ENERGY-ALLOCATION IN AVIAN NESTLINGS FACING SHORT-TERM FOOD SHORTAGE

BØRGE MOE

Dr. scient. thesis

Department of Biology

Faculty of Natural Sciences and Technology

Norwegian University of Science and Technology

2004

Authors address:

Børge Moe
Department of Biology
Faculty of Natural Sciences and Technology
Realfagsbygget
Norwegian University of Science and Technology
NO-7491 Trondheim
Norway
E-mail: Borge.Moe@bio.ntnu.no

- ISBN 82-471-6528-7 (electronic ver.)
- ISBN 82-471-6529-5 (printed ver.)
- ISSN 1503-8181

Contents

Acknowledgments

My work on the present thesis has been supervised by Prof. Claus Bech. His clear ideas, enthusiasm and friendly approach have stimulated my focus into interesting issues and influenced me at all stages during the work with the thesis.

I want to acknowledge that the present thesis has been greatly encouraged and influenced by the ideas in the book *Avian Growth and Development*, edited by J. Matthias Starck and Robert. E. Ricklefs, and from the thesis *The evolutionary significance of developmental plasticity in growing birds*, by William A. Schew.

I am indebted to my co-authors, Einar Stølevik, Siri Brunvoll, Daniel Mork, Trond Einar Brobakk and Claus Bech for their great contributions in the present project. I thank my co-workers from the early start of the kittiwake project, Ingveig Langseth, Geir Wing Gabrielsen, Marianne Fyhn and Claus Bech, for initiating my interest for seabirds. I am grateful to Olivier Chastel, as well as to Marian Førde and Kari Mette Murvoll, for the co-operation during and after the 2002 season on Svalbard and to Bernt Rønning, Einar Stølevik, Pål Olsvik, Henrik Jensen and Claus Bech for different kinds of co-operation on the zebra finch projects. Special thanks go to the staff at the NTNU laboratory, Odd Arne Indset and Bjørn Simensen, for providing the animal housing conditions. I am indebted to Carolyn Baggerud for improving the English language, Rob Barrett, J. Matthias Starck and referees for providing comments on early versions of manuscripts, Thor Harald Ringsby, Ivar Herfindal and Christophe Pélabon for statistical guidance and to the staff at the lighthouse on Sklinna for their hospitality. I want to thank all the students in 'Claus-gruppa', the phd-students and the colleagues at the Department of Biology for providing a friendly and stimulating environment.

The Norwegian Research Council financed the work of this thesis, and I am extremely grateful that they found this project promising. I also thank the Department of Biology, the Faculty of Natural Sciences and Technology and 'Forskningsutvalget' for their support and for providing great working conditions at the Norwegian University of Science and Technology.

Finally, I want to acknowledge Mari, my family and my friends for all the love, support and friendship I have received.

Papers included in the thesis

This thesis is based on the following original papers, which are referred to in the text by their Roman numerals:

I. Moe, B., Stølevik, E. & Bech, C. (2005) Ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. *Physiological and Biochemical Zoology*, **78** (1) (in press).

II. Moe, B., S. Brunvoll, D. Mork, T.E. Brobakk & Bech, C. (2004) Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*). *Journal of Experimental Biology*, **207**, 4067-4076.

III. Moe, B., S. Brunvoll, D. Mork, T.E. Brobakk & Bech, C. (2004) Does food shortage delay development of homeothermy in European shag nestlings (*Phalacrocorax aristotelis*)? *Journal of Comparative Physiology B* (in press).

IV. Moe, B., S. Brunvoll, D. Mork, T.E. Brobakk & Bech, C. (2004) The effect of short-term food shortage on subsequent growth and fledging body size of European shag nestlings. *Journal of Animal Ecology* (submitted).

Introduction

Living organisms have to balance their energy allocation in such a manner that future survival and reproduction is ensured (Stearns 1992). This is particularly challenging for young birds, because they need to get through the vulnerable developmental period as rapidly as possible, while at the same time they should attain a fully developed adult size that ensures both survival and future reproduction (Schew & Ricklefs 1998).

Food availability plays a crucial role during avian development (Martin 1987). Variation in food availability may arise from various environmental factors, such as weather conditions (Bryant 1975; Konarzewski & Taylor 1989; Velando *et al.* 1999), changes in the stocks of prey species (Barrett & Krasnov 1996), rate of food delivery at the nest (Bertram *et al.* 1991), seasonal variation (Lepage *et al.* 1998), habitat differences (Richner 1989) and sibling competition (Magrath 1990; Ricklefs 1993).

It is well documented that systematic food shortage during a breeding season has a devastating effect on growth and survival in seabird nestlings that are dependent on parental provisioning (Barrett *et al.* 1987; Monaghan *et al.* 1989; Anderson 1989; Chastel *et al.* 1993; Harris & Wanless 1997) as well as in self-feeding precocial chicks (Cooch *et al.* 1991; Larsson & Forslund 1992). However, little is known about the effect of short-term and transient food shortage within a breeding season on the ontogenetic development of physiological and morphological characters in birds.

Developmental plasticity

As birds grow from neonate to adult, they may encounter periods of low food availability that cause phenotypic changes from the normal ontogenetic development given by their genotype. Such phenotypic changes (arising from variation in food availability or other environmental conditions) are known as developmental plasticity (Schmalhausen 1949; Bradshaw 1965; Smith-Gill 1983; Schew & Ricklefs 1998; Schlichting & Pigliucci 1998). There has been controversy about the genetic mechanisms and the way phenotypic and developmental plasticity is affected by natural selection (Via *et al.* 1995), but it is well accepted that developmental plasticity is an important life-history-trait that may evolve as a strategy to cope with environmental heterogeneity (Pigliucci 2001). Environmental cues can activate alternative developmental programs (Schmalhausen 1949; Smith-Gill 1983). Smith-Gill (1983) discussed this in terms of multiple, discrete phenotypic states or developmental stages and defined it *developmental conversion*. Schew & Ricklefs (1998) provided a theoretical framework for developmental plasticity in birds, and used the term *induced responses* analogous to Smith-Gill's developmental conversion. They argued that the basic premise, that the organism actively alters development as an adaptive response to environmental cues, should apply equally to continuous measures of metabolism and growth as compared to discrete phenotypic states. Alternatively, the organism may show a passive response, in which the phenotypic changes are imposed by the physical environment. The developmental program is not altered, but the degrees of expression of the developmental program may be modified. Such responses were defined as *imposed responses* and *developmental modulation* by Schew & Ricklefs (1998) and Smith-Gill (1983), respectively.

Growth

The developmental trajectory of chicks involves a co-development of growth and maturation. In this thesis I refer to growth as increase in size and to maturation as changes through which tissues approaches more closely their mature, or adult, level of function.

If avian nestlings are faced with limited food intake during a period of their development, several 'strategies' of energy allocation are possible (Schew & Ricklefs 1998). *First*, the chicks may simply not respond at all. They will consequently maintain the growth rate and the rate of maturation of the organs, muscles etc. at the maximum possible rate, which is allowed with the available food resources and the amount of energy stored at the onset of the food-deprivation period (e.g. Konarzewski *et al.* 1996). *Secondly*, chicks may selectively allocate energy to the growth and development of specific parts of the body important for survival. Less 'important' organs, in terms of immediate survival, will in consequence have their development stalled (e.g. Øyan & Anker-Nilssen 1996). *Thirdly*, the chicks may react to a temporary food-deprivation period by an overall arrest of growth and maturation (e.g. Emlen *et al.* 1991).

The first and the third growth strategy have been classified as imposed and induced responses, respectively, by Schew & Ricklefs (1998). They argued that the first strategy is passive and merely a consequence of the inability to cope with the environmental conditions, whereas the latter is an active and adaptive response. Schew & Ricklefs (1998) advocated that an induced response was characterised by a sharp arrest of overall growth and maturation, whereas an imposed response was characterised by maintained growth within the limits of food intake (usually pathological slowing of growth) and continued maturation. From studies on aerial insectivores (Lack & Lack 1951; Ricklefs 1976; Bryant 1978; Emlen *et al*. 1991), induced growth responses have been characterised as *labile development*, because the growth and maturation has been temporarily stalled. When conditions have improved the nestlings have resumed normal growth, extended developmental time, and fully compensated for the growth perturbation. Highly variable nestling periods have also been reported in many seabirds (Lack 1968; Barrett & Rikardsen 1992; Croxall *et al*. 1988), but it is not known whether these patterns reflect induced growth responses to temporal variation in food availability. Also, developmental time should be subject to an evolutionary trade-off with time dependent mortality, and it is not fully understood to what degree nestlings can increase developmental time to compensate for short-term growth perturbations and attain normal fledging body size.

During realimentation, i.e. the subsequent provisioning of food when conditions have improved, growth may follow three different trajectories. That is accelerated growth (*catch up growth* sensu Bohman 1955), parallel growth or retarded growth, which refers to whether the growth rate is faster, similar or slower, respectively, compared to normal growth relative to chronological age. Among birds, accelerated growth has only been reported for body mass growth (e.g. Schew 1995; Negro *et al*. 1994), whereas parallel growth (e.g. Morse & Vohra 1971; Lepczyk & Karasov 2000) and retarded growth (e.g. Schew 1995;) has been reported for both body mass and structural growth. The occurrence of accelerated growth during realimentation would suggest that normal growth rate is less than the maximum physiological potential and that growth is optimized below a physiological limit rather than maximised.

From studies on development of avian skeletal muscles, it is proposed that growth is inversely related to functional maturation (*growth-maturation hypothesis*, Ricklefs &

Webb 1985; Ricklefs *et al.* 1994). Thus, the growth of an organ can proceed most rapidly when it is less mature. If a period of food shortage therefore results in an overall growth reduction, while the maturation of organs at the same time continues unaffected, the nestling may be in trouble after normal food supply is restored, because the increased level of maturation of the organs will impede the potential for growth.

Development of metabolism and homeothermy

Throughout development the basal level of energy metabolism constitutes a substantial part of the total energy budget of chicks (Weathers 1996). It is generally accepted that the energy budgets of chicks have responded to selection, but little consideration has been given to the evolution of the chick's ability to modify the pattern of energy use and allocation. Recent studies, however, have revealed that some chicks may show energy saving by reducing the resting metabolic rate (RMR) when faced with food shortage (Schew 1995; Kitaysky 1999; Brzek & Konarzewski 2001). Modifying the RMR could occur as an active response. By reducing the energy expenditure, the chicks could lessen the detrimental effects of food shortage and increase survival. Alternatively, any reduction in the RMR could be a direct consequence of the lack of sufficient nutrients during food shortage, and merely reflect the chick's inability to cope with the environment.

Modification of RMR could result from several physiological pathways. Slowing of structural growth has been regarded as one of the means by which RMR can be lowered during food shortage (Schew & Ricklefs 1998). Such a response would represent a shift in the energy allocation from growth to maintenance. Visceral organs, especially the heart, liver, kidneys and intestine, are believed to consume much of the energy used in RMR (Daan *et al.* 1990). Changes in body composition by reductions of the size of these organs should, therefore, lower RMR. Another response that can affect RMR is facultative hypothermia. Regulating the body temperature (T_b) at a lower level results in substantial energy saving.

The chick's ability to defend its T_b during exposure to low ambient temperatures depends on three factors: 1) The peak metabolic rate (PMR), i.e. the capacity for maximum heat production, 2) minimal thermal conductance (MTC) and 3) the heat capacity of the chick (Visser 1998). The transition from neonate to adult includes substantial changes in PMR and MTC, and these changes depend on the position in the altricial-precocial spectrum. I have not taken into account possible changes in heat capacity of the chicks. Precocial chicks are covered with insulating down and are able to show a metabolic response to low ambient temperatures already at hatching (Koskimies & Lahti 1964; Visser & Ricklefs 1993; Starck & Ricklefs 1998a). Altricial nestlings, in contrast, hatch without insulating down and do not respond metabolically to low ambient temperatures (Marsh & Wickler 1982; Starck & Ricklefs 1998a; Visser 1998). Consequently, the degree of homeothermy, measured as the index of homeothermy (HI), is very low in altricial neonates and improves substantially during subsequent growth and development (Ricklefs 1987). Small altricial birds achieve homeothermy at the end of the growth period (Visser 1998), but large altricial birds may develop homeothermy during the early part of the growth period (Dunn 1976; Kirkham & Montevecchi 1982; Montevecchi & Vaughan 1989; Østnes *et al*. 2001).

Food shortage may affect the development of homeothermy for several reasons. Chicks may simply fail to sustain the growth and development of the organs most important for thermoregulatory ability during food shortage. Chicks may also show induced responses to food shortage which involve an overall arrest of growth and maturation. As a consequence, the development of homeothermy will be delayed. Finally, chicks may reduce the RMR to save energy during food shortage. This may in turn negatively affect PMR if RMR is a predictor of PMR.

It is important to separate hypothermia and the development of homeothermy as two distinct features of thermoregulation. The development of homeothermy refers to the development of the physiological properties enabling the defence of the T_b. The occurrence of hypothermia is not necessarily an indication of a poor development of homeothermy. It may occur as a facultative response in concert with a reduction in RMR as a means of energy saving during a period of food shortage, even when homeothermy is fully developed.

Aims of the thesis

This thesis investigates effects of short-term food shortage on growth, body composition and metabolic development of Pekin ducklings (*Anas platyrhynchos domesticus*) and European shag nestlings (*Phalacrocorax aristotelis*), two species representing extremes within the altricial-precocial spectrum. The aims of the thesis were to 1) characterise patterns of growth and development in response to short-term food shortage during early development, 2) examine aspects of energy allocation during these responses with special emphasis on the basal level of energy metabolism, thermoregulatory ability and body composition and 3) investigate the effect of short-term food shortage on subsequent growth and fledging body size.

The individual papers included in the thesis attempt to answer the following questions:

1) Do chicks exhibit any energy saving that can lessen the detrimental effects of short-term food shortage during early development? **Paper I and II**

2) How is energy allocated between growth and maintenance during short-term food shortage? **Paper I and II**

3) Are hypothermia or changes in body composition components of any energy saving response? **Paper I and II**

4) How is thermoregulatory ability and the development of homeothermy affected by short-term food shortage? **Paper I and III**

5) Does short-term and transient food shortage affect subsequent growth and fledging body size? **Paper IV**

The introductory part of the thesis aims to summarise the findings of the papers and provides a general discussion of the findings.

Methods

The corner stone of the experimental approach in this thesis was a diet-restriction treatment. This laboratory treatment aimed to mimic a naturally occurring situation of short-term (4 or 5 days) food shortage which restricted the chicks from following a normal growth trajectory and only allowed a stable body mass (weight-maintenance diet). The diet restriction treatment was applied in all the papers. In Paper IV, a procedure of realimentation (i.e. subsequent provisioning) was used after the diet restriction to monitor the subsequent growth of diet-restricted nestlings in the wild. Here, I briefly describe the procedures of the diet restriction treatment and the realimentation and give information about the animals and their housing conditions. Brief information, relevant for the experimental setup, is provided about metabolic measurements and body composition analyses. Detailed information of that as well as other experimental conditions is provided in the respective papers. The experimental protocols were approved by The National Committee for Animal Research in Norway (‘Forsøksdyrutvalget’).

Animals

European shag nestlings were studied on Sklinna, a small group of islands situated ~50 km off the coast of central Norway (65°12’N, 11°00’E). In 2001, the year of the data collection, the breeding population of European shags consisted of 1750 pairs (N. Røv pers. comm.), and it has increased (6.3% annually) in the period 1984-2001 (Lorentsen 2001). The Norwegian Directorate for Nature Management, Trondheim, gave us permission to work in the shag colony.

The Pekin ducklings were studied at the laboratory of the Department of Biology, NTNU-Trondheim. Eggs were obtained from a farmer and hatched in the laboratory.

Diet restriction and housing conditions

European shag nestlings were collected from their nests in the colony and brought (500 m) to a nearby laboratory at the age of 12 days for the purpose of diet restriction or control-feeding. The nestlings were kept, 4-8 together, in an enclosure (100 x 50 cm) with a heat lamp providing a constant range of operative temperatures (Bakken 1992) of 22-33°C. The diet-restricted and the control-fed nestlings were hand fed with fillets of saithe (*Pollachius virens*) and cod (*Gadus morhua*) and were fed for four days, until they were 16 days old. The diet-restricted nestlings received small portions of food 8-10 times a day to maintain a relatively stable body mass, while the controls were fed every second hour, allowing them to follow a normal body mass growth trajectory.

The ducklings were kept in enclosures (60 X 40 cm) with heat lamps and had *ad libitum* access to water and poultry pellets after hatching. They were progressively given more space with larger enclosures as they grew bigger. The ducklings were randomly assigned to diet restriction or control treatment. Diet restriction consisted of a weight-maintenance diet for 5 days, in which pellets were provided in small portions 4-7 times a day. Two age-groups of ducklings were subject to diet restriction, one at 5-10 and another at 15-20 days of age. Ducklings subject to control-treatment had *ad libitum* access to food. Both treatments had *ad libitum* access to water. There was no mortality during any of the diet-restriction treatments.

Realimentation experiment

After the diet restriction treatment, a sample of European shag nestlings at 16 days of age was assigned to new nests with foster parents in the colony. In this experiment I intended to monitor the growth during realimentation under favourable competitive conditions in the wild. They were swapped with the α-nestlings which were moved to new nests with foster parents. The α-nestlings were heavier than the diet-restricted nestlings whereas the β–nestlings were lighter than the diet-restricted nestlings. With this swapping procedure the diet-restricted nestling got the α-position in the nestling size hierarchy, the brood size (2 or 3) was unchanged and the work load on the parents was presumed to be unchanged.

As controls to the diet-restricted nestlings I assigned a sample of unmanipulated nestlings at 12 days of age to new nests with foster parents. As for the diet-restricted nestlings, they were subject to the same swapping procedure that provided favourable competitive conditions. The controls were assigned to foster parents at the age of 12 days because their body mass was almost the same as the diet-restricted nestlings at 16 days of age. The growth of the controls and the diet-restricted nestlings was monitored until they were 36-48 day old. Growth parameters and fledging body size were estimated by a logistic growth model (Ricklefs 1983).

Metabolic measurements and body composition

Metabolic measurements including measurements of T_b were performed on the diet-restricted chicks on the last day of the diet restriction treatment and on independent groups of controls with ages corresponding to the age of the diet-restricted chicks at the start and at the end of the diet restriction periods. Each chick was only subject to one metabolic measurement and was sacrificed immediately after the metabolic measurement for later analyses of body composition.

Results

Metabolism

Ducklings and European shag nestlings exhibited substantial energy saving in response to short-term food shortage. Relative to their body mass, the RMR of 10 and 20 day old diet-restricted ducklings was 16.4 and 32.1% lower, respectively, compared to controls with *ad libitum* access to food (Paper I), and the RMR of diet-restricted European shag nestlings was 36.5% lower compared to control fed nestlings (Paper II).

The reductions in RMR were accompanied by moderate hypothermia in the youngest-diet restricted ducklings and in the diet-restricted European shag nestlings, but hypothermia did not occur in the oldest diet-restricted ducklings. The T_b of the youngest diet-restricted ducklings was 1.1 ^{o}C lower compared to controls at the same age (Paper I), and the T_b of the diet-restricted European shag nestlings was 2.1 ^{o}C lower compared to controls at the same age (Paper II). Hypothermia accounted for ~50 and ~70% of the reduction in RMR in the youngest diet-restricted ducklings and the diet-restricted European shag nestlings, respectively. However, calculations, assuming a Q_{10} between 2.0-2.5, showed that more than 60% of the reductions in RMR must have been due to other physiological processes than just the passive temperature dependence of RMR.

Analyses were performed to test whether changes in body composition could explain the observed reductions in RMR in the diet-restricted chicks. These analyses revealed that variation in the size of the liver was a significant predictor of the reductions in RMR. However, it was not a strong predictor.

Thermoregulation

PMR was negatively affected by the diet restriction in both species. PMR was ~10% lower in both age-groups of diet-restricted ducklings compared to controls (Paper I), and PMR was 16% lower in the diet-restricted European shag nestlings compared to the controls (Paper III).

In contrast to PMR, absolute metabolic scope (PMR-RMR) and mass-specific MTC were not negatively affected by the diet restriction (Paper I, III). This shows that the portion of PMR available for regulatory thermogenesis and the total insulation developed according to age even though body mass lagged behind during the diet-restriction. Thus, the overall thermoregulatory ability seemed to be well maintained. This was supported by the measurement of an index of homeothermy in European shag nestlings. Diet restricted nestlings did not exhibit a significantly lower index of homeothermy as compared to controls at the same age (Paper III).

Maturation

Maturation, measured as the lean dry fraction (LDF), of skeletal muscles increased during the diet restriction periods in both species (Paper I, III). Thus, maturation of skeletal muscles was similar or higher in diet-restricted chicks compared to controls at the same age. Also, the maturation of the other muscle tissues, i.e. the heart and the gizzard, was similar or higher. Further, diet-restriction had no negative effect on maturation in any visceral organs, except for the intestine of European shag nestlings (Paper II, III) and the liver in the oldest group of ducklings (Paper I). Consequently, except for the latter examples, the results on maturation did not indicate any temporarily stalled maturation of muscles or organs.

Structural growth

Growth of structural elements, i.e. skull (head + bill), tarsus and wings, showed a significantly different response to diet restriction in the ducklings in comparison with the European shag nestlings. Whereas structural growth was sustained at high rates during food shortage in the European shag nestlings (Paper II, IV), slowing of structural growth occurred in the Pekin ducklings (Paper I). The structural growth of the ducklings depended on the structural element in question and on age. The growth of the skull was given the highest priority and increased in size during the diet-restriction period relative to body mass. However, the growth of the skull of the diet restricted ducklings was lower than that of the controls of the same age. The growth of the tarsus and the wings were lower than that of the skull in the diet-restricted ducklings. The tarsus of the diet-restricted ducklings was maintained in proportion to body mass, and so were the wings of the oldest diet-restricted ducklings. However, the wings of the youngest diet-restricted ducklings were ~16% shorter than predicted from the normal relationship between body mass and wing length.

Body composition

Diet restriction had a substantial effect on body composition in both species. A common response to the diet restriction was a reduced mass of the liver and the lipid stores relative to body mass in both species. In the ducklings, the mass of the leg muscles, the heart and the kidneys were maintained in proportion to body mass during the diet-restriction (Paper I). In contrast, the intestine was the only organ in the European shag nestling that was maintained in proportion to body mass during the diet restriction (Paper II, III). The intestine mass of the ducklings was substantially reduced during the diet restriction (Paper I). The mass of the pectoral muscles were reduced in the oldest diet-restricted ducklings but not in the youngest. The mass of the gizzard was heavier than predicted from body mass in the youngest diet-restricted ducklings, but not in the oldest (Paper I).

Growth during realimentation

The diet-restricted European shag nestlings resumed normal growth when they were assigned to foster parents in the wild (Paper IV). Although different statistical methods provided slightly contrasting results for this period of realimentation, it seemed that body mass and structural elements grew according to a parallel trajectory compared to controls. Developmental time was extended in the diet-restricted nestlings, and the asymptotic values for the growth characters did not differ between controls and diet-restricted nestlings. Growth was monitored until the nestlings were 36-48 days of age, and all nestlings had reached or were close to 95% of the asymptotic values for all growth characters. Therefore, the asymptotic values were adequate measures of fledging body size. Accordingly, fledging body size did not differ between controls and diet-restricted nestlings (Paper IV).

General discussion

Induced vs. imposed growth responses

Growth of linear measurements and functional maturation defines a pattern of ontogeny. The pattern that occurs under normal conditions has been suggested as a crucial benchmark for evaluating whether developmental plasticity of growth can be characterised as induced or imposed (Schew & Ricklefs 1998). Several traits and methods have been used to characterise maturation, i.e. the degree of mature, or adult, morphology and level of function. On the tissue level, the lean dry fraction (LDF) is found to be a good measure of the functional maturation in skeletal muscles (Ricklefs & Webb 1985; Ricklefs *et al*. 1994), and it is assumed to reflect functional maturity rather well in visceral organs as well (Ricklefs *et al*. 1998). LDF is a measure that is easy to obtain by adequate drying of tissue samples. For bones and skeletal elements, different histological methods have been used to obtain adequate measures of maturity (Starck 1998; Starck & Chinsamy 2002; de Margerie *et al*. 2004). In my studies (Paper I, II, III) I obtained measures of the LDF of skeletal muscles and visceral organs. These measures should correlate well to overall maturation of the chicks, but I cannot assess how precisely they reflect the maturity of the skeleton. The maturation of skeletal muscles and most visceral organs increased during the periods of diet restriction in the ducklings (Paper I) and in the European shag nestlings (Paper II, III). All the structural elements (skull, tarsus, wings) of the European shag nestlings grew at very high rates, almost in line with that of controls, and were presumably supported by heavily scavenging nutrients from skeletal muscles and visceral organs. The structural growth of the ducklings depended on the structural element and age-group in question. In relation to body mass of the ducklings, the skull grew at the highest rates, and the tarsus and the wings grew at the lowest rates, while the youngest ducklings exhibited higher growth rates compared to the oldest ducklings relative to that of controls.

Schew (1995) suggested that induced responses in structural growth also were characterised by a sharp decrease in growth rate instead of a gradual pathological decrease. Accordingly, the growth of the structural elements of the European shag nestlings should be categorised as an imposed response, rather than an induced response. However, the overall growth response of the European shag nestlings, though, does not seem to be '*passive*'. A passive response should presumably correspond to a relatively proportional growth, whether growth was sustained or relaxed, in all body parts, but this did not occur. The structural elements seemed to have an extreme priority at the expense of visceral organs and skeletal muscles. The structural growth of the ducklings differed compared to the European shag nestlings. The oldest ducklings, but to some degree also the youngest ducklings, seemed to decrease structural growth rates immediately at the onset of diet restriction, but the functional maturation was sustained (Paper 1; own unpublished data). The ducklings, therefore, seem to exhibit some degree of labile development. As Schew & Ricklefs (1998) argued that imposed and induced responses represent a continuum rather than discrete options, it might be correct to place the growth responses of the ducklings at the induced side of that continuum rather than at the imposed side. This is in accordance with a previous study that reports some degree of labile development of mallards (*Anas platyrhynchos*, Street 1978), the species from which the Pekin duck is domesticated.

Although the European shag nestlings did not show an induced growth response in the face of food shortage, their growth potential did not seem to be negatively affected. When conditions improved, the nestlings seemed to follow a parallel growth trajectory to that of controls, and they slightly extended developmental time. Consequently, they attained a fledging body size that was similar to that of the controls (Paper IV). An impeded growth potential should be expected due to the continued maturation of the nestlings during the period of food shortage. Why then, did the nestlings attain normal fledging size? One plausible explanation for this is that the changes in LDF of the skeletal muscles and the visceral organs do not adequately reflect the maturation of the skeleton. If the degree of ossification and the size of the cartilaginous proliferation zones were maintained in normal proportions to the growth of the bones, the potential for growth should not be impeded. Another explanation is that the growth-maturation hypothesis is not valid for the growth of the European shag nestlings. Such an interpretation, however, would be controversial, as morphological data generally support the growth and functional maturity trade-off (Starck 1998). A third explanation is that the food shortage did not last long enough to impose sufficient changes in growth and maturation that would impede the growth potential significantly. As the nestlings were able to sustain high structural growth rates during the food shortage period, even a limited ability to extend developmental time was sufficient to buffer the growth perturbations.

Metabolic responses

Plasticity of the ontogenetic development of energy metabolism can be an adaptation to unpredictable and fluctuating feeding conditions. A reduction in the basal level of energy metabolism might reduce the detrimental effects of food shortage and facilitate survival. Making firm conclusions about the adaptiveness of developmental responses is obviously a dangerous exercise (Gould & Lewontin 1979), but I have argued that the observed reductions in energy metabolism in the diet-restricted ducklings and European shag nestlings may have an adaptive significance (Paper I, II). The experimental setup, however, did not allow tracking of the changes in energy metabolism at the onset of diet restriction or at the onset of realimentation. Consequently, I cannot unequivocally judge the responses as being active or passive, as suggested by Schew & Ricklefs (1998). They proposed that an active response was characterised by a sharp decrease in metabolic rate immediately at the onset of diet restriction, and a sharp increase in metabolic rate at the onset of realimentation. Tracking of plasma levels of uric acid could also be a fruitful measure in assessing possible adaptive energy saving responses. This has been done in studies of the physiological adaptations of king penguin (*Aptenodytes patagonica*) chicks (Cherel *et al*. 1987) and adults (LeMaho 1983; Cherel *et al*. 1988) to fasting during winter and incubation, respectively. Within three days of fasting king penguins switch to a nitrogen-sparing physiology, in which lipids are metabolised and proteins are saved. The daily mass loss is minimised, enabling survival of the chicks through fasting for 5 months during the subantarctic winter. The protein-sparing metabolism of king penguins has been suggested as a clear example of an adaptive metabolic response (Schew & Ricklefs 1998).

Energy saving in response to experimental food shortage has been reported for several species (Table 1). Japanese quail (*Coturnix coturnix japonica*, Schew 1995) and male broilers (*Gallus gallus*, Zubair & Leeson 1994) respond rapidly to diet restriction by decreasing metabolic rate and subsequently increasing it in response to realimentation. In contrast, nestling European starlings (*Sturnus vulgaris*, Schew 1995) and song thrushes (*Turdus philomelos*, Konarzewski & Starck 2000) did not show any metabolic response during short-term diet restriction. Konarzewski & Starck (2000) suggested that a lack of frequent, unpredictable fluctuations in food availability or a strong selection for uniform adult phenotypes could prevent plasticity of the developmental program of nestlings.

The importance of the ecological settings, determining the degree of predictability in the food availability, was tested by Kitaysky (1999). He found that the piscivorous horned and tufted puffins (*Fratercula corniculata* and *Lunda cirrhata*) which rely on fluctuating food resources, showed greater metabolic responses to food shortage compared to the planktivorous crested and parakeet auklets (*Aethia cristatella* and *Cyclorhinchus psittacula*) which rely on continuously available food resources. Sibling competition may also be among the selective factors for metabolic responses to short-term food shortage. Brzek & Konarzewski (2001) demonstrated a reduced RMR in diet-restricted sand martin nestlings (*Riparia riparia*), and showed that this response was amplified by the presence of hungry siblings.

Table 1. Species known to show energy saving (i.e. reduced resting metabolic rate) in response to experimental short-term food shortage during early development.

Species	Developmental mode	Reference
European shag (*Phalacrocorax aristotelis*)	Altricial	Paper II, III
Sand martin (*Riparia riparia*)	Altricial	Brzek & Konarzewski 2001
Horned puffin (*Fratercula corniculata*)	Semi-precocial	Kitaysky 1999
Tufted puffin (*Lunda cirrhata*)	Semi-precocial	Kitaysky 1999
Crested auklet (*Aethia cristatella)*	Semi-precocial	Kitaysky 1999
Parakeet auklet (*Cyclorhinchus psittacula*)	Semi-precocial	Kitaysky 1999
Broiler (*Gallus gallus*)	Precocial	Zubair & Leeson 1994
Pekin duck (*Anas platyrhynchos dom.*)	Precocial	Paper I
Japanese quail (*Coturnix c. japonica*)	precocial	Schew 1995

PMR was negatively affected by diet restriction in both of my study species (Paper I, III). This could occur as a result of negative effects of diet restriction on the capacity for regulatory thermogenesis or as a result of the low RMR, or as a combination of both. The absolute scope, i.e. the proportion of PMR available for regulatory thermogenesis was not negatively affected in the diet restricted chicks, and I revealed that RMR was a significant predictor of PMR. Accordingly, the low PMR seemed to occur as a result of the low RMR of the diet-restricted chicks. This indicates that chicks seem to face a trade off between reducing the basal level of energy metabolism, as an energy saving response, and maintaining the capacity for maximum heat production during temporal food shortage. In contrast to PMR, the MTC developed in line with that of controls of the same age (Paper I, III), and the isolative properties of the diet-restricted chicks were therefore very well developed.

Despite reductions in RMR and PMR, the abilities of the diet-restricted chicks to defend their T_b seemed to be very well developed. This was further supported by measurements of an index of homeothermy in the European shag nestlings, which showed that the degree of homeothermy was not significantly affected in the diet-restricted nestlings compared to that of controls at the same age (Paper III).

The time and energy budgets of chicks and parents are interrelated (Beintema & Visser 1989; Moe *et al.* 2002). If food shortage delays the development of homeothermy in chicks, the need for continued brooding would constrain the available foraging time of the parents. Continued development of homeothermy even during food shortage could therefore be particularly adaptive for species inhabiting harsh environments. In contrast, entering a substantial hypothermic state has been regarded as an adaptive response to substantial food shortage in unattended chicks of fork-tailed storm petrels (*Oceanodroma furcata*, Boersma 1986). This is a pelagic seabird that may experience periods of protracted fasting. Moreover, it nests in burrows with constant and predictable cool ambient temperatures. Bech *et al.* (1991) and Weathers *et al.* (2000), however, found no evidence that Antarctic fulmarine petrels normally experience hypothermia in the field, and suggested that the cold and variable climate precludes these species from using substantial hypothermia. Ducklings and European shag nestlings are not as likely to experience periods of protracted fasting, as are pelagic seabirds, and their ambient temperatures are highly variable. Feeding patterns as well as physical and climatic conditions could be important factors for the evolution of different developmental strategies of thermoregulation in different species.

Differential developmental plasticity

The ducklings and the European shag nestlings exhibited a varying degree of plasticity of different body parts in response to food shortage. Visceral organs and muscles showed generally a higher degree of plasticity compared to that of the structural elements (skull, tarsus, wings). Moreover, the physiological systems (including RMR, PMR and T_b) also showed a high degree of plasticity.

One explanation for such differential developmental plasticity could be that different body components merely have different nutritional requirements. The observed differential developmental plasticity is then merely a product of how the food intake meets the different nutritional requirements. Calcium and phosphorus, for example, are essential inorganic structural nutrients (Murphy 1996). If these nutrients, rather than energy primarily limit the rate of structural growth, it could suggest that the chicks were provided in sufficient amount during the food restriction to sustain growth of all parts of the skeleton (shags, Paper II, III) or to sustain growth of some parts of the skeleton (ducklings, Paper I).

Different degrees of developmental plasticity of different body components may also be due to a competition for nutrients between various growing tissues (O'Connor 1977; Sedinger 1986). Priority should then be given to those tissues or body parts most important for immediate survival. During food shortage, the growth and maturation of the brain is reported to be strictly sustained (Schew 1995), and the mass of the heart is reported to be maintained in proportion to body mass (Schew 1995; Paper I). Amino acids are essential nutrients for energy metabolism and for structural properties of visceral organs, muscles and the skeleton. Judging from the substantial reductions in the size of some visceral organs and muscles (e.g. pectoral), amino acids appeared to be actively scavenged from these organs to support growth or maintenance of the skeleton (Paper I, II, III). In the European shag nestlings, sustained growth of the whole skeleton was given an extreme priority at the expense of visceral organs and some muscles (i.e. pectoral and heart, Paper II, III), whereas the ducklings preferentially allocated resources to a slightly sustained growth of the skull (Paper I). The size of the leg muscles as well as the maturation of the skeletal muscles was given high priority in both species. These results suggest that thermoregulatory ability was given a high developmental priority. However, it could also indicate a high priority of locomotor ability and competitive ability for sibling competition (Brzek & Konarzewski 2001). Also, the combined effect of sustained overall skeletal growth and maturation of skeletal muscles would be particularly beneficial for the competitive abilities of nestling European shags.

Another explanation for differential developmental plasticity is that different body parts may have different abilities to recover when conditions improve after a period of food shortage. Avian visceral organs and muscle tissue are shown to exhibit a highly flexible nature, allowing fast and reversible changes (e.g. Schew 1995; Piersma & Lindstrøm 1997; Starck 1999; Starck & Rahman 2003). These organs and tissues are therefore likely to fully recover if conditions sufficiently improve during realimentation. The skeleton, in contrast, is generally thought to be less flexible compared to visceral organs due to internal physical constraints (Schew & Ricklefs 1998; Pigliucci 2001). Some species exhibit a very rigid development of the skeleton (e.g. Konarzewski *et al.* 1996; Paper II, IV). However, it is evident that other species exhibit a very flexible development of the skeleton (e.g. Emlen *et al.* 1991; Schew 1995). The ability of the skeleton to recover during realimentation should therefore also reflect species-specific development, and not only general properties or constraints of the skeletal tissues. Also, recovery is an integrated process between organ systems. The digestive organs, for example, must fully function before other parts of the body can receive sufficient resources for subsequent growth and recovery. This could be a possible explanation as to why the intestine and the gizzard were given so high priority during food shortage in the European shag nestlings (Paper II) and in the ducklings (Paper I), respectively. The maintenance of these organs could be of major importance for the ability to immediately resume normal growth at the onset of realimentation in both species (Paper IV; unpublished data).

I reported a remarkable example of differential developmental plasticity of the European shag nestlings (Paper II, IV), in which structural growth was rigidly sustained while RMR and body composition showed highly flexible development during food shortage. Lack of flexible development of structural body size has been attributed several evolutionary interpretations. It may evolve if the prevailing environment lacks frequent, unpredictable fluctuations in food availability during the development (Konarzewski *et al.* 1996). Also, if there is strong selection for relatively uniform fledging or adult body size, developmental flexibility might be suppressed even under fluctuating environmental conditions. This may occur if flexibility cannot be compensated during later development, and flexible development consequently would produce a diversity of fledging and adult body sizes (Konarzewski & Starck 2000). Finally, rigid development of body size may evolve as a result of strong selective pressure on nestling competitive abilities and of strong time-dependent mortality (Schew 1995).

The flexible development of RMR and body composition of European shag nestlings (Paper II, III) does not easily support the first interpretation which predicts an overall rigid development. The two latter interpretations are not mutually exclusive. If the ability of European shag nestlings to extend developmental time is rather limited, the rigid development of body size may have evolved as a result of selection on fledging or adult body size, selection on nestling competitive abilities or of strong time-dependent mortality.

Altriciality vs. precociality

The evolution of developmental responses is driven by natural selection and limited by internal constraints (Starck & Ricklefs 1998a; Ricklefs *et al.* 1998; Pigliucci 2001), in which genetic and developmental constraints are important (Pigliucci 2001). Therefore, developmental mode, in the altricial-precocial spectrum, could possibly be a crucial determinant for developmental responses to short-term food shortage. Altricial and precocial chicks differ substantially in the degree of maturity at hatching (Starck & Ricklefs 1998a). Moreover, their thermoregulatory and locomotor abilities, feeding patterns and growth trajectories differ throughout postnatal development (Nice 1962; Starck & Ricklefs 1998b).

The European shag and the Pekin duck represent two extremes within the altricial-precocial spectrum. The responses of structural growth differed between the two study species. However, their physiological responses (including energy metabolism and body composition of visceral organs) were rather consistent. Energy saving in response to food shortage has been revealed in several other species across the altricial-precocial spectrum (Table 1), and labile development of structural growth has also been reported in several species of different developmental modes (see Schew 1995). In agreement with recent literature (e.g. Emlen *et al*. 1991; Schew 1995; Konarzewski *et al*. 1996; Kitaysky 1999; Brzek & Konarzewski 2001), the results of the present thesis suggest that developmental responses to short-term food shortage are not strictly linked to the placement within the altricial-precocial spectrum, but are more likely to be influenced by the degree of unpredictability of the food availability in the environment, rates of provisioning, and the degree of sibling competition and time-dependent mortality.

Conclusions

The following main conclusions can be drawn from the findings of this thesis:

- Pekin ducklings as well as European shag nestlings showed substantial energy savings, by lowering RMR, in response to short-term food shortage. Such energy savings could lessen the detrimental effects of food shortage and enhance survival.
- The RMR reductions were partly explained by hypothermia and reductions in the size of the liver. Hypothermia (modest reductions in T_b) accounted for a large portion of the reductions in RMR in European shag nestlings and in the youngest ducklings, but did not occur in the oldest ducklings. A reduction in the size of the liver was a significant, but not a major, determinant of the observed reductions in RMR.
- PMR was negatively affected by food shortage in both study species. It seemed that the reductions in RMR rather than any decreased capacity for regulatory thermogenesis caused the negative effect on PMR. MTC decreased and absolute scope increased during the period of food shortage in both species. These results indicate that thermoregulatory abilities were given a high developmental priority during food shortage.
- Short-term food shortage corresponded with a substantial change in energy allocation between growth and maintenance, especially in the European shag nestlings. They sustained high rates of structural growth at the expense of the size of most visceral organs and muscles.
- Fledging body size was similar between controls and diet-restricted nestlings after realimentation. Thus, variation in fledging body size does not seem to arise from short-term and transient food shortage during early growth of European shags.
- The development of metabolic rate, body composition and growth showed varying degrees of plasticity in response to short-term food shortage in Pekin ducklings as well as in the European shag nestlings. Moreover, the two study species showed similar as well as different developmental responses to food shortage, indicating that such responses are not strictly linked to the placement within the altricial-precocial spectrum.

References

Anderson, D.J. (1989) Differential responses of boobies and other seabirds in the Galápagos to the 1986-1987 El Niño-souther oscillation event. *Marine Ecology Progress Series*, **52**, 209-216.

Bakken, G.S. (1992) Measurement and application of operative and standard operative temperatures in ecology. *Amercian Zoologist*, **32**, 194-216.

Barrett, R.T. & Rikardsen, F. (1992) Chick growth, fledging periods and adult mass loss of Atlantic puffins *Fratercula arctica* during years of prolonged food stress. *Colonial Waterbirds*, **15**, 24-32.

Barrett, R.T. & Krasnov, Y.V. (1996) Recent changes in stocks of prey species by seabirds breeding in the southern Barents Sea. *ICES Journal of Marine Sciences*, **53**, 713-722.

Barrett, R.T., Anker-Nilssen, T., Rikardsen, F., Valde, K., Røv, N. & Vader W. (1987) The food, growth and fledging success of Norwegian Puffin chicks *Fratercula arctica* in 1980-1983. *Ornis Scandinavia*, **18**, 73-83.

Bech, C., Mehlum, F. & Haftorn, S. (1991) Thermoregulatory abilities of nestlings in the Antarctic petrel (*Thalassoica antarctica*). *Polar Biology*, **11**, 233-238.

Beintema, A.J. & Visser, G.H. (1989) The effects of weather on time budgets and development of chicks of meadow birds. *Ardea*, **77**, 181-192.

Bertram, D.F., Kaiser, G.W. & Ydenberg, R.C. (1991) Patterns in the provisioning and growth of rhinoceros auklets. *Auk*, **108**, 842-852.

Boersma, P.D. (1986) Body temperature, torpor, and growth of chicks of fork-tailed storm petrels (*Oceanodroma furcata*). *Physiological Zoology*, **59**, 10-19.

Bohman, V.R. (1955) Compensatory growth of beef cattle: The effect of hay maturity. *Journal of Animal Science*, **14**, 249-255.

Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115-155.

Bryant D.M (1975) Breeding biology of house martins *Delichon urbica* in relation to aerial insect abundance. *Ibis*, **117**, 180-216.

Bryant, D.M. (1978) Environmental influences on growth and survival of nestling House Martins *Delichon urbica*. *Ibis*, **120**, 271-283.

Brzek, P. & Konarzewski, M. (2001) Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. *Journal of Experimental Biology*, **204**, 3065-3074.

Chastel, O., Weimerskirch, H. & Jouventin, P. (1993) High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel *Pagodroma nivea*- a 27 year study. *Oecologia*, **94**, 278-285.

Cherel, Y., Stahl, J-C. & Le Maho, Y. (1987) Ecology and physiology of fasting in king penguin chicks. *Auk*, **104**, 254-262.

Cherel, Y., Robin, J-P. & Le Maho, Y. (1988) Physiology and biochemistry of long-term fasting in birds. *Canadian Journal of Zoology*, **66**, 159-166.

Cooch, E.G., Lank, D.B., Dzubin, A., Rockwell, R.F. & Cooke, F. (1991) Body size variation in lesser snow geese: Environmentally plasticity in gosling growth rates. *Ecology*, **72**, 503-512.

Croxall, J.P., Hill, H.J., Lidstone-Scott, R., O'Connel, M.J. & Prince P.A. (1988) Food and feeding ecology of Wilson's storm petrel, *Oceanites oceanicus* at South Georgia. *Journal of Zoology London*, **216**, 83-102.

Daan, S., Masman, D. & Groenewold, A. (1990) Avian basal metabolic rates: their association with body composition and energy expenditure in nature. *American Journal of Physiology*, **259**, 333-340.

de Margerie, E., Robin, J.P., Verrier, D., Cubo, J., Groscolas, R. & Castanet, J. (2004) Assessing a relationship between bone microstructure and growth rate: a fluorescent labelling study in the king penguin chick (*Aptenodytes patagonicus*). *Journal of Experimental Biology*, **207**, 869-879.

Dunn, E.H. (1976) Development of endothermy and existence energy expenditure of nestling Double-crested Cormorants. *Condor*, **78**, 350-356.

Emlen, S.E., Wrege, P.H., Demong, N.J. & Hegner, R.E. (1991) Flexible growth rates in nestling white-fronted bee-eaters: a possible adaptation to short-term food shortage. *Condor*, **93**, 591-597.

Gould, S.J. & Lewontin, R.C. (1979) The spandrels of San Marco and the Panglossian paradigm: a critique of the adaptationist programme. *Proceedings of the Royal Society of London B*, **205**, 581-598.

Harris, M.P. & Wanless, S. (1997) Breeding success, diet, and brood neglect in the kittiwake (*Rissa tridactyla*) over an 11-year period. *ICES Journal of Marine Sciences*, **54**, 615-623.

Kirkham, I.R. & Montevecchi, W.A. (1982) Growth and thermal development of Northern Gannets (*Sula bassanus*) in Atlantic Canada. *Colonial Waterbirds*, **5**, 66-72.

Kitaysky, A.S. (1999) Metabolic and developmental responses of alcid chicks to experimental variation in food intake. *Physiological and Biochemical Zoology*, **72**, 462-473.

Konarzewski, M. & Taylor, J.R.E. (1989) The influence of weather conditions on growth of little auk *Alle alle* chicks. *Ornis Scandinavica*, **20**, 112-116.

Konarzewski, M. & Starck, J.M. (2000) Effects of food shortage and oversupply on energy utilization, histology, and function of the gut in nestling song thrushes (*Turdus philomelos*). *Physiological and Biochemical Zoology*, **73**, 416-427.

Konarzewski, M., Kowalczyk, J., Swierubska, T. & Lewonczuk, B. (1996) Effect of short-term feed restriction, realimentation and overfeeding on growth of song thrush (*Turdus philomelos*) nestlings. *Functional Ecology*, **10**, 97-105.

Koskimies, J. & Lahti, L. (1964) Cold-hardiness of the newly hatched young in relation to ecology and distribution in ten species of European ducks. *Auk*, **81**, 281-307.

Lack, D. (1968) Ecological adaptations for breeding in birds. Methuen and Co., London.

Lack, D. & Lack, E. (1951) The breeding biology of the Swith *Apus apus*. *Ibis*, **93**, 501-546.

Larsson, K. & Forslund, P. (1992) Genetic and social inheritance of body size and egg size in the barnacle goose (*Branta leucopsis*). *Evolution*, **46**, 235-244.

Le Maho, Y. (1983) Metabolic adaptations to long-term fasting in Antarctic penguins and domestic geese. *Journal of Thermal Biology*, **8**, 91-96.

Lepage, D., Gauthier, G. & Reed, A. (1998) Seasonal variation in growth of greater snow goose goslings: the role of food supply. *Oecologia*, **114**, 226-235.

Lepczyk, C.A. & Karasov, W.H. (2000) Effect of ephemeral food restriction on growth of house sparrows. *Auk*, **117**, 164-174.

Lorentsen, S.-H. (2001) The national monitoring programme for seabirds. Results including the breeding season 2001. Norwegian Institute for Nature Research. 'NINA Oppdragsmelding'. **726**, 1-36.

Magrath, R.D. (1990) Hatching asynchrony in altricial birds. *Biological Reviews*, **65**, 587-622.

Marsh, R.L. & Wickler, S.J. (1982) The role of muscle development in transition to endothermy in nestling Bank Swallows, *Riparia riparia*. *Journal of Comparative Physiology B*, **149**, 99-105.

Martin, T.E. (1987) Food as a limit on breeding birds: A life-history perspective. *Annual Review of Ecology and Systematics*, **18**, 453-487.

Moe, B., Langseth, I., Fyhn, M. & Bech, C. (2002) Changes in body condition in breeding Kittiwakes *Rissa tridactyla*. *Journal of Avian Biology*, **33**, 225-234.

Monaghan, P., Uttley, J.D., Burns, M.D., Thaine, C. & Blackwood, J. (1989) The relationship between food supply, reproductive effort and breeding success in Arctic Terns *Sterna paradisea*. *Journal of Animal Ecology*, **58**, 261-274.

Montevecchi, W.A. & Vaughan, R.B. (1989) The ontogeny of thermal independence in nestling Gannets. *Ornis Scandinavica*, **20**, 161-168.

Morse, K. & Vohra, P. (1971) The effect of early growth retardation of Coturnix (Japanese Quail) on their sexual maturity. *Poultry Science*, **50**, 283-284.

Murphy, M.E. (1996) Nutrition and metabolism. *Avian energetics and nutritional ecology* (ed. C. Carey), pp. 31-60. Chapman & Hall, New York.

Negro, J.J., Chastin, A. & Bird, D.M. (1994) Effects of short-term food deprivation on growth of hand-reared American kestrels. *Condor*, **96**, 749-760.

Nice, M.M. (1962) Development of behaviour in precocial birds. *Transactions of the Linnean Society of New York*, **8**, 1-211.

O'Connor, R.J. (1977) Differential growth and body composition in altricial passerines. *Ibis*, **119**, 147-166.

Østnes, J.E., Jenssen, B.M. & Bech, C. (2001) Growth and development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*). *Auk*, **118**, 983-995.

Øyan, H.S. & Anker-Nilssen, T. (1996) Allocation of growth in food-stressed Atlantic Puffin chicks. *Auk*, **113,** 830-841.

Piersma, T. & Lindstrøm, Å. (1997) Rapid reversible changes in organ size as a component of adaptive behviour. *Trends in Ecology and Evolution*, **12,** 134-138.

Pigliucci, M. (2001) *Phenotypic plasticity. Beyond Nature and Nurture*. The Johns Hopkins University Press, Baltimore.

Richner, H. (1989) Habitat-specific growth and fitness in carrion crows and their effects on access to food. *Journal of Animal Ecology*, **58**, 427-440.

Ricklefs, R.E. (1976) Growth rates of birds in the humid New World tropics. *Ibis*, **118**, 179-207.

Ricklefs, R.E. (1983) Avian postnatal development. *Avian biology* (eds. D.S. Farner, J.R. King & K.C. Parkes), Vol VII, pp. 1-83. Academic Press, New York.

Ricklefs, R.E. (1987) Characterizing the development of homeothermy by rate of body cooling. *Functional Ecolology*, **1**, 151-157.

Ricklefs, R.E. (1993) Sibling competition, hatchling asynchrony, incubation periods and lifespan in altricial birds. *Current Ornithology*, **11**, 199-276.

Ricklefs, R.E. & Webb, T. (1985) Water content, thermogenesis, and growth rate of skeletal muscles in the European starling. *Auk*, **102**, 369-376.

Ricklefs, R.E., Shea, R.E. & Choi, I.H. (1994) Inverse relationship between functional maturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. *Evolution*, **48**, 1080-1088.

Ricklefs, R.E., Starck, J.M. & Konarzewski, M. (1998) Internal constraints on Growth in Birds. *Avian Growth and Development*. (eds. J.M. Starck & R.E. Ricklefs), pp. 266-287. Oxford University Press, New York.

Schew, W.A. (1995) *The evolutionary significance of developmental plasticity in growing birds*. PhD diss. University of Pennsylvania, Philadelphia.

Schew, W.A. & Ricklefs, R.E. (1998) Developmental plasticity. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 288-304. Oxford University Press, New York.

Schlichting, C. D. & Pigliucci, M. (1998) *Phenotypic Evolution. A Reaction Norm Perspective*. Sinauer Associates, Sunderland.

Schmalhausen, I. I. (1949) *Factors of evolution*. Philadelphia: Blakiston. Reprinted 1986, Chicago University press, Chicago.

Sedinger, J.S. (1986) Growth and development of Canada Goose goslings. *Condor*, **88**, 169-180.

Smith-Gill, S. J. (1983) Developmental plasticity: developmental conversion *versus* phenotypic modulation. *American Zoologist*, **23**, 47-55.

Starck, J.M. (1998) Structural variants and invariants in avian embryonic and postnatal development. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 59-88. Oxford University Press, New York.

Starck, J.M. (1999) Phenotypic flexibility of the avian gizzard: Rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. *Journal of Experimental Biology*, **202**, 3171-3179.

Starck, J.M. & Ricklefs, R.E. (1998a) Patterns of Development: The Altricial-Precicial Spectrum. *Avian Growth and Development*. (eds. J.M. Starck & R.E. Ricklefs), pp. 3-30. Oxford University Press, New York.

Starck, J.M. & Ricklefs, R.E. (1998b) Avian growth rate data. *Avian Growth and Development*. (eds. J.M. Starck & R.E. Ricklefs), pp. 381-423. Oxford University Press, New York.

Starck, J.M. & Chinsamy, A. (2002) Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology*, **254**, 232-246.

Starck, J.M. & Rahman, G.H.A. (2003) Phenotypic flexibility of structure and function of the digestive system of Japanese quail. *Journal of Experimental Biology*, **206**, 1887-1897.

Stearns, S.C. (1992) *The evolution of life histories*. Oxford University Press, Oxford.

Street, M. (1978) The role of insects in the diet of Mallard ducklings- an experimental approach. *Wildfowl*, **29**, 93-100.

Velando, A., Ortega-Ruano, J.E. & Freire, J. (1999) Chick mortality in European shag *Stictocarbo aristotelis* related to food limitations during adverse weather events. *Ardea*, **87,** 51-59.

Via, S., Gomulkiewicz, R., De Jong, G., Scheiner, S.M., Schlicting, C.D. & Van Tienderen, P.H. (1995) Adaptive phenotypic plasticity: consensus and controversy. *Trends in Ecology and Evolution*, **10**, 212-217.

Visser, G.H. (1998) Development of temperature regulation. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 117-156. Oxford University Press, New York.

Visser, G.H. & Ricklefs, R.E. (1993) Temperature regulation in neonates of shorebirds. *Auk*, **110**, 445-457.

Weathers, W.W. (1996) Energetics of postnatal growth. *Avian energetics and nutritional ecology* (ed. C. Carey), pp. 461-496. Chapman & Hall, New York.

Weathers, W.W., Gerhart, K.L. & Hodum, P.J. (2000) Thermoregulation in Antarctic fulmarine petrels. *Journal of Comparative Physiology B*, **170**, 561-572.

Zubair, A.K. & Leeson, S. (1994) Effect of early restriction and realimentation on heat production and changes in sizes of digestive organs of male broilers. *Poultry Science*, **73,** 529-538.

Paper I

Physiological and Biochemical Zoology 78, (1) (in press)

Ducklings Exhibit Substantial Energy-saving Mechanisms as a Response to Short-term Food Shortage

Running title: Physiological Responses of Ducklings to Diet Restriction

Børge Moe, Einar Stølevik and Claus Bech

Department of Biology

Norwegian University of Science and Technology

NO-7491 Trondheim, Norway

Address for correspondence and proof:
Børge Moe
Department of Biology
Norwegian University of Science and Technology, NO-7491 Trondheim, Norway
Phone: +47 73 59 60 30, Fax: +47 73 59 13 09, Email: Borge.Moe@bio.ntnu.no

ABSTRACT

We investigated whether Pekin ducklings (*Anas platyrhyncos domesticus*) exhibited any energy saving mechanisms that could lessen the detrimental effects of reduced food intake during early development. Further, we evaluated the role of body compositional changes behind such potential mechanisms, and the consequences on thermoregulatory capacity.

The ducklings exhibited substantial energy saving mechanisms as a response to diet restriction. After 5 days of diet restriction, the resting metabolic rate (RMR) of 10 and 20 day old ducklings was 16.4 and 32.1% lower, respectively, than predicted from body mass, compared to *ad libitum* fed ducklings (controls). These reductions in RMR could have been adaptive responses in anticipation of a lasting food shortage, or they could have been consequences of the restricted diet and the lack of essential nutrients. We argue that the responses were adaptive. The low RMRs were not a consequence of depleted fuel stores, as the diet-restricted ducklings exhibited substantial amounts of stored lipids at the end of the diet restriction periods. Hypothermia accounted for ~50% of the reduction in RMR in the 10 day old diet-restricted ducklings, but hypothermia did not occur in the 20 day old diet-restricted ducklings. Diet restriction resulted in a reduced liver and intestine size, an unchanged size of the leg muscles and heart, while the length of the skull increased (compared to controls of a given body mass). However, changes in body composition were only minor predictors of the observed changes in RMR.

Peak metabolic rate (PMR) was ~10% lower in the diet-restricted ducklings compared to the controls. We have interpreted the lower PMR as a consequence of the reductions in RMR, rather than a consequence of a decreased function of the thermoregulatory effector mechanisms.

Introduction

Living organisms have to balance their energy allocation in such a manner that future survival and reproduction is ensured (Stearns 1992). This is particularly challenging for young birds, because they need to get through the vulnerable developmental period as rapidly as possible, while at the same time they should attain a fully developed adult size that ensures both survival and future reproduction (Schew and Ricklefs 1998).

Little is known about the effect of temporal changes in food availability on the ontogenetic development in birds. As chicks grow from neonate to adult, they may encounter periods of low food availability that can affect their development (Schew and Ricklefs 1998). Poor feeding conditions can suppress normal growth, affect adult morphology (De Kogel 1997; Birkhead et al. 1999) and result in negative long-term consequences (Lindström 1999; Metcalfe and Monaghan 2001; Dufty et al. 2002).

In order to maximise their survival during poor feeding conditions, chicks should optimise the allocation of their ingested energy to growth and maintenance. At reduced levels of energy intake, the available energy for growth can be allocated to growth and functional maturation of different tissues and organs in the same relative proportions as during normal energy intakes (Konarzewski et al. 1996). Alternatively, it can be specifically allocated to favoured organs and tissues at the expense of others (Øyan and Anker-Nilssen 1996; Schew and Ricklefs 1998).

Developmental plasticity is regarded as adaptive if the animal actively adjusts the ontogenetic processes to a change in the environment (Smith-Gill 1983). Modification of the basal level of energy expenditure could occur as an active response in anticipation of a lasting food shortage. By reducing the energy expenditure, the chicks could increase survival and enable more energy to be allocated to growth. Alternatively, any reduction of the basal level of energy expenditure could be a direct consequence of the lack of sufficient nutrients during food shortage. Also, the lack of nutrients could impose reductions in growth rate and in the size of energy consuming organs which consequently could cause reductions in the basal level of energy expenditure, as a non-adaptive response. However, reductions in the size of energy consuming organs and in growth rate could also be an adaptive response in anticipation of a lasting food shortage.

Any reduction of the resting metabolic rate (RMR) may be associated with severe costs in terms of reduced capacity for peak metabolism (PMR). According to the 'aerobic capacity' hypothesis (Bennett and Ruben 1979; Taigen 1983), the evolution of endothermy and high levels of sustained activity in birds and mammals was accompanied by an increase in the capacity of aerobic pathways of metabolism and in organ systems (digestion, respiration, circulation and excretion) that support this capacity. Assuming a close coupling between RMR and PMR, as has been demonstrated interspecifically in adult birds (Dutenhoffer and Swanson 1996; Rezende et al. 2002) and intraspecifically in chicks (Konarzewski et al. 2000), a decrease in basal metabolism should be accompanied by a decrease in peak metabolism (i.e. thermogenic capacity). However, the functional relationship between basal metabolism and peak metabolism is not yet fully understood (Hayes and Garland 1995; Ricklefs et al. 1996).

Birds are generally believed to rely mainly on muscular shivering for heat production during cold exposure (Hohtola and Visser 1998). In contrast, visceral organs (especially the heart, liver, kidneys and intestine) are believed to consume much of the energy used in basal metabolism (Daan et al. 1990). However, the specific organs and tissues that predict RMR or PMR differ among studies (e.g. Burness et al. 1998; Bech and Østnes 1999; Chappell et al. 1999). Hence, it is not fully understood how body composition functionally relates to RMR and PMR.

It is generally accepted that the energy budgets of chicks have responded to selection, but little consideration has been given to the evolution of the chicks' ability to modify the pattern of energy use and allocation. Precocial birds, like ducklings, are likely to encounter variable food availability during early development, due to foraging inexperience, fluctuations in resources, adverse weather conditions or dominance/sibling interactions. Ducklings are almost thermoregulatory independent of their parents, and they depend on their own thermoregulatory capacity. In this study, we experimentally imposed short-term diet restriction on Pekin ducklings (*Anas platyrhynchos domesticus)*, kept under laboratory conditions, to shed light on the relationship between food availability, energy allocation and metabolism during early development.

In this context, we ask whether ducklings exhibit any energy saving mechanisms that can lessen the detrimental effects of reduced food intake during early development.

Secondly, whether a change in body composition is the physiological mechanism behind such a metabolic response and thirdly whether any energy saving mechanisms (i.e. lowered RMR) result in a negative effect on the thermoregulatory capacity (PMR) of growing chicks.

Material and Methods

Animal Housing and Experimental Design

Pekin duck eggs were obtained from a local farmer and hatched in the laboratory. After hatching (day 0), the ducklings were kept for ~12 h in the incubator at a T_a of 38°C. Thereafter, they were transferred to an enclosure (60 x 40 cm) with *ad libitum* access to food (poultry pellets) and water. A heat lamp provided a constant range of operative temperatures (Bakken 1992) of 24-33°C within the enclosure. As the ducklings grew bigger, they progressively gained access to more space. The oldest ducklings (15-20 days old) were confined in an enclosure of 140 x 125 cm in groups of 5-10 ducklings.

Ducklings were randomly assigned to either diet restriction or control (*ad libitum*) treatment. Water was provided *ad libitum* to all ducklings in both groups. Diet restriction was imposed for 5-day periods on one group of ducklings from the age of 5 to 10 days and on a second group from the age of 15 to 20 days. The diet-restricted ducklings received small portions of food 4-7 times a day to maintain a relatively stable body mass.

Metabolic measurements were made on each duckling at the end of the diet restriction period, at the age of 10 or 20 days, respectively. Ducklings fed *ad libitum* were used as controls, and metabolic measurements were made on four independent groups of controls at the age of 5, 10, 15 and 20 days, respectively. In total, we measured the metabolic rate (MR) of 55 ducklings, of which 33 were postabsorptive and 22 were not (see ‘statistical analyses’). Postabsorptive ducklings did not receive any food for >10 h prior to the metabolic measurement. The National Committee for Animal Research in Norway (‘Forsøksdyrutvalget’) approved the experimental protocols.

Metabolic Measurements

O_2 consumption rates were measured by open-flow respirometry (Withers 1977). A high pressure air outlet in the laboratory facilitated atmospheric air. After drying over silica gel, the actual flow rates (0.85-3.7 l min^{-1}) entering the metabolic chamber were measured with a mass flow controller (Bronkhorst Hi-Tec, F-201C-FA-22-V). Excurrent air was dried over silica gel, before a fraction of the air was directed to the O_2

analyser (Servomex 1100A). The O_2 analyser was calibrated with a) dry atmospheric air (20.95%) before every experiment and with b) pure N_2 after every ~10 experiments. Any changes from the pre- to the post-experiment readings of the O_2 content in dry atmospheric air, was controlled for by assuming a linear drift. Measurements of the O_2 content in excurrent air (accuracy 0.01%) were stored, along with the temperature measurements, on a Squirrel data logger, at 30 sec intervals.

The metabolic rates (MR) were calculated by using formula (1d) in Withers (1977), assuming a constant RQ of 0.79 and corrected for wash-out delays in the system by using the method given by Niimi (1978). In this way, we obtained the instantaneous O_2 consumption rates. Values of the metabolic rates (MR) were calculated from the O_2 consumption rates using 5.5824 W as the caloric equivalent for 1L O_2 h^{-1}.

Resting metabolic rate (RMR) was defined as the lowest MR calculated with 25 min running average during exposure to thermoneutral conditions. The ambient temperature for thermoneutral conditions was set between 33 and 26°C, depending on the ducklings' age (Østnes and Bech 1997). The use of a running average over a 25 min interval was justified after plotting the minimum values of the MR, calculated in five randomly selected experimental runs using intervals which varied from 2 min to 60 min. For a running average lower than 15 min, these curves revealed a very strong positive relationship between the minimum values of RMR and the length of the running average interval. Short intervals resulted in very low minimum values of RMR, thereby underestimating the RMR level. However, at a running average between 15 min and 60 min, the minimum values of RMR changed relatively little (see Meerlo et al. (1997) for a description of this procedure).

Depending on the ducklings' age, the metabolic chamber was 9 or 25 L. A surrounding climatic chamber (Heraeus Vötsch, type VEM 03/500) regulated the T_a inside the metabolic chambers. After 3-6 h exposure to thermoneutral conditions, the T_a was lowered at a constant rate of 0.7°C min^{-1} inside the 9 L and the 25 L chamber, respectively. The lowest T_a imposed in the metabolic measurements was –26.2 °C. The ducklings' MR increased with decreasing T_a. After reaching a peak MR, MR and T_b showed a consistent decrease to a further decrease in T_a, and the experiment was terminated. Peak metabolic rate (PMR) was defined as the highest 10 min running average MR during cold exposure. Some of the 20 days old ducklings (4 controls and 4 diet-restricted) maintained a relatively stable MR and T_b at the lowest ambient temperatures imposed by the climatic chamber. Hence, we can not be sure that they reached the true PMR, and they were excluded from the PMR analyses. One 5 day old duckling was also excluded, due to a power supply failure during the PMR measurement. Each individual was only used once in the experiments. The ducklings were sacrificed with ether immediately after the metabolic measurement and stored at –20°C for subsequent analysis of body composition. Body masses of the ducklings were weighed, to the nearest 0.1 g, before and immediately after each experiment. A linear decrease in body mass during the experiment was assumed when calculating the body mass at the time when RMR and PMR were obtained.

The ambient temperature (T_a) was measured with a thermocouple mounted inside the metabolic chamber. The body temperature (T_b) was measured during the entire metabolic measurement in the cloaca with a copper-constantan thermocouple (California fine wire, type 0.005) surrounded by a polypropylene tubing (PP 50, Portex Ltd.) and secured with adhesive tape over the cloaca. Depending on the duckling's age, the thermocouple was inserted 1.8-4.5 cm into the cloaca.

The minimal 'wet' thermal conductance (MTC) was calculated according to the method originally described by Scholander et al. (1950). However, this method is only valid when T_b is kept constant. Since the T_b of the ducklings decreased during cold exposure, we had to include a correction factor to account for the fall in T_b (see also Visser and Ricklefs 1993). Thus, the following formula was used to calculate minimal thermal conductance:

$$\mathrm{MTC} = (\mathrm{PMR} + \mathrm{A}) / (T_b - T_a) \qquad (1)$$

where A is the correction factor for the decrease in energy content (W $\mathrm{kg^{-1}}$). The calculation of the correction factor was based on the rate of fall in T_b recorded during the last 10 min period before PMR was attained, and a specific heat of 3.45 J $\mathrm{g^{-1}\ {}^{o}C^{-1}}$ (Hart 1951). Thermal conductance (TC) during thermoneutral conditions was calculated according to the following formula:

$$\mathrm{TC} = \mathrm{RMR} / (T_b - T_a) \qquad (2)$$

Body Composition

Dissection was performed on semi-thawed carcasses in order to reduce vaporisation and improve organ separation. We removed heart, liver, kidney, gizzard and intestine (small and large). The entire right breast muscle (*m. supracoracoideus* and *m. pectoralis*) was separated from the skeleton. Also, the entire right leg muscle was separated from the tibiotarsus-tarsometatarsus joint. Gizzard, intestine and heart atrium were emptied of contents, while all organs and muscles were carefully trimmed of fat and weighed (±1 mg, carcasses to ±0.1 g). They were then dried to a constant mass at 56^{o}C and reweighed. Fat content was thereafter removed in baths of petroleum ether, and the samples where again dried and reweighed. The lean dry fraction (LDF) of organs was calculated as the ratio of lipid free dry organ mass to lipid free fresh organ mass. The LDF of most organs and tissues increases during the ontogenetic development due to a build-up of proteins and functional components on the cellular level. Hence, the LDF is regarded as reflecting the functional maturity of an organ.

Statistical Analyses

We used a General Linear Model (GLM) procedure with the type III sum of squares to perform analysis of covariance and variance. The GLM was performed with the ENTER method, in which we excluded insignificant interaction terms, factors or covariates one by one from the null modell. We inspected all variables graphically to ensure linearity before performing GLM. MR and organ mass show allometric relationships to body mass. Hence, $\log_{10}$ –transformation was used to linearize these variables prior to examination.

We analysed the relationship between organ mass and MR, as well as the relationship between PMR and RMR by including body mass as a covariate to remove the effect of body mass (i.e. body mass is held constant; Hayes and Shonkwiler 1996). In order to avoid possible effects of part-whole correlation, we subtracted organ mass from the body mass variable, prior to examination, when organ mass and body mass were included in the same analysis (Christians 1999).

Typically for precocial species, the ontogenetic development of RMR follows a biphasic pattern in relation to body mass. A biphasic pattern also exists for the ontogenetic development of many organs. Hence, we have defined 'age-group' as a factor, in which 5-10 day old and 10-20 day old ducklings constitute two different age-groups. When we have performed statistical analyses on each age-group separately, a two-level factor for 'treatment' (1 = controls, 2 = diet-restricted ducklings) is used. When performing analyses including both age-groups, age-group is included as a factor in the model, and the two-level factor treatment or the three-level factor 'treatment-group' (1 = controls, 2 = 10 days old diet-restricted, 3 = 20 days old diet-restricted) is included in the model and specified in the text.

When two regressions (3) with $\log_{10}$ transformed variables (e.g. metabolic rate (MR) on body mass (BM)) have the same regression coefficient (β), but have different intercepts (α and $\alpha + \gamma$), we have calculated the percentage difference (X) between the non-transformed regressions according to formula (4):

$$\log_{10} \mathrm{MR}_{(1)} = \beta \log_{10} \mathrm{BM} + \alpha$$

$$\log_{10} \mathrm{MR}_{(2)} = \beta \log_{10} \mathrm{BM} + \alpha + \gamma \qquad (3)$$

$$X = 100 \times (1 - \frac{\mathrm{MR}_{(2)}}{\mathrm{MR}_{(1)}}) = 100 \times (1 - \frac{\mathrm{BM}^{\beta} * 10^{\alpha} * 10^{\gamma}}{\mathrm{BM}^{\beta} * 10^{\alpha}}) = 100 \times (1 - 10^{\gamma}) \qquad (4)$$

We measured MR on postabsorptive and non-postabsorptive ducklings. In order to find any effect of the 'absorptive status' (i.e. postabsorptive or non-postabsorptive) on MR (and T_b), we used the GLM procedure with MR as the dependent variable, body mass as covariate and absorptive status and treatment as factors. This revealed that absorptive status and the interactions with body mass and treatment significantly affected MR. Consequently, we adjusted the MR of non-postabsorptive ducklings downward to the postabsorptive levels. The parameter estimates from the GLM were used to make the appropriate equation for the adjustment for each treatment group. The $\log_{10}$-transformed MR of each non-postabsortive duckling was subtracted by ΔMR which was calculated according to the following equation (5) within each treatment group:

$$\Delta \mathrm{MR} = \text{predicted } \log_{10} \mathrm{MR}_{(1)} - \text{predicted } \log_{10} \mathrm{MR}_{(0)}$$

$$= \beta_{(1)} \log_{10} \mathrm{BM} + \alpha_{(1)} - (\beta_{(0)} \log_{10} \mathrm{BM} + \alpha_{(0)})$$

$$= (\beta_{(1)} - (\beta_{(0)}) \log_{10} \mathrm{BM} + \alpha_{(1)} - \alpha_{(0)} \quad (5)$$

where $_{(1)}$ and $_{(0)}$ denote parameters obtained from non-postabsorptive and postabsorptive ducklings, respectively. BM is body mass, and α and β represent the intercepts and slopes, respectively. This correction to postabsorptive levels uses an average value (for each treatment group and controlled for body mass) for metabolic rates to adjust non-postabsorptive metabolic rates. A variance in non-postabsorbtive RMR caused by a variation in heat increment of feeding will still exist after the correction to postabsortive levels. However, we regard this 'noise' as too weak to obscure the conclusions in this study.

We tested the effect of diet restriction on RMR (and PMR) by GLM models with RMR and lean dry body mass as dependent and independent variables, respectively. The effect of diet restriction on body composition was analysed separately for each organ with lean dry organ mass and lean dry body mass (minus organ mass) as the dependent and the independent variable, respectively. Treatment and age-group were entered as factors in these models. The null models included the two interactions 'treatment x body mass' and 'age-group x body mass'. The effect of diet restriction on LDF was analysed separately for each organ and for each age-group.

We analysed the relationship between RMR (and PMR) and organ mass for each organ separately. Organ mass and body mass (minus organ mass) were entered as covariates, and treatment was entered as a factor. We performed separate analyses for each age-group, as well as analyses in which both age-groups were included. In the latter analyses we added age-group and treatment-group as factors.

Biometric measurements (wing length, tarsus length, skull length (head+bill)) were made on 5, 10, 15 and 20 day old ducklings. Growth rate was calculated as the daily growth during the last 5 days (mm day^{-1}). Hence, growth rates of structural elements were obtained for 10, 15 and 20 day old ducklings and were not instantaneous growth rates at these specific ages. We used a principal component analysis to extract a factor score (PC1) of the growth rate of the wing, the tarsus and the skull. Analyses of the relationship between growth rate and RMR were performed separately for each growth rate variable (wing, skull, tarsus, PC1, body mass). Body mass was included as a covariate, and age-group was included as a factor, while treatment was only included as a factor for the analyses where both controls and diet-restricted ducklings were included.

The Bonferroni method was used for post hoc pairwise multiple comparisons (‘pairwise comparisons’ hereafter). It reports adjusted P-values that have been multiplied with the number of pairs tested. All statistical analyses were performed with SPSS v. 11.5.1 (2002).

Results

Body Mass

The diet restriction had a substantial effect on body mass growth (Fig.1). The body mass of the diet-restricted ducklings was maintained at a relatively stable level during the periods of diet restriction, i.e. they only gained 2.9 g d^{-1} between 5 and 10 days of age ($P<0.001$) and 6.3 g d^{-1} between 15 and 20 days of age ($P<0.001$). In contrast, the growth of the *ad libitum* fed ducklings (controls) followed a normal growth curve (Fig. 1).

Metabolism

The development of RMR showed a biphasic pattern in relation to body mass ('age-group x body mass' interaction, $F_{1,49}=4.49$, $P<0.05$, Fig. 2). RMR of 5-10 day old ducklings scaled to body mass by the power of 1.01 (SE=0.08), while RMR of 15-20 day old ducklings scaled to body mass by the power of 0.82 (SE=0.07). The interaction 'treatment x body mass' was also significant ($F_{1,49}=13.21$, $P<0.001$), indicating that 10 day old diet-restricted ducklings showed a different metabolic response to diet restriction compared to 20 day old diet-restricted duckling. Hence, we removed the 2-level treatment factor and entered treatment-group as a factor with 3 levels (1 = controls, 2 = 10d old diet-restricted, 3 = 20d old diet-restricted). The 'treatment-group x body mass' interaction was not significant, while the intercepts of the regressions of the treatment-groups were highly significantly different ($F_{2,49}=56.89$, $P<0.001$, Fig. 2). This shows that diet restriction had a substantial effect on RMR. The parameter estimates from the GLM and pairwise comparisons showed that the 20 days old diet-restricted ducklings exhibited 32.1% lower RMR compared to controls ($P<0.001$), while the 10 days old diet-restricted ducklings exhibited 16.4% lower RMR compared to controls ($P<0.001$).

PMR scaled to body mass by the power of 0.82 (SE=0.03, $F_{1,41}=713.19$, $P<0.001$, Fig. 2). The 'age-group x body mass' interaction was not significant, but for consistency with the RMR analysis, we included the 3-level treatment-group factor in the model. The main effect of treatment-group was highly significant ($F_{2,41}=10.95$, $P<0.001$), while the interaction with body mass was not significant. Hence, PMR was lower in the diet-restricted ducklings compared to the controls. Pairwise comparisons showed that the PMR of the 20 day old diet-restricted ducklings was 11.3% lower compared to controls ($P<0.005$), while the PMR of the 10 day old diet-restricted ducklings was 9.2% lower compared to controls ($P<0.05$).

T_b measured at RMR increased with age (Fig. 3a). The body temperature of 20 day old diet-restricted and 20 day old controls was both 40.5 ^{o}C. In contrast, the body temperature of 10 day old diet-restricted ducklings was significantly lower compared to 10 day old controls ($t=-4.55$, $df=15$, $P<0.001$). The relationship between $\log_{10}$-transformed T_b and lean dry body mass revealed that 10 day old diet-restricted ducklings exhibited 0.7 ^{o}C lower T_b than predicted from controls of the same body mass ($F_{2,51}=11.03$, $P<0.001$).

Ambient temperature (T_a) measured at PMR showed a negative relationship to the age of the ducklings ($F_{1,40}=23.47$, $P<0.001$; Fig. 3b). The ‘age x treatment’ and the ‘age x age-group’ interactions were significant. T_a measured at PMR for 10 day old diet-restricted ducklings was 5.3^{o}C higher compared to 10 day old controls ($P<0.05$), but not significantly different to 5 day old controls. In contrast, T_a measured at PMR was lower for 20 day old diet-restricted ducklings than that of 15 day old controls ($P<0.05$), but not significantly different from that of 20 day old controls. Hence, 20 day old diet-restricted ducklings attained PMR at a lower ambient temperature than expected from body mass ($F_{1,18}=9.1$, $P<0.001$), while 10 day old diet-restricted ducklings attained PMR at an ambient temperature as expected from body mass ($F_{1,21}=0.01$, $P>0.05$). T_b measured at PMR was not different between diet-restricted ducklings and controls ($F_{1,42}=0.02$, $P>0.05$). Average values for T_b at PMR were 37.2 ^{o}C (±0.8), 37.4 ^{o}C (±0.5), 39.0 ^{o}C (±0.3), 38.6 ^{o}C (±0.4) for 5, 10, 15 and 20 day old controls, respectively, and 37.4 ^{o}C (±0.4) and 38.1 ^{o}C (±0.6) for 10 and 20 day old diet-restricted ducklings, respectively.

Minimal thermal conductance decreased with increasing body mass ($F_{1,40}=15.90$, $P<0.001$, Fig. 4a) and age (Fig. 4b). The slopes of the regressions between $\log_{10}$-transformed MTC and lean dry body mass were not significantly different between the treatment groups ($F_{2,37}=0.47$, $P>0.05$, Fig. 4a) or between the age-groups ($F_{1,39}=3.46$, $P>0.05$). The main effect of treatment-group was significant ($F_{2,40}=4.39$, $P<0.05$), and pairwise comparisons showed that the MTC of the 20 day old diet-restricted ducklings was lower (by 15%) compared to controls ($P<0.05$), while the MTC of the 10 day old diet-restricted ducklings was not.

The absolute scope (PMR minus RMR, i.e. the portion of PMR available for regulatory thermogenesis) showed no response to the diet restriction treatment (Fig. 5a). The slopes of the regressions between $\log_{10}$ -transformed absolute scope and lean dry body mass were not significantly different among the treatment groups ($F_{2,39}=2.79$, $P>0.05$, Fig. 5a), and the intercepts of these relationships were not significantly different ($F_{2,41}=0.90$, $P>0.05$). Body mass ($F_{1,41}=273.93$, $P<0.001$) and age-group ($F_{1,41}=4.9$, $P<0.05$) were the only significant predictors of absolute scope. In contrast, the factorial scope (PMR/RMR) were significantly higher for 20 day old diet-restricted ducklings compared to 15 and 20 day old controls ($F_{2,18}=22.74$, $P<0.001$, Fig. 5b). The factorial scope was also different between 5 and 10 day old ducklings ($F_{2,22}=11.00$, $P<0.001$, Fig. 5b), but the factorial scope of 10 day old diet-restricted ducklings was only significantly higher compared to 10 day old controls ($P<0.001$) and not to 5 day old controls ($P>0.05$).

RMR showed a positive relationship to PMR in the 5-10 day old duckling ($F_{1,22}=4.63$, $P<0.05$, $r=0.42$) and in the 15-20 day old ducklings ($F_{1,18}=15.32$, $P<0.001$, $r=0.68$). Body mass was a significant covariate. Treatment and the ‘treatment x RMR’ interaction were included in the null model, but they were not significant. Consequently, they were excluded from the final model. RMR also showed a positive relationship to PMR ($F_{1,42}=16.10$, $P<0.001$, $r=0.53$) in the analysis where both age groups were included.

Body Composition

Diet restriction affected organ size in three different ways. 1) There was no significant effect of the treatment (e.g. Fig. 6a). This was the case for the leg muscles ($F_{1,50}=0.46$, $P>0.05$, Fig. 6a), the heart ($F_{1,52}=0.25$, $P>0.05$) and the kidneys ($F_{1,52}=0.30$, $P>0.05$), diet-restricted ducklings and controls showed the same allometric relationship between organ mass and body mass. 2) There was a significant effect of the treatment, but no significant treatment x body mass interaction (e.g. Fig. 6b). Diet-restricted ducklings had significantly lighter liver mass ($F_{1,50}$=85.76, $P<0.001$, Fig. 6b) and shorter intestine length ($F_{1,51}$=6.61, $P<0.001$) compared to controls. 3) There was a significant effect of the treatment, but the effect of the treatment depended on the body mass and was different between 10 and 20 day old diet-restricted ducklings (i.e. significant 'treatment x body mass' interaction, e.g. Fig 6c and 6d). In these analyses we entered treatment-group as a factor in order to do pairwise comparisons between the intercepts (means controlled for body mass and age-group) of each treatment group. This revealed that the lipid mass of 10 day old diet-restricted ducklings was 30.8% lower compared to controls ($P<0.001$), while the lipid mass of 20 day old diet-restricted was 16.4% lower compared to controls ($P<0.01$). In contrast, the gizzard mass of 10 day old diet-restricted ducklings was 22.7% heavier compared to controls ($P<0.001$), while the gizzard mass of 20 day old diet-restricted was not significantly different from that of controls ($P>0.05$). The mass of the pectoral muscles for 10 day old diet-restricted ducklings was not significantly different compared to controls ($P>0.05$), while the pectoral muscle mass of 20 day old diet-restricted ducklings was 29.2% lighter compared to controls ($P<0.001$). The intestine mass of 20 day old diet-restricted ducklings was 21.3% lower compared to controls ($P<0.001$), while the intestine mass of 10 day old diet-restricted ducklings was 10.9% lower compared to controls ($P<0.05$).

The structural size of the ducklings was also affected by diet restriction. The length of the skull (head + bill) of the diet-restricted ducklings was 6.0% longer compared to controls for a given mass ($F_{1,45}$=54.36, $P<0.001$, Fig 6f). However, the age-specific growth of the skull was not fully sustained during the diet-restriction. For a given age, the diet-restricted ducklings exhibited ~6% shorter skulls compared to controls ($F_{1,45}$=13.39, $P<0.001$). In the analysis of the length of the tarsus, there was a significant 'treatment x body mass' interaction ($F_{1,46}$=4.89, $P<0.05$), indicating that diet restriction affected the tarsus of 10 and 20 day old diet-restricted ducklings differently. However, after entering the 3-level treatment factor, pairwise comparisons revealed that tarsus length did not vary among diet-restricted and control ducklings in either the 10 or the 20 day groups ($P>0.05$). There was also a significant 'treatment x body mass' interaction in the analysis of the length of the wing ($F_{1,44}$=13.18, $P<0.001$). After entering the 3-level treatment factor, pairwise comparisons revealed that the length of the wing of 10 day old diet-restricted ducklings was 15.6% shorter compared to controls

($P<0.001$), while the length of the wing of 20 days old diet-restricted ducklings was not different compared to controls ($P>0.05$).

The diet restriction also affected the LDF of organs. The LDF of the pectoral muscles was higher in 10 day old ($F_{1,22}=12.64$, $P<0.005$) and 20 day old diet-restricted ducklings ($F_{1,26}=69.00$, $P<0.001$) compared to controls (Fig. 7d). The LDF of the gizzard was also higher in 10 day old ($F_{1,23}=9.98$, $P<0.005$) and 20 day old diet-restricted ($F_{1,26}=6.17$, $P<0.05$) compared to controls (Fig. 7b). The LDF of the leg muscles was higher in the 10 day old diet-restricted ducklings ($F_{1,22}=5.31$, $P<0.05$), but not in the 20 day old diet-restricted ducklings ($F_{1,24}=0.81$, $P>0.05$), compared to controls (Fig. 7a). In contrast, the LDF of the liver was lower in 20 day old diet-restricted ducklings ($F_{1,26}=6.75$, $P<0.05$), but not in 10 day old diet-restricted ducklings ($F_{1,23}=0.89$, $P>0.05$), compared to controls. The LDF of the heart, the kidneys (Fig. 7e) and the intestine showed no significant differences between diet-restricted and controls.

Body Composition and RMR

No organ masses (main effects) were significant predictors of RMR in any of the two age groups of ducklings (Table 1). Body mass and treatment were strong predictors of RMR in all the analyses of any relationship between organ mass and RMR. However, there was a significant interaction with treatment for intestine length ($F_{1,21}=6.74$, $P<0.05$) and intestine mass ($F_{1,21}=4.62$, $P<0.05$) of 5-10 days old ducklings. For the controls, the parameter estimates showed a positive relationship between intestine length and RMR ($B=1.1$, $r=0.42$, $P<0.05$; B is the partial regression coefficient) and a positive, but non-significant, relationship between intestine mass and RMR ($B=0.41$, $r=0.31$, $P=0.16$). For the diet-restricted ducklings, the corresponding estimates were negative and non-significant (intestine length, $B=-0.37$, $r=-0.49$ $P>0.4$, intestine mass $B=-0.22$, $r=-0.42$ $P>0.3$).

We also performed analyses in which both age-groups were included. These analyses were consistent with the analyses in which we separated the age-groups, except for one organ. The mass of the liver was a significant predictor of RMR ($F_{1,50}=8.54$, $P<0.005$, $r^2=0.15$).

Growth Rate and RMR

In the controls (*ad libitum* fed ducklings), the growth rate of the wings ($F_{1,22}$=10.20, P<0.005) and the growth rate of the body mass ($F_{1,33}$=10.42, P<0.005) were significant predictors of RMR. When we analysed the controls and the diet-restricted ducklings together, the growth rate of the wings was still significant ($F_{1,22}$=10.20, P<0.005), but the growth rate of the body mass was not. PC1, the factor score extracted from a principal component analysis with wing, skull and tarsus growth rate, showed a tendency towards a positive relationship to RMR in controls ($F_{1,22}$=3.07, P=0.09) and in controls and diet-restricted ducklings ($F_{1,38}$=3.20, P=0.08).

Body Composition and PMR

A general trend was apparent for the analyses of any relationship between organ mass and PMR. The treatment factor and body mass were strong predictors of RMR in almost all the analyses. In addition, some organ masses were significant predictors. In 5-10 day old ducklings, the mass of the leg muscles ($F_{1,20}$=6.53, P<0.05, Table 2) and the total lipid mass ($F_{1,21}$=4.81, P<0.05) were significant predictors of PMR. By using the residuals from these analyses, we found that the mass of the leg muscles was positively correlated to the total lipid mass (r=0.87, N=24, P<0.01).

In 15-20 day old ducklings, the mass of the liver ($F_{1,17}$=9.92, P<0.01) was a significant predictor of PMR. For the heart, the 'treatment x organ mass' interaction was significant ($F_{1,16}$=7.25, P<0.05). The parameter estimates showed a positive relationship between organ mass and PMR in diet-restricted ducklings (B=0.46, r=0.56, P<0.05), but no relationship between organ mass and PMR in controls (B=0.02, r=0.05, P>0.05).

We also performed analyses in which both age-groups were included. With these analyses, we found that the mass of the leg muscles was a significant predictor of PMR ($F_{1,37}$=8.20, P<0.01). The total lipid mass was also a significant predictor of PMR ($F_{1,41}$=10.07, P<0.005). Again, using the residuals from this analysis and from the analyses of the relationship between the mass of the leg muscles, we found that they were strongly correlated (r=0.87, N=45, P<0.01).

The Relationship between LDF and MR (PMR and RMR)

There was a negative and nearly significant relationship between LDF of the leg muscles and PMR in 5-10 day old ducklings, ($F_{1,20}$=4.08, P=0.057). In 15-20 day old ducklings, there was no relationship between LDF of the leg muscles and PMR ($F_{1,15}$=0.73, P>0.05). There were no significant relationships between the LDF of any of the other organs and PMR in any of the two age-groups.

In 5-10 day old ducklings, there was a positive and significant relationship between LDF of the kidneys and RMR ($F_{1,22}$=12.89, P<0.005). In 15-20 day old ducklings, there was no relationship between LDF of the kidneys and RMR ($F_{1,25}$=0.74, P>0.05), or between LDF of any of the organs and RMR.

In 5-10 day old ducklings, the 'LDF of the leg muscles x treatment' interaction was a significant predictor of RMR ($F_{1,20}$=5.15, P<0.05). There was a significant positive relationship between LDF of the leg muscles and RMR in the diet-restricted ducklings (B=2.6, r=0.45, P<0.05), while a negative significant relationship existed between LDF of the leg muscles and RMR in the controls (B=-2.2, r=-0.46, P<0.05).

Discussion

In this study, we asked three main questions. 1) Will ducklings show any energy saving mechanisms that lessen the detrimental effects of reduced food intake during early development? 2) Are changes in body composition the physiological mechanism behind such a metabolic response? 3) Will any energy saving mechanism (i.e. lowered RMR) result in a negative effect on the thermoregulatory capacity (PMR) of growing chicks?

The results of the present study reveal that ducklings exhibit a substantial energy saving mechanism as a response to short term diet restriction. After 5 days of diet restriction, the RMR of 10 and 20 day old duckling was 16.4 and 32.1% lower, respectively, compared to *ad libitum* fed ducklings (controls). The results revealed that diet restriction induced changes in the body composition of the ducklings. The liver, the intestine and the lipid mass were substantially lower than predicted from the body mass. Our analyses of the relationship between RMR and organ masses suggested that the liver mass partly explained the low RMR in diet-restricted ducklings. The results also revealed that diet restriction affected PMR. Diet-restricted ducklings (10 and 20 day old) exhibited ~10% lower PMR than predicted from the body mass.

Adaptive Metabolic Responses to Food Shortage

This study clearly demonstrates developmental plasticity in the ontogenetic development of the ducklings. Developmental plasticity is regarded as adaptive if the animal actively adjusts the ontogenetic processes to a change in the environment (Smith-Gill 1983). Schew and Ricklefs (1998) suggested that temporal changes in the metabolic processes of chicks in response to variation in food intake could be adaptive because it might facilitate survival of chicks during food shortages. We regard the low RMR of the diet-restricted ducklings as an adaptive response, because the reductions in RMR occurred before the stored lipid mass was severely depleted. After 5 days of diet restriction, the ducklings still exhibited substantial amount of stored lipid (lipid mass/lean dry mass, 10 days old, 18.4%; 20 days old, 40.9%), indicating sufficient amount of stored nutrients to fuel the maintenance metabolism. Alternatively, the low RMR could have been non-adaptive. It could have been a consequence of the restricted diet, either through pathological changes in chick metabolism while near starvation (e.g.

through depletion of other essential nutrients than lipids), or through reduced size of the visceral organs or reduced overall growth. Deleterious pathological changes, most likely, did not happen. We have unpublished results (from experiments with the same diet restriction protocols) which show that the ducklings immediately resumed normal growth at the start of realimentation. This also indicates that metabolic rates were rapidly increased, and that the cellular structures responsible for the metabolism were intact. Visceral organs and overall growth were reduced. These changes could have been a consequence of the restricted diet, but they could also have been adaptive changes in anticipation of a lasting food shortage. Nevertheless, changes in organ size or growth were not major predictors of the low RMR in the diet-restricted ducklings (also discussed later). A rapid response upon diet restriction has been proposed to characterise an adaptive response to food shortage (Schew and Ricklefs 1998). We did not monitor the RMR changes (or the changes in body composition or growth) over the course of the diet restriction periods, and therefore, we can not entirely rule out the RMR changes being non-adaptive.

During the last decade, adaptive metabolic responses of chicks to food shortage have been the subject of several studies. Schew (1995) demonstrated that Japanese quail (*Coturnix coturnix japonica*) exposed to diet restriction from the age of 3 to 13 days old, reduced metabolic rate by 40% already by the second day of diet restriction and increased metabolic rate by 87% within the first day of realimentation. Similarly, male broilers (*Gallus gallus*) responded rapidly to diet restriction by decreasing metabolic rate and subsequently increasing it in response to realimentation (Zubair and Leeson 1994). Japanese quails exposed to diet restriction from the age of 20 to 30 days old, also showed a rapid metabolic response, but the reduction in metabolic rate was very small compared to the response of the younger diet-restricted chicks. In contrast, neither young nor old chicks of European starling (*Sturnus vulgaris*) showed any metabolic response to 3 days of diet restriction (Schew 1995). Similarly, nestlings of another passerine species, the song thrush (*Turdus philomelos*), showed no metabolic response to diet restriction (Konarzewski and Starck 2000). Konarzewski and Starck (2000) suggested that a lack of frequent, unpredictable fluctuations in food availability or a strong selection for uniform adult phenotypes could prevent plasticity of the developmental program of nestlings.

Metabolic responses of chicks to food shortage have also been investigated in relation to inherent growth rate. Van der Ziel and Visser (2001) revealed that the level of plasticity of the metabolic development was not determined by the maximum inherent growth rate. Japanese quail chicks from a line selected for high postnatal growth rates did not show a different metabolic response to long-term undernutrition compared to chicks from a line not selected for high postnatal growth rates.

Of the above, it is possible that phylogenetic constraints and the ecological settings of the particular species may determine the metabolic responses of chicks to food shortage. Kitaysky (1999) conducted an experiment on chicks of 4 closely related alcid species, which reduced metabolic rate by 24-47% after 2 days of fasting. He highlighted the ecological settings, determining food provisioning, as an important selective factor. The piscivorous horned and tufted puffins (*Fratercula corniculata* and *Lunda cirrhata*) which rely on fluctuating food resources, showed greater metabolic responses to food shortage compared to the planktivorous crested and parakeet auklets (*Aethia cristatella* and *Cyclorhinchus psittacula*) which rely on continuously available food resources. However, this could also be a phylogenetic response as the puffins behaved more similarly to each other than they did to the auklets.

Plasticity in the ontogenetic development of metabolism can be an adaptation to unpredictable fluctuating feeding conditions. The Pekin duck, the Japanese quail and the broiler are domesticated species. It is unlikely that these species have been artificially selected for plasticity of the metabolic development. However, the ecological settings of their ancestors include factors that could select for such flexibilty. In nature, poor weather and foraging inexperience can impose short-term food limitations on self-feeding precocial chicks, as reported for the willow ptarmigan (*Lagopus l. lagopus,* Erikstad and Andersen 1983; Erikstad and Spidsø 1982), the black-tailed godwit (*Limosa limosa*, Beintema and Visser 1989a) and the northern lapwing (*Vanellus vanellus*, Beintema and Visser 1989b).

Body Composition and Explanations of the Variation in RMR due to Diet Restriction

The changes in body composition indicated that energy was allocated preferentially to parts of the skeletal structure (i.e. head + bill), to promote a higher growth of these parts relative to the growth in body mass. Energy was allocated to the leg muscles, the heart and the kidneys in such a manner that these organs maintained normal size-relationships to body mass. In contrast, the liver, the intestine and the lipid mass were smaller than predicted from their normal allometric relationship to body mass. Liver tissue has a high intrinsic MR, while adipose tissue has a low intrinsic MR (Scott and Evans 1992). Our results showed that the liver size was a significant predictor of RMR when we analysed the data where both age-groups were included. In addition, the significant interaction between intestine mass (and length) and treatment in the RMR comparisons indicates that intestine differences might also play a role in the RMR differences. Statistically, the liver size (and the intestine size) was not a strong predictor of the variation in RMR. Body mass and the treatment-factor were the strong predictors in all the organ mass-RMR analyses. By using 15.1 W kg^{-1} for liver- and intestine MR, as measured for liver in vitro by Scott and Evans (1992), we calculated that the reductions in the liver and intestine size explained 15 and 18 % of the reductions in the overall RMR in 10 and 20 day old diet-restricted ducklings, respectively. However, such a quantitative value of the reduction in RMR should be treated carefully as Scott and Evans (1992) measured the MR of liver samples from adult birds and in different species to ours.

The functioning of tissues may have changed during the diet restriction due to the changes in water content, measured as the LDF. The LDF is regarded to relate to the functional maturity of tissues, and in skeletal muscles the LDF is postively related to the thermoregulatory abilities (Ricklefs et al. 1994). It is less clear how the LDF relates to the tissue-RMR (Ricklefs et al. 1998). Despite the fact that the diet restriction treatment in this study imposed variation in water content in various tissues, LDF was not a good predictor of changes in RMR or PMR.

As neither organ size or tissue water content were major predictors of RMR in the diet-restricted ducklings, other mechanisms related to the intrinsic MR of organs or mechanisms related to the central control of the MR should be more important.

Hypothermia is a well-known energy-saving mechanism in small, adult birds and mammals. In this study, we observed that 10 day old diet-restricted ducklings used hypothermia (to a low degree), but the 20 day old diet-restricted ducklings did not. They regulated their body temperature 0.7°C below the expected value (predicted from body mass). By using the measured values for thermal conductance (0.95 W kg^{-1} $^{o}C^{-1}$) and ambient temperature (31.5 °C) during thermoneutral conditions, we calculated that the hypothermia accounted for ~50% of the observed reduction in RMR of 10 day old diet-restricted duckling. Furthermore, a Q_{10} effect (assuming a Q_{10} of 2.5) explained 76% of the energy savings caused by hypothermia. Hypothermia is also reported for diet-restricted Japanese quail chicks (Schew 1995). They lowered the body temperature by 2-3 °C during the diet restriction period, and both small and large chicks exhibited hypothermia. An extreme example of hypothermia in young birds is reported for the fork-tailed storm petrel (*Oceanodroma furcata*), in which underfed and unattended chicks regulated the body temperature at 10 °C (Boersma 1986).

Organ size changes (15%) and hyporthermia (50%), combined, explained 65% of the observed reductions in RMR of 10 day old diet-restricted ducklings. However, organ size changes were the only revealed energy saving mechanism of the 20 day old diet-restricted ducklings and explained only 18% of the observed reductions in RMR. Although we want to be careful in attributing a quantitative value to the reduction in RMR from the organ mass changes, we believe that we are left with a body of unexplained mechanisms behind the observed reductions in RMR of the 20 day old diet-restricted ducklings.

The growth rate of the wings and the growth rate of the body mass was positively related to RMR in the controls (*ad libitum* fed ducklings). In addition, the factor score extracted from a principal component analysis with wing-, skull- and tarsus growth rate showed a tendency ($P<0.1$) towards a positive relationship with RMR. These results could support the proposed positive relationship between growth rate and RMR, in which RMR include indirect costs of growth, in terms of costs of maintaining organs that support growth or represent a potential for growth (Drent and Klaassen 1989; Klaassen and Drent 1991). It could also suggest that variation in the direct costs of growth, i.e. the costs of biosynthesis, is a significant source of the variation in RMR. However, only the growth rate of the wings (controlled for body mass) was significantly related to RMR when the controls and the diet-restricted were included in the analyses. Furthermore, the diet restriction only imposed reductions in growth rate of the wings in the 10 day old diet-restricted ducklings (and not in the 20 day old). At that age, the growth of the wings is very low, and the potential savings in RMR must also be very low. Hence, variation in the growth rate did not seem to be important predictors of the reductions in RMR of diet-restricted ducklings.

Diet restriction and Thermoregulatory Abilities

The diet-restricted ducklings (both 10 and 20 days old) exhibited ~10% lower PMR compared to controls. In contrast, the absolute scope (PMR minus RMR) was not different between diet-restricted ducklings and controls. Hence, the portion of PMR available for regulatory thermogenesis was similar between the two groups. The leg muscles, which are regarded as the most important organs for shivering thermogenesis in young birds (Hohtola and Visser 1998), and the heart, which is important for maximum oxygen consumption (Chappel et al. 1999), were maintained at the normal size expected from the body mass during the diet restriction. The importance of the leg muscles for the regulatory thermogenesis was also indicated by the positive relationship between the leg muscle mass and PMR. However, the liver mass showed a positive relationship to PMR in 15-20 day old ducklings. The paradigm of muscular shivering as the only source of cold induced thermogenesis has been thrown into debate (Duchamp et al. 1993; Marsh 1993). However, the skeletal muscles, not the liver, have been targeted as the main site of a potential non-shivering thermogenesis. Hence, the statistical relationship between the liver mass and PMR is most likely an indirect relationship through a correlation with RMR. We showed a positive relationship between RMR and PMR and between the liver mass and RMR.

The lower PMR in diet-restricted ducklings could indicate a negative effect of the diet restriction on the thermoregulatory effector mechanisms or it could be a result of the lower RMR. The methods of describing the ducklings' capacity for regulatory thermogenesis, absolute scope and factorial scope, are based on different assumptions of the relationship between RMR and PMR, and they provide contrasting results (Fig. 5). While the absolute scope indicated a conserved capacity (Fig 5a), the factorial scope indicated an increased age-specific capacity for regulatory thermogenesis of the diet-restricted ducklings (Fig. 5b). The calculation of factorial scope assumes a factorial relationship between RMR and PMR. In contrast, the calculation of absolute scope assumes that RMR is a fixed part of PMR (i.e. PMR = RMR +Thermoregulation). RMR is predicted to correlate to PMR if RMR is a fixed part of PMR (Ricklefs et al. 1996), and we found such a positive relationship between RMR and PMR. Hence, we regard absolute scope to provide a better measure of the capacity for regulatory thermogenesis than factorial scope. Consequently, we think that the diet-restricted ducklings have conserved their capacity for regulatory thermogenesis, and we think the low RMR has entailed the lower PMR.

Although the diet-restricted ducklings exhibited a lower PMR, they coped rather well with cold ambient temperatures (Fig. 3b) and attained PMR with body temperatures not different to that of controls. The 20 day old diet-restricted ducklings seemed to compensate for the lower PMR by increasing the insulation (probably increased down thickness), as indicated by the lower MTC (Fig. 4a). Consequently, they attained PMR at an ambient temperature as expected from age. The 10 day old diet-restricted ducklings did not compensate for the lower PMR by decreasing the MTC, and they attained PMR at an ambient temperature as expected from body mass, i.e. at a higher ambient temperature compared to the 10 day old controls (Fig.3b).

Costs of Plasticity

Even small deviations from the normal growth might be expected to produce fitness consequences (Gebhardt-Henrich and Richner 1998). The quantitative and qualitative nature of the food restriction, as well as the duration and the timing, determine these deviations (Schew and Ricklefs 1998). Generally, food restriction delays the schedule of mass and skeleton accretion (Øyan and Anker-Nilssen 1996; Lepczyk and Karasov. 2000). Premature fledging (Kitasky 1999) and permanent stunting of external measurements (Boag 1987; Ohlsson and Smith 2001) have also been reported.

Plasticity of the developmental trajectory can be adaptive and lessen the detrimental effects of food stress during early development. However, plasticity may be associated with short- and long-term costs. The reduced PMR of the diet-restricted ducklings can be regarded as a short-term cost of the plasticity of the development of the RMR. Also, the liver, the intestine and the lipid masses can be energetically costly to restore after food stress. However, studies on growing young (Nir and Nitsan 1979; Schew 1995) and migrating adults (Piersma and Lindstrøm 1997; Piersma 1998) have reported that these organs and tissues can be rapidly rebuilt during sufficient realimentation. However, although body mass, external measurements and visceral organs may fully recover during realimentation, long-term fitness consequences may appear later in life (Metcalfe and Monaghan 2001).

Conclusions

In the present study, we have shown that ducklings exhibit substantial energy-saving mechanisms as a response to short-term food shortage. This physiological response entailed a negative effect on the cold induced peak metabolic rate (PMR), but the overall thermoregulatory abilities were nevertheless very well maintained. Changes in body composition were a minor predictor of the energy saving mechanisms, and in the 10 day old diet-restricted ducklings, hypothermia was an important mechanism. Still, we are left with a body of unexplained mechanisms behind the metabolic responses to food shortage, especially for the 20 day old diet-restricted ducklings. We argue that the observed energy saving mechanisms are adaptive responses, but we can not entirely rule out the responses being non-adaptive. Further investigation of the mechanisms behind physiological responses to food shortage as well as investigations of the long-term fitness consequences deserve attention in future studies.

Acknowledgements

We are grateful to the technicians, Odd Arne Indset and Bjørn Simensen, who skilfully constructed and maintained animal housing conditions. We thank Carolyn Baggerud for improving the English, and we thank two anonymous reviewers for constructive comments and suggestions on an earlier draft of this article. CB/BM was supported by a grant from the Research Council of Norway (138698/410).

Literature Cited

Bakken G.S. 1992. Measurement and application of operative and standard operative temperatures in ecology. American Zoologist 32:194-216.

Bech C. and J.E. Østnes. 1999. Influence of body composition on the metabolic rate of nestling European shags (*Phalacrocorax aristotelis*). Journal of Comparative Physiology B 169:263-270.

Beintema A.J. and G.H. Visser. 1989a. Growth parameter in chicks of Charadriiform birds. Ardea 77:169-180.

Beintema A.J. and G.H. Visser. 1989b. The effects of weather on time budgets and development of chicks of meadow birds. Ardea 77:181-192.

Bennett A.F. and J.A. Ruben. 1979. Endothermy and activity in vertebrates. Science 206:649-654.

Birkhead T.R., F. Fletcher and E.J. Pellatt. 1999. Nestling diet, secondary sexual traits and fitness in the zebra finch. Proceedings of the Royal Society London B 266:385-390.

Boag P.T. 1987. Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). Auk 104:155-166.

Boersma, P.D. 1986. Body temperature, torpor, and growth of chicks of fork-tailed storm petrels (*Oceanodroma furcata*). Physiological Zoology 59:10-19.

Burness G.P, R.C. Ydenberget and P.W. Hochachka. 1998. Interindividual variability in body composition and resting oxygen consumption rates in breeding tree swallows, Tachycineta bicolor. Physiological Zoology 71:247-256.

Chappell M.A., C. Bech and W.A. Buttemer.1999. The relationship of central and peripheral organ masses to aerobic performance variation in House sparrows. Journal of Experimental Biology 202:2269-2279.

Christians J.K. 1999. Controlling for body mass effects: is part-whole correlation important? Physiological and Biochemical Zoology 72:250-253.

Daan S., D. Masman and A. Groenewold. 1990. Avian basal metabolic rates: their association with body composition and energy expenditure in nature. American Journal of Physiology 259:333-340.

De Kogel C.H. 1997. Long-term effects of brood size manipulation on the morphological development and sex-specific mortality of offspring. Journal of Animal Ecology 66:167-178.

Drent R.H. and M. Klaassen. 1989. Energetics of avian growth: the causal link with BMR and metabolic scope. Pp. 349-359 in C. Bech and R.E. Reinertsen, eds. Physiology of Cold Adaptation in Birds. Plenum, New York.

Duchamp C., F. Cohen-Adad, J-L. Rouanet, E. Dumonteil and H. Barré. 1993. Existence of nonshivering thermogenesis in birds. Pp. 529-533 in C. Carey, G.L. Florant, B.A. Wonder and B. Horwitz, eds. Life in the Cold. Ecological, Physiological and Molecular Mechanisms. Westview, Colorado.

Dufty A.M., Jr., J. Clobert and A.P. Møller. 2002. Hormones, developmental plasticity and adaptation. Trends in Ecology and Evolution 17:190-196.

Dutenhoffer M.S. and D.L. Swanson. 1996. Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiological Zoology 69:1232-1254.

Erikstad K.E. and T.K. Spidsø. 1982. Weather influence on food intake, insect prey selection and feeding behaviour in willow grouse chicks in northern Norway. Ornis Scandinavica 13:176-182.

Erikstad K.E. and R. Andersen. 1983. The effect of weather on survival, growth rate and feeding time in different sized willow grouse broods. Ornis Scandinavica 14:249-252.

Gebhardt-Henrich, S. and H. Richner. (1998). Causes of growth variation and its consequences for fitness. Pp. 324-339 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Hart J.S. 1951. Calorimetric determination of average body temperature of small mammals and its variation with environmental conditions. Canadian Journal of Zoology 29:224-233.

Hayes J.P. and T. Garland, Jr. 1995. The evolution of endothermy: testing the aerobic capacity model. Evolution 49:836-847.

Hayes J.P. and J.S. Shonkwiler. 1996. Analyzing mass-independent data. Physiological Zoology 69:974-980.

Hohtola E. and G.H. Visser. 1998. Development of locomotion and endothermy in altricial and precocial birds. Pp. 157-173 in J.M. Starck and R.E. Ricklefs, eds. Avian growth and Development. Oxford University Press, Oxford.

Kitaysky A.S. 1999. Metabolic and developmental responses of alcid chicks to experimental variation in food intake. Physiological and Biochemical Zoology 72:462-473.

Klaassen M. and R.H. Drent. 1991. An analysis of hatchling basal metabolism: in search of ecological correlates that explain deviations from allometric relations. Condor 93:612-629.

Konarzewski M. and J.M. Starck. 2000. Effects of food shortage and oversupply on energy utilization, histology and function of the gut in nestling Song thrushes (*Turdus philomelos*). Physiological and Biochemical Zoology 73:416-427.

Konarzewski M., J. Kowalczyk, T. Swierubska and B. Lewonczuk. 1996. Effect of short-term feed restriction, realimentation and overfeeding on growth of Song thrush (*Turdus philomelos*) nestlings. Functional Ecology 10:97-105.

Konarzewski M., A. Gavin, R. McDevitt and I.R. Wallis. 2000. Metabolic and organ mass responses to selection for high growth rates in the domestic chicken (*Gallus domesticus*). Physiological and Biochemical Zoology 73:237-248.

Lepczyk C.A. and W.H. Karasov. 2000. Effect of ephemeral food restriction on growth of house sparrows. Auk 117:164-174.

Lindström J. 1999. Early development and fitness in birds and mammals. Trends in Ecology and Evolution 14:343-348.

Marsh R.L. 1993. Does regulatory thermogenesis exist in birds? Pp. 535-538 in C. Carey, G.L. Florant, B.A. Wonder and B. Horwitz, eds. Life in the Cold. Ecological, Physiological and Molecular Mechanisms. Westview, Colorado.

Meerlo P., L. Bolle, G.H. Visser, D. Masman and S. Daan. 1997. Basal metabolic rate in relation to body composition and daily energy expenditure in the field vole, *Microtus agrestis*. Physiological Zoology 70:362-369.

Metcalfe N.B. and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254-260.

Niimi A.J. 1978. Lag adjustments between estimated and actual physiological responses conducted in flow-through systems. Journal of the Fisheries Research Board of Canada 35:1265-1269.

Nir I. and Z. Nitsan. 1979. Metabolical and anatomical adaptations of light-bodied chicks to intermittent feeding. British Poultry Science 20:61-71.

Ohlsson T. and H.G. Smith. 2001. Early nutrition causes persistent effects on pheasant morphology. Physiology and Biochemical Zoology 74:212-218.

Østnes J.E. and C. Bech. 1997. Ontogeny of deep-body cold sensitivity in Pekin *ducklings Anas platyrhynchos*. Journal of Comparative Physiology B 167:241-248.

Øyan H.S. and T. Anker-Nilssen. 1996. Allocation of growth in food-stressed Atlantic Puffin chicks. Auk 113:830-841.

Piersma T. 1998. Phenotypic flexibility during migration: optimization of organ size contingent on the risks and rewards of fueling and flight? Journal of Avian Biology 29:511-520.

Piersma T. and Å. Lindstrøm. 1997. Rapid reversible changes in organ size as a component of adaptive behviour. Trends in Ecology and Evolution 12:134-138.

Rezende E.L., D.L Swanson, F. F. Novoa and F. Bozinovic. 2002. Passerines versus nonpasserines: so far, no statistical differences in the scaling of avian energetics. The Journal of Experimental Biology 205:101-107.

Ricklefs R.E., R.E. Shea and I.H. Choi. 1994. Inverse relationship between functional maturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. Evolution 48:1080-1088.

Ricklefs R.E., M. Konarzewski and S. Daan. 1996. The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. American Naturalist 147:1047-1071.

Ricklefs R.E., J.M. Starck and M. Konarzewski. 1998. Internal constraints on growth in birds. Pp. 266-287 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Schew W.A. 1995. The evolutionary significance of developmental plasticity in growing birds. PhD diss. University of Pennsylvania, Philadelphia.

Schew W.A. and R.E. Ricklefs. 1998. Developmental plasticity. Pp. 288-304 in J.M. Starck and R.E. Ricklefs, eds. Avian Growth and Development. Oxford University Press, Oxford.

Scholander P.F., R. Hock, V. Walters, F. Johnson and L. Irving. 1950. Heat regulation in some arctic and tropical mammals and birds. Biological Bulletin 99:237-258.

Scott I. and P.R. Evans. 1992. The metabolic output of avian (*Sturnus vulgaris, Calidris alpina*) adipose tissue liver and skeletal muscle: Implications for BMR/Body mass realtionships. Comparative Biochemistry and Physiology 103A:329-332.

Smith-Gill S.J. 1983. Developmental plasticity: developmental conversion *versus* phenotypic modulation. American Zoologist 23:47-55.

SPSS. 2002. SPSS for Windows. Release 11.5.1. – SPSS Inc. Chicago.

Stearns S.C. 1992. The evolution of life histories. Oxford University Press, Oxford.

Taigen T.L. 1983. Activity metabolism of anuran amphibians: implications for the origin of endothermy. American Naturalist 121:94-109.

Van der Ziel C.E. and G.H. Visser. 2001. The effect of food restriction on morphological and metabolic development in two lines of growing Japanese quail chicks. Physiological and Biochemical Zoology 74:52-65.

Visser G.H. and R.E. Ricklefs. 1993. Temperature regulation in neonates of shorebirds. Auk 110:445-457.

Withers P.C. 1977. Measurement of VO_2, VCO_2 and evaporative water loss with flow-through mask. Journal of Applied Physiology 42:120-123.

Zubair A.K. and S. Leeson. 1994. Effect of early restriction and realimentation on heat production and changes in sizes of digestive organs of male broilers. Poultry Science 73:529-538.

Tables

Table 1: Relationships between resting metabolic rate (RMR) and organ masses (and intestine length) in controls and diet-restricted ducklings[a].

	RMR of 5-10 day old ducklings							RMR of 15-20 day old ducklings						
	Main effects				Interaction mass[b] x treatment			Main effects				Interaction mass[b] x treatment		
	F	df	P	r^2	F	df	P	F	df	P	r^2	F	df	P
Breast	0.1	1,21	ns	0.00	1.5	1,20	ns	0.8	1,25	ns	0.03	0.9	1,24	ns
Leg	0.6	1,21	ns	0.03	2.5	1,20	ns	0.0	1,25	ns	0.00	0.7	1,24	ns
Heart	0.1	1,22	ns	0.00	1.7	1,20	ns	0.3	1,25	ns	0.01	0.5	1,24	ns
Liver	1.0	1,22	ns	0.04	0.6	1,21	ns	0.6	1,25	ns	0.02	0.2	1,24	ns
Gizzard	0.2	1,22	ns	0.01	3.3	1,21	ns	3.8	1,25	0.06	0.13	0.0	1,24	ns
Kidney	0.8	1,22	ns	0.04	3.8	1,21	ns	1.1	1,25	ns	0.04	0.0	1,24	ns
Intestine	0.2	1,21	ns	0.01	4.6	1,21	**<0.05**	0.2	1,25	ns	0.01	0.4	1,24	ns
Int. length	0.8	1,21	ns	0.04	6.7	1,21	**<0.05**	1.5	1,24	ns	0.06	0.2	1,23	ns
Lipid	2.1	1,22	ns	0.41	1.8	1,21	ns	0.0	1,25	ns	0.00	0.6	1,24	ns

[a] The analyses were performed separately on each organ with a GLM on $\log_{10}$ -transformed metabolic rate, lean dry body mass (minus organ mass) and lean dry organ mass. Treatment was included as a factor with 2 levels (1=controls, 2= diet-restricted). The 'organ mass x treatment' interaction was included in the null model. The treatment factor and body mass were strong predictors of RMR in all the analyses. In this table, we focus on the relationship between the organ masses and RMR. Hence, the statistics for treatment and body mass are not included in this table.

[b] Mass refers to the organ mass.

Table 2: Relationships between peak metabolic rate (PMR) and organ masses (and intestine length) in controls and diet-restricted ducklings[a].

	PMR of 5-10 day old ducklings							PMR of 15-20 day old ducklings						
	Main effects				Interaction mass[b] x treatment			Main effects				Interaction mass[b] x treatment		
	F	df	P	r^2	F	df	P	F	df	P	r^2	F	df	P
Breast	0.1	1,20	ns	0.00	2.4	1,19	ns	0.5	1,17	ns	0.03	0.2	1,16	ns
Leg	6.5	1,20	**<0.05**	0.25	1.7	1,19	ns	0.3	1,17	ns	0.02	2.7	1,16	ns
Heart	0.0	1,21	ns	0.00	2.1	1,20	ns	4.0	1,16	0.06	0.20	7.3	1,16	**<0.05**
Liver	0.2	1,21	ns	0.01	0.9	1,20	ns	9.9	1,17	**<0.01**	0.37	0.2	1,16	ns
Gizzard	0.6	1,21	ns	0.03	0.8	1,20	ns	0.8	1,17	ns	0.04	0.3	1,16	ns
Kidney	0.3	1,21	ns	0.01	0.2	1,20	ns	3.4	1,17	0.08	0.17	0.2	1,16	ns
Intestine	1.9	1,21	ns	0.08	0.0	1,20	ns	2.3	1,17	ns	0.12	1.6	1,16	ns
Int. length	0.0	1,21	ns	0.00	0.0	1,20	ns	0.6	1,17	ns	0.03	1.3	1,16	ns
Lipid	4.8	1,21	**<0.05**	0.19	1.1	1,20	ns	0.4	1,17	ns	0.02	2.4	1,16	ns

[a] The analyses were performed separately on each organ with a GLM on $\log_{10}$ -transformed metabolic rate, lean dry body mass (minus organ mass) and lean dry organ mass. Treatment was included as a factor with 2 levels (1=controls, 2= diet-restricted). The 'organ mass x treatment' interaction was included in the null model. The treatment factor and body mass were strong predictors of PMR in almost all the analyses. In this table, we focus on the relationship between the organ masses and PMR. Hence, the statistics for treatment and body mass are not included in this table.

[b] Mass refers to the organ mass.

Figure legends

Figure 1. Body mass growth (g) of Pekin ducklings (*Anas platyrhynchos*). Ducklings fed *ad libitum* (controls) are shown as filled squares and diet-restricted ducklings are shown as open squares. The grey horizontal bars indicate the time of the diet restriction periods. Means ± 1 SE.

Figure 2. Resting and peak metabolic rate (RMR and PMR) of *ad libitum* fed (controls, filled symbols) and diet-restricted ducklings (open symbols). Metabolic rate in watts and lean dry body mass in grams. The axes are $\log_{10}$-scaled. Regression lines are shown separately for controls and diet-restricted ducklings within each of the two age-groups (5-10 day old and 15-20 day old ducklings). The coefficient of determination (r^2) was 0.99 for both the RMR and the PMR model (see the text for details about the statistical models).

Figure 3. Body temperature (T_b) measured at RMR (A) and ambient temperature (T_a) measured at PMR (B) in *ad libitum* fed (controls, filled symbols) and diet-restricted ducklings (open symbols). Temperature in Celsius (^oC) and age in days (d). Temperatures are given as means ± 1 SE.

Figure 4. Minimal thermal conductance (MTC) in relation to lean dry body mass (A) and age (B) in *ad libitum* fed (controls, filled symbols) and diet-restricted ducklings (open symbols). MTC in watts per kilograms per degree Celsius ($W\ kg^{-1}\ {}^oC^{-1}$), lean dry body mass in grams (g) and age in days (d). In figure A the values are $\log_{10}$ –transformed, and regression lines are shown separately for controls and diet-restricted ducklings within each of the two age-groups (5-10 day old and 15-20 day old ducklings). MTC is given as means ± 1 SE in figure B.

Figure 5. Absolute scope in relation to lean dry body mass (A) and factorial scope in relation to age (B) in *ad libitum* fed (filled symbols) and diet-restricted ducklings (open symbols). Absolute scope was calculated as PMR-RMR, and factorial scope was calculated as PMR/RMR. In figure A the values are $\log_{10}$ –transformed, and regression

lines are shown separately for controls and diet-restricted ducklings within each of the two age-groups (5-10 day old and 15-20 day old ducklings). Absolute scope is given in watts (W), lean dry (ld) body mass in grams (g) and age in days (d). Factorial scope is given as means ± 1 SE.

Figure 6. Body composition of *ad libitum* fed (controls, filled symbols) and diet-restricted ducklings (open symbols). The relationship of leg mass (A), liver mass (B), lipid mass (C), gizzard mass (D), intestine mass (E) and skull length (head + bill, F) to body mass. Regression lines are drawn separately for controls and diet-restricted ducklings within each of the two age-groups (5-10 day old and 15-20 day old ducklings). Organ and body masses are lean dry masses in grams and lipid mass is dry mass in grams. The skull length is given in millimetres. The axes are $\log_{10}$-scaled.

Figure 7. Lean dry fraction (LDF) of the leg muscles (A), the gizzard (B), the heart (C), the pectoral muscles (D) and the kidneys (E) in relation to lean dry (ld) organ mass in *ad libitum* fed (controls, filled symbols) and diet-restricted ducklings (open symbols). Regression lines are drawn separately for controls and diet-restricted ducklings within each of the two age-groups (5-10 day old and 15-20 day old ducklings). LDF was calculated as lipid free dry organ mass/lipid free fresh organ mass. Organ masses in grams (g). The axes are $\log_{10}$-scaled.

Figure 1.

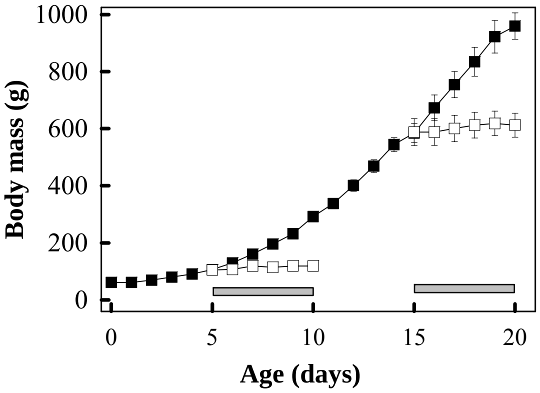


Figure 2.

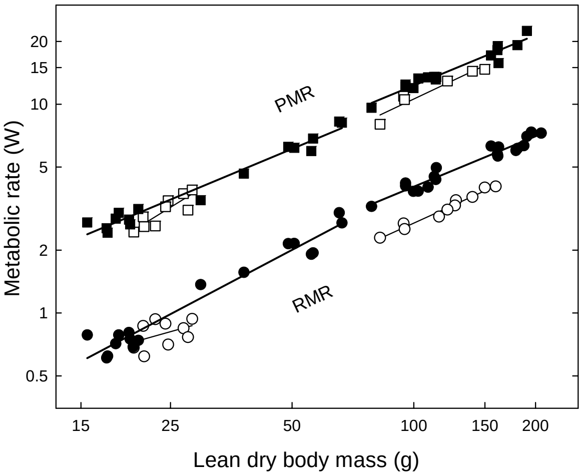
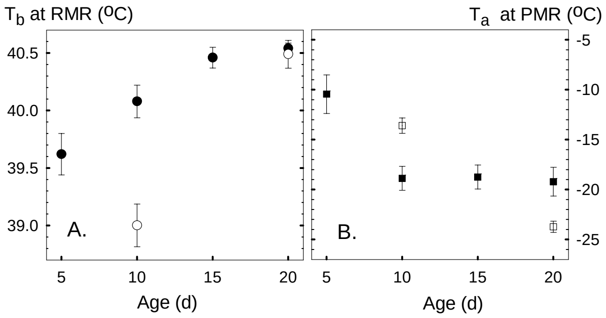

PMR
RMR
Metabolic rate (W)
20
15
10
5
2
1
0.5
Lean dry body mass (g)
15
25
50
100
150
200

Figure 3.



T_b at RMR (°C)
40.5
40.0
39.5
39.0
A.
5
10
15
20
Age (d)
T_a at PMR (°C)
-5
-10
-15
-20
-25
B.
5
10
15
20
Age (d)

Figure 4.

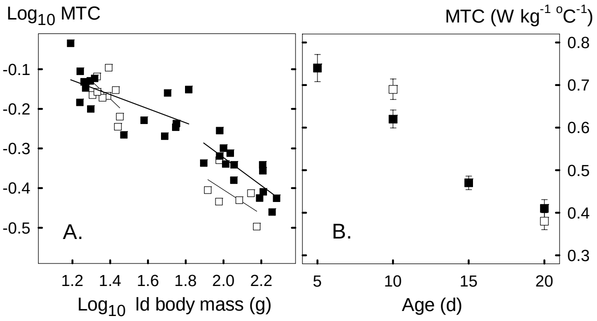

Log10 MTC
-0.1
-0.2
-0.3
-0.4
-0.5
A.
1.2
1.4
1.6
1.8
2.0
2.2
Log10 ld body mass (g)
MTC (W kg-1 oC-1)
0.8
0.7
0.6
0.5
0.4
0.3
B.
5
10
15
20
Age (d)

Figure 5.

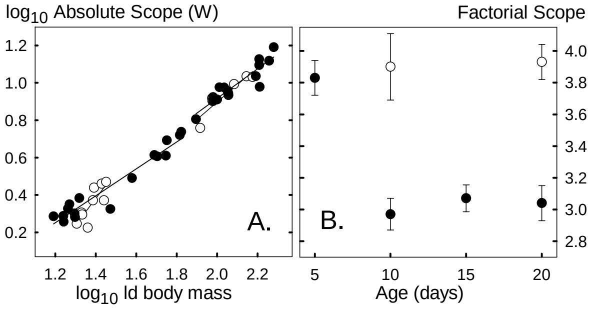

log$_{10}$ Absolute Scope (W)
Factorial Scope
1.2
1.0
0.8
0.6
0.4
0.2
4.0
3.8
3.6
3.4
3.2
3.0
2.8
A.
B.
1.2
1.4
1.6
1.8
2.0
2.2
log$_{10}$ ld body mass
5
10
15
20
Age (days)

Figure 6.

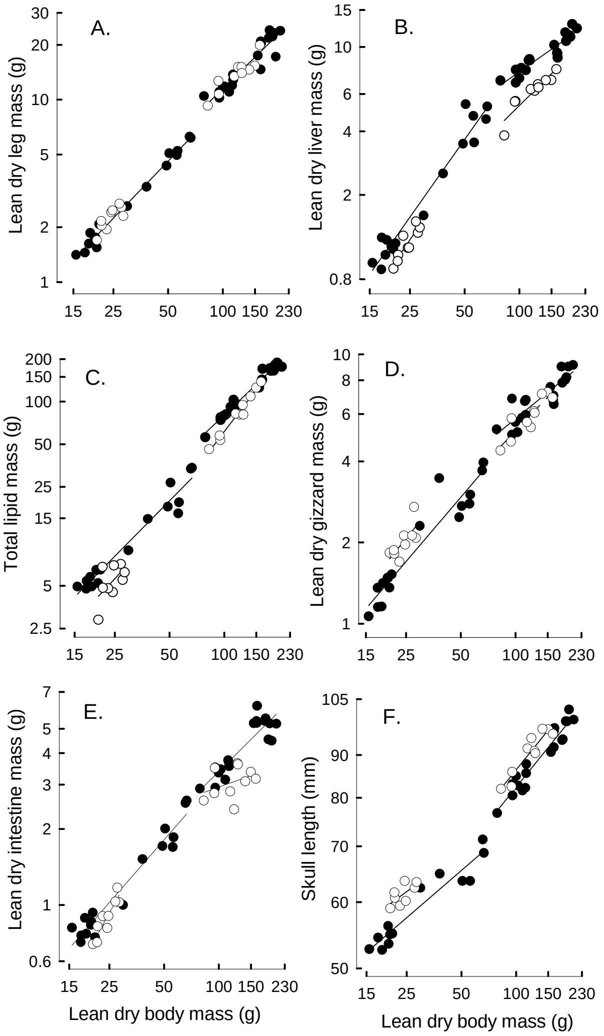
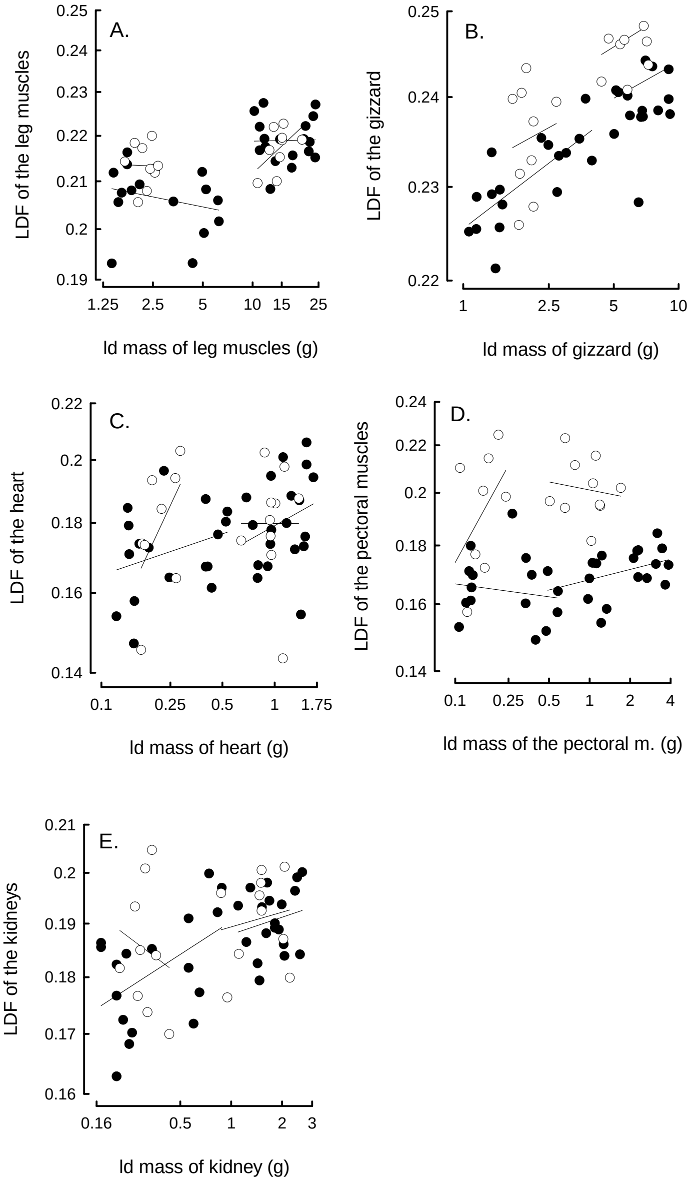

A.
Lean dry leg mass (g)
30
20
10
5
2
1
15
25
50
100
150
230
B.
Lean dry liver mass (g)
15
10
6
4
2
0.8
15
25
50
100
150
230
C.
Total lipid mass (g)
200
150
100
50
25
15
5
2.5
15
25
50
100
150
230
D.
Lean dry gizzard mass (g)
10
8
6
4
2
1
15
25
50
100
150
230
E.
Lean dry intestine mass (g)
7
5
4
3
2
1
0.6
15
25
50
100
150
230
Lean dry body mass (g)
F.
Skull length (mm)
105
90
80
70
60
50
15
25
50
100
150
230
Lean dry body mass (g)

Figure 7.


A.
LDF of the leg muscles
0.25
0.24
0.23
0.22
0.21
0.2
0.19
1.25
2.5
5
10
15
25
ld mass of leg muscles (g)
B.
LDF of the gizzard
0.25
0.24
0.23
0.22
1
2.5
5
10
ld mass of gizzard (g)
C.
LDF of the heart
0.22
0.2
0.18
0.16
0.14
0.1
0.25
0.5
1
1.75
ld mass of heart (g)
D.
LDF of the pectoral muscles
0.24
0.22
0.2
0.18
0.16
0.14
0.1
0.25
0.5
1
2
4
ld mass of the pectoral m. (g)
E.
LDF of the kidneys
0.21
0.2
0.19
0.18
0.17
0.16
0.16
0.5
1
2
3
ld mass of kidney (g)

Paper II

Paper II is not included due to copyright.

Paper III

Journal of Comparative Physiology B (in press)

Does food shortage delay development of homeothermy in European shag nestlings (*Phalacrocorax aristotelis*)?

Børge Moe, Siri Brunvoll, Daniel Mork, Trond Einar Brobakk and Claus Bech
Department of Biology
Norwegian University of Science and Technology
NO-7491 Trondheim, Norway

Address for correspondence and proof:
Børge Moe
Department of Biology, NTNU, NO-7491 Trondheim, Norway
Phone: +47 73 59 60 30
Fax: +47 73 59 13 09
E-mail: Borge.Moe@bio.ntnu.no

Abbrevations GLM general linear model, HI index of homeothermy, LDF lean dry fraction, MR metabolic rate, MTC mass-specific minimal conductance, PMR peak metabolic rate, RMR resting metabolic rate, T_a ambient temperature, T_b body temperature

Abstract

Nestlings seem to face a trade off between reducing the basal level of energy metabolism (RMR), as an energy-saving response, and maintaining thermogenic capacity during temporal food shortage. In the present study we examined developmental responses to short-term diet restriction of 12-16 day old nestling European shags kept under laboratory conditions and tested whether temporal food shortage delay the development of homeothermy.

During food shortage the European shag nestlings substantially reduced RMR, resulting in significant energy savings. The reduction in RMR corresponded with a reduction in peak metabolic rate (PMR). At the same time, the low PMR of diet-restricted nestlings was offset by a lower mass-specific minimal thermal conductance (MTC), and an increased mass-specific absolute scope. Consequently, the insulation and the portion of PMR available for regulatory thermogenesis seemed to develop normally, as expected from age, during the period of food shortage. Further, the degree of homeothermy, measured as the index of homeothermy (HI), was not significantly lower in diet-restricted nestlings compared to controls at the same age. We conclude that temporal food shortage did not significantly delay the development of homeothermy in the European Shag nestlings despite substantial reductions in RMR and PMR.

KEYWORDS: European shag, *Phalacrocorax aristotelis,* Development, Homeothermy, Metabolic rate, Nestlings, Food shortage, Body composition

Introduction

Avian development highly depends on food availability (Martin 1987). As chicks grow from neonate to adult, they may face periods of food shortage that can cause phenotypic changes from the normal ontogenetic development given by their genotype. Such phenotypic changes (arising from variation in food availability or other environmental conditions) are known as developmental plasticity (Schew and Ricklefs 1998; Schlichting and Pigliucci 1998). A number of recent studies have investigated how chicks can modify the pattern of energy use and allocation in response to food shortage during growth and development (e.g. Schew 1995; Kitaysky 1999; Konarzewski and Starck 2000; Brzek and Konarzewski 2001; Moe et al. in press, b). These studies have revealed that growing birds may lower the resting metabolic rate (RMR) in response to food shortage. Such an energy saving response is considered to lessen the detrimental effects and enhance survival during temporal food shortage (Schew and Ricklefs 1998). Moe et al. (in press) demonstrated that a reduction in RMR negatively affected the capacity for maximum heat production (i.e. peak metabolic rate [PMR]) in diet-restricted ducklings, but little is known about how food shortage affects the development of homeothermy in birds.

In this study we investigated the development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*) subject to short-term food shortage. The European shag is very well suited for studying developmental responses to temporal food shortage because of its life-history characteristics and its ecology. The European shag is a large altricial seabird, and nestlings exhibit high growth rates (Østnes et al. 2001) and compete with siblings for food (Amundsen and Stokland 1988; Velando et al. 1999, 2000). Consequently, the nestlings are highly dependent on successful food provisioning to follow the normal developmental trajectory. In this species, variable food provisioning during early development is reported to occur due to adverse weather conditions, which affects the foraging success of the parents (Velando et al. 1999).

The development of homeothermy in European shag nestlings has previously been studied by Østnes et al. (2001). They argued that the rapid increase of the European shag nestlings' homeothermic ability during the first 2-3 weeks of development was mainly due to a rapid increase in mass-specific RMR. We have performed experiments with nestling European shags subject to diet restriction (Moe et al. 2004), and the experiments have revealed that the nestlings substantially reduced the mass-specific RMR in response to the diet restriction. Assuming a close coupling between RMR and PMR, as has been demonstrated interspecifically in adult birds (Dutenhoffer and Swanson 1996; Rezende et al. 2002) and intraspecifically in chicks (Bech and Østnes 1999; Konarzewski et al. 2000; Moe et al. in press), a decrease in RMR should be accompanied by a decrease in PMR. Accordingly, one could expect a delayed development of homeothermy in nestlings subject to food shortage.

The functional relationship between RMR and PMR is not fully understood (Hayes and Garland 1995; Ricklefs et al. 1996). Klaassen and Bech (1992) advocated that a coupling between RMR and PMR in birds does not always imply causality. In arctic tern chicks (*Sterna paradisaea*) with varying growth rates, they found that RMR developed in pace with body mass whereas PMR was more dependent on age. This relationship held true if the body mass of the chicks was not lower than 75% of that expected from their age. Accordingly, one could expect a sustained development of homeothermy in nestlings facing food shortage, if their body masses were not below a critical level.

Østnes et al. (2001) also argued that the homeothermic ability of the European shag nestlings was due to a substantial decrease in mass-specific minimal thermal conductance (MTC). Further, they argued that the decrease in MTC probably represented a passive effect of a decrease in the surface-to-volume ratio (causing an increased thermal inertia) rather than an increase in the insulation from growth of a down coating. In contrast, nestling European shags subject to diet restriction seemed to increase in the surface-to-volume ratio (Moe et al. 2004), due to the combination of a rather stable body mass (weight maintenance diet) and a continued structural growth (tarsus, wings and skull). Accordingly, one could expect a delayed development of homeothermy in nestlings facing food shortage.

In this study, we experimentally imposed short-term diet restriction on 12-16 day old nestling European shags, kept under laboratory conditions, to shed light on the relationship between food availability and development of homeothermy during early development. In this context we tested the hypothesis that food shortage delays the development of homeothermy in altricial nestlings.

Materials and methods

Study area and animals

Data were collected during the 2001 breeding season (June and July) on Sklinna, a small group of islands situated ~50 km off the coast of central Norway (65^{o}12'N, 11^{o}00'E). In 2001 the breeding population of the study species, the European shag, consisted of 1750 pairs (N. Røv pers. comm.). We measured T_a in the colony with a temperature logger placed in the shade of a boulder.

Housing conditions, feeding protocols and treatment groups

A sample of 34 nestlings was brought to the laboratory at the age of 12 days (day of hatching termed day 0) for the purpose of metabolic measurements (RMR and PMR). They were kept, 4-8 together, in an enclosure (100 x 50 cm) with a heat lamp providing a constant range of operative temperatures (Bakken 1992) of 22-33^{o}C. We randomly assigned 12 nestlings to a diet-restriction feeding protocol (hereafter 'diet-restricted nestlings') and 22 nestlings to a control group (hereafter 'controls'). Within the controls, 12 nestlings were subject to metabolic measurements at the age of 12 days, whereas 10 nestlings were subject to a control-feeding protocol. The diet-restricted and the control fed nestlings were hand fed with fillets of saithe (*Pollachius virens*) and cod (*Gadus morhua*), because these gadoids constitute 70% of the diet of shags breeding in the study area (Barrett et al. 1990). They were fed for four days, until they were 16 day old and metabolic rates were measured. The diet-restricted nestlings received small portions of food 8-10 times a day to maintain a relatively stable body mass, while, the controls were fed every second hour, allowing them to follow a normal body mass growth trajectory (Fig. 1). The daily food intake and the body mass growth of 16 day old controls were lower compared to that of the 15 day old controls, because of fasting prior to the metabolic measurements.

This sample of nestlings (N=34) is the same sample of nestlings from which the results of RMR are presented in Moe et al. (2004). Consequently, the housing conditions, the feeding protocols and the methods for RMR measurements are also described in details in Moe et al. (2004).

RMR and PMR measurements

The metabolic measurements were performed on postabsorptive nestlings. The lengths of fasting prior to the measurements were 6.4 ± 0.5, 7.3 ± 0.5 and 9.4 ± 0.4 h for 12 day old controls, 16 day old diet-restricted and 16 day old controls, respectively. The longer length of fasting of the latter group was chosen due to presumed higher gut content. Diet-restricted nestlings and controls were randomly measured with respect to time of the day, but RMR showed no diurnal cycle.

O_2 consumption rates were calculated by using formula 1d in Withers (1977), assuming a constant RQ of 0.72 and corrected for wash-out delays in the system by using the method given by Niimi (1978). In this way, we obtained the instantaneous O_2 consumption rates. Values of MR were calculated from the O_2 consumption rates using 5.4611 W as the caloric equivalent for 1 l O_2 h^{-1}, using gas exchange conversion factors from Schmidt-Nielsen (1990). RMR was defined as the lowest MR calculated with 25 min running average during exposure to thermoneutral conditions (29-31°C).

Outside air was dried using silica gel and pumped through a 10-litre temperature controlled metabolic chamber with a flow rate of 3.3 l min^{-1}. The actual flow rates entering the metabolic chamber were measured with a calibrated mass flow controller (Bronkhorst Hi-Tec [Rurlo, Holland], type F-201C-FA-22-V). Excurrent air was again dried, before a fraction of the air was directed to the O_2 analyser (Servomex [Crowborough, East Sussex, UK], type 244A). The O_2 analyser was calibrated with dry atmospheric air (20.95%) and pure stock nitrogen. Any changes from the pre- to the post-experiment readings of the O_2 content in dry atmospheric air, were controlled for by assuming a linear drift. Measurements of the O_2 content in excurrent air (accuracy 0.001%) were recorded, along with the measurements of body and ambient temperatures (T_b and T_a; accuracy 0.1°C), on a data logger (Grant [Cambridge, UK], type Squirrel), at 30 sec intervals.

The metabolic chamber was a water-jacketed vessel connected to a temperature controller (Grant Instruments, type LT D G) that provided control of the T_a in the inner metabolic chamber. T_a was measured with a copper-constantan thermocouple mounted inside the metabolic chamber, and T_b was measured, during the entire metabolic measurement, in the cloaca with a copper-constantan thermocouple (California fine wire, type 0.005) surrounded by a polypropylene tubing (PP 50, Portex Ltd.). Depending on the nestling's size, the thermocouple was inserted 2 to 4 cm into the cloaca and secured with adhesive tape.

The PMR measurements were obtained as a continuation of the RMR measurements. After 3-5 h exposure to thermoneutral conditions, the T_a in the metabolic chamber was lowered at a constant rate of $0.3^{o}C\ min^{-1}$. The nestlings' MR increased with decreasing T_a. After reaching a peak MR, MR and T_b consistently decreased to a further decrease in T_a, and the experiment was terminated. PMR was defined as the highest 10 min running average MR during cold exposure. One PMR measurement was excluded due to it being disturbed during the cold exposure part of the trial. Body masses of the nestlings were weighed, to the nearest 0.1 g, before and immediately after each experiment. A linear decrease in body mass during the experiment was assumed when calculating the body mass at the time when RMR and PMR were obtained. Each individual was only used once in the experiments to obtain independent measurements.

Minimal thermal conductance

The minimal 'wet' thermal conductance (MTC) was calculated according to the method originally described by Scholander et al. (1950). However, this method is only valid when T_b is kept constant. Since the T_b of the nestlings decreased during cold exposure, we had to include a correction factor to account for the fall in T_b (see also Visser and Ricklefs 1993). Thus, the following formula was used to calculate mass-specific minimal thermal conductance:

$$\mathrm{MTC} = (\mathrm{PMR} + \mathrm{A}) / (T_b - T_a) \qquad (1)$$

where A is the correction factor for the decrease in energy content ($W\ kg^{-1}$). The calculation of the correction factor was based on the rate of fall in T_b recorded during the last 10 min period before PMR was attained, and assumed a specific heat of $3.45\ J\ g^{-1}\ {}^{o}C^{-1}$ for the chicks (Hart 1951).

Body Composition

A sample of 28 nestlings were sacrificed immediately after the metabolic measurements and stored at –20°C, for subsequent analysis of body composition. These analyses produced information on organ masses and the LDF of the organs. The results on body composition are partly presented in Moe et al. (2004), and the methods for analysing body condition were described therein. However, in the present paper we focus on the aspects of body composition which relate to thermoregulatory ability.

Index of homeothermy

For the purpose of measuring the index of homeothermy (HI), another sample of 27 nestlings were brought to the laboratory at the age of 12 days. Housing conditions, including feeding protocols and fasting prior to the measurements, were identical to that of the nestlings subject to the metabolic measurements. The HI characterises the degree of homeothermy (Visser 1998), and it was obtained from cooling rates of 12 day old control (N=8), 16 day old diet-restricted (N=12) and 16 day old control (N=7) nestlings. Prior to the measurement of HI the nestlings were kept under a heat lamp to ensure normal *Tb*s, and subsequently, they were placed in a chamber in which T_a was maintained at 10°C (range 9.0-11.4 °C), consistent to the mean T_a of the colony during June (10.2 °C). The T_b of the nestlings and the T_a of the chamber were measured and stored as described above for the metabolic measurements. The nestlings were subject to cooling for 45 min, and the HI was calculated according to the formula (3) from Ricklefs (1987).

$$HI = (T_f - T_a)/(T_i - T_a) \tag{2}$$

where T_f and T_i are final and initial body temperatures, respectively. The HI is equal to 1 if a nestling maintains its T_b throughout the entire cooling trial, and the HI is 0 if the T_b equals the T_a at the end of the cooling trial. After the HI measurements, the nestlings were brought back to their nest of origin or to a nest with foster parents where they were used for another study. In addition, 6 of the 34 nestlings that were subject to metabolic measurements were also brought back to a nest with foster parents.

Statistics

We used a general linear model (GLM) with the type III sum of squares to perform analyses of covariance and variance. We manually excluded insignificant interaction terms, factors or covariates one by one from the null model (ENTER method). All variables were inspected graphically to ensure linearity, and $\log_{10}$ –transformation was used to linearize the variables (MR, body mass, organ mass) prior to examination.

We analysed the relationship between organ mass and MR, as well as the relationship between RMR and PMR (and absolute- and factorial scope) by including body mass as a covariate to remove the effect of body mass (i.e. body mass is held constant; Hayes and Shonkwiler 1996). In order to avoid possible effects of part-whole correlation, we subtracted organ mass from the body mass variable, when organ mass and body mass were included in the same analysis (Christians 1999). Colinearity diagnostics were used to justify that LDF could be included as a covariate (together with body mass and organ mass) in the analyses of the relationship between organ mass and MR (tolerance > 0.3 for all variables).

When two regressions with $\log_{10}$ transformed variables (e.g. metabolic rate on body mass) have the same slope, but have different intercepts, we have calculated the percentage difference between the non-transformed regressions according to formula (4) in Moe et al. (in press). The Bonferroni method was used for post hoc pairwise multiple comparisons (‘Post Hoc’ hereafter). It reports adjusted P-values that have been multiplied with the number of pairs tested. Means are reported with ±1SE. All statistical tests were performed with SPSS v. 11.5.1 (2002).

Results

RMR, PMR and T_b

The RMR was negatively affected by the diet restriction. With respect to body mass, the RMR of the diet-restricted nestlings was 36.5% lower than the controls ($F_{1,31}$=90.0, P<0.001) and scaled to body mass by the power of 0.84 (SE=0.12, $F_{1,31}$=51.2, P<0.001). With respect to age, the mass-specific RMR was 11.6 ± 0.36, 11.1 ± 0.34 and 7.4 ± 0.37 W kg^{-1} for 12 day old controls, 16 day old controls and 16 day old diet-restricted nestlings, respectively (for further details, see Moe et al. 2004).

PMR was substantially affected by the diet restriction. With respect to body mass, the PMR of the diet-restricted nestlings was 16.4% lower than the controls ($F_{1,30}$=11.5, P<0.002; Fig. 2). PMR scaled to body mass by the power of 1.3 (SE=0.13, $F_{1,30}$=99.1, P<0.001) in both groups (RMR x body mass interaction, $F_{1,29}$=0.0, P=0.91, but see figure 2 for separate linear regression equations). With respect to age, the mass-specific PMR was 14.5 ± 0.5, 17.2 ± 0.4 and 13.0 ± 0.7 W kg^{-1} for 12 day old controls, 16 day old controls and 16 day old diet-restricted nestlings, respectively.

Diet-restricted nestlings exhibited a lower T_b compared to 16 day old controls during thermoneutral conditions at RMR (Post Hoc, P<0.001, Fig. 3A) and during the cooling phase at PMR (P<0.001, Fig. 3A). However, they obtained PMR at the same T_a (P=1.0, Fig. 3B).

Index of homeothermy (HI)

HI was measured in a different sample of nestlings (N=27) than the sample of nestlings (N=34) subject to the metabolic measurements (see methods). HI increased with body mass ($F_{1,24}$=30.8, P<0.001, Fig. 4A) and age ($F_{2,24}$=6.7, P<0.005, Fig. 4B). The effect of diet restriction on HI contrasted to that on PMR. With respect to body mass, the diet-restricted nestlings tended to exhibit a higher degree of homeothermy compared to the controls ($F_{1,24}$=64.2, P=0.052, Fig. 4A). The slopes of the regressions of HI on body mass were not significantly different (HI x body mass interaction, $F_{1,23}$=1.0, P=0.32). With respect to age, Post Hoc tests showed that the diet-restricted nestlings exhibited a HI (0.79 ± 0.02) not significantly different (P>0.1) to 16 day old controls (0.84 ± 0.02), but they tended to exhibit a higher HI (P=0.08) compared to 12 day old controls (0.72 ± 0.02).

Minimal thermal conductance (MTC)

Mass-specific minimal conductance (MTC), calculated from the PMR measurements, decreased with body mass ($F_{1,27}$=12.1, P<0.002, Fig. 5A) and age ($F_{1,27}$=11.5, P<0.001, Fig. 5B). The slopes of the regressions of MTC on body mass were not significantly different ($F_{1,26}$=2.3, P=0.14). With respect to body mass, the diet-restricted nestlings showed a 31% lower MTC compared to controls ($F_{1,27}$=11.2, P<0.002). With respect to age, the MTC was not different between controls and diet-restricted nestlings (Post Hoc, P=1.0).

Metabolic scope

The absolute scope (PMR minus RMR, i.e. the portion of PMR available for regulatory thermogenesis) scaled to body mass by the power of 2.5 (SE=0.3, $F_{1,30}$=55.5, P<0.001, Fig. 6A) in both groups (interaction, $F_{1,29}$=0.0, P=0.91, 6A). With respect to body mass, the diet-restricted nestlings exhibited a 57% higher absolute scope compared to the controls ($F_{1,30}$=10.2, P<0.003). The allometric scaling exponent of 2.5 demonstrates a considerable increase in mass-specific absolute scope as a function of body mass. From Fig. 6B it is evident that this relationship was an effect of age. Mass-specific absolute scope increased from 2.7 ± 0.3 to 5.6 ± 0.3 W kg^{-1} in 12 and 16 day old controls (Post Hoc, P<0.001, Fig. 6B), and the mass-specific absolute scope was not significantly different between 16 day old controls and diet-restricted nestlings (P=1.0).

The factorial scope (PMR/RMR) increased with age ($F_{2,30}$=29.6, P<0.001), and the diet-restricted nestlings exhibited a higher factorial scope (1.75) compared to that of the 16 day old controls (1.56; Post Hoc, P<0.05) and the 12 day old controls (1.25; Post Hoc, P<0.001).

Body composition and organ maturation

With respect to body mass, the pectoral muscle mass ($F_{1,25}$=19.9, P<0.001, Fig. 7A) and the heart mass ($F_{1,25}$=18.2, P<0.001, Fig. 7C) of the diet-restricted nestlings was 18.9 and 17.4 % lower compared to that of the controls, respectively. In addition, the leg muscle mass tended to be slightly lower in diet-restricted nestlings compared to controls ($F_{1,25}$=3.8, P=0.06, Fig. 7B). The total lipid mass, the liver mass, the gizzard mass and the kidney mass was also negatively affected by the diet-restriction, while the intestine mass was strictly maintained with respect to body mass (see Moe et al. 2004).

The lean dry fraction (LDF) increased significantly with age in all organs (e.g. pectoral and leg, $F_{1.25}$>22.3, P<0.001, Fig. 7D,E) except for the liver, the intestine and the heart (e.g. heart, $F_{1.25}$=0.9, P=0.39, Fig. 7F), indicating that the latter organs had already reached a high degree of functional maturation. The lean dry fraction (LDF) was not different between 16 day old diet-restricted and 16 day old controls in any organ or muscles (e.g. pectoral muscles, leg muscles and heart, Post Hoc, P>0.1, Fig. 7D,E,F), except for the intestine. The LDF of the intestine was lower in diet-restricted nestlings compared to controls (Post Hoc, P<0.05).

Relationships between RMR, PMR, absolute and factorial scope

We tested for any relationship between RMR and PMR. RMR was a significant predictor of PMR ($F_{1,28}$=39.8, P<0.001), but the positive correlation between RMR and PMR was stronger in the diet-restricted nestlings compared to the controls (interaction, $F_{1,28}$=5.2, P<0.05). In contrast, RMR was not related to absolute scope ($F_{1,29}$=0.0, P=0.87, body mass and treatment were significant covariate and factor, respectively). Factorial scope was positively related to PMR within both treatment groups ($F_{1,29}$=7.0, P<0.05). Factorial scope related differently to RMR between the treatment groups (interaction, $F_{1,28}$=6.1, P<0.05). It was negatively related to RMR within the controls (r=-0.47, $F_{1,19}$=5.4, P<0.05), while no significant correlation existed for the diet-restricted nestlings (r=0.04, $F_{1,8}$=0.0, P=0.90).

Correlations between organ mass, LDF and metabolic rate

The diet restriction had a substantial effect on body composition. In order to evaluate whether changes in body composition could explain any of the differences in metabolic rates between the treatment groups, we tested whether organ masses correlated with RMR, PMR, absolute scope or factorial scope. In these analyses, we controlled for organ LDF, body mass (minus organ mass) and treatment by including them in the models.

The mass of the liver, the pectoral muscle mass and the lipid mass were significant predictors of RMR ($r>0.4$, $P<0.05$, Table 1). It might be expected that the same organs also correlated to PMR, as RMR was a major predictor of PMR. However, for these organs and for the heart mass, the interaction term (with treatment) was significant, and the organ masses and the intestine length positively correlated to PMR in the diet-restricted nestlings only (Table 1). No main effects of organ masses were significant predictors of absolute or factorial scope, but the leg mass tended to be significantly correlated to absolute and factorial scope ($r>0.37$, $P=0.07$, Tabel 1). The interaction term (with treatment) was significant for the liver mass and the lipid mass, and these variables correlated negatively to factorial scope in the controls only. In addition, the intestine length was positively correlated to factorial scope in the diet-restricted nestlings only.

We did not reveal any significant statistical relationships between the LDF of the thermoregulatory effector organs (e.i., the leg muscles, the pectoral muscles and the heart) and PMR, absolute or factorial scope. However, for the LDF of the leg muscles, the results depended on how we specified the final model in the GLM analysis. If we excluded treatment from the final model, the LDF of the leg muscles tended to be a significant predictor of absolute scope ($F_{1,23}=4.3$, $P=0.051$). Treatment was not a significant factor in the final model ($F_{1,22}=0.9$, $P=0.36$), but the fit of the final model was slightly better when treatment was included ($r^2=0.75$ vs $r^2=0.74$). However, the relationships between LDF of organs and absolute scope seem to depend on age. We specified a model, in which only the 16 day old nestlings were included. This model indicated that absolute scope was positively related to the LDF of the leg muscles

($F_{1,13}$=4.3, P=0.059), the leg muscle mass ($F_{1,13}$=3.9, P=0.071), treatment ($F_{1,13}$=4.4, P=0.057) and body mass ($F_{1,13}$=5.1, $P<0.05$).

Discussion

Food shortage and homeothermic abilities

The diet-restricted nestlings exhibited a lower PMR compared to that of the controls, with respect to body mass and age. The low PMR corresponded with a low RMR and T_b. In a study of ducklings, Moe et al. (in press) also found that PMR was inferior in ducklings subject to food shortage compared to that of *ad libitum* fed ducklings.

The absolute metabolic scope was substantially higher in diet-restricted nestlings compared to the controls with respect to body mass (Fig. 6a), and the mass-specific absolute scope did not differ between the treatments with respect to age (Fig. 6b). This indicates that the capacity for heat production at low T_a improved along with age in diet-restricted nestlings.

The mass-specific minimal thermal conductance (MTC) improved during the period of diet restriction despite an apparent increase in the surface-to-volume ratio in the diet-restricted nestlings (Moe et al. 2004), and MTC was not different between 16 day old diet-restricted nestlings and controls. Consequently, the improved MTC must have occurred as a result of growth of down or improved vasomotor control or both. We observed, but did not measure, that the thickness of the down coating grew and that it did not seem to differ between controls and diet-restricted nestlings. It is, however, difficult to assess whether the growth of the down coating was sufficient, alone, to account for the improvement in MTC.

Despite a substantial reduction in PMR, the food shortage did not significantly delay the development of homeothermy in the European Shag nestlings. With respect to body mass, the diet-restricted nestlings tended to exhibit a higher index of homeothermy (HI) compared to controls, and with respect to age, the HI was not significantly different between 16 day old diet-restricted nestlings and controls. To our knowledge, this is the first study to measure the index of homeothermy in nestlings subject to food shortage. Consequently, comparative data is not available.

We interpret the lower PMR as a consequence of the lower RMR and T_b, rather than a consequence of reduced thermogenic capacity. Moe et al. (in press) also found that the low PMR of diet-restricted ducklings corresponded with a low RMR and T_b, and argued that a lowered RMR rather than any decreased function of the mechanisms underlying regulatory thermogenesis caused the reduced PMR. RMR constitutes a major part of PMR in young shag nestlings, and in the present study we found that RMR was a significant predictor of PMR. The idea of Klaassen and Bech (1992) that RMR and PMR are uncoupled in chicks with body masses deviating from the normal growth trajectory, does not seem to apply to European shag nestlings. The strong correlation between RMR and PMR (after controlling for body mass) which we found within the controls as well as the diet-restricted nestlings, is not consistent with that view.

The nestlings obtained PMR at a lower T_b compared to the T_b at RMR, and this is a common feature of conventional cold-induced PMR measurements on young chicks. Ricklefs and Williams (2003) argued that the measured PMR does not represent the true thermogenic capacity of a chick, if it is measured at a low T_b. According to a suggested procedure of Ricklefs and Williams (2003), we used the simultaneous measurement of T_b to adjust every single value of MR during the entire cooling trail to calculate an adjusted PMR. This PMR was adjusted to a high reference T_b (40°C) with a Q_{10} of 2. This calculation showed that diet-restricted nestlings exhibited the same adjusted PMR as controls with respect to body mass. This finding supports the view that the low PMR in the diet-restricted nestlings was a consequence of the low RMR and T_b , rather than a consequence of a reduced thermogenic capacity.

Body composition, organ maturation and thermoregulation

The size of the pectoral muscles was significantly negatively affected by the diet-restriction, and the leg muscles tended to be slightly smaller in diet-restricted nestlings compared to controls of the same body mass. Hence, one could expect the thermogenic capacity also to be negatively affected in the diet-restricted nestlings (Hohtola and Visser 1998; Chappel et al. 1999). However, it has been shown for neonates that muscles are not capable of shivering thermogenesis unless their LDF is higher than 0.15 (Ricklefs and Webb 1985; Dietz et al. 1997), and the pectoral muscles are not regarded to participate significantly in shivering thermogenesis until the European Shag nestlings are about 21 day old (Østnes et al. 2001). Therefore, a reduction in the mass of the pectoral muscles should not be crucial for thermogenic capacity in the diet-restricted nestlings. The mass of the leg muscles, on the other hand, should be expected to be important. A positive relationship between the mass of the leg muscles and PMR has been found in ducklings (Moe et al. in press) and chickens (Konarzewski et al. 2000), but we found no such relationship in the present study. As the ability to show a metabolic response to a low T_a is poor in young altricial birds (i.e. low factorial scope), RMR rather than regulatory thermogenesis will constitute the major part of PMR. Consequently, the thermoregulatory effector organs should rather show a relationship to absolute scope or factorial scope in young altricial birds. In the present study, the mass of the leg muscles tended to correlate positively to absolute and factorial scope. However, despite the slight reduction in leg muscle mass with respect to body mass in the diet-restricted nestlings (Fig. 7B), the absolute scope or the factorial scope was not negatively affected of the diet restriction.

The LDF of the leg muscles increased in line with that of the controls during the time of diet restriction (Fig. 7E). The capacity for regulatory heat production during low T_a has been found to correlate with functional maturity of the skeletal muscles in altricial and precocial chicks (Ricklefs and Webb 1985; Choi et al. 1993; Dietz et al. 1997). Accordingly, we believe an increase in functional maturity of the leg muscles probably resulted in the high absolute scope of the diet-restricted nestlings.

The present study partly supports the idea of Klaassen and Bech (1992) that '*the maturation of the thermoregulatory system proceeds steadily with time even when body mass lags behind*'. Our results on the development of the LDF of the leg muscles and on the development of the mass-specific absolute scope are consistent with their view. However, their idea was based on the finding that PMR was more dependent on age and less dependent on body mass in Arctic tern chicks with varying growth rates. Rather than PMR per se, we suggest that the mass-specific absolute scope, i.e. the portion of PMR available for regulatory thermogenesis, proceeds steadily with time even body mass lags behind. Results on diet-restricted ducklings support this view (Moe et al. in press). However, the thermoregulatory responses to food shortage could also depend on species-specific developmental priorities, but available comparative data is scarce.

Differences in growth and maintenance of different body components may be due to a competition for nutrients between various growing tissues (O'Connor 1977;

Sedinger 1986). Resources should be allocated to those tissues where they are most needed. During food shortage, the growth and maturation of the brain is reported to be strictly maintained (Schew 1995), and the lean dry mass of the heart is reported to be maintained in proportion to body mass (Schew 1995; Moe et al. in press). Muscle tissue could serve as a crucial source of essential amino acids and energy for maintenance in nestlings during food shortage. However, drawing on stores of amino acids may negatively influence the function of a muscle. Surprisingly, we found that the mass of the heart of the diet-restricted European shag nestlings was 17% lower compared to controls with the same body mass. This corresponded with a substantial mass loss of the pectoral muscles (and all visceral organs except the intestine mass), while the mass of the leg muscles only tended to be lower in diet-restricted nestlings compared to controls. This indicates a high developmental priority of the leg muscles. If the leg muscles (peripheral organ), rather than the heart (central organ), limit maximum heat production in European shag nestlings, this result suggest that thermoregulatory ability was given a high developmental priority. However, it could also indicate a high priority of locomotor ability and competitive ability for sibling competition (Brzek and Konarzewski 2001).

Ecological correlates

The time and energy budgets of chicks and parents are interrelated (Beintema and Visser 1989; Coulson and Johnson 1993; Farner 2000; Moe et al. 2002). The development of homeothermy in the nestlings is a prerequisite for the parents to go on long foraging trips and leave the nestlings alone in the nest (Clark 1984; Tveraa and Christensen 2002). During food shortage, time needed for successful foraging increases. If food shortage delays the development of homeothermy in the nestlings, the need for continued brooding would constrain foraging time of the parents. In contrast, continued development of homeothermy would lessen the constraints on the time for foraging of the parents. The mean T_a was 10°C (range 6 to 18°C) in the shag colony in June, and the climate was typically windy and rainy. Continued development of homeothermy even during food shortage seems particularly adaptive for nestlings inhabiting harsh environments. Another explanation, though not mutually exclusive to the above, is that the observed pattern of continued development of homeothermy may be a typical pattern for seabird species receiving regular feeds with less probability of facing protracted fasting (e.g. inshore feeders). In species receiving more irregular feeds (e.g. pelagic feeders), periods of protracted fasting might induce substantial hypothermia of unattended nestlings (e.g. Boersma 1986). However, Bech et al. (1991) and Weathers et al. (2000) found no evidence that nestling Antarctic fulmarine petrels normally experience hypothermia in the field, and suggested that the cold climate precludes these species from using substantial hypothermia. More studies are needed to assess whether inshore and pelagic feeders exhibit different adaptive patterns of development of homeothermy and whether the prevailing climatic conditions interact with these patterns.

In conclusion, we have shown that short-term food shortage did not significantly delay the development of homeothermy in the European shag nestlings. The PMR was negatively affected by the food shortage, but the mass-specific absolute scope (i.e. the portion of PMR available for regulatory thermogenesis) and minimal thermal conductance were improved during the period of food shortage. However, the duration and the magnitude of the food shortage should be a crucial factor. The nestlings in the present study seemed to be close to a physiological limit for keeping up the development of homeothermy. The functional capacity of the organs most important for regulatory thermogenesis could fail during a longer and a more severe food shortage.

Acknowledgements The Norwegian Directorate for Nature Management, Trondheim, gave us permission to work in the shag colony. The National Committee for Animal Research in Norway (‘Forsøksdyrutvalget’) approved the experimental protocols. We are indebted to the Norwegian lighthouse authorities for permission to use the facilities on Sklinna, the lighthouse keepers on Sklinna for their hospitality. We thank Carolyn Baggerud for improving the English and J. Matthias Starck as well as three anonymous referees for providing helpful comments on an earlier version of this paper. CB and BM were supported by a grant from the Research Council of Norway (138698/410).

References

Amundsen T, Stokland JN (1988) Adaptive significance of asynchronous hatching in the shag- a test of the brood reduction hypothesis. J Anim Ecol 57: 329-344

Bakken GS (1992) Measurement and application of operative and standard operative temperatures in ecology. Am Zool 32: 194-216

Barrett RT, Røv N, Loen J, Montevecchi WA (1990) Diet of Shags *Phalacrocorax aristotelis* and Cormorants *P. carbo* in Norway and possible implications for gadoid stock recruitment. Mar Ecol Prog Ser 66: 205-218

Bech C, Østnes JE (1999) Influence of body composition on the metabolic rate of nestling European shags (*Phalacrocorax aristotelis*). J Comp Physiol B 169: 263-270

Bech C, Mehlum F, Haftorn S (1991) Thermoregulatory abilities of nestlings of the Antarctic petrel (*Thalassoica antarctica*). Polar Biol 11: 233-238

Beintema AJ, Visser GH (1989) The effects of weather on time budgets and development of chicks of meadow birds. Ardea 77: 181-192

Boersma PD (1986) Body temperature, torpor, and growth of chicks of fork-tailed storm petrels (*Oceanodroma furcata*). Physiol Zool 59: 10-19

Brzek P, Konarzewski M (2001) Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. J Exp Biol 204: 3065-3074

Chappell MA, Bech C, Buttemer WA (1999) The relationship of central and peripheral organ masses to aerobic performance variation in House sparrows. J Exp Biol 202: 2269-2279

Choi IH, Ricklefs RE, Shea RE (1993) Skeletal muscle growth, enzyme activities, and the development of thermogenesis: A comparison between altricial and precocial birds. Physiol Zool 66: 455-473

Christians JK (1999) Controlling for body mass effects: is part-whole correlation important? Physiol Biochem Zool 72: 250-253

Clark L (1984) Consequences of homeothermic capacity of nestlings on parental care in the European starling. Oecologia 65: 387-393

Coulson JC, Johnson MP (1993) The attendance and absence of adult kittiwakes Rissa tridactyla from the nest site during the chick stage. Ibis 135: 372-378

Dietz MW, Van Mourik S, Tøien Ø, Koolmees PA, Tersteeg-Zijderveld, MHG (1997) Participation of breast and leg muscles in shivering thermogenesis in young turkeys and Guinea Fowl. J Comp Physiol B 167: 451-460

Dutenhoffer MS, Swanson DL (1996) Relationship of basal to summit metabolic rate in passerine birds and the aerobic capacity model for the evolution of endothermy. Physiol Zool 69: 1232-1254

Farner CG (2000) Parental care: The key to understanding endothermy and other convergent features in birds and mammals. Am Nat 155: 326-334

Hart JS (1951) Calorimetric determination of average body temperature of small mammals and its variation with environmental conditions. Can J Zool 29: 224-233

Hayes JP, Garland T (1995) The evolution of endothermy: testing the aerobic capacity model. Evolution 49: 836-847

Hayes JP, Shonkwiler JS (1996) Analyzing mass-independent data. Physiol Zool 69: 974-980

Hohtola E, Visser GH (1998) Development of locomotion and endothermy in altricial and precocial birds. In: Starck JM, Ricklefs RE (eds) Avian Growth and Development. Oxford University Press, New York, pp 157-173

Kitaysky AS (1999) Metabolic and developmental responses of alcid chicks to experimental variation in food intake. Physiol Biochem Zool 72: 462-473

Klaassen M, Bech C (1992) Resting and peak metabolic rates of Arctic tern nestlings and their relations to growth rate. Physiol Zool 65: 803-814

Konarzewski M, Starck JM (2000) Effects of food shortage and oversupply on energy utilization, histology and function of the gut in nestling Song thrushes (*Turdus philomelos*). Physiol Biochem Zool 73: 416-427

Konarzewski M, Gavin A, McDevitt R, Wallis IR (2000) Metabolic and organ mass responses to selection for high growth rates in the domestic chicken (*Gallus domesticus*). Physiol Biochem Zool 73: 237-248

Martin TE (1987) Food as a limit on breeding birds: A life-history perspective. Ann Rev Ecol Syst 18: 453-487

Moe B, Langseth I, Fyhn M, Bech C (2002) Changes in body condition in breeding Kittiwakes *Rissa tridactyla*. J Avian Biol 33: 225-234

Moe B, Stølevik E, Bech C (in press) Ducklings exhibit substantial energy saving mechanisms as a response to short-term food shortage. Physiol Biochem Zool

Moe B, Brunvoll S, Mork D, Brobakk TE, Bech C (2004) Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*). J Exp Biol 207: 4067-4076

Niimi AJ (1978) Lag adjustments between estimated and actual physiological responses conducted in flow-through systems. J Fish Res Board Can 35: 1265-1269

O'Connor RJ (1977) Differential growth and body composition in altricial passerines. Ibis 119: 147-166

Østnes JE, Jenssen BM, Bech C (2001) Growth and development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*). Auk 118: 983-995

Rezende EL, Swanson DL, Novoa FF, Bozinovic F (2002) Passerines versus nonpasserines: so far, no statistical differences in the scaling of avian energetics. J Exp Biol 205: 101-107

Ricklefs RE (1987) Characterizing the development of homeothermy by rate of body cooling. Funct Ecol 1: 151-157

Ricklefs RE, Webb T (1985) Water content, thermogenesis, and growth rate of skeletal muscles in European starling. Auk 102: 369-376

Ricklefs RE, Williams JB (2003) Metabolic responses of shorebird chicks to cold stress: hysteresis of cooling and warming phases. J Exp Biol 206: 2883-2893

Ricklefs RE, Konarzewski M, Daan S (1996) The relationship between basal metabolic rate and daily energy expenditure in birds and mammals. Am Nat 147: 1047-1071

Schew WA (1995) The evolutionary significance of developmental plasticity in growing birds. PhD Thesis, University of Pennsylvania, Philadelphia

Schew WA, Ricklefs RE (1998) Developmental plasticity. In: Starck JM, Ricklefs RE (eds) Avian Growth and Development. Oxford University Press, New York, pp 288-304

Schlichting CD, Pigliucci M (1998) Phenotypic Evolution. A Reaction Norm Perspective. Sinauer Associates, Sunderland

Schmidt-Nielsen K (1990) *Animal physiology: Adaptation and environment.* Cambridge University Press, Cambridge

Scholander PF, Hock R, Walters V, Johnson F, Irving L (1950) Heat regulation in some arctic and tropical mammals and birds. Biol Bull 99: 237-258

Sedinger JS (1986) Growth and development of Canada Goose goslings. Condor 88: 169-180

SPSS (2002) SPSS for Windows. Release 11.5.1. SPSS Inc, Chicago

Tveraa T, Christensen, GN (2002) Body condition and parental decisions in the Snow Petrel (*Pagodroma nivea*). Auk 119: 266-270

Velando A, Ortega-Ruano JE, Freire J (1999) Chick mortality in European shag *Stictocarbo aristotelis* related to food limitations during adverse weather events. Ardea 87: 51-59

Velando A, Graves J, Freire J (2000) Sex-specific growth in the European shag *Strictocarbo aristotelis*, a sexual dimorphic seabird. Ardea 88: 127-136

Visser GH (1998) Development of temperature regulation. In: Starck JM, Ricklefs RE (eds) Avian Growth and Development. Oxford University Press, New York, pp 117-156

Visser GH, Ricklefs RE (1993) Temperature regulation in neonates of shorebirds. Auk 110: 445-457

Weathers WW, Gerhart KL, Hodum PJ (2000) Thermoregulation in Antarctic fulmarine petrels. J Comp Physiol B 170: 561-572

Withers PC (1977) Measurement of VO_2, VCO_2 and evaporative water loss with flow-through mask. J Appl Physiol 42: 120-123

Table 1 Correlations (r values) between lean dry organ mass and metabolic performance in controls and diet-restricted European shag nestlings. (RMR resting metabolic rate, PMR peak metabolic rate)

	RMR	PMR	Absolute scope	Factorial scope
Pectoral	**0.50****	[a)]	0.28	0.04
Leg	-0.05	0.29	**0.37 (0.07)**	**0.38 (0.07)**
Heart	0.17	[a)]	0.05	-0.08
Liver	**0.64*****	[a)]	0.03	[b)]
Gizzard	-0.26	-0.20	-0.13	0.12
Kidney	0.09	-0.05	0.05	-0.13
Intestine	0.25	0.27	0.08	0.03
Lipid	**0.44***	[a)]	-0.09	[b)]

* P<0.05, ** P<0.01, *** P<0.001

For cells with 0.1>P>0.05, the P level is given in parentheses.

Separate GLM analyses were performed for each organ and each dependent variable (RMR, PMR, Absolute scope and Factorial scope). The null models included organ mass, lean dry fraction (LDF) and lean dry body mass (minus organ mass) as covariates, treatment as factor and the interactions organ mass x treatment and LDF x treatment.

[a)] Significant interaction between treatment and organ mass. Significant positive correlation between organ mass and PMR in diet-restricted nestlings only.

[b)] Significant interaction between treatment and organ mass. Significant negative correlation between organ mass and F scope in controls only.

Figure legends

Figure 1. Daily food intake (A) and body mass (B) as a function of age in controls (black bars and closed symbols) and diet-restricted nestlings (white bars and open symbols) of European shags kept in the laboratory. The regression line of a logistic growth curve calculated from 1645 body mass measurements of nestlings fed by their parents in the colony is shown for comparison in B. Food intake is given as fresh weight of gadoid fish fillets in grams per day. Error bars are 1SE.

Figure 2. Peak metabolic rate (PMR) as a function of body mass (BM) in controls (closed symbols) and diet-restricted nestlings (open symbols) of European shags. The axes are log-scaled, and linear regression lines are shown for each treatment group. (log PMR controls = 1.30 (± 0.12) log BM - 2.56 (± 0.30), log PMR diet-restricted = 1.34 (± 0.45) log BM – 2.74 (± 1.15))

Figure 3. Body temperature (T_b) at resting metabolic rate (upward triangles, A) and at peak metabolic rate (downward triangles, A) and ambient temperature (T_a) at peak metabolic rate (B) as a function of age in controls (closed symbols) and diet-restricted nestlings (open symbols) of European shags. Error bars are 1SE.

Figure 4. Index of homeothermy (HI) as a function of body mass (A) and age (B) in controls (closed symbols) and diet-restricted nestlings (open symbols) of European shags. The axes are log-scaled in A and B, and the linear regression lines are shown for each treatment group in A. Error bars are 1SE.

Figure 5. Minimal thermal conductance (MTC) as a function of body mass (A) and age (B) in controls (closed symbols) and diet-restricted nestlings (open symbols) of European shags. The axes are log-scaled in A and B, and the linear regression lines are shown for each treatment group in A. Minimal thermal conductance is given in watts per kilo per degree Celsius. Error bars are 1SE.

Figure 6. Absolute scope (W) in relation to body mass (A) and mass-specific absolute scope (W kg^{-1}) in relation to age (B) in controls (filled symbols) and diet-restricted nestlings (open symbols) of European shags. Absolute scope was calculated as PMR-RMR. The axes in A are log scaled and linear regression lines are shown for each treatment group. Error bars are 1SE.

Figure 7. The relationship of lean dry (Ld) pectoral muscle mass (A), leg muscle mass (B) and heart mass (C) to lean dry (Ld) body mass, and the relationship of lean dry fraction (LDF) of the pectoral muscles (D), the leg muscles (E) and the heart (F) to age in controls (filled symbols) and diet-restricted nestlings (open symbols) of European shags. The axes are log-scaled, and the linear regression lines are shown for each treatment group in A,B,C. LDF was calculated as lipid free dry organ mass/lipid free fresh organ mass. Error bars are 1SE.

Figure 1.

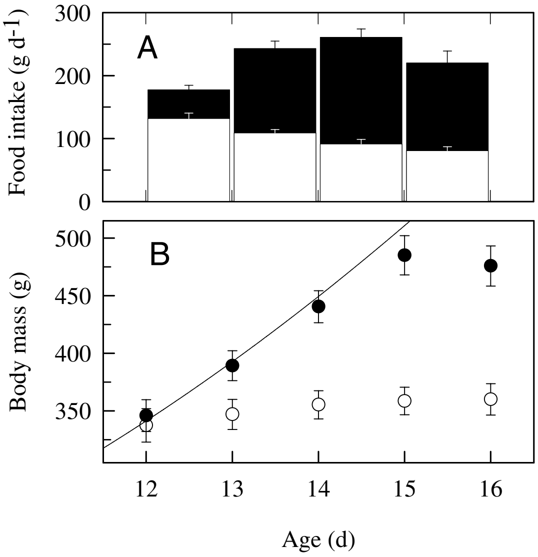

A
B
Food intake (g d-1)
0
100
200
300
Body mass (g)
350
400
450
500
12
13
14
15
16
Age (d)

Figure 2.

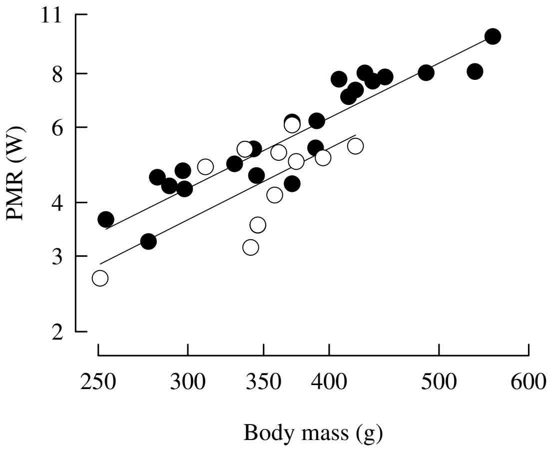
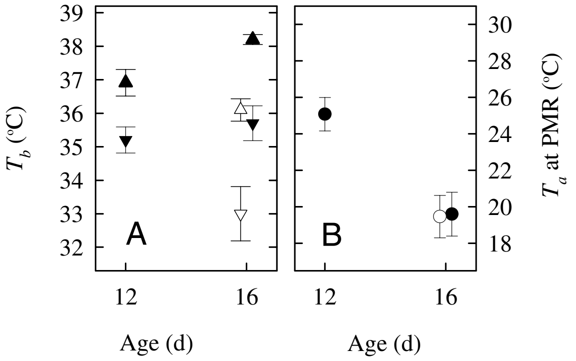


Figure 3.



T_b (°C)
39
38
37
36
35
34
33
32
A
12
16
Age (d)
B
12
16
Age (d)
30
28
26
24
22
20
18
T_a at PMR (°C)

Figure 4.

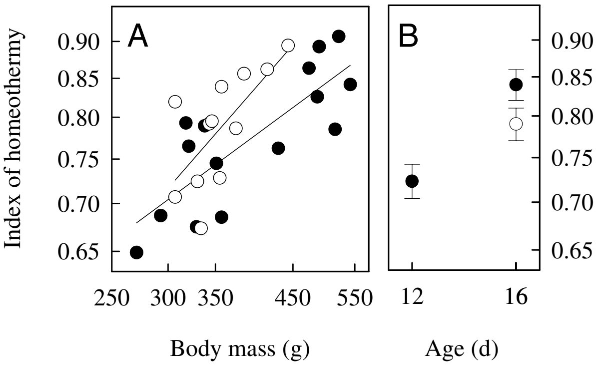

A
B
Index of homeothermy
0.90
0.85
0.80
0.75
0.70
0.65
250
300
350
450
550
Body mass (g)
12
16
Age (d)

Figure 5.

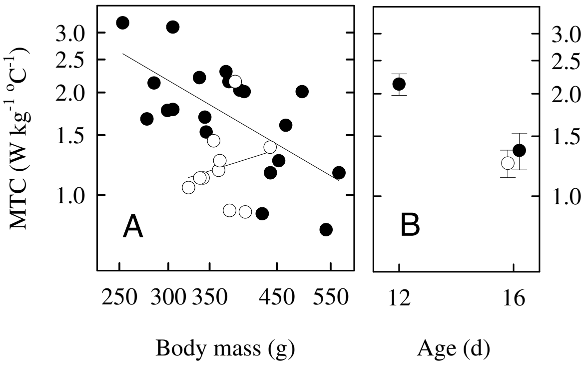

MTC (W kg-1 °C-1)
3.0
2.5
2.0
1.5
1.0
A
B
250
300
350
450
550
12
16
Body mass (g)
Age (d)

Figure 6.

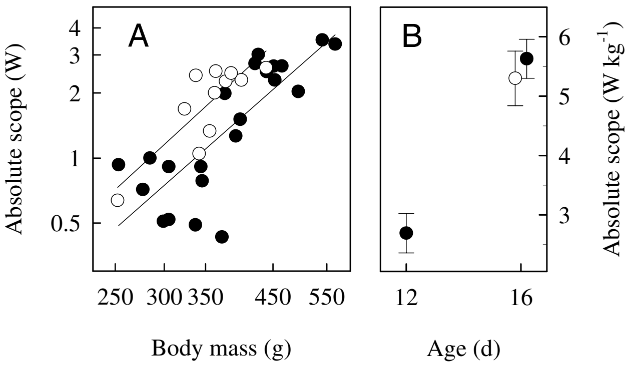
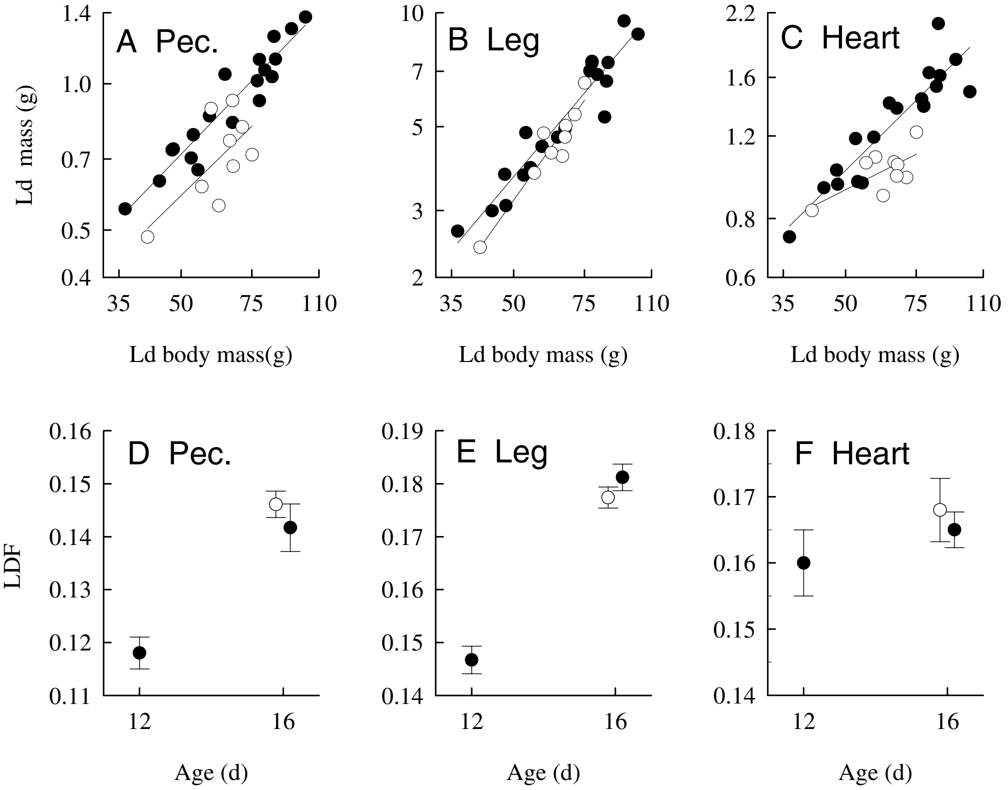

A
B
Absolute scope (W)
4
3
2
1
0.5
250
300
350
450
550
Body mass (g)
12
16
Age (d)
6
5
4
3
Absolute scope (W kg^{-1})

Figure 7.


A Pec.
B Leg
C Heart
D Pec.
E Leg
F Heart
Ld mass (g)
1.4
1.0
0.7
0.5
0.4
10
7
5
3
2
2.2
1.6
1.2
0.8
0.6
35
50
75
110
Ld body mass(g)
Ld body mass (g)
Ld body mass (g)
LDF
0.16
0.15
0.14
0.13
0.12
0.11
0.19
0.18
0.17
0.16
0.15
0.14
0.18
0.17
0.16
0.15
0.14
12
16
Age (d)
Age (d)
Age (d)

Paper IV

Journal of Animal Ecology (submitted manuscript)

The effect of short-term food shortage on subsequent growth and fledging body size of European shag nestlings

Running title: Effect of food shortage on growth of shag nestlings

by
Børge Moe, Siri Brunvoll, Daniel Mork, Trond Einar Brobakk and Claus Bech
Department of Biology
Norwegian University of Science and Technology
NO-7491 Trondheim, Norway

Address for correspondence and proof:
Børge Moe
Department of Biology
Norwegian University of Science and Technology
NO-7491 Trondheim, Norway
Phone: +47 73 59 60 30
Fax: +47 73 59 13 09
E-mail: Borge.Moe@bio.ntnu.no

Keywords: developmental plasticity, development, diet restriction,

Phalacrocorax aristotelis, seabirds

Summary

1. Little is known about the effect of short-term and transient food shortage on growth and subsequent fledging body size in seabirds. This information is important to understand how seabirds are adapted to a stochastic environment. The ontogenetic responses to variability in food availability are potentially important for the fitness prospect of individuals.

2. We examined the developmental plasticity of European shag (*Phalacrocorax aristotelis*) nestlings by subjecting them to short-term food shortage (diet restriction) in the laboratory between 12-16 days of age and realimentation (subsequent provisioning) in the wild (up to 48 days of age) or overfeeding in the laboratory between 16-24 days of age.

3. During the food shortage period, body mass remained rather stable while structural elements (skull, tarsus, wings) continued to grow at high rates.

4. Although different statistical approaches provided slightly contrasting results, body mass and structural elements of diet-restricted nestlings seemed to grow according to a parallel trajectory compared to controls. The European shag nestlings, thus, showed limited capacity for accelerated growth, indicating that growth during natural conditions is close to the limits set by physiological or anatomical constraints. As overfed nestlings did not grow at faster rates than nestlings in the wild, food quantity did not seem to limit growth of the nestlings during realimentation in the wild.

5. Diet-restricted nestlings extended developmental time to compensate for the early growth perturbation. However, for the structural elements, the perturbation was not severe, because the structural elements grew at high rates during the period of diet-restriction.

6. Fledging body size was similar between controls and diet-restricted nestlings, suggesting that variation in fledging body size does not seem to arise from short-term and transient food shortage during early growth of European shags.

Introduction

The stochastic nature of prey availability within the marine environment frequently provides periods of food shortage for breeding seabirds (Ashmole 1971; Suryan *et al.* 2002). It is well documented that chronic food shortage has a devastating effect on growth and survival in cohorts of seabird nestlings (Barrett *et al.* 1987; Monaghan *et al.* 1989; Anderson 1989; Chastel, Weimerskirch & Jouventin 1993; Harris & Wanless 1997), but little is known about the effect of short and transient food shortage within a breeding season on growth and subsequent fledging body size in seabird nestlings. Even small deviations from normal growth might be expected to produce fitness consequences (Gebhardt-Henrich & Richner 1998). Consequently, the ontogenetic responses to variation in food availability are potentially important for the fitness prospects of individuals. In periods where the parents can not adequately provision their young, the phenotypic developmental trajectory of the young may deviate from normal ontogenetic development. Such phenotypic changes are known as developmental plasticity (Schmalhausen 1949; Smith-Gill 1983; Schew & Ricklefs 1998; Schlichting & Pigliucci 1998).

Whether short-term and transient food shortage during growth has an effect on subsequent fledging body size, depends on the developmental responses during 1) the food shortage period and 2) during the realimentation period (i.e. subsequent provisioning).

In the face of temporary food shortage, avian nestlings may use one of three 'strategies' of resource allocation (Schew & Ricklefs 1998). *First*, the chicks may simply not response at all. They will consequently maintain the growth rate and the rate of maturation of the organs, muscles etc. at the maximum possible rate, which is allowed with the available food resources and the amount of energy stored at the onset of the food-deprivation period (e.g. Konarzewski *et al.* 1996; Konarzewski & Starck 2000). *Secondly*, chicks may selectively allocate energy to growth and development of such body parts as wings and head, which are important for nestling and post-fledging survival (e.g. Øyan & Anker-Nilssen 1996; Kitaysky 1999; Moe *et al.* in press). *Thirdly*, the chicks may react to a temporary food shortage by an overall arrest of growth and maturation and resume these processes when conditions improve (e.g. Emlen *et al*. 1991; Schew 1995; Starck & Chinsamy 2002).

During realimentation, growth may follow three different trajectories. That is accelerated growth (*catch up growth* sensu Bohman 1955), parallel growth or retarded growth, which refers to whether the growth rate is faster, similar or slower compared to normal growth relative to chronological age. Among birds, accelerated growth has only been reported for body mass growth (e.g. Schew 1995; Negro, Chastin & Bird 1994), whereas parallel growth (e.g. Morse & Vohra 1971; Lepczyk & Karasov 2000) and retarded growth (e.g. Schew, 1995) have been reported for both body mass and structural growth. It has been questioned whether the reported accelerated growth in birds is real or is an artefact of how growth data are expressed (Schew & Ricklefs 1998). The question is important because accelerated growth during realimentation would suggest that normal growth rate is less than the maximum physiological potential and that growth is optimized below a physiological limit rather than maximised.

Developmental time is an aspect of growth that is a determinant for subsequent fledging body size. A decoupling of chronological and physiological age may occur when functional maturation is temporarily stalled. From studies on aerial insectivores (e.g. Emlen *et al*. 1991), such responses have been characterised as *labile* or *flexible* development, and have been associated with extended developmental time. Extended developmental time is the only means by which a nestling can compensate for previous poor growth, unless it can accelerate growth during realimentation. Highly variable nestling periods have been reported in many seabirds (Lack 1968; Barrett & Rikardsen 1992; Croxall *et al*. 1988). However, developmental time should be subject to an evolutionary trade-off with time dependent mortality, and it is not fully understood to what degree seabird nestlings can increase developmental time to compensate for short-term growth perturbations and attain normal fledging body size.

The different strategies of resource allocation during a period of food shortage may have different consequences on subsequent growth during realimentation (Schew & Ricklefs 1998). From studies on development of avian skeletal muscles, it is proposed that growth is inversely related to the functional maturity (*growth-maturation hypothesis*, Ricklefs & Webb 1985; Ricklefs, Shea & Choi 1994). Accordingly, continued maturation during a period of food shortage may enhance functional capacity (e.g. thermoregulation and locomotor activity) but reduce the potential for subsequent growth. Therefore, it is suggested that the nestlings slowing both growth and maturation during food shortage will have the best probabilities of attaining normal fledging body size (Schew 1995), either through accelerated growth or through parallel growth with extended developmental time during realimentation.

Nestling European shags (*Phalacrocorax aristotelis*) are very well suited for studying growth responses to short-term food shortage. They experience variable environmental conditions and food availability (Velando, Ortega-Ruano & Freire 1999; Rindorf, Wanless & Harris 2000). In contrast to many pelagic seabirds, which raise single nestlings with slow growth rates, European shag nestlings exhibit high growth rates (Østnes, Jenssen & Bech 2001) and compete with 1-3 other siblings for food (Amundsen & Stokland 1988). Consequently, they should depend on successful food provisioning rates to follow their normal developmental trajectory.

Experiments with European shag nestlings subject to diet restriction under laboratory conditions (Moe *et al*. 2004; own unpublished data) have revealed that structural growth was maintained at very high rates whereas several visceral organs and muscles and resting metabolic rate were substantially reduced. Further, the functional maturation of the skeletal muscles and the thermoregulatory ability continued to increase during the period of diet restriction (four days on a weight maintenance diet). According to the *growth-maturation hypothesis* the potential for growth during realimentation and the ability to extend developmental time should be reduced by such a continued maturation of the skeletal muscles. Accordingly, one could expect that European shag nestlings facing a short-term food shortage will not be able to recover completely during realimentation and will not be able to attain normal fledging body size.

The aim of the present study was to investigate the effect of short-term and transient food shortage on subsequent growth and fledging body size in a large altricial seabird, the European shag. Specifically, we tested whether nestlings could compensate for a growth perturbation by accelerating growth rate during realimentation or extending developmental time to attain normal fledging body size. This was done by subjecting nestlings to a diet restriction treatment in the laboratory between 12-16 days of age and to subsequent realimentation in the wild with foster parents or to overfeeding in the laboratory.

Materials and methods

STUDY AREA AND ANIMALS

The study was carried out during the 2001 breeding season (June and July) on Sklinna, a small group of islands situated ~50 km off the coast of central Norway (65^{o}12’N, 11^{o}00’E). In 2001 the breeding population of European shags consisted of 1750 pairs (N. Røv pers. comm.), and it has increased (6.3% annually) in the period 1984-2001 (Lorentsen 2001). Nests (N=355) were marked and visited every second day in order to determine the hatching dates of the nestlings (defined as day 0). Each nestling was identified with ink on one of its legs on day 0 or day 1. The nestlings were banded with standard metal rings at the age of ~18 days.

DIET RESTRICTION AND HOUSING CONDITIONS

A sample of 26 nestlings was brought to the laboratory (500 m from the colony) at the age of 12 days for the purpose of a diet restriction treatment (weight maintenance diet for 4 days). The nestlings were kept, 4-8 together, in an enclosure (100 x 50 cm) with a heat lamp providing a constant range of operative temperatures (Bakken 1992) of 22-33^{o}C. The diet-restricted nestlings were hand fed with fillets of saithe (*Pollachius virens*) and cod (*Gadus morhua*), because these gadoids constitute 70% of the diet of shags breeding in the study area (Barrett *et al*. 1990). They were fed small portions of food 8-10 times a day to maintain a relatively stable body mass, until they were 16 day old. The daily food intake was ~100 g d^{-1} which is less than 50% of the food intake for hand fed nestlings following a normal growth trajectory (Moe *et al*. 2004).

REALIMENTATION EXPERIMENT

After the diet-restriction treatment, 20 of the 26 diet-restricted nestlings at 16 days of age were assigned to new nests with foster parents. In this experiment we intended to monitor the growth during realimentation of diet-restricted nestlings under favourable competitive conditions in the wild. The diet-restricted nestlings could get a competitive disadvantage in their broods of origin. Consequently, they were brought to new nests with foster parents and swapped with the original α-nestlings which were assigned to new nests with foster parents. The subsequent growth of the original α-nestlings was not monitored. Nests were selected in which the original α-nestlings were heavier than the diet-restricted nestlings and the original β–nestlings were lighter than the diet-restricted nestlings. With this swapping procedure the diet-restricted nestling got the α-position in the nestling size hierarchy, the brood size (2 or 3) was unchanged and the work load on the parents was presumed to be unchanged.

As controls to the diet-restricted nestlings we assigned 12 unmanipulated nestlings at 12 days of age to new nests with foster parents. As for the diet-restricted nestlings, they were subject to the same swapping procedure that provided favourable competitive conditions. The controls were assigned to foster parents at the age of 12 days because their body mass was almost the same as the diet-restricted nestlings at 16 days of age.

All the controls and the diet-restricted nestlings received ~30 g of fish fillets before they were assigned to the new nest. All the nestlings were successfully accepted by their foster parents. At the time when we terminated the study, all the nestlings had reached, or were close to, 95% of the asymptotic value for each growth character. It means that we monitored the growth of all the nestlings until they were 36-48 day old, except for two diet-restricted nestlings that disappeared under boulders at 22 and 24 days of age, respectively. The body mass as well as the length of the skull (head + bill), the tarsus and the wings were measured every second day during realimentation.

OVERFEEDING EXPERIMENT

The remaining six of the 26 diet-restricted nestlings at 16 days of age were subject to an overfeeding treatment in the laboratory. In this experiment we intended to investigate whether growth during realimentation in the wild was limited by food availability. Based on previous experience with feeding protocols of shag nestlings (Moe *et al.* 2004), the six diet-restricted nestlings (hereafter called overfed nestlings) were hand fed with fish fillets every second hour. During the first day, from 16 to 17 days of age, the overfed nestlings begged intensively for food. Thereafter, the begging ceased, and they were fed with the maximum portions that did not induce regurgitation. This feeding regime worked well up to the age of 21 days for all the overfed nestlings. Consequently, data are presented for the overfed nestlings up to that age. After day 21, three nestlings started to regurgitate when being fed. As one of these nestlings died, presumably due to digestive problems, the other two were anaesthetised and sacrificed. The remaining three overfed nestlings were in good condition and were assigned to new nests with foster parents. Their subsequent growth was not monitored. The body mass as well as the length of the skull (head + bill), the tarsus and the wings were measured every day during the overfeeding treatment.

STATISTICS

For each nestling in the realimentation experiment, data for each growth character (body mass, skull, tarsus and wings) were fitted to a logistic growth model ($A/[1+e^{(-k(age-T))}]$) by non-linear least square regressions (Ricklefs 1983). In this equation, A is the asymptotic value, k is the growth constant and T is the age at the inflection point. A general linear model (GLM) with the type III sum of squares was used to test for differences between controls and diet-restricted nestlings for each growth character and each parameter (A, k, T) separately. Treatment was entered as a factor, and the value of the growth character at the age of 12 days was included as a covariate to control for any effect of initial size.

A mixed linear model with restricted maximum likelihood was used to analyse growth data including repeated measures on individuals. The null models included age and age^2 as covariates, treatment as factor and the interaction age x treatment. Nestling-id and the interaction nestling-id x age were included as random effects. Akaike's information criteria (AIC) were used to select the appropriate final models (Burnham & Anderson 1998). All statistical tests were performed with SPSS 12·0 (SPSS inc., Chicago), except for the mixed linear models which were performed with S-PLUS 6·2 (Insightful Corp., Seattle). Means are reported with ± 1SE.

ETHICS

The National Committee for Animal Research in Norway ('Forsøksdyrutvalget', S-1278-01) approved the experimental protocols in the present study. The Norwegian Directorate for Nature Management, Trondheim, gave us permission to work in the shag colony.

There was no mortality during the diet restriction treatment. The appearance of the nestlings during the diet restriction treatment as well as the successful growth and development during realimentation were strong indicators of healthy individuals (N=20). Although we carefully aimed to avoid stressing the overfed nestlings (N=6), three of these nestlings received higher quantities of fish than they could digest and their welfare seemed negatively affected. This encourages restrictive use of overfeeding.

Results

DIET RESTRICTION AND SUBSEQUENT REALIMENTATION IN THE WILD

Body mass and structural elements (skull, tarsus, wings) showed different growth trajectories during the four days of diet restriction. Body mass remained virtually stable during this period (i.e. an increase of only 5·3 g d^{-1}, mixed linear model, $t_{1,79}$=8·3, P<0·001, Fig. 1a) compared to the body mass growth of the controls (see Fig. 1a). The structural elements exhibited high daily growth rates (skull, 2·7 mm d^{-1}, $t_{1,63}$=33·6, P<0·001, Fig. 1b; tarsus, 1·9 mm d^{-1}, $t_{1,63}$=32·1, P<0·001, Fig. 1c; wings, 3·2 mm d^{-1}, $t_{1,63}$=27·4, P<0·001, Fig. 1d).

The growth trajectory of the diet-restricted nestlings during realimentation in the wild is shown in comparison with controls in Fig. 1 and Table 2. General linear models (GLM) revealed that the growth constant (K) was not different between diet-restricted nestlings and controls for body mass, skull or wing (Table 1), but it was significantly higher in diet-restricted nestlings compared to controls for the tarsus (Table 1). In addition to the GLM analyses of the growth constants (Table 1) from the individual logistic growth equations, we also used mixed linear models to test for differences in growth rates during realimentation. The mixed models included measurements from the age of 16-24 days for body mass, skull and wing and 16-22 days for tarsus, which corresponded to the start of realimentation and the most linear phases of growth. Results from the mixed models were consistent with the GLM analyses of the growth constant (K) for body mass, indicating no differences in body mass growth rate (age x treatment interaction, $F_{1,126}$=0·7, P>0·1) . The analyses of the growth rate of the structural elements, however, provided contrasting results. The mixed model showed a significant higher growth rate of the skull (age x treatment interaction, $F_{1,123}$=4·8, P<0·05) and a tendency for a higher growth rate of the wings (age x treatment interaction, $F_{1,121}$=2·9, P=0·09) in the controls compared to the diet-restricted nestlings during realimentation, but no significant difference in tarsus growth rate (age x treatment interaction, $F_{1,85}$=2·3, P>0·1).

The age at the inflection point (T) of the logistic growth curves of the diet-restricted nestlings was significantly higher compared to controls for all the four growth characters (Table 2), showing that maximum growth occurred 1·5-2·5 days later in diet-restricted nestlings. Using the logistic growth equations (Table 1), the diet-restricted nestlings reached 95% of their asymptotic values 0·5-3·8 days later compared to controls (body mass, 39·4 vs 38·4 d; skull, 47·2 vs 43·4 d; tarsus, 25·7 vs 25·2 d; wing, 52·4 vs 50·5 d).

Asymptotic values (A) for body mass, skull, tarsus and wings did not differ between controls and diet-restricted nestlings (Table 2). As we monitored the growth until the nestlings were 36-48 days of age, all nestlings had reached or were close to 95% of the asymptotic values for all growth characters. Therefore, the asymptotic values were adequate measures of fledging body size. Accordingly, fledging body size did not differ between controls and diet-restricted nestlings.

DIET RESTRICTION AND SUBSEQUENT OVERFEEDING

Body mass growth and food intake of the diet-restricted nestlings (N=6) that were subsequently subject to overfeeding is shown in Fig. 2. A mixed linear model showed that the body mass growth increased substantially as a response to the overfeeding after the diet restriction was lifted (treatment x age, $F_{1,51}$=118·9, P<0·001, Fig. 2b). In contrast, the growth of the skull, the tarsus and the wings did not increase as a response to the overfeeding (treatment x age, P>0·1, Fig. 3a,b,c). In these analyses we controlled for a significant negative and positive curvature (age^2 as a main effect) for the tarsus and the wings, respectively.

OVERFEEDING VERSUS REALIMENTATION IN THE WILD

We compared the growth of the overfed nestlings to the diet-restricted nestlings that were subject to realimentation in the wild, from the age of 16 to 21 days. The overfed nestlings did not grow at a faster rate (Fig. 2, Fig. 3). On the contrary, a mixed linear model showed that their tarsus grew at a significantly lower rate (treatment x age, $F_{1,73}$=12·6, P<0·001, Fig. 3b) while their body mass ($F_{1,77}$=2·5, P>0·1, Fig. 2b), skull ($F_{1,74}$=1·8, P>0·1, Fig. 3a) and wings ($F_{1,73}$=2·5, P>0·1, Fig. 3c) grew at slower, but not significantly slower, rates.

During the diet restriction period, food intake as well as growth of the diet-restricted nestlings (N=6) that were subsequently subject to overfeeding were similar to that of the diet-restricted nestlings (N=20) that were subsequently subject to realimentation in the wild.

Discussion

Although it was not noticeable from the logistic regression line in Fig. 1a, a period of accelerated body mass growth appeared within 48 hours of realimentation in the wild and overfeeding in the laboratory (Fig. 2b). However, such a response is more likely to be attributed to a gut-fill phenomenon rather than to real growth (Wilson & Osbourn 1960; Lepczyk & Karasov 2000). During the entire course of the realimentation period the growth constant of the diet-restricted nestlings was not significantly higher compared to controls (Table 1). This result was supported by the analysis with the mixed linear model in which repeated individual measurements were included.

The results for the growth of the structural elements during realimentation were not consistent between the two statistical methods that we applied. The mixed model indicated a significantly lower growth rate of the skull and a tendency for a lower growth rate of the wings in the diet-restricted nestlings, whereas the GLM, including individual values of the growth constant, indicated a significantly higher growth rate of the tarsus in the diet-restricted nestlings. As these statistical methods showed no consistent pattern of accelerated or retarded growth in diet-restricted nestlings compared to controls, and the differences in growth rates of structural elements between controls and diet-restricted nestlings were anyhow very small, we refrain from characterising the observed pattern of growth as either accelerated or retarded. Therefore, we consider the pattern of growth as an example of parallel growth. The European shag nestlings, thus, showed limited capacity for accelerated growth, indicating that growth during normal conditions is close to the limits set by physiological or anatomical constraints. Alternatively, growth during realimentation in the wild could have been limited by the quantity of food provided by the foster parents. However, the overfed nestlings in the laboratory did not grow at faster rates, and, apparently, their digestive capacity limited their potential for higher growth. We provided the overfed nestlings with fillets of fish, whereas nestlings in the wild received whole-fish from their foster parents. This difference in diet quality could possibly confound the comparison. However, we think that the difference in diet quality explains why the overfed nestlings tended to grow at slower rates compared to the nestlings in the wild. Since food availability seemed to be good in 2001, as indicated by a high breeding success, and because the nestlings possessed the competitive α-position within the broods, we do not think that the growth of the nestlings in the wild was limited by food availability.

We did not obtain any measure of body composition at the end of realimentation. Hence, we have no data on whether body mass growth was due to true growth, i.e. adding of proteins, or due to accumulation of fat. However, there is no doubt that the structural elements exhibited true growth. Also, we do not know whether the nestlings had recovered from the mass loss of muscles tissue and visceral organs that occurred during the diet restriction (Moe *et al*., in press). However, given the highly flexible nature of avian visceral organs and muscle tissue, allowing fast and reversible changes (e.g. Schew 1995; Piersma & Lindstrøm 1997, Starck 1999, Starck & Rahman 2003), we think it is likely that these organs and tissues fully recovered during the realimentation.

Contrary to the *growth-maturation* hypothesis, the continued functional maturation of skeletal muscles and most visceral organs during the food shortage period (Moe *et al*. 2004; own unpublished data) did not hamper the potential for subsequent growth of body mass and structural elements. The European shag nestlings improved thermoregulatory abilities during the period of diet restriction, probably due to the continued maturation of the skeletal muscles (own unpublished data). The present results suggest that the nestlings improved thermoregulatory abilities without paying a penalty in terms of a reduced growth potential. However, the mauturation of the skeletal muscles may not precisely reflect the maturation of the skeleton. If the the maturation of the skeleton, i.e. the degree of ossification and the size of the cartilaginous proliferation zones (Starck 1998), was maintained in normal proportions to the growth of the bones, the potential for growth of the skeleton should not be impeded.

Fledging body size was similar between controls and diet-restricted nestlings, suggesting that variation in fledging body size does not seem to arise from short-term and transient food shortage during early growth of the European shag. Other experimental studies have provided contrasting results. Whereas short-term food shortage had no effect on final body size in Japanese quails (*Coturnix c. japonica*, Schew 1995) and house sparrows (*Passer domesticus*, Lepczyk & Karasov 2000), final body mass and structural size in European starlings were negatively affected by short-term food shortage (*Sturnus vulgaris,* Schew 1995). From field studies of aerial insectivores (Lack & Lack 1951; Ricklefs 1976; Bryant 1978; Emlen *et al.* 1991), it has been found that naturally occurring transient food shortage can be met with a characteristic labile development, in which growth and development are temporally stalled during the period of food shortage. Developmental time is extended and the subsequent growth during realimentation compensates fully for the growth perturbations. The benefits of extending developmental time, however, should be balanced against the costs arising from time dependent mortality (Bosque & Bosque 1995; Remes & Martin 2002). Moe *et al.* (2004) suggested that predation, together with sibling competition, could be selective factors for the observed rigid pattern of structural growth during food shortage. We have no data on whether the diet restriction treatment affected time of fledging, but the present study indicates that sustained structural growth during a short period of food shortage prevents an extension of the developmental time by as much as the length of the food shortage period.

Although the present study showed no effect of short-term food shortage on fledging body size, long-term food shortage (i.e. >4 d) or variation in diet quality, however, could be potential environmental sources of variation in fledging body size. Morse & Vohra (1971) elegantly showed that the length of the food shortage period was crucial for the ability of Japanese quails to compensate fully for the growth perturbation. Poor diet quality, causing poor feeding conditions throughout the growth period, has been found to cause stunted fledging body size in wild geese (Cooch *et al.* 1991; Larsson & Forslund 1992) as well as in captive zebra finches (Boag 1987; De Kogel 1997; Bech, Rønning & Moe in press).

Further experiments are needed to reveal how long European shag nestlings can sustain high rates of structural growth during food shortage. If structural nutrients such as calcium and phosphorus (Murphy 1996), rather than energy primarily limit the rate of structural growth, it could suggest that the nestlings were provided well in excess during normal conditions and still in sufficient amount during the period of diet-restriction, and the nestlings could consequently show sustainable high rates of structural growth during food shortage. Since the size of most visceral organs and some muscles were substantially reduced during diet restriction (Moe *et al.* 2004), we assume that proteins and perhaps also other essential structural nutrients were actively scavenged from these tissues. If it occurred, it suggests that the high rates of structural growth could not be sustained through a long period of food shortage. Notably, in contrast to the growth rate of body mass (Fig. 2b) the growth rates of structural elements were not higher during overfeeding compared to the diet restriction (Fig. 3). This indicates that structural growth was sustained at maximum rates within the limits of the food intake and the endogenous reserves. It also indicates that the structural growth during the diet restriction and overfeeding was limited by diet quality rather than diet quantity.

Due to the rigid structural growth during the period of food shortage we refrain from characterising the observed growth responses as flexible, although developmental time was slightly extended. Lack of flexible development of body size may have several evolutionary interpretations. It may evolve if the prevailing environment lacks frequent, unpredictable fluctuations in food availability during the development (Konarzewski *et al.* 1996). Also, if there is strong selection for relatively uniform fledging or adult body size, developmental flexibility might be suppressed even under fluctuating environmental conditions. This may occur if flexibility cannot be compensated during later development, and flexible development consequently would produce a diversity of fledging and adult body sizes (Konarzewski & Starck 2000). Finally, as already mentioned, it may evolve as a result of nestling competition or strong time-dependent mortality. The flexible development of resting metabolic rate and body composition of European shag nestlings (Moe *et al.*, 2004) does not easily support the first interpretation which predicts an overall rigid development. If the ability of European shag nestlings of extending developmental time is rather limited, we believe that the rigid development of body size may have evolved as a result of selection on fledging or adult body size, selection on nestling competitive abilities or of strong time dependent mortality.

For obvious reasons, the knowledge of how fledging body size may affect fitness is not extensive for seabirds. Velando (2000) found no effect of fledging body mass on dominance rank within juvenile European shags, and Steinen & Brenninkmeijer (2002) found no effect of fledging body condition on post fledging survival in Sandwich terns (*Sterna sandvicensis*). In contrast, fledging body mass has been found to relate positively to post fledging survival in Cape gannets (*Sula capensis*, Jarvis 1974), sooty shearwaters (*Puffinus griseus*, Sagar & Horning 1998), Manx shearwaters (*Puffinus puffinus*, Perrins, Harris & Britton 1973) and king penguins (*Aptenodytes patagonicus*, Olsson 1997). The body of literature is more comprehensive on passerines. The suggestion that small differences in the size of morphological characters may have consequences for fitness (Boag 1987; Gebhardt-Henrich & Richner 1998) is supported by several studies reporting a positive relationship between fledging body size and post fledging survival (Garnett 1981; Richner 1989; Tinbergen & Boerlijst 1990; Ringsby, Sæther & Solberg 1998).

Fledging body size could possibly affect a whole array of fitness parameters in seabirds, including post fledging survival, mating success and fecundity. Although recent literature has questioned whether compensating for growth perturbations or nutritional stress during early development might be associated with negative fitness consequences that appear later in life (Metcalfe & Monaghan 2001), we believe that the ability to attain full fledging body size, even after a short-term food shortage, could be of major importance for the fitness prospects of European shag nestlings.

In conclusion, we have shown that European shag nestlings exhibited high rates of structural growth as well as continued functional maturation during the period of food shortage. This had apparently no negative effect on the subsequent growth potential. Developmental time was slightly increased and fully compensated for the growth perturbations. Hence, variation in fledging body size does not seem to arise from short-term and transient food shortage in European shags. Flexible developmental programmes have been described in altricial and precocial chicks. The ability to extend developmental time represents some degree of flexible development, but this ability might be very limited, and we do not characterise the observed structural growth of the European shag nestling as flexible development.

Acknowledgments

We are indebted to the Norwegian lighthouse authorities for permission to use the facilities on Sklinna and to the lighthouse keepers on Sklinna for their hospitality. We thank Carolyn Baggerud for improving the English. Rob Barrett and J. Matthias Starck provided valuable comments on an earlier version of this manuscript. CB and BM were supported by a grant from the Research Council of Norway (138698/410).

References

Amundsen, T. & Stokland, J.N. (1988) Adaptive significance of asynchronous hatching in the shag - a test of the brood reduction hypothesis. *Journal of Animal Ecology*, **57**, 329-344.

Anderson, D.J. (1989) Differential responses of boobies and other seabirds in the Galápagos to the 1986-1987 El-Niño-Souther oscillation event. *Marine Ecology Progress Series*, **52**, 209-216.

Ashmole, N.P. (1971) Seabird ecology and the marine environment. *Avian biology* (eds. D.S. Farner & J.R. King), Vol I, pp. 224-286. Academic Press, New York.

Bakken, G.S. (1992) Measurement and application of operative and standard operative temperatures in ecology. *Amercian Zoologist*, **32**, 194-216.

Barrett, R.T. & Rikardsen, F. (1992). Chick growth, fledging periods and adult mass loss of Atlantic puffins *Fratercula arctica* during years of prolonged food stress. *Colonial Waterbirds*, **15**, 24-32.

Barrett, R.T., Anker-Nilssen, T., Rikardsen, F., Valde, K., Røv, N. & Vader W. (1987) The food, growth and fledging success of Norwegian Puffin chicks *Fratercula arctica* in 1980-1983. *Ornis Scandinavia*, **18**, 73-83.

Barrett, R.T., Røv, N., Loen, J. & Montevecchi, W.A. (1990) Diet of Shags *Phalacrocorax aristotelis* and Cormorants *P. carbo* in Norway and possible implications for gadoid stock recruitment. *Marine Ecology Progress Series*, **66**, 205-218.

Bech, C., Rønning, B. & Moe, B. (in press) Individual variation in the basal metabolism of Zebra finches *Taeniopygia guttata*: effect of food quality during early development. *International Congress Series*.

Boag, P.T. (1987) Effects of nestling diet on growth and adult size of zebra finches (*Poephila guttata*). *Auk*, **104**, 155-166.

Bohman, V.R. (1955) Compensatory growth of beef cattle: The effect of hay maturity. *Journal of Animal Science*, **14**, 249-255.

Bosque, C. & Bosque, M.T. (1995) Nest predation as a selective factor in the evolution of developmental rates in altricial birds. *American Naturalist*, **145**, 234-260.

Bryant, D.M. (1978) Environmental influences on growth and survival of nestling House Martins *Delichon urbica*. *Ibis*, **120**, 271-283.

Burnham, K.P. & Anderson, D.R. (1998) *Model Selection and Inference. A Practical Information-Theoretic Approach.* Springer-Verlag, New York.

Chastel, O., Weimerskirch, H. & Jouventin, P. (1993) High annual variability in reproductive success and survival of an Antarctic seabird, the snow petrel *Pagodroma nivea*- a 27 year study. *Oecologia*, **94**, 278-285.

Cooch, E.G., Lank, D.B., Dzubin, A., Rockwell, R.F. & Cooke, F. (1991) Body size variation in lesser snow geese: Environmentally plasticity in gosling growth rates. *Ecology*, **72**, 503-512.

Croxall, J.P., Hill, H.J., Lidstone-Scott, R., O'Connel, M.J. & Prince P.A. (1988) Food and feeding ecology of Wilson's storm petrel, *Oceanites oceanicus* at South Georgia. *Journal of Zoology* (London), **216**, 83-102.

De Kogel, C.H. (1997) Long-term effects of brood size manipulation on the morphological development and sex-specific mortality of offspring. *Journal of Animal Ecology*, **66,** 167-178.

Emlen, S.E., Wrege, P.H., Demong, N.J. & Hegner, R.E. (1991) Flexible growth rates in nestling white-fronted bee-eaters: a possible adaptation to short-term food shortage. *Condor*, **93**, 591-597.

Garnett, M.C. (1981) Body size, its heritability and influence on juvenile survival among great tits, *Parus major*. *Ibis*, **123**, 31-41.

Gebhardt-Henrich, S. & Richner, H. (1998) Causes of growth variation and its consequences for fitness. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 324-339. Oxford University Press, New York.

Harris, M.P. & Wanless, S. (1997) Breeding success, diet, and brood neglect in the kittiwake (*Rissa tridactyla*) over an 11-year period. *ICES Journal of Marine Sciences*, **54**, 615-623.

Jarvis, M.J.F. (1974) The ecological significance of clutch size in the South African Gannet *Sula capensis* (Lichtenstein). *Journal of Animal Ecology*, **43**, 1-17.

Kitaysky, A.S. (1999) Metabolic and developmental responses of alcid chicks to experimental variation in food intake. *Physiological and Biochemical Zoology*, **72**, 462-473.

Konarzewski, M. & Starck, J.M. (2000) Effects of food shortage and oversupply on energy utilization, histology, and function of the gut in nestling song thrushes (*Turdus philomelos*). *Physiological and Biochemical Zoology*, **73**, 416-427.

Konarzewski, M., Kowalczyk, J., Swierubska, T. & Lewonczuk, B. (1996) Effect of short-term feed restriction, realimentation and overfeeding on growth of song thrush (*Turdus philomelos*) nestlings. *Functional Ecology*, **10**, 97-105.

Lack, D. (1968) Ecological adaptations for breeding in birds. Methuen and Co., London.

Lack, D. & Lack, E. (1951) The breeding biology of the Swith *Apus apus*. *Ibis*, **93**, 501-546.

Larsson, K. & Forslund, P. (1992) Genetic and social inheritance of body size and egg size in the barnacle goose (*Branta leucopsis*). *Evolution*, **46**, 235-244.

Lepczyk, C.A. & Karasov, W.H. (2000) Effect of ephemeral food restriction on growth of house sparrows. *Auk*, **117**, 164-174.

Lorentsen, S.-H. (2001) The national monitoring programme for seabirds. Results including the breeding season 2001. Norwegian Institute for Nature Research. '*NINA Oppdragsmelding'*, **726**, 1-36.

Metcalfe N.B. & Monaghan, P. (2001) Compensation for a bad start: grow now, pay later? *Trends in Ecology and Evolution,* **16**, 254-260.

Moe, B., Stølevik, E. & Bech, C. (in press). Ducklings exhibit substantial energy saving mechanisms as a response to short-term food shortage. *Physiological and Biochemical Zoology.*

Moe, B., Brunvoll, S., Mork, D., Brobakk, T.E. & Bech, C. (2004). Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings *Phalacrocorax aristotelis*. *Journal of Experimental Biology,* **207**, 4067-4076.

Monaghan, P., Uttley, J.D., Burns, M.D., Thaine, C. & Blackwood, J. (1989) The relationship between food supply, reproductive effort and breeding success in Arctic Terns *Sterna paradisea*. *Journal of Animal Ecology*, **58**, 261-274.

Morse, K. & Vohra, P. (1971) The effect of early growth retardation of Coturnix (Japanese Quail) on their sexual maturity. *Poultry Science*, **50**, 283-284.

Murphy, M.E. (1996) Nutrition and metabolism. *Avian energetics and nutritional ecology* (ed. C. Carey), pp. 31-60. Chapman & Hall, New York.

Negro, J.J., Chastin, A. & Bird, D.M. (1994) Effects of short-term food deprivation on growth of hand-reared American kestrels. *Condor*, **96**, 749-760.

Olsson, O. (1997) Effects of food availability on fledging condition and post-fledging survival in king penguin chicks. *Polar Biology*, **18**, 161-165.

Østnes, J.E., Jenssen, B.M. & Bech, C. (2001) Growth and development of homeothermy in nestling European shags (*Phalacrocorax aristotelis*). *Auk*, **118**, 983-995.

Øyan, H.S. & Anker-Nilssen, T. (1996) Allocation of growth in food-stressed Atlantic Puffin chicks. *Auk*, **113,** 830-841.

Perrins, C.M., Harris, M.P. & Britton, C.K. (1973) Survival of Manx Shearwaters *Puffinus puffinus*. *Ibis*, **115**, 535-548.

Piersma, T. & Lindstrøm, Å. (1997) Rapid reversible changes in organ size as a component of adaptive behviour. *Trends in Ecology and Evolution*, **12,** 134-138.

Remes, V. & Martin, T.E. (2002) Environmental influences on the evolution of growth and developmental rates in passerines. *Evolution*, **56**, 2505-2518.

Richner, H. (1989) Habitat-specific growth and fitness in carrion crows (*Corvus corone corone*). *Journal of Animal Ecology*, 58, 427-440.

Ricklefs, R.E. (1976) Growth rates of birds in the humid New World tropics. *Ibis*, **118**, 179-207.

Ricklefs, R.E. (1983) Avian postnatal development. *Avian biology* (eds. D.S. Farner, J.R. King & K.C. Parkes), Vol VII, pp. 1-83. Academic Press, New York.

Ricklefs, R.E. & Webb, T. (1985). Water content, thermogenesis, and growth rate of skeletal muscles in the European starling. *Auk*, **102**, 369-376.

Ricklefs, R.E., Shea, R.E. & Choi, I.H. (1994). Inverse relationship between functional maturity and exponential growth rate of avian skeletal muscle: a constraint on evolutionary response. *Evolution*, **48**, 1080-1088.

Rindorf, A., Wanless, S. & Harris, M.P. (2000) Effects of changes in sandeel availability on the reproductive output of seabirds. *Marine Ecology Progress Series*, **202**, 241-252.

Ringsby, T.H., Sæther, B-E. & Solberg, E.J. (1998) Factors affecting juvenile survival in House Sparrow *Passer domesticus*. *Journal of Avian Biology*, **29**, 241-247.

Sagar, P.M. & Horning, D. S. (1998) Mass-related survival of fledging Sooty Shearwaters *Puffinus griseus* at The Snares, New Zealand. *Ibis*, **140**, 329-339.

Schew, W.A. (1995) *The evolutionary significance of developmental plasticity in growing birds.* PhD diss. University of Pennsylvania, Philadelphia.

Schew, W.A. & Ricklefs, R.E. (1998) Developmental plasticity. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 288-304. Oxford University Press, New York.

Schlichting, C. D. & Pigliucci, M. (1998). *Phenotypic Evolution. A Reaction Norm Perspective.* Sinauer Associates, Sunderland.

Schmalhausen, I. I. (1949). *Factors of evolution*. Philadelphia: Blakiston. Reprinted 1986, Chicago University press, Chicago.

Smith-Gill, S. J. (1983) Developmental plasticity: developmental conversion *versus* phenotypic modulation. *American Zoologist*, **23**, 47-55.

Starck, J.M. (1998) Structural variants and invariants in avian embryonic and postnatal development. *Avian Growth and Development* (eds. J.M. Starck & R.E. Ricklefs), pp. 59-88. Oxford University Press, New York.

Starck, J.M. (1999) Phenotypic flexibility of the avian gizzard: Rapid, reversible and repeated changes of organ size in response to changes in dietary fibre content. *Journal of Experimental Biology*, **202**, 3171-3179.

Starck, J.M. & Chinsamy, A. (2002) Bone microstructure and developmental plasticity in birds and other dinosaurs. *Journal of Morphology*, **254**, 232-246.

Starck, J.M. & Rahman, G.H.A. (2003) Phenotypic flexibility of structure and function of the digestive system of Japanese quail. *Journal of Experimental Biology*, **206**, 1887-1897.

Steinen, E.W.M. & Brenninkmeijer, A. (2002) Variation in growth in Sandwich Tern chicks *Sterna sandvicensis* and the consequences for pre- and post-fledging mortality. *Ibis*, **144**, 567-576.

Suryan, R.M, Irons, D.B., Kaufman, M., Benson, J., Jodice, P.G.R., Roby, D.D. & Brown, E.D. (2002) Short-term fluctuations in forage fish availability and the effect on prey selection and brood-rearing in the black-legged kittiwake *Rissa tridactyla*. *Marine Ecology Progress Series*, **236**, 273-287.

Tinbergen, J.M. & Boerlijst, M.C. (1990) Nestling weight and survival in individual great tits (*Parus major*). *Journal of Animal Ecology*, **59**, 1113-1127.

Velando, A. (2000) The importance of hatching date for dominance in young shags. *Animal Behaviour*, **60**, 181-185.

Velando, A., Ortega-Ruano, J.E. & Freire, J. (1999) Chick mortality in European shag *Stictocarbo aristotelis* related to food limitations during adverse weather events. *Ardea*, **87,** 51-59.

Wilson, P.N. & Osbourn, D.F. (1960) Compensatory growth after undernutrition in mammals and birds. *Biological Reviews of the Cambridge Philosophical Society*, **35**, 324-363.

Table 1. Asymptote (A), growth constant (K) and age at inflection point (T) of logistic growth equations ($A/[1+e^{(-K(\mathrm{age}-T))}]$) for different growth characters of controls and diet-restricted European shag nestlings during realimentation in the wild. The logistic regression lines are shown in Fig. 1. Differences in parameter estimates ($\bar{X}$) between controls and diet-restricted nestlings were tested with GLM [a].

Character	Parameter	Diet-restricted (N=18)		Controls (N=12)			
		$\bar{X}$	*SE*	$\bar{X}$	*SE*	$F_{1,27}$	*P*
Body mass	A	1724	35	1693	44	0·3	0·59
	K	0·176	0·006	0·161	0·008	2·4	0·13
	T	22·6	0·36	20·1	0·44	**18·4**	**<0·001**
Skull	A	137·6	0·9	136·0	1·1	1·15	0·29
	K	0·091	0·03	0·098	0·03	2·31	0·14
	T	14·8	0·19	13·3	0·23	**23·9**	**<0·001**
Tarsus	A	64·5	0·39	65·4	0·49	1·98	0·17
	K	0·234	0·007	0·211	0·008	**4·42**	**0·045**
	T	13·0	0·12	11·2	0·15	**88·3**	**<0·001**
Wing	A	253·6	3·0	248·1	3·7	1·35	0·26
	K	0·116	0·002	0·117	0·002	0·1	0·76
	T	27·0	0·35	25·3	0·43	**9·2**	**<0·005**

[a] GLM was performed with initial size (i.e. character at the age of 12 days) as a covariate. The covariate was significant for most of the parameters, but for consistency we included it in all the GLMs. Hence, the parameter estimates are adjusted for the covariate.

Figure legends

Figure 1. Changes in body mass (A) and in the length of the skull (B), tarsus (C) and wings (D) as a function of age in European shag nestlings during diet restriction in the laboratory (age of 12-16 days, filled symbols, N=20 for body mass, N=16 for structural elements) and subsequent realimentation in the wild (age ≥ 16 d, dashed logistic regression line), as well as in controls in the wild (age ≥ 12 d, solid logistic regression line). In the wild, controls (N=12) and diet-restricted nestlings (N=18) were raised in different nests by foster parents. The equations for the logistic regression lines are presented in Table 1.

Figure 2. Daily food intake (A) and body mass (B) as a function of age (days) in European shag nestlings (filled bars and symbols) subject to diet restriction (DR) and subsequent overfeeding (OF) in the laboratory. Error bars are 1SE. For comparison, the logistic regression lines from Fig. 1a are shown in B. The dashed line represents the diet-restricted nestlings during realimentation in the wild (age ≥ 16 d), and the solid line represents the controls (age ≥ 12 d). See text for statistics.

Figure 3. Length of the skull (A), tarsus (B) and wings (C) as a function of age in European shag nestlings (filled symbols) subject to diet restriction (DR) and subsequent overfeeding (OF) in the laboratory. Measurements on day 14 are missing. Error bars are 1SE. For comparison, the logistic regression lines from Fig. 1b,c,d are shown. The dashed line represents the diet-restricted nestlings during realimentation in the wild with the (age ≥ 16 d), and the solid line represents the controls (age ≥ 12 d). See text for statistics.

Figure 1.

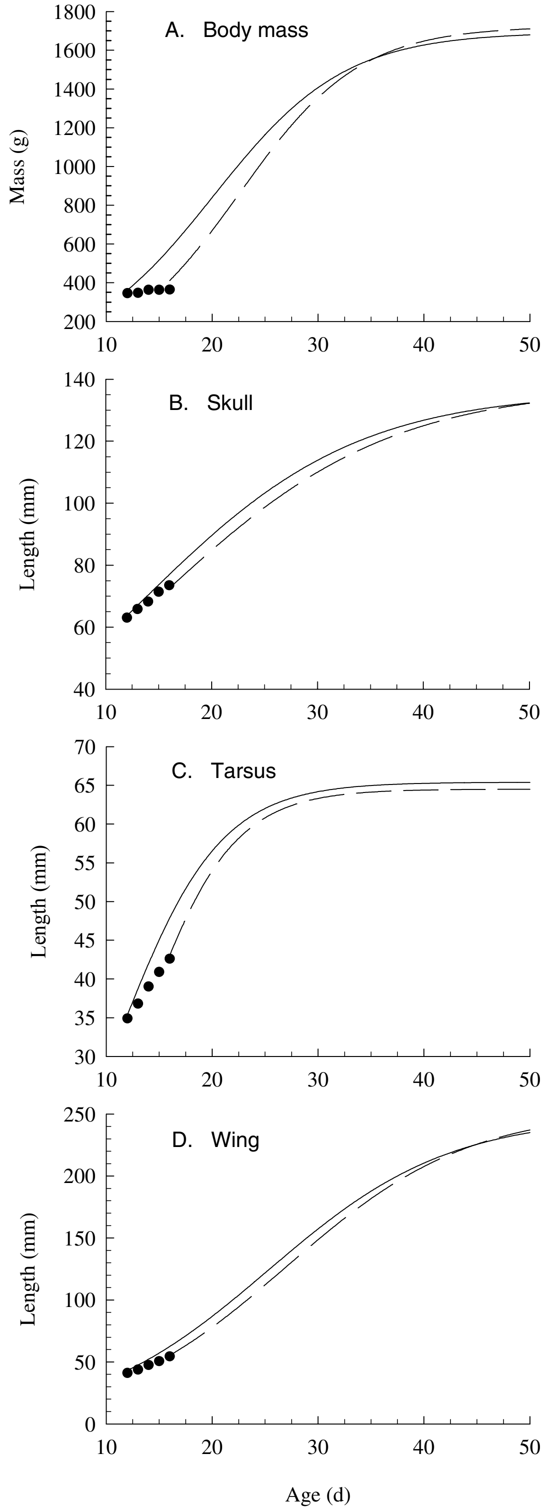

A. Body mass
Mass (g)
1800
1600
1400
1200
1000
800
600
400
200
B. Skull
Length (mm)
140
120
100
80
60
40
C. Tarsus
Length (mm)
70
65
60
55
50
45
40
35
30
D. Wing
Length (mm)
250
200
150
100
50
0
10
20
30
40
50
Age (d)

Figure 2.

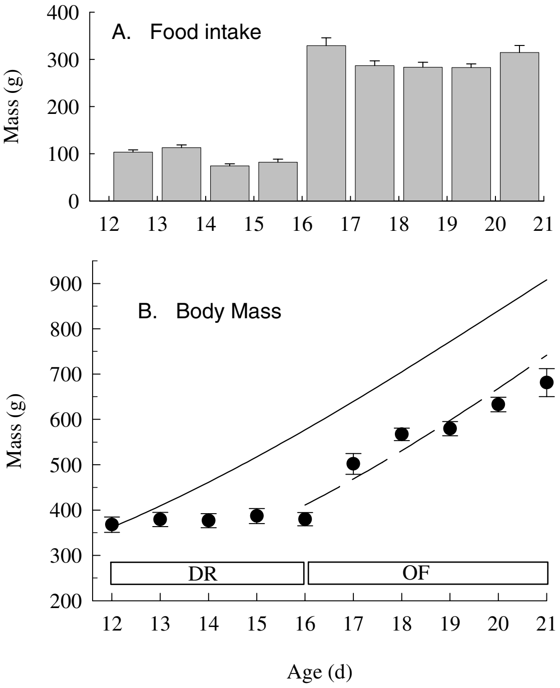
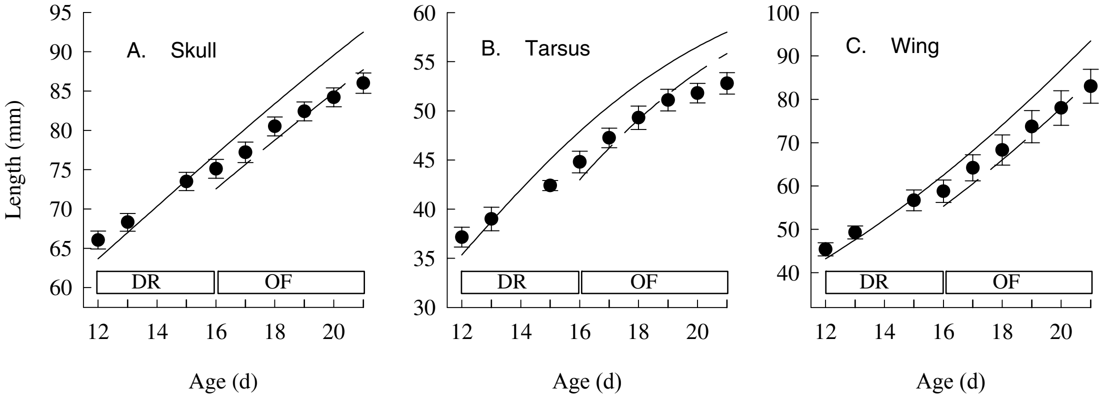

A. Food intake
Mass (g)
400
300
200
100
0
12
13
14
15
16
17
18
19
20
21
B. Body Mass
Mass (g)
900
800
700
600
500
400
300
200
DR
OF
12
13
14
15
16
17
18
19
20
21
Age (d)

Figure 3.


A. Skull
B. Tarsus
C. Wing
Length (mm)
95
90
85
80
75
70
65
60
55
50
45
40
35
30
100
DR
OF
12
14
16
18
20
Age (d)

Doctoral theses in Biology
Norwegian University of Science and Technology

Year	Name	Degree	Title
1974	Tor-Henning Iversen	Dr. philos Botany	The roles of statholiths, auxin transport, and auxin metabolism in root gravitropism
1978	Tore Slagsvold	Dr. philos. Zoology	Breeding events of birds in relation to spring temperature and environmental phenology.
1978	Egil Sakshaug	Dr.philos Botany	"The influence of environmental factors on the chemical composition of cultivated and natural populations of marine phytoplankton"
1980	Arnfinn Langeland	Dr. philos. Zoology	Interaction between fish and zooplankton populations and their effects on the material utilization in a freshwater lake.
1980	Helge Reinertsen	Dr. philos Botany	The effect of lake fertilization on the dynamics and stability of a limnetic ecosystem with special reference to the phytoplankton
1982	Gunn Mari Olsen	Dr. scient Botany	Gravitropism in roots of *Pisum sativum* and *Arabidopsis thaliana*
1982	Dag Dolmen	Dr. philos. Zoology	Life aspects of two sympartic species of newts (*Triturus, Amphibia*) in Norway, with special emphasis on their ecological niche segregation.
1984	Eivin Røskaft	Dr. philos. Zoology	Sociobiological studies of the rook *Corvus frugilegus.*
1984	Anne Margrethe Cameron	Dr. scient Botany	Effects of alcohol inhalation on levels of circulating testosterone, follicle stimulating hormone and luteinzing hormone in male mature rats
1984	Asbjørn Magne Nilsen	Dr. scient Botany	Alveolar macrophages from expectorates – Biological monitoring of workers exosed to occupational air pollution. An evaluation of the AM-test
1985	Jarle Mork	Dr. philos. Zoology	Biochemical genetic studies in fish.
1985	John Solem	Dr. philos. Zoology	Taxonomy, distribution and ecology of caddisflies (*Trichoptera*) in the Dovrefjell mountains.
1985	Randi E. Reinertsen	Dr. philos. Zoology	Energy strategies in the cold: Metabolic and thermoregulatory adaptations in small northern birds.
1986	Bernt-Erik Sæther	Dr. philos. Zoology	Ecological and evolutionary basis for variation in reproductive traits of some vertebrates: A comparative approach.
1986	Torleif Holthe	Dr. philos. Zoology	Evolution, systematics, nomenclature, and zoogeography in the polychaete orders *Oweniimorpha* and *Terebellomorpha*, with special reference to the Arctic and Scandinavian fauna.
1987	Helene Lampe	Dr. scient. Zoology	The function of bird song in mate attraction and territorial defence, and the importance of song repertoires.
1987	Olav Hogstad	Dr. philos. Zoology	Winter survival strategies of the Willow tit *Parus montanus*.
1987	Jarle Inge Holten	Dr. philos Bothany	Autecological investigations along a coust-inland transect at Nord-Møre, Central Norway

1987	Rita Kumar	Dr. scient Botany	Somaclonal variation in plants regenerated from cell cultures of *Nicotiana sanderae* and *Chrysanthemum morifolium*
1987	Bjørn Åge Tømmerås	Dr. scient. Zoology	Olfaction in bark beetle communities: Interspecific interactions in regulation of colonization density, predator - prey relationship and host attraction.
1988	Hans Christian Pedersen	Dr. philos. Zoology	Reproductive behaviour in willow ptarmigan with special emphasis on territoriality and parental care.
1988	Tor G. Heggberget	Dr. philos. Zoology	Reproduction in Atlantic Salmon (*Salmo salar*): Aspects of spawning, incubation, early life history and population structure.
1988	Marianne V. Nielsen	Dr. scient. Zoology	The effects of selected environmental factors on carbon allocation/growth of larval and juvenile mussels (*Mytilus edulis*).
1988	Ole Kristian Berg	Dr. scient. Zoology	The formation of landlocked Atlantic salmon (*Salmo salar* L.).
1989	John W. Jensen	Dr. philos. Zoology	Crustacean plankton and fish during the first decade of the manmade Nesjø reservoir, with special emphasis on the effects of gill nets and salmonid growth.
1989	Helga J. Vivås	Dr. scient. Zoology	Theoretical models of activity pattern and optimal foraging: Predictions for the Moose *Alces alces*.
1989	Reidar Andersen	Dr. scient. Zoology	Interactions between a generalist herbivore, the moose *Alces alces*, and its winter food resources: a study of behavioural variation.
1989	Kurt Ingar Draget	Dr. scient Botany	Alginate gel media for plant tissue culture,
1990	Bengt Finstad	Dr. scient. Zoology	Osmotic and ionic regulation in Atlantic salmon, rainbow trout and Arctic charr: Effect of temperature, salinity and season.
1990	Hege Johannesen	Dr. scient. Zoology	Respiration and temperature regulation in birds with special emphasis on the oxygen extraction by the lung.
1990	Åse Krøkje	Dr. scient Botany	The mutagenic load from air pollution at two work-places with PAH-exposure measured with Ames Salmonella/microsome test
1990	Arne Johan Jensen	Dr. philos. Zoology	Effects of water temperature on early life history, juvenile growth and prespawning migrations of Atlantic salmion (*Salmo salar*) and brown trout (*Salmo trutta*): A summary of studies in Norwegian streams.
1990	Tor Jørgen Almaas	Dr. scient. Zoology	Pheromone reception in moths: Response characteristics of olfactory receptor neurons to intra- and interspecific chemical cues.
1990	Magne Husby	Dr. scient. Zoology	Breeding strategies in birds: Experiments with the Magpie *Pica pica*.
1991	Tor Kvam	Dr. scient. Zoology	Population biology of the European lynx (*Lynx lynx*) in Norway.
1991	Jan Henning L'Abêe Lund	Dr. philos. Zoology	Reproductive biology in freshwater fish, brown trout *Salmo trutta* and roach *Rutilus rutilus* in particular.
1991	Asbjørn Moen	Dr. philos Botany	The plant cover of the boreal uplands of Central Norway. I. Vegetation ecology of Sølendet nature reserve; haymaking fens and birch woodlands
1991	Else Marie Løbersli	Dr. scient Botany	Soil acidification and metal uptake in plants

1991	Trond Nordtug	Dr. scient. Zoology	Reflctometric studies of photomechanical adaptation in superposition eyes of arthropods.
1991	Thyra Solem	Dr. scient Botany	Age, origin and development of blanket mires in Central Norway
1991	Odd Terje Sandlund	Dr. philos. Zoology	The dynamics of habitat use in the salmonid genera *Coregonus* and *Salvelinus*: Ontogenic niche shifts and polymorphism.
1991	Nina Jonsson	Dr. philos.	Aspects of migration and spawning in salmonids.
1991	Atle Bones	Dr. scient Botany	Compartmentation and molecular properties of thioglucoside glucohydrolase (myrosinase)
1992	Torgrim Breiehagen	Dr. scient. Zoology	Mating behaviour and evolutionary aspects of the breeding system of two bird species: the Temminck's stint and the Pied flycatcher.
1992	Anne Kjersti Bakken	Dr. scient Botany	The influence of photoperiod on nitrate assimilation and nitrogen status in timothy (*Phleum pratense* L.)
1992	Tycho Anker-Nilssen	Dr. scient. Zoology	Food supply as a determinant of reproduction and population development in Norwegian Puffins *Fratercula arctica*
1992	Bjørn Munro Jenssen	Dr. philos. Zoology	Thermoregulation in aquatic birds in air and water: With special emphasis on the effects of crude oil, chemically treated oil and cleaning on the thermal balance of ducks.
1992	Arne Vollan Aarset	Dr. philos. Zoology	The ecophysiology of under-ice fauna: Osmotic regulation, low temperature tolerance and metabolism in polar crustaceans.
1993	Geir Slupphaug	Dr. scient Botany	Regulation and expression of uracil-DNA glycosylase and O^6-methylguanine-DNA methyltransferase in mammalian cells
1993	Tor Fredrik Næsje	Dr. scient. Zoology	Habitat shifts in coregonids.
1993	Yngvar Asbjørn Olsen	Dr. scient. Zoology	Cortisol dynamics in Atlantic salmon, *Salmo salar* L.: Basal and stressor-induced variations in plasma levels ans some secondary effects.
1993	Bård Pedersen	Dr. scient Botany	Theoretical studies of life history evolution in modular and clonal organisms
1993	Ole Petter Thangstad	Dr. scient Botany	Molecular studies of myrosinase in Brassicaceae
1993	Thrine L. M. Heggberget	Dr. scient. Zoology	Reproductive strategy and feeding ecology of the Eurasian otter *Lutra lutra.*
1993	Kjetil Bevanger	Dr. scient. Zoology	Avian interactions with utility structures, a biological approach.
1993	Kåre Haugan	Dr. scient Bothany	Mutations in the replication control gene trfA of the broad host-range plasmid RK2
1994	Peder Fiske	Dr. scient. Zoology	Sexual selection in the lekking great snipe (*Gallinago media*): Male mating success and female behaviour at the lek.
1994	Kjell Inge Reitan	Dr. scient Botany	Nutritional effects of algae in first-feeding of marine fish larvae
1994	Nils Røv	Dr. scient. Zoology	Breeding distribution, population status and regulation of breeding numbers in the northeast-Atlantic Great Cormorant *Phalacrocorax carbo carbo.*
1994	Annette-Susanne Hoepfner	Dr. scient Botany	Tissue culture techniques in propagation and breeding of Red Raspberry (*Rubus idaeus* L.)

1994	Inga Elise Bruteig	Dr. scient Bothany	Distribution, ecology and biomonitoring studies of epiphytic lichens on conifers
1994	Geir Johnsen	Dr. scient Botany	Light harvesting and utilization in marine phytoplankton: Species-specific and photoadaptive responses
1994	Morten Bakken	Dr. scient. Zoology	Infanticidal behaviour and reproductive performance in relation to competition capacity among farmed silver fox vixens, *Vulpes vulpes*.
1994	Arne Moksnes	Dr. philos. Zoology	Host adaptations towards brood parasitism by the Cockoo.
1994	Solveig Bakken	Dr. scient Bothany	Growth and nitrogen status in the moss *Dicranum majus* Sm. as influenced by nitrogen supply
1995	Olav Vadstein	Dr. philos Botany	The role of heterotrophic planktonic bacteria in the cycling of phosphorus in lakes: Phosphorus requirement, competitive ability and food web interactions.
1995	Hanne Christensen	Dr. scient. Zoology	Determinants of Otter *Lutra lutra* distribution in Norway: Effects of harvest, polychlorinated biphenyls (PCBs), human population density and competition with mink *Mustela vision*.
1995	Svein Håkon Lorentsen	Dr. scient. Zoology	Reproductive effort in the Antarctic Petrel *Thalassoica antarctica*; the effect of parental body size and condition.
1995	Chris Jørgen Jensen	Dr. scient. Zoology	The surface electromyographic (EMG) amplitude as an estimate of upper trapezius muscle activity
1995	Martha Kold Bakkevig	Dr. scient. Zoology	The impact of clothing textiles and construction in a clothing system on thermoregulatory responses, sweat accumulation and heat transport.
1995	Vidar Moen	Dr. scient. Zoology	Distribution patterns and adaptations to light in newly introduced populations of *Mysis relicta* and constraints on Cladoceran and Char populations.
1995	Hans Haavardsholm Blom	Dr. philos Bothany	A revision of the *Schistidium apocarpum* complex in Norway and Sweden.
1996	Jorun Skjærmo	Dr. scient Botany	Microbial ecology of early stages of cultivated marine fish; inpact fish-bacterial interactions on growth and survival of larvae.
1996	Ola Ugedal	Dr. scient. Zoology	Radiocesium turnover in freshwater fishes
1996	Ingibjørg Einarsdottir	Dr. scient. Zoology	Production of Atlantic salmon (*Salmo salar*) and Arctic charr (*Salvelinus alpinus*): A study of some physiological and immunological responses to rearing routines.
1996	Christina M. S. Pereira	Dr. scient. Zoology	Glucose metabolism in salmonids: Dietary effects and hormonal regulation.
1996	Jan Fredrik Børseth	Dr. scient. Zoology	The sodium energy gradients in muscle cells of *Mytilus edulis* and the effects of organic xenobiotics.
1996	Gunnar Henriksen	Dr. scient. Zoology	Status of Grey seal *Halichoerus grypus* and Harbour seal *Phoca vitulina* in the Barents sea region.
1997	Gunvor Øie	Dr. scient Bothany	Eevalution of rotifer *Brachionus plicatilis* quality in early first feeding of turbot *Scophtalmus maximus* L. larvae.
1997	Håkon Holien	Dr. scient Botany	Studies of lichens in spurce forest of Central Norway. Diversity, old growth species and the relationship to site and stand parameters.

1997	Ole Reitan	Dr. scient. Zoology	Responses of birds to habitat disturbance due to damming.
1997	Jon Arne Grøttum	Dr. scient. Zoology	Physiological effects of reduced water quality on fish in aquaculture.
1997	Per Gustav Thingstad	Dr. scient. Zoology	Birds as indicators for studying natural and human-induced variations in the environment, with special emphasis on the suitability of the Pied Flycatcher.
1997	Torgeir Nygård	Dr. scient. Zoology	Temporal and spatial trends of pollutants in birds in Norway: Birds of prey and Willow Grouse used as Biomonitors.
1997	Signe Nybø	Dr. scient. Zoology	Impacts of long-range transported air pollution on birds with particular reference to the dipper *Cinclus cinclus* in southern Norway.
1997	Atle Wibe	Dr. scient. Zoology	Identification of conifer volatiles detected by receptor neurons in the pine weevil (*Hylobius abietis*), analysed by gas chromatography linked to electrophysiology and to mass spectrometry.
1997	Rolv Lundheim	Dr. scient. Zoology	Adaptive and incidental biological ice nucleators.
1997	Arild Magne Landa	Dr. scient. Zoology	Wolverines in Scandinavia: ecology, sheep depredation and conservation.
1997	Kåre Magne Nielsen	Dr. scient Botany	An evolution of possible horizontal gene transfer from plants to sail bacteria by studies of natural transformation in *Acinetobacter calcoacetius*.
1997	Jarle Tufto	Dr. scient. Zoology	Gene flow and genetic drift in geographically structured populations: Ecological, population genetic, and statistical models
1997	Trygve Hesthagen	Dr. philos. Zoology	Population responces of Arctic charr (*Salvelinus alpinus* (L.)) and brown trout (*Salmo trutta* L.) to acidification in Norwegian inland waters
1997	Trygve Sigholt	Dr. philos. Zoology	Control of Parr-smolt transformation and seawater tolerance in farmed Atlantic Salmon (*Salmo salar*) Effects of photoperiod, temperature, gradual seawater acclimation, NaCl and betaine in the diet
1997	Jan Østnes	Dr. scient. Zoology	Cold sensation in adult and neonate birds
1998	Seethaledsumy Visvalingam	Dr. scient Botany	Influence of environmental factors on myrosinases and myrosinase-binding proteins.
1998	Thor Harald Ringsby	Dr. scient. Zoology	Variation in space and time: The biology of a House sparrow metapopulation
1998	Erling Johan Solberg	Dr. scient. Zoology	Variation in population dynamics and life history in a Norwegian moose (*Alces alces*) population: consequences of harvesting in a variable environment
1998	Sigurd Mjøen Saastad	Dr. scient Botany	Species delimitation and phylogenetic relationships between the Sphagnum recurvum complex (Bryophyta): genetic variation and phenotypic plasticity.
1998	Bjarte Mortensen	Dr. scient Botany	Metabolism of volatile organic chemicals (VOCs) in a head liver S9 vial equilibration system in vitro.
1998	Gunnar Austrheim	Dr. scient Botany	Plant biodiversity and land use in subalpine grasslands. – A conservtaion biological approach.
1998	Bente Gunnveig Berg	Dr. scient. Zoology	Encoding of pheromone information in two related moth species

1999	Kristian Overskaug	Dr. scient. Zoology	Behavioural and morphological characteristics in Northern Tawny Owls *Strix aluco*: An intra- and interspecific comparative approach
1999	Hans Kristen Stenøien	Dr. scient Bothany	Genetic studies of evolutionary processes in various populations of nonvascular plants (mosses, liverworts and hornworts)
1999	Trond Arnesen	Dr. scient Botany	Vegetation dynamics following trampling and burning in the outlying haylands at Sølendet, Central Norway.
1999	Ingvar Stenberg	Dr. scient. Zoology	Habitat selection, reproduction and survival in the White-backed Woodpecker *Dendrocopos leucotos*
1999	Stein Olle Johansen	Dr. scient Botany	A study of driftwood dispersal to the Nordic Seas by dendrochronology and wood anatomical analysis.
1999	Trina Falck Galloway	Dr. scient. Zoology	Muscle development and growth in early life stages of the Atlantic cod (*Gadus morhua* L.) and Halibut (*Hippoglossus hippoglossus* L.)
1999	Torbjørn Forseth	Dr. scient. Zoology	Bioenergetics in ecological and life history studies of fishes.
1999	Marianne Giæver	Dr. scient. Zoology	Population genetic studies in three gadoid species: blue whiting (*Micromisistius poutassou*), haddock (*Melanogrammus aeglefinus*) and cod (*Gradus morhua*) in the North-East Atlantic
1999	Hans Martin Hanslin	Dr. scient Botany	The impact of environmental conditions of density dependent performance in the boreal forest bryophytes *Dicranum majus*, *Hylocomium splendens*, *Plagiochila asplenigides*, *Ptilium crista-castrensis* and *Rhytidiadelphus lokeus*.
1999	Ingrid Bysveen Mjølnerød	Dr. scient. Zoology	Aspects of population genetics, behaviour and performance of wild and farmed Atlantic salmon (*Salmo salar*) revealed by molecular genetic techniques
1999	Else Berit Skagen	Dr. scient Botany	The early regeneration process in protoplasts from *Brassica napus* hypocotyls cultivated under various g-forces
1999	Stein-Are Sæther	Dr. philos. Zoology	Mate choice, competition for mates, and conflicts of interest in the Lekking Great Snipe
1999	Katrine Wangen Rustad	Dr. scient. Zoology	Modulation of glutamatergic neurotransmission related to cognitive dysfunctions and Alzheimer's disease
1999	Per Terje Smiseth	Dr. scient. Zoology	Social evolution in monogamous families: mate choice and conflicts over parental care in the Bluethroat (*Luscinia s. svecica*)
1999	Gunnbjørn Bremset	Dr. scient. Zoology	Young Atlantic salmon (*Salmo salar* L.) and Brown trout (*Salmo trutta* L.) inhabiting the deep pool habitat, with special reference to their habitat use, habitat preferences and competitive interactions
1999	Frode Ødegaard	Dr. scient. Zoology	Host spesificity as parameter in estimates of arhrophod species richness
1999	Sonja Andersen	Dr. scient Bothany	Expressional and functional analyses of human, secretory phospholipase A2
2000	Salvesen, Ingrid	Dr. scient Botany	Microbial ecology in early stages of marine fish: Development and evaluation of methods for microbial management in intensive larviculture
2000	Ingar Jostein Øien	Dr. scient. Zoology	The Cuckoo (*Cuculus canorus*) and its host: adaptions and counteradaptions in a coevolutionary arms race

2000	Pavlos Makridis	Dr. scient Botany	Methods for the microbial econtrol of live food used for the rearing of marine fish larvae
2000	Sigbjørn Stokke	Dr. scient. Zoology	Sexual segregation in the African elephant (*Loxodonta africana*)
2000	Odd A. Gulseth	Dr. philos. Zoology	Seawater tolerance, migratory behaviour and growth of Charr, (*Salvelinus alpinus*), with emphasis on the high Arctic Dieset charr on Spitsbergen, Svalbard
2000	Pål A. Olsvik	Dr. scient. Zoology	Biochemical impacts of Cd, Cu and Zn on brown trout (*Salmo trutta*) in two mining-contaminated rivers in Central Norway
2000	Sigurd Einum	Dr. scient. Zoology	Maternal effects in fish: Implications for the evolution of breeding time and egg size
2001	Jan Ove Evjemo	Dr. scient. Zoology	Production and nutritional adaptation of the brine shrimp *Artemia* sp. as live food organism for larvae of marine cold water fish species
2001	Hilmo, Olga	Dr. scient Botany	Lichen response to environmental changes in the managed boreal forset systems
2001	Ingebrigt Uglem	Dr. scient. Zoology	Male dimorphism and reproductive biology in corkwing wrasse (*Symphodus melops* L.)
2001	Bård Gunnar Stokke	Dr. scient. Zoology	Coevolutionary adaptations in avian brood parasites and their hosts
2002	Ronny Aanes	Dr. scient	Spatio-temporal dynamics in Svalbard reindeer (*Rangifer tarandus platyrhynchus*)
2002	Mariann Sandsund	Dr. scient. Zoology	Exercise- and cold-induced asthma. Respiratory and thermoregulatory responses
2002	Dag-Inge Øien	Dr. scient Botany	Dynamics of plant communities and populations in boreal vegetation influenced by scything at Sølendet, Central Norway
2002	Frank Rosell	Dr. scient. Zoology	The function of scent marking in beaver (*Castor fiber*)
2002	Janne Østvang	Dr. scient Botany	The Role and Regulation of Phospholipase A_2 in Monocytes During Atherosclerosis Development
2002	Terje Thun	Dr. philos Biology	Dendrochronical constructions of Norwegian conifer chronologies providing dating of historical material
2002	Birgit Hafjeld Borgen	Dr. scient Biology	Functional analysis of plant idioblasts (Myrosin cells) and their role in defense, development and growth
2002	Bård Øyvind Solberg	Dr. scient Biology	Effects of climatic change on the growth of dominating tree species along major environmental gradients
2002	Per Winge	Dr. scient Biology	The evolution of small GTP binding proteins in cellular organisms. Studies of RAC GTPases in *Arabidopsis thaliana* and
2002	Henrik Jensen	Dr. scient Biology	Causes and consequenses of individual variation in fitness-related traits in house sparrows
2003	Jens Rohloff	Dr. philos Biology	Cultivation of herbs and medicinal plants in Norway – Essential oil production and quality control
2003	Åsa Maria O. Espmark Wibe	Dr. scient Biology	Behavioural effects of environmental pollution in threespine stickleback *Gasterosteus aculeatur* L.
2003	Dagmar Hagen	Dr. scient Biology	Assisted recovery of disturbed arctic and alpine vegetation – an integrated approach
2003	Bjørn Dahle	Dr. scient Biology	Reproductive strategies in Scandinavian brown bears

2003	Cyril Lebogang Taolo	Dr. scient Biology	Population ecology, seasonal movement and habitat use of the African buffalo (*Syncerus caffer*) in Chobe National Park, Botswana
2003	Marit Stranden	Dr.scient Biology	Olfactory receptor neurones specified for the same odorants in three related Heliothine species (*Helicoverpa armigera, Helicoverpa assulta* and *Heliothis virescens*)
2003	Kristian Hassel	Dr.scient Biology	Life history characteristics and genetic variation in an expanding species, *Pogonatum dentatum*
2003	David Alexander Rae	Dr.scient Biology	Plant- and invertebrate-community responses to species interaction and microclimatic gradients in alpine and Artic environments
2003	Åsa A Borg	Dr.scient Biology	Sex roles and reproductive behaviour in gobies and guppies: a female perspective
2003	Eldar Åsgard Bendiksen	Dr.scient Biology	Environmental effects on lipid nutrition of farmed Atlantic salmon (*Salmo Salar* L.) parr and smolt
2004	Torkild Bakken	Dr.scient Biology	A revision of Nereidinae (Polychaeta, Nereididae)
204	Ingar Pareliussen	Dr.scient Biology	Natural and Experimental Tree Establishment in a Fragmented Forest, Ambohitantely Forest Reserve, Madagascar
2004	Tore Brembu	Dr.scient Biology	Genetic, molecular and functional studies of RAC GTPases and the WAVE-like regulatory protein complex in *Arabidopsis thaliana*
2004	Liv S. Nilsen	Dr.scient Biology	Coastal heath vegetation on central Norway; recent past, present state and future possibilities
2004	Hanne T. Skiri	Dr.scient Biology	Olfactory coding and olfactory learning of plant odours in heliothine moths. An anatomical, physiological and behavioural study of three related species (*Heliothis virescens, Helicoverpa armigera* and *Helicoverpa assulta*)
2004	Lene Østby	Dr.scient Biology	Cytochrome P4501A (CYP1A) induction and DNA adducts as biomarkers for organic pollution in the natural environment
2004	Emmanuel J. Gerreta	Dr. philos Biology	The Importance of Water Quality and Quantity in the Tropical Ecosystems, Tanzania
2004	Linda Dalen	Dr.scient Biology	Dynamics of Mountain Birch Treelines in the Scandes Mountain Chain, and Effects of Climate Warming
2004	Lisbeth Mehli	Dr.scient Biology	Polygalacturonase-inhibiting protein (PGIP) in cultivated strawberry (*Fragaria* x *ananassa*): characterisation and induction of the gene following fruit infection by *Botrytis cinerea*