult snow petrels, we did not find any effect of age on parental behavior, through hatching success, length of the guarding period, chick body condition and chick survival. At a proximate level, young snow petrels exhibited higher baseline corticosterone levels than old ones, suggesting higher energetic constraints (Angelier et al., 2007b). During the brooding period, older snow petrels exhibited lower stress-induced corticosterone levels than younger ones. A decline of the stress response over age has been also found in incubating common terns (*Sterna hirundo*, Heidinger et al., 2006; Heidinger et al., 2008). Similarly, prolactin, a hormone involved widely in regulating parental cares, decreased after a stressor in a age-specific manner: old snow petrels maintained higher stress-induced prolactin levels than young ones (Angelier et al., 2007a). Such age-related stress responses have been interpreted as a mechanism underlying the well-known improvement of reproductive performance over age (Heidinger et al., 2006; Angelier et al., 2007a). Alternatively, our transversal study could not exclude the selection hypothesis: phenotypes excessively sensitive to stress could have progressively disappeared. This implies that the magnitude of the stress response is fixed early in the reproductive life of an individual. However, individuals appear to modulate their hormonal stress response flexibly according to the value of the reproductive event (Lendvai et al., 2007). Stress resistance may have also arisen from a habituation process to handling with age. However known-age snow petrels have been seldom handled prior to this study and a similar study found no effect of recapture history on stress-hormone levels (Heidinger et al., 2006). Such age-related resistance to acute stress was independent of the treatment which only magnified the stress response in delayed birds. Hence, the degree of flexibility in response to reduced brood value did not differ across age. This strengthens the hypothesis that the responsiveness to stressors is adjusted according to the current brood value (timing of breeding) and additionally, to the individual prospect of future breeding opportunities (age of breeders).

Our study provides evidence that snow petrels value the brood less if it hatched later than expected. This adjustment in parental effort, combined with individual (age, body

condition) and environmental factors, might give new insights into the tight link between breeding schedule and reproductive success.

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IV. Age, phénologie et mécanismes hormonaux

IV.1. Organisation du chapitre

Chez les oiseaux longévifs, l'âge influence le succès reproducteur, la décision de ne pas se reproduire et la date de ponte. Les articles C & D ont pour vocation l'étude des processus hormonaux, au niveau individuel, qui sont impliqués dans l'orchestration de la décision de ne pas se reproduire et du timing de la reproduction en fonction de l'âge (7-45 ans).

D'une part, l'article C cible le rôle de la corticostérone et de la sensibilité au stress comme médiateur potentiel entre l'âge et les décisions de ne pas se reproduire et / ou les décisions de date de ponte. Concernant l'âge, les très jeunes ont une probabilité plus élevée de ne pas se reproduire. De plus, les très jeunes et les très vieux sont les plus tardifs. Nous avons mis en évidence que les très jeunes et les très vieux sont les plus sensibles au stress. Les taux basaux de corticostérone influencent la probabilité de ne pas se reproduire, chez les femelles, et la date de ponte chez les mâles et femelles (Article C).

D'autre part et via un test de réponse à la GnRH, la dynamique de sécrétion de la LH a été suivie chez les pétrels des neiges (article D). Les taux basaux de LH augmentent avec l'âge, tandis que la capacité maximale de sécrétion de la LH n'est pas dépendante de l'âge. De façon intéressante, nous avons montré que les très jeunes et les très vieux étaient moins aptes à maintenir des taux élevés de LH après un pic de LH, induit par l'injection de GnRH. Ce fort déclin des taux de LH pendant une période de stress prédit la décision de ne pas se reproduire (Article D).

IV.2. Age, décision de ponte, date de ponte et corticostérone

ARTICLE C

Age and the timing of breeding in a long-lived bird: a role for stress hormones?

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Age and the timing of breeding in a long-lived bird: a role for stress hormones?

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Summary

1. Young birds often reproduce later in the season than older ones, with poorer breeding success, but the proximate mechanisms involved in such a pattern remain poorly studied, especially in long-lived species. One possible mechanism is the endocrine stress response which is accompanied by the release of corticosterone. Elevated corticosterone levels can trigger physiological and behavioural responses that may delay or even suppress reproduction.

2. We tested the hypothesis that the delayed timing of breeding of young birds may be related to a greater susceptibility to stress compared with older ones during the pre-laying period of the breeding season. For this purpose, known-age (7–44 years old) pre-laying snow petrels, *Pagodroma nivea*, were monitored for baseline and acute stress-induced corticosterone levels. We examined whether baseline and stress-induced corticosterone levels were related to age, and whether they could influence the decision to breed and egg-laying date.

3. Young snow petrels were more likely to skip the breeding season and to breed later than middle-aged birds. In addition, the oldest birds bred later than middle-aged ones, suggesting a possible senescence on laying dates. Baseline corticosterone levels were independent of age but young and very old birds were more sensitive to stress than middle-aged ones. However, there was no effect of stress-induced corticosterone levels on breeding decision and egg-laying date. Elevated baseline corticosterone levels during the pre-laying period were associated with a higher probability of skipping breeding in females and a delayed timing of egg-laying in both sexes.

4. These results suggest that the greater susceptibility of young breeders to stress was not the functional mechanism explaining their delayed timing of breeding. Baseline corticosterone levels, although independent of age, appear to be a more likely mediator of breeding decision and egg-laying date. In long-lived birds, the relationship between age and timing of breeding may be rather indirect, as other age-related factors such as breeding experience or foraging skills may have a deeper impact on baseline corticosterone than age *per se*.

Key-words: age, glucocorticoids, laying date, snow petrel (*Pagodroma nivea*), stress response

Introduction

Breeding at the right time is a key factor for successful reproduction in fluctuating environments (Lack 1968; Perrins 1970; Nager & van Noordwijk 1995). The adjustment of breeding schedule allows a good overlap between offspring's energy requirements and local peak in food availability (*match–mismatch hypothesis*; Visser *et al.* 1998; Stenseth & Myrseth 2002; Durant *et al.* 2007). In bird species, although early breeders usually perform better than late breeders, the decision to start breeding greatly differs between individuals. Typically, young breeders reproduce

later in the season than older ones, and have lower reproductive success (Martin 1995; DeForest & Gaston 1996; Gonzalez-Solis *et al.* 2004; Ezard, Becker & Coulson 2007; McCleery *et al.* 2008; Blas, Sergio & Hiraldo 2009). This could be explained by (i) a progressive disappearance of late-breeders over age classes (*selection hypothesis*; Mauck, Huntington & Grubb 2004), (ii) an individual adjustment of life-history strategies: as it gets older, a bird would favour current breeding rather than survival (*restraint hypothesis*; Stearns 1992), (iii) an improvement of breeding skills with age owing to the accumulation of breeding experience (*constraint hypothesis*, Curio 1983) or (iv) a progressive incorporation of early breeders over age classes (*delayed-breeder hypothesis*; Forslund & Pärt 1995; Blas, Sergio & Hiraldo

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2009). Ultimate factors explaining why young breeders reproduce later than older individuals have been discussed widely (Curio 1983; Forslund & Pärt 1995; Blas & Hiraldo 2010). However, the mechanisms underlying this age-specific timing of breeding are still poorly understood. In that respect, endocrine mechanisms deserve special attention as they mediate life-history strategies in relation to environmental conditions and to the physiological state of individuals (Wingfield *et al.* 1998; Ricklefs & Wikelski 2002).

Specifically, the endocrine stress response could play a key role in the age-specific timing of breeding. In vertebrates, stressful conditions such as harsh weather, scarce food availability or presence of predators, trigger an acute secretion of glucocorticoid hormones, that is, cortisol or corticosterone (Wingfield *et al.* 1998). In turn, this stress response allows individuals to cope with the stressors by suppressing reproductive functions (Wingfield & Sapolsky 2003; Landys, Ramenofsky & Wingfield 2006; Breuner, Patterson & Hahn 2008). Although the majority of such studies focused on the parental phase of the reproductive cycle (i.e. incubation and chick rearing in birds), very few studies have investigated relationships between glucocorticoids, known to interfere with the hypothalamic–pituitary–gonadal axis (Wingfield & Sapolsky 2003; Pérez-Rodríguez *et al.* 2006), and the timing of breeding (Salvante & Williams 2003; Schoech *et al.* 2009). These studies suggest a similar inhibiting role of corticosterone during the pre-laying period (i.e. before the onset of breeding). Elevated corticosterone levels were associated with delayed first clutch initiation during unfavourable years in Florida scrub jay (*Aphelocoma coerulescens*; Schoech *et al.* 2009). Furthermore, experimental administration of exogenous corticosterone in zebra finches (*Taeniopygia guttata*) greatly decreased the number of pairs initiating reproduction and delayed clutch initiation (Salvante & Williams 2003). Corticosterone appears therefore to be an excellent candidate adjusting the timing of breeding in response to environmental conditions (Schoech *et al.* 2009). Understanding the functional role of corticosterone implies to distinguish between (i) baseline corticosterone level, which is a marker of activities and energetic state (Kitaysky, Wingfield & Piatt 1999; Love *et al.* 2004; Landys, Ramenofsky & Wingfield 2006), and (ii) stress-induced corticosterone level, which mirrors the sensitivity to stress and less commitment to current reproduction (Lendvai, Giraudeau & Chastel 2007).

According to life-history theory, the stress response should be attenuated when the value of the current reproduction is high relative to the value of future breeding events (Wingfield & Sapolsky 2003). Recent experimental and comparative studies support this assumption (*brood value hypothesis*; Lendvai, Giraudeau & Chastel 2007; Lendvai & Chastel 2008; Bokony *et al.* 2009). Relating this to age-specific reproductive success, it has been predicted and observed that the stress response of old adults is attenuated, probably to ensure that reproduction is not inhibited (Heidinger, Nisbet & Ketterson 2006; Angelier *et al.* 2007a). During the pre-laying period, an attenuation of the stress response might therefore be the endocrine mechanism underlying early breeding in old birds.

Focusing on a long-lived species, the snow petrel (*Pagodroma nivea* Foster), we hypothesized that young birds are more sensitive to stress before the onset of reproduction than older ones. First, we tested whether young birds lay their egg later or whether they were more likely to skip the breeding season than older birds. Secondly, we tested whether baseline or stress-induced corticosterone levels varied with age. We predicted that young birds would exhibit higher baseline and/or stress-induced corticosterone levels than older birds during the pre-laying period. Thirdly, we predicted that individuals with high pre-laying baseline and/or stress-induced corticosterone levels would delay the onset of breeding or even halt their reproduction.

Materials and methods

STUDY SITE AND SPECIES

The study was carried out on Ile des Pétrels, Pointe Géologie Archipelago, Terre Adélie (66°40'S, 140°01'E), Antarctica. Snow petrels (250–500 g; Fig. 1) are very long-lived birds with low annual fecundity and high adult life expectancy (Chastel, Weimerskirch & Jouventin 1993; Jenouvrier, Barbraud & Weimerskirch 2005). Like all Procellariiform species, breeding pairs lay only one egg per season, with no replacement clutch in case of failure (Chastel, Weimerskirch & Jouventin 1993; Barbraud & Weimerskirch 2001). The age at first breeding is 10 years on average and birds breed annually, although a significant proportion of birds skip the breeding season (Chastel, Weimerskirch & Jouventin 1993). Nest and mate fidelity is high (Bried, Pontier & Jouventin 2003). Males and females provide roughly similar amounts of parental care (incubation of the egg and care for the chick). Birds arrive on the breeding ground in early November. The courtship and mating period typically lasts about 2 weeks. Then, individuals of both sexes forage at sea to accumulate resources and females build up reserves for the egg (pre-laying exodus). The laying period ranges from late November to mid-December. Within one breeding season, egg-laying dates differ between individuals over a period of 2 weeks (Barbraud, Lormee & LeNeve 2000).

The ongoing mark-recapture programme of the snow petrels population began in 1963 (see Chastel, Weimerskirch & Jouventin 1993; Barbraud & Weimerskirch 2001 for detailed methodology). Every



Fig. 1. A pair of courting snow petrels during the pre-laying period, in Terre Adélie, Antarctica. Photo by Aurélie Goutte.

chick of the monitored colonies has been banded prior to fledging every year since 1963. Thus, a part of the current population is of known age.

BLOOD SAMPLING, BODY CONDITION AND PHENOLOGY

Adult male and female snow petrels were handled during the 2007 pre-laying period (6–19 November, that is, 26.93 ± 0.76 days before egg-laying). A total of 55 known-age adults (7–40 years old) and 11 unknown-age adults (but they were ringed as adults and they were at least 11 years old) were caught at their nest, in 65 different nests. In one nest, both partners of the pair were of known age and we randomly selected one bird of this pair. A blood sample from the alar vein was taken immediately (157.5 ± 5.8 s) after capture using a 1-mL heparinized syringe and a 25-gauge needle. For nine of them, bleeding time exceeded the time required for baseline corticosterone levels (Romero & Reed 2005), so these nine values were not used for the analysis. Birds were then placed into cloth bags, and subsequent blood samples were collected 30 min later, according to the standardized capture/restraint stress protocol (Wingfield *et al.* 1994). Skull length (head + bill) and tarsus length were measured using a calliper to the nearest 0.5 mm, wing length to the nearest 1 mm and weight to the nearest 2 g using a spring balance. As univariate metrics may be inadequate to estimate overall body size in birds, we used the first component (PC1) of a principal component analysis as an index of size. As male and female snow petrels differ greatly in size (Barbraud & Jouventin 1998), males ($N = 38$) and females ($N = 28$) were analysed separately. The first component accounted for 76 and 72% of the total variance in male and female measurements, respectively, and was highly positively correlated with all variables ($r > 0.83$ for both sexes). PC1 scores increased with the size of the birds. Standardized body condition was then calculated for males and females separately from a least-squares linear regression of body mass against body size (PC1). Petrels were then marked with spots of dye on the forehead to distinguish them from their partner and were released at their nest. Then, we checked the 65 nests every 2 days to monitor the presence or absence of the marked birds, breeding decision (i.e. decision to lay an egg or to skip the breeding season) and the egg-laying date. The time between sampling and egg-laying did not differ between the sexes ($F_{1,52} = 1.078$, $P = 0.304$) and was not related to age ($F_{1,44} = 0.377$, $P = 0.542$).

In addition, we prospected for other known-age birds during the incubating period to increase the sample size. A total of 109 known-age adults (6–44 years old) were indeed monitored for breeding decision, and 96 eggs were followed every day until hatching to assess hatching success and hatching date. As blood sampling and bird handling might disturb the breeding phenology, we compared breeding decision and hatching date of manipulated birds with the sample of non-manipulated birds nesting in the long-term study plots (see Chastel, Weimerskirch & Jouventin 1993), where birds were seldom handled ($N = 221$ for the breeding decision and $N = 142$ for the hatching date). Timing of hatching for handled birds was similar to non-handled ones ($N = 142$; Wilcoxon's $w = 2665.5$, $P = 0.721$). The proportion of breeders tended to be slightly reduced by handling (handled birds: 83.33 ± 4.62%; non-handled birds: 90.95 ± 1.93%; $N = 221$, $\chi^2 = 3.072$, $P = 0.080$).

MOLECULAR SEXING AND HORMONE ASSAY

Blood samples were centrifuged and plasma was decanted and stored at -20 °C until assayed. Red cells were also kept frozen for molecular

sexing at the Centre d'Etudes Biologiques de Chizé (CEBC). Sex was determined by polymerase chain reaction (PCR) amplification of a part of two highly conserved genes (CHD) present on the sex chromosomes (Fridolfsson & Ellegren 1999), as detailed in Weimerskirch, Lallemand & Martin (2005). Plasma concentrations of total baseline and stress-induced corticosterone were determined by radioimmunoassay at the CEBC, as described by Lormée *et al.* (2003). Only one assay was performed and the intra-assay coefficient of variation was 5.6% ($N = 4$ duplicates).

STATISTICAL ANALYSES

All analyses were performed using R 2.8.0 (<http://r-project.org/>). We used generalized linear model (GLM) with binomial error distribution and a logit link function to explain breeding decision, and GLM with normal errors and an identity link function to explain egg-laying date and hormone levels. Dependent continuous variables were previously tested for normality with a Shapiro–Wilk test and were log-transformed when necessary. An information-theoretic approach was used to select the best models (Burnham & Anderson 2002). Model selection criteria included the corrected version of Akaike's information criterion (AICc) for small sample sizes and the difference in AICc between each candidate model and the model with the lowest AICc value (Δ AICc; Burnham & Anderson 2002). AICc weight can be used as a measure of the relative probability of a model for being the best model with a given data set among other potential models. Selected models were then checked for assumptions, that is, constancy of variance and residual normality. Parameters' estimates ± SE were given for models with the highest AICc weight and by removing interactions with covariables (Quinn & Keough 2002).

Age, breeding decision and timing of breeding

As female body size is known to account for a great part of the variation in the egg-laying date of snow petrels (Barbraud, Lormée & LeNeve 2000), we included body size in our analysis. Therefore, we tested whether breeding decision and egg-laying date were influenced by standardized body condition, body size, sex and the interaction body condition × sex and body size × sex in pre-laying snow petrels ($N = 66$). To test for an effect of age, we used known-age snow petrels handled during the pre-laying period and the 54 known-age birds handled after the egg-laying period. As hatching date was monitored for each nest and was highly correlated to egg-laying date (estimate = 0.770 ± 0.077, $F_{1,36} = 99.30$, $P < 0.001$), we used hatching date instead of egg-laying date in this model. We tested whether breeding decision and hatching date were influenced by 'sex', 'age', 'age²' and the interaction 'sex (age + age²)'. Within breeding pairs, the age of the male was positively correlated with that of the female ($F_{1,23} = 6.522$, $P = 0.018$).

Age and stress hormones

Because standardized body condition was negatively related to sampling date (see 'Results'), we first tested whether baseline and stress-induced corticosterone levels were influenced by body condition, sex and the interaction 'sex × body condition' by using linear regressions. Then we analysed whether baseline and stress-induced corticosterone levels were influenced by the sampling date (hereafter 'date'), 'sex', 'age', 'age²' and the interaction 'sex (age + age²)'.

Moreover, we ran the analysis again adding the 'time before egg-laying' variable (i.e. the time between sampling and egg-laying date)

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to take into account a potential hormonal change with advancing time. 'Time before egg-laying' and 'sampling time' were tested separately as they were significantly negatively linked ($F_{1,52} = 39.580$, $P < 0.001$).

Stress hormones, breeding decision and timing of breeding

We tested the influence of pre-laying baseline (or stress-induced) corticosterone on breeding decision and egg-laying date. As baseline and stress-induced corticosterone levels differed between sexes (see 'Results'; Table 1), we could not include the 'sex' and 'baseline (or stress-induced) corticosterone levels' variables simultaneously in a model. Thus, we standardized baseline (or stress-induced) corticosterone levels for each sex by subtracting for each sex the mean baseline (or stress-induced) corticosterone levels from each observed value, and dividing by the SD. Because sex and standardized corticosterone levels were no longer associated, we started our analyses from the general model including the 'date', 'sex', 'baseline (or stress-induced) corticosterone levels' variables and the 'sex \times baseline (or stress-induced) corticosterone levels' interaction. To test for an effect on breeding decision and on egg-laying date, we considered all monitored nests prospected during the pre-laying period and then only eggs which hatched successfully.

Results

Pre-laying body condition was not related to age (estimate = 0.009 ± 0.014 , $F_{1,53} = 0.427$, $P = 0.516$). Pre-laying body condition decreased with the date of sampling (estimate = -0.111 ± 0.029 , $F_{1,64} = 14.961$, $P < 0.001$), but was not affected by the time between sampling and egg-laying date (estimate = 0.047 ± 0.028 , $F_{1,52} = 2.779$, $P = 0.102$).

AGE, BREEDING DECISION AND TIMING OF BREEDING

Breeding decision and egg-laying date were not influenced by pre-laying body condition or body size (for all tests: $P > 0.16$). Breeding decision was influenced by the breeder's age ($N = 109$, $\chi^2 = 8.586$, $P < 0.001$): birds that skipped breeding were mainly younger (< 13 years old; Fig. 2a). There was a significant effect of age on hatching date, in a quadratic way: young (less than 13 years old) and very old (more than 30 years old) birds bred later than the 13–30-year-old birds (Fig. 2b; $N = 60$; age: estimate = -0.484 ± 0.209 , $\chi^2 = 4.976$, $P = 0.026$; age²: estimate = 0.010 ± 0.004 ,

Table 1. Model selection using corrected version of Akaike's information criterion (AICc) to explain (a) baseline corticosterone levels ($N = 47$) and (b) stress-induced corticosterone levels ($N = 53$). K refers to the number of parameters. Candidate models and models without any explanatory variable (the 'intercept model') are ranked according to their AICc. The second part of the table includes the parameter estimates for the selected model: (a) 'Date; Sex' and (b) 'Date; Sex; Age; Age²'

No.	Model	AICc	Δ AICc	AICc weight	K
(a)					
1.	Date; Sex	120.58	0	37.6%	4
2.	Date	121.73	1.15	21.1%	3
3.	Date; Sex; Age	122.93	2.35	11.6%	5
4.	Date; Sex; Age; Sex \times Age	123.64	3.07	8.1%	6
5.	Date; Age	124.01	3.43	6.8%	4
6.	Intercept	124.36	3.78	5.7%	2
7.	Date; Sex; Age; Age ² ; Sex (Age + Age ²)	125.23	4.65	3.7%	8
8.	Date; Sex; Age; Age ²	125.41	4.84	3.4%	6
9.	Date; Age; Age ²	126.38	5.80	2.1%	5
	Selected model	Parameters	Estimate \pm SE	Chi-squared	P
	Date; Sex	Intercept	1.44 \pm 0.48	9.11	0.003
		Date	0.08 \pm 0.04	4.87	0.027
		Sex	0.44 \pm 0.24	3.35	0.067
(b)					
1.	Date; Sex; Age; Age ²	480.42	0	63.2%	6
2.	Date; Age; Age ²	484.21	3.80	9.5%	5
3.	Date; Sex	484.38	3.96	8.7%	4
4.	Date; Sex; Age	485.12	4.71	6.0%	5
5.	Date; Sex; Age; Age ² ; Sex (Age + Age ²)	485.22	4.80	5.7%	8
6.	Date	486.23	5.81	3.5%	3
7.	Date; Sex; Age; Sex \times Age	487.45	7.03	1.9%	6
8.	Date; Age	487.88	7.46	1.5%	4
9.	Intercept	502.98	22.56	0%	2
	Selected model	Parameters	Estimate \pm SE	Chi-squared	P
	Date; Sex; Age; Age ²	Intercept	75.17 \pm 26.58	8.04	0.005
		Date	3.51 \pm 0.94	13.87	<0.001
		Sex	14.37 \pm 5.87	5.99	0.014
		Age	-5.76 \pm 2.06	7.83	0.005
		Age ²	0.12 \pm 0.04	6.94	0.008

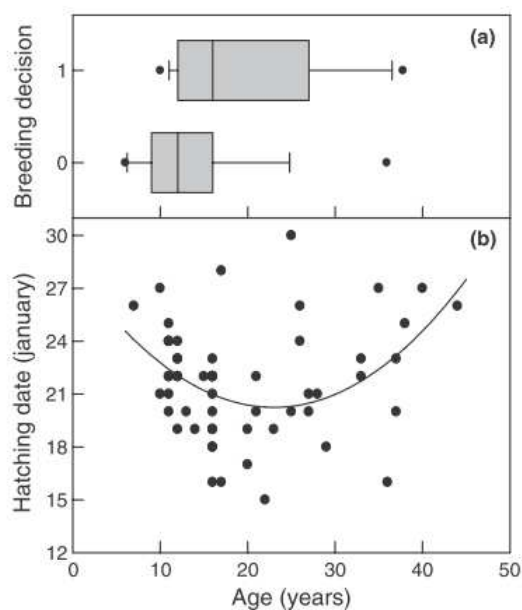


Fig. 2. (a) Breeding decision (0 = non-breeder; 1 = breeder) in relation to age in snow petrels (The median, 10th, 25th, 75th and 90th percentiles are plotted as horizontal boxes with error bars) and (b) hatching date in relation to age in snow petrels. The probability of breeding increased with age, whereas young and very old birds bred later than middle-aged ones.

$\chi^2 = 6.190$, $P = 0.013$). As mentioned, the use of hatching date was more powerful (larger sample size) than the use of egg-laying date ($N = 33$). However, this did not affect the detected trend (egg-laying date = $-0.375 \times \text{age} + 0.009 \times \text{age}^2$ vs. hatching date = $-0.484 \times \text{age} + 0.010 \times \text{age}^2$).

AGE, BODY CONDITION AND STRESS HORMONE LEVELS

Baseline corticosterone levels were not associated with pre-laying body condition (estimate = -0.042 ± 0.631 , $F_{1,55} = 0.006$, $P = 0.940$) even when considering an interaction with sex (estimate = 0.105 ± 0.290 , $F_{1,53} = 0.124$, $P = 0.726$). However, baseline corticosterone levels were higher in males than in females (8.255 ± 0.758 and 6.620 ± 0.972 ng mL⁻¹, respectively), increased with the date of sampling (Table 1a), but were not influenced by age (Table 1a and Fig. 3a).

During the pre-laying period, males and females responded to the stress of capture by increasing their corticosterone levels significantly ($P < 0.001$ for paired Student's *t*-tests). Stress-induced corticosterone levels were independent of baseline corticosterone levels (estimate = 0.003 ± 0.004 , $F_{1,54} = 1.115$, $P = 0.296$). Stress-induced corticosterone levels were negatively correlated to body condition (estimate = -13.690 ± 3.139 , $F_{1,62} = 19.022$, $P < 0.001$). Moreover, stress-induced corticosterone levels were higher in males than in females (77.937 ± 4.213 and 67.860 ± 5.657 ng mL⁻¹, respectively), increased with the date of sampling and were correlated to age in a quadratic

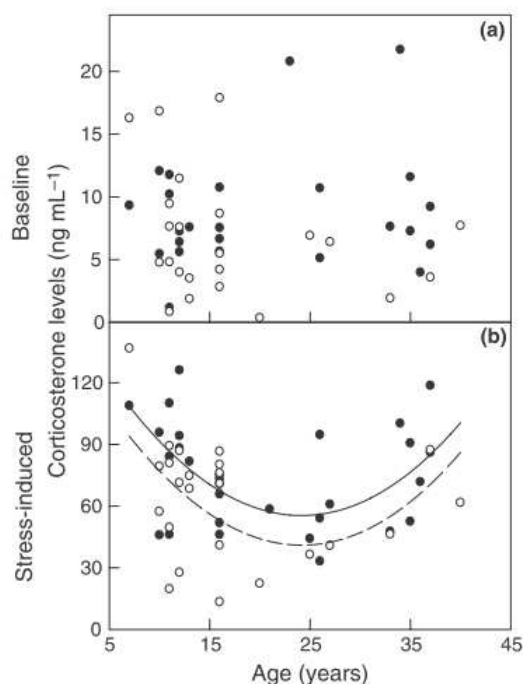


Fig. 3. (a) Baseline and (b) stress-induced corticosterone levels during the pre-laying period in relation to age in males ($N = 29$, closed circles with solid best-fit line), and females ($N = 25$, open circles with dashed best-fit line) snow petrels.

way (Table 1b): young and very old birds were more sensitive to stress than middle-aged ones (Fig. 3b).

After running the analysis with the 'time before egg-laying' variable instead of the 'sampling date' variable, stress hormone levels were not associated with the time before egg-laying ($P > 0.17$ for baseline and stress-induced corticosterone levels).

STRESS HORMONES, BREEDING DECISION AND TIMING OF BREEDING

Elevated baseline corticosterone levels during the pre-laying period were associated with a higher probability of skipping breeding in females (Table 2a and Fig. 4). Breeding decision was not influenced by stress-induced corticosterone levels (Table 2b). When considering all laid eggs (i.e. eggs that hatched successfully and eggs that failed to hatch), egg-laying dates were neither influenced by the standardized baseline corticosterone levels (estimate = 0.123 ± 0.111 , $F_{1,43} = 1.236$, $P = 0.272$), nor by the standardized stress-induced corticosterone levels (estimate = 0.206 ± 0.529 , $F_{1,50} = 0.027$, $P = 0.869$), even when considering an interaction effect 'sex \times standardized corticosterone levels' (baseline: estimate = -1.037 ± 1.148 , $F_{1,41} = 0.817$, $P = 0.371$; stress-induced: estimate = 0.461 ± 1.079 , $F_{1,48} = 0.183$, $P = 0.671$). However, hatching success was negatively correlated with standardized baseline corticosterone levels (estimate = -0.924 ± 0.423 , $N = 40$, $\chi^2 = 9.037$, $P = 0.003$): snow petrels with elevated standardized baseline corticoste-

Table 2. Model selection using corrected version of Akaike's information criterion (AICc) to explain breeding decision (0 = non-breeder; 1 = breeder) in relation to (a) standardized baseline corticosterone levels (Bas. CORT, $N = 57$) and standardized stress-induced corticosterone levels (Max. CORT, $N = 64$) during the pre-laying period. K refers to the number of parameters. Candidate models and models without any explanatory variable (the 'intercept model') are ranked according to their AICc. The second part of the table (a) gives the estimates of parameter (absolute baseline corticosterone levels) for males ($N = 32$) and females ($N = 25$)

No.	Model	AICc	Δ AICc	AICc weight	K
(a)					
1.	Bas. CORT; Sex; Bas. CORT \times Sex	57.62	0	39.6%	4
2.	Intercept	57.92	0.30	34.0%	1
3.	Bas. CORT	59.14	1.52	18.5%	2
4.	Bas. CORT; Sex	60.82	3.20	8.0%	3
Selected model		Parameters	Estimate \pm SE	Chi-squared	P
Bas. CORT; Sex; Bas. CORT \times Sex		<i>Males</i>			
		Intercept	0.55 \pm 1.02	17.301	< 0.001
		Bas. CORT	0.09 \pm 0.12	0.542	0.461
		<i>Females</i>			
		Intercept	3.83 \pm 1.36	89.867	< 0.001
		Bas. CORT	-0.26 \pm 0.12	7.315	0.007
(b)					
1.	T Intercept	61.47	0	50.6%	1
2.	T Max. CORT	62.46	0.98	31.0%	2
3.	T Max. CORT; Sex	64.16	2.68	13.2%	3
4.	T Max. CORT; Sex; Max. CORT \times Sex	66.04	4.56	5.2%	4

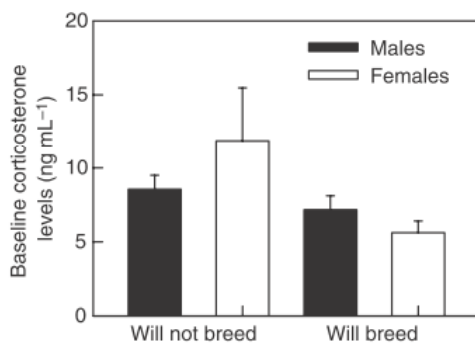


Fig. 4. Standardized baseline pre-laying corticosterone levels (ng mL^{-1} , mean and SE) of snow petrels in relation to breeding decision (will skip or will breed). Black-filled bars denote males and white-filled bars denote females.

rone levels during the pre-laying period were more likely to lose their egg. When only considering eggs that hatched successfully, there was a positive and significant correlation between standardized baseline corticosterone and egg-laying date (Table 3a and Fig. 5a). Pre-laying stress-induced corticosterone levels were not associated with the egg-laying date of hatched eggs (Table 3b and Fig. 5b).

Discussion

As found in other avian species (Forslund & Pärt 1995; Gonzalez-Solis *et al.* 2004; Ezard, Becker & Coulson 2007; McCleery *et al.* 2008), young snow petrels (less than 13 years old) bred later than middle-aged birds (from 13 to 30 years). Moreover, young snow petrels were more likely to skip a breeding attempt than old ones. In addition, the oldest birds

(> 30 years old) bred later than middle-aged ones, suggesting a possible effect of older age on the timing of breeding, a pattern only reported to date in the common tern (*Sterna hirundo*; Ezard, Becker & Coulson 2007) and the mute swan (*Cygnus olor*; McCleery *et al.* 2008). In parallel, young birds and very old birds were more sensitive to stress than middle-aged ones during the pre-laying period. However, there was no link between stress-induced corticosterone levels and egg-laying date. However, elevated baseline corticosterone levels were associated with a higher probability to skip breeding in females and a delayed timing of egg-laying in both sexes.

AGE AND STRESS HORMONES

During the pre-laying period, snow petrels exhibited a 10-fold increase in corticosterone levels related to capture and handling. This magnitude was similar during incubation and was lower during chick rearing in petrel species (Adams *et al.* 2005; Angelier *et al.* 2007a, 2009). Adults in poor body condition were more sensitive to stress than those in good body condition as shown in many other species (Wingfield *et al.* 1998; Angelier *et al.* 2009). Furthermore, baseline and stress-induced corticosterone levels increased with sampling date, whereas body condition decreased with sampling date, but not with the time before egg-laying. During the pre-laying period, early-arrived petrels were indeed in better condition than late-arrived ones (Chastel, Weimerskirch & Jouventin 1995). Birds in poor condition and showing elevated corticosterone levels may then be of lower quality (e.g. low foraging skills) and may not be able to arrive early in the season. Males exhibited slightly higher baseline and stress-induced corticosterone levels than females, possibly in response to different pre-laying energetic constraints and activities (Wingfield *et al.*

Table 3. Model selection using corrected version of Akaike's information criterion (AICc) to explain laying date of hatched egg in relation to (a) standardized baseline corticosterone levels (Bas. CORT, $N = 30$) and standardized stress-induced corticosterone levels (Max. CORT, $N = 37$) during the pre-laying period. K refers to the number of parameters. Candidate models and models without any explanatory variable (the 'intercept model') are ranked according to their AICc. The second part of the table (a) includes the parameter estimates for the selected model 'Bas. CORT'

No.	Model	AICc	Δ AICc	AICc weight	K
(a)					
1.	Bas. CORT	156.41	0	66.8%	3
2.	Bas. CORT; Sex	158.62	2.21	22.2%	4
3.	Bas. CORT; Sex; Bas. CORT \times Sex	161.26	4.85	5.9%	5
4.	Intercept	161.56	5.15	5.1%	2
	Selected model	Parameters	Estimate \pm SE	Chi-squared	P
	Bas. CORT	Intercept	9.74 \pm 0.58	277.020	< 0.001
		Bas. CORT	2.10 \pm 0.75	7.618	0.006
(b)					
1.	Intercept	198.60	0	63.3%	2
2.	Max. CORT	200.59	1.99	23.4%	3
3.	Max. CORT; Sex	202.47	3.87	9.1%	4
4.	Max. CORT; Sex; Max. CORT \times Sex	204.05	5.45	4.1%	5

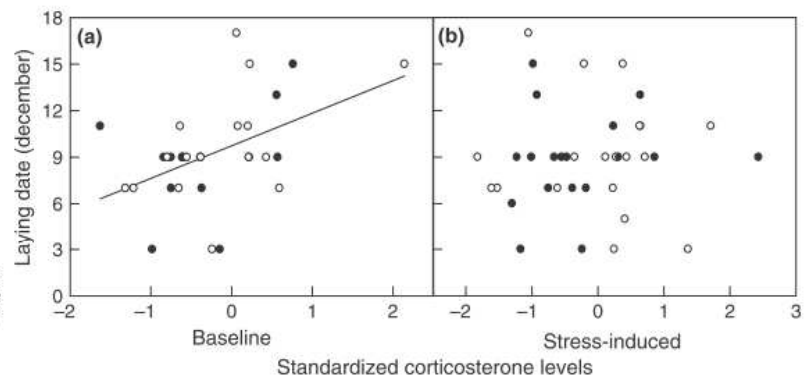


Fig. 5. Relationship between the egg-laying date (December) and standardized pre-laying baseline (a) and stress-induced (b) corticosterone levels of breeders. Closed circles denote males and open circles denote females.

1998; Landys, Ramenofsky & Wingfield 2006) such as fasting and strong intrasexual competition (Barbraud & Chastel 1999).

Contrary to our prediction, pre-laying baseline corticosterone levels were not correlated with age, as reported in other long-lived birds (Angelier *et al.* 2006; Heidinger, Nisbet & Ketterson 2006; Angelier *et al.* 2007a,b). This may suggest that baseline corticosterone is more likely to be influenced daily by short-term stressful events (Landys, Ramenofsky & Wingfield 2006) and be independent of age. However, breeding experience (i.e. number of previous breeding attempts) greatly differs between snow petrels of the same age, because they frequently skip a breeding attempt and the age when first breeding is greatly variable between individuals (Chastel, Weimerskirch & Jouventin 1993). In long-lived birds, breeding experience explains better the variation of baseline corticosterone than age *per se* (Angelier *et al.* 2006, 2007b). This suggests that baseline corticosterone levels are not only influenced by short-term factors but can also be influenced by longer-term factors such as breeding experience.

According to our prediction, young pre-laying birds were more sensitive to stress than middle-aged ones. This has also been observed during incubation in another long-lived bird

(Heidinger, Nisbet & Ketterson 2006) and is commonly interpreted as a mechanism for low commitment into current reproduction (Heidinger, Nisbet & Ketterson 2006; Angelier *et al.* 2007a). Young birds should favour future reproductive opportunities, likely through high pre-laying stress-induced corticosterone levels. Stress resistance may have arisen from a habituation process to handling with age. However, known-age snow petrels have been seldom handled prior to this study and a similar study found no effect of recapture history on stress hormone levels (Heidinger, Nisbet & Ketterson 2006). The lower stress response in middle-aged snow petrels compared with younger ones may originate from a decrease in adrenal capacity as suggested by a recent study of known-age common terns (Heidinger, Ketterson & Nisbet 2008).

Interestingly, we found that the oldest birds (> 30 years) showed a stronger response to stress than the 13–30-year-old birds. To our knowledge, this is the first report of a magnified stress response at an older age in a free-living organism. The stronger stress response in the oldest birds may therefore originate from a possible physiological senescence of the hypothalamo-pituitary-adrenal (HPA) system, as observed in elderly humans and rats (Sapolsky,

Krey & McEwen 1986a,b; Wilkinson, Peskind & Raskind 1997; Sapolsky 1999; Otte *et al.* 2005).

An alternative hypothesis could be a progressive disappearance of adults excessively sensitive to stress over time. This would imply that the magnitude of the stress response is fixed early in the reproductive life of an individual. However, as shown experimentally in the house sparrow (*Passer domesticus*; Lendvai, Giraudeau & Chastel 2007), the same individual can modulate its hormonal stress response flexibly according to the value of the reproductive event. Furthermore, this 'selection hypothesis' would not explain the magnified stress response found in the oldest birds.

AGE, STRESS HORMONES AND TIMING OF BREEDING

As found in Florida scrub jay and zebra finches (Salvante & Williams 2003; Schoech *et al.* 2009), elevated pre-laying baseline corticosterone levels were associated with the decision to skip breeding in female snow petrels, and with a postponed egg-laying date in both sexes. Baseline corticosterone seems therefore to fine-tune the timing of breeding with regard to stress occurring during pre-laying, suggesting energetic constraints.

Male stress hormone levels were important for the timing of breeding, although it is thought that females mainly drive the egg-laying date (Caro *et al.* 2009). This suggests that baseline corticosterone levels of one partner may reflect the global state of the breeding pair (e.g. quality of the nest, male's ability to defend the nest), or an assortative mating according to stress levels. However, male corticosterone levels were not as decisive as in females for breeding decision. This highlights a potential sex difference in the response to environmental cues related to seasonal events (Ball & Ketterson 2008; Blas & Hiraldo 2010).

How could baseline corticosterone orchestrate breeding decision and timing of breeding? In domesticated animals, cortisol suppresses luteinizing hormone (LH, a pituitary hormone involved in the onset of breeding) secretion by inhibiting pituitary response to gonadotropin-releasing hormone (GnRH; Breen & Karsch 2004). Moreover, corticosterone potentially acts via the gonadotropin-inhibiting hormone (GnIH), a recently discovered peptide, that downregulates the hypothalamic–pituitary–gonadal axis (Bentley *et al.* 2006; Ubuka *et al.* 2006; Calisi, Rizzo & Bentley 2008; Greives *et al.* 2008; Kirby *et al.* 2009). Thereby, in snow petrels, stress hormones might activate the GnIH system and postpone the timing of breeding or even cancel the breeding attempt.

However, the positive correlation between baseline corticosterone levels and egg-laying date was only observed in snow petrels that incubated their egg successfully. Failed clutches belonged to parents with high baseline corticosterone levels during the pre-laying period. We did not observe any disappearance of egg owing to predation and all case of failure were because of nest desertion. In snow petrels, incubation requires petrels to fast on the nest. Birds bearing elevated baseline corticosterone levels may then be of lower quality

(e.g. low foraging skills; Angelier *et al.* 2007c) and may not be able to cope with the energetic constraints of incubation. In conclusion, higher baseline corticosterone levels in snow petrels during the pre-laying period were correlated to a higher probability (i) to skip the breeding attempt, (ii) to loose the egg or (iii) to start breeding later in the season.

Our aim was to test whether the often observed age-specific timing of breeding could be related to higher stress sensitivity in young breeders compared with older ones. Contrary to our prediction and in spite of a strong age-specific stress response during the pre-laying period, the sensitivity to stress was not the functional mechanism involved in age-specific timing of breeding pattern observed in snow petrels. Our sample size was sufficient to rule out any hidden effect. Stress-induced levels of corticosterone are not as flexible and reactive to daily environmental cues than baseline corticosterone (Kitaysky, Piatt & Wingfield 2007). Thus, stress-induced corticosterone levels might be inappropriate to rule the adjustment of timing of breeding or the decision to breed.

However, baseline corticosterone levels, which predicted breeding decision and timing of breeding, were independent of age. How can the age-specific differences in timing of breeding be explained? The link between age and timing of breeding could possibly be indirect and originate from other age-related parameters (see Blas & Hiraldo 2010). In snow petrels, timing of breeding is better explained by previous breeding experience than age *per se* (unpublished data). We suggested that baseline corticosterone levels would be higher in inexperienced birds as found in other long-lived birds (Angelier *et al.* 2006, 2007b), and in turn would explain the functional mechanism of late breeding in young birds. Regarding the very old snow petrels, their late breeding could be the consequence of reduced foraging skills (Catry *et al.* 2006) and/or the possible death of their previous mate. Newly formed pairs breed later than long-term partners (snow petrels, unpublished data) and may exhibit higher baseline corticosterone levels, as shown in black-legged kittiwakes (Angelier *et al.* 2007d). Thus age-related factors, like previous breeding experience, foraging ability or change of mates may influence the timing of breeding rather than age *per se*. In turn, these age-related factors could influence the baseline corticosterone levels of pre-laying birds.

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IV.3. Age, décision de ponte et dynamique de la sécrétion de LH

ARTICLE D

Why do some adult birds skip breeding? Age-related hormonal constraints in a long-lived bird

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Biology letters, submitted

Why do some adult birds skip breeding? Age-related hormonal constraints in a long-lived bird

Summary

Skipping of reproduction is often observed in long-lived organisms but proximate mechanisms remain poorly understood. Since young and/or very old snow petrels (*Pagodroma nivea*) commonly skip breeding, we tested whether they are physiologically constrained during the pre-laying stage compared to middle-aged petrels. To do so, we measured the readiness to breed and the ability of known-age [11-45 years old] petrels to release luteinizing hormone (LH, an important driver of breeding), by injecting exogenous GnRH (gonadotropin-releasing hormone). Although young petrels exhibited low baseline LH levels, they were able to release LH after a GnRH challenge. Moreover, young and very old petrels showed a stronger decrease in LH levels after the GnRH-induced LH peak compared to middle-aged petrels. Birds that skipped breeding were as able as breeders to release LH after a GnRH challenge, indicating that they had functional hypothalamo-pituitary-gonadal axes. However, the decision to skip reproduction was linked to a strong LH decrease after the GnRH-induced peak. Our result suggests that the youngest and the oldest petrels fail to maintain elevated baseline LH levels during stressful situations, thereby do not initiate reproductive activities. Skipping of reproduction in long-lived birds probably results from age-related constraints on the dynamic of the HPG axis.

Key words: Intermittent breeding, Age, GnRH challenge, Luteinizing Hormone, Snow petrels

Introduction

Skipping of reproduction (i.e. non-breeding by individuals that previously bred) is often observed in long-lived organisms (Cam et al. 1998). The “prudent parent hypothesis” posits that non-breeding decision should be favoured, when the value of the immediate reproduction is low relative to the value of future reproductive opportunities and survival (Drent & Daan 1980), as it is expected to be in young adults. Moreover, senescent individuals may take advantage of sabbatical years to recover from previous reproductive efforts (Velando et al. 2010). Alternatively, skipped breeding, instead of being strategically decided, might be imposed by physiological constraints in low quality individuals (Cam et al. 1998). Age appears to be the keystone of skipped breeding, since this behaviour is markedly observed in the youngest and in the oldest adult birds (Berman et al. 2009; Goutte et al. 2010). Thus, some of the needed physiological requirements for successful reproduction may be deficient in young and senescent adults (Forslund & Pärt 1995).

Although ultimate causes of skipped breeding have been widely discussed, proximate factors influencing such patterns remain poorly studied (Schoech et al. 2004; Blas & Hiraldo 2010), especially in an age-specific context (Goutte et al. 2010). In birds, the onset of breeding is under strong hormonal control (hypothalamic-pituitary-gonadal [HPG] axis, Dawson 2008). In response to increased day length in spring, GnRH (gonadotropin-releasing hormone) is expressed and triggers the secretion of LH (luteinizing hormone) by the pituitary gland. LH, in concert with follicle-stimulating hormone, promotes gonadal maturation, sexual steroids secretion and in turn, sexual behaviours. Considering that non-breeding events result from physiological constraints (Cam et al. 2008), it is then conceivable that skipping breeders are unable to appropriately release LH.

As very long-lived birds, the snow petrels (*Pagodroma nivea*) provide an excellent model system to study non-breeding decision. Indeed, up to 60% of adults can skip breeding in a given year (Chastel et al. 1993), mainly the youngest and the oldest snow petrels (Berman et al. 2009; Goutte et al. 2010). The present study is designed to test, in this monogamous species, 1) whether age affects the ability to release LH during the pre-laying period and 2) whether skipping petrels fail to release LH. To do so, we used a common and powerful protocol, the injection of exogenous GnRH during the photosensitive stage, and tested the readiness to breed and the ability of known-age petrels to release LH (Schoech et al. 2004). We predicted that young and very old petrels would release less LH after a GnRH injection than middle-aged ones, and that lower LH release would predict high probability to skip breeding. LH levels progressively decrease after the GnRH-induced LH peak and returned to

baseline levels (Schoech et al. 2004). We therefore investigated for the first time whether this LH decrease 1) would be stronger in young and very old petrels than in middle-aged ones and 2) would predict a high probability to skip breeding.

Methods

The study was conducted on Terre Adélie (66°40'S, 140°01'E), Antarctica. Snow petrels are very long-lived philopatric birds with a low fecundity (one egg per year, Chastel et al. 1993). Part of the current population is of known age, because chicks have been banded prior to fledging each year since 1963 (Chastel et al. 1993). Forty-one male and female snow petrels, from 11 to 45 years old, were handled during the pre-laying period (i.e. courtship and mating period), from the 11th to 23rd November 2008. All sampled birds were previously observed as breeders. They were caught at their nest by hand and a first blood sample was collected with a syringe immediately after capture to determine baseline LH levels. Then, birds were immediately injected with 0.1mL of a GnRH solution in the alar vein ([Gin⁸], Sigma Lot 121H04314). The GnRH was dissolved in a physiological solution to yield a dosage of 0.6 µg/0.1mL (1.5 µg/ kg body mass in 1mL of 0.9% saline solution, as validated for seabirds in Jouventin & Mauget 1996). A second blood samples was collected 10 minutes after the injection to measure the LH releasing ability. Then, to monitor the dynamic of LH change after the GnRH-induced peak, a third blood sample was taken 30 minutes after the injection. Blood samples were centrifuged and plasma was stored at -20°C, until LH levels were assayed by heterologous radioimmunoassay at the CEBC (see Chastel et al. 2005). The lowest detectable quantities for LH were 0.05 ng/mL and the intra-assay coefficient of variation was 6.2 % (N = 3 duplicates). The 46 nests were checked every two days to monitor if birds engaged in breeding or if they skipped breeding.

All analyses were performed using R 2.8.0 (R Development Core Team 2008). We used generalized linear model (GLM) with normal errors and an identity link function to explain LH (baseline levels, change following a GnRH injection = $(LH_{t=10} - LH_{t=0}) / LH_{t=0}$, or change after a LH peak = $(LH_{t=30} - LH_{t=10}) / LH_{t=10}$) as a function of sex, age, age² and the interactions sex * age and sex * (age+age²). Similarly, we used GLM with binomial error distribution and a logit link function to test for an effect of sex, LH (baseline levels, LH change following a GnRH injection, or LH change after a peak) and the interactions sex * LH on the probability to skip breeding. Selected models were checked for assumptions.

Results

Baseline LH levels were higher in males than in females, and significantly increased with age (sex: $F_{1,38} = 7.856$, $p = 0.008$; age: $F_{1,37} = 4.389$, $p = 0.043$, Fig. 1A), without quadratic effect for age and/or interaction effects ($p > 0.38$ for all tests). LH release after the

GnRH challenge significantly decreased with age ($F_{1,36} = 6.640$, $p = 0.014$; Fig. 1B), without effect for sex, age² and interactions ($p > 0.15$ for all tests). However, absolute LH levels reached 10 minutes after GnRH challenge were unrelated to age (linear: $F_{1,33} = 0.093$, $p = 0.763$; quadratic: $F_{1,33} = 1.623$, $p = 0.212$). LH decrease after the GnRH-induced LH peak was higher in young and very old petrels than in middle-aged ones (quadratic: $F_{1,35} = 4.569$, $p = 0.040$, Fig. 1C), without effects for sex and interactions ($p > 0.12$). Baseline LH levels and LH change after a GnRH challenge did not explain skipped breeding decision ($p > 0.42$). However, a strong LH decrease after a LH peak predicted high skipped breeding probability ($N = 37$, $\chi^2 = 4.066$, $p = 0.044$, Fig. 2).

Figure 2: Means and standard errors of LH change measured 30 minutes after a GnRH-induced peak of snow petrels in relation to breeding decision (will skip or will breed).

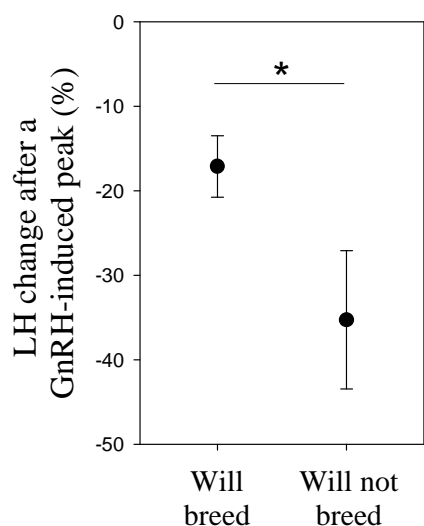
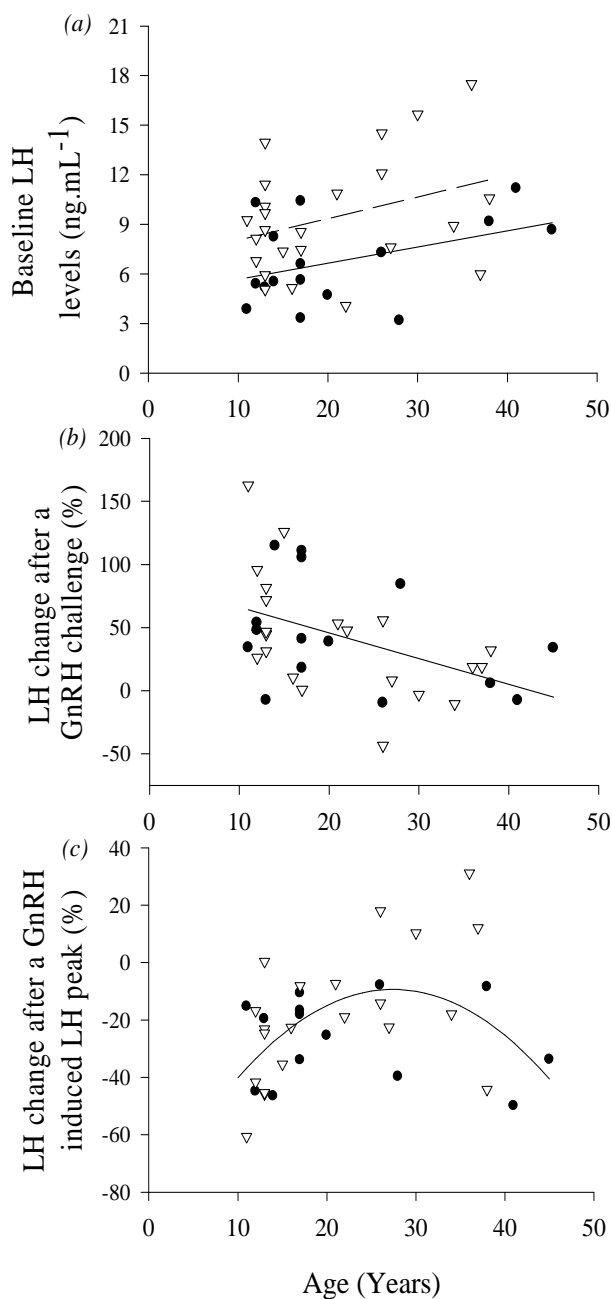


Figure 1: Means and standard errors of LH levels in relation to age in pre-laying snow petrels. (A) Baseline LH levels increased with age in males (open triangles and dashed line) and females (filled circles and solid lines). (B) LH changes measured 10 minutes after the GnRH challenge were negatively linked to age (C) LH changes measured 30 minutes after a GnRH-induced peak were quadratically linked to age.



Discussion

Although young petrels exhibited low baseline LH levels, they strongly released LH after a GnRH challenge and reached similar absolute LH levels compared to older petrels. Indeed, this clearly indicates that young adults had functional HPG axes. How then is it possible to explain their lower baseline LH levels? Although photoperiod is the primary signal controlling seasonal reproductive activities, social interactions can influence central physiology by modifying GnRH neurosecretory cells, which in turn stimulates the adjustment of reproductive physiology and behaviour (Dawson 2008). In the monogamous snow petrel, repeated breeding attempts with the same partner could therefore provide important benefits by improving coordination of breeding activities (Cezilly and Nager 1996). In this context, low mate stimulations in young and newly-paired petrels could explain their lower baseline LH levels. Similarly, young females are thought to reduce reproductive costs by delaying estradiol secretion and ovarian development until establishing pairs bond with a dominant male (Blas & Hiraldo 2010). Contrary to young petrels, old birds showed high baseline LH levels and did not strongly respond to the GnRH challenge. This may suggest that older petrels already secreted LH at their maximum levels, because of strong mate-mate interactions stimulated by long-term partnerships.

There were considerable inter-individual variations in LH change after the GnRH-induced LH peak: LH levels rapidly decreased, did not change or continued increasing from 10 to 30 minutes after the GnRH challenge. Compared to middle-aged, the youngest and the oldest petrels failed to maintain LH release from 10 to 30 minutes after the GnRH challenge. In a previous study, we found that young and very old petrels were more sensitive to stress than middle-age ones (Goutte et al, 2010). We suggest that this high stress response could interfere with the capacity to maintain LH production in an age-specific manner, so that the youngest and the oldest petrels would be likely to down-regulate LH production during stressful conditions. Alternatively, this age-related hormonal pattern may be the consequence of a progressive disappearance over time of adults excessively sensitive to stress and poorly able to maintain LH release. However this 'selection hypothesis' (Forslund & Pärt 1995) would not explain the magnified stress response and the strong LH decrease found in the oldest birds. Indeed, the marked decline in LH levels following the GnRH-induced LH peak in the oldest birds may originate from a possible physiological senescence of the HPG system, as observed in aging poultry (Sharp et al. 1992) but reported here for the first time in a free-living bird.

As found in some cooperative breeding bird species (Schoech et al. 2004), skipped breeding in the monogamous snow petrel was not associated with a physiological suppression of LH secretion, the main trigger of gonadal and behavioural development required for the onset of breeding. However, we found that failure to maintain LH levels after the GnRH-induced peak was the main predictor of the decision to skip reproduction. Specifically, the youngest and the oldest individuals, which appear to be more susceptible to stressors than middle-aged ones (Goutte et al. 2010), fail to maintain elevated LH levels during the photo-sensitive stage and thus would be highly susceptible to skip breeding. As highlighted by Cam et al. (1998), we therefore suggest, that non breeding in long-lived birds is probably the consequence of some constraints acting on the dynamic of the HPG axis and is imposed by age, as a component of intrinsic quality (Angelier et al. 2010).

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V. Rôle fonctionnel de la corticostérone sur l'axe HPG et sur la phénologie

V.1. Organisation du chapitre

Cette partie vise à élucider le rôle fonctionnel de la corticostérone dans les ajustements de la phénologie de la reproduction et dans la régulation de l'axe HPG (sécrétion de la LH et de la testostérone). Les deux articles inclus dans ce chapitre s'efforcent de répondre à cette question mécanistique, de façon descriptive, puis expérimentale.

Dans une démarche descriptive, l'article E cible l'action fonctionnelle de la corticostérone sur la sécrétion de LH et d'hormones sexuelles chez les mouettes tridactyles. Une attention particulière est portée aux différences entre mâles et femelles. Nous avons montré que chez les femelles, des taux élevés de corticostérone basale perturbent la sécrétion de LH, même après un test de réponse à la GnRH. Les femelles qui ne se reproduisent pas présentent, en période prénuptiale, des taux élevés de corticostérone basale et des taux faibles de LH basale. De plus, la date de ponte est influencée par la condition corporelle des femelles mais pas par leur taux d'hormones. Enfin, les mâles présentant des taux de testostérone élevés après une injection de GnRH, se reproduisent le plus tôt (Article E).

Par son approche expérimentale, l'article F permet de conclure sur le rôle joué par la corticostérone dans la régulation de la décision de non-reproduction et dans la date de ponte, en prenant en compte le succès reproducteur immédiat et le taux de retour l'année suivante. Une diminution expérimentale de la sensibilité au stress n'influence pas les décisions de reproduction, mais avance la date de ponte, chez les femelles. Les différences entre mâles et femelles sont très présentes. De plus, les femelles, dont les taux de corticostérone ont été diminués, sont en meilleure condition, et réussissent mieux que les couples témoins. Le taux de retour et la décision de reproduction l'année suivante ne sont pas affectés par la diminution expérimentale de la sensibilité au stress (Article F).

V.2. Liens entre corticostérone, LH, testostérone et phénologie

ARTICLE E

**Stress and the Timing of Breeding: Glucocorticoid - Luteinizing Hormones
relationships in an Arctic Seabird**

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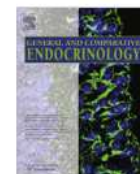
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Stress and the timing of breeding: Glucocorticoid-luteinizing hormones relationships in an arctic seabird

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ABSTRACT

In birds, stressful environmental conditions delay the timing of breeding but the underlying mechanisms are poorly understood. The stress hormone corticosterone appears to be a good candidate for mediating the decision to breed and when to start egg-laying, via a possible inhibition of luteinizing hormone (LH) and sex-steroids production. We used luteinizing hormone releasing hormone (LHRH) challenge in pre-laying male and female Black-legged kittiwakes (*Rissa tridactyla*) to test whether LH and testosterone secretion were depressed by elevated corticosterone levels. Females bearing high baseline corticosterone levels showed reduced baseline LH levels and a low ability to release LH, following LHRH challenge. Further, females bearing low baseline LH levels and elevated baseline corticosterone levels were more likely to skip breeding. However, non-breeding females were physiologically primed for breeding, since they mounted high LHRH-induced LH release. Egg-laying date was advanced in good body condition females but was unaffected by hormones secretion. In males, corticosterone levels had no effect on LH and/or testosterone secretion and did not affect their decision to breed. Interestingly, males with high LHRH-induced testosterone release bred early. Our study highlights clear sex-differences in the HPG sensitivity to stress hormones in pre-laying kittiwakes. Because females have to store body reserves and to build up the clutch, they would be more sensitive to stress than males. Moreover, intrasexual competition could force male kittiwakes to acquire reproductive readiness earlier in the season than females and to better resist environmental perturbations. We suggest that high testosterone releasing ability would mediate behavioural adjustments such as courtship feeding, which would stimulate early egg-laying in females.

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1. Introduction

Breeding at the right time is a key-component of fitness, as successful reproduction requires a good overlap between energy-demanding needs and the peak of available resources (*match-mismatch hypothesis*, Cushing, 1990; Visser et al., 1998; Stenseth and Mysterud, 2002; Durant et al., 2007; review in Visser, 2008). Hence, as adaptive responses to environmental variations, free-living organisms must exhibit considerable behavioural and physiological flexibilities in the timing of their seasonal activities (review in Wingfield, 2008). To do so, they integrated photoperiod, as a fixed cue (Wingfield and Kenagy, 1991; Dawson et al., 2001) and variable cues, such as ambient temperature, food supply, nest sites availability, and/or stimulatory social interactions (Wingfield, 1980;

Wingfield and Kenagy, 1991; Ball, 1993; Visser et al., 1998; Wingfield et al., 2003; Schoech et al., 2004; Ball and Ketterson, 2008; Both et al., 2009). At the endocrine level, the onset of breeding involves the activation of the hypothalamic-pituitary-gonadal axis (hereafter HPG axis): increasing day length activates the expression of a neuro-hormone, the Gonadotropin Releasing Hormone (GnRH) that triggers the release of two pituitary gonadotrophins: the luteinizing hormone and the follicle-stimulating hormone (LH and FSH, reviewed in Dawson et al., 2001). In turn, gonadotrophins activate the gonadal development and the release of sex steroids such as estradiol and testosterone. A wide range of sex steroid hormone-dependent behaviours is then expressed (Ball, 1993), such as nest building, courtship, and mating. However, this hormonal cascade is not only driven by photoperiod, but could also be regulated by additional non-photoperiodic cues. Specifically, pre-laying energetic constraints, such as food shortage and/or environmental cues that enable individuals to anticipate food availability (see Shultz

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et al., 2009) should be involved in the down-regulation of the HPG axis (Wingfield et al., 2003).

At the individual level, the underlying mechanisms have received increased attention (review in Schoech et al., 2009). One potential mediator is the endocrine stress response, which is known to adjust life-history strategies in relation to environmental conditions and to individual physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Indeed, the release of glucocorticoids during stressful perturbations triggers physiological and behavioural adjustments that shift energy investment away from reproduction and redirects it towards survival (Wingfield and Sapolsky, 2003). Relating this to the timing of breeding, elevated pre-laying glucocorticoids levels might postpone or even halt the immediate breeding event, as correlatively shown in Florida scrub-jays (*Aphelocoma coerulescens*, Schoech et al., 2009), Marine iguanas (*Amblyrhynchus cristatus*, Vitousek et al., 2010), and Snow petrels (*Pagodroma nivea*, Goutte et al., 2010). Moreover, experimental administration of glucocorticoids decreased the proportion of breeding females and delayed the onset of egg-laying in captive Zebra finches (*Taeniopygia guttata*, Salvante and Williams, 2003). However, the functional action of glucocorticoids on the HPG axis remains poorly understood in free-living vertebrates (reviewed in Schoech et al., 2009).

In domesticated animals, it has been better documented how stress and stress hormones can act on the HPG system via central and peripheral sites. First, glucocorticoids can suppress LH release by inhibiting pituitary responsiveness to GnRH (e.g. Breen and Karsch, 2006). Second, glucocorticoids can act directly at the hypothalamic level by disrupting the GnRH pulse frequency (e.g. Oakley et al., 2009). Third, glucocorticoids can impair testicular development but not through the inhibition of LH release (e.g. in Common carp, *Cyprinus carpio* L., Consten et al., 2002). A novel down-regulator of the HPG axis, the gonadotropin-inhibitory hormone (GnIH), appears to inhibit the release of LH, FSH and testosterone (review in Bentley et al., 2009; Tsutsui et al., 2009; McGuire and Bentley, 2010), and is especially expressed during stressful conditions and/or glucocorticoid release (Calisi et al., 2008; Kirby et al., 2009). Thus, stress hormones seem to be candidates for the stress-related inhibition of the reproductive axis and associated physiological pathways.

Despite these findings from captive or domesticated species, the functional action of glucocorticoids on gonadotropin release and timing of breeding remains unclear and controversial in free-living vertebrates (review in Schoech et al., 2009). For instance, experimental increased glucocorticoid levels did not suppress LH and testosterone levels in free-living male American Tree sparrows (*Spizella arborea*, Astheimer et al., 2000) and failed to delay breeding in food-supplemented Florida scrub jay (*Aphelocoma coerulescens*, Schoech et al., 2007). In that context, it is crucial to carefully distinguish the two levels of glucocorticoids. The baseline glucocorticoid level is a marker of activities, energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004; review in Landys et al., 2006), while the stress-induced glucocorticoid level mirrors the sensitivity to stress and the commitment into current reproduction (review in Wingfield and Sapolsky, 2003; Lendvai et al., 2007). Moreover, it is difficult to draw a general picture since males and females are expected to differ in the down-regulation of the HPG axis in response to stressful events (review in Ball and Ketterson, 2008).

The aim of the present study was to address the functional action of corticosterone –the main glucocorticoid in birds– on the breeding decision and the adjustment of egg-laying date in an Arctic population of Black-legged kittiwake (*Rissa tridactyla*). At the population level, a long-term (38 years) study in Svalbard showed great inter-annual variation in the timing of breeding (Moe et al., 2009). Moreover, breeding success was higher in years when

breeding was early, and high spring sea surface temperatures were associated with early breeding (Moe et al., 2009). This population is thus well-appropriated for our study, since at the individual level, early (pre-laying) environmental and physiological conditions should be decisive for the breeding schedule. In Svalbard, kittiwakes attend the colony during two months before egg-laying (i.e. pre-laying period), thus offering the opportunity to explore corticosterone/LH relationships in pre-laying males and females, by using luteinizing hormone releasing hormone (LHRH) challenge. LHRH is an equivalent of the GnRH, and a small injection of LHRH is commonly used to assess the readiness of a bird to breed and its ability to release temporary LH and sex-steroid (Schoech et al., 1996). We predicted that elevated baseline and/or stress-induced corticosterone levels during the pre-laying period would be linked to non-breeding decision or postponed egg-laying date, through the disruption of the HPG axis. First, we tested whether baseline and/or LHRH-induced levels of LH or testosterone decreased with increasing baseline and/or stress-induced corticosterone levels. Then, we investigated whether the breeding decision was related to hormonal levels (corticosterone, LH, testosterone). Finally, we tested whether the egg-laying date was related to hormonal levels (corticosterone, LH, testosterone).

2. Materials and methods

2.1. Study area and birds

Our study was conducted on a colony of Black-legged kittiwakes at Kongsfjorden, Svalbard (78°54'N, 12°13'E), 7 km southeast of Ny-Ålesund, Norway. Black-legged kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago (Anker-Nilssen et al., 2000). We studied kittiwakes in one plot of around 116 pairs breeding on cliff ledges at heights of 5–10 m.

2.2. Blood sampling and LHRH challenge

Male and female kittiwakes were sampled from 20 May to 6 June 2008, during the pre-laying period (i.e. copulations and nest building period). In Black-legged kittiwakes, baseline LH in males and females and testosterone in males reach maximal levels during the pre-laying period (Goutte et al., unpublished data). Seventy seven birds were caught on the nests with a noose at the end of a 5 m fishing rod. In 50 of them, a first blood sample (ca. 0.3 mL) was collected immediately after capture, from the alar vein with a 1 mL heparinised syringe and a 25-gauge needle to assess baseline LH, testosterone (in males only) and corticosterone levels. Bleeding time (i.e. time elapsed from capture to the end of the first blood sample: 3 min 31 ± 5 [SE] seconds) exceeded the time recommended by Romero and Reed (2005) for some birds. Corticosterone levels were thus related to bleeding time in males (estimate of the slope: 0.067 ± 0.019, $F_{1,25} = 14.540$, $p < 0.001$) but not in females ($F_{1,21} = 0.012$, $p = 0.914$). We performed statistical tests by using the residuals of corticosterone against bleeding time, then by excluding corticosterone levels, whose bleeding time exceeded 3 min, and then by using absolute corticosterone levels. The three approaches led to similar results. Hence, we reported the results for absolute corticosterone levels to facilitate the comparison with other published results.

Immediately after this first blood sampling, kittiwakes were injected with 0.1 mL of a solution of LHRH ([Gin⁸], Sigma Lot 121H04314) to test the responsiveness of the pituitary gland and the gonads. The LHRH was dissolved in physiological solution to yield a final dosage of 0.6 µg/0.1 mL (1.5 µg/kg body mass in

1 mL of 0.9% saline solution). This dose of LHRH has been shown to be sufficient to elicit the maximal release of LH in other seabird species (Jouventin and Mauget, 1996). We administered 0.1 mL of LHRH solution (LHRH-injected birds, $N = 46$) or saline solution (control birds, $N = 31$) directly into the alar vein. Kittiwakes were then placed into cloth bags and subsequent blood samples (ca. 0.3 mL) were collected from the alar vein at 10 min and 30 min after the injection. Hence we assessed the LHRH-induced release of LH in males and females, as well as the LHRH-induced release of testosterone in males only.

Kittiwakes were individually marked with metal rings and PVC plastic bands engraved with a three-digit code and fixed to the bird's tarsus for identification from a distance. Birds were weighed to the nearest 2 g using a Pesola spring balance, and their skull length (head + bill) was measured to the nearest 0.5 mm with a sliding caliper. A scaled mass index (Peig and Green, 2009) was calculated individually for males and females separately, because of sex-difference in skull length (Moe et al., 2002). Kittiwakes were marked with spots of dye on the forehead to distinguish them from their partner during subsequent observation and were released. Using a mirror at the end of an 8 m fishing rod, we checked the whole plot (ca. 116 nests) every two days to monitor breeding decision (at least one egg is laid or no egg laid) and egg-laying dates. Breeding decision and egg-laying date were not influenced by the LHRH-injection ($p > 0.39$ for all tests).

2.3. Molecular sexing and hormone assay

Blood samples were centrifuged, and plasma and red blood cells were separated and stored at -20°C until used, respectively in hormone assays or molecular sexing, at the Centre d'Etudes Biologiques de Chizé (CEBC). Molecular sexing was performed as detailed in Weimerskirch et al. (2005). LH radioimmunoassay was conducted following the methods previously described for other seabirds (Mauget et al., 1994; Chastel et al., 2005), and validated for Black-legged kittiwake plasma. Pooled plasma samples of kittiwakes produced dose–response curves that paralleled the chicken LH standard curves ("AGM 51122F", sources: LH, Prf. Ishii and Wakabayashi, Wadesa University, Japan, Fig. 1). Parallel curves indicate that the concentration-dependent binding of LH to antibody is similar in kittiwakes and chickens, and that this heterologous RIA can be used to

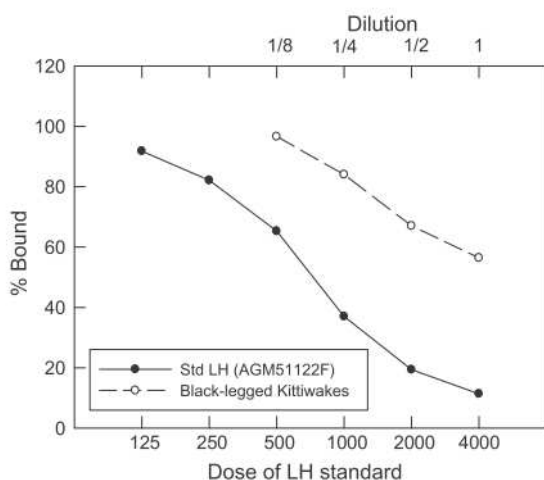


Fig. 1. Dose–response curve for LH in standard LH (AGM 51122F) and Black-legged kittiwakes. LH standard is expressed in pg per tube.

assess relative levels of plasma LH in the Black-legged kittiwakes. The lowest detectable concentration for LH was 0.06 ng/mL and the intra-assay coefficient of variation was 8.7% ($N = 3$ duplicates). Plasma concentrations of testosterone were assayed for males only, by radioimmunoassay, at the CEBC as described by Chastel et al. (2003). The lowest detectable concentration for testosterone was 0.05 ng/mL and the intra-assay coefficient of variation was 7% ($N = 3$ duplicates). Plasma concentrations of corticosterone were determined by radioimmunoassay at the CEBC, as described by Lormée et al. (2003). The lowest detectable concentration for corticosterone was 0.5 ng/mL. Only one assay was performed and the intra-assay coefficient of variation was 6.7% ($N = 5$ duplicates).

2.4. Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team, 2008). We used generalised linear mixed-effects models (GLMM) and included bird identity as a random effect, to test the individual variation of hormone levels over 10 and 30 min after the injection of LHRH or saline solution. Then we used generalised linear models (GLM) with a normal/binomial error distribution and an identity/logit link function to test our biological assumptions (Table 1). Baseline and LHRH-induced LH and testosterone levels were tested as a function of bleeding time, sampling date and scaled mass index in males and females separately (Table 1i). Then, effects for baseline and/or stress-induced corticosterone levels on baseline and LHRH-induced LH levels were tested in pre-laying male and female kittiwakes and on baseline and LHRH-induced testosterone levels in males only (Table 1ii). Lastly, the effects for hormone levels on breeding decision and on first egg-laying date were tested in males and females (Table 1iii and 1iv). Diagnostic plots were assessed whether the data sufficiently met the assumptions of the linear model, and dependent continuous variables were log-transformed when necessary.

3. Results

LHRH-injection had no effect on stress-induced corticosterone levels compared to controls (10 min: $F_{1,72} = 0.295$, $p = 0.589$, 30 min: $F_{1,70} = 0.621$, $p = 0.434$). Corticosterone levels significantly increased after 10 and 30 min (GLMM, $F_{2,143} = 615.911$, $p < 0.001$), without effect of sex (GLMM, $F_{1,75} = 2.217$, $p = 0.141$; interaction: $F_{2,141} = 1.511$, $p = 0.224$). Scaled mass index was neither correlated to sampling date (males: $F_{1,41} = 1.127$, $p = 0.295$, females: $F_{1,32} = 2.146$, $p = 0.153$). Before LHRH-injection, baseline (i.e. at 0 min) hormone levels did not differ between LHRH-injected and control kittiwakes (GLM, LH: $F_{1,41} = 0.690$, $p = 0.411$; testosterone: $F_{1,25} = 0.131$, $p = 0.721$, corticosterone: $F_{1,74} = 0.501$, $p = 0.481$). Following LHRH injection, LH levels reached maximum levels at 10 min and then returned to baseline after 30 min (GLMM, time as factor, $F_{2,50} = 41.684$, $p < 0.001$), without sex difference (sex: $F_{1,42} = 2.263$, $p = 0.140$; interaction: $F_{2,48} = 1.640$, $p = 0.205$, Fig. 2A and B). In control birds, LH levels significantly decreased over 10 and 30 min of handling (GLMM, $F_{2,32} = 8.029$, $p = 0.002$, Fig. 2A and B) and were lower in males than in females at 30 min (GLM, $F_{1,22} = 12.161$, $p = 0.002$) but not at 10 min after the injection of saline solution ($F_{1,21} = 1.302$, $p = 0.267$). In LHRH-injected males, testosterone levels significantly increased over 30 min after injection (GLMM, $F_{2,32} = 5.958$, $p = 0.006$, Fig. 2C), while in control males, testosterone significantly decreased over 30 min (GLMM, $F_{2,28} = 5.648$, $p = 0.009$, Fig. 2C).

Concerning the factors influencing hormonal levels during the pre-laying period (Table 1i), baseline LH levels were not influenced by sampling date (males: $F_{1,25} = 1.135$, $p = 0.297$; females: $F_{1,21} = 3.080$, $p = 0.094$), by scaled mass index (males: $F_{1,24} = 0.200$,

Table 1

Biological assumptions tested and associated generalised linear models. Model selection was performed by a step down approach starting from the global model including all the independent variables. Sample sizes were given for males (Nm) and females (Nf). Corticosterone and luteinizing hormone levels were abbreviated as 'CORT' and 'LH'.

Dependant variable	Independent variables	Nm	Nf
<i>i-Effect of sampling date, body condition and sex on hormone levels</i>			
1-a Baseline LH	Bleeding time, sampling date, scaled mass index	27	23
1-b LHRH-induced LH	Sampling date, scaled mass index	23	17
1-c Baseline testosterone	Bleeding time, sampling date, scaled mass index	27	
1-d LHRH-induced testosterone	Sampling date, scaled mass index	22	
<i>ii-Effect of corticosterone levels on LH and testosterone levels</i>			
2-a Baseline LH	Baseline CORT	27	22
2-b Baseline LH	Stress-induced CORT	27	22
2-c LHRH-induced LH	Baseline CORT	23	16
2-d LHRH-induced LH	Stress-induced CORT	23	16
2-e Baseline Testosterone	Baseline CORT	27	
2-f Baseline Testosterone	Stress-induced CORT	26	
2-g LHRH-induced Testosterone	Baseline CORT	22	
2-h LHRH-induced Testosterone	Stress-induced CORT	22	
<i>iii-Effect of hormone levels on breeding decision (breeding or skipped breeding)</i>			
3-a Breeding decision	Body condition	43	32
3-b Breeding decision	Baseline CORT	27	21
3-c Breeding decision	Stress-induced CORT	42	29
3-d Breeding decision	Baseline LH	27	21
3-e Breeding decision	LHRH-induced LH	23	16
3-f Breeding decision	Baseline Testo	27	
3-g Breeding decision	LHRH-induced Testo	22	
<i>iv-Effect of hormone levels on first-egg-laying date</i>			
4-a First-egg-laying date	Body condition	31	20
4-b First-egg-laying date	Baseline CORT	20	15
4-c First-egg-laying date	Stress-induced CORT	30	20
4-d First-egg-laying date	Baseline LH	20	15
4-e First-egg-laying date	LHRH-induced LH	15	10
4-f First-egg-laying date	Baseline Testo	18	
4-g First-egg-laying date	LHRH-induced Testo	15	

$p = 0.659$; females: $F_{1,20} = 1.425$, $p = 0.247$), and by bleeding time (males: $F_{1,23} < 0.001$, $p = 0.997$; females: $F_{1,19} = 0.014$, $p = 0.908$). LHRH-induced LH levels (10 min) were not influenced by sampling date (males: $F_{1,21} < 0.001$, $p = 0.988$; females: $F_{1,15} = 1.195$, $p = 0.293$) and scaled mass index (males: $F_{1,20} = 0.653$, $p = 0.429$; females: $F_{1,14} = 0.529$, $p = 0.479$). In males, baseline testosterone levels were not related to bleeding time ($F_{1,25} = 0.134$, $p = 0.718$). Baseline and LHRH-induced (30 min) testosterone levels were neither influenced by sampling date ($F_{1,24} = 0.717$, $p = 0.406$ and $F_{1,19} = 0.927$, $p = 0.348$) nor by scaled mass index ($F_{1,25} = 2.355$, $p = 0.138$ and $F_{1,20} = 3.385$, $p = 0.081$).

3.1. Effect of corticosterone levels on LH and testosterone (Table 1ii)

Baseline LH levels decreased significantly with increasing baseline corticosterone levels in females (estimate: -0.405 ± 0.228 , Table 2a, Fig. 3A) but not in males (estimate: 0.136 ± 0.099 , Table 2a, Fig. 3B). In addition, LHRH-induced LH levels (10 min) decreased significantly with increasing baseline corticosterone levels in females (estimate: -0.929 ± 0.474 , Table 2c, Fig. 3C) but not in males (estimate: -0.165 ± 0.224 , Table 2c, Fig. 3D). There was no effect of stress-induced corticosterone levels on baseline and LHRH-induced LH levels in both sexes (Table 2b and d). In males, baseline testosterone levels did not vary with baseline or stress-induced corticosterone levels (Table 2e and f). LHRH-induced testosterone levels did not vary with baseline and/or stress-induced corticosterone levels (Table 2g and h).

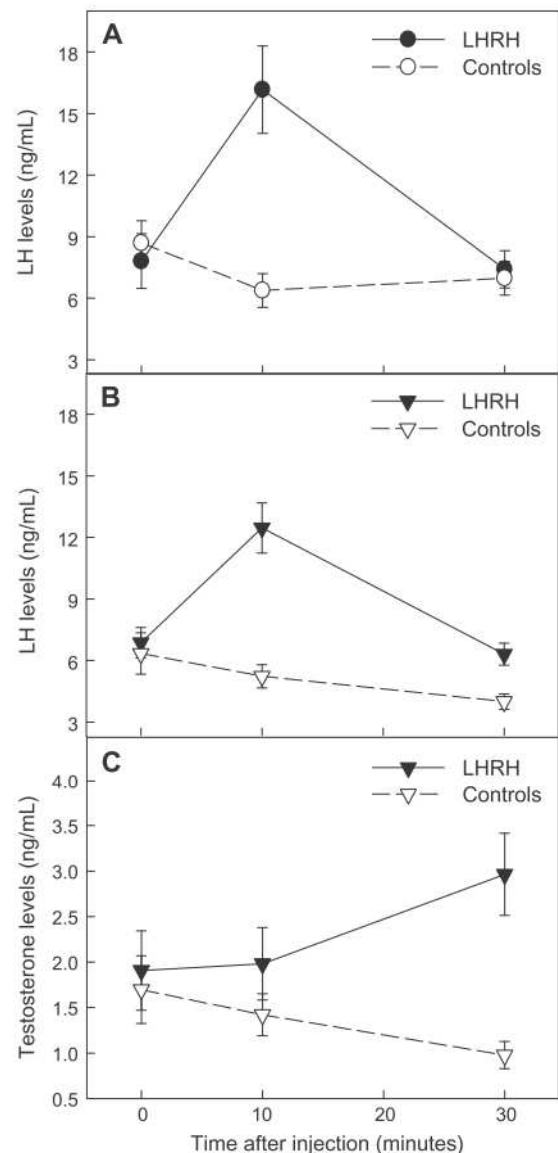


Fig. 2. Hormonal change after the injection of the LHRH solution (solid line, filled symbols) or of the saline solution (control, dashed lines, open symbols). LHRH-injected females (A, circle) and males (B, triangle) significantly released LH over the first 10 min then LH returned to the baseline levels over 30 min, while control levels decreased after injection. (C) Testosterone levels in males following the injection of LHRH (solid line) or saline solution (control, dashed lines). LHRH-injected males showed significantly elevated testosterone over 30 min, while control levels decreased after injection.

3.2. Hormonal levels and breeding decision (Table 1iii)

Breeding decision was not influenced by scaled mass index in both sexes (Table 3a). In females, breeding decision was related to pre-laying baseline LH levels and to pre-laying baseline corticosterone levels: females that will not breed show significantly lower pre-laying baseline LH levels and higher baseline corticosterone levels than females that will breed (Table 3a, Fig. 4A and B). LHRH-induced LH levels and stress-induced corticosterone levels were not linked to female breeding decision (Table 3a, Fig. 4C and D). In males, breeding decision was not related to levels of

Table 2

Modelling baseline (Hyp. 2a and 2b) and LHRH-induced (Hyp. 2c and 2d) LH levels, baseline (Hyp. 2e and 2f) and LHRH-induced (Hyp. 2g and 2h) testosterone (T) levels as a function of baseline (or stress-induced) corticosterone levels (CORT), using GLMs (normal error distribution, identity link function) in pre-laying male and female kittiwakes.

Hyp	Dependant variable	Independent variables	d.f.	F	p-value
2a	Baseline LH levels	Baseline CORT (males)	1,25	1.880	0.183
		Baseline CORT (females)	1,20	4.327	0.050
2b	Baseline LH levels	Stress-induced CORT (males)	1,25	1.188	0.286
		Stress-induced CORT (females)	1,20	0.020	0.890
2c	LHRH-induced LH levels (log)	Baseline CORT (males)	1,21	0.224	0.641
		Baseline CORT (females)	1,14	4.643	0.049
2d	LHRH-induced LH levels (log)	Stress-induced CORT (males)	1,21	0.432	0.518
		Stress-induced CORT (females)	1,14	2.175	0.162
2e	Baseline T levels (log)	Baseline CORT (males)	1,25	1.370	0.253
2f	Baseline T levels (log)	Stress-induced CORT (males)	1,24	0.265	0.612
2g	LHRH-induced T levels (log)	Baseline CORT (males)	1,20	3.221	0.088
2h	LHRH-induced T levels (log)	Stress-induced CORT (males)	1,20	0.164	0.690

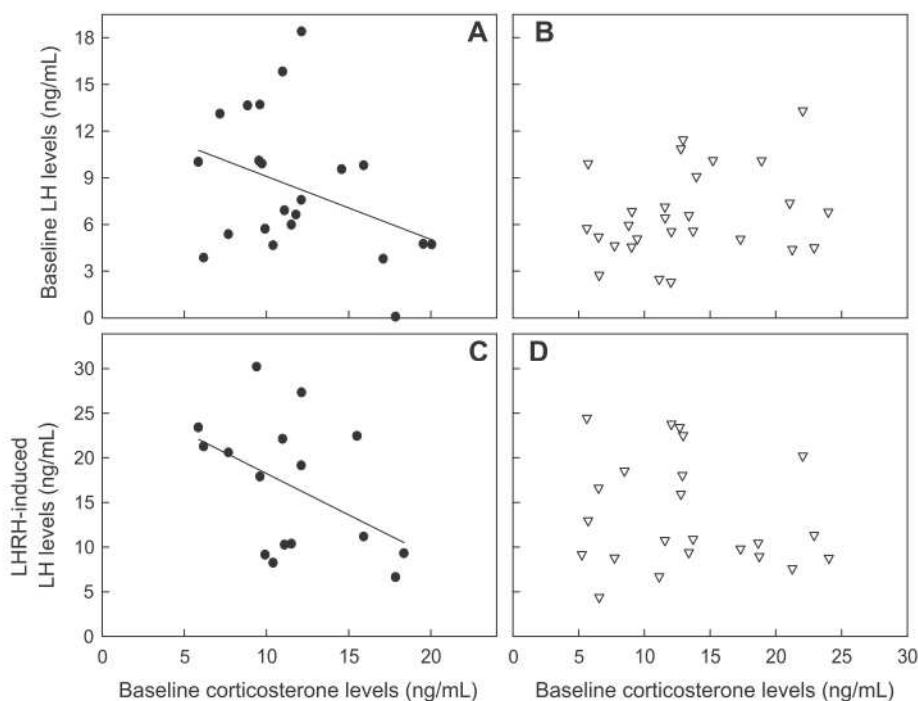


Fig. 3. In pre-laying females (filled circles), baseline (A) and LHRH-induced (C) LH levels decreased with increasing baseline corticosterone levels. In pre-laying males, baseline (B) and LHRH-induced (D) LH levels were not linked to baseline corticosterone levels.

baseline or LHRH-induced LH (Table 3a, Fig. 4A and C), baseline or stress-induced corticosterone (Table 3a, Fig. 4B and D), baseline or LHRH-induced testosterone (Table 3a).

3.3. Hormonal levels and egg-laying dates (Table 1iv)

In females, there was a significant effect of pre-laying scaled mass index on first egg-laying date (Table 3b): females with high scaled mass index start laying their first egg early (Fig. 5A). Males pre-laying scaled mass index had no effect on egg-laying date (Table 3b). Baseline or stress-induced corticosterone, baseline or LHRH-induced LH levels and baseline testosterone levels had no influence on first egg-laying date (Table 3b). On the other hand, males with high LHRH-induced testosterone levels bred significantly earlier than males with low LHRH-induced testosterone levels (Table 3b, Fig. 5B).

4. Discussion

In the present study we showed that elevated baseline, but not stress-induced, corticosterone levels have the potential to disrupt the HPG axis of female Black-legged kittiwakes. Furthermore, females with low baseline LH levels and elevated baseline corticosterone levels had the highest probability to skip breeding. On the contrary, elevated baseline and/or stress-induced corticosterone levels did not disrupt LH release in males, highlighting strong sex-difference in the HPG sensitivity to stress in Black-legged kittiwakes. Concerning the timing of breeding, females' scaled mass index, but not hormone levels, had a positive influence on egg-laying date. Males that exhibited high LHRH-induced testosterone release ability bred earlier than males with low ability to release testosterone.

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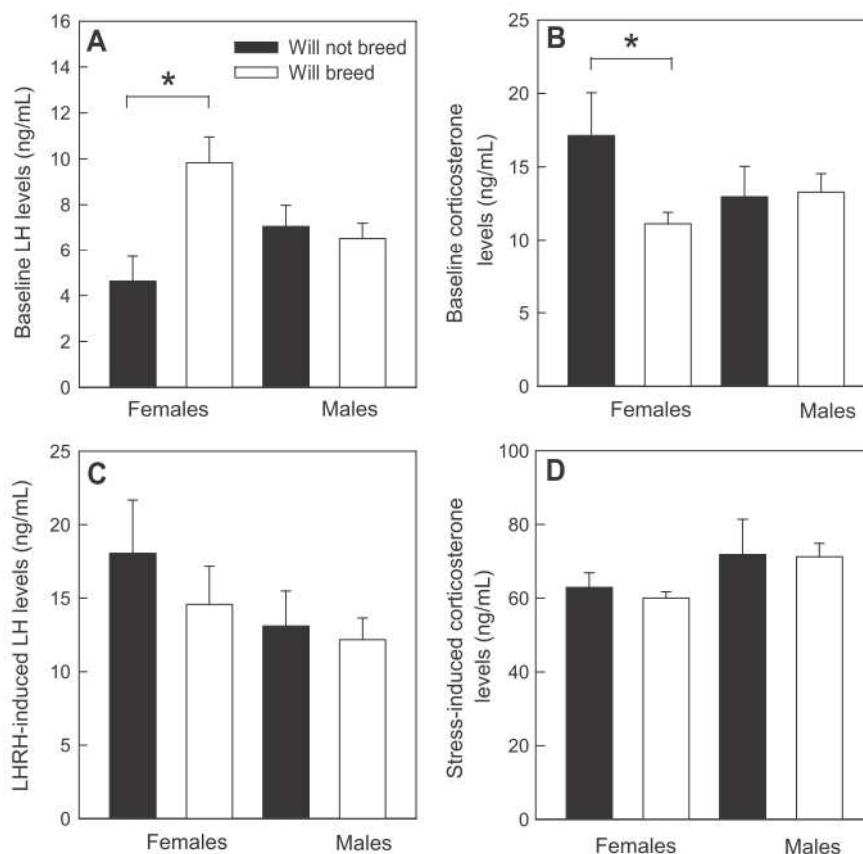
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Table 3

Modelling (a) breeding decision and (b) first-egg-laying date in 2008 as a function of scaled mass index and hormones levels using GLMs with binomial error distribution and logit link function (a) or normal error distribution and identity link function (b).

Independent variables	N	Chi ²	p-value	N	Chi ²	p-value
a-Breeding decision				FEMALES		
Scaled mass index	43	0.087	0.768	32	1.078	0.299
Baseline CORT	27	0.016	0.900	21	7.656	0.006
Stress-induced CORT	42	0.029	0.866	30	0.036	0.850
Baseline LH	27	0.173	0.678	21	7.368	0.007
LHRH-induced LH	23	1.599	0.206	16	0.614	0.433
Baseline Testosterone	27	0.704	0.402			
LHRH-induced Testosterone	22	0.443	0.506			
b-First egg-laying date				FEMALES		
Independent variables	d.f.	F	p-value	d.f.	F	p-value
Scaled mass index	1,29	0.486	0.491	1,18	5.289	0.034
Baseline CORT	1,18	1.622	0.219	1,13	0.004	0.953
Stress-induced CORT	1,28	0.650	0.427	1,18	0.003	0.959
Baseline LH	1,18	0.425	0.523	1,13	0.874	0.367
LHRH-induced LH	1,13	0.987	0.339	1,8	0.323	0.585
Baseline Testosterone	1,16	1.639	0.219			
LHRH-induced Testosterone	1,13	7.365	0.018			

**Fig. 4.** Hormone levels in pre-laying male and female kittiwakes that will breed (white bar) and that will not breed (black bar): (A) baseline LH levels, (B) baseline corticosterone levels, (C) LHRH-induced LH levels and (D) stress-induced corticosterone levels.**4.1. Effect of corticosterone levels on LH and testosterone**

Our study showed, for the first time in free-living birds, that elevated baseline corticosterone was paralleled by low baseline LH levels and by low ability to release LH after a LHRH-injection. Such

down-regulation of the HPG axis by stress hormones was however strongly sex-specific in kittiwakes, since it was only observed in females. In free-living avian species, the link between LH and corticosterone levels has been seldom investigated and to our knowledge, studies were mainly biased towards males (Wilson and Follett,

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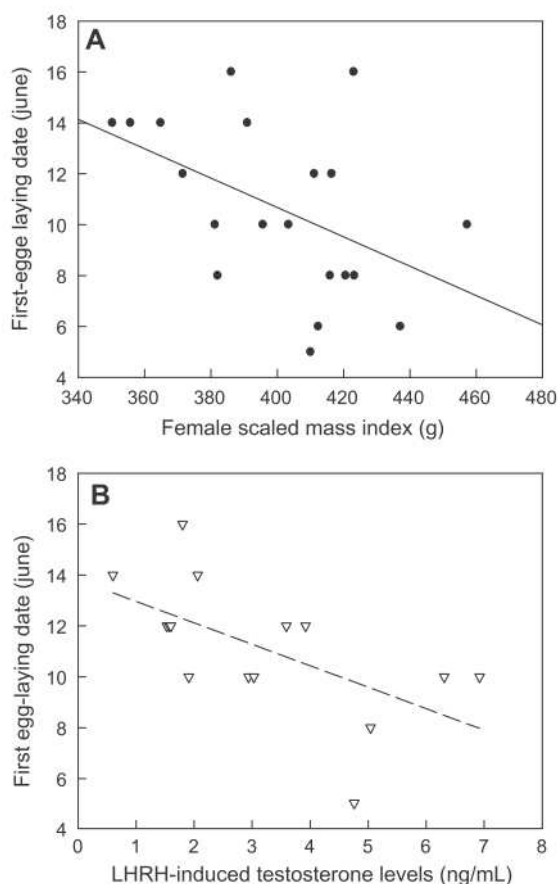


Fig. 5. (A) Egg-laying date (June 2008) advanced with increasing females' scaled mass index; (B) Egg-laying date (June 2008) advanced with increasing males' testosterone release ability.

1975; Astheimer et al., 2000; review in Schoech et al., 2009). Our results highlight that in female kittiwakes, plasma corticosterone may act at the pituitary level, though a down-regulation of LH production and/or a low responsiveness to the GnRH input. This physiological pathway could be related to the stress-induced expression of GnIH and its associated inhibition of LH release, as recently reported in captive house sparrows and laboratory rats (Calisi et al., 2008; Kirby et al., 2009).

In males, baseline corticosterone levels had no effect on baseline LH levels and on LH releasing ability after a LHRH challenge. This supports the idea that the sensitivity of the HPG axis to stressful cues may differ between sexes, as suggested by Ball and Ketterson (2008). For instance, intrasexual competition could force male kittiwakes to acquire reproductive readiness earlier in the season than females and to better resist environmental perturbations. Moreover, females have to extract and store a sufficient amount of energy to build up the eggs, hence being more sensitive to environmental stressors, such as poor food supply, than males (Ball and Ketterson, 2008). The low susceptibility to stress of male HPG axis was further supported by the lack of relationship between baseline corticosterone and testosterone levels as experimentally observed in male American Tree sparrows (Astheimer et al., 2000).

It is also important to notice that stress-induced corticosterone levels had no effect on LH in males and females and on testosterone levels in males. In kittiwakes, corticosterone may need time

to suppress the HPG axis, therefore a short acute release of stress hormones could be inappropriate to trigger strong HPG disruption.

4.2. Hormonal levels and breeding decision

As previously found (Salvante and Williams, 2003; Vitousek et al., 2010; Goutte et al., 2010), elevated pre-laying baseline corticosterone levels were associated with the decision to skip breeding in females. Females bearing elevated baseline corticosterone levels may then be of lower quality (e.g. low foraging skills, Angelier et al., 2007; Kitaysky et al., 2010) and may be unable to cope with the energetic requirements for egg-formation and future incubation effort.

Moreover, LH levels were higher in females that did breed than in females that did not breed. Since the LHRH injection did not reveal an inability to release LH, non-breeding females had a fully functional HPG axis (Schoech et al., 1996) and thus appeared to be physiologically primed for breeding. In that context, how to explain low baseline LH levels in non-breeding females? Our results suggest that non-breeding females failed to express endogenous GnRH input that is required for high baseline LH levels. Poor nest site, weak social stimulations or low mate interactions in females that will not breed could explain their low baseline LH levels (Ball, 1993; Dawson, 2008 for review). This down-regulation of LH levels might have been associated with possible reduced release of other pituitary hormones (FSH) and/or estradiol, which play a major role in the growth of reproductive organs and the expression of sexual behaviours. Estradiol is known to stimulate female begging behaviour (Hunt and Wingfield, 2004) and courtship feeding (Eda-Fujiwara et al., 2003). In pre-laying Black-legged kittiwakes, courtship feeding by the males is commonly observed and is closely linked to successful copulation (Helfenstein et al., 2003; Kempnaers et al., 2007). Hence, females with low baseline LH levels would have exhibited low estradiol levels and low mating effort, thereby skipping the breeding attempt. Therefore, in pre-laying female kittiwakes, elevated corticosterone levels appear to be strongly involved in the disruption of the HPG axis and to negatively affect breeding decision. At the ultimate level, skipped breeding could be an adaptive response to promote females' own survival, especially for long-lived prudent parents (Drent and Daan, 1980).

4.3. Hormonal levels and egg-laying dates

Concerning the timing of breeding, females with low scaled mass index laid their first egg later than females with high one. However, baseline corticosterone levels were not related to the timing of breeding, contrary to recent correlative and experimental findings in another long-lived seabird, the Snow petrel (Goutte et al., 2010). Why is the hormonal regulation of the timing of breeding species-dependant? Energetic constraints associated with egg formation should differ between these two seabird species. Indeed, small Larids, like kittiwakes, build up an egg in about 6–8 days (Astheimer and Grau, 1990), while female petrels have to accumulate energy reserves at sea during an extended period of three weeks (pre-laying exodus, Warham, 1996). Therefore, the timing of breeding should be under strong energetic constraints in petrels, and thus should be greatly influenced by early environmental conditions and physiological state (baseline corticosterone levels, Goutte et al., 2010). On the contrary, kittiwakes should experience lower energetic constraints during egg formation, and in turn the timing of egg-laying would be poorly mediated by elevated baseline corticosterone levels. The functional action of corticosterone on the egg-laying date could also be environment-dependant. Indeed, favourable conditions may overcome the effect of stress hormones on the first egg-laying date, as it was shown in females Florida scrub-jays (Schoech et al., 2009). The observed inhibition of HPG axis by high baseline

corticosterone levels in females could postpone the breeding schedule, but only during poor years.

In male kittiwakes, corticosterone levels were not as decisive as for females concerning the breeding decision, thereby highlighting sex differences in the response to environmental cues related to seasonal events (Ball and Ketterson, 2008; Blas and Hiraldo, 2010; Goutte et al., 2010). Furthermore, baseline and/or LHRH-induced LH and testosterone levels did not differ between breeders and non breeders, as found in Florida scrub jays (Schoech et al., 1996). Therefore, in male kittiwakes, pre-laying energetic and hormonal states appear to poorly influence the HPG system and the breeding decision.

Interestingly, the timing of breeding was closely linked to males' testosterone profile. Female kittiwakes bred earlier when their mates exhibited higher testosterone release ability after a LHRH challenge. How could males' testosterone drive the egg-laying date? High testosterone levels after a LHRH input could mediate behavioural adjustments in males, such as aggressiveness to obtain and defend a high quality nest, and/or high quality courtship display. In turn, provisioning effort was suggested to be a sexually selected trait on which female kittiwakes base decisions about timing and frequency of copulations (Kempnaers et al., 2007). Additionally, males with high testosterone release ability may enhance high body condition in pre-laying female kittiwakes, thereby triggering early breeding.

In conclusion, our study highlights clear sex-differences in the HPG sensitivity to stress hormones in pre-laying kittiwakes. This may originate from the specific constraints faced by males and females during the pre-laying period. Experimental manipulations of corticosterone levels coupled with behavioural observations (courtship feeding effort and copulation rate in corticosterone-implanted males and females) have to be conducted to confirm this hypothesis.

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V.3. Manipulation expérimentale de la corticostérone et conséquences sur la fitness

ARTICLE F

Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes

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Short title: corticosterone and timing of breeding

Experimentally reduced corticosterone release promotes early breeding in black-legged kittiwakes

Summary

Breeding at the right time is one of the most challenging issues for reproductive success. In birds, stressful environmental conditions are known to delay the timing of breeding but the underlying mechanisms are poorly understood. The stress hormone corticosterone appears to be a good candidate for mediating egg-laying date according to early environmental conditions and physiological state. By altering the perception of stress in Black-legged kittiwakes during the pre-laying period, we tested whether egg-laying date was mechanistically linked to corticosterone levels. To do so, we implanted male and female kittiwakes with a low dose of exogenous corticosterone to inhibit endogenous corticosterone production. According to our predictions, the experimental reduction of corticosterone release was paralleled by a significant advancement of egg-laying in females (around 4 days earlier). This effect was strongly sex-specific since it was not observed in corticosterone-treated males. In addition, females with experimentally reduced corticosterone release gained mass during the pre-laying period and had a higher breeding success compared to control females. The corticosterone treatment did not influence immediate breeding decision and clutch size, as well as return rate and breeding decision the following year. Our results confirm that corticosterone mediates egg-laying dates, possibly through the accumulation of energy reserve and body mass gain. Moreover, we suggest that the enhanced breeding success was triggered by the advancement of egg-laying in females with experimentally reduced corticosterone levels. At last, the corticosterone treatment and its associated immediate fitness consequences did not compromise survival and breeding opportunities the following year.

Keywords: glucocorticoid, implants, phenology, fitness, arctic seabird

INTRODUCTION

Breeding at the right time is one of the most challenging issues for reproductive success in a fluctuating environment (e.g. Lack, 1968; Perrins, 1970; Lyon et al., 2008). In birds, late breeding is usually associated with low breeding success, because of a possible mismatch between offspring's energy needs and the peak of food supply (*match-mismatch hypothesis*, Visser et al., 1998; Stenseth and Mysterud 2002; Durant et al., 2007). In addition, the timing of egg-laying has long-term fitness consequences since an experimental advancement and/or delay of breeding compromise future reproduction and survival (Nilsson and Svensson, 1996; Brinkhof et al., 2002). The adjustment of the timing of breeding is thus crucial and is possible through some behavioural and physiological flexibilities in response to environmental and internal factors (reviewed in Wingfield, 2008; McNamara and Houston, 2008). In that context, free-living birds are thought to track pre-breeding environmental cues that enable them to *anticipate* the peak of food availability and then, to *adaptively adjust* their breeding schedule (e.g. Frederiksen et al., 2004). However, this hypothesis is not always supported by correlative and experimental evidences (Hipfner et al., 2008, Shultz et al., 2009). An alternative but not exclusive hypothesis states that the timing of breeding is *imposed* by pre-laying energetic constraints associated with the clutch formation (Durant et al., 2005, Visser and Both, 2005). In that context, it is essential to clarify the underlying mechanisms that link pre-laying environmental cues, physiological state and phenological decisions at the individual level.

What are the mechanism underlying the onset of breeding? Increasing day length activates the hypothalamic–pituitary–gonadal axis (hereafter HPG axis). The expression of the Gonadotropin Releasing Hormone (GnRH) triggers the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH, reviewed in Dawson et al., 2001). In turn, gonadal development is promoted and sex-steroids (estradiol and testosterone) are released, thereby mediating the adoption of sexual behaviours, such as courtship, mating, and nest building (Ball, 1993). In addition to this fixed photoperiodic cue, many environmental variables, such as temperature, food supply, and/or stimulatory social interactions may interact to modulate the onset of breeding (Wingfield, 1980; Wingfield and Kenagy, 1991; Ball, 1993 ; Wingfield et al., 2003; Dawson, 2008). The underlying mechanisms that regulate the HPG axis according to pre-laying non-photoperiodic cues have received growing attention but remain still poorly understood (review in Schoech et al., 2009). One potential mediator is the release of glucocorticoids in response to stressors (such as inclement weather, food

shortage, or predator attacks), which is known to adjust life-history strategies according to environmental conditions and physiological state (Ricklefs and Wikelski, 2002; Wingfield and Sapolsky, 2003). Indeed in Vertebrates, the acute secretion of glucocorticoids (corticosterone and/or cortisol) via the Hypothalamic-Pituitary-Adrenal (HPA) axis, promotes the immediate survival through the mobilization of stored energy, but at the expense of the current reproduction (review in Breuner et al., 2008). During the pre-laying period, elevated corticosterone (hereafter CORT) levels appeared to be associated with delayed breeding in females (Salvante and Williams, 2003; Schoech et al., 2009; Goutte et al., 2010a), via a possible perturbation of the HPG axis (reviewed in Schoech et al., 2009). In Black-legged kittiwakes (*Rissa tridactyla*, Linnaeus, 1758), we recently found that females bearing high baseline CORT levels showed reduced baseline LH levels and a low ability to release LH, following LHRH challenge (Goutte et al., in press). Thus, CORT appears to be a potential candidate that mediates the timing of breeding according to pre-laying environmental and physiological conditions. In that respect, it is crucial to carefully distinguish the baseline and the stress-induced corticosterone levels. Baseline CORT levels are considered as a marker of activities, energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004; review in Landys et al., 2006), whereas stress-induced CORT levels mirror the sensitivity to stress and the commitment into current reproduction (review in Wingfield and Sapolsky, 2003; Lendvai et al., 2007, Bokony et al., 2009).

Conversely, a low release of CORT in response to stress would be a possible mechanism to ensure early breeding through a fully expression of the HPG axis. We tested this hypothesis for the first time, by down-regulating the CORT release of Black-legged kittiwakes during the pre-laying period. Our aim was to alter the perception of environmental stress in Black-legged kittiwakes during the pre-laying period. To do so, we implanted pre-laying male and female kittiwakes with a low dose of exogenous CORT to inhibit the HPA axis and endogenous corticosterone production. When exposed to low doses of CORT for a prolonged period, the HPA axis compensates by reducing ACTH production, adrenal capacity to produce CORT, and adrenal activity (Akana et al., 1992; Wolkowitz, 1994). Following this CORT treatment, we addressed the following issues: (1) what were the phenological consequences of the CORT treatment? Since the HPG axis of females appear to be more sensitive to stressful events than males' ones in black-legged kittiwakes (Goutte et al., in press), we predict that females, with reduced CORT stress response, would breed earlier than control ones. (2) What were the fitness consequences of the CORT treatment? Immediate fitness parameters (hatching and breeding success) and residual fitness (return rate and

breeding decision the next year), were thus monitored. Since early breeding is associated with high breeding success (Perrins, 1970) and since an attenuated CORT stress response favours reproductive investment (Lendvai et al., 2007), we predict that females with reduced CORT stress response, would have a higher breeding success than control ones.

2. MATERIALS AND METHODS

Study area and birds

Our study was conducted between 19 may to 8 august 2009 in a colony of Black-legged kittiwakes at Kongsfjorden, Svalbard (78°54'N, 12°13'E), 7 km east of Ny-Ålesund, Norway. Black-legged kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago. We studied kittiwakes in one plot of ca. 100 pairs breeding on cliffs at a height of 5-10 m. Birds were individually marked with white PVC plastic bands engraved with a three-digit code and fixed to the bird's tarsus. Thus, kittiwakes could be identified from a distance without perturbation.

Experimental treatment of stress-induced corticosterone levels

The corticosterone implantation was conducted from the 19th to 31st may 2009, during the pre-laying period. 21 females and 20 males were captured at their nest with a noose on the end of a 5 m fishing rod (day 0). Immediately after capture, a blood sample was collected from the alar vein with a 1 mL heparinized syringe and a 25 gauge needle, within 3 minutes (3 minutes and 2 ± 5 seconds) to determine baseline CORT levels. In 8 birds, handling time exceeded the time recommended by Romero and Reed (2005) to assess baseline CORT levels and these values were excluded for the analysis. Immediately after this first blood sample, we implanted the kittiwakes subcutaneously (between shoulders) with one 25-mm silastic tubes (internal diameter 1.47 mm, external diameter 1.96 mm, Dow Corning, Michigan) sealed at the ends with medical grade silicon (Dow Corning) and either filled with crystallized CORT (C2505, Sigma Chemical Co., St. Louis, USA, N = 13 CORT-blocked females and 10 CORT-blocked males) or empty (N = 8 control females and 10 control males). Just prior to implantation, a 1 mm incision was made with scissors in both ends of the implant. The implant site was disinfected with povidone iodine and then glued (Vetbond, 3M SANTÉ). Administration of exogenous CORT is known to down-regulate the endogenous CORT production in birds during a prolonged period (Vandenborne et al., 2005; Romero et al., 2005;

Busch et al., 2008; Müller et al., 2009). Two 25-mm silastic implants significantly elevate baseline CORT levels in Black-legged kittiwakes during the first three days after implantation (Kitaysky et al., 2001; Angelier et al., 2007, 2009). We choose half of this dose (one implant) to inhibit CORT release, without strongly elevating baseline CORT levels (Akana et al., 1992; Wolkowitz, 1994).

After implantation, birds were weighed to the nearest 2 g using a Pesola spring balance and their skull length (head+bill) was measured to the nearest 0.5 mm using a sliding calliper. An index of body condition was calculated from a regression of body mass against body size (GLM, $F_{1,38} = 15.934$, $p < 0.001$). Kittiwakes were then marked with spots of dye on the forehead to facilitate subsequent observations and were released.

Physiological effects of the corticosterone treatment

During the 26 first days after the CORT implantation (day 1 to day 26, still during the pre-laying period), 18 CORT-blocked kittiwakes and 19 controls were recaptured opportunistically. The number of days between the treatment and the recapture did not differ between CORT-blocked (11.72 ± 1.83 days) and control kittiwakes (11.11 ± 1.54 days, student's test, $t = 0.258$, $df = 34.277$, $p = 0.798$). A blood sample was collected immediately (2 minutes and 54 ± 5 seconds) after capture to assess baseline CORT levels. Kittiwakes were then weighed and body mass change was calculated as the difference between the body mass at the day of recapture (BMt) and the body mass at the day of implantation (BM0), corrected by BM0, that is, $(BMt - BM0)/BM0$. A second blood sample was collected 15 minutes (15 minutes and 46 ± 19 seconds) after handling to assess the stress-induced CORT levels. Although maximum levels of CORT are reached 30 minutes after capture and handling in black-legged kittiwakes (Chastel et al., 2005), we decided to minimize the time of capture.

Timing of breeding and fitness consequences of the corticosterone treatment

During the egg-laying period, we checked the nests every two days, using a mirror at the end of an 8 m fishing rod. Breeding decision (at least one egg is laid or no egg laid), egg-laying dates, clutch size (1 or 2 eggs) were thus monitored. Then, we checked the nests every 2 or 3 days to monitor the number of lost eggs per active nest, and the number of chicks that reached 12 days old per active nest (hereafter called breeding success). During the following year (from the 20th May 2010 to 13th June 2010), we monitored the return rate and the breeding decision of CORT-blocked and control kittiwakes in order to investigate possible long-term fitness consequences of the CORT treatment.

Hormones assay

The blood samples were immediately put into ice until centrifugation at the end of the day. Plasma was separated and stored at -20°C . Molecular sexing was performed as detailed in Weimerskirch et al. (2005). Plasma concentrations of CORT were determined by radioimmunoassay at the Centre d'Etudes Biologiques de Chizé (CEBC) as previously described (Lormée et al., 2003). All samples were run in one assay; the coefficient of intra-assay variation was assessed using several reference plasmas within the assay (coefficient of variation = 5.8%, N = 5 duplicates). Minimal detectable CORT level was 0.5 ng/mL

Statistical analyses

All statistical analyses were performed using R 2.8.0 (R Development Core Team 2008). Concerning baseline corticosterone levels, the first day after treatment was particular compared to day 2 and more (ref). Thus we first tested for an effect of CORT treatment (CORT-blocked or control), period of sampling (day 0, day 1 and period between day 2 to day 26), sex and the interactions CORT treatment * period and treatment * sex on baseline CORT levels. Because birds were sampled twice (day 0 and day of recapture), we used generalized linear mixed model (GLMM) by including bird identity as a random effect. Then we focussed from day 2 to day 26. We tested for an effect of treatment, day of sampling (day 2 to day 26), sex and the interaction treatment * day of sampling, treatment * sex on baseline CORT levels, by using a generalized linear model (GLM) with normal errors and an identity link function. Secondly, we investigated whether stress-induced CORT levels were dependant on CORT treatment, period (day 1 and the period between days 2-26), or day of sampling (from the 1st to the 26th day after treatment), sex and the interactions CORT treatment * day of sampling, treatment * sex on. Indeed stress-induced CORT levels were not assessed at day 0, and we used a GLM normal errors and an identity link function. Third we tested for an effect of the CORT treatment, body condition and the interaction CORT treatment * body condition on 1) breeding decision by using GLM with binomial error distribution and a logit link function, on 2) laying date of the first egg, 3) clutch size, 4) number of lost eggs, 5) breeding success, by using GLMs with Poisson errors and a log link function, 6) return rate and 7) breeding decision the following year by using GLM with binomial error distribution and a logit link function. Males and females were analysed separately. Finally, we tested for an effect of treatment on body mass change before egg-laying by using wilcoxon tests. Only pre-laying females that *did* breed were analysed for body mass change, because non-breeding females

are not constrained to acquire and store energy for the eggs building. Males' body mass change were analysed for all and then for breeders only. Dependant variables were log-transformed when necessary and models were checked for assumptions.

RESULTS

Validation of the corticosterone treatment

At day 0, CORT-blocked kittiwakes and controls did not differ in baseline CORT levels ($F_{1,31} = 2.209$, $p = 0.148$) even when considering an interaction effect with sex (sex: $F_{1,30} = 1.813$, $p = 0.189$; treatment * sex: $F_{1,29} = 0.019$, $p = 0.892$); in body condition ($F_{1,39} = 0.002$, $p = 0.964$), even when considering an interaction effect with sex (sex: $F_{1,38} = 0.001$, $p = 0.971$; treatment * sex: $F_{1,37} = 0.530$, $p = 0.471$); in date of treatment ($F_{1,39} = 0.330$, $p = 0.569$), even when considering an interaction effect with sex (sex: $F_{1,38} = 0.737$, $p = 0.396$; treatment * sex: $F_{1,37} = 1.557$, $p = 0.220$).

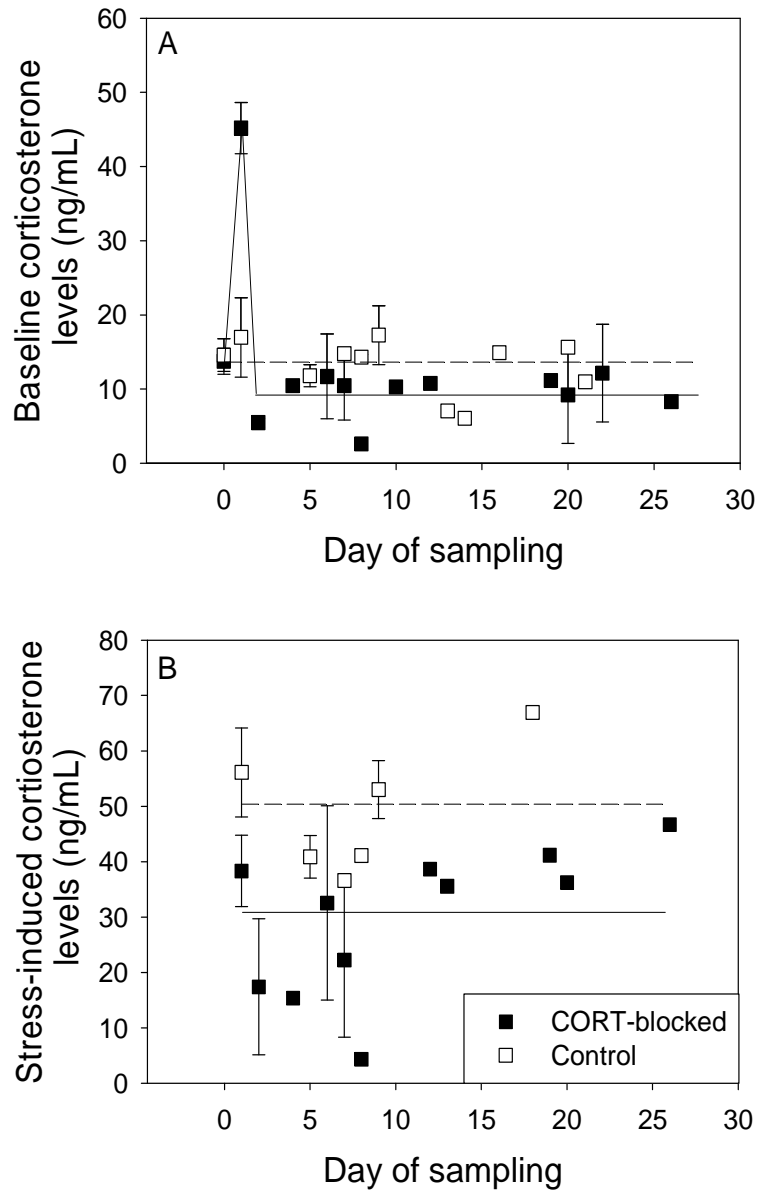
Baseline CORT levels were influenced by the period (day 0, day 1, days 2-26) but in a different way between CORT-blocked and control groups (Table 1a, Fig. 1A). Indeed, within the control group, baseline CORT levels were not affected by the period (GLMM, $F_{2,12} = 0.342$, $p = 0.717$), or by the day of sampling (GLMM, $F_{1,13} = 1.1001$, $p = 0.313$). Within the CORT-blocked group, baseline CORT levels significantly increased from day 0 to day 1 ($W = 0$, $p = 0.009$) and then significantly decreased between day 1 and days 2-26 ($W = 32$, $p = 0.013$). From the 2nd to the 26th days after the treatment, baseline CORT levels were not affected by the day of sampling (Table 1b). At day 1, baseline CORT levels tended to be higher in CORT-blocked than in control kittiwakes but it was not significant (Wilcoxon, $W = 0$, $p = 0.2$). After day 1, baseline CORT levels were significantly lower in the CORT-blocked group (9.47 ± 1.28 ng.mL⁻¹) than in the control ones (13.58 ± 1.26 ng.mL⁻¹, Table 1b). In addition, males had significantly lower baseline CORT than females, from the 2nd to the 26th days (Table 1b)

Stress-induced CORT levels were significantly lower in the CORT-blocked group (30.78 ± 3.76 ng.mL⁻¹) than in controls (50.35 ± 3.61 ng.mL⁻¹, Table 1c,d, Fig. 1B). Stress-induced CORT levels were unaffected by the period or the day of sampling, the sex and the interactions (Table 1c,d).

Table 1: Effect for the CORT treatment, period (day 0, day, 1, days 2 to 26), sex and the interactions CORT treatment * period, CORT treatment * sex on the levels of a) baseline corticosterone and c) stress-induced corticosterone using GLMMs with bird identity as a random effect. Effect for the CORT treatment, days (from the second to the 26th days after CORT treatment), sex and the interactions CORT treatment * days, CORT treatment * sex on the levels of b) baseline corticosterone and d) stress-induced corticosterone, using GLMs using normal errors and an identity link function.

Dependant variable	Independant variables	d.f.	F	p
a-Baseline corticosterone (ng/mL) N = 68	Treatment	1,35	1.531	0.224
	Period	2,25	7.436	0.003
	Treatment * Period	2,25	6.503	0.005
	Sex	1,35	0.303	0.586
	Treatment * Sex	1,35	0.003	0.959
b- Baseline corticosterone (ng/mL) between day 2 and 26 N = 32	Treatment	1,30	9.378	0.005
	Days after treatment (>1)	1,29	0.087	0.770
	Sex	1,28	13.743	0.001
	Treatment * Days after treatment	1,27	0.002	0.962
	Treatment * Sex	1,26	2.898	0.101
c-Stress-induced corticosterone (ng/mL) N = 29	Treatment	1,27	13.101	0.001
	Period	1,26	2.616	0.119
	Sex	1,25	0.096	0.759
	Treatment * Period	1,24	1.604	0.218
	Treatment * Sex	1,23	1.604	0.218
d- Stress-induced corticosterone (ng/mL) N = 29	Treatment	1,27	11.174	0.003
	Days after treatment	1,26	1.318	0.263
	Sex	1,25	0.153	0.700
	Treatment * Days after treatment	1,24	0.001	0.981
	Treatment * Sex	1,23	0.180	0.675

Figure 1: Evolution of (A) baseline corticosterone levels and (B) stress-induced corticosterone levels with day of sampling after treatment (day 0 to day 26) for CORT-blocked kittiwakes (filled square, solid line) and controls (open square, dashed line). Baseline corticosterone levels increased at day 1 compared to day 0 and then returned to lower levels at days 2-26 compared to controls.



Corticosterone treatment and immediate phenological and fitness consequences

The breeding decision did not differ between CORT-blocked females and control females (Table 2a, Fig. 2A). However, the proportion of non-breeding females increased with decreasing body condition (Table 2a). CORT-blocked females laid their eggs around 4 days earlier than control ones (Table 2b, Fig. 2B), without an effect of body condition. Clutch size was not influenced by the corticosterone treatment and by body condition in males and females (Table 2c). In nests with a CORT-blocked female, around ¼ of the clutches were predated by Glaucous gulls, *Larus hyperboreus*, far less than in nests with a control females: the whole clutches were predated (Table 2d, Fig. 2C). Breeding success was significantly higher in nest with CORT-blocked females than in control ones (Table 2e, Fig. 2D). In males, a higher proportion of CORT-blocked males tended to skip the breeding event than control males, without an interaction effect for body condition (Table 2a, Fig. 2A). Our experimental treatment had no effect on egg-laying date, number of lost eggs and breeding success in males (Table 2b,d,e, Fig. 2B,C, D).

During incubation, our CORT treatment had a significant effect on body mass changes: CORT-blocked breeding females gained mass (N = 5, +6.31 ± 1.56%) whereas control breeding females lost mass (N = 4, -4.05 ± 2.89%, Wilcoxon W = 1.5, p = 0.032, Fig. 3). Body mass change did not differ between CORT-blocked males and control ones (W = 22, p = 0.805), even when considering only breeders (W = 10, p = 0.762).

Corticosterone treatment and long-term fitness consequences

The return rate in 2010 did not differ between CORT-blocked kittiwakes and control ones (Table 2f, Fig. 2E). For the ones observed at the colony in early 2010, the decision to breed the year following the treatment 2010 did not differ between CORT-blocked kittiwakes and control ones (Table 2g, Fig. 2F).

Table 2: Modelling a) breeding decision, b) egg-laying date, c) clutch size, d) number of predated eggs, e) breeding success, f) Return rate in early 2010, g) breeding decision in 2010, as functions of treatment and body condition, using GLM with binomial error distribution and logit link function for (a, f, g) and GLMs with poisson error distribution, log link function for (b, c, d, e), in CORT-blocked and control kittiwakes. Females and Males were analysed separately. Significant variables were indicated in bold.

Dependant variables	Independante variables	Statistics (Females)			Statistics (Males)		
		N	Chi ²	p	N	Chi ²	p
a- Breeding decision	Treatment	21	1.129	0.288	20	3.439	0.064
	Body condition	21	4.338	0.037	20	0.249	0.618
	Treatment * Body condition	21	0.566	0.452	20	0.052	0.819
b- Egg-laying date	Treatment	13	4.035	0.045	12	0.517	0.472
	Body condition	13	0.265	0.607	12	0.235	0.628
	Treatment * Body condition	13	0.544	0.461	12	0.052	0.820
c- Clutch size	Treatment	13	0.036	0.849	12	0.080	0.777
	Body condition	13	< 0.001	1.000	12	0.002	0.965
	Treatment * Body condition	13	0.002	0.964	12	0.009	0.925
d- Lost eggs	Treatment	13	4.010	0.045	12	0.439	0.508
	Body condition	13	< 0.001	1.000	12	0.010	0.920
	Treatment * Body condition	13	0.125	0.723	12	0.205	0.650
e- Breeding success	Treatment	13	7.355	0.007	12	0.299	0.584
	Body condition	13	< 0.001	1.000	12	0.350	0.554
	Treatment * Body condition	13	0.111	0.739	12	2.943	0.086
f- Return rate	Treatment	21	1.044	0.307	20	2.848	0.092
	Body condition	21	1.737	0.188	20	0.965	0.326
	Treatment * Body condition	21	2.648	0.104	20	0.185	0.667
g- Breeding decision the next year	Treatment	16	0.760	0.383	15	0.043	0.835
	Body condition	16	2.497	0.114	15	0.161	0.689
	Treatment * Body condition	16	2.048	0.152	15	0.018	0.894

Figure 2: (A) Breeding decision (at least one egg is laid : 1, no egg is laid : 0), (B) first egg-laying date, (C) Number of predated eggs per active nest (0, 1 or 2), (D) Breeding success (number of chick > 12 days old per active nest), (E) return rate the year following the treatment (not seen: 0, seen: 1), (F) breeding decision the year following the treatment for CORT-blocked (filled symbols), and control kittiwakes (open symbols). Circles denote females and triangles denote males.

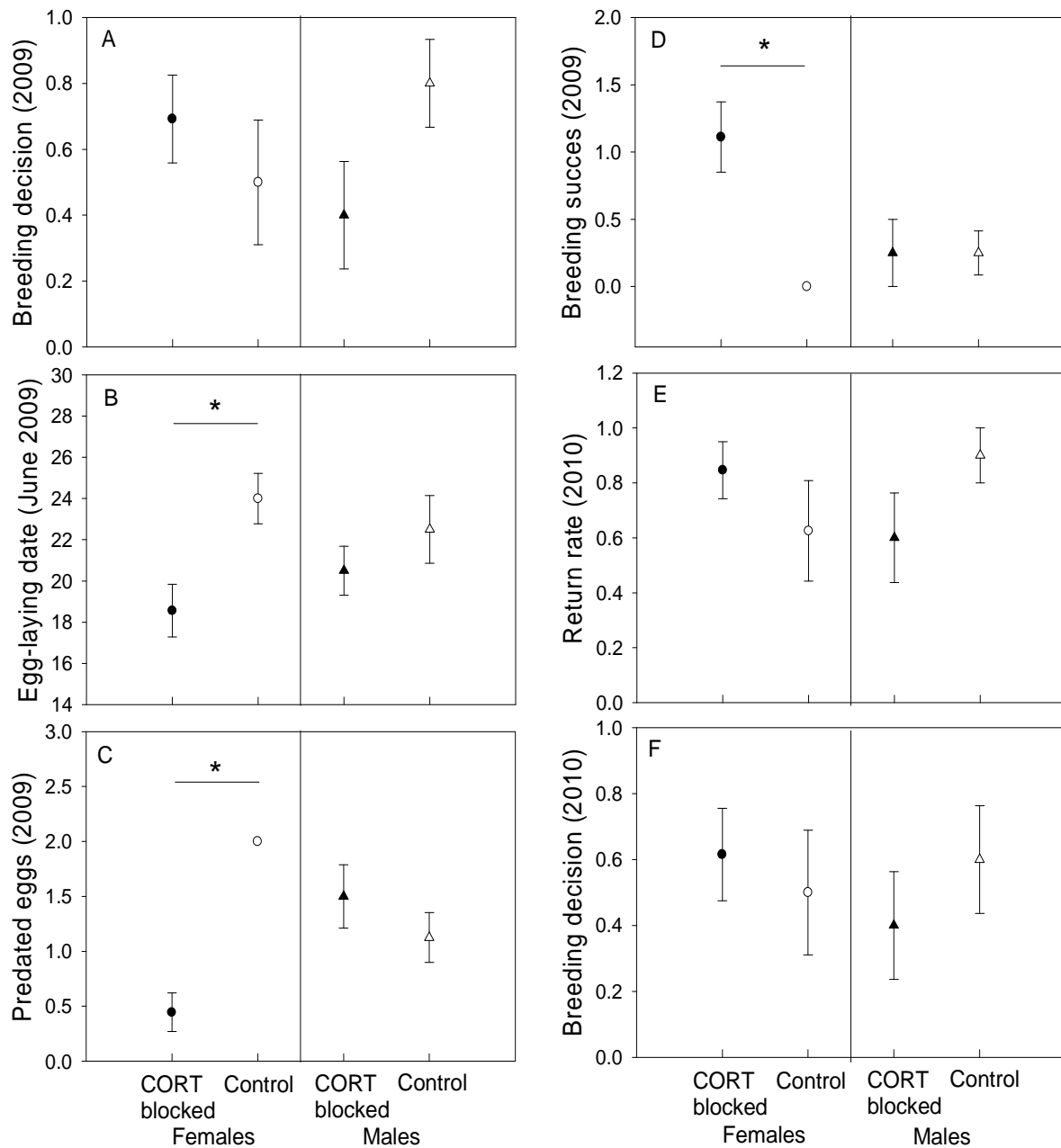
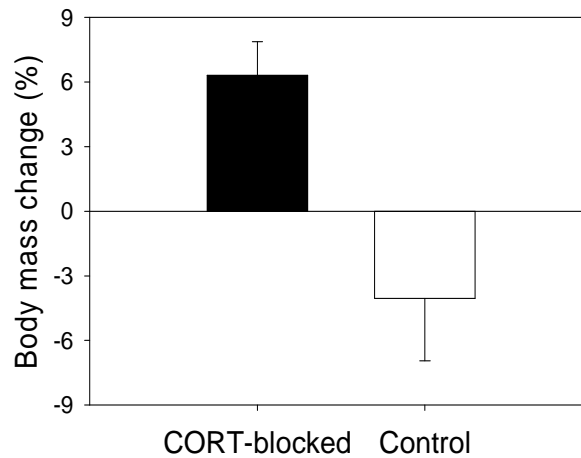


Figure 3: Body mass change between day of implantation (day 0) and day of recapture (day 1-26) in CORT-blocked breeding females (N = 5) and control breeding females (N = 4)



DISCUSSION

In this study of pre-laying black-legged kittiwakes, we showed for the first time that a prolonged reduction of baseline and stress-induced CORT levels was accompanied by an advancement of the egg-laying date and by an improvement of breeding success. This effect was strongly sex-specific since it was observed only in females. Moreover, breeding females with experimentally reduced CORT levels gained body mass, whereas control ones lost mass. Finally, the return rate and the breeding decision the following year were not affected by the CORT treatment.

CORT treatment and CORT secretion

Our aim was to alter the perception of stress during the pre-laying period in kittiwakes. To do so, a low dose of exogenous CORT was used to down-regulate the HPA axis and the release of endogenous CORT in response to the stress of capture (Akana et al., 1992; Wolkowitz, 1994). Thus in male and female kittiwakes, the stress-induced CORT levels were around 60% lower after a CORT implantation than after a sham-implantation. This down-regulation of CORT release was long-lasting since it was observed at least 26 days after the CORT treatment. In addition, baseline CORT levels were reduced by about 70% from the second to the 26th day. At day 1, crystallised corticosterone was released into the bloodstream so that plasma corticosterone levels were 3.5 times higher than before the CORT treatment. This short CORT increase was within the physiological range observed for an acute stress response in black-legged kittiwakes and was much lower than the 10-time increase of baseline

CORT levels observed with two CORT implants (Kitaysky et al., 1999; Chastel et al., 2005). Finally, baseline CORT levels were lower in males than in females, from day 2 to day 26, but without an interaction effect with the CORT treatment.

Corticosterone treatment and the timing of breeding

According to our predictions, we found that the experimental reduction of CORT release during the pre-laying period was paralleled by a significant advancement of egg-laying in female kittiwakes (around 4 days earlier). This experimental CORT treatment strongly supports the hypothesis that CORT levels and the timing of egg-laying are mechanistically linked, as previously suggested by correlative observations : high baseline CORT levels were associated with late breeding in female Florida scrub-jays (*Aphelocoma coerulescens*, Schoech et al., 2009) and in snow petrels (*Pagodroma nivea*, Goutte et al., 2010a). Moreover in captive female Zebra finches (*Taeniopygia guttata*, Salvante and Williams, 2003), an experimental elevation of plasma CORT levels decreased the proportion of breeding females and delayed the onset of egg-laying. As high baseline CORT levels is a marker of energetic state and food availability (Kitaysky et al., 1999; Love et al., 2004; review in Landys et al., 2006), CORT may thus mediate breeding phenology according to energetic constraints. However, this relation appears complex since high baseline CORT levels were not associated to delayed breeding during favourable conditions breeding in Florida scrub-jays (Schoech et al., 2009) and in kittiwakes (Goutte et al., in press). Furthermore, an experimental CORT administration did not delay the timing of breeding when females Florida scrub-jays were fed *ad libitum* during the pre-laying period (Schoech et al., 2007). In this study, we investigated for the first time to our knowledge, whether the suppression of CORT release influence the timing of breeding in free-living birds. Our results support the idea that low baseline and/or stress-induced CORT levels were mechanistically linked to early egg-laying in female kittiwakes.

How to explain the causal link between experimentally reduced CORT levels and early breeding in female kittiwakes? In a previous study, we found that high baseline CORT levels were associated with low baseline LH levels and low LH releasing ability after a GnRH injection (Goutte et al., in press). By experimentally reducing CORT levels, LH secretion and gonadal development could have been promoted, thereby triggering early breeding. In that respect, we also found a high body mass gain in breeding females with reduced CORT levels. As body mass gain could result from the rapid gonadal growth, this would support the hypothesis of an accelerated reproductive physiology, induced by the experimentally reduced

CORT levels. Alternatively, body mass gain could reflect the accumulation of energetic reserves in females with experimentally reduced CORT levels, instead of gonadal development. Since high body condition in pre-laying female kittiwakes predicts early breeding (Goutte et al., in press), females with high body mass gain would have laid their eggs earlier in the season. Consequently, females with experimentally reduced CORT levels may have bred earlier, because of a higher accumulation of energy reserves compared to control ones. Indeed, at the proximate level, the primary function of CORT is to facilitate glucose release for utilization during challenges. It is thus conceivable that females with experimentally reduced corticosterone levels would have saved more energy reserves than control ones. Moreover, courtship feeding is a common behaviour in kittiwakes during the pre-laying period (Kempeneers et al., 2007). Although we did not record female begging during our experiment, it is conceivable that females with experimentally reduced CORT levels would have begged more for food. In turn, their mates might have fed them more and/or earlier than usual, hence allowing them to accumulate energy reserves and to breed earlier. Further behavioural observations have to be conducted to confirm this hypothesis.

The effect for reduced CORT release on the timing of breeding was strongly sex-specific since males with experimentally reduced CORT levels did not breed earlier than control ones. Indeed the sensitivity of the HPG axis to stressful cues is thought to differ between sexes (Ball and Ketterson, 2008). Supporting this, we have recently shown that the inhibition of LH release by high baseline CORT levels was only observed in female kittiwakes and not in males (Goutte et al., in press). Pre-laying constraints should be strongly sex-specific in free-living seabirds. In one hand, male-male competition could force male kittiwakes to acquire reproductive readiness earlier in the season than females and to better resist environmental perturbations. In the other hand, females have to extract and store a sufficient amount of energy to build up the eggs, and could be strongly sensitive to environmental stressors, such as a depletion of food supply early in the season (Ball and Ketterson, 2008).

Fitness consequences of the corticosterone treatment

First, it is important to notice that the experimental reduction of CORT levels did not influence breeding decision in females. Only females with low initial pre-laying body condition did not breed, which suggests that the reduction of CORT levels could not counteract energetic constraints. However, in males, an experimental reduction of CORT levels tended to decrease the breeding probability, without an effect of body condition. It is

conceivable that males need to maintain relatively high baseline CORT levels to ensure sustained reproductive activities (Landys et al., 2006). During the pre-laying period, nest defence, foraging activities and courtship feeding should require moderate levels of baseline CORT, so that a prolonged reduction of CORT levels would have led to nest and/or mate lost.

Secondly, females with experimentally reduced CORT levels had a higher breeding success than control ones, although clutch size did not differ between groups. Indeed, females with experimentally reduced CORT levels managed to incubate successfully their eggs (around 0.5 vs 2 lost eggs in the control group). As frequently observed in birds, early breeders succeed better than late ones (review in Verhulst and Nilsson, 2008), particularly during the incubation period in seabirds (e.g. DeForest and Gaston, 1996). In that context, we suggest that females with experimentally reduced CORT levels would have benefited from better environmental conditions than controls, thanks to the advancement of egg-laying. Alternatively, being heavier, CORT-blocked females may have benefited from higher body reserves during the challenging brooding period compared to control females (Moe et al., 2002). The CORT treatment might have also lasted longer than the pre-laying period and might have been effective during the incubation and chick-rearing period. Since the attenuation of CORT stress response is known to favour high parental effort (*brood value hypothesis*, Lendvai et al., 2007), females with experimentally reduced stress-induced CORT levels would have attend the nest more, hence having a higher reproductive success than control females. However, this should have been observed in CORT-blocked males, since incubating and chick-rearing roles, as well as CORT levels during these periods are largely similar between males and females in kittiwakes (Kitaysky et al., 1999; Chastel et al., 2005). Since male kittiwakes with experimentally reduced CORT levels did not have a better reproductive success than controls, this contests the hypothesis of a very long attenuation of CORT release.

Finally, the CORT treatment had no long-term consequences, neither in males nor in females. Indeed, kittiwakes with experimentally reduced CORT levels during the pre-laying period did not suffer from a low return rate the following year. As two CORT implants during the chick-rearing period decreased the survival probability of kittiwakes (Goutte et al., 2010b), our result suggests that the number of CORT implants and/or the period of CORT implantation had contrasted long-term consequences. Moreover, females with experimentally reduced CORT levels had an identical return rate and breeding decision the following years, although they bred earlier than usual. Brinkhof et al. (2002) demonstrated that female coots (*Fulica atra*) with experimentally advanced breeding had a lower survival rate than controls.

However, the advancement of hatching date in coots was higher (10 days), than in our study (ca. 4 days). Moreover, the production of a second brood in the experimentally advanced coot pairs could have resulted in higher reproductive costs and associated higher mortality rate (Brinkhof et al., 2002). Finally, the higher breeding success and hence higher breeding investment in females with experimentally reduced CORT levels did not compromise their breeding opportunities the following year, contrary to the hypothesis of Golet et al. (2004).

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Discussion



Tout vient à point à qui sait se détendre.

Guillaume Aldebert

VI. Discussion et perspectives

Ce doctorat visait à identifier les mécanismes hormonaux impliqués dans le contrôle des décisions de non-reproduction et des dates de ponte en fonction des paramètres environnementaux et individuels. Notre étude, à l'interface entre l'écologie et la physiologie, s'est concentrée sur deux espèces d'oiseaux marins en milieux polaires, présentant, comme caractéristiques majeures, une forte longévité (~ 20 ans pour les mouettes tridactyles, ~ 50 ans pour les pétrels des neiges) et une étroite fenêtre de reproduction liée à l'extrême saisonnalité de ces régions. Notre travail s'est appuyé sur des approches descriptives et expérimentales.

Nous avons pu mettre en évidence le rôle clé de la corticostérone, comme médiateur des décisions de non-reproduction et de dates de ponte, en réponse à un stress environnemental, et en fonction des caractéristiques individuelles.

Dans ce chapitre, les résultats sont tout d'abord replacés dans un contexte écologique, puis les mécanismes physiologiques impliqués dans la décision et la phénologie de la reproduction sont discutés et nuancés en fonction des effets individuels et des différences interspécifiques, pour terminer sur les limites de ce travail et les perspectives envisagées.

VI.1. La date de ponte comme composante de la fitness

VI.1.1. Succès reproducteur et survie

En accord avec des études antérieures (Verhulst & Nilsson 2008), nous avons retrouvé un déclin saisonnier du succès reproducteur au niveau individuel. De plus, une manipulation expérimentale de la phénologie confirme le lien direct entre un retard de la reproduction, un fort échec à l'éclosion et une faible motivation à maintenir les soins parentaux pendant l'élevage du jeune poussin (Article B). Enfin, l'avancée des dates de ponte, par l'intermédiaire d'une manipulation hormonale, est associée à un plus fort succès reproducteur chez les mouettes tridactyles (Article F). Ainsi, la date de ponte apparaît comme une composante clé de la fitness chez ces deux espèces. L'échec a principalement lieu au moment

de l'incubation avec une plus forte perte des œufs chez les individus naturellement tardifs et / ou expérimentalement « retardés », ce qui a été observé chez la majorité des oiseaux marins (e.g. DeForest & Gaston 1996).

Ce déclin saisonnier du succès reproducteur découle de différents facteurs, comme l'illustre la figure 26 (modifiée de la figure 3, en introduction). Tout d'abord, chez les couples « avancés » de pétrels des neiges, le succès reproducteur est inchangé, ce qui supporte l'hypothèse de la qualité individuelle (Article B, voir Verhust & Nilsson 2008, Fig. 26). Par exemple, chez les pétrels des neiges, nous avons montré que les très jeunes et les très vieux pétrels se reproduisent plus tard dans la saison (Article C) et montrent, par ailleurs, une forte probabilité d'échec pendant l'incubation (Angelier *et al.* 2007b). En plus de la qualité intrinsèque du couple, l'hypothèse de la date *per se* (voir Verhust & Nilsson 2008) peut expliquer 1) le fait que les mouettes réussissent mieux, lorsque la date de ponte est avancée (Article F), et 2) le fait que les pétrels des neiges échouent plus, lorsque la date d'éclosion est retardée (Article B, Fig. 26). Enfin, nos résultats ne confortent pas l'hypothèse d'optimisation individuelle proposée par Drent (2006), selon laquelle chaque couple 'déciderait' de la date de ponte optimale en fonction de leurs caractéristiques intrinsèques et de son environnement. En effet, les pétrels et les mouettes « avancées » ne souffrent pas d'une baisse de la fitness immédiate, ni apparemment de coûts sur le long terme (Article B, F). Au contraire, les mouettes « avancées » ont un meilleur succès reproducteur, tandis que leur taux de survie et de probabilité de reproduction l'année suivante sont identiques à celles des témoins (Article F, Fig. 26).

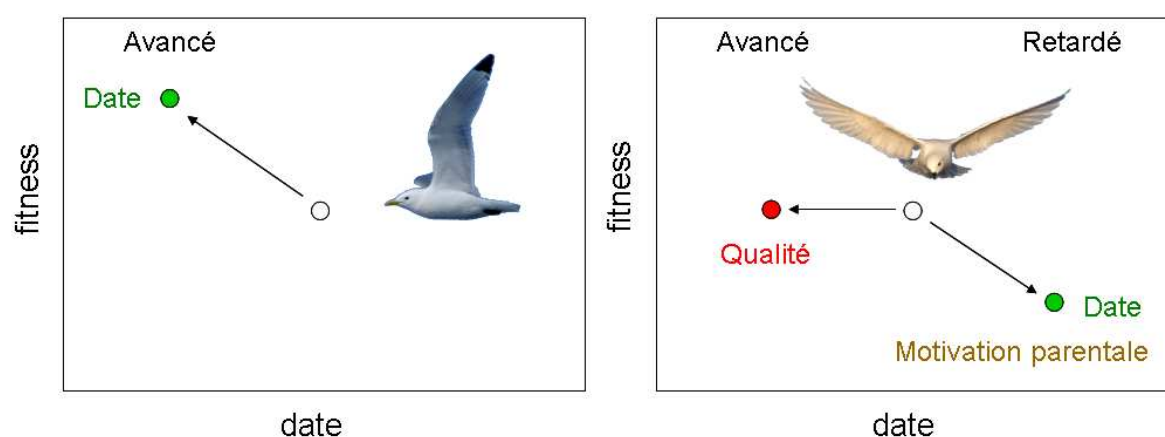


Figure 26 : Synthèse des conséquences d'une avancée ou d'un retard de la reproduction en termes de fitness, chez la mouette tridactyle (Article F) et le pétrel des neiges (Article B). Les hypothèses de date et de qualité sont concluantes. Nous proposons en plus un déclin saisonnier de la motivation parentale.

VI.1.2. Motivation parentale

Via la mesure de la sensibilité au stress, nous avons pu tester un déclin de la motivation parentale lorsque la reproduction a lieu plus tard que prévu (Fig. 26, Article B). En effet, suite à un décalage expérimental des dates de reproduction, nous avons montré que des parents « retardés » présentent une plus forte sécrétion de corticostérone suite à un protocole de stress, et abandonnent plus fréquemment leur œuf, ce qui engendre un plus fort échec reproducteur (Article B). Sachant qu'une réponse au stress élevée est communément associée à une redirection des ressources allouées à la reproduction, vers la survie immédiate (Wingfield & Sapolsky 2003), nos résultats physiologiques et comportementaux étayent donc l'hypothèse d'une baisse de la valeur de la reproduction (*brood value hypothesis* ; Lendvai *et al.* 2007; Lendvai & Chastel 2008), lorsque celle-ci a lieu plus tard que prévue. Les parents d'espèces longévives apparaissent donc moins motivés à s'investir dans une reproduction tardive.

Cet ajustement de la motivation parentale s'applique-t-il à une reproduction ayant lieu plus tôt que prévu ? Etant donné qu'une reproduction précoce est synonyme d'une forte valeur de la nichée, les parents « avancés » devraient être d'autant plus motivés à élever les jeunes et à atténuer leur sensibilité au stress. Or nos travaux montrent que les parents « avancés » ne diffèrent pas des « témoins », ni par leur taux basaux de corticostérone, ni par leur réponse au stress, ni par leur comportement, ni par leur succès reproducteur final (Article B). Nos résultats contestent donc l'hypothèse qu'une reproduction « avancée » engendre une augmentation de la motivation parentale, du moins chez le pétrel des neiges. Sachant qu'une avancée de la reproduction augmente la mortalité chez les femelles de foulques (*Fulica atra*, Brinkhof *et al.* 2002), des parents prudents, et notamment des espèces très longévives, comme le pétrel des neiges, devraient ne pas compromettre leur survie en investissant trop dans la reproduction en cours (Drent & Dann 1980). Ce résultat suggère que les espèces très longévives présentent une moindre flexibilité de la motivation parentale et de la sensibilité au stress (Bokony *et al.* 2009), tandis que chez des espèces peu longévives, comme les moineaux, la réponse au stress apparaît être plus modulable (accentuée et atténuée), en fonction de la valeur de la nichée en cours (*Brood value hypothesis*, Lendvai *et al.* 2007).

VI.2. Mécanismes hormonaux en période prénuptiale

La problématique principale de ce doctorat consistait à déterminer les mécanismes hormonaux orchestrant la décision de ne pas se reproduire et la date de ponte.

En comparant deux saisons de reproduction très contrastées chez la mouette tridactyle, nous avons observé des différences marquées en termes d'ajustements physiologiques, comportementaux et phénologiques (Article A). En effet, une année tardive (plus de 8 jours de décalage entre les moyennes des pontes de 2009 par rapport à 2008) avec une proportion de nids actifs moindre (environ 25% de couples reproducteurs en moins) et une faible taille de ponte (14% d'œufs en moins) est caractérisée par des trajets alimentaires prénuptiaux, éloignés de la colonie et par des taux élevés de corticostérone basale, en particulier pour les mâles (Article A). Une activité de recherche alimentaire soutenue et une faible disponibilité des ressources ont pu causer cette forte sécrétion de corticostérone (Kitaysky *et al.* 1999; Wingfield & Kitaysky 2002; Kitaysky, Piatt & Wingfield 2007; Benowitz-Fredericks *et al.* 2008). De plus, les taux de LH sont plus faibles en 2009 qu'en 2008 pour les mâles et les femelles, ainsi que les taux de testostérone pour les mâles (Article A). Une absence au nid prolongé, un manque de stimulation sociale et un niveau de stress élevé sont autant d'arguments qui peuvent expliquer ces faibles taux de LH et de testostérone en 2009, comparés à 2008 (Helm *et al.* 2006). Ces différences interannuelles laissent pressentir de forts liens entre les conditions environnementales (disponibilité des ressources, distance à parcourir), des taux élevés de corticostérone, de faibles taux de LH / testostérone et une ponte retardée.

Par le biais d'approches descriptives et expérimentales, nous avons tenté d'établir les liens entre les taux de corticostérone et l'ajustement de la date de ponte ; les taux de corticostérone et la décision de non-reproduction et entre les taux de corticostéron et la sécrétion de LH et / ou d'hormones sexuelles. L'objectif de ce paragraphe est donc de synthétiser et discuter les différents résultats (Fig. 27).

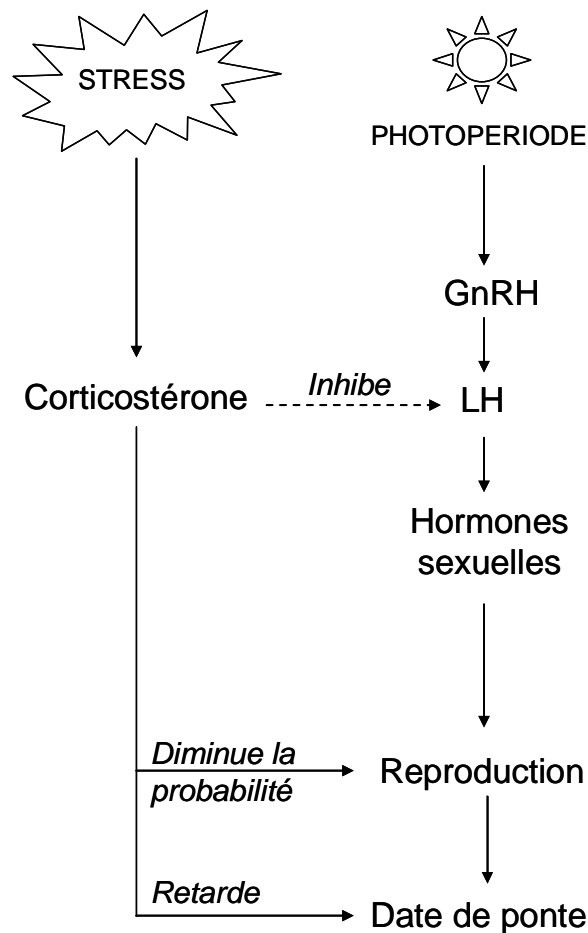


Figure 27 : Schéma synthétique des relations entre le stress environnemental en période prénuptiale, la sécrétion de corticostérone et le retard de ponte, voire l'absence de la reproduction. La corticostérone agit probablement en inhibant la sécrétion de LH, avec cependant des différences entre espèces et entre mâles et femelles.

VI.2.1. La corticostérone

i. Décision de ne pas se reproduire

La décision de ne pas se reproduire est caractérisée, chez les femelles de pétrels des neiges et de mouettes tridactyles, par des taux basaux élevés de corticostérone pendant la phase prénuptiale (Article C & E), comme cela a été montré précédemment chez les diamants mandarins en captivité (Salvante & Williams 2003) et chez les iguanes marins (Vitousek *et al.* 2010). Dans la mesure où des taux élevés de corticostérone basale peuvent être la conséquence d'un faible succès de pêche (Angelier *et al.* 2007c ; Lecomte *et al.* 2010), nos résultats suggèrent que les femelles présentant, pendant la phase prénuptiale, de forts taux

basaux de corticostérone sont probablement de moindre qualité (Angelier *et al.* 2010), avec, par exemple, une faible aptitude de recherche alimentaire (Angelier *et al.* 2007c), échouant alors à subvenir aux besoins énergétiques requis pour la formation des œufs (Article C & E).

En plus des taux basaux, nous avons étudié les taux de corticostérone induits par un stress, qui reflètent la perception individuelle du stress et la motivation des parents à abandonner ou à poursuivre l'investissement parental en cas de perturbations sévères (Ricklefs & Wikelski 2002; Wingfield & Sapolsky 2003; Lendvai *et al.* 2007; Lendvai & Chastel 2008; Breuner *et al.* 2008). Cependant, les taux de corticostérone induits par un stress n'influencent pas la décision de se reproduire, ni chez les mouettes, ni chez les pétrels (Article C & E). De même, une baisse expérimentale de la réponse au stress ne modifie pas la probabilité de ponte chez les femelles de mouettes tridactyles (Article F). Ces résultats laissent supposer que le fait de ne pas se reproduire est une contrainte (taux basaux de corticostérone, *i.e.* ratio énergétique entre les besoins nécessaires à la réalisation d'une activité spécifique par rapport aux ressources disponibles, Kitaysky *et al.* 1999; Love *et al.* 2004; Landys *et al.* 2006) et non une stratégie adaptative (réponse au stress, *i.e.* degré de motivation parentale dans la reproduction en cours, par rapport aux opportunités de reproductions futures).

ii. Date de ponte

Les dates de ponte semblent aussi être sous le contrôle de la corticostérone, mais les relations sont plus complexes, comme évoqué récemment par Schoech *et al.* (2009) : chez les pétrels des neiges, la reproduction est retardée, lorsque les taux basaux de corticostérone sont élevés (Article C). Des conditions environnementales difficiles lors de la période pré-nuptiale (éloignement des ressources par exemple, Article A) et / ou un état physiologique moindre d'un membre du couple (condition corporelle des femelles, Article E) pourraient donc retarder la formation de l'œuf via des taux basaux de corticostérone élevés. Ce résultat étaye donc l'hypothèse d'une date de ponte contrainte et imposée très tôt dans le cycle annuel par les conditions environnementales et la capacité individuelle à extraire efficacement l'énergie nécessaire à la mise en place de la reproduction (Hipfner *et al.* 2008; Shultz *et al.* 2009; Both *et al.* 2009). Au contraire, les taux de corticostérone induits par un stress n'influencent pas la date de ponte (Article C). Cela suggère, encore une fois, qu'une ponte tardive résulterait

davantage d'une contrainte énergétique (taux basaux de corticostérone élevés, Kitaysky *et al.* 1999; Love *et al.* 2004; Landys *et al.* 2006) et ne serait pas stratégiquement « décidée ».

Chez les mouettes tridactyles, les liens entre la corticostérone et la date de ponte du premier œuf semblent plus complexes. Via une approche corrélative, les dates de ponte semblent indépendantes des taux de corticostérone basaux ou induits par un stress (Article E). Via une manipulation expérimentale, une réduction des taux basaux de corticostérone et des taux de corticostérone induits par un stress, chez les femelles en période pré-nuptiale, sont associées à une avancée de la date de ponte (Article F). Les deux niveaux de corticostérone (basale et induit par un stress) semblent donc avoir un rôle subtil dans les variations de dates de ponte et apportent des éléments de réflexion sur la décision des dates de ponte. La corticostérone pourrait agir indirectement sur la date de ponte, via une modification des réserves énergétiques. En effet, les femelles dont la sécrétion de corticostérone est diminuée prennent du poids et pondent plus tôt que les femelles témoins (Article F). La corticostérone pourrait aussi agir sur les interactions au sein du couple, en agissant par exemple sur la quémante des femelles et sur le taux d'approvisionnement des mâles. En effet, le nourrissage de la femelle par le mâle est fréquent chez les mouettes tridactyles en phase pré-nuptiale (Kempnaers *et al.* 2007). Au contraire, si la date de ponte est anticipée et stratégiquement ajustée (Frederiksen *et al.* 2004; Reed *et al.* 2009), une reproduction précoce pourrait être contrôlée par l'atténuation de la réponse au stress, comme cela a été montré dans l'article F. Si la date de ponte est contrainte par les conditions environnementales et / ou par l'état physiologique de l'individu, la date de ponte serait contrôlée par les taux de corticostérone basale (Article C). Ces deux hypothèses n'étant pas mutuellement exclusives, la date de ponte pourrait être contrainte chez les individus en mauvaise condition, et anticipée chez les individus en bonne condition.

Par ailleurs, il n'est pas exclu que des mécanismes autres que la corticostérone soient impliqués dans la phénologie de la reproduction (Schoech *et al.* 2009), comme par exemple la leptine, une hormone peptidique produite par les cellules adipeuses. La leptine régule la prise alimentaire, le métabolisme et semblerait affecter la reproduction en agissant sur l'axe HPG (Henson & Castracane, 2003). Il a d'ailleurs été montré récemment que la décision de produire une seconde couvée, chez les mésanges, était contrôlée par la leptine (Löhmus & Björklund 2009).

VI.2.2. La LH et la testostérone

La LH est le déclencheur hormonal des processus comportementaux et physiologiques, qui initie la reproduction (Dawson *et al.* 2001). Les taux basaux de LH diminuent progressivement lors d'une période de stress, sans injection de GnRH/LHRH (30 minutes de capture, Article E). De plus, la testostérone diminue progressivement pendant une phase de stress (30 minutes de capture, Article E). Cette réponse au stress d'hormones, autres que la corticostérone, est encore mal connue. En plus de l'augmentation des taux de corticostérone (Wingfield & Sapolsky 2003), les taux d'autres hormones diminuent en réponse à un stress, telles que la LH, la testostérone (Article E, Lynn *et al.* 2010), ou la prolactine, l'hormone impliquée dans les soins parentaux (Chastel *et al.* 2005 ; Angelier & Chastel 2009).

i. Décision de ne pas se reproduire

Chez les mouettes tridactyles, nos résultats montrent tout d'abord que les femelles non-reproductrices présentent des taux basaux de LH plus faibles. Cependant, elles sont capables de sécréter de LH à des niveaux comparables aux reproducteurs, suite à une injection de GnRH/LHRH (Article E). Les non-reproducteurs ont donc un axe HPG fonctionnel, comme montré chez les 'helpers' d'une espèce coopérative, le geai à gorge blanche (Schoech *et al.* 1996) et chez le milan noir, un rapace dont les populations sont caractérisées par une part importante d'individus non-reproducteurs ('floaters', Blas & Hiraldo 2010). Comment expliquer les plus faibles taux basaux de LH chez les femelles non-reproductrices par rapport aux reproductrices? La sécrétion de LH est principalement initiée par l'allongement de la durée du jour mais aussi par d'autres facteurs, tels que les interactions sociales (Ball 1993). Parce qu'elles interagissent moins avec un partenaire, la sécrétion de LH chez les femelles non-reproductrices est probablement moins stimulée et leurs follicules ovariens sont moins développés (Article E, Schoech *et al.* 1996). Chez les mouettes tridactyles femelles, nous avons aussi analysé quelques taux d'œstradiol en période pré-nuptiale : les femelles qui ne se reproduisent pas ont tendance à présenter des taux d'œstradiol plus faibles que les femelles qui vont se reproduire (N = 8, Wilcoxon W = 12, p = 0.057). Chez les mâles, les taux basaux de LH et ceux induits par une injection de GnRH/LHRH ne diffèrent pas entre reproducteurs et non reproducteurs, ce qui laisse supposer que la sécrétion de LH des mâles n'est pas

impliquée dans la décision de non-reproduction et que la sécrétion de LH ne dépend pas des interactions / stimulations sociales (Article E). En ce qui concerne les hormones sexuelles, la décision de non-reproduction n'est pas liée au profil de testostérone (taux basaux testostérone et taux induit par une injection de GnRH et un pic de LH) chez les mâles de mouettes tridactyles (Article E).

De plus, nous avons mis en évidence l'importance de la dynamique de LH dans la médiation de la décision de non-reproduction (Article D). Suite à un test de réponse hypophysaire à la GnRH, les pétrels ont des taux de LH maximum à 10 minutes puis, entre 10 et 30 minutes après l'injection de GnRH, les taux de LH diminuent progressivement. Le taux de déclin de LH, entre 10 et 30 minutes après l'injection de GnRH / LHRH, est particulièrement important dans la probabilité de non-reproduction (Article D). En effet, l'incapacité à maintenir des taux élevés de LH après le pic est associée à une faible probabilité de ponte chez les pétrels des neiges, pour les mâles et les femelles (Article D). La capacité à maintenir des taux élevés de LH, lors d'un évènement stressant, pourrait donc moduler les ajustements physiologiques et comportementaux liés à l'initiation de la reproduction.

ii. Date de ponte

Les taux de LH n'expliquent pas la date de ponte au niveau individuel (Article E, pas de relation pour les pétrels des neiges non plus). Bien qu'ils semblent fortement impliqués dans la décision de ne pas se reproduire, ils n'ont surement peu ou pas d'impact sur les ajustements de la ponte à fine échelle temporelle.

La date de ponte est cependant corrélée au profil de testostérone (Article E). Après une injection de GnRH/LHRH et donc après un pic de LH, les taux de testostérone augmentent progressivement (30 minutes) chez les mâles de mouettes tridactyles. Plus les taux de testostérone atteints 30 minutes après l'injection de GnRH sont importants, plus les dates de ponte sont précoces. Ce résultat suggère des ajustements comportementaux et sociaux liés à la sécrétion de testostérone, tels que l'agressivité, la conquête et la défense d'un nid de qualité et / ou l'élaboration de parades nuptiales. Chez les mouettes tridactyles, les mâles apportent de la nourriture à la femelle avant la ponte (e.g. Kempenaers *et al.* 2007), ce qui peut assurer 1) le gain de masse des femelles (*immediate material benefits*, Hunter *et al.* 1993), 2) un investissement maternel plus important en raison de la qualité du mâle (*genetic benefits for the offspring*, Jennions & Petrie 2000; *future material benefits for the offspring*, Birkhead &

Møller 1992) et / ou 3) des copulations plus fréquentes (*mating effort*, Kempnaers *et al.* 2007). Une forte capacité à sécréter de la testostérone peut être liée à des interactions plus fréquentes avec la femelle, et / ou à un gain de masse, ce qui peut expliquer une ponte précoce. Ce résultat dénote, de surcroît, l'implication du mâle dans la phénologie de la reproduction. Il apparaît logique que la femelle décide de la date de ponte, comme cela a été montré chez les mésanges (Caro *et al.* 2009). Cependant, cette décision apparaît être fortement influencée par la qualité du mâle et / ou les interactions avec le partenaire (Helm *et al.* 2006).

VI.2.3. Inhibition de la sécrétion de LH par la corticostérone

La corticostérone agit-elle sur les traits d'histoire de vie, via l'inhibition de la sécrétion de LH et / ou d'hormones sexuelles ? Existe-il un lien fonctionnel entre la corticostérone et la sécrétion de LH et / ou de testostérone ? Nos résultats confirment ce lien : les taux basaux de corticostérone des femelles de mouettes tridactyles sont négativement corrélés aux taux basaux de LH (Article E). De plus, suite à un test de réponse hypophysaire à la GnRH, des forts taux de corticostérone basale sont associés à une faible capacité à sécréter la LH (Article E). Bien que le rôle inhibiteur de la corticostérone sur la sécrétion de LH soit connu chez les animaux domestiques (brebis ayant subi une ovariectomie, Breen & Karsch 2006), cette régulation hormonale est peu étudiée et peu connue chez les animaux sauvages, notamment les femelles. Cette inhibition de la sécrétion de la LH par la corticostérone est probablement le mécanisme clé de la décision de non reproduction chez les mouettes tridactyles (Article E). En ce qui concerne la phénologie, l'inhibition de la sécrétion de LH par la corticostérone ne semble pas contrôler les variations individuelles de date de ponte (Article E, pas de corrélation non plus chez les pétrels des neiges). Il est important de souligner de plus que les taux de corticostérone induits par un stress n'influencent pas les taux de LH (Article E). Chez les mâles de mouettes tridactyles, nous n'avons pas trouvé de liens entre corticostérone et LH (Article E). Les différences mâles-femelles et leurs causes possibles sont détaillées plus bas (§ VI.3.3.)

Chez les pétrels des neiges, nous n'avons pas mis en évidence d'effet inhibiteur de la corticostérone (taux basaux et / ou induit par un stress) sur la sécrétion de LH (taux basaux de LH, taux de LH après une injection de GnRH, et / ou taux de LH 20 minutes après un pic de LH, $p > 0.9$ pour toutes les corrélations, Fig. 28). Il n'est pas exclu que la corticostérone

agisse non pas sur la LH mais sur la FSH ou bien, directement sur les taux d'hormones sexuelles (testostérone, e.g. Consten *et al.* 2001). Nous n'avons pas pu tester cette hypothèse à cause d'une quantité de plasma trop faible. Il est aussi envisageable que la corticostérone ne perturbe pas l'axe HPG mais que ces hormones (corticostérone, LH, testostérone) aient des actions complémentaires sur la médiation de la décision de ne pas se reproduire et de la date de ponte. Ainsi, chez les femelles de pétrels des neiges, les non-reproducteurs ont des taux de corticostérone plus élevés que les reproducteurs (Article C) et présentent une moindre capacité à maintenir des taux de LH élevés après un pic de LH (Article D), sans que les taux de LH soient corrélés aux taux de corticostérone (Fig. 28).

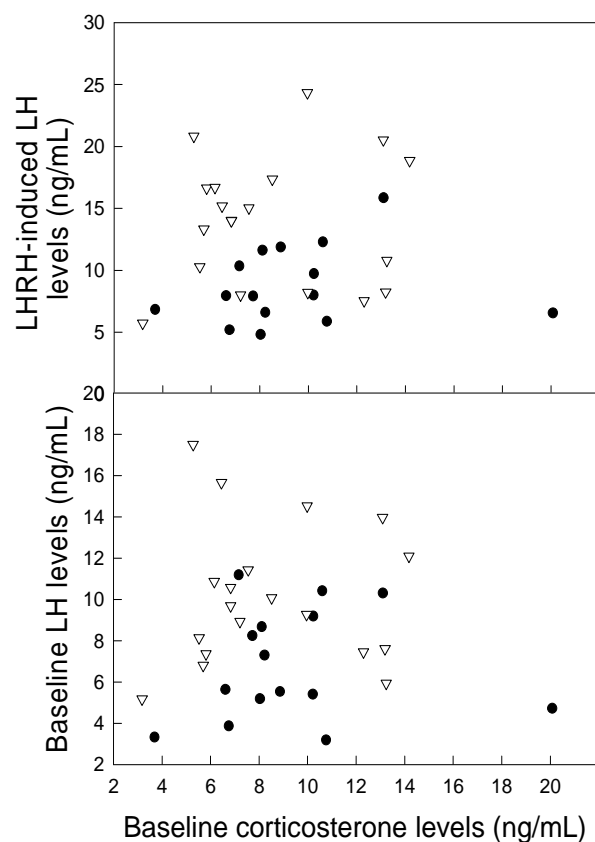


Figure 28 : Relations entre les taux basaux de corticostérone et les taux basaux et induits par une injection de LHRH / GnRH chez les pétrels des neiges mâles (triangles blancs) et femelles (ronds noirs) pendant la période pré-nuptiale

VI.2.4. Récentes avancées en neuroendocrinologie

Pour aller plus loin, il a été récemment mis en évidence qu'une classe de peptides, appelée RFamide, est impliquée dans les activités de reproduction au niveau neuroendocrinien (revue dans Kriegsfeld *et al.* 2006; Tsutsui & Ukena 2006). Deux de ces RFamides, la kisséptine et la GnIH, de l'anglais gonadotropin-inhibitory hormone (GnIH, revue dans Bentley *et al.* 2009; Tsutsui 2009) ont été identifiés comme des régulateurs majeurs de l'axe HPG, avec des effets opposés (revue dans Kriegsfeld *et al.* 2006; Tsutsui *et al.* 2006). La kisséptine joue un rôle crucial dans le développement et la régulation positive du système reproductif chez les mammifères tandis que la GnIH a une action inhibitrice (revue dans Greives *et al.* 2008). La GnIH pourrait être tout particulièrement impliquée dans la terminaison de la reproduction (Bentley *et al.* 2003).

Chez les mammifères, amphibiens, oiseaux et poissons, la GnIH agit directement au niveau central en inhibant la sécrétion de la LH et de la FSH (revue dans Greives *et al.* 2008; Bentley *et al.* 2009; Tsutsui 2009). De plus, la GnIH est exprimée et agit au niveau des testicules en réduisant la production de testostérone chez le mâle de moineau domestique (*Passer domesticus*, McGuire & Bentley 2010). Existe-il un lien fonctionnel entre les taux de glucocorticoïdes et la GnIH ou ont-ils simplement des actions complémentaires ? Il a été récemment montré que l'expression de la GnIH est non seulement activée par un stress aigu suite à la capture chez les moineaux domestiques (Calisi, Rizzo, & Bentley 2008), mais aussi par des taux élevés de glucocorticoïdes chez les rats (Kirby *et al.* 2009). Ces découvertes neuroendocriniennes ouvrent de nouvelles perspectives à la compréhension des mécanismes proximaux liés à l'initiation de la reproduction chez les animaux en milieu sauvage.

VI.3. Sources de variation : de l'espèce à l'individu

Comme cela a été évoqué, l'action de la corticostérone sur les traits d'histoire de vie (décision de ne pas se reproduire et date de ponte), et sur l'axe HPG n'est pas généralisable et varie non seulement entre les deux espèces étudiées, mais aussi en fonction de certains caractères individuels, tels que les différences entre mâles-femelles, la condition corporelle et / ou l'âge.

VI.3.1. Variations interspécifiques

Chez les pétrels des neiges, la corticostérone agit clairement sur la décision de non-reproduction et la date de ponte (Article C), tandis que les corrélations entre les taux de corticostérone et sur la date de ponte semblent plus complexes chez les mouettes tridactyles (Fig. 30, Articles E & F). Ceci peut être dû aux contraintes énergétiques associées à la formation des œufs. En effet, les mouettes fabriquent un œuf en 6 - 8 jours (Astheimer & Grau 1990) et pondent jusqu'à trois œufs au Svalbard, tandis que les pétrels des neiges accumulent des réserves énergétiques en mer pendant 3 semaines pour produire un œuf unique (exode prénuptial, Warham 1990). Ainsi, les conditions environnementales prénuptiales et l'état physiologique doivent être plus contraignants chez les pétrels des neiges par rapport aux mouettes tridactyles, ce qui expliquerait un fort lien entre les taux basaux de corticostérone et la date de ponte chez les pétrels des neiges, mais pas chez les mouettes tridactyles (Articles C, E & F). Des facteurs autres qu'énergétiques peuvent intervenir, tel que la durée de recherche d'un partenaire avant la ponte. En effet, le taux de fidélité au partenaire est plus élevé chez les pétrels des neiges (Bried *et al.* 2003) que chez les mouettes tridactyles.

VI.3.2. Variations interindividuelles : l'âge et la condition corporelle

i. La condition corporelle

Chez les oiseaux, la condition corporelle, notamment des femelles, joue un rôle primordial dans la décision de se reproduire et la date de ponte (e.g. Perrins 1970; Bêty *et al.* 2003). La

probabilité de ponte est d'autant plus faible, et les dates de ponte sont d'autant plus tardives que la condition corporelle des femelles de mouettes tridactyles est faible (Article E, Fig. 30). Ainsi, l'état des réserves énergétiques des femelles semblent fortement déterminer ces deux traits d'histoire de vie, comme cela a été montré précédemment (e.g. Chastel *et al.* 1995; Bety *et al.* 2003). Ces liens n'ont toutefois pas été validés chez les pétrels des neiges (Article C)

Cependant, nous n'avons pas trouvé de liens entre la condition corporelle et les taux basaux de corticostérone ; entre la condition corporelle et les taux de LH (basale ou maximale) ; ou entre la condition corporelle et les taux de testostérone (basale ou maximale, Article C & E). Le mode d'action fonctionnelle des réserves énergétiques sur les traits d'histoire de vie ne semble donc pas se faire via la sécrétion d'hormones de stress et / ou via l'inhibition de la LH et des hormones sexuelles. Le rôle potentiel de la leptine, hormone peptidique produite par les cellules adipeuses, pourrait être étudié comme mécanisme complémentaire entre l'état des réserves (condition corporelle) et la décision de se reproduire et la date de ponte. Seule la réponse au stress (taux maximum de corticostérone) est atténuée pour des individus en bonne condition (Article C). Ce résultat peut refléter une augmentation de l'investissement parental pour la reproduction immédiate lorsque les réserves énergétiques sont élevées.

ii. L'âge

Quant aux effets de l'âge (Fig. 30), les très jeunes et les très vieux individus ont une probabilité plus forte de ne pas se reproduire et / ou de se reproduire plus tard dans la saison, bien qu'ils soient sexuellement matures (Article C). Une analyse longitudinale a de plus été menée et souligne l'importance de l'expérience de reproduction sur la date d'éclosion (Goutte *et al.* manuscrit en préparation, Fig. 29).

Au niveau proximal, les très jeunes et les très vieux individus semblent être plus sensibles à un stress, étant donné que leurs taux de corticostérone induits par un stress sont plus élevés que ceux des individus d'âge moyen (Article C). Quant aux taux basaux de corticostérone, pourtant impliqués dans le contrôle des traits d'histoire de vie (décision de ponte et date de ponte des pétrels), ils ne varient pas avec l'âge (Article C). Il n'est pas exclu que les taux de corticostérone basale soient mieux expliqués par l'expérience de reproduction que par l'âge, comme cela a été montré précédemment (Angelier *et al.* 2006; Angelier *et al.* 2007d). De plus, les taux basaux de LH sont plus faibles chez les jeunes pétrels que chez les

vieux, bien qu'ils soient physiologiquement capables de produire et de sécréter de la LH après une injection de GnRH, à des niveaux équivalents aux vieux individus (Article D). Ceci suggère un manque de stimulation sociale chez les jeunes couples et / ou un nid de plus mauvaise qualité. Enfin, le déclin des taux de LH après un pic induit par l'injection de GnRH est plus prononcé chez les très jeunes et les très vieux (Article D). D'après ces résultats, la sénescence endocrine chez les pétrels des neiges pourrait être caractérisée par une plus forte sensibilité au stress (taux de corticostérone induits par un stress très élevés chez les vieux individus) et des contraintes au niveau hypophysaire (incapacité à maintenir des taux de LH élevés suite à un pic et pendant une phase de stress).

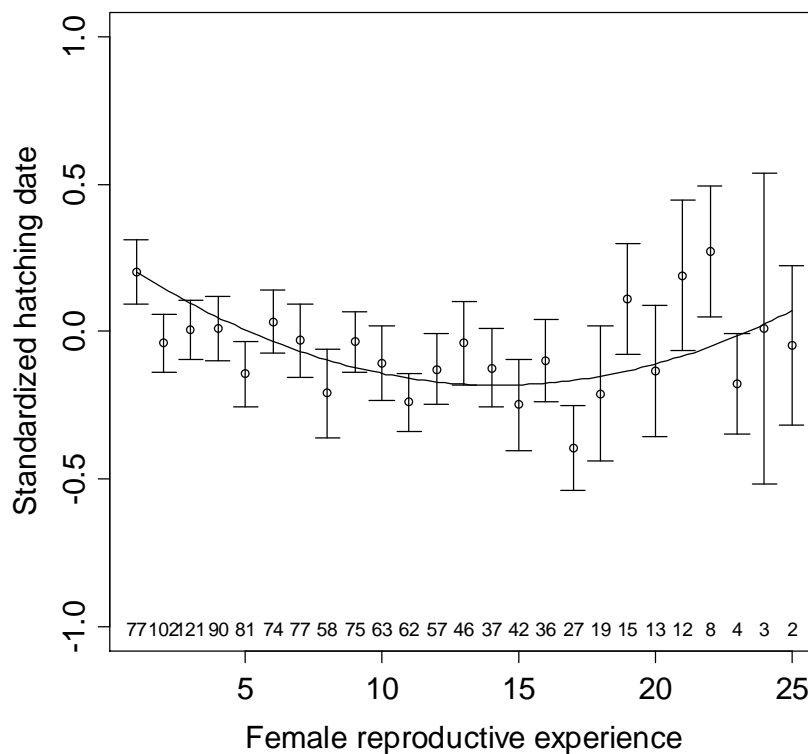


Figure 29 : Date d'éclosion standardisée (entre 1993 et 2007, moyenne \pm SE) en fonction de l'expérience de reproduction des femelles ($N_{\text{observations}} = 1201$, $N_{\text{individus}} = 344$). Les effectifs pour chaque expérience sont donnés.

VI.3.3. Différences entre sexes

Comme prédit par Ball & Ketterson (2008), mâles et femelles diffèrent grandement dans les mécanismes régulant les activités saisonnières en fonction des conditions environnementales (Fig. 30). Tout d'abord, la condition corporelle des mâles n'influence pas

les décisions de reproduction et les dates de ponte, alors que celle des femelles est corrélée à ces deux traits d'histoire de vie. Ce constat peut s'expliquer par des contraintes énergétiques différentes, notamment liées à la formation des œufs par les femelles.

En outre, les liens entre les taux basaux de corticostérone et la décision de ne pas se reproduire sont inhérents aux femelles, et inexistantes chez les mâles, quelle que soit l'espèce considérée (Article C & E). Ces différences entre mâles et femelles persistent après une manipulation expérimentale des taux de corticostérone (Article F). Chez les femelles, les taux basaux de corticostérone font clairement le lien entre l'état physiologique et / ou les ressources disponibles dans l'environnement et la décision de ne pas se reproduire (Article C & E). La corticostérone agirait par l'inhibition de la sécrétion de LH basale et induit par une injection de GnRH/LHRH (Article E). Chez les mâles, cette décision est sûrement orchestrée par des mécanismes autres. Les mâles ont tout intérêt à être prêt à se reproduire tôt dans la saison et à être résistant face à un événement stressant, à cause de la compétition entre mâles (Ball & Ketterson 2008). Il est concevable que les mâles aient des activités plus soutenues pendant la phase pré-nuptiale, comme par exemple, le nourrissage de la femelle pour les mouettes tridactyles (Kempnaers *et al.* 2007) et que ces activités, mobilisant de l'énergie, soient associées à des taux élevés de corticostérone. Cependant, ces forts taux de corticostérone basale ne doivent pas bloquer l'initiation de la reproduction. Ainsi cela expliquerait qu'au niveau physiologique, l'inhibition de la LH par la corticostérone ne soit pas observée chez les mâles (Article E).

D'après Blas & Hiraldo (2010), les mâles de milans noirs ont intérêt à investir dans la recrudescence totale des gonades bien qu'ils soient contraints par un accès limité aux territoires de bonne qualité. Les femelles, au contraire, n'atteignent pas une maturation complète du système reproducteur à cause d'un manque de stimulation sociale. Elles ont intérêt à réduire les coûts physiologiques de la reproduction, en retardant la sécrétion d'œstradiol et le développement ovarien, jusqu'à ce qu'elles soient en couple avec un mâle dominant.

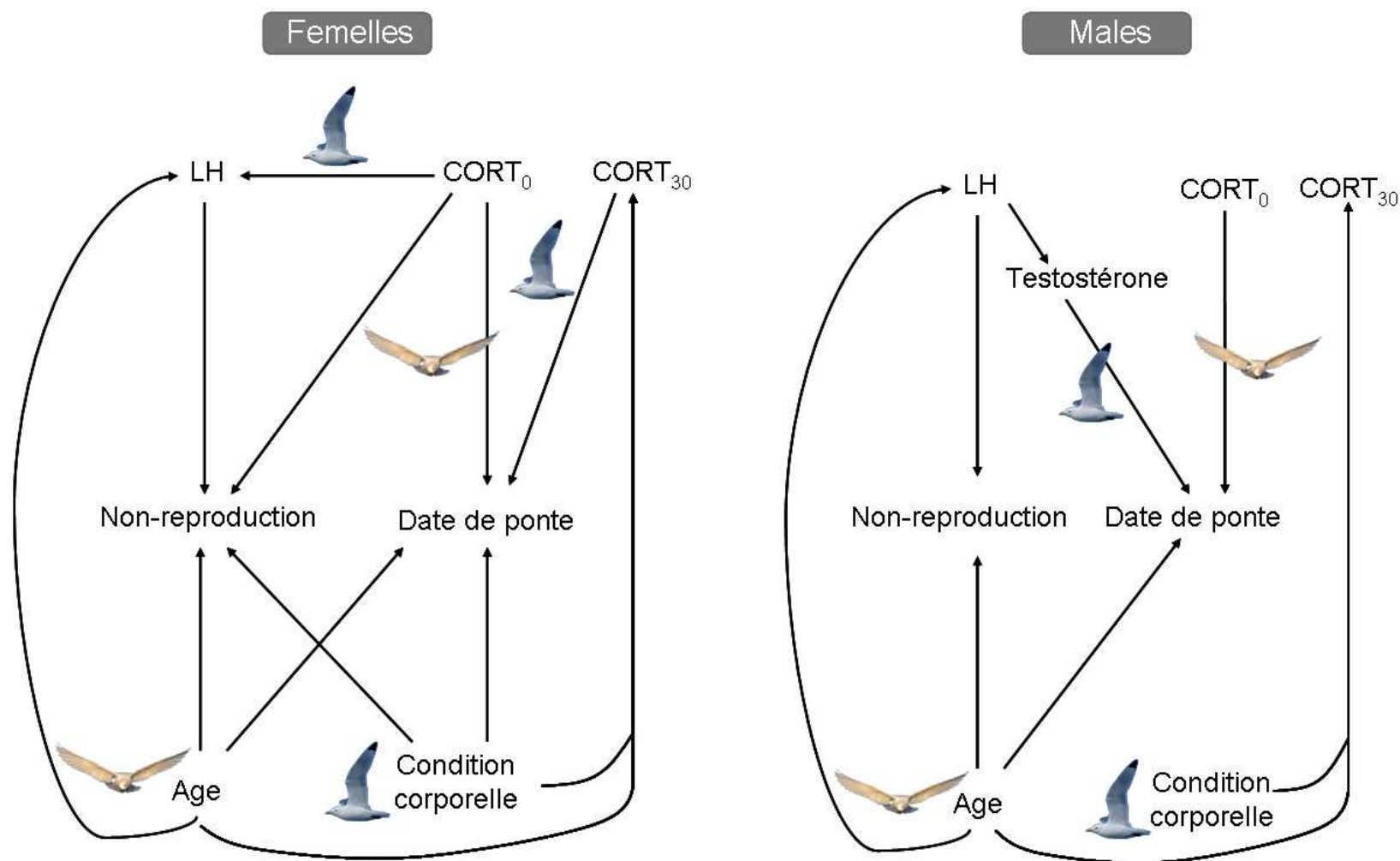


Figure 30: Synthèse des relations trouvées entre les taux de LH, de corticostérone basale (CORT₀), de corticostérone après 30 minutes de stress (CORT₃₀), la décision de non reproduction, la date de ponte et les paramètres individuels (âge, condition corporelle) selon le sexe et l'espèce étudiée (mouette tridactyle et pétrel des neiges).

VI.4. Limites et perspectives

VI.4.1. Hormones non dosées et protéines de transport

La GnRH n'a pas été dosée puisqu'elle ne circule pas dans le sang et n'est présente que dans l'hypothalamus, nécessitant alors le sacrifice des individus. De plus, la FSH, qui circule dans le sang, n'a pas été analysée, car le dosage n'a pas été mis au point. Cette gonadotrophine a des effets légèrement différents de la LH et il serait intéressant de comprendre comment la FSH est régulée par des signaux environnementaux non-photopériodiques et par l'état physiologique individuel. Enfin, les quantités restantes de plasma étaient insuffisantes pour doser l'œstradiol. Seul un faible effectif d'échantillons a été analysé, ne permettant pas d'aboutir à des résultats concluants. La progestérone n'a pas non plus été considérée dans ce travail, malgré des effets inconnus qui demanderaient à être explorés chez les oiseaux. Ces différentes informations manquent à la compréhension globale de l'action fonctionnelle de la corticostérone sur l'axe HPG (GnRH → LH / FSH → œstradiol / testostérone). On sait par exemple, que chez les brebis, il existe des rétrocontrôles entre les hormones sexuelles et l'inhibition de l'axe HPG par les glucocorticoïdes (Oakley *et al.* 2009).

De plus, en ce qui concerne la corticostérone, seuls les niveaux de corticostérone totale ont été considérés dans ce travail de thèse. Or, la corticostérone circule dans le sang en étant liées à des protéines de transport, les CBG, de l'anglais Corticosterone Binding Globuline. Seule la corticostérone libre serait active, alors que la corticostérone liée aux CBG n'aurait aucune action physiologique et comportementale (*Free hormone hypothesis*, Breuner & Orchinick 2002, Breuner & Hahn 2003, Lynn *et al.* 2003, Love *et al.* 2004). Il aurait été intéressant de discerner le rôle de la corticostérone libre sur la décision de non-reproduction, sur les dates de ponte et sur les taux de LH, en prenant en compte les caractéristiques individuelles, telles que l'âge et / ou la condition corporelle.

VI.4.2. Interactions sociales

Les observations comportementales dans ce doctorat se sont limitées à la présence d'un ou des membres du couple, au nid, tous les jours ou tous les deux jours. Un suivi plus régulier, à intervalle de temps plus courts, aurait permis d'intégrer l'expression d'une plus

large gamme de comportements, tel que les parades nuptiales, les nourrissages, les squats de nid par les non-reproducteurs, les accouplements, les constructions de nids et / ou les interactions avec les conspécifiques. Les stimuli sociaux constituent, en effet, une source d'information cruciale (Conradt & Roper 2003; Danchin *et al.* 2004; Dall *et al.* 2005), notamment dans le contexte des cycles annuels (Helm *et al.* 2006). Ces informations peuvent non seulement être transmises activement, par l'intermédiaire de communications visuelles, vocales et / ou olfactives entre les deux membres du couple (Gil & Gahr 2002; Dehley *et al.* 2003; Bonadonna & Nevitt 2004), mais peuvent aussi être obtenues passivement, par l'intermédiaire des conspécifiques (Boulinier & Danchin 1997).

Il a été montré que l'information sociale peut affecter la date et la synchronisation de la reproduction (Darling 1938; Waas *et al.* 2005). Chez les mouettes tridactyles notamment, la densité du voisinage influence la synchronisation des pontes (Coulson & White 1960). Au niveau proximal, chez des espèces coloniales, la synchronisation de la reproduction peut s'expliquer par le niveau de stress du voisinage : la femelle pond lorsque ses voisins sont peu agités, réduisant alors la probabilité de perdre la couvée (Jovani & Grimm 2008). Enfin, les stimuli sociaux, en plus d'induire une ponte précoce synchrone, influencent les taux d'hormones sexuelles (e.g. Setiawan *et al.* 2007). Ce travail de doctorat aurait donc pu être enrichi par la prise en compte des aspects sociaux au sein du couple et entre conspécifiques (en particulier, les interactions avec le voisinage proche), sur la décision de ponte, sur la date de ponte, et sur les ajustements hormonaux. Enfin, les effets de l'âge (jeunes et sénescents) sur la perception et la traduction des stimuli sociaux auraient pu être explorés, en particulier dans le contexte de la dynamique des taux de LH (Article D).

En outre, l'expérience avec le même partenaire est primordiale dans les performances reproductrices (Cézilly & Nager 1996). Chez les pétrels des neiges, les couples se reproduisant avec un nouveau partenaire pondent plus tard que les couples se reproduisant avec le même partenaire (Goutte *et al.* manuscrit en préparation, Fig. 31). La date de la reproduction suite à un changement de partenaire n'est pas significativement différente de celle des individus se reproduisant pour la première fois (Fig. 31). Au niveau hormonal, le fait de changer de partenaire est perçu comme un stress, comme cela a été mesuré via l'élévation des taux de corticostérone chez les mouettes tridactyles pendant la période de reproduction (Angelier *et al.* 2007e).

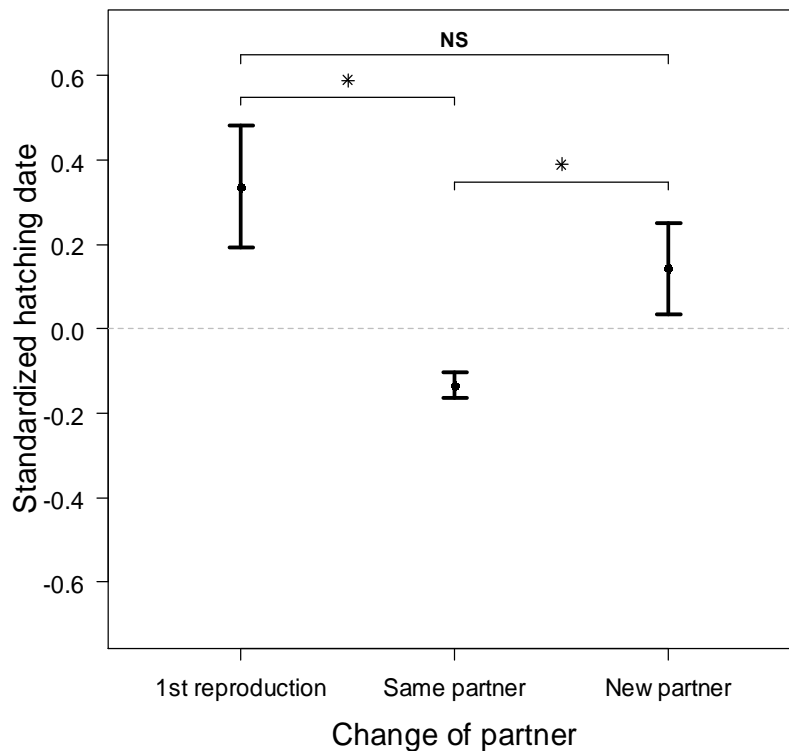


Figure 31 : Date d'éclosion standardisée des couples se reproduisant pour la première fois, s'étant reproduit au moins une fois et sans changement de partenaire par rapport au dernier épisode de reproduction, ou s'étant reproduit au moins une fois et ayant changé de partenaire par rapport au dernier épisode de reproduction.

VI.4.3. Déterminisme génétique

Nous avons implicitement considéré que tous les individus au sein d'une même population avaient la même capacité génétique d'ajustement de leur date de ponte. Or, la date de ponte est un trait héritable (Charmentier *et al.* 2006, 2008 ; Brommer *et al.* 2008). Les individus diffèrent considérablement dans le degré de plasticité phénotypique, en réponse aux variations de l'environnement (e.g. la température, Brommer *et al.* 2008). Par exemple, il a été montré que les reproducteurs généralement tardifs sont moins flexibles et ne sont pas capables de se reproduire tôt lors des années favorables (printemps « chauds »), tandis que les reproducteurs généralement précoces présentent un spectre plus large de modulation de la date de ponte en fonction des conditions environnementales (Brommer *et al.* 2008). Ces différences génétiques engendrent de fortes disparités phénologiques et n'impliquent pas forcément de régulations hormonales sous-jacentes

VI.4.4. Approche multi-stress

L'intégration des conditions environnementales, en particulier pendant la migration et l'hivernage (enregistrés par des GLS), mais aussi de la date d'arrivée sur le site de reproduction et de la date de terminaison de la saison de reproduction permettrait d'avoir une vision plus globale de l'impact du stress environnemental sur la succession des traits d'histoire de vie (e.g. Schroeder *et al.* 2009).

De plus, d'autres facteurs de stress peuvent être pris en compte tels que les taux de polluants. Il a été montré que des taux élevés de contaminants organochlorés sont associés à une ponte tardive chez les goélands marins (*Larus marinus*, Helberg *et al.* 2005; Bustnes *et al.* 2008) et chez les skuas antarctiques (*Catharacta maccormicki*, Bustnes *et al.* 2007). De plus, les taux de polluants sont associés aux taux basaux élevés de corticostérone et à une inhibition de la réponse au stress chez le goéland bourgmestre (*Larus hyperboreus*, Verboven *et al.* 2010). Les contaminants organochlorés perturbent les mécanismes endocriniens (revu dans Dawson 2000; Wingfield & Mukai 2009). En particulier, certains polluants interfèrent avec la sécrétion de LH (Khan & Thomas 2001). Des analyses préliminaires chez les mouettes tridactyles montrent que les non reproducteurs ont des taux plus élevés de c-Nonachlorine en période prénuptiale, comparé aux reproducteurs (Goutte *et al.* manuscrit en préparation).

Bibliographie



*Les citations, c'est de la pensée en conserve:
c'est pas cher, c'est pas toujours très bon, mais tout le monde en mange.*

Anonyme

VIII. Bibliographie

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Annexe



Quand les mouettes ont pied, il est temps de virer

Proverbe Breton

VII. Annexe

ARTICLE G

Long-term Survival effect of Corticosterone Manipulation in Black-Legged Kittiwakes

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Long-term survival effect of corticosterone manipulation in Black-legged kittiwakes

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ABSTRACT

The secretion of corticosterone in response to stress is thought to be an adaptive mechanism, which promotes immediate survival at the expense of current reproduction. However, at the individual level, the hypothesis of a corticosterone-related survival appears to be complex. In this study, we tested this hypothesis by combining for the first time an experimental manipulation of corticosterone levels and capture-mark-recapture (CMR) models. To do so, we increased corticosterone levels of chick-rearing Black-legged kittiwakes (*Rissa tridactyla*) via subcutaneous implants. Then, we monitored the long-term survival of kittiwakes over the 2 consecutive years. Corticosterone-implanted birds showed a significantly lower apparent annual survival than sham-implanted ones (46.9% vs 77.8%). This result is supported by the well-known deleterious effects of elevated corticosterone levels on cognitive and immune functions. Alternately and in the light of recent studies, our experimental manipulation may have down-regulated the endogenous secretion of corticosterone through a prolonged negative feedback. If so, the corticosterone-implanted kittiwakes may have failed to trigger an appropriate stress response during subsequent life-threatening perturbations, hence being unable to adjust their behavior and physiology toward immediate survival. This study highlights the complex long-term consequences of corticosterone manipulation on fitness in free-living vertebrates.

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1. Introduction

Reproduction imposes the challenge to allocate an optimal amount of energy and time to the offspring's requirements without jeopardizing long-term survival (life-history theory, Stearns, 1992). This is especially predicted for long-lived species that should behave as "prudent parents" (Drent and Daan, 1980; Stearns, 1992) because their lifetime reproductive success is primarily a function of adult survival (Williams, 1966). At the proximate level, endocrine mechanisms are at the helm of such life history decisions (Wingfield et al., 1998; Ricklefs and Wikelski, 2002). Specifically, glucocorticoid hormones (GC) are released under life-threatening perturbations, such as food shortage, harsh weather or predation risk, and in turn, trigger the adoption of an emergency stage (reviewed in Landys et al., 2006). The behavior and physiology exhibited during this emergency stage enhance the animals' ability to cope with the stressor (Wingfield and Kitaysky, 2002; Wingfield and Sapolsky, 2003) at the expense of non-vital functions such as

current reproductive effort (Silverin, 1986; Wingfield and Kitaysky, 2002; Love et al., 2004).

The adrenocortical stress response is thus expected to be an adaptive mechanism, which redirects energy allocation towards immediate survival (Wingfield and Sapolsky, 2003). The stress response may have evolved due to its short-term selective advantages (Wingfield and Sapolsky, 2003; Landys et al., 2006), and occurs despite its adverse long-term effects (reviewed in Sapolsky, 2000). Supporting this, a recent comparative study has shown that long-lived bird species with low fecundity have a stronger acute stress response than short-lived, high fecundity ones (Bókony et al., 2009). However, although stress response has been measured in many species (Romero, 2002; Bókony et al., 2009), few studies have tested this assumption at the individual level and the results are rather variable (reviewed in Breuner et al., 2008). For example, a recent study of American redstarts (*Setophaga ruticilla*) showed that high stress-induced GC were linked to a higher return rate, but this was only observable for birds wintering in low quality habitats (Angelier et al., 2009b). However, other studies showed the opposite pattern. Under stressful events, marine iguanas (*Amblyrhynchus cristatus*) with greater stress-induced GC had a lower survival (Romero and Wikelski, 2001). In white stork (*Ciconia*

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ciconia), nestlings with strong GC reactivity showed a lower return rate (Blas et al., 2007). Song sparrow (*Melospiza melodia*) with greater stress responses were less likely to return to breed the following year (MacDougall-Shackleton et al., 2009). In addition, the hypothesis of GC-related survival appears to be complex because elevated baseline GC levels have also been associated with either low (Kitaysky et al., 2007, in press; Romero and Wikelski, 2002; Brown et al., 2005) or high return rate (Cote et al., 2006; Cabezas et al., 2007). Moreover, it is difficult to draw a general picture on the link between GC and fitness (Breuner et al., 2008; Bonier et al., 2009), since the above studies included measurements of stress responses made in developing chicks (Blas et al., 2007), non-breeding (Cabezas et al., 2007; Angelier et al., 2009b) and breeding adults (Romero and Wikelski, 2001; Brown et al., 2005; MacDougall-Shackleton et al., 2009).

Experimental manipulations of GC levels are thus needed and should be one of the best ways to test the effects of GC on survival. However, the link between manipulated GC levels and subsequent survival has been rarely investigated in free-living vertebrates (Kitaysky et al., 2001; Cote et al., 2006). Furthermore, relating GC levels and survival at the individual levels is rarely achieved because of the need to sample marked individuals from long-term capture-mark-recapture (CMR) studies (Brown et al., 2005). The CMR approach explicitly allows taking into account capture probability and temporary absence from the study area, and thus provides unbiased estimators of adult survival (Lebreton et al., 1992).

In 2005, we manipulated adult Black-legged kittiwakes (*Rissa tridactyla*), a long-lived seabird, with corticosterone implants (Angelier et al., 2007a, 2009a). To do so, we increased plasma corticosterone levels during the chick-rearing phase (Angelier et al., 2007a, 2009a), and within the physiological range observed for this species (Kitaysky et al., 1999b, 2001; Chastel et al., 2005; Angelier et al., 2007a,b, 2009a). Following the hormonal manipulation, corticosterone (hereafter Cort) implanted birds invested poorly into current reproduction (Angelier et al., 2007a, 2009a). The apparent survival rates were estimated using CMR models over the following 2 years (2006 and 2007). Therefore, this experimental manipulation offers the possibility to test for the first time with CMR models whether corticosterone administration affect subsequent survival of a long-lived bird.

2. Materials and methods

2.1. Study area and birds

Our study was conducted during the breeding seasons 2005, 2006 and 2007 with a colony of Black-legged kittiwakes at Kongsfjorden, Svalbard (78°54'N, 12°13'E), 7 km east of Ny-Ålesund, Norway. Black-legged kittiwakes are colonial seabirds that breed on cliffs throughout the northern parts of the Pacific and Atlantic, including the Barents Sea region up to the Svalbard Archipelago. Birds were individually marked with metal rings. Their sex was already known by molecular sexing (Weimerskirch et al., 2005) carried out previously at the Centre d'Etudes Biologiques de Chizé (CEBC).

2.2. Manipulating corticosterone levels

We manipulated adult kittiwakes between 23 July and 7 August 2005, during the mid chick-rearing period (chick age 15–20 days). In order to reduce variance related to sex differences, we focused our study on males only. At day 0, 43 chick-rearing males were captured at their nest with a noose at the end of a 5 m fishing rod. Blood samples were collected immediately after capture for baseline corticosterone (Romero and Reed, 2005) from the alar

vein with a 1-ml heparinized syringe and a 25-gauge needle. All birds were then weighed to the nearest 2 g using a Pesola spring balance. Their skull length (head + bill) was measured to the nearest 0.5 mm. Immediately after taking these measurements, each bird was subcutaneously implanted between the shoulders with two 25 mm silastic tubes (internal diameter 1.47 mm, external diameter 1.96 mm, Dow Corning, Michigan) either filled with crystallized corticosterone (C2505, Sigma Chemical Co., St. Louis, MO, $n = 22$ Cort-implanted birds) or empty ($n = 21$ sham-implanted birds). Knowing that corticosterone cannot diffuse through silastic, we cut both ends of the silastic tube to allow rapid release of corticosterone over a limited time period. Our aim was to increase plasma corticosterone levels (2 days, see Angelier et al., 2007a, 2009a) within the physiological range (stress-induced levels in male kittiwakes, Kitaysky et al., 1999a; Chastel et al., 2005). However, we did not remove the implants after this experimental increase, and thus, we were not able to monitor the diffusion rate of crystallized corticosterone (see Kitaysky et al., 2003). At day 0, both groups (Cort-implanted birds and sham-implanted ones) did not differ significantly with respect to body condition, baseline corticosterone levels, date of treatment, and brood size (see Angelier et al., 2007a, 2009a for details).

2.3. Subsequent blood samples

Following implantation, kittiwakes were recaptured opportunistically when they were present at the colony (Angelier et al., 2007a, 2009a). Specifically, birds were recaptured 1, 2, 3 and 8 days after the implantation and were sampled for blood as described above. One part of the birds was sampled at day 1 (Cort-implanted birds, $N = 9$, sham-implanted birds, $N = 4$), another part at day 2 (Cort-implanted birds, $N = 5$, sham-implanted birds, $N = 3$) to confirm that our corticosterone manipulation was effective. In addition, 14, then 10 Cort-implanted birds and 18, then 9 sham-implanted birds were sampled at days 3 and 8, respectively. Moreover, baseline corticosterone levels the day after Cort-implantation were compared to average stress-induced corticosterone levels of chick-rearing males sampled in 2000 and 2004 ($N = 75$, see Chastel et al. (2005) and Angelier et al. (2007b) for detailed methodology). This was done to ensure that the experimental increase of corticosterone was within the physiological range observed for Black-legged kittiwakes. In the sham-implanted birds, the corticosterone level was not significantly changed in the days following implantation, whereas in the Cort-implanted birds, the corticosterone value was significantly increased the first and second days after implantation. From days 3 to 8 the corticosterone values were not different from the baseline level in both groups of birds (see Angelier et al. (2009a) for detailed statistical analysis, Fig. 1). The corticosterone level of Cort-implanted birds one day after implantation was not different from the acute stress-induced corticosterone level measured in kittiwakes from the same study site after an acute stress protocol (Chastel et al., 2005; Angelier et al., 2007b; $df = 21$, $t = -1.72$, $p = 0.099$, Fig. 1).

2.4. Hormone assays

Within 6 h from sampling, blood samples were centrifuged and the plasma was stored at -20°C for later analysis. All laboratory analyzes were performed at the Centre d'Etudes Biologiques de Chizé (CEBC). Plasma concentrations of corticosterone were determined by radioimmuno-assay as detailed in Lormée et al. (2003). The intra-assay variations was 7.7% and the minimal detectable corticosterone levels was 0.5 ng. No samples fell below this limit.

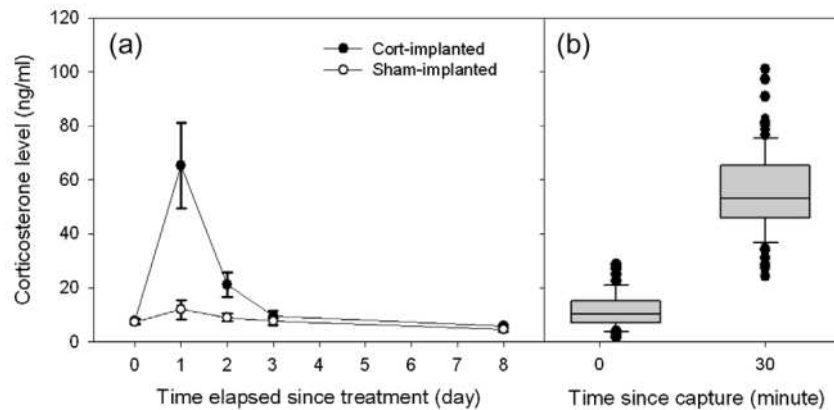


Fig. 1. (a) Effect of corticosterone and empty implants (sham) on plasma levels of corticosterone 1–8 days after implantation in male Black-legged kittiwakes during the chick-rearing period 2005 (mean \pm SE). (b) Baseline and stress-induced corticosterone levels reached after an acute stress protocol in Black-legged kittiwakes in the same site (Chastel et al., 2005; Angelier et al., 2007b). Plasma corticosterone measured one day after corticosterone administration (Fig. 1a) where similar to those measured after 30 min of handling protocol (Fig. 1b).

2.5. Survival monitoring

All birds were marked with a plastic ring with a code of three letters allowing identification from a distance without perturbation. Information on survival and local dispersal were gathered during the 2 following years (2006 and 2007), through intensive and extensive field surveys of ringed birds. Field surveys were conducted daily between 26th of June and 9th of August in 2006 and from 26th of April to 4th of August in 2007. Local breeding dispersal was monitored by extending re-sighting effort to four other colonies of kittiwakes (from 120 to 200 pairs), located in the immediate vicinity of the study plot and up to 10 km apart. However, long-scale breeding dispersal was not estimated because other colonies in the region were inaccessible and hence not surveyed. Annual survival rate estimated for non-manipulated males from the same colonies between 2001 and 2007 was 75.4% (range 65.4–83.2%), while mean annual recapture rate was 91.4% (range 76.9–97.2%, unpublished data).

2.6. Data analysis

The re-sighting at time $t + 1$ of a ringed individual manipulated at t depends on three events and their associated probabilities: the probability of surviving from t to $t + 1$, called the survival rate and written Φ ; the probability, if alive, of being present in the studied colonies at t , called the presence probability; and a probability, if alive and present, of being resighted: the recapture probability (p). We therefore estimated annual apparent survival and recapture probabilities using the general methods of Lebreton et al. (1992) and Burnham and Anderson (2002). Four models were thus built:

- (1) Φ (CORT); p (CORT) considers an effect of corticosterone treatment (CORT) on survival rates from 2005 to 2006 (Φ) and on recapture rates in 2006 (p).
- (2) Φ (CORT) considers an effect of CORT on Φ but no effect on p .
- (3) p (CORT) considers an effect of CORT on p but no effect on Φ .
- (4) Null model considers no effect of CORT on Φ and p .

Program MARK (White and Burnham, 1999) was used to assess the fit of these candidates' models to the data set, using the Akaike Information Criterion, corrected for small sample size (AICc). The model with the lowest AICc is considered as the best one (Burnham and Anderson, 2002). If Δ AICc (difference between the AICc value

of one model relative to the AICc value of the best model) is lower than 2.0, the two models are indistinguishable. AICc weight measures a model's relative probability of being the best model for the data among the set of candidate models.

Before comparing the fit of the candidate models, program RELEASE (Burnham et al., 1987) was used to perform a goodness of fit test for the data set (test 1, between groups test: $\chi^2 = 5.529$, $p = 0.063$). Because of only three capture events, tests 2 and 3 of the GOF could not have been performed.

3. Results

The best-fitting model is the second one (called " Φ (CORT)", Table 1). This model postulates that the survival rate of Cort-implanted birds differs significantly from the survival rate of sham-implanted ones, whereas recapture rates in 2006 are similar for the both groups. An effect of corticosterone implantation in 2005 on the apparent survival rate from 2005 to 2006 is highly supported (91.38% of the sum of AICc weights, Table 1) by model selection. Models including effects of the treatment on recapture rate in 2006 were not well supported (25% of the sum of AICc weights, Table 1).

The estimated survival rate from 2005 to 2006 derived from the best-fitting model, i.e. Φ (CORT) was 39.7% lower for Cort-implanted birds than for sham-implanted ones (Fig. 2). The recapture rate of Cort and sham-implanted kittiwakes in 2006 was estimated to 100% by MARK software.

4. Discussion

4.1. Apparent survival, dispersal and skipped breeding

In the present study, we estimated apparent survival rate. In mark-recapture studies, the presence probability is included in

Table 1

Model selection to test the influence of hormonal manipulation (CORT) in 2005 on survival rates (Φ) and recapture rates (p) in 2006. The best model was selected by using the Δ AICc and AICc weights.

#	Model	AICc	Δ AICc	AICc weights (%)	No. parameters
(2)	Φ (CORT)	88.750	0.000	69.07	3
(1)	Φ (CORT); p (CORT)	91.010	2.260	22.31	4
(4)	Null model	93.631	4.881	6.02	2
(3)	p (CORT)	95.307	6.557	2.60	3

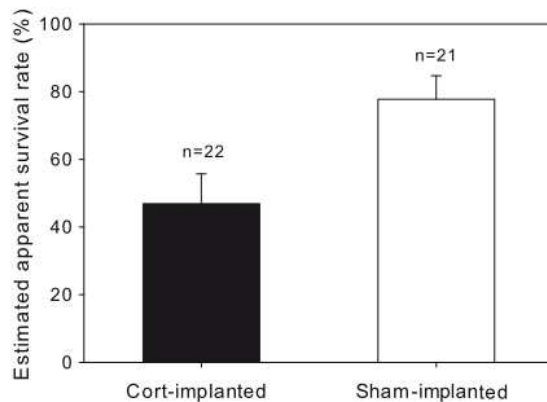


Fig. 2. Effects of the hormonal manipulation in 2005 on estimated apparent survival rate between 2005 and 2006 in males Black-legged kittiwakes (means \pm SE). Means and standard errors of survival rates were estimated using MARK software, and are based on the best-fitting model, i.e. model (2) Φ (CORT) (see text for details).

survival rate or in recapture probability, if absence is permanent or temporary, respectively (Lebreton et al., 1992; Kendall and Nichols, 1995). The observed negative effect of corticosterone administration on apparent survival could then be attributed to mortality or to absence. The Cort-implanted kittiwakes had a lower reproductive success in 2005 (Angelier et al., 2009a), which is well-known to reduce breeding propensity and to enhance breeding dispersal the next year in kittiwakes (Cam et al., 1998; Naves et al., 2006; Boulinier et al., 2008). Furthermore, corticosterone is also known to trigger irruptive migration and dispersal (Astheimer et al., 1992; Belthoff and Dufty, 1998; Meylan et al., 2002). One may suggest that the lower apparent survival of Cort-implanted kittiwakes may be the result of multiple skipped breeding events or permanent breeding dispersal. Concerning skipped reproductions, Cort-implanted birds observed in 2006 were all seen incubating or caring for chicks. This suggests that the breeding probability of resighted kittiwakes was not affected by our treatment. Regarding dispersal movement, we only recorded two small scale movements between study plots, involving one Cort-implanted bird and one sham-implanted bird. However, we cannot exclude the possibility that some Cort-implanted birds established permanently in other colonies outside the study area. The apparent low survival rate (47%) of Cort-implanted kittiwakes may therefore be a result of mortality and possible large-scale breeding dispersal.

4.2. Possible effects of experimentally increased corticosterone levels on survival

Our experimental setup resulted in a significant reduction of the apparent annual survival in kittiwakes (46.9% vs 77.8%). It is important to notice that we did not observe any mortality of Cort-implanted birds over a 10-day period (Angelier et al., 2009a). In a similar study, Cort- and sham-implanted kittiwakes showed the same survival during a 3-week period after the implantation (Kitaysky et al., 2001). Hence, mortality would have occurred during the post-breeding period, long after the implantation.

In our study, corticosterone implants were not removed and the crystallized corticosterone could not be metabolized in few days. A similar study conducted in the chicks of the same species (Kitaysky et al., 2003) pointed out that the implants were not totally depleted 4 weeks after the treatment and still contained approximately 2/3 of the initial amount of crystallized corticosterone.

Although plasma corticosterone levels only increased over a 2-day period (Angelier et al., 2007a, 2009a), the remaining exogenous corticosterone could have diffused over a long-term period. In that context, our corticosterone manipulation could rather mimic GCs effects within a chronic framework (Breuner et al., 2008). Chronically elevated corticosterone levels are known to compromise cognitive and learning abilities (McEwen and Sapolsky, 1995; Wingfield et al., 1998; McEwen, 1999 but see Pravosudov, 2003), immune functions and recovery capacities (Dhabhar and McEwen, 1997, 1999; McEwen and Sapolsky, 1995; Saino et al., 2003; Berger et al., 2005), and also provoke the waste of muscle tissue (reviewed in Sapolsky, 2000). Ultimately, long-term exposure to high corticosterone levels had detrimental effects on survival in long-lived vertebrates (Romero and Wikelski, 2001; Brown et al., 2005; Kitaysky et al., 2007, in press). If our corticosterone manipulation had consisted in the release of exogenous corticosterone over a long-term period, this would have caused the deleterious effects described above, ultimately reducing survival. The reason why baseline corticosterone levels decreased the 3th and 8th days after the treatment could result from a negative down-regulation of endogenous corticosterone release (see below), or a high clearance rate of exogenous corticosterone from the blood system. Moreover, we only measured total corticosterone levels, i.e. free and bound hormone levels. The binding protein Corticosteroid Binding Globulin (CBG) binds corticosterone with high affinity in circulation (e.g. Breuner and Orchinik, 2001; Shultz and Kitaysky, 2008). Although the primary role of CBG is under debate, the Free Hormone Hypothesis suggests that unbound, or free, concentration of hormone in the plasma is biologically active. It is conceivable that free corticosterone levels may have been higher in Cort-implanted kittiwakes compared to the control ones, while total corticosterone levels were similar between groups.

Other endocrine processes may also be implied in the low apparent survival of the Cort-implanted birds. Administration of exogenous GC is known to inactivate the HPA axis in birds (Vandenborne et al., 2005). Specifically a negative feedback down-regulates corticotropin-releasing factor (CRF) gene expression in the hypothalamus (Kretz et al., 1999; Ma et al., 2001; Feldman and Weidenfeld, 2002). This may reduce CRF receptors production in the pituitary (Pozzoli et al., 1996; Aguilera et al., 2001) resulting in an inhibition of the adrenocorticotropin ACTH secretion (Dallman et al., 1987). Recent lab and field studies in birds highlighted a down-regulation of the HPA system following corticosterone administration, through dermal application (Busch et al., 2008), pellets (Müller et al., 2009) or silastic implants (Romero et al., 2005). Thus, GC administration results in a reduction of the magnitude of the adrenocortical response (Romero et al., 2005; Busch et al., 2008; Müller et al., 2009). This effect was relatively long-lasting since for example, acute stress response was absent 8 days after corticosterone administration in kestrel (*Falco tinnunculus*) nestlings (Müller et al., 2009). ACTH challenge confirmed that the sensitivity of the HPA axis was down-regulated at the hypothalamo-pituitary level or higher (Busch et al., 2008). In the present study, exogenous corticosterone may have down-regulated endogenous corticosterone secretion so that HPA axis was deactivated or impaired. Thus, life-threatening perturbations (e.g. poor food condition, predation or inclement weather) might not have induced acute endogenous corticosterone releases in Cort-implanted kittiwakes. This inability to adopt an emergency coping response could have resulted in a high mortality (Wingfield et al., 1998; Wingfield and Kitaysky, 2002; Angelier et al., 2009b). Because Cort-implanted birds were not seen dead over a 10-day period (Angelier et al., 2009a), mortality of kittiwakes might have occurred during fall or winter following Cort-implantation. This would suggest that our Cort-treatment resulted in a longer down-regulation of endogenous stress response (more than 1 week

after implantation). Although this glucocorticoid negative feedback is still poorly studied in free-living individuals (Busch et al., 2008), it has been shown to last less than 20 days by using corticosterone pellets (Müller et al., 2009). The possible effects of GC administration on fitness have thus to be better understood (Breuner et al., 2008).

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Résumé

La phénologie de la reproduction a des conséquences majeures sur la valeur sélective, et témoigne de la flexibilité des individus à répondre aux conditions environnementales, en fonction de leur état physiologique. Les mécanismes proximaux sous-jacents restent cependant très peu connus. Ce doctorat vise à explorer les processus hormonaux impliqués dans l'ajustement de la date de ponte chez deux oiseaux marins polaires, la mouette tridactyle et le pétrel des neiges. Dans un premier temps, une contrainte nutritionnelle en période pré-nuptiale nous a permis d'associer une année tardive à une élévation des taux de corticostérone (hormone de stress) et à une diminution des taux de LH (hormone lutéinisante, déclencheur principal de la reproduction). Par ailleurs, un retard expérimental de la reproduction provoque une sensibilité accrue au stress, un déclin de la motivation parentale et un échec reproducteur. Grâce à des approches descriptives et expérimentales, nous avons ensuite pu identifier le rôle de la corticostérone sur l'ajustement individuel des dates de ponte et sur l'abstention de la reproduction, via l'inhibition de la sécrétion de LH. Ce mode d'action fonctionnelle est toutefois à nuancer entre mâles et femelles et entre espèces étudiées. Enfin, sachant que les très jeunes et les très vieux oiseaux pondent tard dans la saison, voire s'abstiennent de se reproduire, nous avons décrit les effets de l'âge (7-45 ans) et de la sénescence sur la dynamique de la corticostérone et de la LH. À la lumière de ces résultats, nous discutons de la possible orchestration hormonale de la phénologie, au carrefour entre le stress environnemental et l'état individuel, dans le cadre théorique des stratégies évolutives.

Mots clés : phénologie, date de ponte, hormone, corticostérone, réponse au stress, hormone lutéinisante, test de réponse à la GnRH, âge, succès reproducteur, manipulation expérimentale, oiseaux marins polaires

Abstract

Breeding at the right time is a key-component of fitness, and requires flexible responses to environmental conditions and physiological state. However, the proximate mechanisms underlying this pattern remain poorly understood. This doctoral research aims to explore the hormonal mechanisms that mediate the onset of egg-laying in two polar seabirds, the black-legged kittiwake and the snow petrel. First of all, a food-related stress during the pre-laying period allowed us to associate a late-breeding year with high levels of corticosterone (stress hormone) and with low levels of LH (luteinizing hormone that triggers reproduction). Moreover, an experimentally delayed breeding promoted an accentuated stress response, a decline of parental effort, and a high reproductive failure. Descriptive and experimental approaches supported the role of corticosterone in the individual adjustment of egg-laying dates and in the non-breeding decision, through the inhibition of LH release. However, this functional action of corticosterone differed between sex and between species. At last, we investigated the effects of age (7-45 years old) and senescence on the dynamics of corticosterone and LH, since very young and very old birds did not breed or bred late in the season. We discussed the possible hormonal orchestration of reproductive phenology, as an interaction between environmental stress and individual state, in the theoretical framework of evolutionary strategies.

Key words: phenology, egg-laying date, corticosterone, stress response, luteinizing hormone, GnRH challenge, age, reproductive success, experimental manipulation, polar seabirds