Exxon Valdez Oil Spill
Restoration Project Annual Report

Effects of Food Stress on Survival and Reproductive Performance of Seabirds

Restoration Project 00479
Annual Report

This annual report has been prepared for peer review as part of the *Exxon Valdez* Oil spill Trustee Council restoration program for the purpose of assessing project progress. Peer review comments have not been addressed in this annual report.

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Study History: Food stress can be quantified by measuring base levels of stress hormones such as corticosterone in the blood of birds, or the rise in blood levels of corticosterone in response to a standardized stressor: capture, handling and restraint. We applied these techniques to seabirds breeding in Lower Cook Inlet and also used captive birds for controlled experiments. This study uses a unique opportunity for a concurrent field and captive study of the behavioral and physiological consequences of stress in birds. Moreover, in coordination with restoration projects 00163 and 00338 it links stress physiology to changes in foraging conditions and demographics, which provides the basis for management of seabird populations in the areas affected by the *Exxon Valdez* oil spill, and it will have broader applications for seabird monitoring programs.

Abstract: In previous studies funded by the *Exxon Valdez* Oil Spill Trustee Council, we found that parent kittiwakes and their dependent chicks respond to food shortages by increasing circulating levels of corticosterone. To examine the behavioral significance of corticosterone release, we experimentally increased levels of circulating corticosterone in parents and chicks up to the levels observed during food shortages. We found that corticosterone-implanted chicks begged more frequently than sham-implanted controls. Corticosterone-implanted chicks in broods of two begged more frequently than singletons. Parent kittiwakes then responded to the increase in corticosterone levels in their chicks by increasing chick feeding rates. However, feeding rates were not different among corticosterone-implanted chicks in broods of two and singletons. We also found that corticosterone-implanted parents spent more time away from the nest – perhaps foraging, and less time brooding/guarding chicks than sham-implanted controls. Untreated mates of the corticosterone-implanted bird did not compensate for the change in their partner's behavior, consequently chicks were left unattended about 20% of the time compared to 1% at the control nests. However, corticosterone-implanted parents did not decrease their chick feeding rates and suffered an increased post-breeding mortality. Our findings suggest two functional implications of the increased corticosterone secretion during food shortages in kittiwakes: (1) it facilitates begging in chicks; and (2) it affects time allocated by parents to guarding young at the nest. Thus, release of corticosterone might provide a mechanistic link between physiological condition and behavioral interactions among adults and their young.

In a previous study, we suggested that the physiological condition of Black-legged kittiwake chicks could be assessed reliably by measuring circulating levels of corticosterone. However, that conclusion was based on sampling food-restricted chicks only once at the end of 4-week dietary treatment. The temporal dynamics of corticosterone release in response to prolonged food shortages were not known for seabird chicks, and a possibility of detecting increased secretion of corticosterone in kittiwake chicks *during* food shortages was not previously examined. In coordination with NPMR project (University of Alaska Fairbanks) we examined temporal dynamics of corticosterone release in Red-legged kittiwake chicks exposed to prolonged restriction in energy content and/or nutritional quality (low vs. high fat content) of their food. We found that food-restricted chicks had lower body mass, chronically (during 2-3 weeks depending on severity of food restriction) elevated baseline and higher acute stress-induced levels of corticosterone compared to chicks fed *ad libitum*. Low fat content of food further exacerbated these effects. An increase in baseline levels of corticosterone was observed shortly after energy requirements of food-restricted chicks exceeded their daily energy intake. Our findings justify that the physiological condition of kittiwake chicks can be assessed reliably by measuring circulating levels of corticosterone during food shortages. Our findings also suggest that the release of corticosterone in response to food shortage is not confined to Black-legged kittiwake chicks.

Finally, in preparation for the final report, we have reviewed available literature and data collected during EVOS funded studies on endocrine responses of birds to unpredictable environmental events, such as food shortages and inclement weather. In addition to seasonal changes in physiology and behavior that occur in predictable annual cycles, there are facultative responses to unpredictable events. These rapid behavioral and physiological changes have been termed the "emergency" life history stage (ELHS) and serve to enhance life-time fitness. Glucocorticoids (e.g. corticosterone) interacting with other hormones initiate and orchestrate the ELHS within minutes to hours. Components of the ELHS include: redirection of behavior from a normal life history stage to increase foraging, irruptive-type migration, elevated gluconeogenesis and recovery once the perturbation passes. These physiological and behavioral changes allow an individual to avoid the deleterious effects of stress that may result from chronically elevated levels of circulating corticosterone over days and weeks. In other words, acute rises in glucocorticoids following perturbation of the environment may actually allow animals to avoid chronic stress and serve primarily as anti-stress hormones. However, if food shortages persist over long periods (weeks), chronic elevation of corticosterone results in long-lasting deleterious effects on fitness of individuals.

Keywords: Corticosterone, ecology, food-stress, reproduction, survival, seabirds

Project Data: (will be addressed in final report of 2002)

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The annual report consists of two published papers (attached), three papers accepted for publication upon satisfactory revision (attached) and also results and preliminary conclusions to date as organized in various manuscripts.

Published papers:

1) Kitaysky A.S., J.C. Wingfield and J.F. Piatt. In press. Corticosterone facilitates begging and affects resource allocation in the Black-legged Kittiwake. Behavioral Ecology.

   We found that release of corticosterone in hungry chicks facilitates begging and allows them to restore depleted energy reserves by increasing parental food provisioning. Parents with experimentally elevated levels of corticosterone spent more time away from the nest – perhaps foraging, and less time brooding/guarding chicks than controls. At the same time, experimental parents fed their chicks at similar rates as controls. All experimental and control birds were observed at the colony during four weeks following the experiment. However, about 80% of corticosterone treated individuals failed to return to the nesting colony in the following reproductive season. **Conclusion: During food shortages, hungry kittiwake chicks increase begging. In turn, stressed parent kittiwakes continue to provide food at chick demands thereby decreasing the probability of their post-breeding survival and increasing the likelihood of chick predation.**

2) Kitaysky, A.S., G.L. Hunt, E.N. Flint, M.A. Rubega, and M.B. Decker. 2000. Resource allocation in breeding seabirds: responses to fluctuations in their food supply. Marine Ecology Progress Series, 206: 283-296.

   In this study we have examined how breeding success and resource allocation of surface-foraging kittiwakes and pursuit-diving murres varied with fluctuations in their food supply. While this paper was about studies conducted at the Pribilof Islands, it was written by Kitaysky while supported by the EVOSTC (as noted in acknowledgements). More importantly, the results offer direct comparisons with Cook Inlet studies and will ultimately enhance our interpretation of these data. We found that fluctuations in food supply affect resource allocation in seabirds. However, a decrease in food abundance is likely to cause an increase in energy expenditures of parent kittiwakes, whereas growth rates in their chicks are less affected. For murres, food shortages are likely to cause a decrease in growth of the chicks, but not an increase in energy expenditures of the parents. **Conclusion: behavioral and physiological responses of seabirds to food shortages depend on species-specific strategies and result in either decreased reproductive performance (such as chick growth and development) or in a decreased post-breeding survival of parents.**

Papers conditionally accepted for publication:

1) Kitaysky, A.S., E.V. Kitaiskaia, J.C. Wingfield, and J.F. Piatt. Dietary restriction causes chronic elevation of corticosterone and enhances stress-response in Red-legged Kittiwake chicks. Journal of Comparative Physiology B.

   This study was done in coordination with NPMR project (University of Alaska Fairbanks) and at no additional cost to the EVOSTC. We examined temporal dynamics of corticosterone release in Red-legged kittiwake chicks exposed to prolonged restriction in energy content and/or nutritional quality (low vs. high fat content) of their food. We found that food-restricted chicks had lower body mass, chronically (during 2-3 weeks depending on treatment) elevated baseline and higher acute stress-induced levels of corticosterone compared to chicks fed ad *libitum*. Low fat content of food further exacerbated these effects. An increase in baseline levels of corticosterone was observed shortly after energy requirements of food-restricted chicks exceeded their daily energy intake. **Conclusion: Our findings fully justified that the physiological condition of kittiwake chicks can be assessed reliably by measuring circulating levels of corticosterone. Our findings also suggest that the release of corticosterone in response to food shortage is not confined to Black-legged kittiwake chicks.**

2) Wingfield, J.C. and A.S. Kitaysky. Endocrine response to unpredictable environmental events: stress or anti-stress hormones? American Zoologist.

   A review article on endocrine responses of birds to environmental perturbations such as inclement weather and food shortages. This review contains some original, unpublished data on murres in Cook Inlet. **Conclusion: Corticosterone secretions in response to environmental perturbations not only trigger physiological and behavioral responses but also allow flexibility so that response is integrated in relation to time of year as well as individual differences owing to body condition and life-history stage.**

3) Pravosudov, V.V., Kitaysky A.S., Wingfield, J.C. and Clayton, N.S. Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees. Journal of General and Comparative Endocrinology.

   The understanding of bird physiological responses to food shortages outside of the reproductive season is critical for interpretation of results obtained in Cook Inlet. Indeed, we requested funds from the EVOSTC to study captive seabirds outside the breeding season, but this work was not funded. Therefore, we cooperated with scientists at UC Davis (at no additional costs to EVOS) on studying the adrenocortical response of photorefractory birds to low and unpredictable food supply. In this captive experimental study we examined adrenocortical responses of adult non-breeding birds to long-term deterioration in foraging condition. We found that food-restricted birds had significantly higher baseline levels of corticosterone compared to those maintained on ad libitum food. **Conclusion: Release of corticosterone in response to long-term food shortages is not confined to adult seabirds and release of corticosterone is a general mechanism maintaining homeostastis in birds during and outside of the reproductive season.**

The following manuscript is submitted and in review:

1. Pravosudov, V.V., Kitaysky, A.S., Wingfield, J.C., and Clayton, N.S. Effects of photoperiod on adrenocortical response of adult resident birds. In review, Journal of General Comparative Endocrinology.

   In a previous study of Black-legged kittiwakes we found that baseline levels of corticosterone vary significantly with changing foraging conditions and reproductive stage. To examine a possibility of the direct influence of photoperiod on adrenocortical response of birds, we examined baseline and maximal stress-induced levels of corticosterone in captive mountain chickadees (in cooperation with scientists at U.C. Davis and at no additional costs to EVOS). We found that baseline and acute stress-induced levels of corticosterone were not significantly different between photo-refractory and photo-stimulated birds fed ad libitum. **Conclusion: our results suggest that seasonal changes of adrenocortical activity might have an ecological basis (e.g. changes in food availability) rather than are associated with development/regression of the reproductive system in birds.**

Manuscripts in preparation are:

1. Kitaysky, A.S., Piatt, J.F. and J.C. Wingfield. Functional significance of seasonal elevation of circulating levels of corticosterone in breeding Common Murres. In preparation, Functional Ecology.

   The results of this study were similar to the study of Black-legged Kittiwakes described above, and also resulted from pilot work. Baseline and acute-stress induced levels of corticosterone may be useful for monitoring foraging conditions in breeding Common Murres, but more study was needed to confirm these results and to measure annual variability. In addition, Common Murres responded to an experimental increase of corticosteroid levels by fledging their chicks at younger age compared to controls. We had planned to submit this paper (per last year's annual report), but delayed submission because a longer time series was needed to justify the claim that the seasonal dynamics of foraging conditions, rather than the regression of reproductive system of common murres toward the end of reproductive season, is a major factor causing the observed increases in corticosterone secretion. **Conclusion: Baseline and acute-stress induced levels of corticosterone are useful parameters for monitoring foraging conditions in breeding Common Murres. Increases in corticosterone secretion cause a**

decrease in reproductive performances of murres, but might facilitate their survival and a probability of successful reproduction in the future.

2. Kitayksy et al. Endocrine correlates of dietary restrictions in Tufted Puffin chicks. In preparation, Physiological and Biochemical Zoology.

   This study was done in conjunction with Romano et al.'s experimental study of chick growth under differing dietary regimes. We examined plasma levels of corticosterone in captive Tufted puffin chicks exposed to variable rates of daily energy intake. Baseline levels of corticosterone were not significantly different among the treatments. These results are not consistent with the results obtained for Black-legged Kittiwake chicks (see above) exposed to similar dietary restrictions. From other studies, we know that puffin chicks can respond to food deprivation by decreasing their metabolic rates. **Conclusion: The stress-response may be lacking or greatly diminished in puffin chicks, which are adapted to highly variable rates of provisioning. The difference in endocrine responses to food stress between puffins and kittiwakes may be phylogenetically determined and/or reflect a physiological adaptation of young puffins to intermittent type of food provisioning.** We also delayed a submission of this manuscript due to a necessity of publishing the results of the most recent experiment on development of the adrenocortical response in tufted puffin chicks in relation to dietary treatment as a satellite paper. We are working on a submission of both papers for back-to-back publication.

3. Kitaysky et al. Adrenocortical stress-response of Common murre chicks in relation to dietary restrictions.

   In this manuscript we present the results of captive study on adrenocortical stress response of common murre chicks. The results obtained for the Tufted Puffin and kittiwake chicks (above) suggest that physiological responses of seabird chicks to food shortages might be determined phylogenetically or is related to parental food provisioning strategy. Common murres are closely related to puffins, but parents provision their chicks with food in a continuous manner. We exposed murre chicks to the standardized feeding protocol (same as used in our captive studies black- and red-legged kittiwakes and tufted puffins) and assessed temporal changes in baseline and acute stress-induced levels of corticosterone. We found that adrenocortical responses of food-restricted common murre chicks are similar to those observed in kittiwakes. Specifically, food-stressed common murre chicks had significantly elevated baseline and acute stress-induced levels of corticosterone compared to ad libitum fed controls. **Conclusion: the adrenocortical stress-response of seabird chicks is likely to be related to parental food-provisioning strategy. Nutritional status of common murre chicks can be reliably determined by measuring circulating levels of corticosterone. Our finding also suggests that the release of corticosterone in**

response to food shortage is not confined to Black-legged and Red-legged kittiwake chicks.

Other manuscripts in preparation are:

Kitaysky A.S., Kitaiskaia E.V., Piatt, J.F., Wingfield J.C. Effects of early nutritional stress on cognitive abilities of Black-legged kittiwakes.

This manuscript was indicated as "in preparation" in last year's annual report. We delayed preparation of this paper because reviewers raised a concern that the impaired cognition of artificially stressed kittiwake chicks may just be a pharmacological effect of the treatment with exogenous corticosterone. We tested for this with a captive study of the effects of nutritional stress (where only food restriction treatment was applied to experimental chicks) on cognition of Red-legged kittiwakes, and have now demonstrated a direct relationship between chronic elevation of corticosterone during development and a decreased learning in kittiwakes. The captive study of Red-legged kittiwake chicks was done in cooperation with NPMR project and at no additional cost to EVOS. Both studies are in preparation for back-to-back publication.

Kitaysky A.S., Piatt, J.F., Wingfield J.C. Seasonal dynamics of corticosterone and LH in breeding Common Murres in relation to fluctuations in food supply.

Completion and submission of this paper is also postponed, because a successful publication of this paper is contingent on an acceptance of manuscript #1 ("in preparation" above).

Corticosterone facilitates begging and affects resource allocation in the Black-legged kittiwake

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Abstract

Parent Black-legged kittiwakes (*Rissa tridactyla*) and their dependent chicks respond to food shortages by increasing circulating levels of corticosterone. To examine the behavioral significance of corticosterone release, we experimentally increased levels of circulating corticosterone in parents and chicks up to the levels observed during food shortages. We found that corticosterone-implanted chicks begged more frequently than sham-implanted controls. Corticosterone-implanted chicks in broods of two begged more frequently than singletons. Parent kittiwakes then responded to the increase in corticosterone levels in their chicks by increasing chick feeding rates. However, feeding rates were not different among corticosterone-implanted chicks in broods of two and singletons. We also found that corticosterone-implanted parents spent more time away from the nest -- perhaps foraging, and less time brooding/guarding chicks than sham-implanted controls. Untreated mates of the corticosterone-implanted bird did not compensate for the change in their partner's behavior, consequently chicks were left unattended about 20% of the time compared to 1% at the control nests. However, corticosterone-implanted parents did not decrease their chick feeding rates. Our findings suggest two functional implications of the increased corticosterone secretion during food shortages in the Black-legged kittiwake: (1) it facilitates begging in chicks; and (2) it affects time allocated by parents to guarding young at the nest. Thus, release of corticosterone might provide a mechanistic link between physiological condition and behavioral interactions among adults and their young.

Key words: begging, corticosterone, food stress, parent-offspring conflict, seabirds

Introduction

Nest-dependent chicks communicate their needs by begging and parents use this information to adjust their investment in food provisioning. Parent-offspring conflict theory predicts that chicks should be selected to solicit a greater investment from their parents than the parents have been selected to provide (Trivers, 1974). Alternatively, signal selection theories suggest that begging reliably conveys chick nutritional needs and parents respond correspondingly (Godfray, 1991, 1995a,b; Zahavi, 1987). Chick begging intensity increases with food deprivation (e.g., Cotton et al., 1996; Iacovides and Evans, 1998; Kilner, 1995; Stamps, 1993) and, at least in some species, parents provision more in response to elevated begging (Henderson, 1975; Price and Ydenberg, 1995; Leonard and Horn, 1996). Although the relationship between hunger levels and chick begging seems well established, the causal mechanism(s) regulating parent-offspring feeding interactions during food shortages remains to be shown. Finding a mechanistic link between hunger and changes in behavior is essential to a realistic assessment of the theoretical models and, eventually, for a better understanding of the evolution of chick begging behavior and parental provisioning strategies.

When food resources are limiting, long-lived parent birds are expected to allocate available resources to body maintenance rather than to reproduction (Cody, 1966). Evidence is accumulating that a decision of parents on the allocation of available resources might be based on their physiological condition (Chaurand and Weimerskirch, 1994; Ricklefs and Schew, 1994; Weimerskirch et al., 1994). Long-lived birds can accumulate fat as energy reserves for self-maintenance during reproduction (Drent and Daan, 1980). As fat reserves are depleted, parents should rely more on amino acid metabolism (mostly from muscle protein; Cherel, Robin and Le Maho, 1988), which is stimulated by secretion of corticosterone, a steroid hormone released by the adrenal glands in response to stress (Veiga, Roselino and Migliorini, 1978).

In adults, increased plasma levels of corticosterone facilitate foraging behavior, trigger irruptive migration, and mobilize stored energy resources to fuel increased locomotory activities (Astheimer et al., 1992; Bray 1993; Wingfield et al., 1997). These behavioral responses can improve adult survival during food shortages (Astheimer et al., 1992). Increased secretion of corticosterone might also change the allocation of available resources between body maintenance and reproductive processes to facilitate the survival of affected individuals (Silverin, 1986; Wingfield and Silverin, 1986; Wingfield et al., 1997; Wingfield et al., 1998).

In contrast to adult birds, nest-bound chicks are limited in their behavioral responses to food-related stress. A hungry chick can compete with nest-mates and increase its share of parental resources at the expense of current siblings. A hungry chick can also intensify its begging for food from a parent, which would respond by feeding a chick more frequently. Therefore, chick behavioral strategies during food shortages may reflect both the selective pressure of competition between siblings and chick nutritional requirements (but see Cotton et al., 1996). Experimental studies have shown that in nest-bound chicks, food shortages are associated with depleted fat reserves and an increase in circulating levels of corticosterone (Kitaysky et al., 1999a; Nunez-de la Mora et al., 1996), though little is known about behavioral responses of chicks to increased corticosterone.

In this study we examine the behavioral responses of Black-legged kittiwake (*Rissa* *tridactyla*) to increased corticosterone. Black-legged kittiwakes are colonial cliff-nesting gulls with a maximal brood size of three and their chicks are nearly constantly brooded or guarded at the nest by one of the parents (Braun and Hunt, 1983; Roberts and Hatch, 1993). Both sexes provision young with food throughout chick-rearing; usually five to six weeks. Parent kittiwakes exchange their duties -- while one parent is brooding chicks, another is at sea foraging for itself and collecting food for the young (Braun and Hunt, 1983). Males and females do not show differences in nest attendance or reproductive effort (Coulson and Wooller, 1984). When a foraging parent returns to the nest, a brooding parent leaves for the ocean. During good foraging conditions, this synchronized behavior results in the constant presence of one of the adults at the nest until chicks are about 34 days old (Braun and Hunt, 1983). If food supply is poor, however, parents start to leave their chicks unattended at an earlier age (Roberts and Hatch, 1993). Thus, experimentally increased levels of corticosterone might cause a kittiwake raising young chicks to increase time spent foraging at the expense of leaving chicks unattended.

In Black-legged kittiwakes, a hungry chick appears to have only two behavioral options to improve its chances of survival (Braun and Hunt, 1983): either eliminate nest-mates (siblicide) or intensify its begging for food. Begging behavior is probably the only form of foraging behavior available to nest-bound chicks. Because increased levels of corticosterone facilitate foraging behavior in adult birds, it is reasonable to hypothesize that experimentally increased levels of corticosterone might affect begging in nest-bound chicks.

Elsewhere, we have shown that a seasonal decrease in parent kittiwake body condition is associated with a seasonal increase in their circulating levels of corticosterone (Kitaysky et al., 1999b). Furthermore, the seasonal increase in baseline levels of corticosterone is stronger among birds rearing young under poor foraging conditions compared to those breeding under favorable feeding conditions (Kitaysky et al., 1999b). We also have shown that Black-legged kittiwake chicks increase circulating levels of corticosterone in response to food-related stress (Kitaysky et al., 1999a). However, the functional role of corticosterone release in regulating feeding interactions of parents and their chicks has not yet been investigated.

In the present study, we experimentally increased levels of circulating corticosterone in kittiwake parents and chicks at a food-rich colony. Our objectives were to test (1) the behavioral responses of parents and chicks to the experimentally increased circulating levels of corticosterone, (2) the behavioral responses of parents to the corticosterone-induced changes in behavior of their offspring and *vice versa*, and (3) the behavioral responses of intact parents to the corticosterone-induced changes in behavior of their mates.

Methods

Study area

We carried out the experimental manipulations and behavioral observations from July 24 to July 27, 1997, at a colony of seabirds on Gull Island in the lower Cook Inlet, Alaska (*59°35'*N, *151°19'*W). Foraging conditions were favorable for Black-legged kittiwakes nesting at the colony in 1997 (Kitaysky et al., 1999b; J.F. Piatt et al., unpubl). The study plot consisted of a 30 m x 25 m vertical wall that contained 40 active nests of Black-legged kittiwakes. We monitored all nests at the study plot at 3-day intervals from the egg-laying stage to fledging of chicks. We observed birds from a blind that had been placed at the colony before kittiwakes started egg-laying; at the time of the experiment (mid chick-rearing), birds were habituated to the presence of observers in the blind. The blind faced the study plot at a distance of about 15 meters.

Experimental design

We randomly selected a total of 24 nests for four experimental treatments (in two treatments we manipulated chicks and in two treatments we manipulated parents). Each treatment had equal numbers of nests with one and two chicks.

Experimental manipulations with chicks. At experimental nests we implanted chicks subcutaneously with a single 25 mm silastic tube (Dow Corning) filled with crystallized corticosterone. At control nests we implanted chicks with a single 25 mm empty silastic tube. Both chicks from broods of two were treated similarly. We individually marked chicks using spots of colored dyes on the forehead and breast. This method has been used in previous experimental studies of birds and does not observably affect behavior of chicks or parents (e.g. Cotton *et al.* 1999 and references therein). Estimated mean distances between nests within a treatment were 1.1±0.52 (*SD*) and 1.0±0.54 m for nests with cort-implanted and sham-implanted chicks, respectively. The average age of cort-implanted chicks (14.8±3.71(*SD*) days after hatching) was similar to sham-implanted chicks (15±3.35 days). In this study we were not always able to distinguish between α and β chicks within a brood, therefore we could not examine behavioral differences between siblings according to their hierarchical status.

Experimental manipulations with parents. At experimental nests we implanted one of the parents subcutaneously with two 25 mm silastic tubes filled with crystallized corticosterone. At control nests we implanted one of the parents with two 25 mm empty silastic tubes. Estimated mean distances between nests within a treatment were 1.7±1.38 (*SD*) and 2.1±0.96 m for nests with cort-implanted and sham-implanted parents, respectively. Age of chicks was similar between the experimental treatments, averaging 16.3±4.08(*SD*) and 16.5±4.37 days-old for nests with cort-implanted and sham-implanted parents, respectively. We individually marked each manipulated bird with a unique combination of color leg bands and spots of colored dyes on the forehead and breast.

Nest observations. We conducted observations of all nests from the blind with 8 x 40 binoculars over a 2-day period beginning 24 hours after implant placement. We watched nests continuously from 0700 to 1800 h (by two observers recording simultaneously during 2 hr shifts). We recorded begging rates of chicks, food provisioning rates and nest attendance of parents. We also recorded aggression between siblings. Color markings allowed us to follow the behavior of individual birds. We defined begging rates as the number of begs per chick per hour at each nest. We defined begging as a chick solicitation (frequent vertical movements of the head accompanied by a high-pitch vocalization) for food from a parent. We considered begging series with pauses of more than 1 minute as separate begging signals. We defined feeding rates as the number of feeds per chick per hour at each nest. We considered consecutive feedings that occurred more than 5 minutes apart as separate meals. We calculated the number of trips away from the nest performed by parents as the mean number of trips per parent per nest per 2-day study period.

After the experiment, we monitored the experimental birds until chicks fledged. In 1998 and

1999, to resight the experimental adult kittiwakes, we conducted regular surveys from June 1 to July 1 at the colony.

Effect of implantation and corticosterone analyses

In parallel to the experiment we tested the effects of subcutaneous corticosterone implantation on birds captured elsewhere at the colony. For that, we captured undisturbed birds and collected the initial baseline blood samples by puncture of the alar vein and collection of blood in heparinized microhematocrit 100μl tubes. After collecting blood sample, blood flow was stopped by application of cotton. We banded captured birds with a unique combination of color bands, implanted (as described above) and adults were released at the colony and chicks placed back in their nests. Three days later, birds were recaptured and blood samples were collected (as described above). All blood samples were collected within 0-3 minutes after capture and were considered to reflect baseline levels of corticosterone (Kitaysky et al., 1999b).

After collection of blood, we emptied hematocrit tubes into 0.5 ml vials, which were stored on ice. Within 12 hours, blood samples were centrifuged and plasma was collected. Plasma samples were frozen at -20°C and transported to J.C.W.'s laboratory at the University of Washington for radioimmunoassay analyses. We measured corticosterone concentrations in duplicate for each plasma sample in one assay after extraction in dichloromethane. Prior to extraction, we added tritiated corticosterone (2000 cpm) to each plasma sample to control for a loss of corticosterone during extraction. Recovery values of the labeled steroid following extraction ranged from 80 to 90% and were used to adjust assayed concentrations of corticosterone. For a detailed description of the radioimmunoassay analysis see Wingfield and

Farner (1975) and Wingfield et al. (1992).

Radioimmunoassay analysis revealed that in 15-day old Black-legged kittiwakes, a single 25 mm silastic tube filled with crystallized corticosterone approximately tripled the initial baseline levels of corticosterone (Fig. 1). The heightened corticosterone levels were similar to the increase of baseline levels of corticosterone (assayed as described above) observed in kittiwake chicks that were reared in captivity under conditions of moderate food deprivation (Fig. 1; Kitaysky et al., 1999a). In parent kittiwakes, the administered amount of crystallized corticosterone (two 25 mm implants) increased baseline concentrations by about 10 ng ml^{-1} (Fig. 1) and was expected to be metabolized within a two-three week period after implantation (J.C. Wingfield, personal observations). Thus, in this study the implantation elevated levels of corticosterone to a concentration and for a period similar to those observed in parent kittiwakes rearing their young during food shortages (Fig. 1; Kitaysky et al., 1999b).

Statistical analyses

We considered each individual nest as an independent sample unit. Therefore, we calculated chick begging rates and food provisioning rates as mean values per each nest. Likewise, we calculated parameters of nest attendance by parents as mean values per each nest. Age of chick was not significantly different among the treatments and did not significantly affect any of the measured parameters of chick and parent behaviors, and therefore we excluded age from further statistical analyses. We compared begging rates among all four treatments using two-way ANOVA (blocked by nest) with experimental treatment and brood size as factors (followed by LSD planned-comparison Post-Hoc test, which includes adjustments for multiple tests). We compared food provisioning rates among all four treatments using two-way ANOVA (blocked by nest) with experimental treatment and brood size as factors (followed by LSD planned-comparison Post-Hoc test). We compared proportions of time chicks were unattended by parents among all four treatments using median test (blocked by nest) with experimental treatments as factor. We compared the behavioral characteristics of parents between nests with cort-implanted and sham-implanted chicks using statistical tests for independent samples, where the experimental treatment (blocked by nest) was used as a grouping variable. We compared the behavioral characteristics of cort-implanted and sham-implanted parents using statistical tests for independent samples. We examined the effects of the experimental treatments on the behavior of mates within a pair using paired-sample comparisons (paired by nest).

If data violated the assumptions for parametric tests (Sokal and Rohlf, 1981) we used nonparametric equivalents. We completed computation of statistical tests using the STATISTICA statistical package.

Results

Chick's responses to experimental treatments

Begging rates

Experimental treatments had a significant effect on chick begging rates (two-way ANOVA: $F_{3,16}=21.76$, $p<0.001$, Fig. 2). Cort-implanted chicks begged more frequently than sham-implanted controls and chicks in the other two treatments (Post-Hoc tests $p<0.001$ for all cases between the nests with cort-implanted chicks and the nests either with sham-implanted chicks, cort-implanted parents or sham-implanted parents; Fig. 2). Furthermore, there was a significant interaction between treatment and brood size ($F_{3,16}$=4.095, p=0.025), cort-implanted chicks begged almost twice as much in broods of two than in broods of one (Post-Hoc test: p<0.001; Fig. 2). Brood size did not affect begging in the nests with sham-implanted chicks, cort-implanted parents or sham-implanted parents (Post-Hoc tests $p \geq 0.273$ for all cases; Fig. 2).

Aggression

We did not observe a significant number of aggressive contacts in the nests with two siblings. The only attacks of a smaller chick by its bigger sibling were observed in one of the nests with sham-implanted chicks.

Parent's responses to experimental treatments

Feeding rate

Experimental treatments had a significant effect on the feeding of chicks by the parents (two-way ANOVA: $F_{3,16}$=6.22, p=0.005, Fig. 3). Cort-implanted chicks were fed more frequently than sham-implanted chicks (Post-Hoc test p<0.001), chicks at the nests with cort-implanted parents (Post-Hoc test p=0.028) and sham-implanted parents (Post-Hoc test p=0.016). Feeding rates were not significantly different between the nests with cort-implanted and sham-implanted parents (Post-Hoc test p=0.787). Brood size did not affect feeding rate ($F_{1,16}$=0.74, p=0.402, Fig. 3).

Nest attendance

Parents of cort-implanted chicks performed more trips (5.3±0.95[SE] trips per parent during 2-

day period, n=6) away from the nest than did parents of sham-implanted chicks (3.5±0.26, n=6) (Kruskal-Wallis test: H_1=6.23, n=12, p=0.013). Cort-implanted parents performed more trips (4.5±0.34[SE] trips per 2-day period, n=6) away from the nest than did sham-implanted parents (3.2±0.41, n=6) (ANOVA: $F_{1,10}$=12.31, p=0.006), and their untreated mates (3.0±0.26, n=6) (paired t-test: t=6.71, df=5, p=0.001).

Cort-implanted parents spent less time (298.2±27.24[SE] min per 2-day period, n=6) brooding/guarding chicks compared to sham-implanted parents (668.5±76.85, n=6) (Kruskal-Wallis test: H_1=8.31, n=12, p=0.004). Cort-implanted parents also spent significantly less time brooding/guarding chicks compared to their untreated mates (760.0±106.55, n=6) (Wilcoxon matched pairs test: Z=1.199, n=6, p<0.05). Sham-implanted parents spent a similar amount of time in brooding/guarding chicks as their untreated mates (617.33±78.95, n=6) (paired t-test: t=0.329, df=5, p=0.756).

Chicks of cort-implanted parents spent more time unattended by either of the parents compared to chicks in all other treatments (Fig. 4; Median test: χ^2=9.33, df=3, p=0.025).

Chick provisioning rates by corticosterone-implanted parents

The experimental increase in corticosterone levels did not alter chick provisioning rates of cort-implanted parents, which fed their chicks at rates (0.174±0.012[SE] feeds hr^{-1}, n=6) similar to those of sham-implanted parents (0.167±0.015, n=6) (ANOVA: $F_{1,10}$=0.122, p=0.734). Feeding rates were also similar between implanted and untreated mates within a pair (paired t-tests: t=0.349, df=5, p=0.741, and t=0.466, df=5, p=0.661, for nests with sham-implanted and cort-implanted parents, respectively).

Survival and resighting of experimental birds

All experimental and control chicks survived until fledgling. All experimental and control parents survived during a three-week period following the implantation and reared their chicks successfully until fledging. During the reproductive season of 1998, 5 out of 6 cort-implanted parents failed to return to the nesting colony, whereas all other parents (except one of the sham-implanted birds) returned to the colony. During the reproductive season of 1999, the individuals that were missing in 1998 were also not resighted at the colony, whereas all other birds returned to the colony. The proportion of cort-implanted parents that failed to return to the breeding colony was significantly larger than in sham-implanted parents (Fisher exact test, p=0.04).

Discussion

Behavioral responses to the experimental increased levels of corticosterone in chicks

Experimental studies have shown that hunger in the nest-bound chick results in increased begging of that chick (e.g. Bengtsson and Ryden, 1983; Cotton et al., 1996; Henderson, 1975). Furthermore, recent empirical studies showed that blue-footed booby (*Sula nebouxii*) chicks release corticosterone in response to short-term food deprivation (Nunez-de la Mora et al., 1996), as do food-restricted Black-legged kittiwake chicks (Kitaysky et al., 1999a). In the present study, we have taken these observations a step further to show that high corticosterone levels increase chick begging rates and increase rates of food provisioning by parents.

Our study suggests the regulation of begging behavior through secretion of corticosterone.

Corticosterone secretion likely maintains and restores animal homeostasis in response to environmental changes (e.g., Silverin, 1998). This current study shows clearly that the release of corticosterone in hungry kittiwake chicks allows them to restore depleted energy reserves by modifying the behavior of their parents. However, such regulation is not without costs. Chronic elevation of corticosterone is known to suppress memory and immune systems, promote wasting of muscle tissue, and cause neuronal cell death (Sapolsky et al., 1986; Sapolsky, 1992). Thus, the regulation of begging through secretion of corticosterone is likely to be associated with benefits and costs, which must be balanced by a begging chick.

The regulation of begging through secretion of corticosterone might represent an evolutionary stable signaling system as a resolution of parent-offspring conflict in birds. Our study suggests that parent kittiwakes probably assess the physiological condition of their chicks by monitoring begging. In such a system, a chick can misrepresent its requirements in order to acquire more food than it needs (Godfray, 1995b). Cheating may be prevented if there is a cost associated with begging that is larger than the benefits of the extra food obtained by a cheating chick (Godfray, 1995b). If continuous begging is associated with a prolonged secretion of corticosterone, then a cheating chick would suffer detrimental effects of chronically elevated levels of corticosterone. Thus, if cheating is associated with high levels of corticosterone, exaggerated begging might be costly and a cheating chick may endanger its future survival. Our conclusion hinges on the assumption that continuous begging requires continuous secretion of corticosterone, which remains to be shown. Clearly, future studies addressing hormonal regulation of begging and potential long-term effects of chronic elevation of corticosterone levels in nest-dependent chicks are needed.

In addition to effects of corticosterone, interactions between chicks within a brood probably increase chick begging rates. In particular, we found that corticosterone-implanted chicks in broods of two begged more frequently than singletons. On the other hand, we did not record any aggression between siblings in the nests with cort-implanted chicks. This supports earlier observations that competition for food between food-stressed siblings is initially expressed by increased begging (Muller and Smith, 1978; Smith and Montgomerie, 1991). In the Black-legged kittiwake, Braun and Hunt (1983) observed higher begging rates of hungry chicks when they occurred in broods of two compared to singletons. Similar observations were reported for some species of birds (e.g., Harper, 1986; for cotingas, Cotingidae), but in other species (Cotton et al., 1996; Kacelnik et al., 1995; for European starling, *Sturnis vulgaris*) a chick tends to beg in relation to its own condition regardless of the behavior of its nest mates. It is not known yet how important are phylogenetic constraints in determining chick begging strategies, and differences among different studies might reflect that phenomenon. Nevertheless, our observations are consistent with the theoretical prediction that the begging rate of a chick depends on its own condition and on conditions of its nest mates (Godfray, 1995a). Thus, although high levels of corticosterone increase begging in Black-legged kittiwake chicks, further escalation of a chick's begging probably depends on the begging levels of its sibling.

Although corticosterone-implanted chicks in broods of two were begging at almost twice the rate of singletons, parents did not feed them at twice the rate of corticosterone-implanted singletons (mean feeding rates differed by 13% between chicks in broods of two and singletons).

In contrast to these results, other experimental studies have shown that parental provisioning is proportional to chick begging (e.g., Kacelnik et al., 1995; and references therein). However, parental ability to increase feeding rates is likely to be limited, and it is possible that parents of all corticosterone-implanted chicks were probably provisioning food at, or near maximal rates. On the other hand, we cannot exclude the possibility that somehow parent kittiwakes are able to discriminate between changes in a chick's begging behavior due to the change in its physiological condition from changes reflecting social interactions between siblings within a brood.

Foraging conditions were favorable for kittiwakes breeding in the study area in 1997 (Kitaysky et al., 1999b; J.F. Piatt, unpubl.). Thus, we observed parental responses to the corticosterone-induced begging of chicks in a situation when parents *could* provide more food. Responses of parent kittiwakes to chick demands might differ under less favorable foraging conditions, thereby changing provisioning rates. If parents cannot provide food in response to chick demands, then aggression leading to siblicide would probably occur (Braun and Hunt, 1983). Before reaching this point, however, begging behavior would offer some evolutionary advantages over aggressive behavior. Begging behavior probably requires less energy (McCarty, 1996; Soler et al., 1999) and entails less risk of injury than aggressive behavior.

Behavioral responses to the experimental increased levels of corticosterone in parents

This study also suggests a physiological mechanism for the regulation of resource allocation by the adult Black-legged kittiwake during chick-rearing. We found that corticosterone-implanted parent kittiwakes performed more trips away from the nests than did sham-implanted parents. We assumed when birds were away from the nests, they were foraging. This assumption seems to be reasonable (e.g. Monaghan et al., 1996) and was justified by direct observations of chick-rearing Black-legged kittiwakes (Irons, 1998). Kitaysky et al. (1999b) have shown that parent kittiwakes respond to food shortages by increased secretion of corticosterone. Moderate increases in corticosterone secretion are known to increase foraging activities (Astheimer et al., 1992; Wingfield et al., 1998) and food intake (Wingfield and Ramenofsky, 1999; Kitaysky et al., unpublished). Thus, we suggest that frequent trips of the corticosterone-implanted parent kittiwakes away from the nests were probably due to an increase in their food demands.

Our results show that in response to the experimental increase in circulating levels of corticosterone adult kittiwakes increased foraging on the expense of guarding their chicks. This resulted in a considerable increase in the amount of time that chicks were unattended and potentially vulnerable to predation. A similar increase in the time that young kittiwake chicks spent unattended during poor foraging conditions was observed by Roberts and Hatch (1993). High corticosterone levels also affect parental behavior in other species (Silverin, 1986; Wingfield et al., 1997; Wingfield et al., 1998). In contrast to other studies of the experimentally increased corticosterone levels in chick-rearing birds (e.g. Silverin, 1986), our results do not demonstrate an effect of high corticosterone on the breeding success of Black-legged kittiwakes. Yet, we examined the relationship between high corticosterone and parental behavior in the context of a food-rich colony where non-experimental parents were not off foraging as much, thereby protecting both their own chicks and the implanted birds' chicks from predators. However, food shortages are likely to affect the behavior of all birds breeding at a particular colony in a similar manner, and all affected birds would be leaving their chicks unattended. In this manner, increased corticosterone secretion can be an important factor determining breeding success of Black-legged kittiwakes during food shortages.

The major prediction of life history theory states that long-lived birds should balance a survival of their current offspring with their own survival (Linden and Moller, 1989). Current reproductive effort affects residual reproductive value in the Black-legged kittiwake (Golet et al., 1998; Hatch et al., 1993; 1994). Kitaysky et al. (2000) have shown that a decrease in food abundance causes an increase in energy expenditures of parent kittiwakes, whereas growth rates of their chicks are not affected. An increase in parental effort of Black-legged kittiwakes results in a decrease of their body condition (through a depletion of fat reserves), which may affect their post-reproductive survival (Golet and Irons, 1999). A depletion of fat reserves in parent kittiwakes results in elevation of circulating levels of corticosterone (Kitaysky et. al., 1999b), which might affect their return rate to the breeding colony (this study). In this current study we established that the individuals with experimentally elevated levels of corticosterone increased foraging on the expense of brooding/guarding their chicks, but did not alter their chick provisioning rates and could suffer a long-term effect of chronically-elevated levels of corticosterone.

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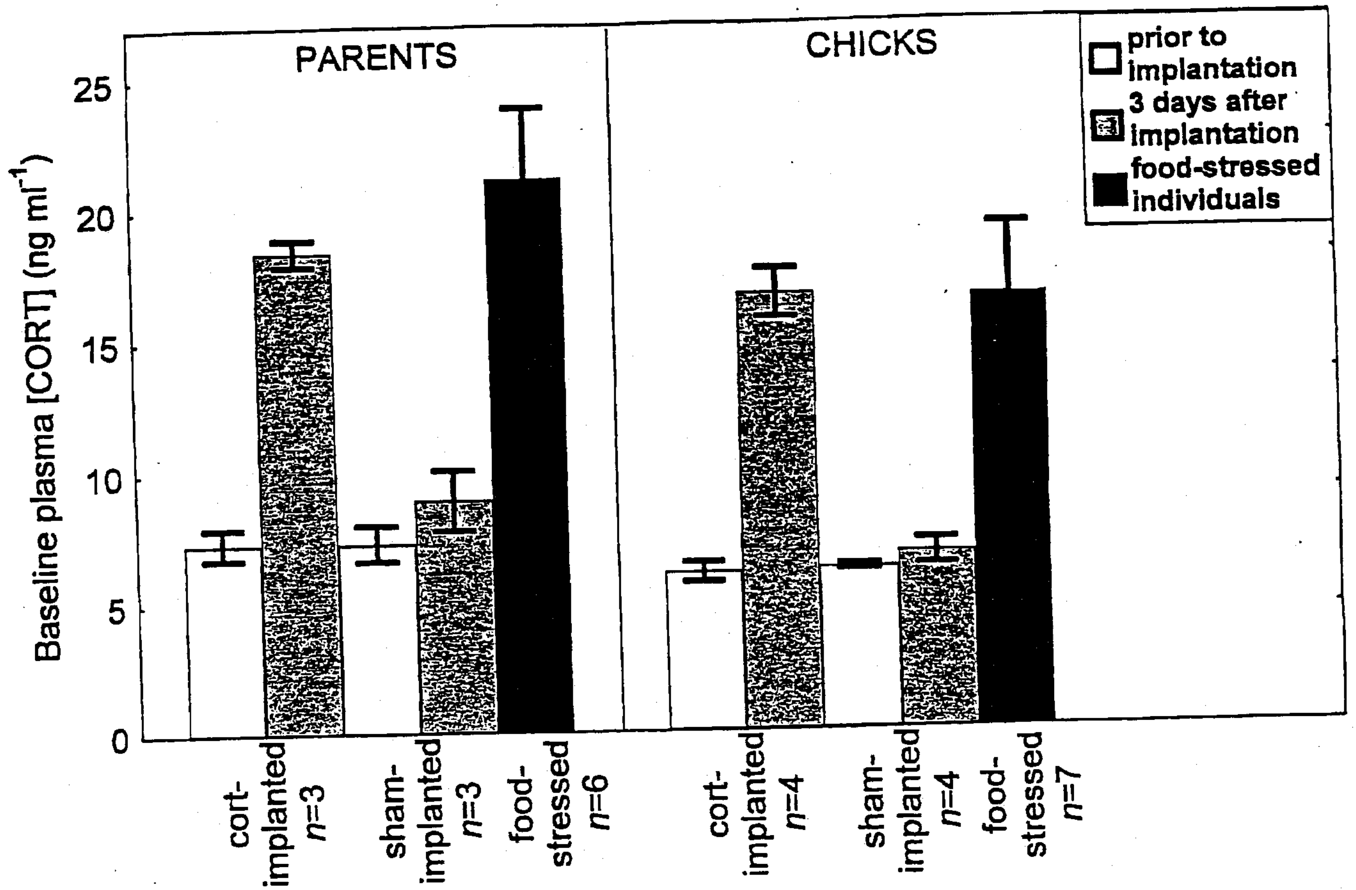
Figure legends

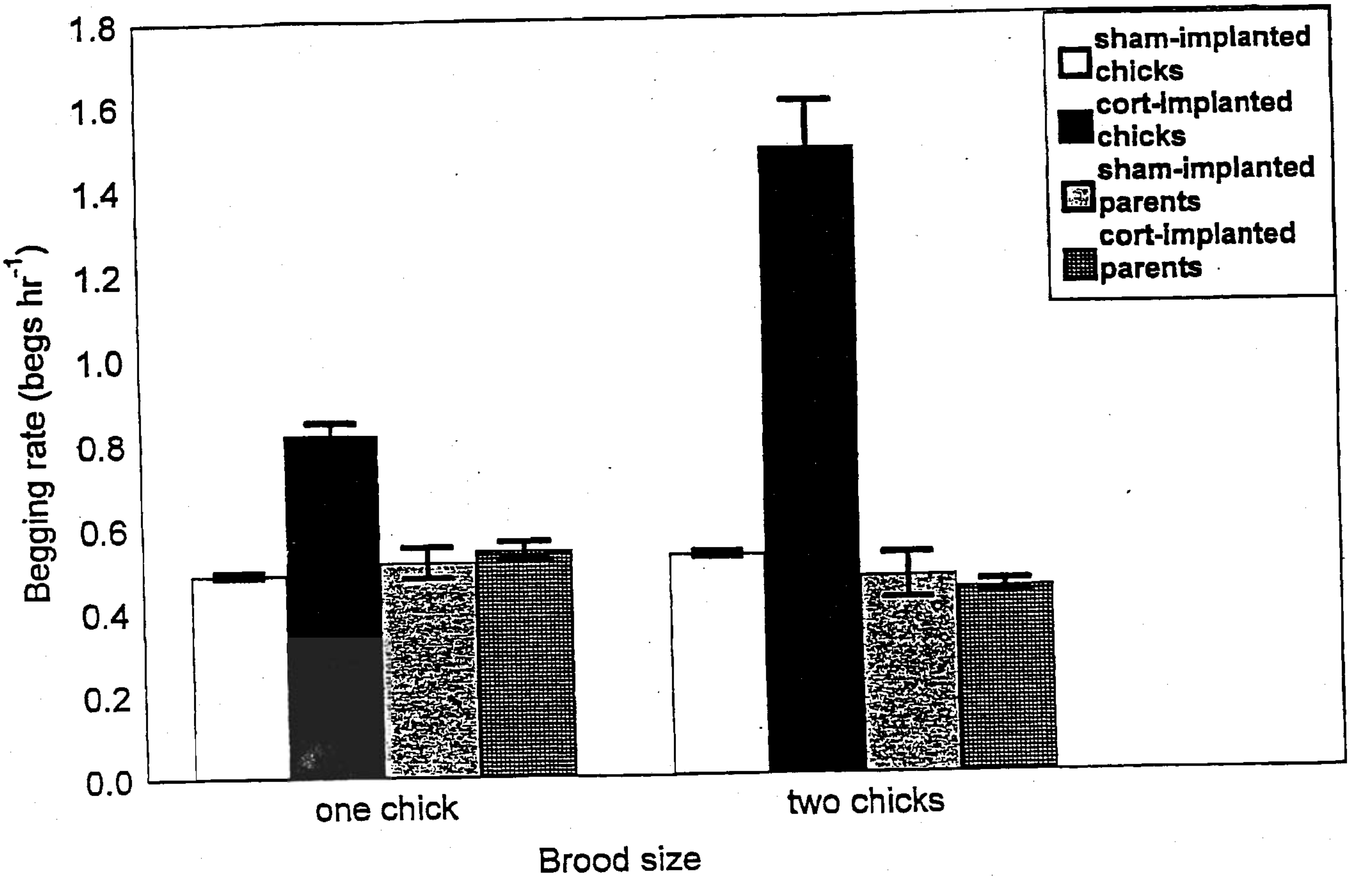
Figure 1. Effects of corticosterone implants on baseline plasma levels of corticosterone in Black-legged kittiwakes (mean ± SE). Cort-implantation caused a significant increase in baseline corticosterone in parents (paired t-test: t=58.26, 2 *df*, p<0.001) and chicks (paired t-test: t=6.41, 3 *df*, p=0.008). Sham-implantation did not cause a significant change in baseline levels of corticosterone in parents (paired t-test: t=1.59, 2 *df*, p=0.253) and chicks (paired t-test: t=0.64, 3 *df*, p=0.568). Baseline levels of food-stressed parent and chick Black-legged kittiwakes are shown (as solid-black bars) for comparative purposes (data from Kitaysky et al. 1999ab).

Figure 2. Behavioral responses of Black-legged kittiwake chicks to experimentally increased circulating levels of corticosterone (mean ± SE; n=3 nests per each treatment).

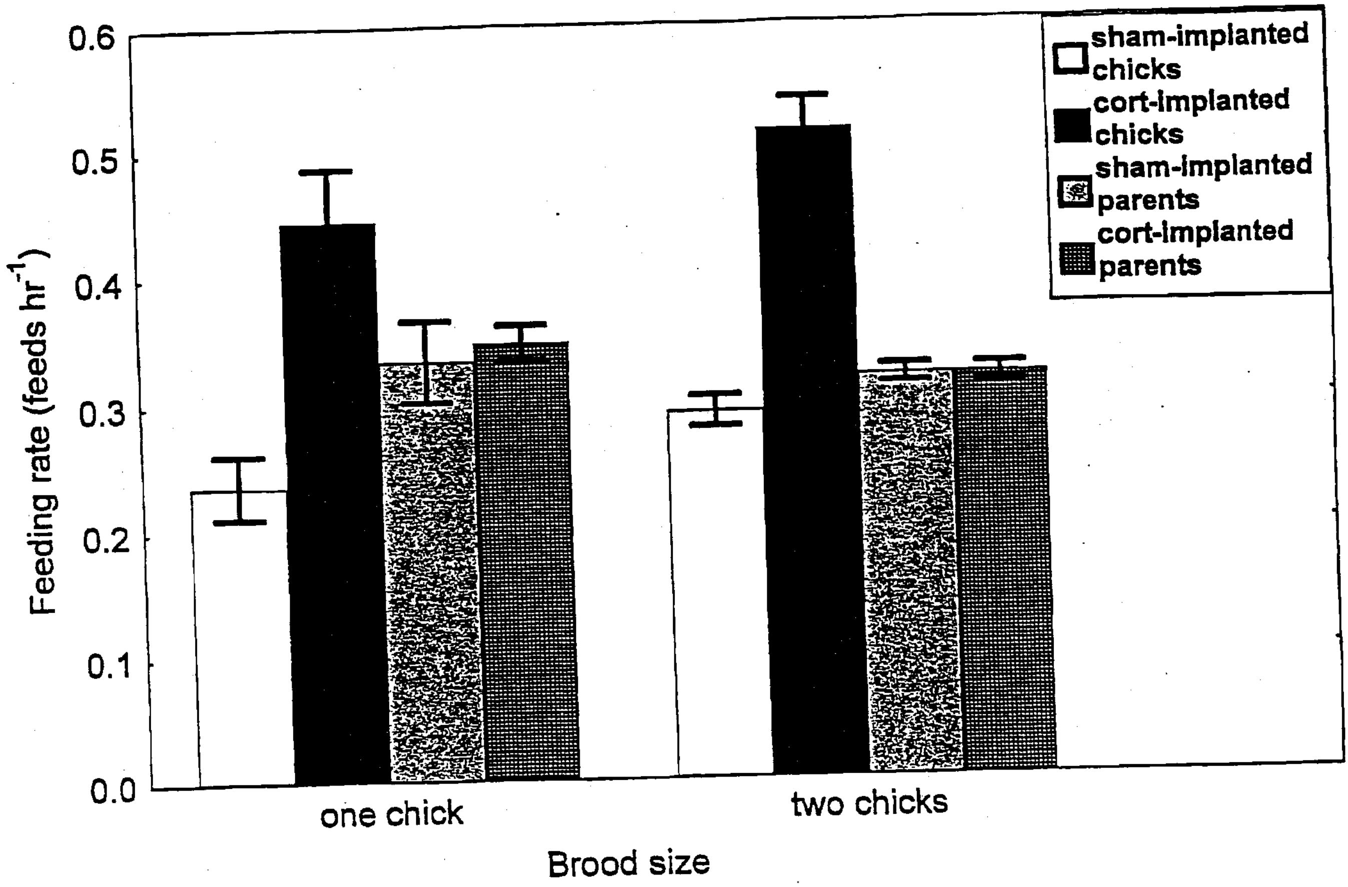
Figure 3. Behavioral responses of parent Black-legged kittiwakes to experimentally increased circulating levels of corticosterone in their chicks (mean ± SE; n=3 nests per each treatment).

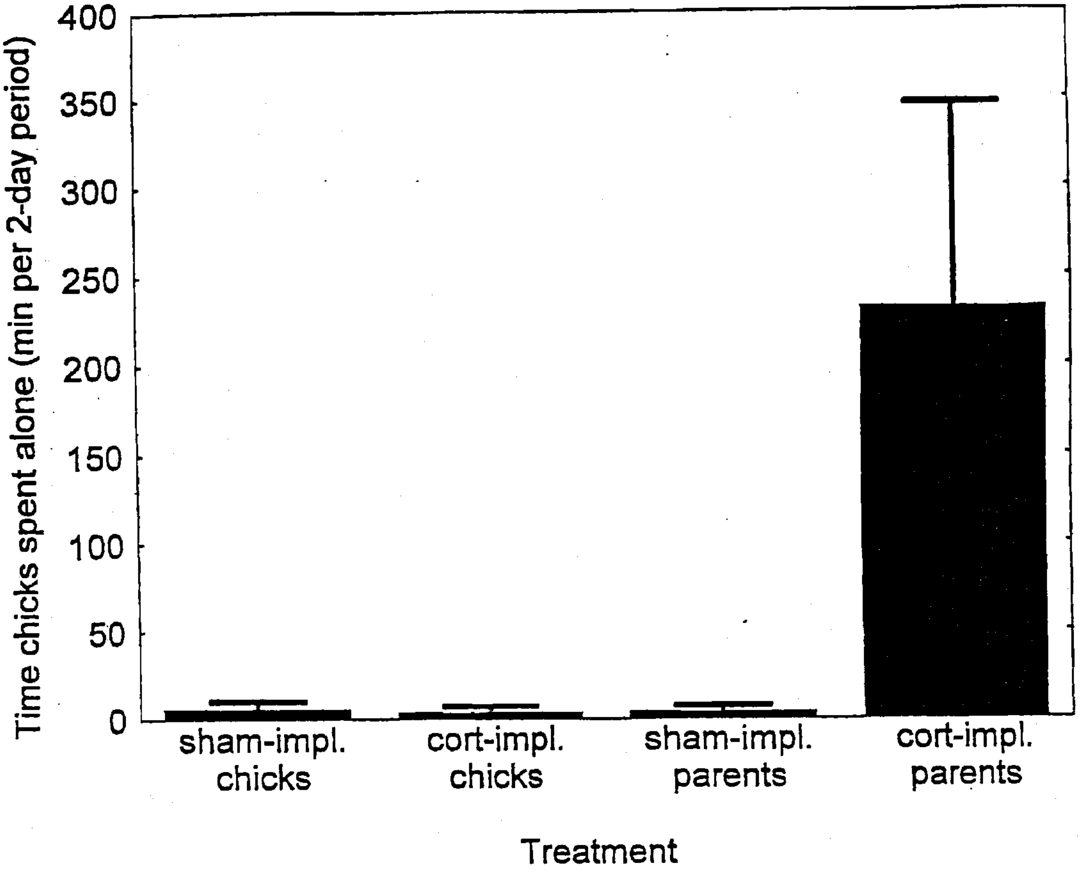
Figure 4. Time spent by Black-legged kittiwake chicks unattended by parents (mean ± SE; n=6 nests per each treatment).


PARENTS
CHICKS
prior to implantation
3 days after implantation
food-stressed individuals
Baseline plasma [CORT] (ng ml^{-1})
0
5
10
15
20
25
cort-implanted *n*=3
sham-implanted *n*=3
food-stressed *n*=6
cort-implanted *n*=4
sham-implanted *n*=4
food-stressed *n*=7



Begging rate (begs hr^{-1})
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
one chick
two chicks
Brood size
sham-implanted chicks
cort-implanted chicks
sham-implanted parents
cort-implanted parents


0.6
0.5
0.4
0.3
0.2
0.1
0.0
Feeding rate (feeds hr^{-1})
one chick
two chicks
Brood size
sham-implanted chicks
cort-implanted chicks
sham-implanted parents
cort-implanted parents


Time chicks spent alone (min per 2-day period)
400
350
300
250
200
150
100
50
0
sham-impl. chicks
cort-impl. chicks
sham-impl. parents
cort-impl. parents
Treatment

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Resource allocation in breeding seabirds: responses to fluctuations in their food supply

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ABSTRACT: In the vicinity of the Pribilof Islands in the Bering Sea, abundance of food available to surface-foraging seabirds was greater during the chick-rearing period in 1988 than in 1987, whereas abundance of food available to pursuit-diving seabirds was greater in 1987. Here we examine how breeding success and resource allocation of surface-foraging black-legged kittiwakes *Rissa tridactyla* (BLKI) and pursuit-diving thick-billed murres *Uria lomvia* (TBMU) varied with the fluctuations in their food supply. We also examine a difference in resource allocation among parents raising chicks at the large colony on St. George Island and those at the nearby small colony on St. Paul Island. We studied breeding success (BS), field metabolic rates (FMR, assessed by using doubly labeled water), foraging distribution, and nest attendance of parents and growth rate (GR) of chicks. The BS of BLKIs was lower in 1987 (a season of less abundant food for kittiwakes) than in 1988 (a season of more abundant food), and parents had higher FMRs in 1987 than in 1988. At-sea distributions and nest attendance suggested that in 1987 BLKIs foraged farther from the colonies, which could have resulted in the higher FMR of the parents. GR of BLKI chicks did not vary between 1987 and 1988. The BS of TBMUs was not significantly different between 1987 (a season of more abundant food for TBMUs) and 1988 (a season of less abundant food). Parent TBMUs had similar FMRs between the seasons. Densities of foraging TBMUs were higher within 20 km around colonies in 1987 than in 1988. Although the total time parent TBMUs spent foraging did not vary inter-seasonally, they performed more foraging trips of a shorter duration in 1987 than in 1988, and the GR of TBMU chicks was higher in 1987 than in 1988. Inter-colony comparisons do not suggest that parents reproducing at the large colony work harder to raise their young compared to parents breeding at the small colony. In 1987 parent BLKIs failed in raising young at the large colony, whereas one-third of BLKIs fledged their chicks at the small colony. In 1988, however, RS and FMRs of parent BLKIs were not significantly different between the colonies. Also, TBMUs at the large colony had higher BS than those at the small colony in both 1987 and 1988. Furthermore, in both years parent TBMUs feeding young at the small colony foraged farther from the colony and had significantly higher FMRs than at the large colony. These results suggest that fluctuations in food supply affect resource allocation in seabirds. However, a decrease in food abundance is likely to cause an increase in energy expenditures of parent BLKIs, whereas growth rates of their chicks are less affected. For the TBMUs, food shortages are likely to cause a decrease in growth of the chicks, but not an increase in energy expenditures of the parents.

KEY WORDS: Doubly labeled water · Field metabolic rates · Food abundance · Kittiwakes · Murres

INTRODUCTION

Field observations imply the possibility of a species-specific response of parent seabirds to variation in food resources (sensu Obst et al. 1995). Species-specific differences probably reflect behavioral/phylogenetic and ecological characteristics of seabirds and their prey, as was suggested for planktivorous and piscivorous alcids, Alcidae (Kitaysky 1996, Kitaysky & Golubova

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2000). Results of field studies have shown that in some species of pelagic seabirds parents were willing and/or able to compensate for variation in food availability through an increase in effort devoted to foraging for the young (Burger & Piatt 1990, Mognahan et al. 1994, Uttley et al. 1994). In other species, parents maintained constant foraging effort despite high variation in food availability (e.g., Obst et al. 1995).

Variations in the availability of food can also influence the reproductive performance of birds in a density-dependent fashion (Ashmole 1963, Furness 1984, Arcese & Smith 1988, Both 1998). Several studies have shown that seabirds in large colonies have reduced chick survival or growth rates compared to birds in smaller colonies, suggesting greater depletion of food resources around large colonies (Gaston & Nettleship 1981, Furness & Birkhead 1984, Hunt et al. 1986). Reduced reproductive performance could indicate that adults at large colonies were not able to compensate fully for a depletion of food through an increase in their foraging effort. The lower rates of chick growth or survival in larger colonies could also reflect an unwillingness of adults to compensate for food shortages by increasing their reproductive investment. Instead, these parents may conserve resources to apply to future reproductive effort (Stearns 1992). Although there is considerable data demonstrating changes in seabird reproductive output as a function of prey resources (e.g., Ainley & Boekelheide 1990, Hunt et al. 1996, Greenstreet et al. 1999, Hunt & Byrd 1999), the behavioral responses of parent seabirds to variation in food resources are not well understood (Furness & Nettleship 1991, Montevecchi 1993). Theoretical considerations of parental effort in reproduction versus allocation of resources to maintenance suggest that even high environmental variability would not change optimal effort of long-lived parent seabirds in reproduction (e.g., Cooch & Ricklefs 1994).

In this study we examined (1) whether parent seabirds adjust their reproductive effort to inter-annual variations in food resources, and (2) whether adult birds at a very large colony work harder to raise their chicks than parents at a smaller nearby colony. Our goal was to determine the relationship among field metabolic rates, foraging and nest attendance behaviors of parents, and growth rates and survival (until fledging) of their chicks following a change in food abundance.

MATERIAL AND METHODS

Study system. We studied the breeding biology of black-legged kittiwakes *Rissa tridactyla* and thick-billed murres *Uria lomvia* on St. Paul and St. George Islands, Pribilof Islands, Bering Sea. These 2 colonies are 88 km apart and differ by an order of magnitude in the number of seabirds breeding on them (Table 1). Black-legged kittiwakes are surface-foraging birds that prey on pelagic fishes and invertebrates during chick-rearing, and provision their young by regurgitating stomach-loads of partially digested food (Decker et al. 1995). On the Pribilof Islands, kittiwakes lay 1 to 2 eggs but rarely raise 2 young (Braun & Hunt 1983). Thick-billed murres are pursuit-divers, and at the Pribilof Islands they concentrate their foraging within 30 to 60 km of each island (Hunt et al. 1982, Schneider & Hunt 1984). Murres raise a single chick per successful breeding bout. Parent murres typically provision their chick with a single prey item that they deliver to the breeding site in the bill (Gaston & Jones 1998).

An interannual change in food distribution and abundance observed at the Pribilof Islands during 1987 and 1988 (Coyle & Cooney 1993, G.L.H. et al. unpubl. data) allowed us to study a change in resource allocation of kittiwake and murre parents in response to this inter-annual fluctuation. Overall, there were no significant inter-annual changes in the diet composition of black-legged kittiwakes and thick-billed murres during 1987 and 1988 (Decker et al. 1995). However, food available to surface-foraging kittiwakes was more abundant in 1988 compared to 1987, whereas food available to pursuit-diving murres was more abundant in 1987 than in 1988 (Table 2).

Breeding success. To examine the contributions of the interannual change in food abundance on the

Table 1. Estimates of the numbers of seabirds breeding on the Pribilof Islands, Bering Sea

	St. Paul Island		St. George Island	
	1976[a]	1980s[b, c]	1976	1980s
All seabirds	267 200	229 400	2 460 500	2 446 000
Black-legged kittiwake *Rissa tridactyla*	42 000	–20 % decline	94 000	–20 % decline
Thick-billed murre *Uria lomvia*	110 000	–51 % decline	1 400 000	–21 % decline

[a]Data from Hickey & Craighead (1977); [b]data from Craighead & Oppenheim (1982); [c]data from Hunt & Byrd (1999)

Table 2. Estimates of abundance of prey potentially available to surface-foraging seabirds (determined by neuston trawls) and pursuit-diving seabirds (acoustically determined) during chick-rearing in 1987 and 1988 at the Pribilof Islands. Sampling by both methods was simultaneously performed in the vicinity of the Pribilof Islands in July and August of each year; data for 43 neuston trawl (0 to 1 m layer of water was sampled) tows are presented as number of trawls in which a particular type of prey was present or absent (G.L.H. unpubl. data); acoustically determined data are from Coyle & Cooney (1993) (collected by 38 kHz echo integration system; probably reflect abundance of fish and euphausiids [Coyle & Cooney 1993], which were the main prey of thick-billed murres nesting on the Pribilof Islands in 1987 and 1988 [Decker et al. 1995]. Measures of prey abundance that were significantly different between years are in **bold** ($p < 0.001$, chi-square tests for neuston trawls, G.L.H. unpubl. data, and Student's *t*-tests for acoustically determined biomass: Coyle & Cooney 1993)

Prey	Neuston trawls (present/absent)		Acoustically determined biomass (g m^{-3})		
	1987	1988	(Depth)	1987	1988
Fish	**3/40**	**18/25**	(5–30 m below surface)	**3.75**	**1.33**
Gelatinous zooplankton	**5/38**	**26/17**	(Strata within 20 m above bottom)	**2.22**	**1.47**
Crab larvae	**0/43**	**33/10**			

breeding performance of seabirds, we used available data on reproductive performance in 1987 and 1988 as summarized by Climo (1993) and Dragoo & Dragoo (1994). For black-legged kittiwakes and thick-billed murres, breeding success was defined as the percentage of nests in which an egg was laid from which a chick fledged successfully. Consequently, to determine season and colony effects on bird breeding success, we used χ^2-tests on relative frequences of nests where parents failed to fledge chicks or fledged chicks successfully.

Field metabolic rates. We determined field metabolic rates of parent kittiwakes and murres by using the doubly labeled water technique (Nagy 1983). We captured adults rearing young at their nests by net and/or noose pole. We then injected isotopically labeled water containing both ^{3}H and ^{18}O into the *pectoralis major* of the birds. The injection solution was suitably mixed to deliver 1 mCi ^{3}H kg^{-1} bird (i.e., 0.4 mCi kittiwake^{-1}, 1.0 mCi murre^{-1}) and 3 ml of 90 to 99 atom% $H_2^{18}O$ kg^{-1} bird (i.e., 1.2 ml kittiwake^{-1}, 3 ml murre^{-1}) in a single injection of 3.2 ml kg^{-1} per bird. Injections of this size are unlikely to disturb the birds' water balance, and are well below the accepted threshold for ^{3}H toxicity (Nagy & Costa 1980). Injected individuals were held for 1 h (kittiwakes) or 1.5 h (murres) to allow the isotopes to equilibrate throughout the body-water space. During this period the individual was weighed, banded and marked with dye for later recognition. At the end of the equilibration period, an initial blood sample was collected from the brachial vein in a 70 µl hematocrit tube and the tube was immediately flame sealed. The birds were then released back at their nests. After 1.5 to 4.5 d (kittiwakes) and 2 to 6 d (murres) following the injection, each bird was recaptured at its nest. A second blood sample was taken and sealed as before.

Blood samples were chilled (2 to 4°C) in the field and transported to the laboratory at University of California, Irvine. There, water was distilled from the blood samples according to the microdistillation technique described by Nagy (1983). Isotope analysis of the distilled water was performed in 2 steps at the laboratory of K. A. Nagy at University of California at Los Angeles. ^{3}H activity was determined via liquid scintillation spectrometry with samples counted to 1% errors. Levels of ^{18}O were measured via proton activation analysis (Wood et al. 1975, Nagy 1983).

To determine total body water, we used ^{18}O levels in initial blood samples via the isotope dilution-space method (Nagy 1983). CO_2 production was calculated from initial and final isotope levels and total body water (Roby & Ricklefs 1986). Field metabolic rates (FMRs) were calculated from CO_2 production by using the energy equivalent of each species' diet composition at each colony during the 1987 and 1988 breeding seasons (Decker et al. 1995).

FMR samples at the 2 colonies/years were controlled for brood size and age of chicks. For statistical comparisons of adult FMRs between colonies and reproductive seasons, we used ANOVA on mass-independent FMRs, where year and colony were the factors. To calculate mass-independent FMRs, we followed the ANCOVA method described by Packard & Boardman (1987a,b). Specifically, to eliminate the effects of body mass on field metabolic rates, we used the ANCOVA on $\log_{10}$ FMR with colony/year as factors and $\log_{10}$-transformed body mass as a covariate. Then, the mass-independent FMR was calculated by using the common slope as the basis for FMR adjustments for body mass.

Nest-attendance observations. In this study, we considered each individual nest as a sampling unit. We assumed that, when parents were not observed at the nest site, they were foraging. Therefore, for each nest

Table 3. *Rissa tridactyla* and *Uria lomvia*. Dates (mo/d) and sample sizes (No. of nests) of nest watches in this study. Min/nest = observation time

Year	St. Paul Island Date	St. Paul Island No. of nests	St. Paul Island Min/nest	St. George Island Date	St. George Island No. of nests	St. George Island Min/nest
Black-legged kittiwake						
1987	8/9–8/11	5	2460			
1988	7/29–7/31	7	2880	7/20–7/22	5	2870
	8/10–8/12	4	3570			
Thick-billed murre						
1987	8/4–8/5	3	1590	8/10–8/12	4	3450
	8/14–8/16	4	2760	8/11–8/18	4	3200
1988	8/6–8/7	12	2045	8/4–8/6	9	3540
	8/13–8/14	6	860	8/11–8/14	7	3600

we calculated: (1) mean number of trips per parent per day, (2) mean duration of foraging trip (time between consecutive departure and arrival of a parent), (3) time chicks spent alone (with no parents present at the nest site), and (4) parent loafing time (time both parents were simultaneously observed at the nest site). We conducted 9 direct watches (each of 48 h or longer duration) of 49 kittiwake and 21 murre nests (Table 3). No nests were observed twice during this study. At least 1 member of a pair was individually marked, which allowed us to determine arrival and departure time for each individual parent. Because on some occasions (see Table 3) nest watches were carried out twice (each time using different nests) during the chick-rearing period, we tested for the effect of date on nest-attendance parameters. Date of observations did not affect the measured parameters significantly, so we combined the data for statistical purposes. Finally, we analyzed the data with Student's *t*-tests, where colony and year were used as factors.

At-sea observations. To determine the foraging distribution of adult birds at sea around the Pribilof Islands, we conducted surveys from 26 July to 17 August 1987 and from 28 July to 21 August 1988. At-sea observations were made twice during each year along 74.2 km-long transects radiating out from each island (Fig. 1). Continuous counts were made from the bridge of the RV 'Alpha Helix' while underway at 14.8 to 18.5 km h^{-1}. All birds within an arc from 300 m ahead of the ship to 90% off the side with the best visibility were counted, and data were entered into a hand-held computer. Birds were recorded as flying, on water, feeding, or ship-following; however, ship-following birds were excluded from the analyses presented here.

We calculated the densities of murres (common, thick-billed and unidentified) recorded on water and feeding, and black-legged kittiwakes recorded as flying, on water and feeding in 9.3 km 'bins' along a given transect. At this spatial scale, bird densities in adjacent areas were not statistically autocorrelated and can be considered independent samples (Decker 1995). To compare bird distribution between the colonies and reproductive seasons, we carried out ANOVA, where year, colony, and distance from island were used as factors.

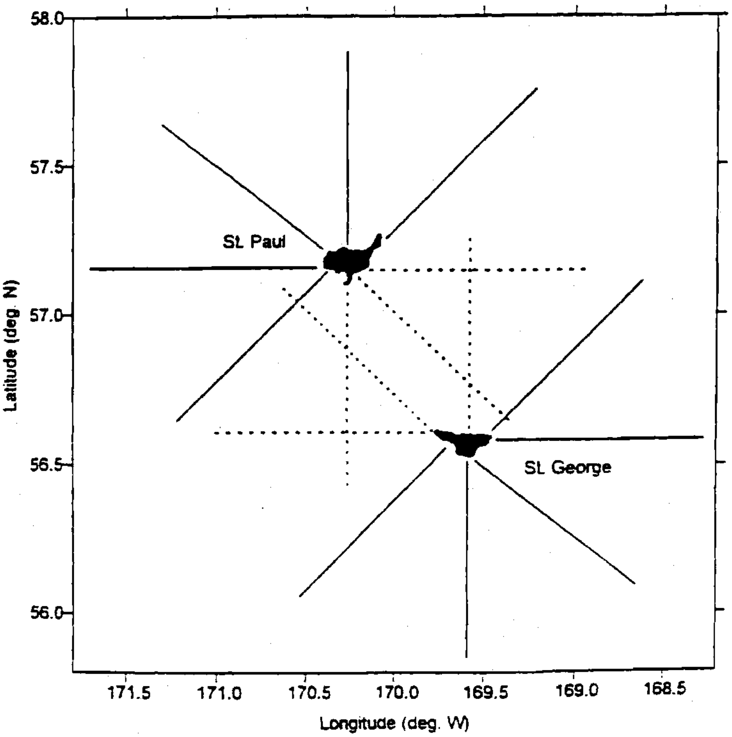

Fig. 1. Map of at-sea transects. Dotted lines = transects that overlap in relation to colony position and thus were excluded from statistical analyses

Chick growth rates. Chick growth rates were measured at each colony by weighing chicks every 3 to 4 d. For each chick, we estimated an increment in body-mass as a regression slope of mass on age during the linear phase of chick growth. The linear phase of body-mass increments occurs between 6 and 22 d post-hatch in the black-legged kittiwake (Barret & Runde 1980), and between 0 and 14 d post-hatch in the thick-billed murre (Gaston & Jones 1998). Accordingly, we restricted our analyses of chick growth to these periods. We restricted our analyses of chick growth to the nests that contained single-chick broods and considered each individual chick as a sample unit. In addition to examining variation in chick growth in 1987 and 1988, we also examined inter-annual and inter-colony variations in chick growth (data were collected as described above) for the time period 1976 to 1986. We analyzed these data as they help us to interpret the relationships between resource allocation and variation in chick growth that were observed in 1987 and 1988.

Statistical procedure. During the statistical analyses, data were tested for the assumptions required by a statistical test (according to Sokal & Rohlf 1981). If data violated assumptions for parametric tests, then their non-parametric equivalents were used. For all computations, we used the SYSTAT statistical package (Wilkinson 1992). Statistical significance was assumed if $p < 0.05$.

RESULTS

Breeding success

In both colonies in 1987 (lower food abundance for surface-foraging seabirds), black-legged kittiwakes *Rissa triclactyla* had lower reproductive success than in 1988 (higher food abundance) (Table 4). In 1987, breeding success was higher at the small colony on St. Paul Island than at the large colony on St. George Island. In 1988, breeding success was not significantly different between the colonies. Breeding success of thick-billed murres *Uria lomvia* did not vary inter-annually during 1987 and 1988, but in both years it was significantly higher among the birds nesting on St. George Island than for those on St. Paul Island (Table 4). Variation in breeding success was not related to predation. Nests at the sites studied were not vulnerable to arctic foxes *Alopex lagopus* and no avian predators were present in the study area in either year.

Field metabolic rate

During the 1987 breeding season when black-legged kittiwakes had low breeding success at St. Paul Island and completely failed in their breeding attempt at St. George Island, parents at St. Paul Island showed higher FMRs than in 1988 ($F_{1,21} = 14.431$, $p = 0.001$, Fig. 2). FMRs were similar between the colonies in 1988 ($F_{1,23} = 1.969$, $p = 0.174$, Fig. 2), when breeding success of these birds was high at both islands relative to historic values (Climo 1993, Dragoo & Dragoo 1994).

For thick-billed murres, FMRs of parent birds were similar between the 1987 and 1988 breeding seasons (Fig. 3) (year effect: $F_{1,33} = 1.60$, $p = 0.215$). However, FMRs of murres breeding at St. Paul Island were consistently higher than those of birds at St. George Island (Fig. 3) (colony effect: $F_{1,33} = 1352.38$, $p < 0.001$; colony × year interaction term: $F_{1,33} = 2.541$, $p = 0.120$).

Nest attendance

There were significant inter-seasonal differences in nest attendance of black-legged kittiwakes and thick-billed murres breeding on the Pribilof Islands in 1987 and 1988. For parent black-legged kittiwakes on St.

Table 4. *Rissa tridactyla* and *Uria lomvia*. Reproductive success (no. of nests where parents failed or fledged their young successfully) of black-legged kittiwakes and thick-billed murres nesting on the Pribilof Islands during 1987 and 1988. Statistically significant effects are shown in **bold**

Area	1987		1988		Inter-seasonal comparison	
	Failed	Fledged	Failed	Fledged	χ^2	p
Black-legged kittiwake						
St. George Island	24	0	29	34	**21.26**	**<0.0001**
St. Paul Island	254	120	60	108	**49.32**	**<0.0001**
Inter-colony comparison	**$\chi^2 = 11.02$**	**p <0.001**	$\chi^2 = 2.06$	p = 0.151		
Thick-billed murre						
St. George Island	125	252	69	126	0.28	0.594
St. Paul Island	395	397	159	158	0.01	0.932
Inter-colony comparison	**$\chi^2 = 28.90$**	**p <0.0001**	**$\chi^2 = 10.67$**	**p = 0.001**		

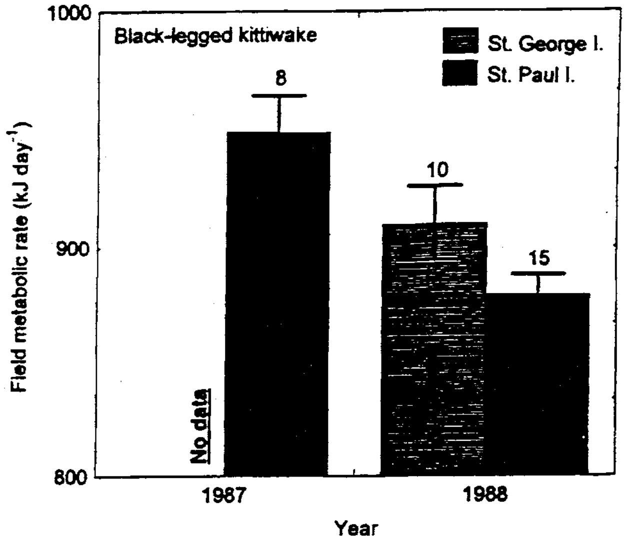

Fig. 2. *Rissa tridactyla*. Mass-independent field metabolic rates (means ± SE) of parent black-legged kittiwakes on the Pribilof Islands during 1987 (season of food shortages) and 1988 (season of abundant food for kittiwakes) reproductive seasons. Sample sizes shown above columns

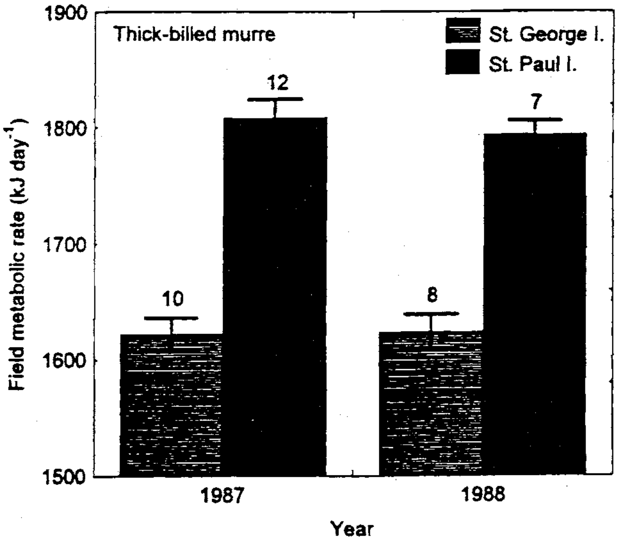

Fig. 3. *Uria lomvia*. Mass-independent field metabolic rates (means ± SE) of parent thick-billed murres on the Pribilof Islands during 1987 (season of abundant food for murres) and 1988 (season of food shortages) reproductive seasons. Sample sizes shown above columns

Paul Island, foraging trips were longer in 1987 (lower food abundance) compared to 1988 (higher food abundance) (Table 5). This difference resulted in a significant increase of time spent by parents in foraging in 1987, even though the number of foraging trips per parent per day were similar in the 2 years (Table 5). Also, in 1987 kittiwakes on St. Paul Island left their chicks unattended for a greater proportion of time than in 1988 (Table 5). In 1988, the parameters of nest attendance by parent kittiwakes were similar on St. Paul and St. George Islands (Table 5). It was not possible to measure foraging trips at St. George Island in 1987 because black-legged kittiwakes completely failed to fledge young at the large colony (Table 4).

Thick-billed murres rearing chicks on St. George Island in 1987 performed more foraging trips per day compared to those on St. Paul Island in 1988 and 1987 and on St. George in 1988 (see colony effect in Table 6). The frequency of foraging trips did not change inter-seasonally on St. Paul Island, which resulted in a significant colony × year interaction term as shown in Table 6. The duration of foraging trips was not significantly different between the 2 islands, and it was significantly shorter in 1987 than in 1988 (Table 6). Also, parent murres spent significantly less time loafing at the St. George colony during 1988 compared to 1987 and to the loafing time of murres on St. Paul Island in both years (colony effect in Table 6). In contrast to other parameters of nest attendance, the total time parent murres spent foraging was not significantly different between either colonies or years (Table 6).

Foraging distribution of birds

We found significant differences in the distribution of seabirds around the 2 islands. As expected, in 1988 black-legged kittiwakes were present in higher densities around St. George Island than St. Paul Island (colony effect as shown in Table 7 and Fig. 4). However, in 1987, kittiwake densities were similar around the 2 islands despite the higher number of birds breeding on St. George Island (a significant colony × year interaction term; Table 7, Fig. 4). Overall, more black-legged kittiwakes were present around St. George Island in 1988 than in 1987 (year effect; Table 7, Fig. 4). Although there was no difference in overall kittiwake abundance around St. Paul Island in 1987 and 1988, more kittiwakes were found within the innermost survey interval (0 to 9.3 km) of St. Paul Island in 1988 than in 1987 ($U = 311.0$, $p = 0.010$) (Fig. 4). The distribution of black-legged kittiwakes with respect to distance from colony showed no significant differences between either the 2 islands or years (colony × distance, year × distance, colony × year × distance interaction terms; Table 7, Fig. 4).

Foraging murres were more abundant around the larger colony on St. George Island than they were around the smaller colony on St. Paul Island in both 1987 and 1988, as expected (colony effect; Table 8, Fig. 5). However, murres from St. Paul Island tended to

Table 5. *Rissa tridactyla.* Nest attendance (means [SE]) patterns of parent black-legged kittiwakes on the Pribilof Islands n = number of nests. Statistically significant effects are shown in **bold**

Colony	Year	No. of foraging trips[a] (trips d^{-1})	Foraging trip duration[a] (min $trip^{-1}$)	Time chicks spent alone[b] (%)	Total foraging time[a] (min d^{-1})
St. Paul Island (n = 5)	1987	1.8 (0.35)	673.1 (131.59)	39.5 (5.10)	1050.2 (45.01)
St. Paul Island (n = 11)	1988	2.7 (0.30)	289.7 (23.42)	0.1 (0.08)	728.1 (7.89)
St. George Island (n = 5)	1988	3.2 (0.31)	235.9 (20.18)	0	718.1 (1.05)
Statistical tables:					
Factor		t p			
Inter-seasonal (St. Paul Island)		1.83 0.088	**$U = 53.0$ p = 0.004**	**$U = 55.0$ p < 0.001**	**$F = 129.4$ p < 0.001**
Inter-colony (for 1988)		0.82 0.428	$t = 1.42$ p = 0.176	$U = 25.0$ p = 0.500	$F = 0.694$ p = 0.419

[a] Mean for each nest was used as sample unit
[b] % of total time each nest was observed during which 2 parents present

forage at a greater distance from the colony than did murres from St. George Island (colony × distance interaction term; Table 8, Fig. 5). Within the 74 km radius around each island, the densities of feeding murres were similar during 1987 and 1988 (year effect; Table 8, Fig. 5). However, more murres were foraging within 9.3 km of St. George Island in 1987 than in 1988 ($U = 174.0$, p = 0.033, Fig. 5) but not at St. Paul Island (Fig. 5), which was reflected in a significant interaction term among colony, year and distance from colony (Table 8).

Chick growth rates during 1987 and 1988

Growth rates of black-legged kittiwake chicks were not significantly different between 1987 and 1988 on St. Paul Island ($F_{1,25} = 1.28$, p = 0.270, Fig. 6), or between the islands in 1988 ($F_{1,19} = 0.08$, p = 0.783, Fig. 6). As with foraging trip duration in 1987, we could not measure the growth rate of black-legged kittiwake chicks on St. George Island because black-legged kittiwakes failed in raising chicks at this large colony.

Growth rates of thick-billed murre chicks were higher in 1987 compared to 1988 (year effect, $F_{1,48} = 41.28$, p < 0.001, Fig. 7) at both colonies (year × colony interaction, $F_{1,48} = 0.01$, p = 0.932, Fig. 7). There were no differences in chick growth rates between the small and the large colonies ($F_{1,48} = 0.891$, p = 0.349, Fig. 7).

Inter-annual variation in chick growth during 1975 to 1988

Growth rates of black-legged kittiwake chicks varied inter-annually (CV [coefficient of variation] = 10.0%, n = 10) between 13.0 ± 0.61 (SE) g d^{-1} (in 1978 on St. George Island) and 16.7 ± 0.57 (SE) g d^{-1} in 1978 on St. Paul Island (Fig. 8).

Table 6. *Uria lomvia.* Nest attendance (means [SE]) patterns of parent thick-billed murres on the Pribilof Islands. n = number of nests. Statistically significant effects are shown in **bold**

Colony	Year	No. of foraging trips[a] (trips d^{-1})		Foraging trip duration[a] (min d^{-1})		Loafing time[b] (%)		Total foraging time[a] (min d^{-1})	
St. Paul Island (n = 7)	1987	2.5 (0.62)		272.9 (63.35)		6.6 (3.27)		503.6 (68.17)	
St. Paul Island (n = 8)	1988	2.9 (0.46)		261.1 (40.07)		5.3 (1.34)		654.7 (51.19)	
St. George Island (n = 18)	1987	5.9 (0.75)		162.0 (23.53)		7.8 (1.97)		766.1 (81.65)	
St. George Island (n = 16)	1988	2.9 (0.43)		325.6 (66.18)		2.6 (0.62)		641.9 (38.20)	
ANOVA tables:									
Factor	df	F	p	F	p	F	p	F	p
Colony	1, 45	**5.38**	**0.025**	1.57	0.216	**5.22**	**0.027**	2.19	0.146
Year	1, 45	3.20	0.080	**5.42**	**0.024**	0.21	0.646	0.02	0.896
Colony × Year	1, 45	**5.62**	**0.022**	2.51	0.120	2.47	0.123	2.48	0.123

[a] Mean for each nest was used as sample unit
[b] % of total time each nest was observed

Table 7. *Rissa tridactyla*. Densities of black-legged kittiwakes around the Pribilof Islands, based on surveys conducted along transects in Fig. 1. n = number of 9.3 km adjacent bins along all transects (see 'Material and methods'). Statistically significant effects are shown in **bold**

Year	Bird density (birds km^{-2})						
		St. George Island				St. Paul Island	
	Mean	SE	n		Mean	SE	n
1987	3.7	(0.45)	37		3.4	(0.38)	38
1988	8.2	(0.91)	40		3.5	(0.40)	40
Statistical tables:							
Factor		*F*		p		df	
Colony		**18.9**		**<0.001**		**1, 123**	
Year		**17.9**		**<0.001**		**1, 123**	
Distance		**3.0**		**0.006**		**7, 123**	
Colony × Year		**14.2**		**<0.001**		**1, 123**	
Colony × Distance		0.7		0.704		7, 123	
Year × Distance		1.4		0.209		7, 123	
Colony × Year × Distance		0.8		0.597		7, 123	

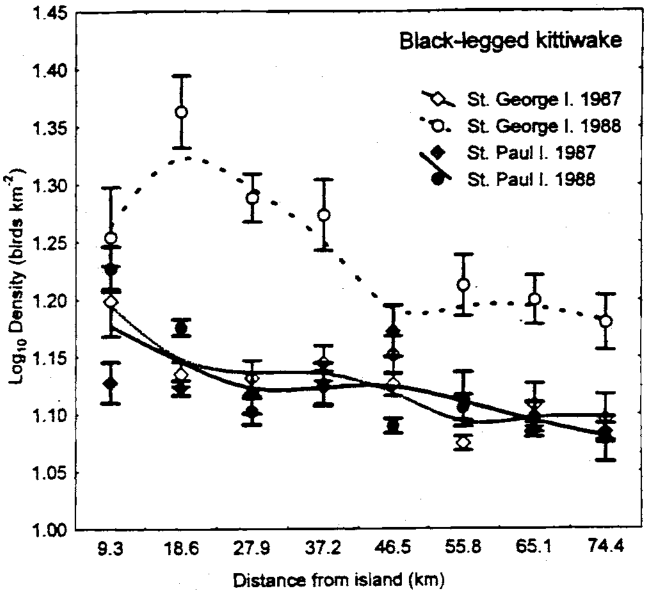

Fig. 4. *Rissa tridactyla*. Foraging distributions (means ± SE) in the vicinity of St. Paul and St. George Islands during 1987 and 1988. Each distance category is 9.3 km interval from each island (e.g. 9.3 on the graph = distance interval of 0 to 9.3 km from the island). Lines = linear regression slopes

Growth rates of thick-billed murre chicks varied inter-annually (CV = 25.4 %, n = 12) between 6.1 ± 0.37 (SE) g d^{-1} (in 1977 on St. George Island) and 13.1 ± 0.73 (SE) g d^{-1} in 1978 on St. Paul Island (Fig. 9).

DISCUSSION

The goal of this study was to determine how a change in food abundance influences the allocation of resources by parent seabirds. We used an integrative approach relating the reproductive, behavioral and physiological responses of ecologically distinct species of seabirds to variation in food abundance and colony size. We found that resource allocation of parent seabirds reflected changes in food resources in a species-specific fashion.

At the Pribilof Islands, parents of black-legged kittiwakes *Rissa tridactyla* and thick-billed murres *Uria lomvia* feed their young with similar types of food (Decker et al. 1995). However, food available to surface-foraging kittiwakes was more abundant in 1988 than in 1987, whereas food available to pursuit-diving murres was more abundant in 1987 than in 1988 (Coyle & Cooney 1993, G.L.H. unpubl. data summarized in Table 2). The results of our study of the reproductive biology and foraging distributions of kittiwakes and murres at the Pribilof Islands also suggest that food abundance and/or its availability varied in opposite ways for black-legged kittiwakes and thick-billed murres during 1987 and 1988.

Resource allocation of black-legged kittiwakes

Food resources available to foraging kittiwakes were more abundant in the vicinity of St. Paul Island in 1988 compared to 1987 (G.L.H. et al. unpubl. data, see Table 2). The results of this study suggest that during the year of less abundant food supply, parent black-legged kittiwakes expanded their foraging range around the large colony at St. George Island. We found that black-legged kittiwake densities in 1988 were greater around St. George Island than St. Paul Island. The breeding colony on St. George Island is an order of magnitude larger than that at St. Paul Island. Thus, the

Table 8. *Uria lomvia*. Densities of thick-billed murres around the Pribilof Islands, based on surveys conducted along transects in Fig. 1. n = number of 9.3 km adjacent bins along all transects (see 'Material and methods'). Statistically significant effects are shown in **bold**

Year	Bird density (birds km^{-2})						
	St. George Island				St. Paul Island		
	Mean	SE	n		Mean	SE	n
1987	46.1	(23.3)	37		3.5	(0.63)	38
1988	19.2	(4.51)	40		6.5	(1.59)	40
Statistical tables:							
Factor		*F*		p		df	
Colony		**49.1**		**<0.001**		**1, 123**	
Year		0.7		0.414		1, 123	
Distance		**19.9**		**<0.001**		**7, 123**	
Colony x Year		1.3		0.253		1, 123	
Colony x Distance		**7.9**		**<0.001**		**7, 123**	
Year x Distance		0.8		0.618		7, 123	
Colony x Year x Distance		**2.5**		**0.022**		**7, 123**	

at-sea distribution of birds around the islands reflected the abundance of birds breeding at the colonies. However, there was no difference in black-legged kittiwake densities around the islands in 1987. We also found that around St. Paul Island there was no overall difference in black-legged kittiwake densities between years. These results suggest that during the year of less abundant food supply, kittiwakes breeding on the large colony may have foraged at greater distances from the colony than during the year with good foraging conditions.

Earlier reported measurements of FMRs in free-living black-legged kittiwakes that were obtained with the double-labelled water technique (Gabrielsen et al. 1987) are similar to those measured in our study. In particular, Gabrielsen et al. reported daily energy expenditures of black-legged kittiwakes feeding chicks of between 596 kJ d^{-1} (for non-foraging birds) and 992 kJ d^{-1} (for foraging birds). The FMRs observed in our study are in the same range, from 831 kJ d^{-1} (for kittiwakes on St. Paul Island in 1988) to 903 kJ d^{-1} (for

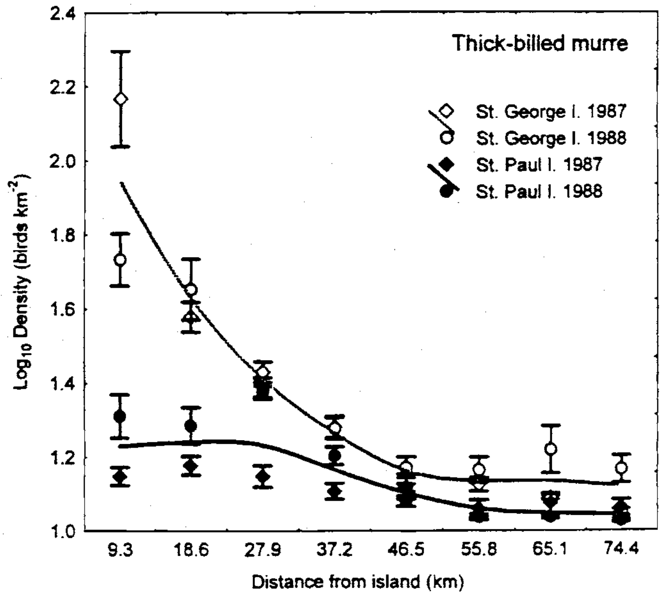

Fig. 5. *Uria lomvia*. Foraging distributions (means ± SE) around St. Paul and St. George Islands in 1987 and 1988. Thick-billed murres consitute approx. 90% of the murres breeding on these islands. Each distance category is 9.3 km interval from each island (e.g. 9.3 on the graph = distance interval of 0 to 9.3 km from the island). Lines = linear regression slopes

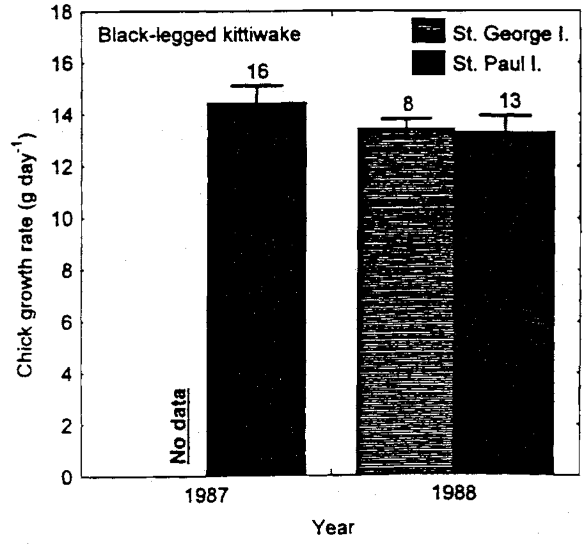

Fig. 6. *Rissa tridactyla*. Daily increments (means ± SE) of body mass of chicks on the Pribilof Islands during 1987 and 1988. Sample sizes shown above columns

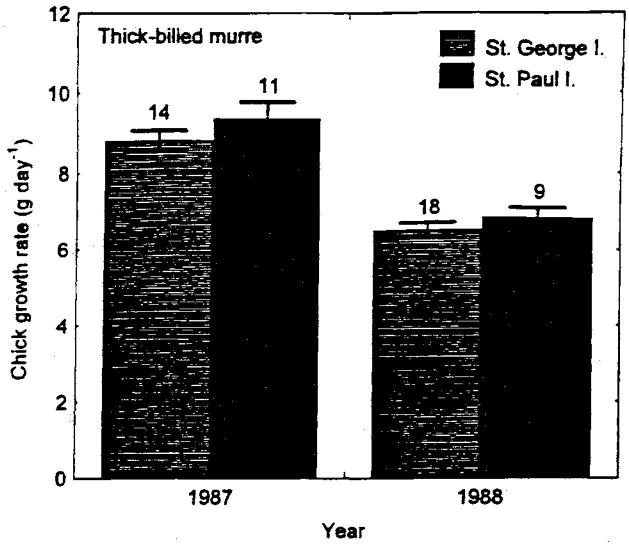

Fig. 7. *Uria lomvia*. Daily increments (means ± SE) of body mass of chicks on the Pribilof Islands during 1987 and 1988. Sample sizes shown above columns

kittiwakes on St. Paul Island in 1987). Therefore, we have confidence that our measurements were within a reasonable range for this species. We found a significant change in the FMRs of parent black-legged kittiwakes between the 1987 and 1988 reproductive seasons. Changes in FMRs and nest attendance of parent kittiwakes observed in this study were in parallel with the change in food availability. Furthermore, our analysis did not indicate a significant difference in kittiwake chick growth rates between the 1987 and 1988 breeding seasons on St. Paul Island. These findings imply that parent kittiwakes were able to compensate for food shortages in 1987 by allocating about 10% more energy to foraging per day.

In contrast to a significant inter-seasonal variation in FMRs of parent kittiwakes on St. Paul Island, we did not find a difference in the FMRs and nest attendance of black-legged kittiwakes breeding on St. Paul and St. George Islands in 1988. We suggest that, when food was abundant, kittiwakes from the small and large colonies allocated similar amounts of available resources to reproduction and achieved similar results, as reflected in the similarity of overall breeding success between the colonies in 1988. However, during food shortages in 1987, black-legged kittiwakes breeding in the large colony failed, whereas kittiwakes breeding in the small colony were able to raise their chicks successfully, suggesting that colony size (Hunt et al. 1986) and/or intrinsic differences in distribution of food resources around the colonies (Schneider & Hunt 1984) might have an important influence on the reproductive performance of these birds.

In black-legged kittiwakes, all parameters measured in this study point to a similar conclusion — when food is short, parents work harder, spend less time attending/guarding their chicks, and expand their foraging range in search for food. Similar changes in food availability might affect birds that breed in large colonies more strongly than those in small colonies. However, in this study we were able to compare only data from 2 colonies in 2 years. We could not and have not controlled for all factors affecting reproductive performance of black-legged kittiwakes. Thus, the importance of density-dependent factors for reproductive performance of kittiwakes remains to be shown.

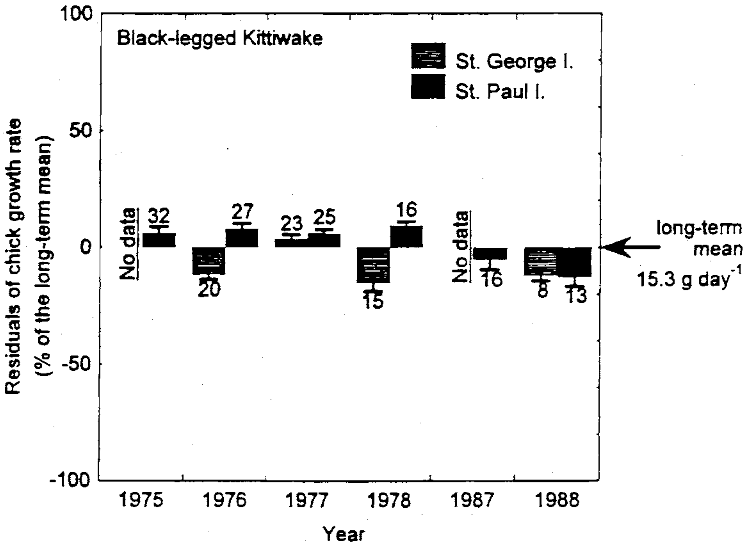

Fig. 8. *Rissa tridactyla*. Long-term variations in daily increments of chick body mass on the Pribilof Islands. 1987 and 1988 were 'poor' years for black-legged kittiwakes, especially on St. Paul Island

Resource allocation of thick-billed murres

In contrast to black-legged kittiwakes, food resources available to pursuit-diving murres were more abundant during 1987 compared to 1988 (Coyle & Cooney 1993, see Table 2). At-sea distributions indicate that murres foraged closer to St. George Island in 1987 than in 1988. We also recorded a longer duration of loafing time, more foraging trips per day, and shorter durations of foraging trips performed by parent thick-billed murres on St. George Island in 1987 than in 1988. This result also indicates that murres were foraging closer to the colony in 1987 than in 1988. Zador &

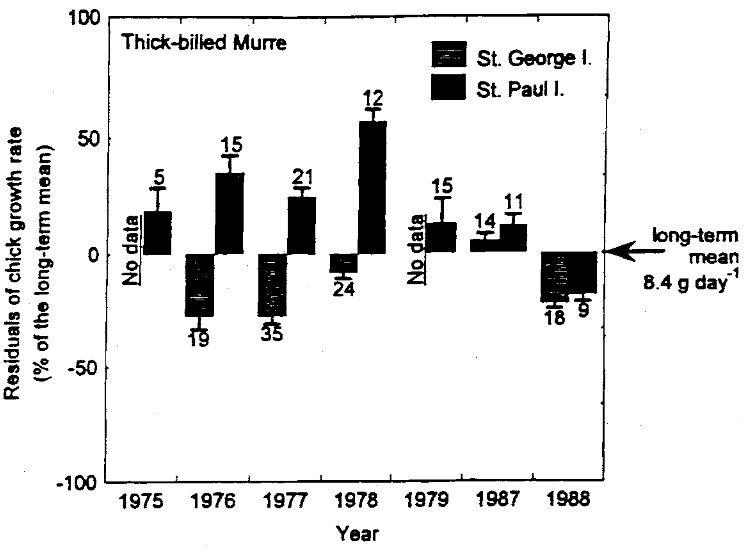

Fig. 9. *Uria lomvia*. Long-term variations in daily increments of chick body mass on the Pribilof Islands. 1988 was a 'poor' year for birds, especially on St. Paul Island, compared to the 1970s. Conversely, 1987 was an exceptionally 'good' year for birds on St. George Island

Piatt (1999) reported a similar result for the relationship between colony attendance and variation in food abundance in parent common murres *Uria aalge*. Thick-billed murres foraging closer to St. George Island in 1987 may explain why growth rates of chicks were higher in this year. One would expect growth rates of chicks to be higher if adults foraged closer to shore and were able to increase the delivery rates of food to the chicks. Comparisons of the amount of time parent thick-billed murres allocated to foraging showed no inter-annual and no inter-colony differences, suggesting that the growth rates of thick-billed murre chicks was determined mostly by variations in parental food provisioning rates.

The inter-seasonal change in food abundance did not affect the FMRs of parent thick-billed murres, suggesting that the reproductive effort of parent thick-billed murres was independent of inter-seasonal variation in foraging conditions. On the other hand, thick-billed murres breeding on St. Paul Island had higher daily energy expenditures than on St. George Island. Using a simple and robust model, Gaston (1985) predicted field metabolic rates of thick-billed murres breeding on Prince Leopold Island in relation to different estimates of foraging range and energy expenditure while foraging. Furthermore, predictions of this model were verified by direct measurments of FMRs of murres with doubly labelled water (A. Gaston unpubl. data, cited in Gaston 1985). Values predicted by this model are very similar to the measures of FMRs and foraging distributions of thick-billed murres observed in our study. According to the Gaston model (for birds spending 125 kJ h^{-1} while feeding), thick-billed murres with an FMR of 1623.4 kJ d^{-1} (as measured for birds nesting at St. George Island) could forage at a distance of about 4 km from nesting colonies. Thick-billed murres with an FMR of 1801.7 kJ d^{-1} as measured for birds at St. Paul Island) could forage at a distance of about 43 km from nesting colonies. These expected foraging distances generally match the observed foraging distribution of murres in our study. In particular, major densities of foraging murres were observed within a 10 km distance around St. George Island, and foraging murres were most abundant within 40 km distance around St. Paul Island (Fig. 5). These comparisons give us confidence in our FMR data, which suggest that thick-billed murres breeding on St. Paul Island invested approximately 12 % more energy in reproduction than those breeding on St. George Island.

The distributions of murres at sea shows that birds breeding on St. Paul Island foraged farther from the colonies than did the birds breeding on St. George Island. Schneider & Hunt (1984) suggested that birds from the 2 islands are exposed to different foraging habitats. In particular, birds on St. George Island have ready access to highly productive areas of the outer shelf of the Bering Sea and a relatively short commute to the shelf edge compared to birds on St. Paul Island. Larger distances between foraging grounds and colonies are in parallel with the higher FMRs of parent thick-billed murres breeding on St. Paul Island compared to St. George Island reported here. Perhaps the successful reproduction of thick-billed murres at the colonies on St. Paul Island requires an additional energy expenditure in foraging, which resulted in the increased energy expenditure of parent murres on St. Paul compared to St. George Island.

Another possible explanation for differences in FMRs found between murres on St. Paul and St. George Islands is that the measured differences might result from 'sampling error'. Several studies (deForest & Gaston 1996, Hipfner 1997, Hipfner & Gaston 1999) have shown that parent thick-billed murres nesting at the same colony differ in their quality and reproductive performance. Coulson & Porter (1985) reported similar differences in the quality of black-legged kittiwake parents nesting at North Shields in England. In our study, all birds were chosen randomly and, ideally, should have represented all individuals including those of poor, intermediate and high quality. However, the greater foraging distances that birds had to travel from the colonies of St. Paul Island could cause an early reproductive failure of poor-quality parents. Because we measured FMRs of birds feeding chicks, a majority of poor-quality parents could either have never laid eggs, or had already lost their eggs/chicks by the time of the FMR measurements. The proportion of good-quality birds sampled, which still retained their chicks, could be then relatively higher at St. Paul Island. Thus, our measurements of FMRs in thick-billed murres on St. Paul Island may represent reproductive investment of high-quality birds that could endure a significant increase in energy expenditures associated with large foraging distances. At the same time, murres breeding on St. George Island foraged in close vicinity to the island, and a difference in reproductive failure between poor- and high-quality parents may have been expressed less than on St. Paul Island. Consequently, at St. George Island we may have measured field metabolic rates of both poor- and high-quality parent thick-billed murres, which could have resulted in a lower average FMRs among thick-billed murres breeding on St. George Island than on St. Paul Island. The finding that larger proportions (Climo 1993, Dragoo & Dragoo 1994) of thick-billed murres lost their eggs on St. Paul Island (36% in 1987, 37% in 1988) than on St. George Island (25% in both years) provides indirect evidence for this hypothesis. It is not known whether poor- and high-quality parent thick-billed murres differ in daily energy expenditures during chick-rearing, and this question requires thorough investigation.

Parent murres do not provide food at a rate that would support chick maximal growth and development (Harris & Wanless 1995, Kitaysky 1996). On the other hand, it is also likely that parent murres do not provide food at a rate lower than some lowest possible rate, which would still allow a chick to survive and fledge successfully. A cost to a parent murre of keeping such a rate of food provisioning might vary with fluctuations in foraging conditions, and could reflect the longer foraging distances of parent thick-billed murres breeding on St. Paul Island compared to those on St. George Island. Our analysis of long-term variability of growth of thick-billed murre chicks at the Pribilof Islands provides indirect evidence for this. In particular, the maximal chick growth rate was 60% above the long-term mean (8.4 g d^{-1}), whereas the minimal chick growth rate was only 25% below the long-term mean (Fig. 9).

Reproductive strategies

Fluctuations in food abundance influence different parameters of reproductive performance in black-legged kittiwakes and thick-billed murres, which should be taken into account during ecological studies of these birds. In particular, the results of our study show that parent black-legged kittiwakes are able/willing to compensate for food shortages by expanding their foraging ranges and investing more energy in reproduction. This parental strategy results in a relatively low inter-seasonal variation of chick growth rates (Fig. 8). For example, the daily increment of captive black-legged kittiwake chicks fed ad libitum varied between 13.3 g d^{-1} (M. Romano unpubl.) and 14.1 g d^{-1} (Kitaysky & Belogubova 1988). These values are within the range of growth rates of wild black-legged kittiwake chicks recorded in our study (Fig. 6). Thus, when food availability varies, parent kittiwakes adjust their provisioning efforts to compensate for these variations. Similar evidence for low variation in chick growth rates were reported in a long-term study of black-legged kittiwakes in England (Coulson & Porter 1985) and among tropical seabirds feeding their young during food shortages (Shea & Ricklefs 1996), suggesting that this parental strategy is not confined to black-legged kittiwakes nesting at the Pribilof Islands.

The functional importance of the food-provisioning strategy of black-legged kittiwakes is not known. Several explanations seem possible. First, kittiwake chicks are physiologically sensitive to variations in daily food intake and respond to moderate food shortages by a chronic elevation of stress hormones (Kitaysky et al. 1999). Chronic elevation of stress hormones is known to suppress memory and immune systems, promote wasting of muscle tissue, and cause neuronal cell death (Sapolsky et al. 1986, Sapolsky 1992, also reviewed in Wingfield 1994). Impaired physiological conditions of under-nourished black-legged kittiwake chicks might decrease their chances of survival. This would force their parents to provide food on chick demand (Kitaysky et al. 1999). Second, the food-provisioning strategy of parent black-legged kittiwakes may be phylogenetically determined. Although pelagic-foraging kittiwakes rely on more variable food resources compared to their inshore foraging counterparts, chick-provisioning behavior of kittiwakes is still similar to that of other species of gulls (e.g., Harris 1970, Henderson 1975, for references see also Ydenberg & Bertram 1989).

In contrast to kittiwakes, parent murres seem to provision young with food in accordance to variations in foraging conditions rather than in response to chick demands (Houston et al. 1996, and references therein). This parental strategy results in large fluctuations of chick growth rates (Fig. 9) and probably in a variable duration of chick development in the nest (Houston et al. 1996). In particular, Gaston & Nettleship (1981) and Hunt et al. (1986) have shown that the duration of murre chick development in the nest is shorter in large colonies than in small colonies. These authors hypothesized that if depletion of food resources occurs faster in the vicinity of a large colony compared to a small colony, parent murres can avoid some of the negative effects of food shortages on their reproduction by fledging their chicks and moving with them to regions of the ocean where food is abundant. Hunt et al. (1986) provided correlative evidence for this hypothesis by comparing fledging age of thick-billed murre chicks between St. Paul and St. George Islands. When food for parent thick-billed murres is scarce, the short duration of their chick development and a reduced daily energy expenditure in feeding their young would preserve available resources and probably increase post-breeding survival of those parents.

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Dietary restriction causes chronic elevation of corticosterone and enhances stress-response in Red-legged Kittiwake chicks.

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Abstract

Release of corticosterone in hungry kittiwake chicks facilitates begging and allows them to restore depleted energy reserves by increasing parental food provisioning. However, in order to avoid detrimental effects of chronic elevation of corticosterone, chicks might suppress adrenocortical activity in response to prolonged food shortages. In this study we examined temporal dynamics of corticosterone release in Red-legged Kittiwake (*Rissa brevirostris*) chicks exposed to prolonged restrictions in energy content and/or nutritional quality (low vs. high lipid content) of their food. Starting at the age of 15 days, chicks were fed either high or low lipid fish at 40, 65 and 100% of *ad libitum* energy intake. Body mass measurements and baseline plasma samples were taken on a weekly basis after beginning of the treatment. After three weeks of treatment, chicks were exposed to a standardized acute handling and restraint stress protocol, where in addition to a baseline sample, three plasma samples were taken at intervals up to 50 minutes. We found that food-restricted chicks had lower body mass, chronically (during 2-3 weeks) elevated baseline and higher acute stress-induced levels of corticosterone compared to chicks fed *ad libitum*. Low lipid content of food further exacerbated these effects. An increase in baseline levels of corticosterone was observed within a week after energy requirements of food-restricted chicks exceeded their daily energy intake. A tendency for suppression of adrenocortical activity was observed in treatments fed low-lipid diets only at the end of the experiment. We suggest that food-stressed, nest-bound, chicks might suffer deleterious effects of chronic elevation of corticosterone in addition to impaired growth and development.

Keywords: corticosterone, food-stress, nest-bound chicks, seabirds

Introduction

Nest-bound chicks of several species of seabirds respond to food shortages by increasing corticosterone secretion (e.g. Nunez-de la Mora et al. 1996 for Blue-footed Boobies, *Sula nebouxii*; Kitaysky et al. 1999a for Black-legged Kittiwakes, *Rissa tridactyla*). Release of corticosterone (at concentrations similar to baseline levels of food-restricted individuals and/or stress-induced levels of corticosterone in individuals fed *ad libitum*) in hungry kittiwake chicks allows them to restore depleted energy reserves by facilitating begging and thereby increasing parental food provisioning (Kitaysky et al., in press). However, chronic elevation of corticosterone at stress-induced levels can be detrimental (Sapolsky et al. 1986; Sapolsky 1992; recent review in Welberg and Seckl 2001). In order to avoid possible detrimental effects of chronic elevation of corticosterone, nest-bound seabird chicks might suppress adrenocortical activity in response to prolonged food shortages.

As a group, seabirds have evolved multiple behavioral and physiological traits allowing them to cope with high variability in food resources (Lack 1968). It might be beneficial for seabird chicks to decrease corticosterone secretion in response to prolonged food shortages. Seabird parents are not always able (or willing) to compensate for a decrease in food availability by increasing their effort in foraging for the young and chick provisioning with food may remain low for extended periods of time (e.g., Kitaysky 1996; Kitaysky et al. 2000). When this is the case, seabird chicks are able to adjust their developmental rate to the rate of energy provisioning by parents and fledge successfully despite severe food shortages (Kitaysky 1999). It is not known whether reduced production and/or release of corticosterone is characteristic for slowly developing under-nourished seabird chicks, but a recent study of the Domestic Turkey (*Meleagris gallopavo*) suggests that a long-term (four weeks) nutritional stress might cause a diminished adrenocortical activity (Carsia and McIlroy 1998). A suppression of adrenocortical response to an acute stressor was also documented in adult Black-legged Kittiwakes breeding during long-term food shortages (Kitaysky et al. 1999b). Elevated secretion of corticosterone can change metabolic pathways so that nutritionally stressed individuals rely on catabolism of proteins to fuel their activities (Axelrod and Reisine 1984; Le Ninan et al. 1988; Cherel et al. 1992). As a short-term benefit, a reduced production of corticosterone would allow under-nourished seabird chicks to avoid loss of skeletal muscle proteins. As a long-term benefit, a reduced production of corticosterone would allow slowly developing individuals to elude lasting detrimental effects of chronically increased corticosterone such as a suppressed immune system and neuronal cell death (Sapolsky et al. 1986; Sapolsky 1992; also reviewed in Sapolsky, Romero and Munck 2000).

The temporal dynamics of corticosterone release in response to prolonged food shortages are not well studied. In the King Penguin, *Aptenodytes patagonica*, 3-4 months old chicks are able to tolerate 100 days of natural winter fast without a substantial increase in corticosterone secretion (Le Ninan et al. 1988). However, in seabirds that provision their chicks with food in a continuous manner, elevated levels of corticosterone in chicks were documented within two days of fasting (Nunez-de la Mora et al. 1996), and after three weeks of moderate food restriction (Kitaysky et al. 1999a). Although increased corticosterone secretion was shown to be strongly negatively correlated with endogenous fat reserves at the end of three week food restriction in kittiwake chicks (Kitaysky et al. 1999a), it remains to be shown whether nest-bound seabird chicks experience chronic elevation of corticosterone during prolonged food-shortages.

The functional relationship between diet composition and the physiological condition of young seabirds can be measured using well-characterized responses of adrenocortical hormones to stress (Kitaysky et al. 1999a). Controlled experiments have shown that food-related stress can account for the lower body mass, depleted fat reserves, and elevated baseline levels of corticosterone of the young at fledging when compared to the young raised on *ad libitum* nutritional regimes (Boag 1987; Kitaysky 1999; Kitaysky et al. 1999a; Romano 2000). If baseline levels of corticosterone appear normal, the pattern and extent of corticosterone secretion following the application of a standardized stressor, such as capture, handling and restraint, indicates increased potential for stress (Wingfield 1994). For example, in Black-legged Kittiwake chicks, prolonged dietary restrictions enhance adrenocortical response to a standardized stressor even though baseline levels of corticosterone appear normal (Kitaysky et al. 1999a).

In this study we examined the relationship between energy intake, diet quality, baseline levels of corticosterone and standardized acute stress-response (*sensu* Wingfield et al. 1992) in captive Red-legged Kittiwake (*Rissa brevirostris*) chicks. The major goal of this study was to assess temporal dynamics of corticosterone release in relation to the long-term alterations in quality or energy content of diet and short-term energy demands of growing chicks. For comparison with a natural system, we also measured adrenocortical response of wild Red-legged Kittiwake chicks exposed to a standardized stressor.

Methods

Partially incubated eggs of free-living Red-legged Kittiwakes were collected on St. George I. in the south-eastern Bering Sea and transported to the University of Washington facilities. Eggs were incubated using Lyon incubators at 37.2°C and 56% relative humidity until hatching. Chicks were raised in individual nests at 30°C and 80% relative humidity (newly-hatched to 10 days post-hatch), at 25°C and 70% relative humidity (10 to 25 days post-hatch), and at outdoor ambient temperatures and humidity until fledging (42-55 days post-hatch). Chicks were hand-fed a mixture of high-lipid forage fish given *ad libitum* until the experiment. Starting at the age of 15 days post-hatch, either low quality fish, rainbow smelt, *Osmerus mordax,* (lipid to protein ratio, LPR=0.61, hereafter called "low-lipid diet"), or high quality silverside, *Menidia menidia* (LPR=1.47, hereafter called "high-lipid diet") were fed to chicks for 21 days (Table 1). The energy content of silverside was calculated from the results of proximate analyses of whole fish (for details of the analysis see Romano 2000 and Kitaysky et al. 1999a). The energy content of rainbow smelt was also calculated from the results of proximate analyses of whole fish (as reported by a commercial provider Shoreline Inc.). Chicks received either 565.1 kJ day^{-1} of high-lipid diet (80 gram day^{-1}, wet fish biomass), 353.2 kJ day^{-1} of high-lipid diet (50 gram day^{-1}), 356.3 kJ day^{-1} of low-lipid diet (80 gram day^{-1}), or 222.7 kJ day^{-1} of low-lipid diet (50 gram day^{-1}). Daily food intake of seven to eight week old captive Red-legged kittiwake chicks (n=5) fed high-lipid diet *ad libitum* was on average 83.9±12.54 (SD) gram day^{-1} (Kitaysky et al. unpublished). Therefore, in this study chick energy intake was at ~40, 65 and 100% of the *ad libitum* ration. To control for possible effects of dietary restrictions other than lipids to protein ratio and daily energy intake, all chicks received an excess of multi-vitamin/mineral supplement (one tablet of USP quality Kirkland Signature on daily basis) in their food. To control for possible effects of captivity on the stress-response of the experimental birds, free-living Red-legged Kittiwake chicks (similar to the experimental birds' ages) were taken from their nests at St. George I. and exposed to handling stress, and blood samples were taken as described below.

Body mass of post-absorptive chicks was measured every five days. To compare chick's energy requirements with its daily energy intake during treatment, we used the equation [$\log_{10}$Resting metabolic rate (kJ day^{-1}) = 0.87+0.70•$\log_{10}$Body mass (gram, wet)] experimentally derived for growing post-absorptive seabird chicks (Kitaysky 1999). Metabolizable energy of forage fish used in this study was assumed at 87% of total energy intake, as determined earlier for growing Black-legged Kittiwake chicks (Romano 2000).

A blood sample of undisturbed (collected immediately after taking a chick from the nest) post-absorptive (after overnight fast) chicks was taken on weekly basis starting at the beginning of the experiment. Blood samples were collected by puncturing the alar vein and collecting blood in 100μl heparinized hematocrit tubes. At the end of the experiment, post-absorptive chicks were exposed to a standardized acute handling and restraint stress protocol, where in addition to the first blood sample (baseline) three blood samples (acute stress response) were taken at intervals of 10, 30 and 50 minutes after capture. All captive and wild chicks were bled between 10.00-14.00 hr according to the following standardized technique. After each sample, blood flow was stopped by the application of cotton, and birds were placed individually into cloth bags. After blood collection, hematocrit tubes were emptied into 0.5 ml vials, which were stored on ice (in the field) or in a refrigerator at 4°C (in the laboratory).

Blood samples were centrifuged and plasma collected within 6 hours.

Plasma samples were frozen at -20°C until radioimmunoassay analyses (for details see Wingfield and Farner 1975, Wingfield et al. 1992). The concentrations of corticosterone in plasma samples were measured from 20 μl of plasma. Steroid concentrations were measured in duplicate for each sample after extraction in 4 ml of dichloromethane. Recovery values (ranging from 85 to 97%) following extraction were used to adjust assayed concentrations of steroids. All baseline plasma samples were processed in one assay, and all plasma samples collected during stress-series were processed in the second assay. Intra- and inter-assay coefficients of variation were 5% and 8%, respectively.

All chicks survived the experiment, were fed *ad libitum* after dietary restriction was lifted, and two months after fledging were transferred to Alaska SeaLife Center (Seward, Alaska).

Statistical analyses

Body mass and baseline levels of corticosterone prior to the experiment were tested with one-way ANOVA, with treatment as a factor. The effects of the experimental treatments on body mass of chicks at the end of treatment were examined with two-way ANOVA (foolowed by Tukey Post Hoc test for pairwise comparisons), where daily energy intake and quality of food (LPR) were used as factors. The effects of daily energy intake and quality of the food on temporal dynamics of baseline levels of corticosterone were examined with repeated measures two-way ANOVA, where daily energy intake and LPR were used as factors and a duration of treatment as a repeated measure. The effects of the experimental treatments on acute stress-induced levels of corticosterone were examined with repeated-measures ANOVA, where experimental treatments were used as factors and samples obtained from the same chick during the acute stress protocol as repeated measures. The effects of chicks' body mass on baseline levels of corticosterone and stress response to handling (expressed as a maximal level of corticosterone achieved during 50 min of restraint) were examined by using regression analyses.

Wild chicks were infested with hard ticks (*Exodes sp.*) making a statistical comparison with not-infested captives invalid. An effect of tick infestation on baseline levels of corticosterone in wild chicks was examined by using linear regression analysis where number of ticks (found feeding on the individual chick) was used as an independent variable.

During statistical analyses, initial data were tested for assumptions required by parametric statistical tests according to Sokal and Rohlf (1981). If these data violated assumptions they were $\log_{10}$-transformed and examined again. All computations were performed by using SYSTAT statistical package (Wilkinson 1992). Statistical significance was assumed at $p<0.05$.

Results

Body mass and baseline levels of corticosterone were similar among treatments prior to the experiment ($F_{3,17}=0.15$, $p=0.930$ for body mass, Fig. 1; and $F_{3,17}=1.73$, $p=0.199$ for baseline corticosterone, Fig. 2 upper panel).

Effects of food-stress on growth of body mass

The diets significantly affected body mass of chicks at the end of treatment (Daily food intake effect: $F_{1, 17}$=282.10, p<0.0001; LPR effect: $F_{1, 17}$=547.70, p<0.0001, Table 1). Also, there was a significant effect of interaction between daily food intake and quality (LPR) of food on chick's body mass ($F_{1, 17}$=13.58, p<0.002). In particular, chicks that received 356.3 kJ day^{-1} of low-lipid diet were significantly lighter at the end of treatment compared to chicks fed 353.2 kJ day^{-1} of high-lipid diet (Tukey Post Hoc test: p=0.001; Fig. 1).

Daily energy requirements vs. daily energy intake

Energy requirements of chicks in the different treatments exceeded their energy intake at different times after the beginning of treatment (Fig. 2, low panel). In particular, metabolizable energy of 50 gram•day^{-1} low-lipid diet was lower then chick's energy requirements immediately after beginning of treatment (Fig. 2, low panel). Energy requirements of chicks fed either 80 gram•day^{-1} of low-lipid or 50 gram•day^{-1} of high-lipid diets first exceeded metabolizable energy of their food during the second week of treatment (Fig. 2, low panel). In contrast, metabolizable energy of 80 gram•day^{-1} high-lipid diet exceeded energy requirements of controls during the treatment (Fig. 2, low panel).

Effects of food-stress on temporal dynamics of baseline levels of corticosterone

The experimental treatments had a highly significant effect on baseline levels of corticosterone during treatment (Daily food intake effect: $F_{1, 17}$=14.26, p=0.002; LPR effect: $F_{1, 17}$=51.27, p<0.001; Daily food intake x LPR interaction term: $F_{1, 17}$=0.212, p=0.651, Fig. 2 upper panel).

Baseline levels of corticosterone were significantly elevated during the experiment in all food-restricted chicks compared to controls (Treatment duration effect: $F_{2,34}$=11.39, p<0.001, Fig. 2 upper panel). However, the elevation in baseline levels of corticosterone was significantly higher in chicks that were fed low-lipid diet than in chicks that were fed high-lipid diet (Treatment duration x LPR interaction term: $F_{2,34}$=5.04, p=0.012, Fig. 2 upper panel). Also, an elevation of baseline levels of corticosterone appeared significantly earlier in chicks that received 50 g day-1 of low-lipid diet compared to chicks that were fed either 80 g day-1 of low-lipid or 50 g day-1 of high-lipid diets (Treatment duration x LPR x Daily food intake interaction term: $F_{2,34}$=3.71, p=0.035, Fig. 2 upper panel). In particular, elevation in baseline levels of corticosterone occurred during the first week in the most restricted (50 g day-1 of low-lipid diet) treatment, and during the second week in the two other food-restricted treatments (Fig. 2 upper panel).

A decrease in baseline levels was observed between day 14 and day 21 of the experiment in the low-lipid treatments but not in the high-lipid treatments (repeated measures ANOVA on baseline levels at days 14 and 21 of treatment; Treatment duration x LPR interaction term: $F_{1,18}$=7.7, p=0.012, Fig. 2 upper panel).

Acute stress-induced levels of corticosterone in captive chicks

All captive chicks responded to an acute standardized stressor with a rapid increase in secretion of corticosterone (Fig. 3 left panel).

Captive chicks fed low-lipid diet responded to acute stress significantly faster and achieved significantly higher levels of corticosterone than the chicks fed high-lipid diet (Fig. 3 left panel;

LPR effect: $F_{1,17}=19.46$, $p<0.001$; Acute stress effect: $F_{3,51}=12.29$, $p<0.001$; Acute stress x LPR interaction term: $F_{3,51}=3.48$, $p=0.028$). The adrenal responses were statistically indistinguishable among the captive chicks that were fed either 50 or 80 g day^{-1} of the same diet (Fig. 3 left panel; Daily food intake effect: $F_{1,17}=2.22$, $p=0.155$; Daily food intake x LPR interaction term: $F_{1,17}=1.39$, $p=0.255$; Acute stress x Daily food intake x LPR interaction term: $F_{1,17}=1.76$, $p=0.174$).

Baseline and acute stress-induced levels of corticosterone in wild chicks

Degree of infestation with hard ticks was significantly positively correlated with baseline levels of corticosterone in wild Red-legged Kittiwake chicks ($R^2=0.926$, $F_{1,5}=62.8$, $p=0.001$, Fig. 4).

All wild chicks responded to a standardized stressor by an increased secretion of corticosterone (Fig. 3 right panel), but the adrenal response of five chicks infested with ticks tended to be higher than that of two chicks with no ticks (Fig. 3 right panel).

Body mass and circulating levels of corticosterone

Among the captive chicks the baseline and maximal acute stress-induced levels of corticosterone were significantly negatively correlated with chick body mass ($R^2 = 0.29$, $F_{1,19}=7.6$, $p=0.013$ and $R^2 = 0.25$, $F_{1,19}=6.46$, $p=0.02$ for the baseline and maximal levels, respectively).

Discussion

In this study we examined whether long-term experimental variations in energy content and quality of food result in a chronically elevated corticosterone secretion in Red-legged Kittiwake chicks.

We found that nutritionally stressed chicks had lower body mass and chronically higher baseline levels of corticosterone than chicks fed high-lipid food *ad libitum*. Baseline levels of corticosterone were elevated for a longer period of time (three weeks) in chicks that were restricted in both quantity and quality of food, and for shorter period (two weeks) in chicks that were fed either reduced quantity or quality of food. Timing of an increase in baseline levels of corticosterone in general reflected the time at which energy demands of food-restricted chicks exceeded their energy intake. In particular, metabolizable energy of low-lipid diet given at 50 gram day-1 was lower then chick's energy requirements immediately at the beginning of treatment (Fig. 2, low panel), and a significant increase of baseline levels of corticosterone was observed during first week of the experiment (Fig. 2, upper panel). Similarly, energy requirements of chicks fed 80 g day-1 of low-lipid and 50 g day-1 of high-lipid diets exceeded metabolizable energy of their food during second week of the experiment and an increase in baseline levels of corticosterone was observed during the second week of treatment (Fig. 2 upper panel). In contrast, metabolizable energy of high-lipid diet given *ad libitum* exceeded energy requirements of control chicks throughout treatment, which was reflected in consistently low baseline levels of corticosterone of those chicks (Fig. 2 upper panel). We conclude that increased secretion of corticosterone in kittiwake chicks reflects negative energy balance experienced by those chicks. Increased secretion of corticosterone probably allows under-nourished chicks rely on protein catabolism to fuel their increased activity levels (e.g. begging rates and aggression [Kitaysky et al. in press; Kitaysky et al., unpublished]) during food shortages.

The effects of chronic elevation during early neonatal development of birds are poorly known. However, chronic elevation of corticosterone during prenatal development has long-term deleterious effects on the affected mammals (reviewed in Welberg and Seckl 2001). Specifically, chronic elevation of corticosterone (at concentrations similar to stress-induced levels) is known to suppress memory and immune systems, promote wasting of muscle tissue, and cause neuronal cell death (Sapolsky et al. 1986, Sapolsky 1992; also reviewed in Wingfield 1994; Sapolsky et al. 2000). It is not known whether the observed chronic elevation of baseline levels of corticosterone in food-restricted kittiwake chicks was high enough to cause long-term deleterious effects. However, the baseline levels of corticosterone observed in low-lipid diet treatments at the end of second week of the experiment were similar to maximal levels of corticosterone achieved during acute stress procedure by controls. Thus, the observed chronic elevation of corticosterone was potentially damaging and could cause long-term deleterious effects on food-restricted kittiwake chicks. We recently confirmed this prediction by showing an impaired cognition of young kittiwakes exposed to nutritional stress or/and moderate chronic elevation of corticosterone during early development (Kitaysky et al. unpublished).

At the end of food-restriction period, baseline levels of corticosterone decreased in chicks fed low-lipid diet. A physiological mechanism(s) that would allow chronically stressed chicks to reduce baseline levels of corticosterone is not known. It is possible that either clearance rate of plasma corticosterone was higher among chicks on low-lipid diet, or long-term nutritional stress reduced adrenal capacity or adrenal sensitivity (e.g. Rees et al. 1985; Romero, Soma and Wingfield 1998). However, although baseline levels of corticosterone decreased between second and third week of experiment in low-lipid dietary treatments, they were still higher than in controls. Moreover, chicks on low-lipid dietary treatments also had stronger and quicker adrenocortical stress-response to a standardized acute stressor than controls. Therefore, it is unlikely that either adrenal capacity or sensitivity of chronically food restricted Red-legged Kittiwake chicks were impaired. Regardless of causal factors, a temporal decrease in baseline levels of corticosterone in nest-bound kittiwake chicks exposed to prolonged food shortages might represent a mechanism that allows affected individuals to ameliorate deleterious effects of chronically high concentrations of corticosterone.

Among food-restricted Red-legged Kittiwake chicks, low-lipid diet enhanced adrenocortical function compared to high-lipid diet. In particular, diets that were iso-caloric but different in nutritional quality and fed to chicks at 65 % of *ad libitum* energy intake, resulted in higher baseline and acute stress-induced levels of corticosterone among chicks raised on the low-lipid diet compared to those among chicks raised on the high-lipid diet. Similar results were obtained in an experimental study of Black-legged Kittiwake chicks (Kitaysky et al. 1999a). Several studies of birds have established that the amount of metabolizable energy is positively correlated with lipids to proteins ratio of the diet (e.g. Buchsbaum et al. 1986, Castro et al. 1989, Romano 2000). Romano (2000) has found that Black-legged Kittiwake chicks are able to metabolize ~12% more energy from high-lipid compared to low-lipid forage fish and, as a result, they are able to accumulate large fat deposits if fed high-lipid diets. Baseline and acute stress-induced levels of corticosterone reflect total body lipids in kittiwake chicks (Kitaysky et al. 1999a), thus, it is not surprising that in this study we found lower levels of corticosterone in Red-legged Kittiwake chicks fed high-lipid diet (relatively high body mass) compared to chicks fed low-fat diet (low body mass).

The results of this and earlier studies (Nunez-de la Mora et al. 1996; Kitaysky et al. 1999a) suggest that low endogenous energy reserves in nest-bound semi-precocial seabird chicks cause an elevation in baseline levels of corticosterone and enhance corticosterone secretion following the application of a standardized stressor. This is in contrast to observations of chicks and juveniles of altricial species of birds, where a relationship between body condition and corticosterone secretion was not found (Romero et al. 1998; Schwabl 1999; Sims and Holberton 2000). It has been suggested that development of the hypothalamic-pituitary-adrenal axis and consequently a chick's ability to respond to acute stressors is associated with chick developmental modes (Schwabl 1999; Sims and Holberton 2000). Specifically, the hypothalamic-pituitary-adrenal axis probably becomes functional later in a chick's life in altricial compared to precocial species of birds. This would explain the discrepancy in the results obtained for different groups of birds.

Among birds that have precocial young, such as the Domestic Fowl (*Gallus gallus domesticus*) and the Mallard (*Anas platyrhynchos*), the baseline plasma concentration of corticosterone in chicks fed *ad libitum* does not change significantly during the first four weeks after hatching (reviewed in Holmes et al. 1990). In mallard chicks fed *ad libitum*, a restraint for 30 min caused a significant increase in corticosterone concentration and the adrenal stress response was stronger in the chicks during first two days post-hatch than between three to four weeks of post-hatch (Holmes et al. 1990). Similarly, we did not find a significant change in baseline levels of corticosterone among Black-legged and Red-legged Kittiwake chicks fed *ad libitum* between two and five weeks of postnatal life (Kitaysky et al. 1999a, this study). Thus, it is likely that hypothalamic-pituitary-adrenal axis of precocial and semi-precocial chicks is fully developed shortly after hatching. However, studies of adrenocortical stress response of semi-precocial seabird chicks at early ages are needed to confirm this prediction.

Adrenocortical responses of precocial and semi-precocial chicks to nutritional stress and depleted energy reserves are not uniform among different species. Freeman and co-authors (1981) found that reducing food intake to 75% of *ad libitum* causes an increase in plasma corticosterone concentration after one week of restriction in the precocial chicks of domestic fowl. Increased levels of corticosterone were also recorded in protein-restricted young of the domestic chicken in vivo, and ACTH challenged adrenal steroidogenic cells in vitro (McIlroy et al. 1999, Carsia and Weber 2000). In semi-precocial nest-bound chicks of Blue-footed Boobies, a short-term food deprivation results in an increase of baseline levels of corticosterone, which was associated with a low body mass (Nunez-de la Mora et al. 1996). In nest-bound chicks of semi-precocial Black-legged and Red-legged Kittiwakes, long-term dietary restriction also causes elevation of baseline levels of corticosterone and enhances adrenocortical response to acute stress (Kitaysky et al. 1999a, this study). In contrast to the results of these studies, Rees and co-authors (1985) found that adrenocortical responses of immature chicken to an acute stressor and ACTH challenge were reduced by food deprivation. Similarly, increased adrenocortical response to an acute stressor was not associated with dietary restrictions in *in vitro* study of precocial Domestic Turkey (*Meleagris gallopavo*, Carsia and McIlroy 1998). Furthermore, in semi-precocial young of American Kestrels (*Falco sparverius*), baseline levels of corticosterone were not affected and acute stress-induced levels of corticosterone were reduced by long-term food-restriction (Heath and Dufty 1998). Thus, modulation of adrenocortical activity of chicks in response to food restriction can reflect not only precocial-altricial spectrum of chick development, but other ecological, physiological and behavioral traits of a particular species (e.g. temporal variability in food resources, rate of chick provisioning with food, metabolic responses of chicks to food restrictions, and/or phylogenetic constraints [e.g. Kitaysky 1999]). Also, in the above mentioned experimental studies, the duration of food restriction ranged from two days to four weeks, and the severity of dietary restriction ranged from complete food deprivation to alterations of quality of diet fed *ad libitum*. As the results of this current study show, the temporal dynamics of adrenocortical response to nutritional stress are complex, which might account for the discrepancy in the results obtained by different studies of related/same species of birds.

We are convinced that the results found in this study were not merely a consequence of captive conditions, but in fact reflect responses we could expect to see in free-living Red-legged Kittiwakes. Other studies of adrenal response in young birds to a standardized acute stressor did not indicate that those birds habituated to frequent handling (Freeman et al. 1981; Dufty and Belthoff 1997; Heath and Dufty 1998, Kitaysky et al. 1999a). Baseline and acute-stress induced levels of corticosterone recorded for captive chicks in this study were within the range observed in free-living Red-legged Kittiwake chicks. Furthermore, baseline and acute stress-induced levels of corticosterone in free-living (not tick-infested) and captive chicks fed *ad libitum* were similar, whereas baseline and acute stress-induced levels of corticosterone were similar between nutritionally stressed captive chicks and free-living chicks infested with ticks. It is possible that tick infestation causes severe metabolic challenges to young Red-legged Kittiwakes, as has been reported for chicks of other species of birds (reviewed in Duffy 1983), which could result in enhanced adrenocortical function of tick-infested wild chicks similar to under-nourished captive chicks. Finally, captive chicks in all treatments were subject to similar controlled conditions. The only differences between them were the energy content and nutritional quality of their food. Thus, it is reasonable to conclude that the results of this study demonstrate differences in the functional responses of Red-legged Kittiwake chicks to variations in energetic content and nutritional quality of their food.

To the best of our knowledge, this paper represents the first experimental demonstration of the temporal dynamics of adrenocortical response of nest-bound semi-precocial chicks in relation to long-term dietary restrictions. The results of this study suggest that for a realistic assessment of adrenocortical response of chicks to nutritional stress it is crucial to understand the temporal dynamics of corticosterone release in relation to diet composition and a chick's energy requirements. Non-manipulative observations and a single assessment of adrenocortical activity at the end of long-term food-restriction experiments might lead to equivocal results. Specifically, our results indicate that there are three phases of adrenocortical response of semi-precocial nest-bound kittiwake chicks to long-term food shortages, which are different from previously described phases of fasting in young and adult birds (e.g. Cherel et al. 1988; Le Ninan et al. 1988). Phase 1 – initial elevation of baseline levels of corticosterone, occurs shortly after chicks started experiencing negative energy balance and is associated with a decrease in growth of body mass; Phase II – chronically elevated baseline levels of corticosterone, no change or slight decrease in body mass; Phase III – a suppression of corticosterone secretion, no change in body mass. An extent and duration of each phase probably depends on a chick's ability to accumulate fat reserves prior to a food shortage, how severe dietary restrictions are, and developmental and physiological responses to food shortages specific for a particular group/species of birds.

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Table 1. Experimental protocols and body mass of Red-legged Kittiwake chicks.

(LPR represents lipid to protein ratio)

Diet composition	LPR	Food intake wet, g d^{-1}	Energy intake kJ d^{-1}	Body mass at 15 d.o. mean	SE	Body mass at 35 d.o. mean	SE	n
Silverside	1.47	80	565.1	181.8	12.4	338.0	3.5	5
Rainbow smelt	0.61	80	356.3	177.2	13.0	237.5	4.9	5
Silverside	1.47	50	353.2	185.3	10.9	262.0	3.7	6
Rainbow smelt	0.61	50	222.7	187.4	9.6	188.9	2.0	5
Lanternfish [1]	2.96	?	?	-	-	339.2*	42.2	5
Lanternfish [2]						373.5*	11.5	2

1- ~30 days old wild chicks infested with ticks; 2- ~30 days old wild chicks with no ticks.
LPR ratio for Lanternfish (*Stenobrachius sp.*) from Van Pelt et al. 1997.

*- It was not possible to control for the post-absorptive condition of wild chicks and their body mass reflects weight of the bird as well as mass of ingested food and feces.

Figure legends

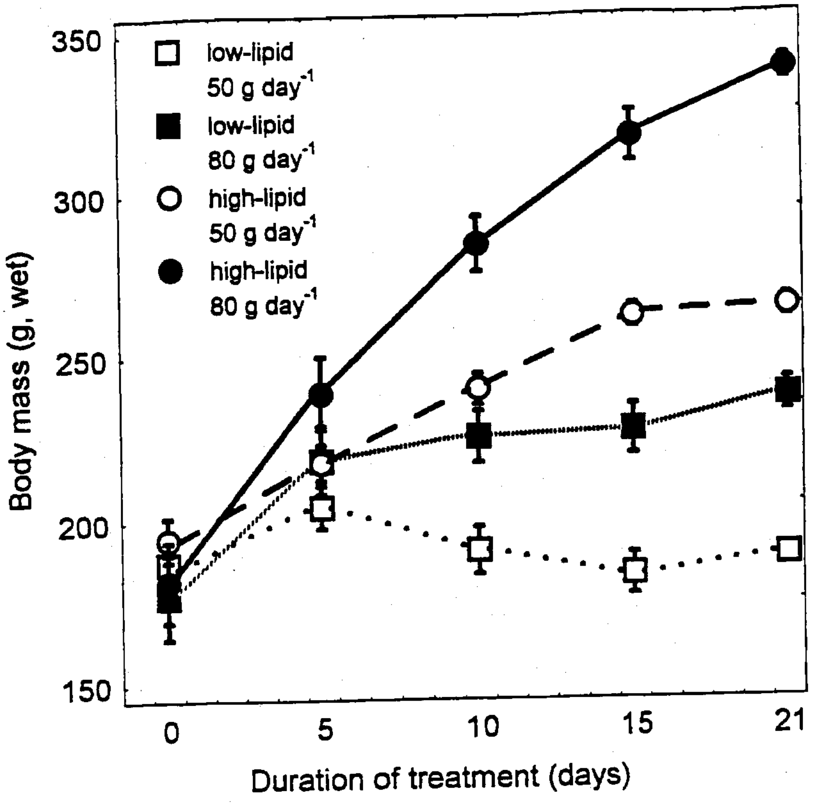
Fig. 1. Growth rate of body mass (means, ±SE) of Red-legged Kittiwake chicks in relation to daily energy intake (40, 65 and 100% of the *ad libitum*), diet composition (LPR: low-lipid=0.61, high-lipid=1.47), and duration of treatment.

Fig. 2. Upper panel: Baseline levels of corticosterone (means, ±SE) of Red-legged Kittiwake chicks in relation to daily energy intake (40, 65 and 100% of the ***ad libitum***), diet composition (LPR: low-lipid=0.61, high-lipid=1.47), and duration of treatment. Low panel: Estimated (see Methods) chicks daily energy requirements (means, ±SE; symbols are as in upper panel) vs. chicks daily energy intake (indicated with lines for each experimental treatment, as in upper panel).

Fig. 3. Adrenal response to a standardized handling and restraint stressor in captive five week-old Red-legged Kittiwake chicks in relation to dietary treatments (left panel), and in wild Red-legged Kittiwake chicks of ages similar to captives (right panel).

Fig. 4. The relationship between infestation with ticks and baseline levels of corticosterone in five week-old wild Red-legged Kittiwake chicks (n=7).

Fig. 1



350
300
250
200
150
Body mass (g, wet)
□ low-lipid 50 g day^{-1}
■ low-lipid 80 g day^{-1}
○ high-lipid 50 g day^{-1}
● high-lipid 80 g day^{-1}
0
5
10
15
21
Duration of treatment (days)

Fig. 2

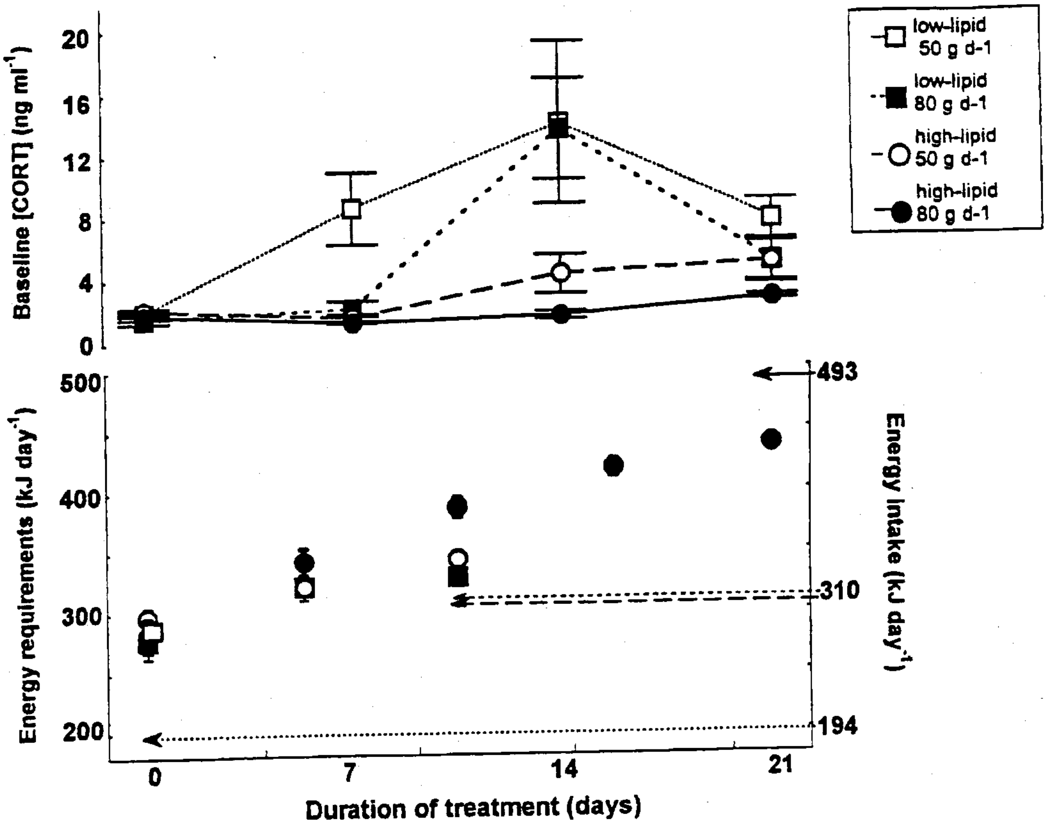

Baseline [CORT] (ng ml^{-1})
20
16
12
8
4
0
low-lipid 50 g d-1
low-lipid 80 g d-1
high-lipid 50 g d-1
high-lipid 80 g d-1
Energy requirements (kJ day^{-1})
500
400
300
200
Energy intake (kJ day^{-1})
493
310
194
0
7
14
21
Duration of treatment (days)

Fig. 3

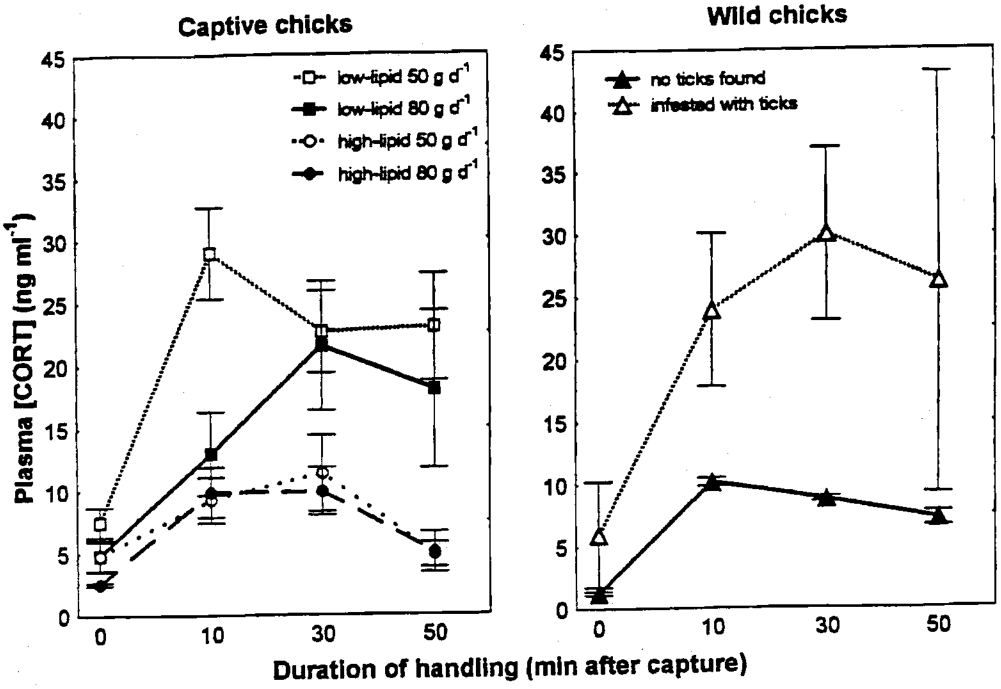
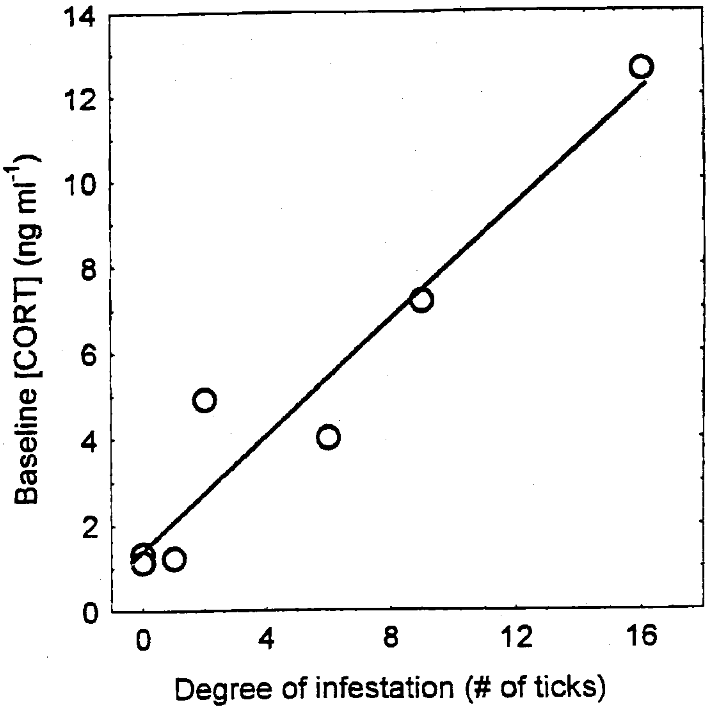

Captive chicks
Wild chicks
-□- low-lipid 50 g d⁻¹
-■- low-lipid 80 g d⁻¹
··○· high-lipid 50 g d⁻¹
-●- high-lipid 80 g d⁻¹
-▲- no ticks found
-△- infested with ticks
Plasma [CORT] (ng ml⁻¹)
0
5
10
15
20
25
30
35
40
45
0
10
30
50
Duration of handling (min after capture)

Fig. 4


14
12
10
8
6
4
2
0
0
4
8
12
16
Baseline [CORT] (ng ml^{-1})
Degree of infestation (# of ticks)

Endocrine Responses to Unpredictable Environmental Events: Stress or Anti-Stress Hormones?[1]

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Running Head: **Stress or Anti-Stress Hormones?**

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[1] From the Symposium on

Synopsis

In addition to seasonal changes in morphology, physiology and behavior that occur in predictable annual cycles, there are facultative responses to unpredictable events known as labile (i.e. short-lived) perturbation factors (LPFs). These rapid behavioral and physiological changes have been termed the "emergency" life history stage (ELHS) and serve to enhance life-time fitness. Glucocorticosteroids interacting with other hormones in the hypothalamo-pituitary-adrenal (HPA) cascade, initiate and orchestrate the ELHS within minutes to hours. Components of the ELHS include: redirection of behavior from a normal life history stage to increased foraging, irruptive-type migration during the day, enhanced restfulness at night, elevated gluconeogenesis and recovery once the perturbation passes. These physiological and behavioral changes allow an individual to avoid potential deleterious effects of stress that may result from chronically elevated levels of circulating glucocorticosteroids over days and weeks. In other words, acute rises in glucocorticosteroids following perturbations of the environment may actually avoid chronic stress and serve primarily as “anti-stress” hormones. Several field studies in diverse habitats indicate that free-living populations have elevated circulating levels of corticosteroids when in an ELHS. However, expression of an ELHS may not always be advantageous and there is accumulating evidence from birds that the adrenocortical responses to LPFs are modulated both on seasonal and individual levels. These data suggest that glucocorticosteroid secretions in response to LPFs not only trigger physiological and behavioral responses but also allow flexibility so that the response is integrated in relation to time of year (normal LHS) as well as individual differences owing to body condition, disease and social status.

INTRODUCTION

I've lived in a good climate, and it bores the hell out of me. I like weather rather than climate.

John Steinbeck, "Travels With Charlie" (1962)

There is no habitat on Earth that is entirely constant, or changes in a perfectly predictable manner. On the other hand it is also probably true to say that no habitat is completely unpredictable or chaotic. Therefore, organisms must be able to adjust their morphology, physiology and behavior to predictable changes in the environment, but also be ready to deal with unpredictable perturbations with little or no prior warning. The concept of predictability and unpredictability in both physical and social environments is illustrated well by the phrase from Steinbeck cited above. We all are familiar with the vagaries of weather. However, climate refers to the average regional environmental conditions that can be expected over the year. Even the most severe climate has a strong predictable component (e.g. the changing seasons) and organisms can thus prepare for future changes. In contrast, weather represents the local conditions at any time that may, or may not, be consistent with the predicted climate (Wingfield and Ramenofsky, 1999). Weather is not the only source of unpredictable events (labile perturbation factors – LPFs). Other examples include sudden changes in social status, increased predator numbers, decreased food resources, and disease (Wingfield et al., 1998; Wingfield and Ramenofsky, 1999; Wingfield, 2001).

Animals use environmental cues such as changing day length, temperature and rainfall to predict future events and adjust life history stages (LHSs) accordingly (Fig. 1). However, responses to LPFs require more rapid responses without possibility of anticipatory changes. This response has been collectively termed the emergency life history stage (ELHS) and serves to direct the individual away from normal LHSs into a physiological and behavioral state that will allow survival in the best condition possible (Fig. 1, Wingfield et al., 1998). If the individual is unable to acclimate to the LPF, or move away from it, then symptoms of stress and eventually death will occur (Wingfield and Ramenofsky, 1999; Wingfield and Romero, 2000). In this chapter we suggest that the ELHS is a mechanism by which animals not only deal with LPFs, but also integrate adjustments in life history cycles to accommodate the stochastic nature of the interrelationships of predictability and unpredictability.

Changes in Responsiveness to LPFs May Have an Ecological Basis

The frequency and magnitude of unpredictable perturbations vary along environmental gradients. Behavioral responsiveness (or latency of response) of animals to LPFs might reflect this variability. For instance, behavioral responses of seabirds to variability in food resources reflects their phylogenetic and ecological characteristics as well as that of their prey (Kitaysky, 1999; Kitaysky and Golubova, 2000; Kitaysky et al., 2000). In animals relying on continuously available food resources, even a short-term decrease in food availability might trigger an ELHS. In animals relying on highly variable food resources, only a severe long-term food shortage should trigger ELHS. So, the more predictable the environment (less stochastic), the quicker physiological and behavioral response to LPFs would be, whereas in less predictable environments (more stochastic) those responses are expected be delayed.

In contrast to variability of LPFs and diversity of life history traits that allow animals to cope with them, the ELHS is a remarkably consistent trait among all vertebrates, and is aimed to maximize lifetime fitness. However, animals are faced with contrasting trade-offs in different LHSs. For example, outside of the reproductive season, survival seems paramount, whereas when breeding, the number of viable offspring produced during current versus future reproductive attempts must be maximized. Thus, the strategy that animals are pursuing when responding to LPFs should reflect which specific component of lifetime fitness is currently being maximized.

Components of the Emergency Life History Stage

The life cycle of vertebrates consists of a series of life history stages (LHSs) each with unique set of sub-stages (Fig. 1). The temporal sequence of LHSs is fixed, although the number of LHSs varies from species to species and each stage is expressed at a time of year for which the stage has evolved to maximize fitness (Jacobs and Wingfield, 2000). State of an individual at any time is a function of the set of sub-stages expressed within a LHS. The emergency life history stage (ELHS) can be expressed at any time in the life cycle. The LPFs that trigger an ELHS are diverse, but the sub-stages within the ELHS are remarkably constant in all vertebrates studied to date (Fig. 1). They serve to direct the individual into a survival mode and then allow it to return to the normal LHS once the LPF passes. Behavioral and physiological components that make up the sub-stages of an ELHS are:

1. "Leave-it" strategy - movements away from LPFs
2. "Take-it" strategy - switch to an alternate set of energy conserving behavioral and physiological traits
3. "Take at first and then leave-it" strategy - switch to energy conserving mode first and then move away if conditions do not improve

Once a "strategy" has been adopted, then mobilization of stored energy sources such as fat and protein to fuel movement away from the source of the LPF, or to provide energy while sheltering in a refuge becomes critical. Finally, once the LPF passes, or the individual has moved away, then it must settle in alternate habitat once an appropriate site is identified, or, return to the original site and resume the normal sequence of LHSs.

These dramatic changes in behavior and physiology can occur within minutes to hours of exposure to a LPF and have been the subject of many experiments to determine the hormonal mechanisms underlying them (Wingfield and Ramenofsky, 1999).

THE EMERGENCY LIFE HISTORY STAGE AND ITS CONTROL: STRESS OR ANTI-STRESS HORMONES?

Vertebrates generally undergo marked activation of the hypothalamo-pituitary adrenal (HPA) axis that is remarkably similar across taxa (Greenberg and Wingfield, 1987). In all tetrapods, LPFs in the environment result in release of adrenocorticotropin (ACTH) from the precursor molecule pro-opiomelanocortin in the anterior pituitary. ACTH release is regulated by corticotropin releasing hormone (CRH). Arginine vasotocin (AVT) and mesotocin (MT) also may be potent secretogogues of ACTH in birds (Gorbman et al., 1983; Norris, 1997). ACTH acts primarily on the adrenocortical cells to promote synthesis and secretion of glucocorticosteroids (Carsia, 1990). The hypothalamic-pituitary-adrenal (HPA) axis of vertebrates, particularly Aves, has many of the same feedback controls as in mammals (Carsia, 1990; Harvey and Hall, 1990).

There is massive evidence that chronic elevation of glucocorticosteroids over weeks or longer, has dramatic and debilitating effects including: inhibition of the reproductive system, suppression of the immune system, promotion of severe protein loss from skeletal muscle, disruption of second cell messengers, particularly the arachidonic acid cascade, neuronal cell death, and suppression of growth (e.g. Munck et al., 1984; Sapolsky, 1987; McEwen et al., 1993; Sapolsky et al., 2000). Although it is clear that elevated secretion of glucocorticosteroids allows an animal to survive many "stressful" events, there is a severe cost of prolonged high blood glucocorticoid levels. Therefore it is possible that the "stress" response system only increases fitness during relatively short-term responses (hours to days) to LPFs, and is detrimental to the animal during protracted challenges to homeostasis (days to weeks).

Rapid effects of glucocorticosteroids in response to LPFs have received much less attention. Accumulating experimental evidence in birds and mammals indicates that corticosterone has a number of behavioral and physiological effects that promote fitness at least in the short term. These have been reviewed extensively by Sapolsky et al. (2000); Wingfield and Romero (2000) and include: suppression of reproductive behavior without inhibiting the reproductive system; regulation of the immune system (e.g. Dhabhar, 1998); increased gluconeogenesis; increase foraging behavior – a permissive role rather than direct; promotion of escape (irruptive) behavior during day; promotion of night restfulness; facilitation of recovery on return to normal life history stage. These short term effects of corticosterone during a response to LPFs suppress "unnecessary" physiological and behavioral functions, activate alternate behavioral and physiological patterns that promote survival (i.e. temporary emergency behavior) and collectively serve to avoid the long-term, detrimental effects of stress-induced high levels of corticosterone.

The basic principles of the ELHS and its control may be widespread among vertebrate taxa, and many aspects of the stress response are nearly stereotypical. Nonetheless, there are large differences in the responses of individuals to the same LPF (e.g., Sapolsky, 1988; Meaney et al., 1993a,b). Some of this variation is based on genetic differences among individuals (e.g., Flaherty and Rowan, 1989; Satterlee and Johnson, 1988; Carsia and Weber, 1986; Gross and Siegel, 1985), whereas other variation is associated with differences in physiological condition and/or sub-stages within a LHS, e.g. reproductive status in the breeding LHS (Wingfield et al., 1994a,b).

Given that the short-term responses to elevated corticosterone induced by a LPF are highly adaptive, then it is pertinent to re-define stress. LPFs can trigger an increase in adrenocortical secretions, but may not be stressful per se. They certainly have the potential to be stressors, but if the animal is able to compensate by activating the ELHS, then the deleterious effects associated with chronic stress are avoided. When in an ELHS, the individual is not stressed but in a transitory stage maximizing chances of survival in best condition possible (Wingfield and Ramenofsky, 1999; Wingfield and Romero, 2000).

This concept is worth formalizing in a more theoretical sense. It has long been known that baseline levels of glucocorticosteroids are essential for energy and salt management and without this absolute baseline (e.g. in adrenalectomized animals), the individual will die (Gorbman et al., 1983; Norris, 1997). We have called this baseline level A (Fig. 2, Wingfield et al., 1997, 1998). Blood levels of glucocorticosteroids also change predictably over the day and also in relation to seasons. Again, it is thought that these fluctuations are involved in metabolism and osmoregulatory processes as the individual changes physiology and behavior from one LHS to the next (Fig. 1). These we have called level B (Fig. 2). Examples include increases of baseline corticosterone in breeding birds, and surges of cortisol in salmonids migrating from fresh to salt water (see Wingfield and Romero, 2000). Superimposed on these predictable changes in glucocorticosteroids at levels A and B, are further surges in circulating levels to much higher and usually transitory peaks that we have called level C (Fig. 2). These transitory surges are what we propose activate the ELHS. Only if they become prolonged because of repeated LPFs, or because the individual is not able to move away, do typical symptoms of stress become apparent (Wingfield et al., 1997,1998). Thus, transitory elevation of corticosterone to level C, above levels A and B for the predictable life cycle, (Fig. 2) may be integrating physiological and behavioral strategies that maximize fitness in the face of unpredictable environmental events. Not only may the HPA axis be an integrator of mechanisms maximizing fitness, it may also be a subject of on-going selection.

GLUCOCORTICOSTEROIDS: INTEGRATORS OF FACULTATIVE BEHAVIORAL AND PHYSIOLOGICAL RESPONSES TO THE UNPREDICTABLE.

The growing evidence that early effects (minutes to hours) of rising glucocorticosteroid levels in response to LPFS trigger an ELHS to avoid severe stress, suggests an integrating role for behavioral and physiological responses to these unpredictable events. The generalized ELHS allows a consistent and adaptive suite of responses regardless of time of year and LHS, and is flexible enough to orchestrate different combinations of behavioral strategies and energy mobilization according to the type of perturbation the individual experiences. Particularly intriguing is the ability to customize the response not only to different LPFS (physical and social), but also integrate them with individual traits such as body condition, disease and social status. Next, we outline several very different examples of corticosterone integration of life history strategies that allow individuals to cope with unpredictability without becoming stressed.

The "Leave it" Strategy

It has long be known that severe weather and other LPFs result in abandonment of nests by birds (e.g. Elkins, 1983). However, this abandonment occurs very quickly after onset of a severe LPF, i.e. well before adults become debilitated trying to forage to feed growing young when trophic resources are declining ("leave-it" strategy). This strategy appears to be cease breeding immediately, despite young being present, and survive the LPF in the best condition possible so that individuals can return to breeding and renest promptly after the LPF passes and trophic resources return to normal. In this way reproductive success may be maximized over the alternate strategy of feeding young despite declining resources and depleting energy stores of the parents (Wingfield et al., 1983). However, this tactic is only likely to succeed if the breeding season is long enough and provides flexibility in timing so that renesting, or multiple broods, are possible.

Evidence from field studies shows that birds abandoning territories and nests have high levels of corticosterone in blood (Fig. 3, Wingfield et al., 1983). Over the breeding season of white-crowned sparrows, Zonotrichia leucophrys pugetensis, there is an increase in plasma levels of corticosterone within level B (Fig. 3) that is associated with actual onset of nesting and feeding young. During a severe storm, however, circulating levels of corticosterone are much higher (level C) and birds abandon their territories and nests and range over a large area in loose flocks. Corticosterone titers return to level B once the LPF passes and birds are renesting (Fi.g 3). Moreover, plasma levels of reproductive hormones such as luteinizing hormone and testosterone are not affected. Thus, high concentrations of plasma corticosterone (level C) redirect these birds away from breeding but the reproductive system remains functional, just behaviorally suppressed. When the LPF subsides and corticosterone concentrations return to level B, renesting could begin immediately and is not delayed by a period of recrudescence that would have been necessary if the gonads had been suppressed and regression had occurred (Wingfield et al., 1983).

Exactly how elevated corticosterone may trigger abandonment of the nest remains to be clarified. A role of CRF has been implicated because injection of this peptide into the third ventricle of Z.l. gambelii rapidly increases perch hopping activity in a dose dependent manner (Maney and Wingfield, 1998). Furthermore, non-invasive treatment of white-crowned sparrows with corticosterone (via ingestion of hormone-treated meal worms) results in a rapid increase in blood levels (within minutes) and an increase in activity (within 15 minutes, Breuner et al., 1998). The loci of these behavioral actions and the receptors involved are currently under investigation.

Another example of LPFs redirecting birds away from breeding involves the effects of an El-Niño southern oscillation event of 1997-1998 that decreased abundance of food resources in the vicinity of breeding colonies of seabirds in the Lower Cook Inlet, Alaska (Piatt J.F. et al. unpublished). Body fat reserves of common murres, Uria aalge, appear to be normal at the pre-egg laying stage of reproductive cycle, but deteriorated during the incubating and chick-rearing stages (Fig. 4). This decrease in body condition coincided with moderately elevated baseline levels of corticosterone (level B) and abandonment of eggs and young chicks (Fig. 4). The results of experimental manipulations with corticosterone in breeding common murres suggest that parents with elevated baseline corticosterone leave breeding colonies (A.S. Kitaysky et al. unpublished). Thus, it is likely that food-shortages during the El-Niño of 1997-98 resulted in an increase in corticosterone secretion that directly caused the abandonment of eggs and young chicks by murres. Though increased baseline levels of corticosterone were detected in most of examined birds, a large variability in corticosterone secretion in response to acute stress was also detected. At the same time, not all murres abandoned their eggs and/or chicks. Furthermore, higher adrenocortical stress response was negatively correlated with current reproductive success. Finally, re-sighting data of breeding common murres at the same colonies during subsequent years show that parents that had higher adrenocortical responses to acute stress during the current reproductive attempt were more likely to reproduce at the same colonies in the future than parents that had had lower stress response (A.S. Kitaysky et al., unpublished). This example demonstrates that the elevation of baseline and acute stress-induced levels of corticosterone reflects current environmental conditions but by no means is a sign of physiological stress. Instead, the elevation of corticosterone secretion during food shortages is a part of the ELHs, that allows common murres to avoid stress. These results also provide a clue that under current conditions, choosing the most appropriate ELHs might be a product of on-going selection.

The "Take-it" Strategy

Not all individuals abandon the territory and adopt a leave-it strategy. Some may seek a refuge and ride out the LPF ("take-it" strategy, Wingfield and Ramenofsky, 1999). A "refuge" can be provided either by using a shelter (use of a micro-habitat) or by using an alternate set of "energy conserving" behavioral and physiological traits. For instance, during reproduction these traits will also depend on life-history stage. Thus juvenile traits are expected to be different from adults setting the stage for evolutionary interactions (e.g. parent-offspring conflict), e.g. in chicks, increases of begging and food delivery by parents, whereas in parents – accumulation of fat/protein reserves, brood reduction, decrease of chick-provisioning.

One form of refuge seeking is to go into torpor by decreasing body temperature. This has been studied in the rufous hummingbird, Selasphorus rufus, that enters nocturnal torpor to conserve energy in the face of environmental challenges, especially during spring migration when trophic resources may be particularly unpredictable. In this species, plasma levels of corticosterone rise in response to capture handling and restraint, and this rise is followed by an increase in urine (cloacal fluid) concentrations. Thus collection of cloacal fluid is a non-invasive way of measuring changes in corticosterone levels in these three gram birds (Hiebert et al., 2000b). Corticosterone treatment via ingestion of cyclodextran in nectar resulted in hummingbirds going into torpor more frequently. However, although corticosterone levels rose in the evening regardless of whether the individual developed a torpid state, an additional factor is probably involved for regulation of this strategy (Hiebert et al., 2000a).

Another form of the "take-it" strategy is to accumulate extra energy reserves in response to a LPF. Field observations have shown that during early stages of reproduction, black-legged kittiwakes, Rissa tridactyla, accumulate large fat deposits in response to a decline in food availability (Kitaysky et al., 1999a). This accumulation of fat reserves was not associated with a change in baseline levels of corticosterone, that were still at level A. Interestingly, accumulation of fat reserves in response to food shortage was not found in kittiwakes raising young. Instead, a strong decline of fat reserves was observed, in parallel with an increase in baseline levels of corticosterone. However, elevated baseline levels were lower (i.e. level B) than acute stress-induced levels of corticosterone (level C), suggesting that kittiwakes feeding young were not physiologically stressed but relied on stored energy reserves to fuel their activities.

At different LHSs birds are likely to be faced with different trade-offs and apparently might use different mechanisms allowing them to cope with the LPF. At early stages of the reproductive cycle, black-legged kittiwakes alternate long incubation shifts with long-duration foraging trips when they are able to accumulate large fat reserves to buffer declines in food availability. At the chick-rearing stage, kittiwakes have to commute frequently between foraging grounds and breeding colonies to feed their young. As a result, energy expenditure of chick-rearing kittiwakes increases during food shortages (Kitaysky et al. 2000). This may preclude accumulation of extra fat reserves and parent kittiwakes partially rely on mobilization of stored energy reserves such as fat and proteins to fuel their increased activities (Kitaysky et al. 1999a). Thus, moderate (level B) increases of baseline levels of corticosterone might represent yet another form of the "take-it" strategy – the mobilization of stored energy sources such as fat and proteins to ride out LPFs.

The "Take it at First and Then Leave-it" Strategy.

Black-legged kittiwakes provide an example of an intriguing combination of ecological and physiological factors shaping emergency life history strategy. During prolonged food shortages breeding black-legged kittiwakes suppress their adrenocortical response to acute stress (Kitaysky et al. 1999a). At early stages of reproduction, a suppressed stress response is associated with increased fat deposits and normal (at level A) baseline corticosterone. At the chick-rearing sub-stage, however, a suppressed stress response coincides with depleted energy reserves and an increased (level B) baseline corticosterone. These physiological changes during reproduction under poor foraging conditions are associated with changes in kittiwakes' behavior. Specifically, in response to food shortages, parent kittiwakes allocate more time to foraging and less time to guarding/brooding their chicks (Kitaysky et al. 2000). Recent experimental studies have shown that a moderate elevation (within level B) of baseline corticosterone directly causes this change in the resource allocation by parent kittiwakes (Kitaysky et al., in press). The change in resource allocation results in a considerable increase of the amount of time that kittiwake chicks are unattended by parents and usually those chicks succumb to predators. However, this loss of offspring during food shortages increases post-breeding survival of parent kittiwakes (Golet et al., 1998). Thus, modulation of stress response by parent black-legged kittiwakes in response to LPFs is a part of the ELHS strategy that increases life-time fitness.

Black-legged kittiwakes with experimentally elevated levels of corticosterone increase foraging at the expense of brooding/guarding their chicks, but do not alter their chick provisioning rate and may suffer long-term effects of chronically-elevated levels of corticosterone as a result (Kitaysky et al., in press). The functional importance of the food-provisioning strategy of black-legged kittiwakes is not known. Several explanations seem possible. First, the food-provisioning strategy of parent black-legged kittiwakes maybe phylogenetically determined. Although pelagic-foraging kittiwakes rely on more variable food resources compared to their inshore foraging counterparts, chick-provisioning behavior of kittiwakes is still similar to that of other species of gull (e.g., Harris 1970; Henderson 1975; reviewed in Ydenberg and Bertram 1989). Second, kittiwake chicks are physiologically sensitive to variations in daily food intake and respond to moderate food shortages by a chronic elevation of corticosterone (Kitaysky et al., 1999b). Timing of an increase in baseline levels of corticosterone (level B) in general reflects the point when energy demands of food-restricted chicks exceed their energy intake and a chronically elevated secretion of corticosterone (at dangerously high concentrations - level C) might persist over period of weeks (Kitaysky et al., 1999b; Kitaysky et al., submitted). Chronic elevation of corticosterone during early development can have long-term deleterious effect (reviewed in Welberg and Seckl, 2001). Impaired physiological condition of black-legged kittiwake chicks under-nourished during early development might decrease their chances of survival. This would force their parents to provide food on chick demands. In fact, recent experimental studies have shown that high corticosterone levels increase chick begging rates and increase rates of food provisioning by parents (Kitaysky et al., in press). Thus, the release of corticosterone in hungry kittiwake chicks represents an ELHS that allows them to restore depleted energy reserves by modifying the behavior of their parents and thereby avoiding nutritional stress.

Modulation of Adrenocortical Responses to LPFS

Facultative expression of behavior and physiology in an ELHS to combat LPFs is adaptive for many populations throughout their life cycle. However, there is growing evidence that the ELHS may not be adaptive in all cases. It has been postulated that birds breeding in severe environments in which the breeding season is brief may reduce the adrenocortical response to LPFs temporarily (Wingfield et al., 1995, 1998). Indeed, several species of arctic birds breeding on the Alaskan tundra near Barrow (71°N) showed liitle or no correlation of baseline (i.e. level B) and stress-induced (level C) corticosterone levels with snow storms accompanied by below freezing temperatures during the breeding season. After breeding had terminated, such storms resulted in a much stronger correlation with corticosterone concentrations in blood (Table 1, Romero et al. 2000). It has also been shown that not only is the adrenocortical response to LPFs reduced in these birds, but the behavioral responses to experimentally implanted corticosterone levels (to mimic level C) were suppressed (Astheimer et al., 2000). These data suggest that although behavioral insensitivity to elevated corticosterone levels develops, metabolic responses indicated by loss of protein in flight muscles are not affected. Thus in response to an LPF, energy can still be mobilized but the behavioral components of the ELHS that would redirect the individual away from breeding (resulting in zero reproductive success for that season) are turned off. Clearly, modulation of the stress responses and effects of corticosterone at level C indicate a high degree of integration of metabolic demands and behavioral strategies to maximize lifetime fitness.

If storms in spring and summer become severe enough and prolonged (e.g. 3-4 days), then at least some species, e.g. Lapland longspurs, Calcarius lapponicus, will eventually abandon the nest and upregulate their adrenocortical response to stress by almost an order of magnitude (Astheimer et al., 1995). In other words it appears that suppression of the adrenocortical response to LPFs is only adaptive up to a point. If the individual's life is threatened by severe weather and the potential for starvation, then it is highly unlikely that young could be raised to independence that season. Abandonment of the breeding attempt and a return of the HPA axis to normal would then make sense. The mechanisms by which the adrenocortical response to LPFs are suppressed and then re-instated are currently under investigation. Particularly intriguing are the mechanisms by which behavioral responses to high circulating levels of corticosterone are turned off while metabolic responses in the periphery remain intact.

The Role of Corticosterone in the Resolution of Parent-Offspring Conflict.

The regulation of begging through secretion of corticosterone might represent an evolutionary stable signaling system as a resolution of parent-offspring conflict in birds. The results of field experiments suggest that parent kittiwakes assess the physiological condition of their chicks by monitoring begging and provide food on chick demands. Chicks given implants of corticosterone beg more than controls resulting in parents foraging to bring more food (Kitaysky et al., in press). In such a system, a chick can misrepresent its requirements in order to acquire more food than it needs (Godfray, 1995). Cheating might be prevented if there is a cost associated with begging that is larger than the benefits of the extra food obtained. If continuous begging is associated with a prolonged secretion of corticosterone, then a cheating chick would suffer detrimental effects of chronically elevated levels of corticosterone. Thus, exaggerated begging might be costly and a cheating chick may endanger its future survival. However, this conclusion hinges on the assumption that incessant begging requires continuous secretion of corticosterone. Recent experimental studies of black-legged and red-legged, Rissa brevirostris, kittiwake chicks confirmed this prediction. Baseline levels of corticosterone are significantly elevated in chicks of both species within 10 minutes of presenting them with a food stimulus to trigger begging. These data suggest strongly that the actual act of begging, or perhaps anticipation of food, do indeed elevate secretions of the HPA axis (A.S. Kitaysky et al., unpublished).

CONCLUSIONS

One of the most critical functions within an individual's life cycle is to respond to unpredictable events in the environment with appropriate behavioral and physiological adjustments. These strategies must integrate management of energy balance in relation to body stores and food available, changes in the physical and social environment that determine access to food and other resources such as shelter, and then trigger individual behavioral responses that will minimize the cost in terms of survival and future potential to reproduce. The emergency life history stage (ELHS) is a suite of such facultative behavioral and physiological responses that provides great flexibility to combat the effects of LPFs at any time of year and within normal LHSs. Plasticity of the ELHS occurs at both the population and individual levels. Secretions of glucocorticosteroids above the normal baseline levels in relation to season and LHSs (level C) integrate these facultative responses, probably in conjunction with many other hormones and paracrine secretions within the brain. The result is a highly customized individual response to LPFs regardless of time of year, LHS, and individual state.

The facultative responses may be as diverse as torpor in the face of reduced food resources at specific times of year, abandonment of a territory and nest resulting in extended movements over a large area, or altered behavioral interactions between parents and offspring that may have profound implication for survival of adults and young. The cellular and molecular mechanisms underlying these specific responses tailored for individual circumstances present a major challenge for the future. Currently it appears that glucocorticosteroids provide an initial integrating signal, and interactions with other hormones and paracrine secretions may determine specific behavioral and physiological responses in different environmental and social contexts.

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FIGURE LEGENDS

Figure 1. Temporal sequence of life history stages (LHSs, left hand side) in a typical non-migratory bird. Each box contains examples of behavioral and physiological sub-stages that are unique to each LHS. The temporal sequence of LHSs is unidirectional and each LHS is timed by environmental cues transduced through neuroendocrine and endocrine secretions. Note that this cycle progresses as a function of the predictable life cycle, e.g. the seasons. Superimposed on this predictable life cycle are unpredictable events (labile perturbation factors, LPFs) in the environment such as presence of predators, inclement weather, sudden changes in social status. These LPFs are disruptive

to the normal temporal progression of LHSs and many have the potential to be stressful. If the LPF is severe, or prolonged (several days or more) then the individual may have to abandon its normal LHS and adopt temporary emergency physiology and behavior to endure the LPF in the best condition possible. The emergency life history stage (ELHS, right hand box) is a facultative LHS that can be triggered by LPFs at any time in the life cycle (arrows with solid lines). It also comprises unique sub-stages that allow the individual to combat the LPF and maximize fitness. Once the LPF passes, the animal can return to the appropriate LHS (arrows with broken lines). From Wingfield et al. (1997); Jacobs and Wingfield (2000).

Figure 2. A theoretical view of different levels of hormone secretion according to the levels of physiological and behavioral response. Level A is the absolute baseline required for existence of the individual. In the case of corticosterone, below this level is typical of adrenalectomy and the individual will die because it cannot manage glucose and salts. Secretion of hormone at this baseline may be constitutive, but some regulatiuon may also occur (e.g. when negative feedback set points change seasonally). Level B is the range of circulating concentrations of corticosterone that occur on a daily basis (or tidal rhythm in marine species) and in relation to seasons. These changes are part of the predictable life cycle and are regulated periodically by responses to environmental cues such as photoperiod. Changes in secretion at levels A and B make up the predictable life cycle of individuals in relation to glucose management, salt regulation, and associated behaviors. Superimposed on this predictable life cycle are transient increases in circulating levels of corticosterone to level C. This marked increase is triggered by a labile perturbation factor (LPF), thus is regulated facultatively, and if prolonged will trigger the emergency life history stage (ELHS, Fig. 1). If the individual is unable to

combat the LPF there is potential for chronic stress and the debilitating effects of chronically high levels of glucocorticosteroids. If the ELHS can be triggered, then plasma levels of corticosterone decline to within level B. From Wingfield et al., (1997).

Figure 3. An example of the effects of a labile perturbation factor (LPF, in this case severe weather), in breeding male white-crowned sparrows, Zonotrichia leucoprhys pugetensis. Changes in plasma levels of corticosterone are arranged in relation to stages and sub-stages in the life cycle (x axis). The circles connected by solid lines represent changes in corticosterone levels in the blood of males breeding normally (i.e. levels A and B) and with no exposure to LPFs (e.g. Wingfield and Farner, 1978). The vertical bars (cross hatched) represent plasma levels of corticosterone in a year (1980) when severe weather interrupted breeding resulting in males abandoning their nests and territories. At this time corticosterone levels (left hand bar) were significantly elevated and in the range of level C. Later in the season the weather ameliorated and these birds returned to their territories and renested. Note that by this time plasma levels of corticosterone returned to the range of level B (right hand bar). It is useful here to compare with Fig 4. In this figure, the different theoretical levels of secretion fit well with an actual response to an LPF. Redrawn from Wingfield et al., (1983).

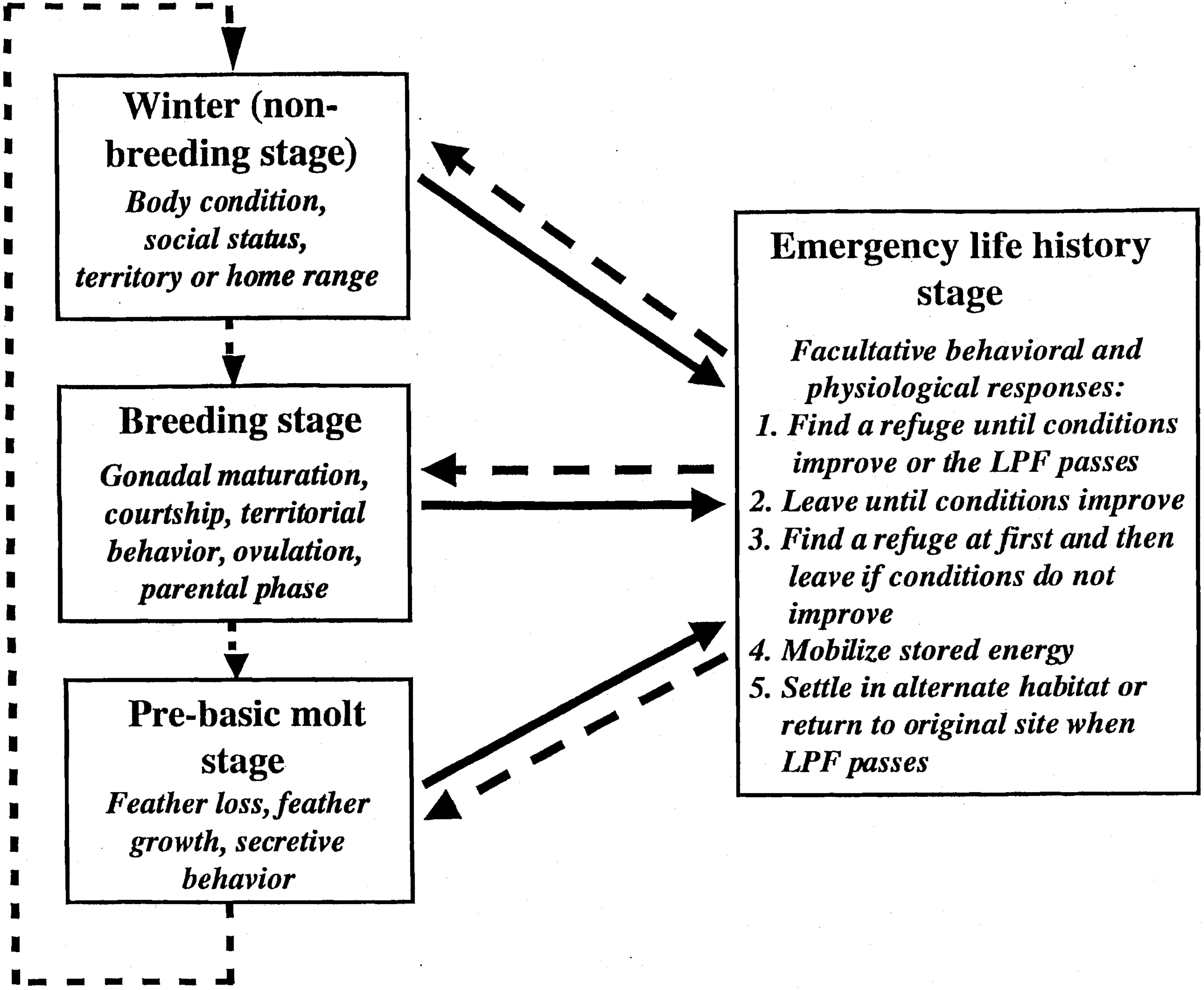
Figure 4. Comparison of the seasonal dynamics of body fat reserves (calculated as body condition index, upper panel) and baseline levels of corticosterone (low panel) in breeding common murres, Uria aalge, between "normal" seasons and an El-Niño in the Low Cook Inlet, Alaska (A.S. Kitaysky et al. unpublished).

TABLE 1

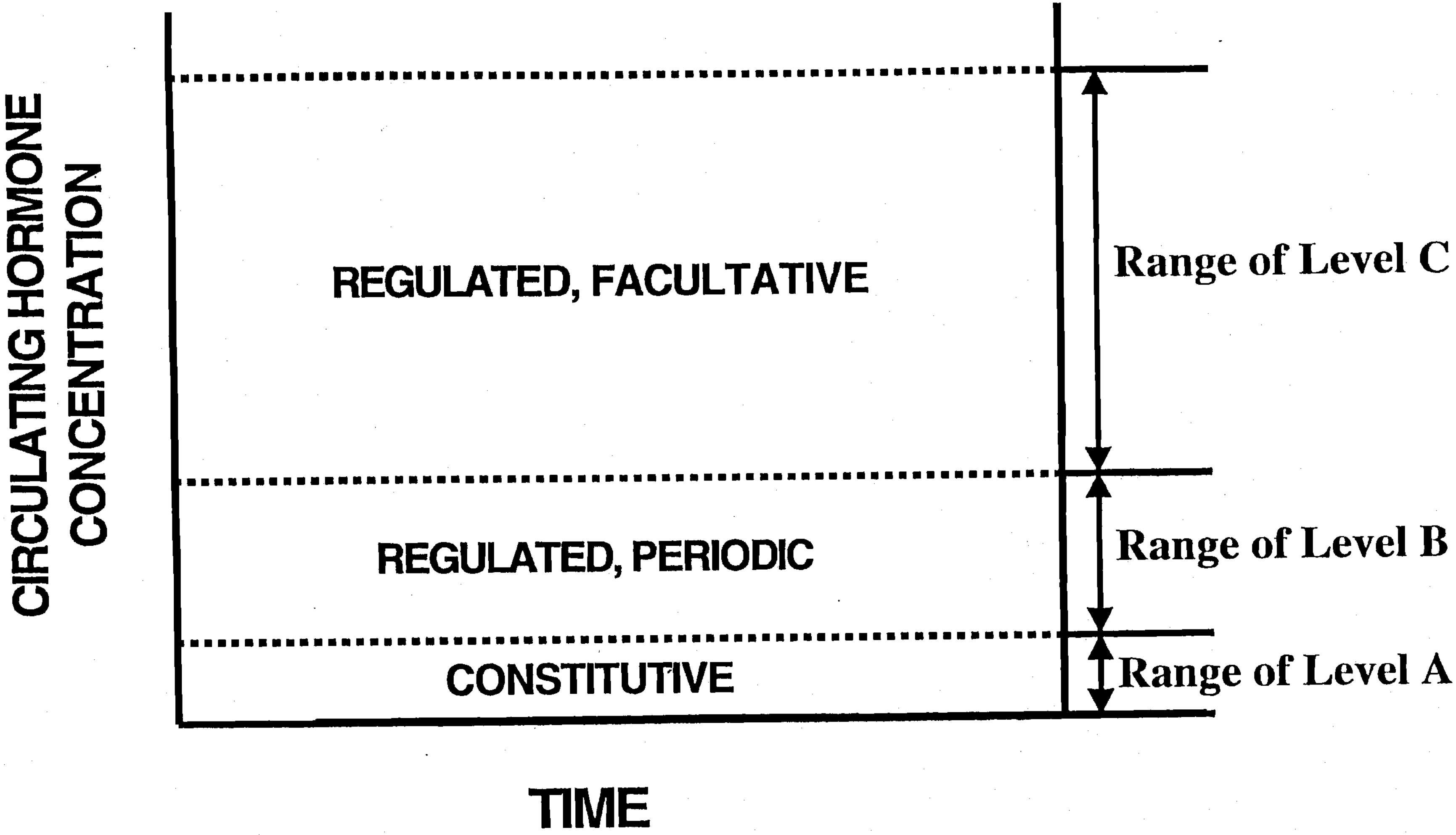
Corticosterone Levels in Relation to Weather at Barrow, Alaska.

	Breeding		Molt	
	Baseline	Stress	Baseline	Stress
Lapland Longspur	No	Yes 37-39%	Yes 47-53%	Yes 41-73%
Snow Bunting	No	No	Yes 19%	Yes 25-30%
Common Redpoll	No	No	Yes 35-59%	Yes 36-60%

Figures represent percentage of the variation in corticosterone levels from breeding and molting passerines that could be explained by weather conditions 24 and 72 hours preceduing capture and sampling. From Romero et al., 2000.


Winter (non-breeding stage)
Body condition, social status, territory or home range
Breeding stage
Gonadal maturation, courtship, territorial behavior, ovulation, parental phase
Pre-basic molt stage
Feather loss, feather growth, secretive behavior
Emergency life history stage
Facultative behavioral and physiological responses:
1. Find a refuge until conditions improve or the LPF passes
2. Leave until conditions improve
3. Find a refuge at first and then leave if conditions do not improve
4. Mobilize stored energy
5. Settle in alternate habitat or return to original site when LPF passes

Levels of Hormone Secretion



CIRCULATING HORMONE CONCENTRATION

REGULATED, FACULTATIVE

Range of Level C

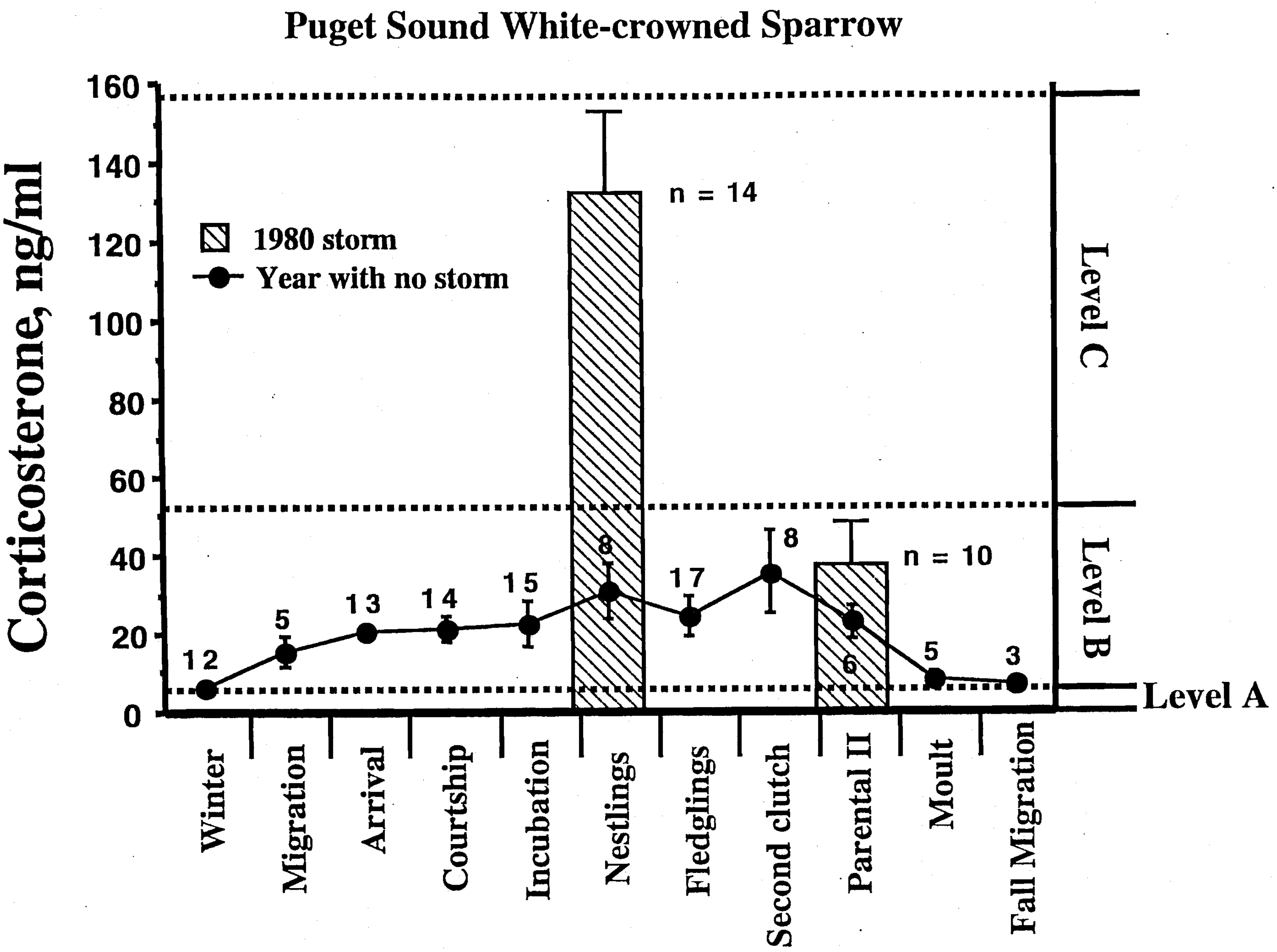
REGULATED, PERIODIC

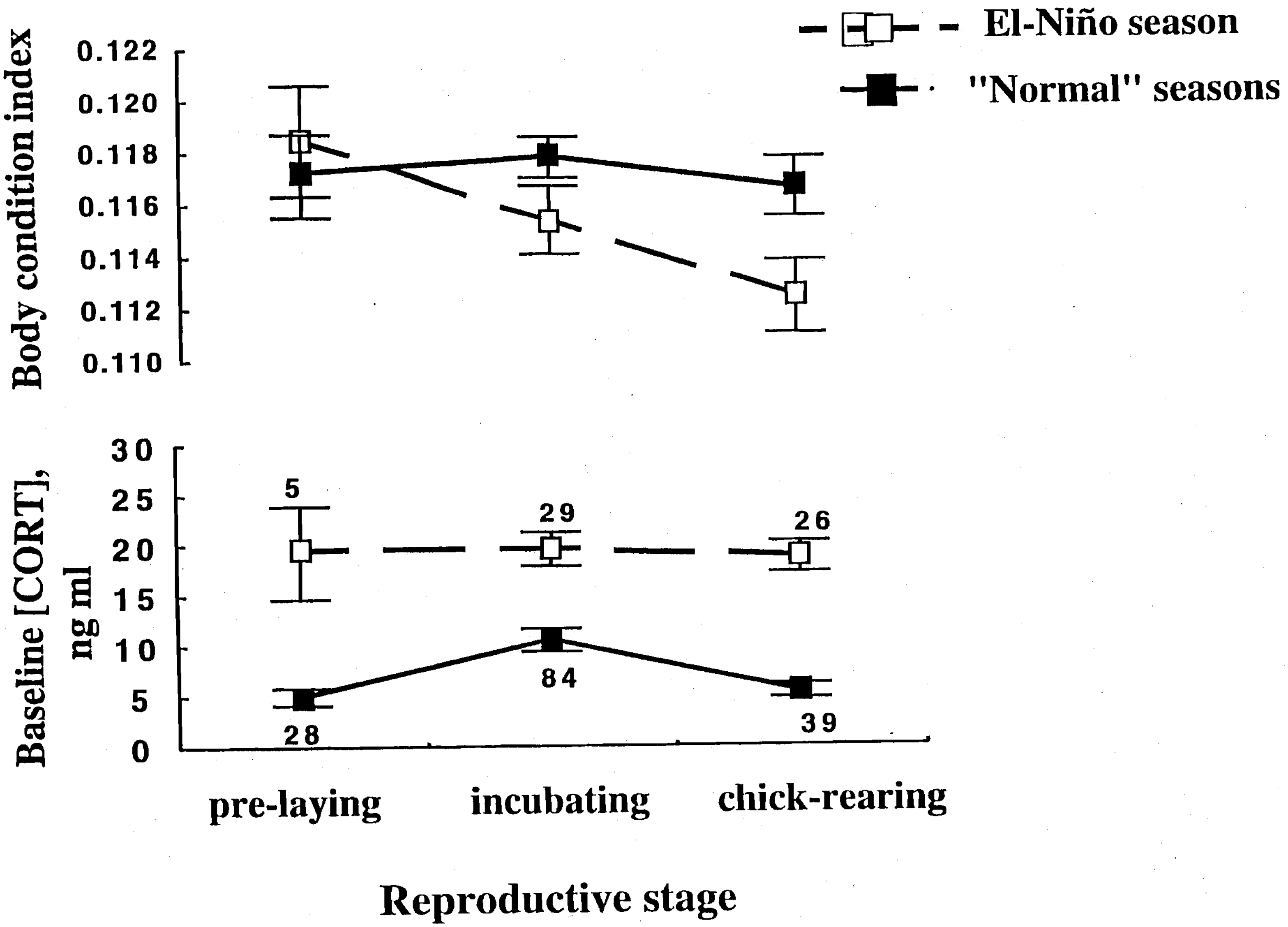
Range of Level B

CONSTITUTIVE

Range of Level A

TIME


Puget Sound White-crowned Sparrow
Corticosterone, ng/ml
160
140
120
100
80
60
40
20
0
▨ 1980 storm
● Year with no storm
n = 14
n = 10
12
5
13
14
15
8
17
8
6
5
3
Level C
Level B
Level A
Winter
Migration
Arrival
Courtship
Incubation
Nestlings
Fledglings
Second clutch
Parental II
Moult
Fall Migration


El-Niño season
"Normal" seasons
Body condition index
0.122
0.120
0.118
0.116
0.114
0.112
0.110
Baseline [CORT], ng ml
30
25
20
15
10
5
0
5
29
26
28
84
39
pre-laying
incubating
chick-rearing
Reproductive stage

GCE

conditionally. accepted
May, 2001

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Long-term unpredictable foraging conditions and physiological stress response in mountain chickadees *(Poecile gambeli)*

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Running title: unpredictable foraging and stress response

Birds respond to short-term deterioration in foraging conditions by increasing their plasma level of corticosterone but the physiological effects of long-term deterioration in food supplies are not well known. In resident passerine birds that winter in temperate climates, such as the mountain chickadee *(Poecile gambeli)*, the food supply may be limited and unpredictable over long periods of time. Whether the long-term limited and unpredictable food supply has an effect on (a) baseline levels of corticosterone and (b) the adrenocortical stress response to a standardized acute stress of handling and restraint in mountain chickadees was assessed. For a period of 94 days, one group of chickadees was maintained on a limited and unpredictable food (food-restricted) and the other group was maintained on *ad libitum* food supply. The food-restricted birds had significantly higher baseline levels of corticosterone compared to those maintained on *ad libitum* food. All birds responded to the acute stressor by increasing secretion of corticosterone but there were no differences between the treatment groups in their stress response. There was a significant effect of sex on the stress response with females reaching higher levels of corticosterone and responding at a faster rate than males. These results suggest that permanent resident birds wintering in harsh environments may have elevated levels of corticosterone on a long-term basis. Whereas other factors such as day length and ambient temperature may contribute to energetic hardship during the winter, the results showed that limited and unpredictable food alone can trigger significant changes in baseline levels of plasma corticosterone. The potential costs and benefits of having long-term increased corticosterone levels in resident food-caching birds are discussed.

Key Words: corticosterone, unpredictable food, food-caching birds, mountain chickadee, stress response.

During the winter, many resident bird species have to cope with harsh environmental conditions characterized by low ambient temperatures, a limited and unpredictable food supply, and only a few hours of daylight in which to replenish the energy reserves that are essential for surviving the long, cold winter nights. These conditions occur for a couple of months at moderate latitudes but may last many months of the year in some areas such as Alaska or northern Siberia. Birds have evolved several behavioral and physiological mechanisms facilitating their survival in these harsh conditions. All birds increase their fat reserves during the winter and some species also create numerous external food caches (see Witter and Cuthill 1993; Pravosudov and Grubb 1997a). It has been shown experimentally that many conditions associated with winter season (day length, unpredictable food supply, ambient temperature) cause birds to increase their fat reserves and the number of food caches (Ekman and Hake 1990; Bednekoff et al. 1994; Pravosudov and Grubb 1997a,b) but the physiological mechanisms of this energy management are not well understood.

It has been suggested that one of the proximate mechanisms regulating energy management in wintering birds could be related to plasma levels of corticosterone (Rohwer and Wingfield 1981; Schwabl et al. 1985, 1988; Astheimer et al. 1992; Rogers et al. 1993; Wingfield et al. 1998). Most of the research to date suggests that increases in corticosterone occur on a short-term basis, as a result of inclement weather such as snow storms and sudden decreases in ambient temperature (Rogers et al. 1993; Wingfield et al. 1998).

Short-term food deprivation elicits elevated levels of corticosterone in migratory and/or non-resident birds (Harvey et al. 1984; Astheimer et al. 1992). Wingfield et al. (1998) referred to these short-term increases in corticosterone levels and associated changes in behavior as "emergency life history stages" in which a response to a sudden change in environmental conditions might interrupt the normal life history cycle and re-direct behavior and physiology towards survival. In particular, increased levels of corticosterone might facilitate foraging behavior and trigger irruptive migration and mobilization of stored energy reserves to fuel increased locomotory activities. During the winter, however, "survival emergency" may last for many months while the resident birds devote most of their time to foraging (Pravosudov 1985). For permanent residents such as food-caching parids, food may be limited and/or unpredictable for a long period of time during the winter, however, these species do not show eruptive migration in response to food shortages (Pravosudov 1985). Furthermore, an increased secretion of corticosterone during the winter was previously documented in resident parids (Silverin 1998), but experimentally increased levels of corticosterone did not trigger irruptive migration (Silverin 1997). This raises the question of whether permanent resident birds respond to prolonged conditions of limited and unpredictable foraging conditions by an increased secretion of corticosterone, or whether observed elevation in corticosterone during the winter is not directly related to foraging conditions. Prolonged, elevated levels of corticosterone resulting from long-term stress has been suggested to cause muscular atrophy, structural damage to the brain, and cognitive impairments (McEwen and Sapolsky 1995, Sapolsky 1996). It is not clear, however, if relatively small long-term increases in baseline levels of corticosterone similar to ones reported in Silverin (1998) have the same negative consequences.

During the winter, in addition to limited and unpredictable food supply, other factors such as day length and ambient temperature make birds' survival energetically challenging. It is important to know if each one of these factors may affect birds' adrenocortical response to stress separately or if all these factors have to be combined to have an effect. This study tested whether long-term unpredictable food conditions alone can affect baseline corticosterone levels as well as adrenocortical responses to acute stress in a permanent resident food-caching passerine, mountain chickadee *(Poecile gambeli).*

MATERIALS AND METHODS

Adrenocortical responses to long-term unpredictable foraging conditions were examined in wild-caught mountain chickadees (*Poecile gambeli*). Twenty four mountain chickadees were caught during November 1999 near the Sage Hen area of Tahoe National Forest in northern California using mistnets near the feeders and decoy birds. After capture, all birds were transported to the laboratory and placed individually in wire-mesh cages (60 × 42× 60 cm). After one week in captivity, the birds were randomly assigned into two groups, with 12 birds (3 females and 9 males) in each group. All birds were maintained on a 8:14-h light:dark cycle corresponding to the shortest day length in the area at a constant temperature of 20^0 C and fed with a mixture of shelled sunflower seeds, crushed peanuts and mealworms. One group was maintained on *ad libitum* food and the other group was given a limited and unpredictable food schedule (food-restricted). Birds in the food-restricted schedule were given three or four 20-min intervals of unlimited access to food per day, resulting in either 60 or 80 min of access to food per day. Each day, we randomly determined whether birds received three of four 20-min feeding intervals, which we randomly distributed within each day. Birds in both treatment groups received the same amount of human disturbance. Every time food had to be added or removed from the cages of one treatment, birds in the other treatment were disturbed similarly. Both groups were maintained in the same room, separated only by a small observation chamber, thus all birds experienced exactly the same physical parameters of the room with the exception of different feeding schedule. As a result, any changes expressed by the birds in either group could be attributed only to different feeding treatments.

Birds were maintained on their feeding schedules for 94 days, from December 21, 1999 until March 23, 2000. Between 60 and 94 days from the beginning of the experimental treatment, all birds were tested on several spatial memory tests (Pravosudov and Clayton 2001). Four blood samples were collected from a wing vein during March 24-27, 2000. The first sample was collected within 3 min of entering the cage, the second sample at 5 min, the third sample at 20 min, and the fourth sample was collected at 50 min after entering the cage. This is standard procedure for testing adrenocortical stress response in small birds with body mass less than 15 g (Wingfield et al. 1995). The total amount of blood collected during the stress series did not exceed 1.5% of bird body mass, which should not cause an additional physiological stress to birds. All chickadees were held in cloth bags between sample collections. We used samples collected within 3 min of entering the cage to determine a baseline level of corticosterone because it has been shown that corticosterone levels usually do not start to elevate until 3 min after capture (Wingfield et al. 1982; Kitaysky et al. 1999). In this study the corticosterone levels did not increase significantly in response to handling within 0-3 min interval after capture (regression analysis of corticosterone concentration on time since entering the cage, $F_{1,19} = 3.14$, $P = 0.092$). Blood was collected at the same time of day for both treatments. Blood was collected into heparinized capillary tubes and then emptied into 0.3ml vials which were kept on ice. All samples were centrifuged within 2 hours of blood collection, the collected plasma samples were frozen at –20°C and then shipped in dry ice to University of Washington for radioimmunoassay analyses (Wingfield and Farner 1975; Wingfield et al. 1992).

We measured concentrations of corticosterone after extraction of 5 – 20 μl samples in dichloromethane. Recovery values of the extraction averaged 80.77 % (range 74.5 – 93.2%). To avoid an inter-assay variation we analyzed all samples during a single assay. An intra-assay variance was 8% and sensitivity of the analysis was 7.8 pg/ml.

After collecting blood samples, all birds were killed for further analyses and their sex was determined by dissection. We also measured wing length and body mass prior to blood collection. Fatness index was used to assess body condition of individual birds, and it was calculated as a ratio of body mass to wing length cubed (Pravosudov and Grubb 1997b). Previous studies demonstrated that fatness index is a good indicator of overall fat reserves in birds (for more discussion on fatness index see Pravosudov and Grubb 1997b).

An ANCOVA compared baseline levels of corticosterone between the two treatment groups and to assess any potential sex differences. Because time of first blood collection varied from 2 to 3 min between the birds we used time since entering the cage until the first bleeding as a covariate when comparing baseline levels of corticosterone. A repeated-measures ANCOVA (PROC MIXED; SAS Institute, 1994) compared the entire stress response including all four samples taken within 50 min of entering the cage. All assumptions of statistical tests were upheld and significance level was set at 0.05.

RESULTS

The baseline levels of corticosterone were significantly higher in food-restricted birds compared to birds maintained on *ad libitum* food (ANCOVA, $F_{1,16} = 6.69$, $P = 0.019$, Fig. 1), but there were no significant differences between males and females (ANOVA, $F_{1,16} = 1.27$, $P = 0.28$). A sex by treatment interaction was not significant ($P > 0.9$) and it was dropped from the final analysis. There were no statistically significant differences between the treatments in time of collecting blood for baseline levels of corticosterone since entering the cage ($t = 0.50$, $df = 20$, $P = 0.62$).

There were no statistically significant differences between the two treatment groups in the adrenocortical response to a standardized acute stress protocol (repeated-measures ANOVA, $F_{1,19} = 0.92$, $P = 0.35$, Fig. 2). Birds significantly increased their levels of corticosterone during the stress protocol (repeated-measures ANOVA, Time effect, $F_{3,55} = 15.70$, $P < 0.001$, Fig. 2). Independent contrasts analysis showed that there were no significant differences between 3 and 5 minutes after entering the cage ($P = 0.26$), but corticosterone levels were significantly higher at 20 min after entering the cage ($P < 0.001$, Fig. 2). Corticosterone concentration was not significantly different between 20 and 50 min after entering the cage ($P = 0.18$). Thus, the highest levels of corticosterone were reached at 20 min after entering the cage but treatments did not statistically differ in their rates of corticosterone change over time (repeated-measures ANOVA, treatment × time interaction, $F_{3,55} = 0.25$, $P = 0.86$, Fig. 2). There were significant sex differences, however, in the adrenocortical response to stress, with females reaching higher levels of corticosterone than males (repeated-measures ANOVA, $F_{1,19} = 9.39$, $P = 0.046$, Fig. 2). Females also showed higher rates of increase in the level of corticosterone than males (repeated-measures ANOVA, time × sex interaction, $F_{3,55} = 2.72$, $P = 0.05$, Fig. 2). Males and females in the two treatment groups responded to the stress differently (repeated-measures ANOVA, treatment × sex interaction, $F_{1,19} = 4.52$, $P = 0.047$, Fig. 2). Females in food-restricted group had significantly higher mean levels of corticosterone during the entire stress response than males ($P < 0.05$) whereas there was no significant differences between males in females in *ad libitum* food group ($P > 0.05$). An interaction between sex, time and treatment was not statistically significant ($F_{3,52} = 0.47$, $P = 0.71$).

At the beginning of the experiments, there were no differences between the two treatment groups in either body size (wing length, ANOVA, $F_{1,20} = 0.32$, $P = 0.59$) or body mass (ANOVA, $F_{1,20} = 0.003$, $P = 0.96$) but females were significantly smaller (wing length, ANOVA, $F_{1,20} = 4.86$, $P = 0.04$) and lighter (ANOVA, $F_{1,20} = 14.45$, $P = 0.001$) than males. A repeated-measures ANOVA showed that birds significantly increased their body mass during the experiment ($F_{1,21} = 12.89$, $P < 0.01$) with no significant difference between the two experimental groups ($F_{1,21} = 0.29$, $P = 0.59$), and an interaction between time and treatment being not significant ($F_{1,21} = 0.96$, $P = 0.34$). Separate analyses, however, indicated that whereas over the course of the experiment, food-restricted birds increased their mass significantly by 4.6% (Paired t-test, $df = 10$, $t = - 4.25$, $P = 0.002$), a mass increase in the *ad libitum* food group was not significant (Paired t-test, $df = 11$, $t = - 1.59$, $P = 0.14$). There were no statistically significant effect of either body mass or body condition measured as fatness index on adrenocortical response to stress (repeated-measures ANCOVA, body mass: $F_{1,17} = 0.36$, $P = 0.55$; fatness index: $F_{1,17} = 0.50$, $P = 0.48$).

DISCUSSION

These results show that mountain chickadees maintained on a short-day photoperiod respond to long-term limited and unpredictable food supply by increasing baseline circulating levels of corticosterone. However, there were no differences in the adrenocortical response to a standardized acute stress of capture and restraint between food-restricted and control groups.

Males and females responded differently to the acute stressor, with females reaching higher levels of corticosterone and more rapidly than males.

These results suggest that birds wintering in temperate climates with harsh winters may have elevated baseline levels of circulating corticosterone over long periods of time as opposed to only short-term elevations caused by limited and unpredictable food supply. Certainly other factors such as day length and ambient temperature could also contribute to the harshness of the environment during the winter. Our study, however, kept temperature and day length constant and as a result we were able to isolate the effect of limited and unpredictable food. Considering that elevated levels of corticosterone appear to be responsible for increased feeding activity and/or increased fat stores (Silverin 1985; Wingfield and Silverin 1986; Gray et al. 1990; Astheimer et al. 1992), our findings are not surprising. Numerous studies have demonstrated that, although fat reserves in birds might fluctuate during the winter in correspondence with short-term environmental stress caused by factors such as snow storms, birds have heightened fat reserves during the entire winter (see Witter and Cuthill 1993; Pravosudov and Grubb 1997a). Whereas many factors can cause birds to increase their fat reserves during the winter (Witter and Cuthill 1993; Pravosudov and Grubb 1997a), limited and unpredictable food supply has been shown to independently affect fattening strategies in birds (Ekman and Hake 1990; Pravosudov and Grubb 1997b). Feeding rates in parids during the winter are also much higher than during the rest of the year (e.g. Pravosudov 1985). Our experiment also demonstrates that food-restricted birds increased their mass significantly over the course of the experiment while maintaining increased levels of corticosterone.

This study demonstrates that limited and unpredictable food supply affects baseline levels of corticosterone independent of other factors. It would be important to find out, however, if other factors associated with winter conditions, i.e. ambient temperature, day length can trigger similar responses.

Mountain chickadees, like many other parids, cache food during the winter (Haftorn 1974, pers. obs.). In food-caching birds, it has been demonstrated that unpredictable food conditions result in increased caching rates (Hurly 1992; Pravosudov and Grubb 1997b). It is possible that an increase in caching rate may also be mediated by elevated levels of corticosterone and increased levels of corticosterone may also have an effect on memory for food caches. For instance, there are some indications that short-term elevation in corticosterone may result in a better memory for caches in mountain chickadees (Saldanha et al. 2000). During the spatial memory tests, it has been found that birds maintained on limited and unpredictable food supply were more accurate in cache retrieval and they performed better on spatial memory tests compared to birds on *ad libitum* food supply (Pravosudov and Clayton 2001). It is possible that better spatial memory performance of food-restricted birds was facilitated by the elevated baseline levels of corticosterone demonstrated in this study.

It has been reported that in some species increased levels of corticosterone might initiate an eruptive migration to more favorable areas (Wingfield et al. 1998). It does not appear, however, to occur in resident food-caching parids, which have been known not to move in the middle of the winter even if conditions are extremely harsh (Pravosudov 1985; Silverin 1997). In food-caching willow tits (*Parus montanus*), increased levels of corticosterone stimulated dispersal behavior of juveniles but not adults during the autumn, but increased levels of corticosterone did not cause either juvenile or adult birds to leave their territories during the winter (Silverin 1997). In wintering food-caching birds, instead of triggering eruptive migration, increased corticosterone levels might mediate an entire suite of adaptive energy management behaviors including gaining extra fat reserves, creating more food caches, and facilitating more accurate memory about the locations of their caches. Thus, the moderate elevation of baseline levels of corticosterone may actually increase the birds' probability of survival during harsh winter conditions.

Although food-restricted birds have significantly elevated baseline levels of corticosterone, those levels are lower than the maximal levels of corticosterone achieved by the birds during the acute stress protocol suggesting that there may be a threshold below which increased levels of corticosterone enhances survival and above which deleterious effects are produced over the long term. The recorded changes in baseline corticosterone could have been within the range normally found in wild birds so that birds were able to compensate for food shortages for example by eating more and/or being more efficient in extracting food energy (Wingfield et al. 1997). If so, slightly increased levels of corticosterone might be an "anti-stress mechanism" facilitating behaviors directed towards maximizing the probability of survival during energetically demanding conditions.

The results of this study also show significant differences between sexes in the adrenocortical response to a standardized acute stressor. It is not clear why female mountain chickadees have a stronger stress response during the winter. A study of wintering dark-eyed juncos (*Junco hyemalis*), for example, did not find any differences between males and females (Rogers et al. 1993). It is also interesting that the difference between males and females in our study was not related to their mass or condition index, although females were lighter than males. From studies of other free-ranging parids, it is known that females usually carry more fat reserves than males (Gosler 1996; Pravosudov et al. 1999) and that these differences are thought to be a result of social dominance. In winter flocks, female parids always assume a subordinate position and thus they might be in a more vulnerable position when energetic emergency appears

(Pravosudov et al. 1999). It is possible that females have a stronger adrenocortical stress response so that their behavior can be re-directed toward energy saving and accumulation at a faster rate than that of dominant males who always have a priority of access to all available food resources.

One concern about the results of this study is that plasma corticosterone was measured only once at the end of the experiment. It is possible that concentration of corticosterone changed with time during the experiment and that the birds have adjusted their corticosterone levels to existing conditions. However, because there was a significant difference in baseline levels of corticosterone between the treatments at the end of the experiment, we believe that birds indeed differed for a long period of time. If anything, such differences could have been larger during the beginning of the experiment. There is no reason to think that there were no differences between the treatments in baseline concentration of corticosterone during most of the experiment and that then such differences would suddenly appear during the last few days. Birds experienced the same conditions for duration of the entire experiment, so the differences were clearly caused by the treatment.

Another concern is that all birds have experienced some stress because of captivity. However, both groups had identical disturbance because every time we would remove or add food to the birds maintained on limited and unpredictable food schedule, we would provide similar disturbance to the birds on *ad libitum* food. There were also no significant differences between the groups in time needed to capture them inside the cage to collect blood samples for baseline levels of corticosterone. Even if all birds experienced stress levels higher than natural, we found that our treatment had an additional significant effect on baseline levels of corticosterone. It is not likely however that captivity resulted in constantly elevated levels of corticosterone because baseline and stress-induced levels of corticosterone in hand-raised mountain chickadees (Pravosudov et al., unpublished) were very similar to values observed in this study.

In conclusion, it is suggested that increases in corticosterone secretion are not limited to short-term deterioration of environmental conditions but can occur over long periods of time. It is possible that some moderate increases in baseline corticosterone levels may not be deleterious for birds but instead be highly beneficial by mediating behaviors directed toward survival over long periods of time. Local foraging conditions do not appear to affect the adrenocortical stress response, which suggests that this response may be regulated seasonally. Elevated levels of corticosterone can result in increased feeding rates and increased fat reserves in all birds, but non-caching birds may have to respond by leaving their territories for more favorable areas whereas food-caching resident passerines can respond by improving their spatial memory for food caches. More direct studies are needed to ascertain the role of corticosterone in food-caching behavior, and memory for caches in particular as well as more generally for understanding the costs of long-term elevated levels of corticosterone in birds.

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FIGURE LEGENDS

Figure 1. Baseline levels of corticosterone (mean and SE) in mountain chickadees maintained on food-restricted schedule (food-restricted birds, solid bar) and in mountain chickadees maintained on *ad libitum* food schedule (hatched bar).

Figure 2. Adrenocortical response to stress (mean and SE) in males and females mountain chickadees maintained on food-restricted schedule and on *ad libitum* food schedule at 0-3, 5, 20, and 50 min after entering the cage. Circles – females, squares – males, open symbols food-restricted treatment, filled symbols – *ad libitum* food treatment.

Figure 1.

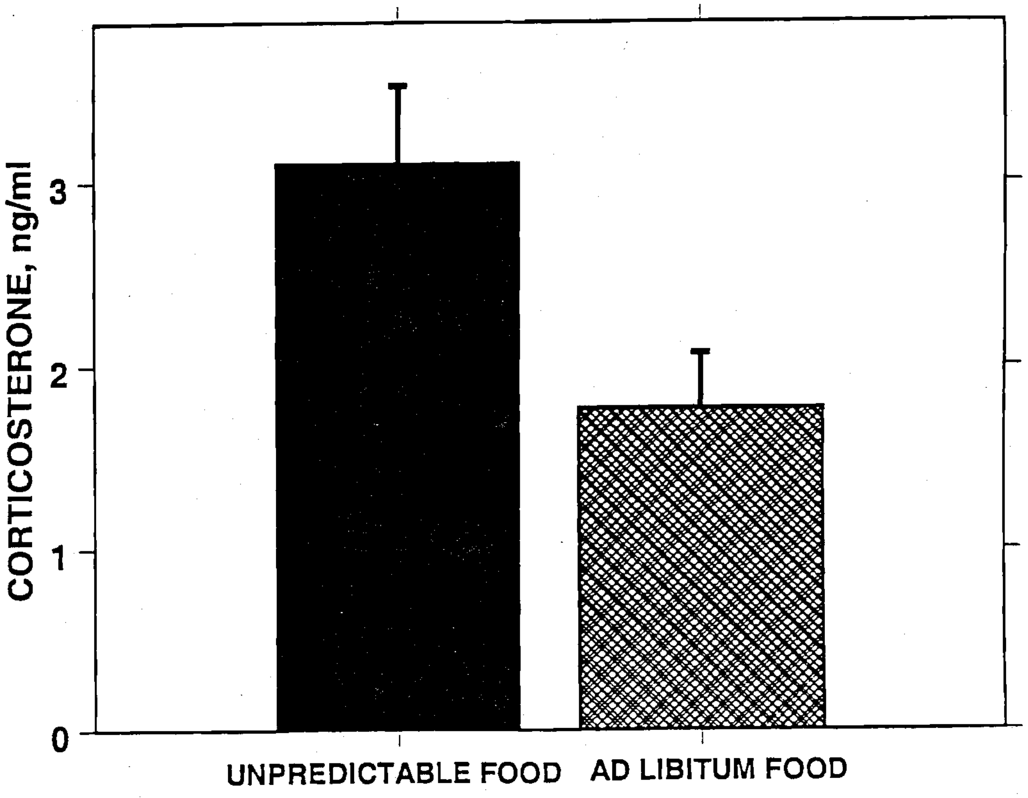
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Figure 2.