The Journal of Experimental Biology 207, 4067-4076
Published by The Company of Biologists 2004
doi:10.1242/jeb.01226

Developmental plasticity of physiology and morphology in diet-restricted European shag nestlings (*Phalacrocorax aristotelis*)

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Accepted 4 August 2004

Summary

Growing animals may exhibit developmental plasticity as an adaptation to variability in the environmental conditions during development. We examined physiological and morphological responses to short-term food shortage of 12–16-day-old European shag nestlings kept under laboratory conditions. After 4 days on a weight maintenance diet, the resting metabolic rate (RMR) of diet-restricted nestlings was 36.5% lower compared with control fed nestlings, after controlling for body mass. This response was accompanied by a reduction in body temperature (T_b) and by reductions in the size of several visceral organs, muscles and lipid stores, while the overall structural growth was maintained almost in line with the age-specific growth rate of controls. Hence, the pattern of energy allocation reflected a very high priority to structural growth at the expense of visceral organs, lipid deposits and muscles. The reduced T_b and size of the liver served as important physiological processes behind the observed reductions in RMR. We discuss the possible adaptive significance of this differential developmental plasticity during temporal food shortage. This is the first study of avian developmental plasticity to report substantial energy saving in combination with a high structural growth rate.

Key words: developmental plasticity, metabolism, growth, development, diet restriction.

Introduction

Food availability plays a crucial role during avian development (Martin, 1987). As nestlings 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 and Ricklefs, 1998; Schlichting and Pigliucci, 1998). Environmental cues can activate alternative, genetically determined, developmental programs (Schmalhausen, 1949; Smith-Gill, 1983). Smith-Gill discussed this in terms of multiple, discrete phenotypic states (‘developmental conversion’; Smith-Gill, 1983). However, the basic premise, that the organism actively alters development as an adaptive response to environmental cues, applies equally to continuous measures of metabolism or growth (‘induced responses’; Schew and Ricklefs, 1998). Alternatively, the organism shows a passive response, usually non-adaptive, in which the phenotypic changes are ‘imposed’ by the environment.

Developmental plasticity, caused by poor feeding conditions, can affect adult morphology (De Kogel, 1997; Birkhead et al., 1999) and result in long-term consequences (Lindström, 1999; Metcalfe and Monaghan, 2001; Dufty et al., 2002). However, developmental plasticity can also show reversible patterns (Schew and Ricklefs, 1998). Energy expenditure and body temperature (Prinzinger and Siedle, 1988; Schew, 1995), but also morphology (Emlen et al., 1991; B.M., S.B., D.M., T.E.B. and C.B., unpublished data), may show considerable reversible short-term responses to temporal variation in environmental conditions during the development. A number of recent studies have investigated how growing birds can modify the pattern of energy use and allocation as a response to short-term diet restriction (e.g. Schew, 1995; Kitaysky, 1999; Konarzewski and Starck, 2000; Brzek and Konarzewski, 2001; Moe et al., in press). Physiological and morphological responses of nestlings to short-term diet restriction form a practical experimental system for studying developmental plasticity, an important aspect of life-history.

Fluctuations in food availability (Konarzewski and Starck, 2000) and sibling competition (Brzek and Konarzewski, 2001) are among the factors that may have selected for adaptive developmental responses to temporal food shortage (Schew and Ricklefs, 1998). During periods of food shortage, lasting less than some critical proportion of the chick’s growth period and longer than a short period that can be easily buffered by stored energy reserves, a reduction in metabolism is expected

to enhance survival (Schew and Ricklefs, 1998). Sibling competition has also been suggested to select for reductions in metabolic rate (MR) as a response to temporal food shortage (Brezek and Konarzewski, 2001), but it has been suggested to select against slowing of growth and maturation of the parts of the skeleton most important in competing with nest mates for food (Schew and Ricklefs, 1998). This is apparently conflicting if growth rate and metabolism is positively related (Drent and Klaassen, 1989; Klaassen and Drent, 1991), and it would require a substantial change in the energy allocation from maintenance to growth.

Modification of the basal level of energy expenditure could occur as an adaptive response to food shortage. 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 (Piersma and Lindstrøm, 1997) and in growth rate (Emlen et al., 1991) could also be adaptive responses. 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), but the specific organs and tissues that predict RMR differ among studies (e.g. Burness et al., 1998; Bech and Østnes, 1999; Chappell et al., 1999; Moe et al., in press). Hence, it is not fully understood how body composition functionally relates to RMR.

Despite the view that the skeleton and the nervous system are regarded as less flexible compared with visceral organs and physiological processes (Schew and Ricklefs, 1998; Pigliucci, 2001), several distinct growth patterns in response to food shortages have been reported. At reduced levels of energy intake, the structural growth rate can be maintained rigidly within the limits of the food intake (e.g. Konarzewski et al., 1996). By contrast, growth and development can be temporally stalled (e.g. Emlen et al., 1991; Schew, 1995; Starck and Chinsamy, 2002). Alternatively, energy can be specifically allocated to growth of favoured structural elements at the expense of others (e.g. Øyan and Anker-Nilssen, 1996; Kitaysky, 1999; Moe et al., in press).

Nestling European shags (*Phalacrocorax aristotelis* L.) are very well suited for studying physiological and morphological responses to temporal food shortage. As individual nestlings exhibit high growth rates (Østnes et al., 2001) and compete with siblings for food (Amundsen and Stokland, 1988; Velando et al., 1999, 2000), they depend on successful food provisioning rates to follow their normal developmental trajectory. Owing to a very low deposition of lipids (Bech and Østnes, 1999), the nestlings have a limited capacity for buffering temporal food shortages. In the study area, the European shag is an inshore and offshore benthic feeder, and relies on gadoids (Barrett et al., 1990). It is reported that nestling European shags are likely to encounter variable food provisioning during early development due to adverse weather conditions, which affects the foraging success of the parents (Velando et al., 1999). Adverse weather also increases the need for brooding at the expense of foraging (Beintema and Visser, 1989).

The evolution of developmental responses is driven by natural selection and limited by internal constraints (Starck and Ricklefs, 1998; Ricklefs et al., 1998; Pigliucci, 2001), of which genetic and developmental constraints are important (Pigliucci, 2001). Hence, developmental mode, in the altricial–precocial spectrum, could possibly constrain or determine the physiological and morphological responses to temporal food shortage. However, only a few altricial species, of which all were passerines, have been investigated in this context. So far, contrasting patterns of physiological responses have been revealed. Sand martins (*Riparia riparia*; Brzek and Konarzewski, 2001) and house martins (*Delichon urbica*; Prinzinger and Siedle, 1988) use hypothermia and lower their basal metabolism, while song thrushes (*Turdus philomelos*; Konarzewski and Starck, 2000) and starlings (Schew, 1995) do not show any energy-saving responses to temporal food shortage. In addition, contrasting patterns of structural growth have been revealed (e.g. white-fronted bee-eaters, *Merops bullockoides*, *versus* song thrushes; Emlen et al., 1991; Konarzewski et al., 1996). This study is the first study to investigate physiological and morphological developmental responses to temporal food shortage in an altricial seabird.

In the present study, we experimentally imposed short-term diet restriction on 12–16-day-old nestling European shags, kept under laboratory conditions. Mass-specific RMR is very high during this age period (Bech and Østnes, 1999; Østnes et al., 2001). We reveal whether nestling European shags exhibit any energy saving that can lessen the detrimental effects of reduced food intake during early development, and reveal how the nestlings allocated the energy between maintenance and growth. We also assess whether hypothermia or changes in body composition are components of any energy saving processes. Information about the effect of diet restriction on thermoregulatory capacity and on subsequent growth during re-alimentation will be published elsewhere.

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°12′N, 11°00′E). In 2001, the breeding population of European shags (*Phalacrocorax aristotelis* L.) consisted of 1750 pairs (N. Røv, personal communication), and it has increased (6.3% annually) in the period 1984–2001 (Lorentsen, 2001). We marked 355 nests that were visited every second day to determine the exact 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.

Housing conditions, feeding protocols and treatment groups

A sample of 34 nestlings was brought to the laboratory at the age of 12 days for the purpose of subsequent metabolic measurements. The nestlings were kept, 4–8 together, in an enclosure (100×50 cm) with a heat lamp providing a constant range of operative temperatures (Bakken, 1992) of 22–33°C. We randomly assigned 12 nestlings to a diet-restricted 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 4 days, until they were 16 days 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. The National Committee for Animal Research in Norway ('Forsøksdyrutvalget') approved the experimental protocols.

Metabolic measurements

Oxygen consumption rates were measured by open-flow respirometry (Withers, 1977). 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 dried before a fraction of the air was directed to the oxygen analyser (Servomex, Crowborough, East Sussex, UK; type 244A). The oxygen (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 stored, 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 s intervals.

The metabolic measurements were performed on post-absorptive nestlings. The lengths of fasting before 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 higher gut content.

The metabolic measurements were performed at different times of the day, but diet-restricted nestlings and controls were randomly measured with respect to time of the day. More importantly, RMR showed no diurnal cycle.

O_2 consumption rates were calculated by using formula 1d in Withers (1977), assuming a constant respiratory quotient 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 MRs 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 a 25 min running average during exposure to thermoneutral conditions. 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 that varied from 5–60 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).

The metabolic chamber was a water-jacketed vessel connected to a temperature controller (Grant Instruments, Royston, UK; type LT D G) that provided control of the T_a in the inner metabolic chamber. The T_a was set between 29–31°C for thermoneutral conditions (Østnes et al., 2001). T_a was measured with a copper–constantan thermocouple (California Fine Wire Company, Grover City, CA, USA; type 0.005) mounted inside the metabolic chamber, and T_b was measured in the cloaca with a Cu–Co thermocouple surrounded by a polypropylene tubing (outer diameter 0.96 mm). Depending on the nestling's size, the thermocouple was inserted 2–4 cm into the cloaca and secured with adhesive tape. Thermal conductance (*TC*) during thermoneutral conditions was calculated according to the following formula:

$$TC = \mathrm{RMR}/(T_b - T_a)\ . \quad (1)$$

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 was obtained. To obtain independent measurements, each individual was only used once in the experiments, and all nestlings originated from different nests.

Body composition

A sample of 28 of the 34 nestlings was anaesthetised with ether (inhalation) and sacrificed by suffocation immediately after the metabolic measurements and stored at –20°C for later analysis of body composition. The remaining six nestlings were brought back to the nest of origin or to a nest with foster parents. Dissection was done on semi-thawed carcasses to reduce vaporisation and to 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. The mass of these muscles was multiplied by two to get the total breast and leg muscle mass.

Gizzard, intestine and heart atrium was 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°C and reweighed. Fat content was subsequently removed in baths of petroleum ether for a minimum of 24 h. Baths were changed until the yellow colour (lipid) of the solution disappeared and became clear, 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 a tissue (Ricklefs et al., 1994).

Morphology and growth

Biometry [wing length, tarsus length, skull length (head + bill)] and body mass of the nestlings were measured every day in the laboratory. Growth rates were calculated as the daily growth (mm day^{-1} and g day^{-1}) during the 4 days (from 12–16-day old). Hence, growth rates of structural elements and body mass were obtained for 16-day-old diet-restricted and 16-day-old control nestlings. We used a principal component analysis to extract a factor score (PC1) from the growth rate of the wing, tarsus and skull.

For comparison, we measured the growth of nestlings that were fed by their parents in the colony. Measurements of body mass (N=1645) and biometry (N=1050) were fitted to a logistic equation. Specific growth rates (g day^{-1} and mm day^{-1}) from the age of 12–16 days were obtained from eight nestlings of which we had repeated measures.

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) before examination.

We analysed the relationship between organ mass and MR 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). 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). Co-linearity 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 GLM procedure was performed unless otherwise specified. The Student's t-test was used for comparison of means of two groups. The Bonferroni method was used for *post hoc* pairwise multiple comparisons ('post hoc' hereafter). This method reports adjusted P-values that have been multiplied with the number of pairs tested. Values reported are means ± 1 S.E.M. All statistical tests were performed with SPSS version 11.5.1 (2002).

Results

Food intake and body mass

The feeding protocols resulted in considerable differences in the daily food intake. The total food intake of the diet-restricted nestlings was only 46% of that of the control fed nestlings. The daily food intake, calculated over the preceding 24 h, of diet-restricted nestlings decreased through the diet-restriction period and ranged from 132±9 to 81±6 g day^{-1} for 13- and 16-day-old nestlings, respectively (Fig. 1A). By contrast, the control fed nestlings increased their food intake during the first 3 days being 177±7 and 261±13 g day^{-1} at the age of 13 and 15 days, respectively. The daily food intake at the age of 16 days (220±19 g d^{-1}) was lower compared with that at 15 days, because of the fasting before the metabolic measurement on day 16.

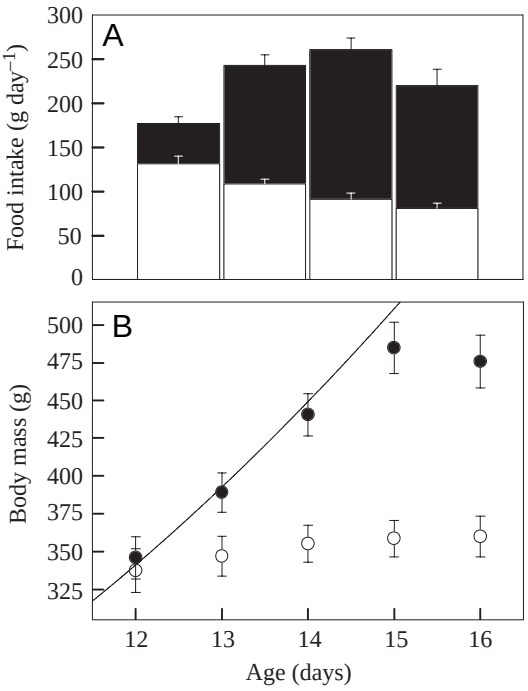

Fig. 1. Daily food intake (A) and body mass (B) as a function of age in controls (black bars and black symbols) and diet-restricted nestlings (open bars and open symbols) of European shags kept in the laboratory. The regression line of a logistic growth curve calculated from 1645 body mass measurements 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 g day^{-1}. Values are means ± 1 S.E.M.

The differences in daily food intake had a huge effect on the body mass (Fig. 1B). The body mass of the diet-restricted nestlings was maintained at a relatively stable level, but with a significant gain of 5.7±0.8 g day^{-1} (P<0.001). By contrast, the control fed nestlings followed a normal body mass growth trajectory, to the age of 15 days, close to that of the nestlings fed by parents in the colony (Fig. 1B). However, at the age of 16 days the body mass growth of the control fed nestlings deviated substantially from that of the nestlings fed by parents, mainly due to the fasting before the metabolic measurements.

Resting metabolic rate and body temperature

RMR scaled to body mass by the power of 0.84±0.12 (mean ± S.E.M.; $F_{1,31}$=51.2, P<0.001; Fig. 2) in both groups (RMR × body mass interaction, $F_{1,30}$=0.5, P>0.1). RMR was substantially 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; Fig. 2). 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. The mass-specific RMR of the 16-day-old diet-restricted nestlings was lower compared with the 16-day-old controls (*post hoc*, P<0.001) and the 12-day-old controls (*post hoc*, P<0.001), whereas that of 16-day-old controls and 12-day-old controls was not significantly different (*post hoc*, P>0.1).

Diet-restricted nestlings exhibited a lower T_b compared with controls. A *post hoc* comparison showed that the T_b of 16-day-old diet-restricted nestlings (36.1±0.34°C) was 2.1°C lower compared with 16-day-old controls (38.2±0.15°C; P<0.001; Fig. 3). The diet-restricted nestlings also exhibited a lower T_b than expected from body mass ($F_{1,28}$=8.58, P<0.007). The T_b of diet-restricted nestlings of 355 g (the mean body mass of the 16-day-old diet-restricted nestlings) was 1.3°C lower than predicted for controls of the same body mass.

Structural growth

The growth of the skull, tarsus and wings is given in Fig. 4A–C for the nestlings of which we had biometric measurements every day. The growth rates of the skull (*t*-test, t=–2.7, d.f.=10, P<0.05; Fig. 4D) and the wings (*t*-test, t=–2.3, d.f.=10, P<0.05; Fig. 4F) were slightly lower in the 16-day-old diet-restricted nestlings compared with the 16-day-old controls. By contrast, the growth rate of the tarsus (*t*-test, t=0.1, d.f.=10, P>0.1; Fig. 4E) was not significantly different between the controls and the diet-restricted nestlings. Thus, the structural growth of the diet-restricted nestlings was almost in line with the age-specific growth of the control fed nestlings, and it contrasted to the vast reductions in body mass growth rate (Fig. 4D–F). With respect to body mass, the 16-day-old diet-restricted nestlings exhibited 17.8% longer wings ($F_{1,20}$=38.5, P<0.001), 13.0% longer tarsus ($F_{1,20}$=36.4, P<0.001) and 10.4% longer skull ($F_{1,20}$=84.7, P<0.001) compared with the controls (12 and 16 days old).

The growth of the nestlings that were fed by their parents in the colony is also shown in Fig. 4. Comparisons between diet-restricted nestlings and nestlings fed by their parents were consistent with the results above. Diet-restricted nestlings exhibited a lower growth rate of the skull (*post hoc*, P<0.05; Fig. 4D) and the wings (*post hoc*, P<0.001; Fig. 4F), while the growth rate of the tarsus was not significantly different (*post hoc*, P>0.1; Fig. 4E) compared with that of the nestlings fed by their parents. The structural growth trajectory of the control fed nestlings in the laboratory was slightly different to that of the nestlings fed by their parents in the colony (Fig. 4A–C). While the growth rate of the skull (*post hoc*, P>0.1; Fig. 4D) and the tarsus (*post hoc*, P>0.1; Fig. 4E) did not differ significantly, the growth rate of the wings was significantly lower in the control fed nestlings compared with that of the nestlings fed by their parents (*post hoc*, P<0.01; Fig. 4F).

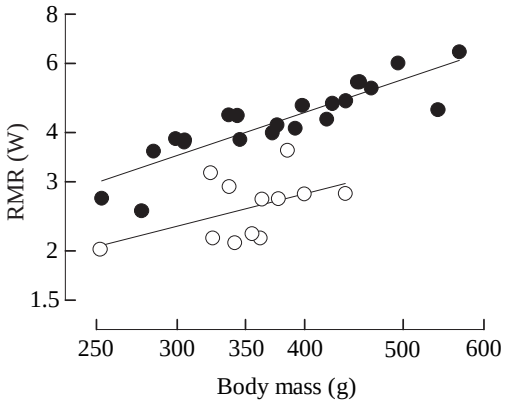

Fig. 2. Resting metabolic rate (RMR) as a function of body mass (M_b) in controls (filled symbols) and diet-restricted nestlings (open symbols) of European shags. The axes are log-scaled, and linear regression lines are shown for each treatment group [log RMR controls=0.87(±0.11)×log M_b–1.62(±0.28), r=0.88; log RMR diet-restricted=0.66(±0.37)×log M_b–1.27(±0.95), r=0.49].

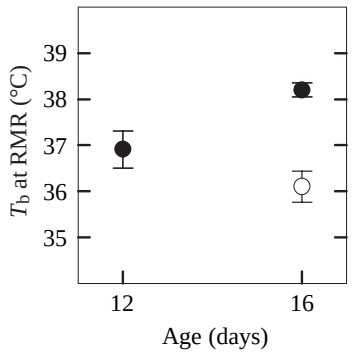

Fig. 3. Body temperature at resting metabolic rate as a function of age in controls (filled symbols) and diet-restricted nestlings (open symbols) of European shags. Values are means ± 1 S.E.M.

Body composition

In contrast to the structural components, organs and muscles were either reduced or maintained with respect to body mass as a response to the diet restriction (Fig. 5). With respect to body mass, the total lipid mass ($F_{1,25}$=69.6, P<0.001; Fig. 5A), the liver mass ($F_{1,25}$=97.4, P<0.001; Fig. 5B), the pectoral muscle

mass ($F_{1,25}$=19.9, P<0.001; Fig. 5C), the heart mass ($F_{1,25}$=18.2, P<0.001; Fig. 5D), the gizzard mass ($F_{1,24}$=25.9, P<0.001) and the kidney mass ($F_{1,25}$=4.9, P<0.05) of the diet-restricted nestlings were 41.4, 29.2, 18.9, 17.4 and 9.8% lower compared with that of the controls, respectively. In addition, the leg muscle mass tended to be slightly lower in diet-restricted nestlings compared with controls ($F_{1,25}$=3.8, P=0.06; Fig. 5E). However, one visceral organ, the intestine, was strictly maintained with respect to body mass as a response to the diet restriction. Both the mass ($F_{1,25}$=0.1, P>0.1; Fig. 5F) and the length of the intestine ($F_{1,25}$=0.4, P>0.1) of the diet-restricted nestlings was not different to that of the controls. The lean dry fraction (LDF) was not different between 16-day-old diet-restricted and 16-day-old controls in any organ or muscles, except for the intestine. The LDF of the intestine was lower in diet-restricted nestlings compared with controls (*post hoc*, P<0.05).

To control for age-dependent effects on body composition, we also performed separate analyses of body composition (organ mass/body mass) in relation to age and treatment. The results from those analyses were consistent with the analyses that were performed in relation to body mass.

Correlations between organ masses and metabolic rate

To evaluate whether the changes in body composition could explain any of the differences in RMR between the treatment groups, we tested whether organ masses correlated to RMR (Table 1). The lean dry mass of the liver (r=0.64, $F_{1,22}$=15.2, P<0.001), the pectoral muscles (r=0.50, $F_{1,23}$=7.8, P<0.01) and the lipid mass (r=0.44, $F_{1,24}$=5.8, P<0.05) correlated significantly and positively with RMR, while the lean dry mass of the leg muscles, heart, gizzard, kidney and intestine did not. We controlled for organ LDF, body mass (minus organ mass) and treatment in these analyses (Table 1). Treatment was a strong and significant factor in all the models.

Growth rates and RMR

The extracted factor score (PC1) from a principal component analysis explained 57% of the variance in the $\log_{10}$-transformed growth rates of the skull (r=0.93), tarsus (r=0.45), and wings (r=0.80). The PC1 correlated positively with RMR ($F_{1,8}$=8.5, P<0.05) and the interaction (treatment×PC1) was not significant ($F_{1,7}$=0.5, P>0.1), indicating that structural growth and RMR was positively related within both treatment groups. Body mass and treatment were controlled for by including them in the analyses as a covariate and a factor, respectively.

Discussion

Energy saving

The European shag nestlings showed substantial energy saving

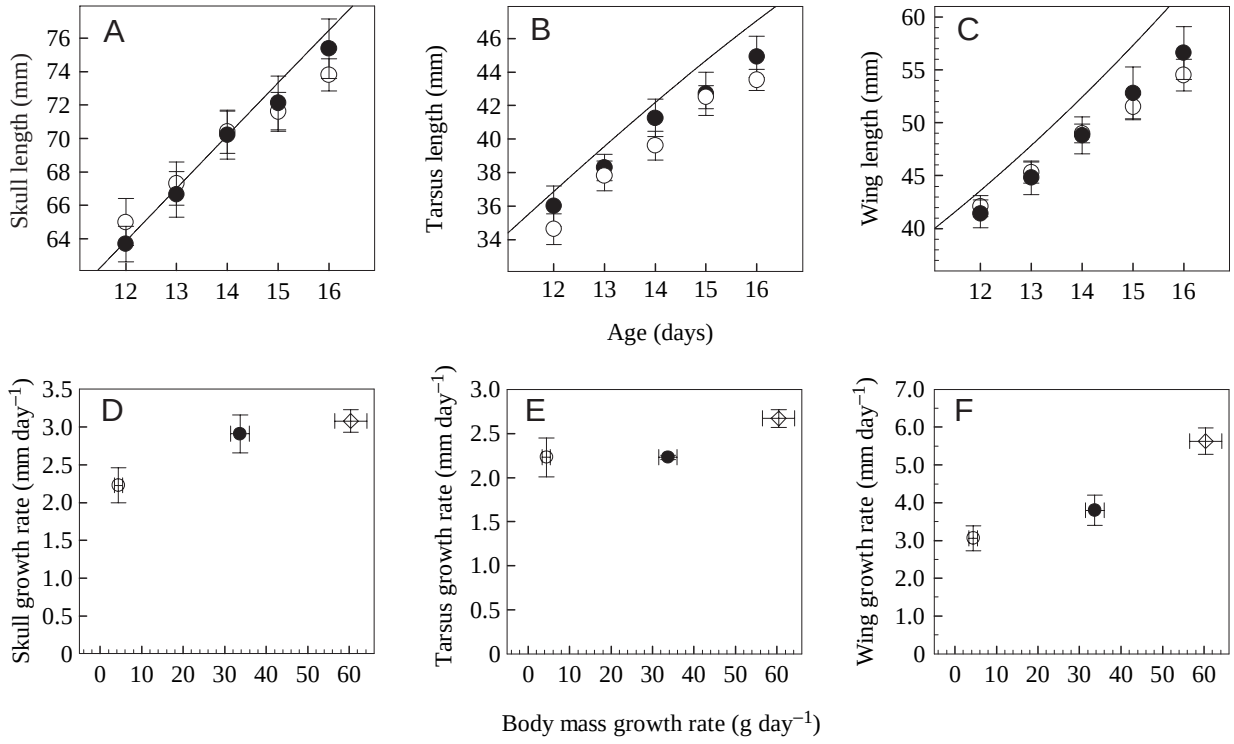

Fig. 4. Length of skull (A), tarsus (B) and wing (C) as a function of age, as well as growth rates of the skull (D), tarsus (E) and wings (F) as a function of the body mass growth rate in control fed (filled symbols, N=5) and diet-restricted nestlings (open symbols, N=7) kept in the laboratory. The regression line of a logistic growth curve calculated from 1050 biometric measurements of nestlings fed by their parents in the colony is shown for comparison in A–C. In D–F, the growth rate of a sample of eight nestlings fed by their parents in the colony, of which we had biometric measurements at the age of 12 and 16 days, is shown for comparison (open diamonds). The growth rates were calculated for the period from the age of 12–16 days. Values are means ± 1 S.E.M.

in response to short-term food shortage. The RMR was 36.5% lower in the diet-restricted nestlings compared with the control fed nestlings after controlling for body mass. In several bird species, hypothermia is suggested to play an important part of an energy saving response to food shortage during ontogenetic development (*Oceanodroma furcata*, Boersma, 1986; *Delichon urbica*, Prinzinger and Siedle, 1988; *Coturnix coturnix japonica*, Schew, 1995; *Riparia riparia*, Brzek and Konarzewski, 2001; *Anas platyrhyncos domesticus*; Moe et al., in press). However, hypothermia is not necessarily a prerequisite for a hypometabolic developmental response, as hypothermia only occurred in the youngest age group of diet-restricted ducklings (Moe et al. in press). In this study, the 16-day-old diet-restricted nestlings showed a moderate hypothermic response and regulated their T_b 2.1°C below the T_b of the 16-day-old controls. By using the measured value for thermal conductance (1.12 W kg^{-1} deg.$^{-1}$; where deg. are Celsius) of the diet-restricted nestlings during thermoneutral conditions and the measured value for T_b and T_a of 16-day-old controls (38.3 and 29.4°C, respectively), we calculated that the hypothermia accounted for 68% of the observed difference in mass-specific RMR between 16-day-old controls and diet-restricted nestlings. Furthermore, a Q_{10} effect, assuming a Q_{10} between 2 and 2.5, explained 46–63% of the energy savings caused by hypothermia. Hence, a major part (57–69%) of the reduction in RMR must be due to other physiological processes than just the temperature dependence of RMR.

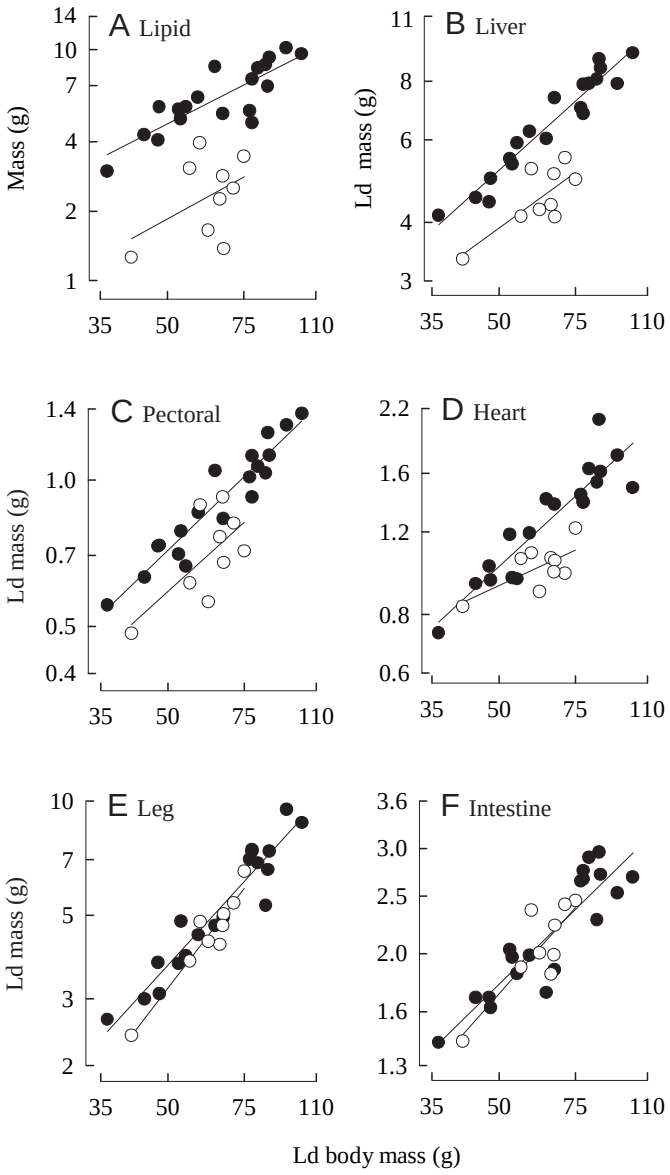

Fig. 5. Body composition of controls (filled symbols) and diet-restricted nestlings (open symbols) of European shags. The relationship of lipid mass (A), liver mass (B), pectoral muscle mass (C), heart mass (D), leg muscle mass (E) and intestine mass (F) to body mass. The axes are log-scaled, and the linear regression lines are shown for each treatment group. Organ and body masses are lean dry (Ld) masses in g, and lipid mass is dry mass in g.

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

	r	$F_{1,23}$[a]	*P*
Pectoral muscle	0.50	7.8	<0.01
Leg muscle	–0.05	0.3	NS
Heart	0.17	0.7	NS
Liver	0.64	15.2	<0.001
Gizzard	–0.26	1.7	NS
Kidney	0.09	0.2	NS
Intestine	0.25	1.5	NS
Lipid	0.44	5.8	<0.05

Separate general linear model (GLM) analyses were performed for each organ. The null models included lean dry organ mass, organ lean dry fraction (LDF) and lean dry body mass (minus organ mass) as covariates, treatment as factor and the interactions organ mass × treatment and LDF × treatment. [a]d.f. Liver were 1, 22 and d.f. lipid were 1, 24. NS, not significant.

The visceral organs are believed to consume much of the energy used in basal metabolism (Daan et al., 1990; Chappell et al., 1999). In our study, the mass of the liver, the pectoral muscles and the lipid mass were positively correlated to RMR. Liver tissue has a high intrinsic MR (Scott and Evans, 1992), and Bech and Østnes (1999) suggested the liver to have a great influence on RMR of nestling European shags. In a study on metabolic responses to food-shortage, the liver size was a significant predictor of the differences in RMR between diet-restricted and *ad libitum* fed ducklings (Moe et al., in press). The positive correlation between the liver mass and RMR in the present study, indicate that the reductions in the liver mass of the diet-restricted nestlings could be an important predictor of the observed differences in RMR. However, liver mass and treatment were a strong and significant covariate and factor, respectively, in the GLM model. Consequently, variation in liver mass together with other physiological changes induced by the diet-restriction treatment must have affected RMR. By using 2.71 ml O_2 g^{-1} h^{-1} for liver MR (Scott and Evans, 1992), liver mass changes explained only 6% of the difference in the

overall RMR between the controls and the diet-restricted nestlings. Such a quantitative value of the reduction in RMR should, however, be treated carefully as Scott and Evans (1992) measured the MR of liver samples from adult birds and in different species to ours.

We also revealed a positive correlation between the pectoral muscles and RMR, which has also been found in juvenile and adult house sparrows (*Passer domesticus*; Chappell et al., 1999) and in migrating knots (*Calidris canutus*; Weber and Piersma, 1996). However, in contrast to the juvenile and adult birds mentioned above, the pectoral muscles of the young shag nestlings are very small, constituting only 2% of the total body mass. Hence, the variation in the mass of the pectoral muscles should have a negligible impact on the variation in total RMR in this study. The total lipid mass also correlated positively with RMR, but adipose tissue has a very low intrinsic MR (Scott and Evans, 1992) and constitutes <2% of the total body mass of the shag nestlings. Consequently, the lipid mass should not contribute significantly to the total RMR in the shag nestlings. If the pectoral muscles and the lipid mass do not contribute directly to the total RMR, but still correlate positively to total RMR, they should correlate to other physiological processes with direct impact on RMR. We suggest that the lipid mass and the pectoral muscle mass could play a possible role as an internal signal on nutritional status (i.e. body condition) to which the basal level of energy expenditure could be regulated.

Energy allocation to growth

The diet-restricted nestlings maintained structural growth very well despite a food intake of only 46% of that of the control fed nestlings. The growth rate of the tarsus was not different to that of control fed nestlings, and the skull and wings showed only a slightly lower growth rate. This rigid pattern of structural growth was accompanied by a rigid development of maturity (LDF) of the muscles and the visceral organs (except intestine). The energy devoted to maintenance and growth constitute substantial parts of the total energy budget during postnatal development (Weathers, 1996). Slowing of structural growth has been regarded as one of the means to lower RMR during temporal food shortage (Schew and Ricklefs, 1998). The high structural growth rate combined with the low RMR, observed in the diet-restricted nestlings in the present study, could support the suggested independent (Ricklefs and White, 1981) or negative (Olson, 1992) relationship between RMR and growth rate. However, the principal component for structural growth rate was positively correlated to RMR, indicating an energetic cost of high structural growth rate within both treatments. The positive relationship is expected if RMR includes 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) or if RMR includes direct cost of growth in terms of cost of biosynthesis. Although the maintenance of the high structural growth rate may have been energetically cheap (Ricklefs and White, 1981), the observed response must have required a substantial change in the energy allocation from maintenance to growth.

However, it is difficult to evaluate the relative importance of structural nutrients and energy nutrients as limiting factors for structural growth. Calcium and phosphorus are essential inorganic structural nutrients during growth (Murphy, 1996). If these nutrients, rather than energy, primarily limit the rate of structural growth it suggests that the nestlings were provided well in excess during normal conditions and still in sufficient amount during the food restriction. Energy nutrients, such as amino acids, may also limit structural growth, but they appear to be actively scavenged from most visceral organs and the skeletal muscles during the diet restriction.

Differential developmental plasticity

This study clearly demonstrates differential plasticity in the development of the physiology and morphology of nestling European shags in response to food shortage. The substantial energy saving was accompanied by a pattern of energy allocation reflecting a rigid priority of the structural growth at the expense of visceral organs, lipid deposits and muscles. Our results contrast with studies on nestling song thrushes (Konarzewski et al., 1996; Konarzewski and Starck, 2000) and nestling European starlings (*Sturnus vulgaris*; Schew, 1995) that showed limited plasticity both in the physiological (metabolism) and in the morphological (structural growth) responses to temporal food shortages. Several studies on other species, however, have revealed flexible development of RMR and body temperature (e.g. Schew, 1995; Brzek and Konarzewski, 2001), body composition (e.g. Moe et al., in press) and skeletal growth (e.g. Emlen et al., 1991) in response to temporal food shortage.

We did not monitor the changes in RMR and body temperature over the course of the diet restriction period, which is purported to be necessary to detect adaptive responses (Schew and Ricklefs, 1998). However, the observed reduction in RMR may have an adaptive significance in lessening the detrimental effects of food shortage and increasing survival. Alternatively, the low RMR resulted from pathological changes or as a passive effect of lack of nutrients (‘imposed response’; Schew and Ricklefs, 1998). Deleterious pathological changes probably did not occur. Diet-restricted shag nestlings resumed normal body mass growth immediately at the onset of re-alimentation (B.M., S.B., D.M., T.E.B. and C.B., unpublished data), indicating that the cellular structures responsible for growth and metabolism were intact. Lack of nutrients also seems unlikely because the structural growth was maintained so well, indicating that nutrients could have been devoted to basal metabolism at the expense of structural growth. However, different nutrients may limit basal metabolism compared with structural growth (energy nutrients *versus* structural nutrients).

If the observed differential developmental plasticity is adaptive and results from adaptations, what could be the selective factors for low RMR and high skeletal growth rates in response to food shortages? Frequent unpredictable

fluctuations in food availability (Schew and Ricklefs, 1998; Konarzewski and Starck, 2000) are purported to select for low RMR. A recent experiment by Kitaysky (1999) showed greater metabolic responses to food shortage in the piscivorous horned and tufted puffins (*Fratercula corniculata* and *Lunda cirrhata*), which rely on fluctuating food resources, compared with the planktivorous crested and parakeet auklets (*Aethia cristatella* and *Cyclorhinchus psittacula*), which rely on continuously available food resources. However, this could also have a phylogenetic explanation because the puffins also behaved more similarly to each other than they did to the auklets. Brzek and Konarzewski (2001) demonstrated a reduced RMR in diet-restricted sand martin nestlings, a response that was amplified by the presence of hungry siblings. Therefore, they suggested a link between developmental flexibility of RMR and sibling competition.

Sibling competition has also been suggested to select against slowing of growth and maturation, especially the parts of the skeleton most important in competing with nest mates for food, because slowing of growth of such parts would decrease the competitive abilities of the individual nestling (Schew and Ricklefs, 1998). Within broods with established size hierarchies due to hatching asynchrony (Stokland and Amundsen, 1988), structural size may determine the ability to obtain the optimal position in the nest for begging (Ryden and Bengtsson, 1980; Bengtsson and Ryden, 1981; Gottlander, 1987; McRae et al., 1993).

By contrast, it has been argued (Ricklefs, 1993; Schew and Ricklefs, 1998) that hatching asynchrony could relax the selection to maintain a rigid growth trajectory in response to temporal food shortage, because hatching order predetermines the rank in the competitive hierarchy within the brood. However, an established rank in the hierarchy does not necessarily prevent competition within asynchronous broods. Amundsen and Stokland (1988) manipulated the degree of asynchrony in nestling European shags and emphasised the importance of the magnitude of the size disparities within the brood, and not only the rank in the hierarchy. Therefore, we believe the competitive abilities of European shag nestlings are sensitive to growth changes as a response to temporal food shortage.

Sibling competition is not the only possible selective factor for a rigid skeletal growth trajectory, since it has also been reported in species producing a single chick. Chicks of the grey-headed albatross (*Diomedea chrysostoma*; Reid et al., 2000) showed a general rigid structural growth, while Atlantic puffin chicks (*Fratercula arctica*; Øyan and Anker-Nilssen, 1996) showed a high priority to growth of feathers and skull in response to food shortage. Both studies, however, emphasised the importance of structures responsible for early fledging and early post-fledging survival.

Slowing of structural growth as a response to food shortage usually delays developmental time (e.g. Emlen et al., 1991; Lepczyk and Karasov, 2000). Delayed fledging is disadvantageous in species with high risk of nest predation, and nestling European shags are exposed to predation (e.g. from the great black-backed gull, *Larus marinus*). Because predation rates are regarded as a selective factor for growth rate and developmental time (Remes and Martin, 2002), predation may also be a selective factor for reduced plasticity in structural growth in the European shag.

In conclusion, we have shown that nestling European shags exhibit substantial energy saving as a response to temporal food shortage, and that reductions in T_b and in the size of the liver serve as important physiological processes behind the energy saving. In contrast to reductions in most visceral organs and muscles, the overall structural growth was very well maintained, showing nearly the same age-specific growth rate as the controls. These physiological and morphological responses demonstrate differential developmental plasticity in the European shag nestlings.

The Norwegian Directorate for Nature Management, Trondheim, gave us permission to work in the shag colony. We are indebted to the Norwegian lighthouse authorities for permission to use the facilities on Sklinna, the lighthouse keepers on Sklinna for their hospitality and to Carolyn Baggerud for improving the English. We thank J. Matthias Starck and two anonymous referees for providing helpful comments on an earlier version of the manuscript. C.B. and B.M. were supported by a grant from the Research Council of Norway (138698/410).

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