The Journal of Experimental Biology 204, 3065–3074 (2001)
Printed in Great Britain © The Company of Biologists Limited 2001
JEB3701

Effect of food shortage on the physiology and competitive abilities of sand martin (Riparia riparia) nestlings

Paweł Brzęk* and Marek Konarzewski

Institute of Biology, University of Białystok, Świerkowa Street 20B, 15-950 Białystok, PO Box 109, Poland

*Author for correspondence (e-mail: brzekpaw@cksr.ac.bialystok.pl)

Accepted 26 June 2001

Summary

We examined developmental and behavioural plasticity of sand martin (*Riparia riparia*) nestlings hand-reared under laboratory conditions. We created six broods of six 4-day-old nestlings and randomly assigned them to one of the two following feeding regimens, each lasting for 3 days: (1) all nestmates fed a similar, limited amount of food (FR nestlings). This simulated synchronous hatching under conditions of food restriction. (2) Half the brood were food-restricted (FR/AL nestlings), and half were fed *ad libitum* (AL nestlings), as in asynchronously hatched broods with differential food allocation. Under both regimens, food restriction resulted in a reduction in body mass, intestinal mass, pectoral muscle mass, fat reserves, body temperature and resting metabolic rate (RMR). However, it simultaneously triggered a significant increase in intestinal uptake rates of L-proline and locomotor activity, quantified as frequency of crawling into the artificial nest tunnel by individual nestlings. Locomotor activity and intestinal uptake rates of L-proline by FR nestlings were higher than those of FR/AL young, while body temperature and RMR of FR nestlings were lower.

We conclude that food-restricted nestlings responded actively to food shortages by upregulating their gut function, reducing the energy costs of maintenance and increasing locomotor activity. These behavioural and physiological responses were strongest in broods of similar-sized FR nestlings, which can be interpreted as an escalation of sibling competition. Thus, developmental and behavioural plasticity may be an important factor in the evolution of sibling rivalry.

Key words: hatching asynchrony, sibling competition, energetics, digestive physiology, sand martin, *Riparia riparia*.

Introduction

Variation in fluctuation of food supplies experienced during ontogeny has important consequences for life history (Gebhardt-Henrich and Richner, 1998). The response of a growing organism to food shortage is largely determined by the plasticity of its developmental program (Smith-Gill, 1983; Schlichting and Pigliucci, 1998). In principle, such plasticity may take one of two forms. It may encompass a passive response to the stressful situation, which reflects invariability of the genetic programs controlling the rate of development (phenotypic modulation, *sensu* Smith-Gill, 1983). Some organisms, however, are capable of activating alternative developmental programs, which allows them to respond actively to adverse environmental conditions (developmental conversion, *sensu* Smith-Gill, 1983; for a review, see Schlichting and Pigliucci, 1998). Both forms of plasticity are well exemplified by the different reactions of altricial nestling birds to food shortage. Young starlings *Sturnus vulgaris* (Schew and Ricklefs, 1998), house sparrows *Passer domesticus* (Lepczyk et al., 1998) or song thrushes *Turdus philomelos* (Konarzewski et al., 1996; Konarzewski and Starck, 2000) attempt to continue their normal pace of growth and postembryonic development within the limits imposed by undernourishment. In contrast, the nestlings of aerial insectivorous birds (such as Meropidae, Apodidae and Hirundinidae) are capable of slowing the rate of development during periods of inclement weather and resuming the normal rate when the feeding conditions improve (Lack and Lack, 1951; Bryant, 1975; O'Connor, 1978; Emlen et al., 1991).

The fate of an altricial nestling is determined, however, not only by variability of the environmental conditions outside the nest but also, critically, by its ability to compete for limited nourishment with its nestmates (Mock and Parker, 1997). It is therefore likely that developmental plasticity increases the competitive abilities of the nestlings. The chances of successful fledgling can be further increased if developmental plasticity enhances behavioural adaptations that help to monopolise food supplies (Bengtsson and Ryden, 1981; McRae et al., 1993; Malacarne et al., 1994; Kacelnik et al., 1995; Koelliker et al., 1998). To test this, we studied developmental and behavioural plasticity in sand martin *Riparia riparia* nestlings, hand-reared under laboratory-controlled conditions. Sand martins are cavity-nesters, suffering little from predatory pressure (Martin and Li, 1992; Martin, 1995). Their growth and developmental rates are therefore primarily shaped by two other factors:

environmental variability outside the nest and sibling competition. We focused on the association between behaviour, energy expenditure and digestive capacity of the gut, which should be particularly sensitive to feeding conditions mediated by within-brood competition.

Sand martin nestlings are unusually mobile for an altricial species. At the age of 5–6 days they are capable of actively leaving the nest cup and crawling up to 30 cm within the nest tunnel towards its entrance (M. Konarzewski, unpublished data). We therefore hypothesised that the young can use this unique locomotory ability to intercept incoming parents and monopolise feeding bouts. We expected that such behaviour should be triggered by an increased number of competing, hungry nestmates (Godfray, 1995). More importantly, we also hypothesised that physiological responses to food shortages should be amplified by sibling rivalry.

When faced with food shortage, hirundinid nestlings are capable of reducing their metabolic rate and eventually entering torpor (Prinzinger and Siedle, 1988). However, such an option can presumably be chosen only at the cost of some impairment of locomotor abilities. We therefore hypothesised that the nestling's optimal response would depend on the choices of other nestmates. A hungry individual could save energy by reducing activity levels, but then risks the loss of competitive abilities in confrontation with its nestmates that have chosen to stay active. Finally, we also expected that a particular decision would be reflected in the modulation of the activity of the digestive system. According to the 'digestive adaptation paradigm' (Karasov, 1990; Karasov, 1996; Starck, 1999), a hungry nestling can either increase the gut's digestive capacity, compensating for food shortage by its more efficient processing or, alternatively, it can downregulate the gut's activity to save energy.

Materials and methods

Animals and their maintenance

Thirty-six, 4-day-old sand martin (*Riparia riparia* L., 1758) nestlings were collected in June 1999 from a breeding colony near Białystok, northeast Poland. To avoid the possible effects of relatedness and hatching order, we did not use last-hatched young, collected no more than two nestlings from each nest, and always assigned them to different experimental groups. The nestlings were collected with permission from the nature conservancy authorities (permit no. 4201/296/99).

Nestlings were taken to the laboratory and placed in artificial nest cavities made of transparent plastic. The cavity consisted of a cylindrical nest chamber (12 cm diameter, height 15 cm) with the bottom modelled to mimic the nest cup, and the attached nest tunnel (a 30 cm long tube, diameter 5 cm). The cavities were surrounded with cardboard, such that the tunnel entrance was the only place through which light could enter. The experiment was carried out in a climatic chamber at 33 °C, 90 % relative air humidity and 16 h:8 h L:D photoperiod. These conditions were found in a pilot experiment to ensure normal growth and development of nestlings.

Experimental procedures

In the colony studied, typical broods consist of 4–5 asynchronously hatched nestlings. However, we created artificial broods of six nestlings, which allowed us to accommodate the broods within the limited space of our climatic chamber and reduce the workload of hand-feeding. We created six such broods and randomly assigned them to two, 3-day-long feeding regimens. Twelve nestlings (i.e. two whole broods, hereafter referred to as FR-nestlings) were fed every 45 min between 08:00 h and 20:00 h with a limited amount of food. This resulted in growth retardation, of the magnitude frequently observed in the wild for undernourished nestlings (P. Brzęk, personal observations). In the remaining four broods, birds were assigned into two subgroups. In each of these broods, three young were fed until they stopped begging every 45 min between 06:00 h and 22:00 h (hereafter referred to as fed *ad libitum*, AL nestlings), while their three nest-mates (hereafter referred to FR/AL nestlings) were fed according to the same feeding protocol as FR nestlings. Here, we attempted to simulate differences in food supply occurring in natural, asynchronously hatching broods facing food shortage. Under natural conditions, 1–2 nestlings hatch 1–2 days later than their nestmates and these individuals typically experience undernourishment when food is scarce (P. Brzęk, personal observations).

Each day, we weighed the nestlings, to the nearest 0.1 g, to estimate daily body mass increments. Upon completion of the third day of experiments the metabolic rates of the young were measured. 18 (five FR, seven FR/AL and six AL nestlings) were subsequently killed; the remaining birds were returned to the breeding colony. During the course of the experiment, one of the FR nestlings was excluded from the trial because of abnormally low body temperature; this individual was returned to the natal nest.

Diet composition and food intake

Nestlings were hand-fed alternately with equal amounts of crickets and a special formula designed for the nestlings of insectivorous birds: fresh, soft cheese, glucose, rice flour and maize flour, mixed in the mass proportions 300:30:15:12 (J. Desselberger, personal communication). This mixture was subsequently added to hard-boiled hens' eggs in the proportion 3:1 and enriched with the vitamin mixture Vitaral (Polfa, Poland). Fresh portions of food were prepared twice a day.

Daily food intake was measured individually for each nestling. Subsamples of food were dried at 60 °C to determine water content, and energy content was determined in a Berthelot-type calorimeter. Regrettably, because of the high locomotor activity of the nestlings, we were unable to measure their individual daily faecal output.

Experimental measurements

Oxygen consumption

Oxygen consumption was measured with a positive-pressure open-circuit respirometry system fitted with two-

mass flow controllers (Sable Systems TR-1 oxygen analyser, Henderson, UT, USA). Depending on the mass of the nestlings, the air flow rate was set to 100 or 200 ml min^{-1}. The air temperature was maintained using a water bath at 33 °C. The air stream vented from the metabolic chamber (100 ml volume) was dried and scrubbed of CO_2 before measuring O_2 levels. For each individual nestling the trial lasted 1 h, and measurements were collected during the second 30 min of this period. We estimated the resting metabolic rate (RMR) by taking the lowest 4 min value recorded. Metabolic data were analysed using Sable Systems DATACAN V software (Sable Systems, 1991). We calculated oxygen consumption rates using equation 4 of Hill (Hill, 1972). For estimation of RMR, we took the lowest 4 min value that did not change by >0.01 % in O_2 concentration.

Body temperature

To monitor the health of the nestlings, we measured cloacal temperature of each individual at the artificial nest three times a day to the nearest 0.1 °C using a 0.5 cm long thermocouple probe attached to a BAT-12 (Physitemp Instruments, USA) electronic thermometer. However, for the sake of brevity below, we report only the body temperatures for each nestling measured at the nest, after 2.5 days of experiments and at the end of the RMR trial.

Morphometrics and determination of carcass fat content

Immediately after measurement of RMR, the nestlings were killed by overdose with ether, and their intestines and pectoral muscles were dissected out, cleared of adherent fat and weighed to the nearest 0.001 g. Leg and pectoral muscles were also dissected and frozen for later analyses. The remaining carcasses were dried at 70 °C to a constant mass, and then homogenised with an electric mill. Fat was extracted from homogenates with petroleum ether in a Soxhlet extractor. The residues were then redried, and the fat content was calculated as the mass lost during extraction (Sawicka-Kapusta, 1975).

Intestinal digestive capacity

We quantified the intestinal digestive activity as brush-border uptake of L-proline using the everted sleeve technique (Karasov and Diamond, 1983). We were not able to evert very tiny and fragile sleeves from the distal intestinal region. We therefore used sleeves from the proximal and middle regions only. However, some were torn during eversion. For this reason, the final sample size for the proximal region of the intestines of AL nestlings and the middle region of FR/AL young was five.

Briefly, small intestines were washed out with cold avian Ringer's solution and cut into sections 1 cm long. The sleeves were everted, mounted on metal rods and pre-incubated for 5 min in oxygenated avian Ringer's solution at 37 °C. They were subsequently incubated for 2 min at 37 °C in Ringer's solution containing 50 mmol l^{-1} L-proline plus the same ^{3}H-labelled nutrient as the radioactive tracer, and [^{14}C]polyethyleneglycol as an adherent fluid marker. Sleeves were then removed from the rods, put into vials, weighed, solubilised with Soluene-350 (Packard Bioscience Company) mixed with scintillation cocktail (Hionic-Fluor, Packard Bioscience Company), and counted for both radiolabels. Uptake rate was expressed as nmol L-proline min^{-1} mg^{-1} intestinal tissue.

Behavioural observations

The locomotor activity of the nestlings was monitored during the second and third day of the experiment, and quantified for each brood and feeding regime as the ratio of the number of feeding bouts when the young were found in the nest tunnel to the total number of bouts. Young found in the nest tunnel were always returned to the artificial nest cup upon completion of the feeding bout.

Statistical analyses

Within-individual values of all measured traits were highly autocorrelated between subsequent days of life (e.g. in the case of locomotor activity r=0.77, P<0.001) and, therefore, they were analysed for the whole 3-day period (except for behavioural observations, which were analysed for 2-day periods).

Many physiological and morphological variables (e.g. metabolic rate, size of internal organs) need to be corrected for body mass prior to comparisons. This is usually performed by means of analysis of covariance (ANCOVA), with body mass as a covariate. However, the almost twofold differences between body mass (*BM*) of nestlings fed *ad libitum* and food-restricted might potentially bias statistical comparisons, because the calculation of *BM*-adjusted means in ANCOVA would require extrapolation of within-treatment relationships between *BM* and the variable of interest outside their range of *BM* variation (e.g. Fig. 1). This could confound the analysis (Cochran, 1957), since the grand mean of *BM* lies beyond the range of its within-treatment variation.

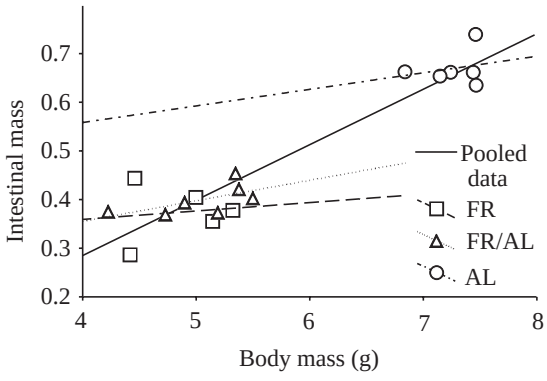

Fig. 1. Intestinal mass *versus* body mass in the three experimental groups of nestlings. The solid line is the regression line for the pooled data set. The broken lines depict within-group regressions, as indicated in the key. FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

Unfortunately, to our knowledge there is no commonly accepted remedy to this problem. Thus, we restricted the use of ANCOVA to statistical comparisons between the food-restricted groups, which did not differ with respect to *BM* (Fig. 1). To analyse the differences between nestlings food-restricted and fed *ad libitum*, we computed mass-specific values of observations for the traits potentially affected by *BM* and analysed them using a Mann–Whitney *U*-test. Although the use of ratios is rightly criticised (e.g. Packard and Boardman, 1987; Jasienski and Bazzaz, 1999), we think that in our case it allowed us to account satisfactorily for the effect of *BM* (Tracy and Sugar, 1989). In all statistical analyses, we subtracted the mass of the organ under consideration from *BM*, to avoid the possibility of autocorrelation.

For some variables (e.g. intestinal uptake rates of L-proline), we used either parametric analysis of variance (ANOVA) or, when a skewed distribution was detected, a non-parametric Kruskall–Wallis test (body temperature, locomotor activity). Accordingly, the significance of between-group differences was tested at P=0.05 by a least-significant difference test (LSD test) or pairwise Mann–Whitney *U*-test. In the case of multi-group comparisons using Mann–Whitney *U*-tests, the conventional P level of 0.05 was adjusted by applying a Bonferroni correction. To do this, we divided P=0.05 by the number of inter-group tests performed. All tests were carried out using the STATISTICA statistical package (StatSoft, Inc., 1997).

Results

Energy intake and growth: validation of the experimental conditions

As expected, the different feeding regimens resulted in significant between-treatment differences in energy intake (ANOVA, $F_{2,32}$=41.8, P<0.001), which was significantly higher in AL nestlings than in the FR and FR/AL groups (LSD test, P<0.001 in both cases); the energy intake of the latter two treatment groups did not differ (LSD test, P=0.41; Table 1).

Although the initial *BM* of the nestlings did not differ between all three groups (ANOVA, $F_{2,33}$=0.12, P=0.89), the high energy intake of AL nestlings resulted in their *BM* increments being larger than those observed in the food-restricted young (ANOVA, $F_{2,33}$=86.06, P<0.001; initial *BM* was not a significant covariate; LSD test, P<0.001 for both cases) (Table 1; Fig. 2). However, the comparable energy intake of FR/AL and FR nestlings was reflected by a lack of any difference in *BM* increments between the two groups (LSD test, P=0.52).

Resting metabolic rate

Feeding regime and the energy intake level of the nestmates significantly affected RMR. FR nestlings had significantly lower RMR than the FR/AL group (ANCOVA, $F_{1,16}$=5.96, P=0.027; *BM* was a significant covariate; Table 1; Fig. 3A). However, both food-restricted groups had a lower mass-specific RMR than AL young (Mann–Whitney test, for AL

Table 1. *Energy intake, body mass increment, resting metabolic rate (RMR), body temperature, fat content, pectoral muscle mass, mass of small intestines, intestinal uptake rate of L-proline and locomotor activity of nestlings from the three experimental groups*

	Group		
Experimental measurement	FR	FR/AL	AL
Energy intake (kJ 72 h^{-1})*	45.80±0.99^{a}	39.77±1.57^{a}	100.87±9.23^{b}
Body mass increment (g 72 h^{-1})*	1.08±0.16^{a}	0.93±0.13^{a}	3.58±0.19^{b}
RMR (ml O_2 h^{-1})‡	11.95±1.14^{a}	16.12±1.21^{b}	–
RMR (ml O_2 (h^{-1} g^{-1})§,[1]	2.16±0.21^{a}	3.03±0.22^{a}	4.58±0.21^{b}
Body temperature after 2.5 days of rearing (°C)§	35.27±0.25^{a}	36.52±0.29^{b}	37.92±0.38^{b}
Body temperature after RMR measurement (°C)§	35.90±0.56^{a}	36.08±0.20^{a}	39.07±0.29^{b}
Carcass fat content (g)‡	0.04±0.003^{a}	0.06±0.003^{b}	–
Carcass fat content (g LDBM^{-1})§,[1]	0.06±0.005^{a}	0.09±0.004^{a}	0.16±0.02^{b}
Pectoral muscle mass (g)‡	0.12±0.01^{a}	0.10±0.01^{a}	–
Pectoral muscle mass (g *BM*$^{-1}$)§,[1]	0.02±0.002^{a}	0.02±0.001^{a}	0.03±0.002^{b}
Small intestine mass (g)‡	0.38±0.02^{a}	0.40±0.02^{a}	–
Small intestine mass (g *BM*$^{-1}$)§,[1]	0.08±0.006^{a}	0.08±0.002^{b}	0.09±0.002^{a}
Uptake rate of L-proline in a proximal part of small intestine (nmol mg^{-1} min^{-1})*	6.21±0.41^{a}	4.19±0.21^{b}	3.01±0.20^{c}
Uptake rate of L-proline in a middle part of small intestine (nmol mg^{-1} min^{-1})*	6.52±0.44^{a}	3.61±0.21^{b}	3.60±0.15^{b}
Locomotor activity§,[2]	0.32±0.03^{a}	0.13±0.04^{b}	0.02±0.009^{b}

All values are means ± S.E.M.

Different superscript letters (a,b,c) indicate values that differ significantly by *ANOVA, ‡ANCOVA (least significant difference test) or §Mann–Whitney tests.

[1]Comparison carried out for FR *versus* AL and FR/AL *versus* AL groups only.

[2]Values are the ratio of the number of feeding bouts when the young were found in the nest tunnel to the total number of bouts.

LDBM, lean dry body mass; *BM*, body mass; FR, FR/AL, food-restricted groups; AL, fed *ad libitum* group.

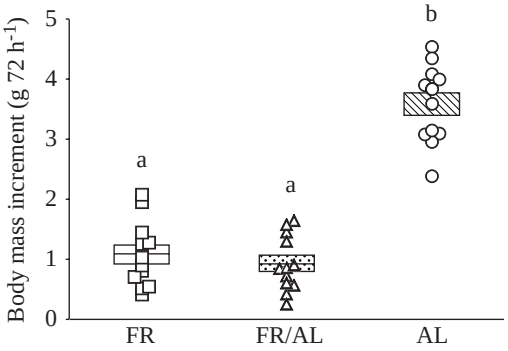

Fig. 2. Body mass increments in the three experimental groups of nestlings. Horizontal lines denote mean values, while boxes denote S.E.M. Different letters indicate a significant difference among all groups (ANOVA). FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

versus FR comparison: U=0, P<0.005; for AL *versus* FR/AL comparison: U=4, P<0.005; Table 1; Fig. 4A).

Body temperature

Nestlings' body temperature measured at the artificial nest (after 2.5 days of experiment) differed significantly between treatment groups (Kruskal–Wallis test, H=18.07, P<0.001), (Table 1; Fig. 5). It was marginally higher in AL than in FR/AL nestlings (the difference did not reach statistical significance using a Mann–Whitney test: U=31, P=0.018). The body temperature of FR nestlings was lower than that of the other two treatment groups (Mann–Whitney test, for FR *versus* FR/AL nestlings: U=25, P=0.0067; for FR *versus* AL nestlings: U=6.5, P=0.00016).

We also detected between-group differences in body temperature measured following completion of the metabolic trials (Kruskal–Wallis test, H=13.78, P=0.001) (Table 1). AL nestlings were characterised by a higher body temperature than both food-restricted groups (Mann–Whitney test, for AL *versus* FR group: U=0, P=0.0027; for AL *versus* FR/AL group: U=0, P=0.0012). There was no difference between FR and FR/AL nestlings (Mann–Whitney test, U=17, P=0.37).

Fat content and mass of internal organs

Marked differences in energy intake and *BM* increments were also reflected in significant, relative differences in fat content normalised to lean dry body mass (LDBM). The AL group had significantly larger LDBM-specific fat stores than did both food-restricted groups (Mann–Whitney test, for AL *versus* FR group: U=0, P=0.006; for AL *versus* FR/AL group: U=0, P=0.0027; Table 1; Fig. 4B). Despite the similar energy intake of the FR and FR/AL nestlings, FR young had significantly smaller fat stores than the FR/AL group (ANCOVA, $F_{1,9}$=20.12, P<0.005; LDBM was a significant covariate; Table 1; Fig. 3B).

The food-restricted groups did not differ in pectoral muscle

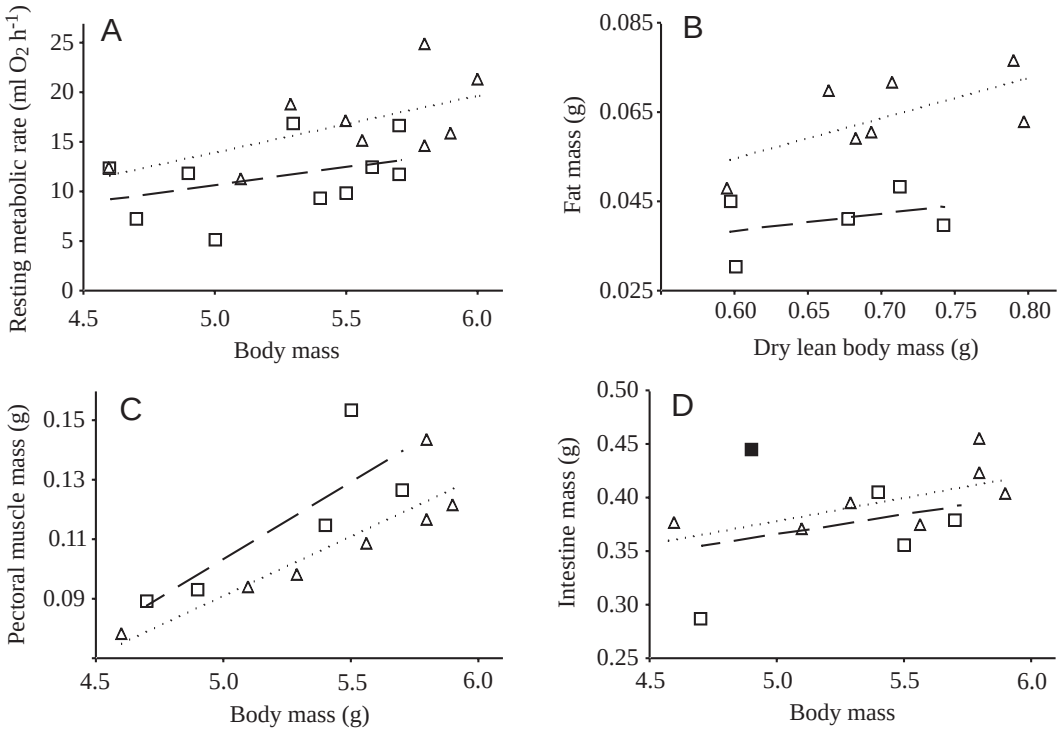

Fig. 3. (A) Resting metabolic rate *versus* body mass, (B) fat mass *versus* dry lean body mass, (C) pectoral muscle mass *versus* body mass and (D) mass of intestines *versus* body mass in the two food-restricted groups (FR and FR/AL nestlings). Dashed lines and squares denote FR nestlings, while dotted lines and triangles denote FR/AL young. The filled square in D denotes the outlying data point from the FR group (see Results).

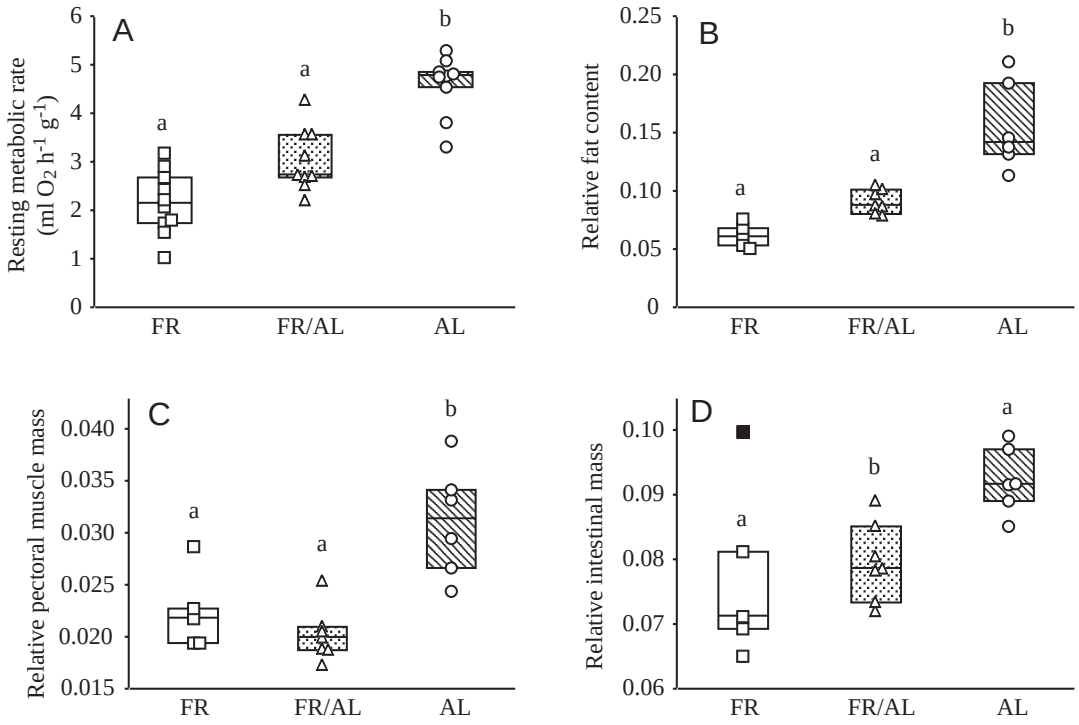

Fig. 4. (A) Mass-specific resting metabolic rate (RMR), (B) lean-dry-mass-specific fat mass, (C) body-mass-specific pectoral muscle mass and (D) body-mass-specific intestinal mass in the three treatment groups. Different letters indicate significant differences between FR *versus* AL, and FR/AL *versus* AL groups only (Mann–Whitney *U*-tests). Horizontal lines denote median values, while boxes denote 25th and 75th centiles. The filled square denotes the outlying data point from the FR group (see Results). FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

mass (ANCOVA, $F_{1,9}$=4.04, P=0.075; *BM* was a significant covariate; Table 1; Fig. 3C). However, AL nestlings had significantly higher *BM*-specific pectoral muscle mass than the other groups (Mann–Whitney test, for AL *versus* FR group: U=2, P=0.018; for AL *versus* FR/AL group: U=1, P<0.005; Fig. 4C).

Mass-specific intestinal mass of the FR/AL group was significantly lower than that of AL nestlings (Mann–Whitney test, U=1, P=0.0043), whereas no difference was found between the FR and AL group (Mann–Whitney test, U=6, P=0.1; Table 1; Fig. 4D). However, when one outlying data point from the FR group (see Fig. 4D) was omitted in the analysis, the latter difference became significant (Mann–Whitney test, U=0, P=0.01). By contrast, there was no difference in intestinal mass between FR and FR/AL nestlings (ANCOVA, $F_{1,9}$=0.52, P=0.49; *BM* was not a significant covariate; Table 1; Fig. 3D). This difference was still not significant when the outlying data point from the FR group was omitted from the computation.

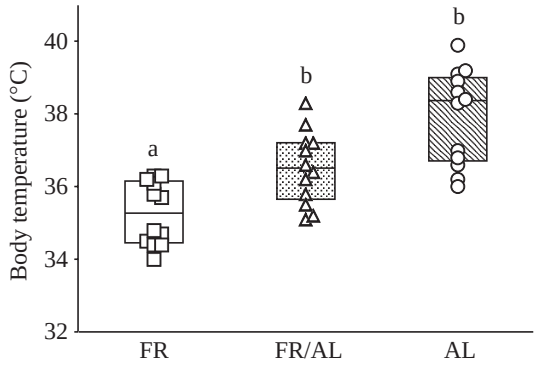

Fig. 5. Body temperature after 2.5 days of rearing in the three experimental groups of nestlings. Horizontal lines denote median values, while boxes denote 25th and 75th centiles. Different letters indicate a significant difference among all groups (Mann–Whitney *U*-test). FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

Intestinal brush-border uptake of L-proline

Two-way ANOVA revealed that treatment group significantly affected intestinal uptake rate of proline ($F_{1,27}$=63.87, P<0.001), whereas the position along the intestinal length had no effect on uptake rates ($F_{1,27}$= 0.22, P=0.65). One-way ANOVA with treatment group as the main factor performed for the proximal intestine revealed that the proline uptake rate of the FR group was significantly higher than that of both the FR/AL and AL groups (LSD test, P<0.005 in both comparisons). The uptake rate of the FR/AL group was also significantly higher than that of AL nestlings (LSD test, P=0.008; Table 1; Fig. 6A). A similar analysis performed for the middle part of the intestines indicated that the uptake rates in FR nestlings were again significantly higher than in the FR/AL and AL groups (LSD test, P<0.001 in both cases),

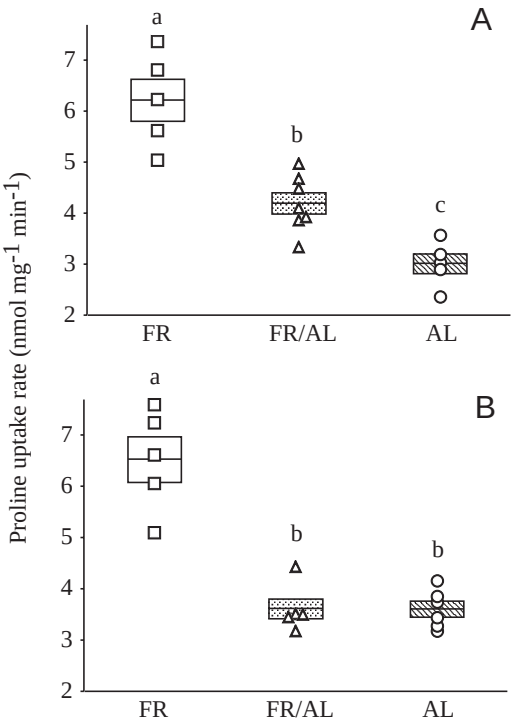

Fig. 6. Intestinal L-proline uptake rate in the proximal (A) and middle (B) part of the gut. Horizontal lines denote mean values, while boxes denote S.E.M. Different letters indicate a significant difference among all groups (ANOVA). FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

whereas the FR/AL and AL groups did not differ (LSD test, P=0.99; Table 1; Fig. 6B).

Locomotor activity

Locomotor activity differed significantly between treatment groups (Kruskal–Wallis test, H=20.03, P<0.001), (Table 1; Fig. 7). FR-nestlings were more active than both FR/AL (Mann–Whitney test, U=22, P=0.0039) and AL nestlings (Mann–Whitney test, U=0, P=0.00003). Locomotor activities of FR/AL and AL nestlings were not significantly different (Mann–Whitney test, U=38, P=0.05).

Discussion

Locomotor activity and sibling competition

An association between begging, locomotor activity and within-brood competition for food has been found in the nestlings of many altricial species (Ryden and Bengtsson, 1980; Bengtsson and Ryden, 1981; Reed, 1981; Greig-Smith, 1985; McRae et al., 1993; Malacarne et al., 1994; Mock and Parker, 1997; Koelliker et al., 1998). However, their locomotor activity usually is limited to a few begging movements (such as gaping, stretching the neck or jostling for a particular position inside the nest cup), which are unlikely to incur high energy costs (McCarty, 1996; Bachman and Chappell, 1998). This limited activity is no match to the striking locomotor

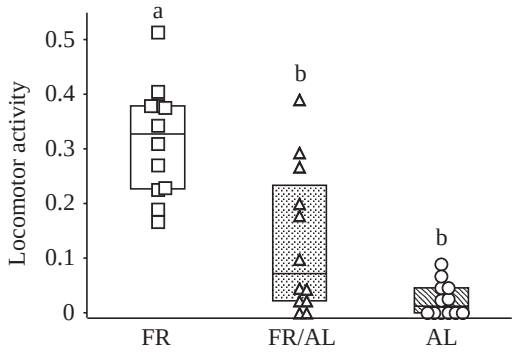

Fig. 7. Locomotor activity, quantified as the ratio of the number of feeding bouts when the young were found in the nest tunnel to the total number of bouts. Horizontal lines denote median values, while boxes denote 25th and 75th centiles. Different letters indicate a significant difference among all groups (Mann–Whitney U-test). FR, FR/AL, food-restricted groups; AL, group fed *ad libitum*.

abilities of 5- to 6-day-old, still ectothermic sand martin nestlings observed in our study, which repeatedly crawled the 30 cm distance within the tunnels of artificial nest burrows. Note that this distance travelled by the nestlings was limited by the length of the tunnel and, therefore, potentially did not reflect their full locomotory ability. Although we were not able to measure the energy expenditure of nestlings crawling towards the tunnel entrance, it seems safe to assume that they are substantial. Furthermore, it is clear that under natural conditions a nestling leaving the nest cup is exposed to increased heat loss, further increasing the possible costs of such behaviour.

Signalling models of parent-offspring conflict suggest that food-restricted nestlings should be more active than their satiated nestmates (Godfray, 1995). Indeed, locomotor activity of food-restricted nestlings was much higher than that of young fed *ad libitum* (Fig. 7; Table 1). This result strongly, although indirectly, suggests that the striking locomotor activity of sand martin nestlings is a means of monopolising feeding bouts by underfed young. However, by creating broods of FR nestlings and mixed broods of FR/AL and AL young, we also attempted to simulate the effect of within-brood competition resulting from different levels of hatching asynchrony. Despite similar food intakes and body mass increments, the locomotor activity of FR nestlings was significantly higher than in the FR/AL group (Fig. 7; Table 1). Thus, the presence of hungry, similar-sized siblings, rather than fully fed, bigger nestmates presumably created a competitive environment that stimulated the nestlings to show particularly high locomotor activity.

A possible explanation for this is that sibling competition would increase the survival prospects of the most mobile FR nestlings, competing against their nestmates of similar size. Increased locomotor activity, however, would be less beneficial for FR/AL young, since they have little chance of outcompeting larger/older siblings. FR/AL nestlings would benefit more from their reducing energy expenditure and thus maximising their survival prospects in the hope of an

improvement in feeding conditions. This interpretation of our results supports Ricklefs' suggestion that hatching asynchrony is an effective parental strategy to eliminate benefits to offspring from sibling competition (Ricklefs, 1993).

How can an individual nestling evaluate the level of nourishment of its nestmates? We cannot offer an unequivocal answer to this question, but we suggest that the high locomotor activity of FR broods was stimulated by the vigorous vocalisation of each individual in those broods. Although we did not quantify the level of vocalisation, it was noticeably less intense in FR/AL nestlings, presumably because of the lack of stimulation from their AL nestmates, which did not vocalise at all. Indeed, in some bird species, begging intensity appears to be stimulated by the begging of hungrier nestmates (Smith and Montgomerie, 1991; Price and Ydenberg, 1995). Particularly relevant is the behaviour of food-restricted tree swallow *Tachycineta bicolor* nestlings, whose begging intensity increases with the number of hungry competitors (Leonard et al., 2000). Furthermore, begging intensity increased in food-deprived broods but did not change in broods that contained some full-fed siblings (Leonard and Horn, 1998).

Intestinal mass and function

If high locomotor activity serves as a means for monopolising limited food resources, one would expect that it should be associated with digestive adaptations that enable the efficient utilisation of scarce food supplies. In principle, this can be achieved either by enlargement of the surface of the intestinal epithelium or by increasing the density of epithelial transporters, or both (Karasov, 1990; Karasov, 1996). In either case, enlargement of the intestinal epithelium should lead to increased intestinal mass. This was not found in our study; food-restricted nestlings had relatively lighter intestines than young fed *ad libitum*. Such a reduction, however, cannot be interpreted as downregulation of intestinal function, because the mass-specific intestinal uptake of L-proline was much higher in food-restricted nestlings than in nestlings fed *ad libitum*. This finding corroborates the results on song thrush (*Turdus philomelos*) nestlings (Konarzewski and Starck, 2000), who also reported higher intestinal uptake rates of L-proline in food-restricted, rather than in overfed, young. Furthermore, although the intestinal mass of FR/AL and FR young did not differ, the latter group was characterised by significantly higher uptake rates of L-proline. Interestingly, this difference was in the same direction as the difference in locomotor activity, possibly suggesting a functional link between the intensity of sibling competition and the digestive physiology of the nestlings.

The validity of our conclusions related to intestinal function clearly relies on the integrity of the tissue samples subject to the everted sleeve technique used to estimate uptake rates of L-proline. Starck et al. (Starck et al., 2000) have recently reported intestinal tissue damage resulting from handling of the samples during the everted sleeves method. In the present study, we were not able to evaluate histologically the effect of tissue damage on intestinal uptake rates, as suggested by Starck et al. (Starck et al., 2000). However, for the following reasons, we believe that our estimates of uptake rates are reliable. First, our most critical comparisons involved two groups of food-restricted nestlings subject to identical feeding regimes. Any bias due to tissue damage would therefore equally affect both groups. Second, the intestines of sand martin nestlings are of comparable size to the intestines of the smallest song thrush (*Turdus philomelos*) nestlings, in which the tissue damage was shown to have only second-order effects on intestinal histology compared with feeding regime (Konarzewski and Starck, 2000). Moreover, the intestinal proline uptake rates of young sand martins were very similar to those of the song thrushes, which would be unlikely to occur if the damage was extensive (Starck et al., 2000). Although we are confident that our findings represent reliable estimates of uptake rates of L-proline within our experimental design, caution must, however, be exercised if one wishes to use absolute values for between-species comparisons.

Fat mass and pectoral mass

The high locomotor activity of FR nestlings was associated with their fat reserves being lower not only than those of AL young, but also of the FR/AL group. It is therefore likely that their low fat stores were due to the energetic expenditure incurred by crawling into the artificial nest tunnel, since energy intake did not differ between the two food-restricted groups.

Food restriction also appeared to be associated with reduced growth of the pectoral muscles. This is in contrast to other studies demonstrating that underfed altricial nestlings preferentially invest in pectoral muscles and other body parts that enhance the chances of successful fledgling (Donazar and Ceballos, 1989; Nilsson and Svensson, 1996; Konarzewski et al., 1996). However, young sand martin are characterised by a relatively long nestling period of approximately 22 days (Turner and Bryant, 1979). This long duration may mean that a delay in the growth of the pectoral muscles during the first week of life can be compensated for later, prior to fledgling. This, therefore, allows a reduction of energy expenditures in the early stages of post-hatching growth.

Body temperature and resting metabolic rate

The nestlings subject to food restriction responded with a reduction in body temperature and RMR. A decrease in metabolic rate in response to food shortage has also been reported for the nestlings of another hirundinid aerial insectivore, the house martin (*Delichon urbica*; Prinzinger and Siedle, 1988). Young house martins are also capable of entering torpor when undernourished (Prinzinger and Siedle, 1988). The sand martin nestlings used in our experiment were partly poikilothermic (Marsh, 1979), whereas the ability to enter torpor requires strict control of body temperature. Furthermore, in our experiment they were reared at an ambient temperature of 33 °C, which did not allow for conspicuous reduction of body temperature and metabolic rate. However, food-deprived song thrush *Turdus philomelos* and starling *Sturnus vulgaris* nestlings, reared under similar conditions to

the present study, maintained body temperature and RMR at the same level as overfed young (Schew and Ricklefs, 1998; Konarzewski and Starck, 2000). Thus, unlike song thrush and starling nestlings, young sand martins responded to food shortage by reducing their body temperature and energy expenditure. Such an important difference suggests that under natural conditions, aerial insectivores face frequent, unpredictable fluctuations in food abundance that could select for mechanisms enhancing energy savings.

The high locomotor activity of FR nestlings is especially puzzling because of their reduced RMR and body temperature (Table 1; Fig. 3A, Fig. 5). Strikingly, both RMR and body temperature (measured at the nest) were lower than those of the FR/AL group. The higher body temperature of FR/AL nestlings at the nest can possibly be attributed to the effect of huddling with warmer nestmates fed *ad libitum*. This is presumably why the difference in body temperature between FR and FR/AL young disappeared when measured immediately after 1 h long metabolic trials. However, RMR was measured individually for each nestling, during exposure to the same temperature, and therefore the lower value in FR nestlings cannot be attributed to their lower temperature at the nest. Thus, the low body temperatures of FR nestlings measured at the nest were likely to be due to the reduced RMR, not *vice versa*. However, the magnitude of the reduction of RMR and body temperature in FR/AL and FR nestlings was inversely related to the satiation level of the nestmates and therefore, the level of their locomotor activity. Thus, at least under the present experimental conditions, a lowered metabolic rate did not impair the nestlings' locomotor activity.

Energy costs of maintenance of the gut are an important component of RMR (Kersten and Piersma, 1987; Konarzewski et al., 2000). Food-restricted song thrush nestlings, whose growth was reduced to a similar extent as in the FR young of the present study, maintained both high intestinal uptake rates of L-proline and high RMR (Konarzewski and Starck, 2000). It is therefore surprising that food-restricted sand martins were able to downregulate RMR, and upregulate energy-dependent, intestinal uptake rates of L-proline. This suggests that the energy costs of such upregulation are relatively low.

In conclusion, our experiment demonstrated that undernourished nestlings reduced energy expenditure, upregulated digestive capacity and increased locomotor activity. These behavioural and physiological responses were clearly amplified by the presence of hungry siblings. We interpret such an amplification as an attempt to increase competitive abilities. This may lead to an escalation of indirect sibling competition among undernourished nestmates of similar size. It is therefore likely that developmental and behavioural plasticity played an important role in the evolution of sibling rivalry.

Our study would not have been finished without the help of Emilia Burak, Malgorzata Lewoc, Anna Roszkowska and Urszula Szymska. Andrzej Gebczyński and Jan Taylor helped us skilfully in laboratory trials. Mariusz Cichoń, Kimberly A. Hammond, Wiliam H. Karasov, Regina McDevitt, Thenius Piersma, Robert Ricklefs, Jan Taylor, J. Matthias Starck and an anonymous reviewer made many helpful comments on the manuscript. The study was supported by Polish KBN grant 6 PO4F 054 14 to P.B. and KBN grant 6 P204 072 to M.K.

References

Bachman, G. C. and Chappell, M. A. (1998). The energetic cost of begging behaviour in nestling house wrens. *Anim. Behav.* **55**, 1607–1618.

Bengtsson, H. and Ryden, O. (1981). Development of parent-young interactions in asynchronously hatched broods of altricial birds. *Z. Tierpsychol.* **56**, 255–272.

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

Cochran, W. G. (1957). Analysis of covariance: its nature and uses. *Biometrics* **13**, 261–281.

Donazar, J. A. and Ceballos, O. (1989). Growth rates of nestling Egyptian Vultures *Neophron percnopterus* in relation to brood size, hatching order and environmental factors. *Ardea* **77**, 217–226.

Emlen, S. E., Wrege, P. H., Demong, N. J. and 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.

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

Godfray, H. J. C. (1995). Signaling of need between parents and young: parent-offspring conflict and sibling rivalry. *Am. Nat.* **146**, 1–24.

Greig-Smith, P. (1985). Weight differences, brood reduction, and sibling competition among nestling Stonechats *Saxicola torquata* (*Aves*: *Turdidae*). *J. Zool., Lond.* **205**, 453–465.

Hill, R. W. (1972). Determination of oxygen consumption by use of the paramagnetic oxygen analyzer. *J. Appl. Physiol.* **33**, 261–263.

Jasienski, M. and Bazzaz, F. A. (1999). The fallacy of ratios and the testability of models in biology. *Oikos* **84**, 321–326.

Kacelnik, A., Cotton, P. A., Stirling, L. and Wright, J. (1995). Food allocation among nestling starlings: sibling competition and the scope of parental choice. *Proc. R. Soc. Lond.* **259**, 259–263.

Karasov, W. H. (1990). Digestion in birds: chemical and physiological determinants and ecological implications. *Stud. Avian Biol.* **13**, 391–415.

Karasov, W. H. (1996). Digestive plasticity in avian energetics and feeding ecology. In *Avian Energetics and Nutritional Ecology* (ed. C. Carey), pp. 61–84. New York: Chapman & Hall.

Karasov, W. H. and Diamond, J. M. (1983). A simple method for measuring intestinal solute uptake in vitro. *J. Comp. Physiol.* **152**, 105–116.

Kersten, M. and Piersma, T. (1987). High levels of energy expenditure in shorebirds: Metabolic adaptations to an energetically expensive way of life. *Ardea* **75**, 175–188.

Koelliker, M., Richner, H., Werner, I. and Heeb, P. (1998). Begging signals and biparental care: nestling choice between parental feeding locations. *Anim. Behav.* **55**, 215–222.

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

Konarzewski, M. and Starck, J. M. (2000). Effects of food shortage and overabundance 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. and Wallis, I. R. (2000). Metabolic and organ mass responses to selection for high growth rates in the domestic chicken (*Gallus domesticus*). *Physiol. Biochem. Zool.*, **73**, 237–249.

Lack, D. and Lack, E. (1951). The breeding biology of the swift *Apus apus*. *Ibis* **93**, 501–546.

Leonard, M. L. and Horn, A. G. (1998). Need and nestmates affect begging in Tree Swallows. *Behav. Ecol. Sociobiol.* **42**, 431–436.

Leonard, M. L., Horn, A. G, Gonza, A. and Ramen, S. (2000). Brood size and begging intensity in nestling birds. *Behav. Ecol.* **11**, 196–201.

Lepczyk, C. A., Vidal, E. and Karasov, W. H. (1998). Digestive responses during food restriction and realimentation in nestling house sparrows *(Passer domesticus*). *Physiol. Zool.* **71**, 561–573.

Malacarne, G., Cucco, M. and Bertolo, E. (1994). Sibling competition in asynchronously hatched broods of the Pallid Swift (*Apus pallidus*). *Ethol. Ecol. Evol.* **6**, 293–300.

Marsh, R. L. (1979). Development of endothermy in nestling Bank Swallows (*Riparia riparia*). *Physiol. Zool.* **52**, 340–353.

Martin, T. E. (1995). Avian life-history evolution in relation to nest sites, nest predation, and food. *Ecol. Monogr.* **65**, 101–127.

Martin, T. E. and Li, P. (1992). Life history traits of open- vs. cavity-nesting birds. *Ecology* **73**, 579–592.

McCarty, J. P. (1996). The energetic cost of begging in nestling passerines. *Auk* **113**, 178–188.

McRae, S. B., Weatherhead, P. J. and Montgomerie, R. (1993). American Robin nestlings compete by jockeying for position. *Behav. Ecol. Sociobiol.* **33**, 101–106.

Mock, D. W. and Parker, G. A. (1997). *The Evolution of Sibling Rivalry*. Oxford: Oxford University Press.

Nilsson, J.-A. and Svensson, M. (1996). Sibling competition affects nestling growth strategies in marsh tits. *J. Anim. Ecol.* **65**, 825–836.

O'Connor, R. J. (1978). Structure in avian growth patterns: a multivariate study of passerine development. *J. Zool., Lond.* **185**, 147–172

Packard, G. C. and Boardman, T. J. (1987). The misuse of ratios to scale physiological data that vary allometrically with body size. In *New Directions in Ecological Physiology* (ed. M. E. Feder, A. F. Benett, W. W. Burggren and R. B. Huey), pp. 216–239. Cambridge: Cambridge University Press.

Price, K. and Ydenberg, R. (1995). Begging and provisioning in broods of asynchronously hatched yellow-headed blackbird nestlings. *Behav. Ecol. Sociobiol.* **37**, 201–208.

Prinzinger, R. and Siedle, K. (1988). Ontogeny of metabolism, thermoregulation and torpor in the house martin *Delichon u. urbica* (L.) and its ecological significance. *Oecologia* **76**, 307–312

Reed, J. R. (1981). Song sparrow 'rules' for feeding nestlings. *Auk* **98**, 828–831.

Ricklefs, R. E. (1993). Sibling competition, hatching asynchrony, incubation period, and lifespan in altricial birds. In *Current Ornithology*, vol. 11 (ed. D. M. Power), pp. 199–276. New York: Plenum Press.

Ryden, O. and Bengtsson, H. (1980). Differential begging and locomotory behaviour by early and late hatching nestlings affecting the distribution of food in asynchronously hatched broods of altricial birds. *Z. Tierpsychol.* **53**, 209–224.

Sable System (1991). *Instruction Manual Version 1.0 for DATACAN V*. Salt Lake City, UT, Sable Systems.

Sawicka-Kapusta, K. (1975). Fat extraction in Soxhlet apparatus. In *Methods for Ecological Energetics* (ed. W. Grodzinski, R. Z. Klekowski and A. Duncan), pp. 288–293. Oxford: Blackwell Scientific Publications,.

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

Schlichting, C. D. and Pigliucci, M. (1998). Phenotypic Evolution: A Reaction Norm Perspective. Sunderland, MA: Sinauer.

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

Smith, H. G. and Montgomerie, R. (1991). Nestling American Robins compete with siblings by begging. *Behav. Ecol. Sociobiol.* **29**, 307–312.

Starck, J. M. (1999). Phenotypic flexibility of the avian gizzard: rapid, reversible and repeated changes in organ size in response to changes in dietary fiber content. *J. Exp. Biol.* **202**, 3171–3179.

Starck, J. M., Karasov, W. H. and Afik, D. (2000). Intestinal nutrient uptake measurements and tissue damage: Validating the everted sleeves method. *Physiol. Biochem. Zool.* **73**, 454–460.

StatSoft, Inc. (1997). *STATISTICA for Windows (Computer Program manual*). Tulsa, OK: StatSoft, Inc.

Tracy, C. R. and Sugar, J. (1989). Potential misuse of ANCOVA: comment on Packard and Boardman. *Physiol. Zool.* **62**, 993–997.

Turner, A. K. and Bryant, D. M. (1979). Growth of nestling sand martins. *Bird Study* **26**, 117–122.