Ca ses a d c se e ces f s-g h age-de e de diffe c es i s a i esi e si ei a ig a sa d i e (*Calidris mauri*, We e Sa d i e)

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1. 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.

I d c i

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

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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 gavauge needle was sutured to the proximal end of the SI and its contents were purged with ice-cold physiological saline (350 mosmol/kg H$_2$O). 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 gavauge needle attached to the proximal end. The gavauge 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).

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 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-μm 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) 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.

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 2(an e0C)0(nl048hh)]602Tw[(th40528 Tw)64/o4-p0nhl5l-7 th

$(F_{1,43} = 9\cdot7$, $P < 0\cdot01$; mean (g) $\pm$ SEM, premigrants: juveniles $25\cdot8 \pm 0\cdot5$ and adults $28\cdot8 \pm 1\cdot0$, and migrants:

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

Parameter	Premigrants		Migrants		Stage of migration	
	Juveniles	Adults	Juveniles	Adults	$F_{1,17}$	P-value
Circumference (mm)	$6{\cdot}6 \pm 0{\cdot}2$	$6{\cdot}5 \pm 0{\cdot}3$	$7{\cdot}6 \pm 0{\cdot}2$	$6{\cdot}9 \pm 0{\cdot}2$	$7{\cdot}3$	$0{\cdot}02$
Muscle width (μm)	102 ± 11	94 ± 11	79 ± 12	93 ± 10	$0{\cdot}6$	$0{\cdot}44$
Villus length (μm)	757 ± 22	747 ± 23	792 ± 25	800 ± 21	$3{\cdot}4$	$0{\cdot}08$
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Circumference values are means $\pm$ SEM. Muscle width and villus length values are least-square means $\pm$ SEM, with circumference as a covariate ($F_{1,16}$).

Structural size (PC1) was independent of age, cestode infection and year ($P \geq 0{\cdot}2$ in each case). There was a significant interaction between age and year for capture mass ($F_{1,63} = 10{\cdot}5$, $P < 0{\cdot}01$); 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 ($F_{1,63} = 3{\cdot}8$, $P \geq 0{\cdot}05$; ls mean (g) $\pm$ SEM, juveniles $= 29{\cdot}4 \pm 0{\cdot}6$, adults $= 27{\cdot}0 \pm 0{\cdot}8$), but varied with cestode infection ($F_{1,63} = 7{\cdot}0$, $P < 0{\cdot}01$; uninfected $= 27{\cdot}1 \pm 0{\cdot}5$, infected $= 29{\cdot}3 \pm 0{\cdot}7$) and year ($F_{1,63} = 10{\cdot}1$, $P < 0{\cdot}01$; 1999 $= 26{\cdot}2 \pm 0{\cdot}8$, 2000 $= 30{\cdot}1 \pm 0{\cdot}6$). The interaction between age and cestode infection was not significant for capture mass ($F_{1,62} = 3{\cdot}6$, $P \geq 0{\cdot}05$; ls mean (g) $\pm$ SEM, uninfected: juveniles $27{\cdot}8 \pm 0{\cdot}7$ and adults $26{\cdot}3 \pm 0{\cdot}9$; infected: juveniles $31{\cdot}2 \pm 0{\cdot}9$ and adults $26{\cdot}3 \pm 1{\cdot}3$). Again, capture mass and dissection mass were strongly correlated ($n = 68$, $r = 0{\cdot}96$, $P < 0{\cdot}001$).
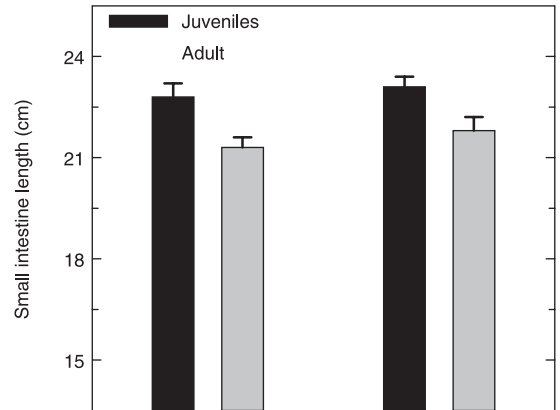
Age and time to dissection accounted for 35% of the variation in SI length (Table 3). Juveniles had SIs that were 8·5% longer on average than those of the adults ($F_{1,63} = 20{\cdot}9$, $P < 0{\cdot}001$; ls mean (cm) $\pm$ SEM, juveniles: $23{\cdot}0 \pm 0{\cdot}2$, adults: $21{\cdot}2 \pm 0{\cdot}3$ 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).

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.

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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 canutus* 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 canutus* 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 canutus* 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 canutus*, 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.

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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 canutus* 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 hyperregenerative response, and hypertrophy of the muscularis (Jones *et al.* 1996a,b). In *Calidris canutus*, 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 ⬛, 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.

Acknowledgements

References

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