

Causes and consequences of post-growth age-dependent differences in small intestine size in a migratory sandpiper (*Calidris mauri*, Western Sandpiper)

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Summary

1. *Calidris mauri* Cabanis (Western Sandpiper) exhibits a pronounced post-growth age-dependent difference in small intestine size during southward migration, such that the later-migrating juveniles have larger small intestines than do the adults. Potential causes and consequences of this age-dependent difference are examined.

2. Premigrant juveniles of full structural size had small intestines that were 10% longer than those of the premigrant adults, even though the juveniles had not attained asymptotic body mass. The elongated small intestines of premigrant juveniles appear to be growth-related.

3. Adults and juveniles exhibited parallel increases in intestinal length (7.0%) and circumference (9.5%) in association with the initiation of migration; these effects are consistent with migratory hyperphagia.

4. Refuelling juveniles had small intestines that were 8.5% longer than those of the refuelling adults. Retaining an enlarged small intestine during migration confers increased digestive capacity to the juveniles, which may be under selection to minimize stopover duration.

5. Refuelling juveniles had a higher prevalence of cestode infection than refuelling adults in one of two years, and the length-corrected mass of the small intestine was 9.7% heavier in infected individuals. Cestode infection may be an important determinant of migration-related mortality for juveniles, by compromising their health and increasing stopover duration.

Key-words: Cestode, Charadriiformes, digestive system, gut size, refuelling

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Introduction

The combination of short growing seasons and strict migration schedules results in particularly high energy demands (Schekkerman & Visser 2001; Schekkerman *et al.* 2003) and stringent selection regimes for the juveniles of long-distance migrants that breed at northern latitudes (Owen & Black 1989, 1991). This results in a unique set of selection pressures early in the life of long-distance migrants, which do not abate until after the juveniles have completed their first southward migration. Mass-specific energy demands are near maximal during the growth phase of avian ontogeny (Murphy 1996), and the digestive system is integral to meeting these demands (Starck 1998). In a few species of passerine birds there is substantial ‘over-shooting’ of small intestine (SI) size during growth, such that

fully grown fledglings have larger digestive systems than adults (Neff 1972; Hume & Biebach 1996). An enlarged digestive system confers increased volumetric digestive capacity. However, little attention has been paid to circumstances in which juveniles could benefit from increased digestive capacity. Maintaining an enlarged digestive system could be important in species where juveniles are confronted with particularly high postgrowth energy requirements, such as long-distance migrants that breed at northern latitudes. Once structural growth is complete, juveniles must quickly deposit lipid and protein to fuel their first southward migration. An enlarged SI that was initially associated with growth could be exploited by juvenile migrants to maintain high digestive capacity during their first southward migration.

To ensure survival and to maximize fitness, migratory birds must optimize refuelling behaviour in regard to both energy acquisition and time expenditure (Alerstam & Lindström 1990); they must also optimize digestive

system size in association with fuelling and flight (Piersma 1998). As a consequence, the digestive system is dynamic during migration (McWilliams & Karasov 2001), and interspecific variation in flight duration may be an important determinant of digestive system dynamics in long-distance migrants. In the Charadriiformes, variation in flight distance has led to categorizing species or subspecies as either short- or long-hop migrants. To reduce transport costs, some long-hop migrants, such as *Calidris canutus* (Red Knot), facultatively down-size the digestive system prior to initiating migratory flights (Piersma 1998). After arrival at a stopover site, long-hop migrants must rebuild the digestive system before maximal refuelling rates can be attained (Karasov & Pinshow 2000). Digestive system dynamics are partially responsible for the extended stopover duration (20–25 days) of some long-hop migrants (Piersma 1998). When flying the same total distance, short-hop migrants make an increased number of shorter flights, carry smaller relative fuel loads and refuel more frequently than long-hop migrants. Short-hop migrants might benefit from maintaining an enlarged digestive system during migration, particularly if it allowed them to begin refuelling rapidly after arrival (Guglielmo & Williams 2003). Therefore, we might expect short-hop migrants to maintain an enlarged digestive system during migration.

Intraspecific variation in digestive system size may be directly related to temporal and age-dependent variation in selection regimes that occur during migration. Selection regimes may vary temporally, owing to changes in food quality, food availability or predation pressure; selection regimes may vary by age, or because of experience, susceptibility to disease or age-dependent differences in migration schedules. If age-dependent differences in migration schedules result in increased predation pressure on juveniles, then juveniles might benefit from maintaining an enlarged digestive system, particularly if additional digestive capacity results in a shorter stopover duration. However, refuelling hyperphagia places increased energetic demands on the digestive system (McWilliams & Karasov 2001), and could result in increased exposure to food-borne pathogens, such as cestodes. Juveniles have minimal foraging experience (Le V. Dit Durell 2000), a naive immune system (Apanius 1998), and consequently, may be at a higher risk of contracting food-borne parasites than adults (Gray 1972). However, age-dependent differences in digestive system size and cestode prevalence have not been considered in studies of refuelling physiology for either long- or short-hop migrants.

Calidris mauri Cabanis (Western Sandpiper) is a small-bodied (22–35 g) long-distance migrant that generally uses a short-hop migration strategy to move between breeding grounds in arctic and subarctic Alaska, and wintering grounds along the Pacific coast of the Americas, primarily between California and Peru (Wilson 1994). Juveniles initiate their first southward migration approximately 1 month after adults

(Wilson 1994) and, while refuelling at the first major stopover site south of the breeding grounds, have SIs that are 20% larger than those of the adults (Guglielmo & Williams 2003). In the present study, we test the predictions of three hypotheses that might explain this marked age-dependent difference in SI size. Hypothesis 1, that selection for rapid growth has resulted in a disproportionately large SI in growing birds (Konarzewski *et al.* 1990; Starck 1998), predicts: (1) that premigrant juveniles should have larger SIs than premigrant adults. Hypothesis 2, that SI size is closely related to energy intake rate (Karasov 1996; Dykstra & Karasov 1992), predicts: (2) that migrants should have larger SIs than premigrants, due to migration-related hyperphagia. Hypothesis 3, that the age-related difference in SI size is associated with cestode infection, predicts: (3) that cestode-infected individuals should have larger SIs owing to the pathological effects of infection, and (4) that cestode prevalence should be higher in juveniles than in adults. Finally, we investigate the influences of the initiation of migration and age on the ultrastructure of the SI (circumference, muscle width and villus length), which might indicate fine-scale differences in digestive function.

Materials and methods

SAMPLE COLLECTION

Calidris mauri were captured with mist nets (1¼ in. (31 mm) mesh, Avinet, Dryden, NY) and collected in accordance with permits from Environment Canada, the US Fish and Wildlife Service and the Alaska Department of Fish and Game. Animal handling protocols were approved by the Simon Fraser University Animal Care Committee (Permit B529) and conformed to the Canadian Committee for Animal Care Guidelines. Female *Calidris mauri* were collected at two stages of migration (stage): premigrants were collected on the breeding grounds at Safety Sound, Alaska (64°27' N, 164°56' W) and migrants were collected while refuelling at the first major stopover site south of the breeding grounds, Boundary Bay, British Columbia (49°10' N, 123°05' W). These sampling sites are separated by approximately 3000 km, and it is likely that *Calidris mauri* fuel up at Bristol Bay, Alaska, before embarking on the 2400 km flight across the Gulf of Alaska, which brings them to Boundary Bay. In 1999, 9 adult (3–16 July) and 8 juvenile (16 July) premigrants were collected, as well as 7 adult (10–13 July) and 23 juvenile (8 August–9 September) migrants. In 1999, the mean hatch date for *Calidris mauri* nests at Safety Sound, Alaska, was 24 June (Neville 2002), which indicates that the premigrant juveniles were approximately 22 days of age when collected. In 2000, 22 adults (4–28 July) and 18 juveniles (9–27 August) migrants were collected. Immediately after capture, individuals were weighed (capture mass; ±0.01 g), and exposed culmen length was measured to assign sex (Page & Fearis 1971).

TISSUE COLLECTION

Immediately prior to dissection, exposed culmen length was re-measured with digital callipers (± 0.01 mm) and each individual was re-weighed (dissection mass; ± 0.01 g). Sandpipers were anaesthetized with an intramuscular injection (4 ml/25 g) of a 1:1 mixture of ketamine hydrochloride (100 mg ml⁻¹) and xylazine (20 mg ml⁻¹), and euthanized by exsanguination. The SI was separated from the gizzard at the pylorus and from the large intestine immediately proximal to the caeca. A gavage needle was sutured to the proximal end of the SI and its contents were purged with ice-cold physiological saline (350 mosmol/kg H₂O). SI length was measured using a modified version of Brambell's method (Freehling & Moore 1987); the SI was held by the distal end with a pair of forceps and suspended vertically beside a ruler (± 0.5 mm), with the gavage needle attached to the proximal end. The gavage needle (2.2 g) applied a moderate and consistent force that incompletely straightened the SI and did not stretch it. Subsequently, the SI was blotted dry and weighed (± 1 mg). Cestodes were collected from the purged contents of the SI and identified to genera by rostellar hook length (Czaplinski & Vaucher 1994). After dissection, the sex of each bird was verified anatomically, and tarsometatarsus and sternum lengths were measured with digital callipers (± 0.01 mm).

INTESTINAL HISTOLOGY AND MORPHOMETRY

To determine the influence of the initiation of migration on SI morphology, histological sections of the proximal duodenum were prepared for juvenile and adult premigrants and migrants collected in 1999. Birds selected for this component of the study were free from cestode infection at the time of capture. Duodenal sections were fixed in 10% formalin in 0.1 M phosphate-buffered saline, pH 7.4, for 48–72 h at room temperature. Fixed sections were divided into three subsections with a razor blade, dehydrated in 70% ethanol and 30% xylene, followed by 100% xylene, and embedded in paraffin. A rotary paraffin microtome was used to cut 5- μ m cross-sections, which were mounted on glass slides and stained with haematoxylin and eosin.

Digital images of cross-sections were obtained at 4-power magnification with an Olympus Vanox microscope (Olympus America Inc., Melville, NY). Images were converted to 8-bit grey scale and analysed in Northern Eclipse 6.0 (Empix Imaging Inc., Mississauga, ON, Canada). Total and inner cross-sectional areas (determined by the distinction between the mucosa and smooth muscle [*tunica muscularis*]) and villus length were measured on 8–10 cross-sections from the three subsections of each tissue section. The circumference of each circular section was calculated from its cross-sectional area. Muscle width was not uniform in

the proximal duodenum; therefore, the mean muscle width was calculated as the radial difference between circles with areas equal to the total and inner cross-sectional areas. To avoid pseudo-replication, statistical analyses were performed on the mean values of each tissue section.

STATISTICAL METHODS

Two complete two-factor designs were analysed and the migrants collected in 1999 were represented in both analyses. Premigrants were sampled in 1999 only, so stage-related comparisons were restricted to this year. Age- and cestode-related comparisons among migrants were conducted on migrants collected in 1999 and 2000, where year effects could be controlled statistically. To exploit the full power of these designs, specific tests considering only portions of the data, for example age-related comparisons among premigrants, were conducted using contrast statements. Interaction terms were examined in each set of analyses and *F*-statistics, *P*-values and least-square (ls) means are based on type III sums of squares. Experimental error rate was controlled by Bonferroni correction ($\alpha = 0.05$), to balance the probabilities of committing type I and type II errors. In the generalized linear models associated with these designs, main effects were entered as dummy variables (age: juveniles = 0 and adults = 1; stage: premigrants = 0 and migrants = 1; year: 1999 = 0 and 2000 = 1; and cestode infection: uninfected = 0 and infected = 1). The time that elapsed between capture and dissection (time to dissection; mean: 8.0 h and range: 4.1–10.7 h) was used as a covariate in the analysis of SI length. In birds, SI length increases after feeding (Robel *et al.* 1990), and, as a result, is expected to decrease while fasting. Time to dissection is an index of fast duration. To avoid part-whole correlation in the analysis of SI mass, variation in body mass was controlled by including dissection mass–SI mass (body mass) as a covariate (Christians 1999). Finally, as a measure of structural body size, the first principal component (PC1) was extracted from a principal components analysis of culmen, tarsus and keel lengths (Rising & Somers 1989), which included all of the birds in the study. Each of the univariate measures of structural body size had a large positive loading (exposed culmen = 0.60, tarsometatarsus = 0.63 and sternum = 0.50) on the PC1 (eigenvalue = 1.61). Analyses were conducted in SAS 8.1 (SAS, Cary, NC).

Results

AGE- AND STAGE-RELATED VARIATION AMONG PREMIGRANTS AND MIGRANTS, 1999

Body size and mass

Structural size (PC1) was independent of age and stage ($P \geq 0.1$ in both cases). There was a significant interaction, however, between age and stage for capture mass

($F_{1,43} = 9.7$, $P < 0.01$; mean (g) \pm SEM, premigrants: juveniles 25.8 ± 0.5 and adults 28.8 ± 1.0 , and migrants: juveniles 28.0 ± 0.5 and adults 25.8 ± 1.0). Although premigrant juveniles had attained full structural size, they were 10.5% lighter than premigrant adults ($F_{1,43} = 5.5$, $P < 0.025$). Dissection mass was used as the basis for the body mass covariate in the analysis of SI mass since capture mass and dissection mass were highly correlated ($n = 47$, $r = 0.95$, $P < 0.001$).

SI size

We used generalized linear models to explain variation in SI length and mass, and generated ls means from these models to test our predictions. Age, stage and time to dissection accounted for 56% of the variation in SI length (Table 1). Juveniles had SIs that were 8.0% longer on average than those of the adults ($F_{1,43} = 20.5$, $P < 0.001$, ls mean (cm) \pm SEM, juveniles: 21.6 ± 0.2 , adults: 20.0 ± 0.3 , Fig. 1a); the mean length difference was 10% for premigrants ($F_{1,43} = 13.2$, $P < 0.001$) and 6.5% for refueling migrants ($F_{1,43} = 8.0$, $P < 0.01$). Migrants had SIs that were 7.0% longer on average than those of the premigrants ($F_{1,43} = 15.2$, $P < 0.001$, ls mean (cm) \pm SEM, premigrants: 20.1 ± 0.3 , migrants: 21.5 ± 0.2). SI length, body mass and stage accounted for 51% of the variation in SI mass (Table 1). The length-corrected mass of the SI was 10.2% larger in migrants than in premigrants ($F_{1,42} = 4.0$, $P < 0.05$, ls mean (g) \pm SEM, premigrants: 0.98 ± 0.04 , migrants: 1.08 ± 0.03 , Fig. 1b). The length-corrected mass of the SI was independent of age ($F_{1,42} = 0.38$, $P \geq 0.6$, ls mean (g) \pm SEM, juveniles: 1.05 ± 0.03 , adults: 1.01 ± 0.05); this is a direct result of controlling for SI length.

Morphometry of the proximal duodenum

Circumference, smooth muscle width and villus length were independent of age ($P \geq 0.2$ in each case). There was, however, significant stage-related variation in the circumference of the proximal duodenum, which was

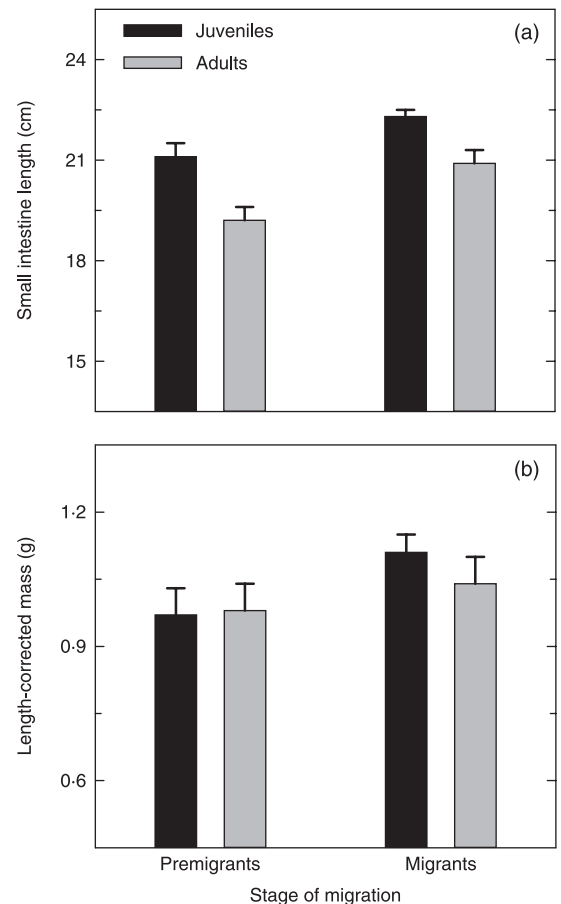


Fig. 1. Stage- and age-related comparisons of small intestine length (a) and length-corrected mass (b) of premigrant (Safety Sound, 1999) and refuelling migrant (Boundary Bay, 1999) female *Calidris mauri*. Time to dissection was a covariate in the analysis of intestine length. Intestine length and body mass were covariates in the analysis of length-corrected mass. Values are least-square means \pm SEM. Sample sizes are as follows: premigrants, 8 juveniles and 9 adults; migrants, 23 juveniles and 7 adults.

9.5% larger in migrants than in premigrants (Table 2). After controlling for variation in circumference, smooth muscle width and villus length were independent of stage.

Table 1. Generalized linear models explaining variation in the length and mass of the small intestines of premigrant (Safety Sound, 1999) and migrant (Boundary Bay, 1999) female *Calidris mauri* ($n = 47$)

Parameter	Coefficient	t_{43}	P -value	Adj. r^2
Intestine length (cm)	–	20.2*	< 0.001	0.56
Intercept	20.51	47.1	< 0.001	
Age	1.59	4.5	< 0.001	
Stage of migration	1.42	3.9	< 0.001	
Time to dissection (h)	–0.11	–3.4	< 0.001	
Intestine mass (g)	–	17.1*	< 0.001	0.51
Intercept	–1.02	–2.6	< 0.01	
Intestine length (cm)	0.07	4.8	< 0.001	
Body mass (g)	0.02	2.0	< 0.05	
Stage of migration	0.11	2.0	< 0.05	

* F -statistics for the full models intestine length and mass are $F_{3,43}$.

Table 2. Morphometry of the proximal duodenum of premigrant (Safety Sound, 1999) and migrant (Boundary Bay, 1999) female *Calidris mauri*

Parameter	Premigrants		Migrants		Stage of migration	
	Juveniles	Adults	Juveniles	Adults	$F_{1,17}$	P -value
Circumference (mm)	6.6 ± 0.2	6.5 ± 0.3	7.6 ± 0.2	6.9 ± 0.2	7.3	0.02
Muscle width (µm)	102 ± 11	94 ± 11	79 ± 12	93 ± 10	0.6	0.44
Villus length (µm)	757 ± 22	747 ± 23	792 ± 25	800 ± 21	3.4	0.08
n	5	5	5	5		

Circumference values are means ± SEM. Muscle width and villus length values are least-square means ± SEM, with circumference as a covariate ($F_{1,16}$).

AGE- AND CESTODE-RELATED VARIATION AMONG MIGRANTS, 1999 AND 2000

Body size and mass

Structural size (PC1) was independent of age, cestode infection and year ($P \geq 0.2$ in each case). There was a significant interaction between age and year for capture mass ($F_{1,63} = 10.5$, $P < 0.01$); we controlled for this interaction by including it in the analysis of capture mass, with age, year and cestode infection as main effects. Capture mass was independent of age ($F_{1,63} = 3.8$, $P \geq 0.05$; ls mean (g) ± SEM, juveniles = 29.4 ± 0.6, adults = 27.0 ± 0.8), but varied with cestode infection ($F_{1,63} = 7.0$, $P < 0.01$; uninfected = 27.1 ± 0.5, infected = 29.3 ± 0.7) and year ($F_{1,63} = 10.1$, $P < 0.01$; 1999 = 26.2 ± 0.8, 2000 = 30.1 ± 0.6). The interaction between age and cestode infection was not significant for capture mass ($F_{1,62} = 3.6$, $P \geq 0.05$; ls mean (g) ± SEM, uninfected: juveniles 27.8 ± 0.7 and adults 26.3 ± 0.9; infected: juveniles 31.2 ± 0.9 and adults 26.3 ± 1.3). Again, capture mass and dissection mass were strongly correlated ($n = 68$, $r = 0.96$, $P < 0.001$).

Table 3. Generalized linear models explaining variation in small intestine length and mass of refuelling female *Calidris mauri* collected during southward migration (Boundary Bay), in 1999 and 2000 ($n = 68$)

Parameter	Coefficient	t_{65}	P -value	Adj. r^2
Intestine length (cm)	–	19.2*	< 0.001	0.35
Intercept	22.53	71.2	< 0.001	
Age	1.57	4.6	< 0.001	
Time to dissection (h)	–0.14	–5.0	< 0.001	
Intestine mass (g)†	–	37.2*	< 0.001	0.68
Intercept	–1.41	–5.2	< 0.001	
Intestine length (cm)	0.09	8.0	< 0.001	
Body mass (g)	0.03	4.1	< 0.001	
Year	0.12	3.0	< 0.005	
Cestode infection	0.11	2.9	< 0.005	

* F -statistics for the full models accounting for variation in intestine length and mass are $F_{2,65}$ and $F_{4,63}$, respectively.

† t statistics for parameters in the model explaining variation in intestine mass are t_{63} .

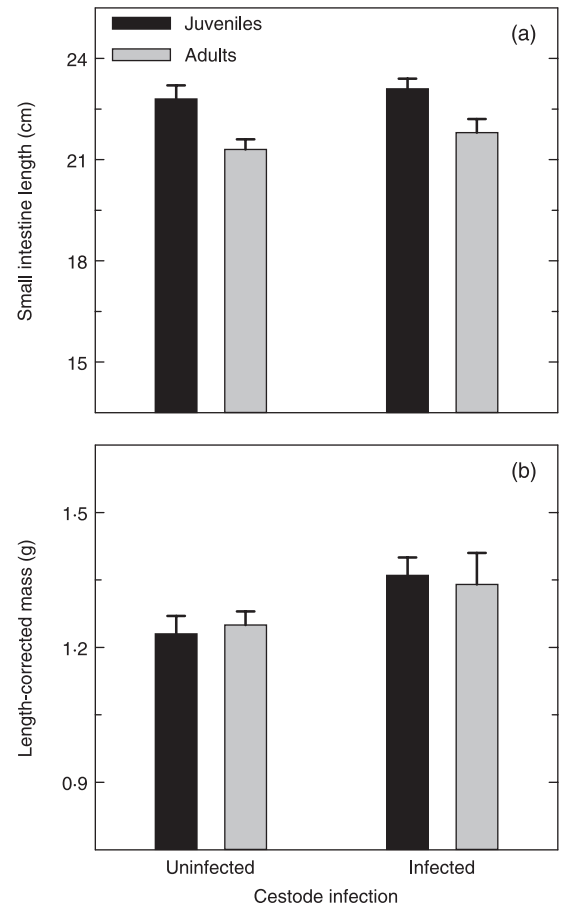


Fig. 2. Age- and cestode-related comparisons of small intestine length (a) and length-corrected mass (b) of refuelling female *Calidris mauri* (Boundary Bay, 1999 and 2000). Time to dissection was a covariate in the analysis of intestine length. Intestine length, body mass and year were covariates in the analysis of length-corrected mass. Values are least-square means ± SEM. Sample sizes are as follows: uninfected, 24 juveniles and 24 adults; infected, 15 juveniles and 5 adults.

SI size

Age and time to dissection accounted for 35% of the variation in SI length (Table 3). Juveniles had SIs that were 8.5% longer on average than those of the adults ($F_{1,63} = 20.9$, $P < 0.001$; ls mean (cm) ± SEM, juveniles: 23.0 ± 0.2, adults: 21.2 ± 0.3 Fig. 2a). SI length

was independent of cestode infection ($F_{1,63} = 0.3$, $P \geq 0.7$; ls mean (cm) \pm SEM, uninfected: 22.2 ± 0.2 , infected: 22.0 ± 0.3). SI length, body mass, year and cestode infection accounted for 68% of the variation in SI mass.). The length-corrected mass of the SI was 9.7% heavier in cestode-infected individuals ($F_{1,62} = 7.5$, $P < 0.01$; ls mean (g) \pm SEM, uninfected: 1.24 ± 0.02 , infected: 1.36 ± 0.04 , Fig. 2b). The length-corrected mass of the SI was independent of age ($F_{1,62} = 0.02$, $P \geq 0.9$; ls mean (g) \pm SEM, juveniles: 1.30 ± 0.04 , adults: 1.30 ± 0.03).

Cestode prevalence

Cestodes were identified from two genera, *Aploparaksis* and *Nadejdolepsis*; further identification to species was not possible owing to contraction of the reproductive organs, which occurred prior to fixation. There was a significant interaction between age and year for cestode prevalence ($\chi^2 = 4.6$, $P < 0.05$; 1999: juveniles 22% and adults 29%; 2000: juveniles 63% and adults 14%). In 2000, juveniles had a higher prevalence than adults ($\chi^2 = 9.8$, $P < 0.01$); juveniles also had a higher prevalence in 2000 than in 1999 ($\chi^2 = 6.6$, $P < 0.01$).

Discussion

The results of the present study confirm and extend the findings of Guglielmo & Williams (2003), that juvenile *Calidris mauri* making their first southward migration have substantially enlarged SIs, relative to adult migrants. Guglielmo & Williams (2003) concluded that this age-dependent difference in SI size was associated with mass, as opposed to length. The present study demonstrates the opposite, that the mass difference is due primarily to a length difference and that there is no age-related difference in length-corrected mass when cestode infection is controlled statistically. This discrepancy was due to a difference in measurement methodology, and highlights the benefits of Brambell's method for measuring SI length, over Leopold's (Freehling & Moore 1987), as well as the importance of measuring SI size in several dimensions. A difference in mass could reflect a difference in length or circumference, which have different implications for digestive function and/or pathology.

We proposed three hypotheses to explain the post-growth age-dependent difference in SI size previously observed in *Calidris mauri* by Guglielmo & Williams (2003), and the four associated predictions received either full or partial support. The prediction associated with hypothesis 1, that an enlarged SI is associated with the growth phase of ontogeny, was supported. Premigrant juveniles that had attained full structural size had SIs that were 10.0% longer than those of premigrant adults, even though the juveniles were 10.5% lighter than the adults. The prediction associated with hypothesis 2, that SI size is closely associated with intake rate, was also supported. In association

with the commencement of migration, the length and circumference of the SI increased in both age-classes. Although we do not report any data on intake rate, these results indicate a marked change in SI size that is associated with the onset of migration and are consistent with the effects of migratory hyperphagia. There was partial support for the predictions associated with hypothesis 3, that the age-related difference in SI size is associated with cestode infection. Although the length of the SI was independent of cestode infection, the length-corrected mass of the SI was 9.7% heavier in infected migrants, and juveniles had a higher prevalence of infection than adults in one of two years.

Guglielmo & Williams (2003) suggested five hypotheses to account for their observation of a larger SI in refuelling juvenile *Calidris mauri*. In light of further research aimed at exploring this observation (Stein *et al.* 2005; this study), we are now able to evaluate their hypotheses, which fall into four general categories: growth, foraging proficiency, digestive strategy and endoparasites. Their first hypothesis, that larger SIs in juveniles indicate continued growth during migration, was not supported by their morphometric data or by ours. Juveniles achieve full structural size before leaving the breeding grounds. Their second hypothesis, that juveniles might be consuming a lower-quality diet, remains untested. Although it is possible, and in some regards likely, that diet quality declines seasonally and that the later-migrating juveniles have a lower-quality diet (Stein *et al.* 2005), there is no direct evidence to support this at Boundary Bay. If this was true, then we would expect Julian date to be a significant predictor of SI size within each age class and it was not. Their third hypothesis, that juveniles ingest more sediment than adults, and that this might explain their heavier but not longer SIs, was not supported. In the present study we demonstrate that the age-dependent difference in SI mass is due to length, not length-corrected mass. In addition, Stein *et al.* (2005) demonstrated that faecal samples from juveniles contained a smaller proportion of ash, which would include any ingested sediment, than those from adults. Their fourth hypothesis, that juveniles employ a digestive strategy that maximizes extraction efficiency by having large guts and slow passage rates, also remains untested. Stein *et al.* (2005) suggest, however, that refuelling juveniles might be processing large volumes of food quickly, thereby sacrificing efficiency, with the aim of maximizing the net rate of energy gain. Stein *et al.* (2005) report data that are consistent with this idea, but are not able to rule out the possibility that diet quality is lower for the later-migrating juveniles. In addition to having a longer SI, refuelling juveniles also have a substantially smaller (27%) proventriculus, and this led Stein *et al.* (2005) to conclude that juveniles probably do process prey differently from adults. Guglielmo & Williams (2003) fifth hypothesis, that the enlarged SIs of juveniles might be associated with higher intestinal parasite loads, receives some support from the present

study. Although cestode infection had no influence on SI length, which is the primary age-related difference in SI size, the length-corrected mass of the SI was heavier in infected individuals.

ONTOGENY OF INTESTINE SIZE IN ASSOCIATION WITH MIGRATION

In birds that have self-feeding precocial chicks, the relative size of the SI (to body mass) is larger during growth than it is in adults (Konarzewski *et al.* 1990; Starck 1998). In addition to growth-specific energy requirements, precocial chicks have high thermoregulatory and activity costs, which result in higher total metabolizable energy requirements than predicted by allometry (Schekkerman & Visser 2001), and this deviation is particularly large in long-distance migrants that breed at northern latitudes (Schekkerman *et al.* 2003). The self-feeding precocial chicks of *Calidris canutus* meet the high mass-specific energy requirements of growth and self-maintenance through hyperphagia, and are able to achieve extremely high growth rates despite high thermoregulatory and foraging costs (Schekkerman *et al.* 2003). For long-distance migrants with precocial young, such as *Calidris* sandpipers, an enlarged SI in fledglings that have attained full structural size is probably the result of selection for high intake rates during growth. Consistent with this idea, the sample of premigrant juvenile *Calidris mauri* reported on here had SIs that were 10.0% longer than those of premigrant adults. These premigrant juveniles were in the final stages of growth at the time of capture. They were approximately 22 days of age, had achieved full structural body size, and their primary feathers were fully grown (R. W. Stein, personal observation). However, the juveniles had not yet reached the pre-departure mass exhibited by adults. The results presented here indicate that premigrant juvenile *Calidris mauri* retain an elongated SI that was initially associated with growth, and that there is an additional increase in length associated with the onset of migration.

Age-dependent differences in SI size have received little attention in studies of refuelling physiology and organ dynamics during migration (but see Guglielmo & Williams 2003; Stein *et al.* 2005); this is somewhat surprising because migration-related mortality can be quite high (Owen & Black 1989; Sillett & Holmes 2002), particularly for juveniles (Owen & Black 1991). Juvenile *Calidris mauri* initiate southward migration 1 month after adults, and navigate the same route but under a more severe predation regime (Lank *et al.* 2003). The costs of carrying an enlarged SI during their first migration could be substantial for juveniles, but the benefits of doing so have not been considered until recently. Stein *et al.* (2005) investigated the possible benefits of maintaining an enlarged SI to refuelling juvenile *Calidris mauri*, and determined that juveniles exhibited: (1) larger volumetric digestive capacity, (2) lower total enzymic digestive capacity and (3) a higher

proportion of residual dietary energy in their faeces. This led Stein *et al.* (2005) to suggest that refuelling juveniles might be rapidly processing large volumes of food with the aim of maximizing the net rate of energy gain, rather than digestive efficiency. Their results, however, could not rule out the alternative explanation that juveniles were simply consuming a lower-quality diet (Stein *et al.* 2005). In either case, the later-migrating juveniles are confronted with additional energetic costs associated with maintaining an enlarged SI and it appears that the only benefit from doing so is increased volumetric digestive capacity. An enlarged SI may reflect a somewhat counter-intuitive response to selection acting to minimize the duration of migration for the later-migrating juveniles.

ENDOPARASITIC INFECTION AND INTESTINE SIZE

In addition to being confronted with increased predation pressure (Lank *et al.* 2003), later-migrating juveniles may also be at a higher risk of contracting food-borne parasites, such as cestodes, than adults. Consistent with this idea, controlled experiments on age-dependent susceptibility of *Gallus gallus* (domestic chickens) to cestode infection demonstrate that host resistance increases with age, even under benign conditions (Gray 1972). Juvenile *Calidris mauri* migrants had a higher prevalence of cestode infection in one year, and this is probably the more typical situation. Annual variation in cestode prevalence in juveniles could result from variation in the abundance of the intermediate invertebrate hosts or the infection rates of these prey items. Once ingested, immature cestodes anchor into the intestinal wall with rostellar hooks, thereby producing pathological effects that impair the host's health and energy assimilation. In the most extreme case, cestode infection leads to intestinal obstruction and can result in the death of the host (Bailey *et al.* 1996). Sub-lethal pathologies associated with cestode infection include localized inflammation at the site of attachment, raised lymphoid nodules, denuded mucosal epithelium accompanied by hyper-regenerative response, and hypertrophy of the muscularis (Jones *et al.* 1996a,b). In *Calidris mauri*, sub-lethal effects of cestode infection were apparent in the length-corrected mass of the SI, which was 9.7% heavier in infected individuals; it is likely that this was the result of hypertrophy of the muscularis and inflammation of the SI. Cestode infection, however, was not associated with the post-growth age-dependent difference in SI length.

Another important sub-lethal effect of cestode infection is competition between the host and parasite for food energy, which impairs nutrient assimilation by the host. Munger & Karasov (1989) demonstrated that cestode infection can have negative impacts on the energy budget of *Peromyscus leucopus* (White-Footed Mouse), and suggested that the magnitude of the

energy losses could compromise host performance during periods of peak energy demand, such as reproduction. For *Calidris mauri*, sub-lethal effects of cestode infection might decrease the overall speed of migration by increasing the time required to refuel. A decrease in the overall speed of migration could result in increased mortality, and this would have a disproportionate impact on juveniles owing to a higher prevalence of cestode infection in some years. The present study is the clearest example of a cestode-related effect on the morphology of the SI in a wild bird (see Shutler, Alisauskas & McLaughlin 1999), and we were able to resolve its effect on length-corrected mass because we measured the SI size in several dimensions. The interaction between age-dependent susceptibility to intestinal parasites and refuelling performance warrants further consideration in studies of refuelling physiology and organ dynamics during migration.

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