Kidney, liver and bone cadmium content in the western sandpiper in relation to migration

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Cadmium content was measured in kidney, liver and tarsus bones of western sandpipers (*Calidris mauri*) at a temperate migratory stopover site (Fraser Delta, British Columbia, Canada) and a wintering site (Playa el Agallito, Chitre, Panama) over a two year period. Cadmium content in liver and kidney was age and sex dependent. Adult females generally had lower kidney and liver cadmium than adult males ($P < 0.05$), but a sex difference was not detected in juveniles. Cadmium increased with age in kidney, liver and to a lesser extent in bone ($P < 0.001$) with average "steady-state" kidney and liver content being reached within the sandpipers first year. In general, tissue cadmium residues in adult males and females were independent of sampling location although for bone, site-specific differences did occur ($P < 0.001$). Bone cadmium was lower in females sampled from their wintering grounds as compared to temperate stopover sites suggesting that bone cadmium may be mobilized during periods of feather molt. Comparison of cadmium residues among sandpipers of increasing age suggest that exposure is occurring along the Pacific Coast, at stopover sites as the birds migrate north to Alaska and south to Panama. This study points to the importance of considering the ecology of the species (*e.g.*, in this case migratory behavior) in interpreting trace metal residues.

Introduction

Coastal muddy intertidal and estuarine environments frequently exhibit elevated levels of cadmium.[1] The submerged macrophytes and benthic invertebrates that inhabit these intertidal regions can accumulate significant amounts of metals *via* absorption or through ingestion of metal bearing sediments.[1] These sediment-dwelling organisms in turn are primary food sources and thereby a route of metal exposure for many migratory bird species, including the western sandpiper (*Calidris mauri*).

The western sandpiper migrates along the Pacific Flyway between its wintering grounds, which extend mainly along the Pacific Coast of northern South America, Central America, Mexico and California, and its breeding grounds, which include portions of Siberia and Alaska.[2] One of the prominent temperate stopover sites used by the western sandpiper is the Fraser Delta, British Columbia.[3] At these stopover sites, the sandpipers exhibit hyperphagia, where they consume more food than normally required for maintenance in order to sustain the high metabolic rates of migration.[3] Stopover sites, therefore, are essential habitats for ensuring the survival of the western sandpiper and other migratory shorebirds. It is unknown if metals such as cadmium are accumulated during these periods of hyperphagia and secondly if amounts accumulated pose a toxicological risk to these birds.

A further complication specifically with respect to the interpretation of trace metal residues in migratory species such as the sandpiper is that metal accumulation could be site specific *i.e.*, one specific migratory stopover route could be high in trace metals whereas other stopover sites relatively low. This is especially true for the sandpiper as its migratory route takes it from the remote intertidal regions of Alaska, down the Pacific coast where stopover sites include highly urbanized regions such as the Fraser River Delta to Panama where the degree of metal contamination is at best uncertain.

Hence, the objectives of our study were two-fold: first, through a comparison of cadmium residues in the liver, kidney and bone of sandpipers collected from specific points on the migratory route, to determine if differences in metal exposure among the stopover sites exist; and, secondly, to determine if amounts of cadmium accumulated in the organs of the sandpiper were of toxicological concern. An important and unique aspect of our study was that we were able to compare cadmium residues in known aged sandpipers from specific locations which allowed us to determine the dependence of cadmium residues on bird age, as well as the dependence of cadmium residues on migratory site.

Methods

Collection

Migrating western sandpipers were sampled during stopover refueling for two years (1995 and 1996) at the Fraser Estuary, British Columbia, Canada (49°10′N, 123°05′W). In each spring (25 April–10 May) adults of each sex were collected during their northward migration (these birds included a small number of first-years, and birds 2+ years of age since we could not age birds at this stage). In each fall birds of each sex were taken in July (adults) and August (juveniles) during their southward migration. Wintering (non-migratory) (December–January) and pre-migratory birds (March) were sampled at Playa el Agallito, Chitre, Panama (8°N, 79°W). We studied only females in Panama due to permit limitations on the total number of

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Table 1 Sampling date, age, sex, sample size, mean weight (grams) and standard deviation of sampled birds

Sampling period: season 'year	Age	Sex	*n*	Average weight/g	*s*
Fall '95	Adult	Female	8	24.7	2.7
Fall '95	Adult	Male	12	25.8	3.5
Fall '95	Juvenile	Female	15	26.8	2.6
Fall '95	Juvenile	Male	13	25.0	3.8
Fall '96	Adult	Female	14	29.1	2.8
Fall '96	Adult	Male	19	25.2	1.9
Fall '96	Juvenile	Female	15	29.5	2.7
Fall '96	Juvenile	Male	12	26.3	2.9
Spring '95	Adult	Female	13	25.8	1.9
Spring '95	Adult	Male	17	25.8	1.9
Spring '96	Adult	Female	15	29.3	1.8
Spring '96	Adult	Male	15	25.4	2.0
Winter '95	Adult	Female	14	24.5	1.3
Winter '95	Juvenile	Female	14	23.4	1.1
Winter '96	Adult	Female	15	27.2	2.5
Winter '96	Juvenile	Female	15	22.6	0.8

collections. In Panama, only birds 2+ years of age (*i.e.* adults) molt into breeding plumage, gain mass and prepare to migrate north by late March.[4] We collected adult and juvenile females in winter (15 December, 1995–8 January, 1996), and females of each age class during premigration (4–18 March, 1996). Sampling periods, age, sex, number of birds sampled, and average weight of each group of birds is presented in Table 1. We captured sandpipers in mist nets (Avinet, Dryden, NY, USA) under permits from the Canadian Wildlife Service and INRENARE (Panama) and birds were anaesthetized and killed by exsanguination.[5] Samples used in the current paper were obtained from birds used by Guglielmo and Williams.[6] Simon Fraser University Animal Care Committee approved the animal handling protocols, which met the Canadian Committee for Animal Care guidelines.[7]

In British Columbia, the birds were double-wrapped in plastic and packed on ice in the field and within hours of capture the birds were dissected in the laboratory. In Panama, the birds were double-wrapped in plastic and immediately frozen at −20 °C. Within two weeks the birds were transferred to −80 °C for storage. Once in the laboratory kidneys, liver and tarsus bone were removed and weighed. Tissues were frozen and stored in eppendorf tubes until lyophilization to a constant mass (3–7 days). The liver and a sub-sample of kidneys were fat extracted with petroleum ether. Sampled tissues were dried for 48 h, weighed and repackaged.

Analysis

Dried kidneys (fat extracted and non-fat extracted), liver (fat extracted) and tarsus bone samples were weighed to the nearest 0.1 mg and digested to near dryness (approximately 2–3 h on hot plates) in open 25 mL acid-washed Erlenmeyer flasks with 5.0 mL of 70% analytical grade HNO_3 acid. Procedural blanks and two standard certified reference materials obtained from the National Research Council of Canada (NRC), TORT-2 (lobster hepatopancreas) and DOLT-2 (dogfish muscle and liver), with known certified cadmium values were digested simultaneously with the kidney and liver samples for quality assurance/quality control. Unlike liver and kidney, bone samples are high in $CaCO_3$. For these analyses, the TORT-2 and DOLT-2 were spiked with 250 μg mL^{-1} $CaCO_3$ to account for possible matrix effects due to the high concentrations of calcium present in the samples. Following complete digestion, 1.0 mL of ddH_2O (double distilled dionized water) was added to each flask. The liquid samples were transferred into labeled falcon tubes. The flasks were then rinsed 2 more times each with 2.0 mL ddH_2O to ensure complete transfer of the sample. Although the liver samples had been previously fat extracted,[5,6] enough fat residue remained after the digestion process to hinder the complete transfer from the flask to the falcon tube. Consequently, after acid digestion was completed the liver samples were subjected to the process of fat oxidation: 5.0 mL ddH_2O and 100 μL of 50% hydrogen peroxide were added to each liver sample, the samples were evaporated to dryness, and the flasks rinsed three times with a total of 5.0 mL ddH_2O (as stated after an acid digestion). The samples were then stored in 15 mL polypropylene Falcon tubes at 4 °C until analysis (<3 weeks). All equipment used during the process of analysis was acid washed in 10% nitric acid for a minimum of 24 h and rinsed 7 times with ddH_2O and then rinsed a final time with ddH_2O. Tissue content of cadmium was determined by atomic absorption spectrophotometry, at a wavelength of 228 nm, and a slit width of 0.7 nm, (Perkin Elmer® AAnalyst 100) with an air–acetylene flame.

Statistical analysis

All data prior to statistical analysis was $\log_{10}$ transformed. The log-transformed arithmetic means of each subpopulation were then compared using parametric tests (Student's *t*-test, one-way ANOVA (General Linear Model (GLM)), and Tukey Honestly Significantly Difference test, and, linear regression) (SAS; Statistical Analysis Systems, SAS Institute, 1998). Significance for all tests was accepted at $P < 0.05$ (significance of *t*-tests were Bonferroni corrected for the number of comparisons made).

Results

Experimental recovery of cadmium in the standard reference materials was within the standard error of certified values (in μg g^{-1} experimental recovery *versus* certified value ± one standard error respectively): 30.2 ± 2.52 and 26.7 ± 3.6 for TORT-2 and 23.9 ± 2.45 and 20.8 ± 3.5 for DOLT-2. RSD for the analysis (*i.e.*, precision of the analysis as determined by the variation in the recoveries of cadmium from reference materials in the sample analysis) were 8.3% and 10.2% for TORT-2 and DOLT-2 respectively. Cadmium content in fat extracted and non-fat extracted kidneys was compared between adult females collected during spring 1995 and spring 1996. No significant difference was found between the fat extracted and non-fat extracted kidney cadmium content ($P = 0.5$; Student's *t*-test) hence values were pooled for further analysis. Contents of cadmium in kidney and to a lesser extent in liver were found to be dependent on sex, ($P < 0.05$; Student's *t*-test), hence, for consistency in analysis for the three tissues, sexes were kept separate.

Cadmium in liver, kidney and bone

Kidney and liver cadmium content were positively correlated ($r = 0.83$; $P < 0.0001$), with an average renal to hepatic ratio of 3.17. This corresponds well with the renal to hepatic ratios measured in dunlin (*Calidris alpina*, 3.10 and curlew sandpipers, *Calidris ferruginea*, 2.80).[8] Of the three tissues, bone contained the lowest cadmium content. As a point of reference, bone cadmium in the sandpipers were on average 2.5 fold lower that those reported for the herbivorous redknobbed coot (*Fulica cristata*).[9] Generally, liver, kidney and bone cadmium content for both adult females and males were independent of sampling location (Fig. 1a, 2a and 3a) although there were exceptions. Adult females collected from their northward stopover, Spring '96 contained significantly greater kidney cadmium content as compared to all other sites ($P = 0.018$; Fig. 1a); adult females collected from their pre-migratory wintering stage contained significantly lower bone cadmium content as compared to those collected in Spring '95 ($P = 0.0001$; Fig. 3a).

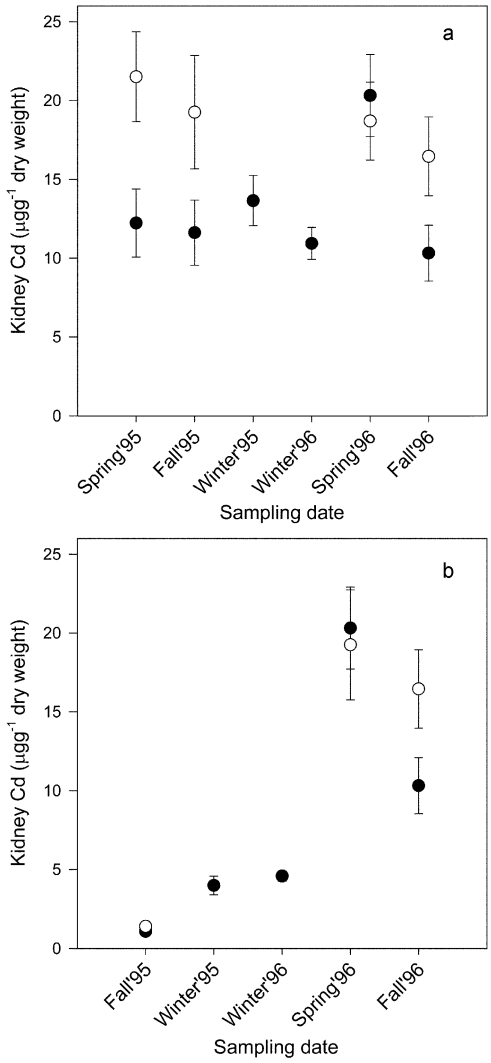

Fig. 1 (a) Kidney cadmium content in adult male (open circle) and adult female (closed circle) western sandpipers (values are means ± 1 SE). Male kidney cadmium contents are not dependent on site whereas female kidney cadmium contents are significantly greater in Spring '96. Note that Winter '95 is December–January, post-migratory and Winter '96 is March, pre-migratory. (b) Kidney cadmium content as a function of bird age (values are means ± 1 SE). An increase in sample date represents an increase in bird age. Kidney content for each age class are significantly different from each other except for female samples during Winter '95 and Winter '96. Symbols as in Fig. 1a.

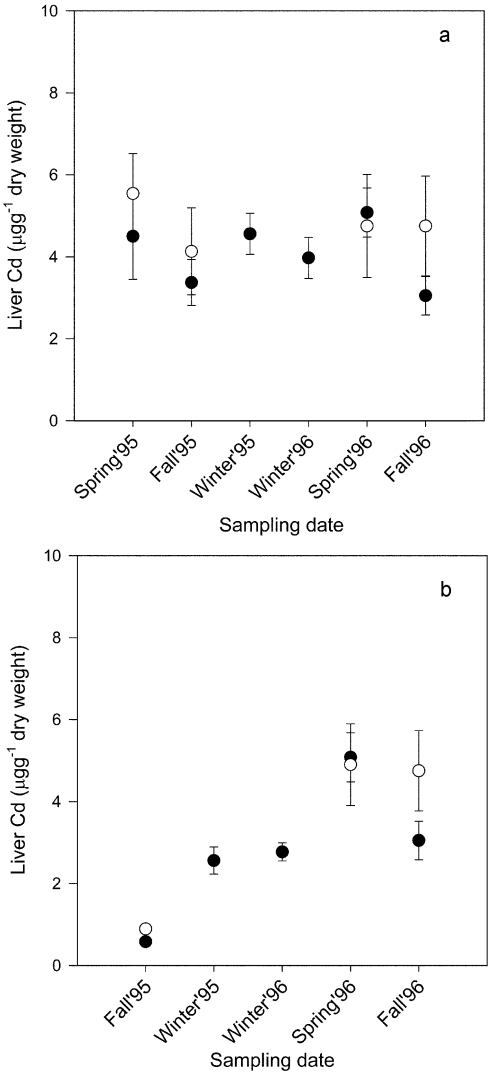

Fig. 2 (a) Liver cadmium content in adult male (open circle) and adult female (closed circle) western sandpipers (values are means ± 1 SE). Male and female liver cadmium contents are not dependent on site ($P > 0.05$; one-way ANOVA). (b) Liver cadmium content as a function of bird age (values are means ± 1 SE). An increase in sample date represents an increase in bird age. As with kidney, liver cadmium is age dependent, except for juvenile females sampled during Winter '95 and Winter '96 where no difference occurs. Symbols as in Fig. 2a.

All adult females and males (2+ years of age) contained on average (in μg g^{-1}) the following: 11.59 ± 0.11 (with Spring '96 omitted) and 18.98 ± 0.26 in kidneys respectively; 4.08 ± 0.08 and 4.79 ± 0.07 in livers respectively; and 1.94 ± 0.02 (with Winter '95 and Winter '96 omitted) and 1.99 ± 0.03 in bone respectively.

Cadmium content in μg g^{-1}, in kidney, liver and bone was dependent on bird age (Fig. 1b, 2b and 3b; one-way ANOVA $P < 0.001$). Juvenile sandpipers captured soon after leaving the breeding grounds at their first stopover site (Fraser Estuary) contained on average 1.53 ± 0.03 and 0.78 ± 0.017 μg g^{-1} in kidney and liver prior to arrival at the wintering grounds. These averages represent both male and female birds sampled in two subsequent years (*i.e.*, Fall '95 and Fall '96). By the time the juvenile birds arrived at the wintering area in Panama, kidney and liver content had increased significantly ($P < 0.0001$; one-way ANOVA) by three-fold. No further increases in tissue residues occurred during the over-wintering period. This implies that minimal exposure to cadmium occurs on the wintering grounds. Indeed there was a net loss of bone cadmium during this period (Fig. 3b). However, by the time the birds are 2+ years of age and are captured on the northward migration, kidney and liver content again increased significantly by three-fold (in kidney, $P < 0.0001$ in liver, $P < 0.0001$ and $P < 0.006$, for females and males respectively; one-way ANOVA). Bone cadmium also increased with age, however, not as dramatically as noted for liver and kidney ($P < 0.01$; Fig. 3b)

Discussion

Cadmium in kidney, liver and bone of the western sandpiper

Adult females *versus* **adult males.** Female adult sandpipers contained in general, lower kidney cadmium content as compared to males. This difference also occurred for liver cadmium although not as pronounced as was noted for the kidney. This sexual dependence may be in part a result of female sandpipers ability to excrete cadmium *via* the process of egg production.[10] Mallards (*Anas platyrhynchos*), royal terns (*Sterna maxima*) and sandwich terns (*Sterna sandvicensis*) were found to produce

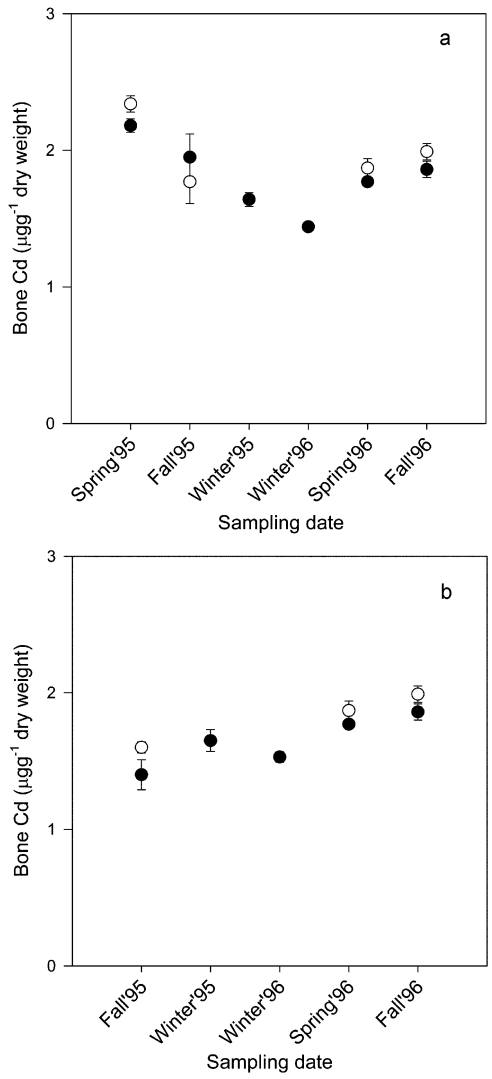

Fig. 3 (a) Bone cadmium content in adult male (open circle) and adult female (closed circle) western sandpipers (values are means ± 1 SE). Female bone cadmium content are site dependent being significantly lower in females sampled during Winter '95 and Winter '96 as compared to all other sampling dates. (b) Bone cadmium content as a function of bird age (values are means ± 1 SE). An increase in sample date represents an increase in bird age. As with kidney and liver, bone cadmium is age dependent increasing with bird age. Symbols as in Fig. 3a.

eggshells containing between 0.003–0.01 μg g^{-1} cadmium.[11,12] Sandpipers lay a clutch of four eggs; hence egg production could be a viable explanation for the observed differences in cadmium content between the two sexes.

Site dependence. Adult male and female sandpipers did not show any consistent difference in kidney and liver tissue cadmium residues among the stopover sites sampled in this study. Bone cadmium content was significantly lower in adult females sampled during Winter '95 and Winter '96. During this over wintering period, adults molt. Hence one possible reason for lower bone cadmium residues could be the mobilization of the metal from bone deposits concurrent with minerals required for feather development as has been observed for mercury.[13]

Age dependence. In contrast to site-specific differences, cadmium residues in all three tissues demonstrated strong age dependences. Age dependent increases in cadmium tissue residues have also been observed for dunlin[8] and for spruce grouse (*Falcipennis canadensis*).[14] Juveniles sampled on their first southward migratory stopover site (Fall '95 and '96) contained the lowest levels of cadmium in liver and kidney. These values most likely represent background content or content as a result of items consumed on the breeding grounds in Alaska. These would include both freshwater (*e.g.*, dipterans) and marine invertebrates.[15] Liver and kidney cadmium contents, however, are three-fold greater in the same cohort of juveniles sampled in their tropical wintering area six months later. This content does not change while the birds over winter; however, birds sampled at their northern migratory stopover site (a mix of first years and 2+ year adults) have three fold greater liver and kidney cadmium content as compared to over wintering juveniles.

The age dependent patterns observed for liver and kidney suggest that migratory birds accumulate about 3 μg g^{-1} cadmium during their southward migration, with no further accumulation occurring during their wintering months. As birds sampled on the northward and southward migratory stopover in British Columbia are aged 2+, to a maximum of 9 years,[15] these cadmium residues represent an "average" for adult birds. This average appears to reach a "steady-state" after year 2+ in the birds, with females containing a lower kidney and liver "steady-state" concentration for reasons previously discussed, as compared to males. Cadmium is known to accumulate in liver and kidney tissue as a function of age, hence this apparent steady-state is likely an artefact of pooling cadmium residues from birds representing an age span from 2–9 years. Importantly, sandpipers do not accumulate cadmium from either their breeding grounds, or their over-wintering sites, but rather from migratory stopover sites along the Pacific coast. These likely include the major stopover sites of Grays Harbour, Washington, and Humboldt and San Francisco Bay, California, USA.[16,17]

We have previously sampled both sediments and invertebrates from the temperate stopover site at Boundary Bay, BC. Cadmium levels were found to be significantly elevated in this bay as compared to other regions of the Fraser River delta, in both invertebrates (*Macoma balthica*) and surface sediments.[1] Sampled invertebrates are known prey items for the sandpiper. As well, we have shown that sediment consumed as grit can be an important trace metal exposure route in birds.[14,18] Content of cadmium in sediment and prey items in Boundary Bay are within the same range for the San Francisco estuary, also a stopover site for the sandpiper.[19] Hence, it is feasible that cadmium residues are a consequence of sandpipers foraging in estuaries located in the highly urbanized metal contaminated regions of the coast.

Toxicological significance. In experimental dosing studies, kidney damage has been demonstrated in fulmars, manx shearwaters (*Puffinus puffinus*), and Atlantic puffins (*Fratercula arctica*) at kidney cadmium contents (wet wt) between 10–120 μg g^{-1} and in starlings between 20–55 μg g^{-1}.[20] It has been suggested that, in general, cadmium poisoning may be expected in bird laboratory studies where birds exhibit cadmium contents above 40 μg g^{-1} in the liver or 100 μg g^{-1} in the kidney.[21] Thus, relative to laboratory derived threshold tissue contents, cadmium does not appear to be accumulating to levels of toxicological concern in the tissues of western sandpipers. However, laboratory derived toxicological effect levels are not able to account for the numerous stresses posed to wild bird populations. Recent studies have indicated that cadmium toxicity may be exacerbated by the presence of other non-essential metals (*e.g.* mercury), organic contaminants, and by food shortage.[22] Hence, the possibility of cadmium-induced toxicological effects cannot be entirely excluded as a potential impact on western sandpipers. Further studies which determine

how the toxic metals, mercury and lead in addition to cadmium vary in relation to western sandpiper migratory behaviour, would be of extreme use in helping to evaluate the toxicological significance of trace metal residues in migratory shore birds.

Summary and conclusions

Our coastal ecosystems continue to be under constant pressure of increased urbanization with the resultant increased emissions of metals such as cadmium. We have shown that the western sandpiper accumulates significant amounts of cadmium, most likely from food and sediments ingested along the northward and southward migratory routes. Given the ability of cadmium to bioaccumulate through trophic levels, the observed increase in cadmium content with age, the relatively long half-life of cadmium in the tissues of *Calidris* species, and the apparent susceptibility of migratory shorebird species, the possible role of environmental cadmium contamination on threatening the survival of western sandpipers and other migratory shorebird species should not be ignored.

References

1 C. Thomas and L. I. Bendell-Young, *Mar. Ecol.: Prog. Ser.*, 1998, **173**, 197–213.
2 American Ornithologists' Union, AO, Washington, DC, 6th edn., 1983.
3 R. W. Butler, F. Delgado, H. de la Ueva, U. Pulido and B. Sandercock, *Wilson Bull.*, 1996, **108**, 656–677.
4 P. O'Hara, PhD Thesis, Simon Fraser University, Burnaby, BC, Canada, 2002.
5 C. G. Guglielmo, N. H. Haunerland and T. D. Williams, *Comp. Biochem. Physiol., B: Biochem. Mol. Biol.*, 1998, **119**, 549–555.
6 C. G. Guglielmo and T. D. Williams, *Physiological and Biochemical Zoology*, in the press.
7 CCAC, Canadian Council on Animal Care, Ottawa, Ontario, Canada, 2nd edn., 1993.
8 S. Blomqvist, A. Frank and L. R. Petersson, *Mar. Ecol.: Prog. Ser.*, 1987, **35**, 1–13.
9 P. H. VanEeden and H. J. Schoonbee, *Water Sa.*, 1996, **22**, 351–358.
10 J. Burger and M. Gochfeld, *Environ. Monit. Assess.*, 1991, **16**, 253–258.
11 D. H. White and M. T. Finley, *Environ. Res.*, 1978, **17**, 53–59.
12 J. L. Maedgen, C. S. Hacker, G. D. Schroder and W. W. Francis, *Arch. Environ. Contam. Toxicol.*, 1982, **11**, 99–102.
13 P. H. Becker, D. Henning and R. W. Furness, *Arch. Environ. Contam Toxicol*, 1994, **27**, 162–167.
14 L. I. Bendell-Young and J. F. Bendell, *Environ. Pollut.*, 1999, **106**, 405–413.
15 H. Wilson, Western Sandpiper (*Calidris mauri*) in *The Birds of North America*, ed. A. Poole and F. Gill, The Academy of Natural Sciences and The American Ornithologists' Union, Philadelphia and Washington DC, 1994, No. 90.
16 N. Warnock and M. A. Bishop, *Condor*, 1998, **100**, 456–467.
17 C. A. Hui, J. Y. Takekawa and S. E. Warnock, *Environ. Monit. Assess.*, 2001, **71**, 107–201.
18 J. King and L. I. Bendell-Young, *J. Wildlife Manage.*, 2000, **192**, 181–193.
19 J. P. Meador, R. C. Clarke, P. A. Robisch, Jr., D. W. Ernest, J. T. Landahl, U. Varanasi, S.-L. Chan and B. B. McCain, *NOAA Technical Memorandum NMFS-NWFSC-16*, NOAA, Seattle, WA, 1994.
20 J. K. Nicholson, M. D. Kendall and D. Osborn, *Nature (London)*, 1983, **304**, 633–635.
21 R. W. Furness, Cadmium in Birds, in *Environmental Contaminants in Wildlife: Interpreting Tissue Content*, ed. W. N. Beyer, G. H. Heinz and A. W. Redmon-Norwood, CRC Press Inc., Boca Raton, FL, 1996, p. 389–404.
22 R. T. Di Giulio and P. F. Scanlon, *Environ. Res.*, 1985, **37**, 433–444.