Seasonal and biogeographical patterns of gastrointestinal parasites in large carnivores: wolves in a coastal archipelago

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SUMMARY

Parasites are increasingly recognized for their profound influences on individual, population and ecosystem health. We provide the first report of gastrointestinal parasites in gray wolves from the central and north coasts of British Columbia, Canada. Across 60 000 km^2, wolf feces were collected from 34 packs in 2005–2008. At a smaller spatial scale (3300 km^2), 8 packs were sampled in spring and autumn. Parasite eggs, larvae, and cysts were identified using standard flotation techniques and morphology. A subset of samples was analysed by PCR and sequencing to identify tapeworm eggs ($n=9$) and *Giardia* cysts ($n=14$). We detected $\geqslant 14$ parasite taxa in 1558 fecal samples. *Sarcocystis* sporocysts occurred most frequently in feces (43·7%), followed by taeniid eggs (23·9%), *Diphyllobothrium* eggs (9·1%), *Giardia* cysts (6·8%), *Toxocara canis* eggs (2·1%), and *Cryptosporidium* oocysts (1·7%). Other parasites occurred in $\leqslant 1\%$ of feces. Genetic analyses revealed *Echinococcus canadensis* strains G8 and G10, *Taenia ovis krabbei, Diphyllobothrium nehonkaiense,* and *Giardia duodenalis* assemblages A and B. Parasite prevalence differed between seasons and island/mainland sites. Patterns in parasite prevalence reflect seasonal and spatial resource use by wolves and wolf-salmon associations. These data provide a unique, extensive and solid baseline for monitoring parasite community structure in relation to environmental change.

Key words: gastrointestinal parasites, gray wolves, *Canis lupus*, feces, Coastal British Columbia, *Echinococcus canadensis*, islands, season, disease monitoring.

INTRODUCTION

Parasites have profound effects on individuals, populations, and ecosystems and may be considered indicators of ecosystem health (Hudson *et al*. 2006) or threats to conservation of wildlife (Daszak, 2000). Parasite-host relationships are shaped by a multitude of interacting factors, including host availability, parasite community structure (Jolles *et al*. 2006; Telfer *et al*. 2010), and environmental heterogeneity at multiple scales (Biek and Real, 2010).

Islands are one source of environmental heterogeneity. Similar to trends in host populations, parasite communities on islands have reduced diversity and increased niche breadth compared with mainland communities (Goüy de Bellocq *et al*. 2003; Nieberding *et al*. 2006). Season also exerts strong influences on parasite-host dynamics and has complex consequences on host populations (Altizer *et al*. 2006). Current and on-going changes in climate and landscape use have the potential to alter parasite-host relationships (Giraudoux *et al*. 2003; Kutz *et al*. 2005; Despommier *et al*. 2006; Greer *et al*. 2008). Consequently, baseline knowledge is important in monitoring parasite-host relationships for changes that could affect or reflect wildlife, human or ecosystem health, particularly in island environments.

As top predators, wolves (*Canis lupus*) host diverse gastrointestinal parasite communities that vary primarily in relation to prey (Mech, 1970; Kreeger, 2003; Craig and Craig, 2005). Wolf parasites have been well-studied throughout much of their range; however, there are no data from the central and north coasts of British Columbia (BC), Canada. Here, wolves are considered 'evolutionarily significant subunits' based on genetic, ecological, behavioural

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and morphological differences compared with interior wolf populations (Muñoz-Fuentes *et al*. 2009). This distinction makes knowledge of potential conservation threats such as parasites or other pathogens important. Relative to wolf populations elsewhere, wolves in coastal BC have not experienced recent large-scale changes in habitat and are rarely hunted. Therefore, these wolves might provide insight into host-parasite relationships in a landscape relatively unaltered by modern anthropogenic disturbances. Moreover, coastal wolves consume numerous marine resources (Darimont *et al*. 2008*a*, 2009), potentially exposing them to parasites not often encountered in a terrestrial diet.

Feces are often used to reflect gastrointestinal parasite assemblages in wildlife, especially when collected to examine temporal and spatial trends (e.g., Turner and Getz, 2010; Stronen *et al*. 2011). In accordance with our own ethical framework and that of local First Nations, we considered fecal samples the only option for studying parasites in this distinct coastal population (Darimont *et al*. 2008*b*). Objectives of this study were to (1) generate a comprehensive profile of gastrointestinal helminths and protozoans in wolves from a naturally structured population across an extensive, remote area and (2) investigate spatial and seasonal factors that affect parasite occurrence in wolf feces. This information comes at a critical time; new parasites could be introduced or disease dynamics altered by dramatic increases in economic activity in coastal BC (Price *et al*. 2009) combined with climate change (Greer *et al*. 2008).

MATERIALS AND METHODS

Study area

The study area extends from the northern end of Vancouver Island (51°46′N, 127°53′W) to north of Prince Rupert (55°37′N, 129°48′W), British Columbia (Fig. 1). The coastal landscape can be generally classified as mountainous mainland, hilly inner islands, and lower outer islands (Darimont *et al*. 2004). Islands vary in size from <1 to >2200 km^2 and are separated from other islands and mainland areas by <100 m to >13 km (Darimont *et al*. 2004; Paquet *et al*. 2006). Prey species available to wolves include a subspecies of mule deer known as Sitka black-tailed deer (*Odocoileus hemionus sitkensis*), 5 species of Pacific Salmon (*Onchorhynchus* spp.), mustelids, ursids, birds, rodents, marine mammals and invertebrates (Darimont *et al*. 2004, 2009).

To investigate spatial and seasonal patterns in prevalence of parasite stages in feces, we collected wolf feces at 2 spatial scales (Table 1; Fig. 1). Across a 60000 km^2 study area, we collected feces (n=718) haphazardly as encountered from 34 wolf packs in the autumn of 2005, 2007 and 2008 (Table 1; Fig. 1). Due to logistical limitations and the expanse of the study area we could not sample all packs in all years, nor were sample collections equally productive from each pack. At a finer spatial scale (3300 km^2), we collected feces (n=923) along established transects from wolf packs with home ranges on 4 islands (n=467) and 4 mainland areas (n=456) in spring and autumn 2007 and 2008 (Table 1). These 8 packs were sampled because they have known home ranges with established transects and are logistically feasible to sample (Darimont *et al*. 2008*a*). We assumed the 8 packs were representative of the larger population.

Within eight hours of collection, fecal samples were frozen at −20 °C and later transported to the University of Saskatchewan for subsequent diagnostic processing. There, samples were kept at −80 °C for 3 days to kill any *Echinococcus* eggs (Hildreth *et al*. 2004). Although freezing may affect recovery of parasite stages from feces (Foreyt, 2001*b*), we considered these steps necessary for practical and safety reasons.

Morphological analysis of parasite stages

For the purposes of this paper, we define: 'parasites' as gastrointestinal helminths and protozoans that shed larval stages in feces; 'parasite stages' as eggs, oocysts, sporocysts, cysts, and larvae shed in feces; and fecal prevalence as the proportion of fecal samples in which we detected parasite stages.

Quantitative parasitological analysis was conducted on 4 g of feces using a modified Wisconsin Sugar Flotation Method, which is suitable for detecting many common parasite stages in canine feces (Foreyt, 1989; Foreyt, 2001*b*; Stronen *et al*. 2011). Most parasites were identified under X100 total magnification with the exceptions of *Sarcocytis* sp. sporocysts and *Isospora* sp. oocysts, which were counted at X400 total magnification. To determine the presence or absence of *Cryptosporidium* sp. and *Giardia* sp. (oo)cysts, we used a commercial immunofluorescent assay (Cyst-a-glo™, Waterborne, Inc. 6045 Hurst Street, New Orleans, LA 70118, USA) with modifications described by Stronen *et al*. (2011). All observers were trained in parasite identification for a minimum of 10 days by a parasitologist (B.W.) and scored >90% when tested for correct identification of parasite stages.

PCR and sequencing of parasite stages

To complement morphological parasite data, we performed genetic analyses on a small subset of fecal samples (Table 1). Taeniid and *Diphyllobothrium* sp. eggs were isolated from fecal samples and identified by sequencing of NAD1 as previously described (Himsworth *et al*. 2010). Amplifications were

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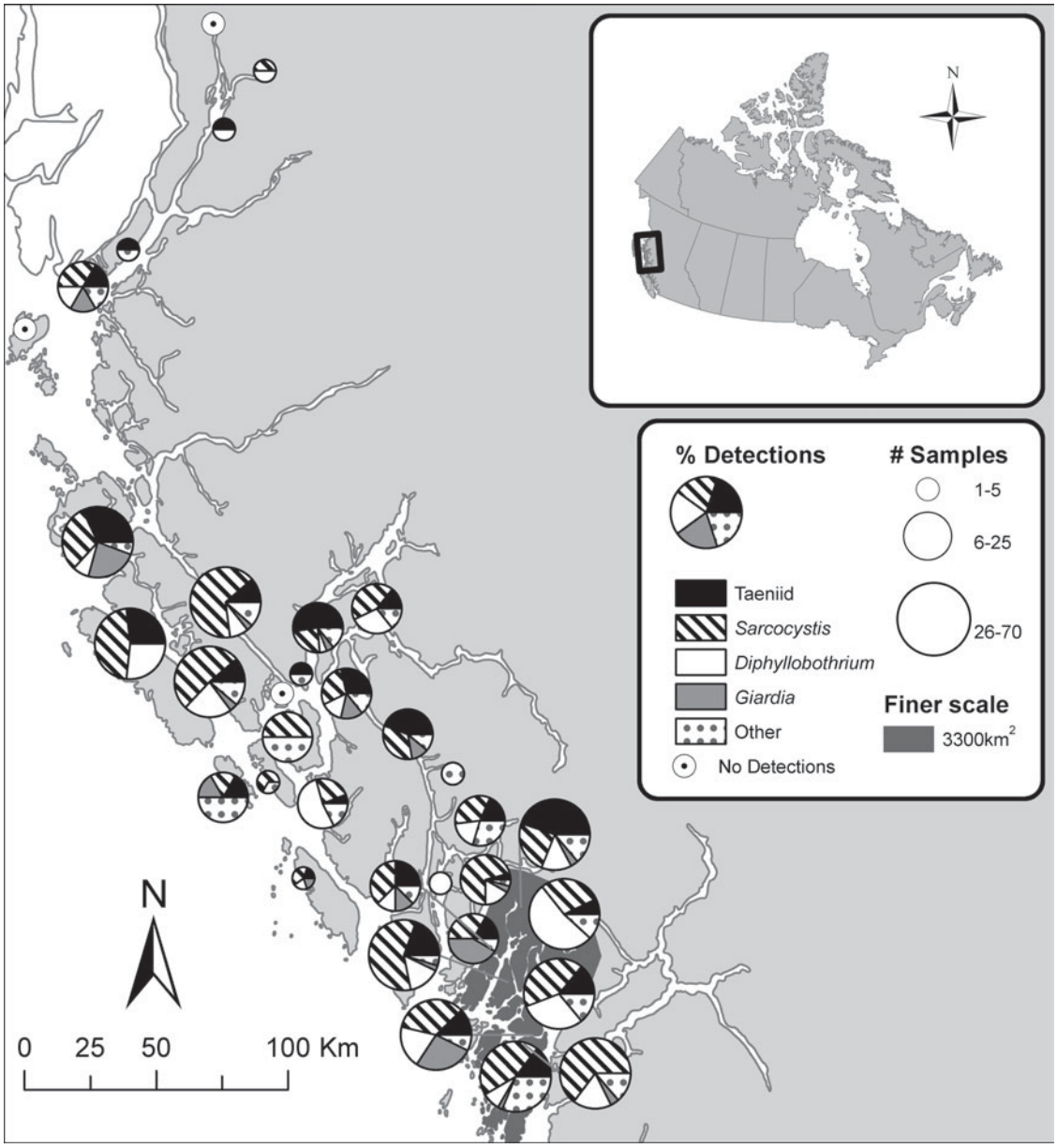

Fig. 1. Map of fecal collection locations from 34 wolf packs across a 60 000 km^2 area on the central and north coasts of British Columbia, Canada, in 2005–2008. Data from these feces were used to compare parasite prevalence on island and mainland areas. Eight of these packs occupying known home ranges over 3300 km^2 (area in dark grey) were sampled consistently in 2 years and 2 seasons to allow for seasonal comparisons. Circles represent wolf packs sampled during autumn collections. Pie charts show the proportion of parasite detections per pack attributed to taeniids, *Sarcocystis* sp., *Diphyllobothrium* sp., *Giardia* sp., and all other taxa combined.

performed with primers JBl 1 (5′-AGA TTC GTA AGG GGC CTA ATA-3′) and JB12 (5′-ACC ACT AAC TAA TTC ACT TTC-3′) (Bowles and McManus, 1993), which amplify a portion of the NADH dehydrogenase subunit I (NAD1) gene of helminths. A 509 bp PCR product was obtained from 12 fecal samples, then purified and sequenced using the amplification primers. High quality, nearly complete or full-length sequences were obtained for 8 of 12 samples and a short (139 bp) sequence was obtained for 1 sample. The PCR products from 3 samples were not detectable or not interpretable due to the presence of multiple sequences. Sequences were compared to the National Center for Biotechnology Information Genbank non-redundant nucleotide database using BLASTn (Altschul *et al*. 1990). Representative NAD1 sequences were deposited in Genbank (Accession numbers HQ423292-HQ423300).

Samples positive for *Giardia* spp. were sent to Murdoch University, Australia, where 19 were sequenced to determine the predominant *Giardia* sp. genotypes. Polymerase chain reaction (PCR) and sequencing was performed on 14 samples as previously described to amplify a segment of the *G. duodenalis* β-giardin gene (Covacin *et al*. 2011).

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Table 1. Summary of datasets shown in this paper, including sampling procedure, feces examined and packs sampled

(Fecal samples (n=1558) were collected from the central and north coasts of British Columbia, Canada, in autumn (September–October) and spring (May–June), 2005–08. Fecal flotations and *Giardia/Cryptosporidium* assays were done on all samples and genetic analyses on a subset.)

Dataset	Sampling of feces	Year	Season	Habitat	Number of feces	Packs sampled	Median feces/pack (range)
Parasite survey[a] (Table 2)	Haphazard as encountered over 60 000 km^2, packs not sampled every year	2005–2008	Autumn & spring	Island	914	25	10 (1–175)
				Mainland	644	20	6 (1–205)
Large spatial scale (Figs 1, 3)	Same as above. Includes 349 samples also used in finer scale dataset	2005–2008	Autumn	Island	473	21	20 (1–70)
				Mainland	245	13	10 (1–67)
Finer spatial scale (Fig. 2)	As encountered along established transects over 3300 km^2, known packs	2007, 2008	Autumn & spring	Island	467	4	23 (9–89)
				Mainland	456	4	22 (0–77)
Tapeworm genetics (Table 3)	Sub-selected by number and type of tapeworm eggs, location, season	2008	Autumn & spring	Island	7	6	1 (1–3)
				Mainland	5	3	1 (1–3)
Giardia genetics (text only)	Subselected by location, season, positive immunofluorescent assay	2005, 2007	Autumn & spring	Island	10	4	2·5 (1–4)
				Mainland	4	4	1 (0)

[a] Dataset includes 23 samples collected in August 2006 and 6 collected in August 2007.

Statistical analysis

To examine the effects of season, year, and pack on parasite presence or absence in feces, we used logistic regression models on data collected from the 3300 km^2 spatial scale. Separate models were tested for *Diphyllobothrium* sp., taeniids, and *Sarcocystis* sp. One wolf pack (My) was analysed with an adjacent pack (BC) because only 1 sample was collected in 2007. Interaction terms and habitat (mainland versus island) were not included in models to avoid excessive zero categories. Variance inflation factors ranged from 1·0 to 1·7. We did not conduct logistic regression on large spatial scale data because of uneven sample sizes per pack and because not all packs were sampled each year. Moreover, we pooled all data from islands and mainland because of these limitations and performed Fisher's exact tests to compare the proportion of parasites in wolf feces on islands and mainland areas. All analyses were carried out using R statistical software (http://www.R-project.org). We consider these analyses to be hypothesis generating due to non-independence of fecal samples.

RESULTS

There was evidence of at least 1 parasitic infection in 975 of 1558 (62·6%) wolf fecal samples. Morphological identification of eggs and (oo)-cysts revealed ⩾14 distinct parasite taxa (Table 2). Of these, 4 occurred in >5% of fecal samples (*Sarcocystis* sp., Taeniidae, *Diphyllobothrium* sp., and *Giardia* sp.)

Genetic sequencing of parasite eggs from 9 feces revealed at least 2 species of taeniid tapeworms, *Echinococcus canadensis* and *Taenia ovis krabbei*, as well as the fish tapeworm, *Diphyllobothrium nihonkaiense* (Table 3). Of 6 taeniid egg isolates, 5 most closely matched *E. canadensis* strain G8 and 1 *E. canadensis* strain G10. Genetic analysis of *Giardia* cysts revealed *Giardia duodenalis* assemblage A in 3 samples and assemblage B in 11 samples.

Season emerged as an important factor affecting parasite prevalence in wolf feces, although the effect varied across parasite species, packs and years (Fig. 2). Based on a logistic regression model including season, year and pack as main effects, *Diphyllobothrium* sp. eggs were 7·57 times more likely in feces collected in autumn compared with those collected in spring (Fig. 2a,b; $P<0·01$, 95% Confidence Interval 4·35–13·15). The magnitude of the difference was not consistent across packs and years but the prevalence of *Diphyllobothrium* sp. was consistently higher in autumn in all but 1 pack over both years. In contrast, taeniid eggs were 0·31 times less likely to occur in autumn (Fig. 2c,d; $P<0·01$, 95% Confidence Interval 0·21–0·45). This trend was consistent in direction across 7 of 8 packs sampled in 2007 (Fig. 2c) and 5 of 7 packs in 2008 (Fig. 2d).

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Table 2. Prevalence, median intensity and range of parasite stages (eggs, oocysts, sporocysts, cysts and larvae) detected in 1558 wolf feces collected from the central and north coasts of British Columbia, Canada, between 2005 and 2008

Taxonomic group	Fecal prevalence (%)	Intensity (propagules/g)	
		Median	Range
Protozoa			
Cryptosporidium[a]	28 (1·7)	—	—
Giardia[a]	106 (6·8)	—	—
Isospora/Eimeria[b]	16 (1·0)	323	15–2880
Sarcocystis[d]	681 (43·7)	135	10–18 945
Unknown Coccidean	63 (4·0)	15	3–1140
Trematoda			
Metorchis[c]	9 (0·6)	5	3–55
Unknown Trematode	9 (0·6)	258	0
Cestoda			
Diphyllobothrium[c]	142 (9·1)	45	3–38 968
Taenia/Echinococcus[a,b,d]	373 (23·9)	23	3–2618
Nematoda			
Capillaria	9 (0·6)	5	3–50
Dorsal-spined larvae[e]	4 (0·3)	3	1–383
Soboliphyme	10 (0·6)	5	3–1208
Spiroidea	2 (0·1)	14	3–25
Toxascaris leonine	10 (0·6)	15	3–338
Toxocara canis[a]	33 (2·1)	73	3–2288
Trichuris	4 (0·3)	6	3–10
Arthropoda			
Demodex (Ectoparasite)	2 (0·1)	—	—
Other			
Unknown	7 (0·4)	5	3–58

[a] Taxa in which some species have zoonotic potential from contact with infected feces.
[b] Taxa identifiable only to family.
[c] Parasites with indirect lifecycles likely transmitted to wolves via fish.
[d] Parasites with indirect lifecycles likely transmitted to wolves via deer.
[e] One sample identified as *Parelaphostrongylus odocoilei* (Bryan *et al*. 2010).

Sarcocystis sp. sporocysts were 0·48 times less likely to occur in autumn (Fig. 2e,f; $P<0·01$, 95% Confidence Interval 0·36–0·64). The direction of this trend was the same in 7 of 8 packs sampled in 2007 (Fig. 2e) and 5 of 7 packs in 2008 (Fig. 2f). At least 2 levels of pack were significant for each parasite but the effect of year was significant only for *Sarcocystis* sp. ($P=0·02$). *Toxocara canis* eggs were 2·95 times more likely to be detected in autumn (Fisher's exact test $P<0·01$; 95% Confidence Interval 1·30–7·29).

Across the entire study area, islands showed a higher fecal prevalence of *Giardia* sp. infections and a lower prevalence of *Diphyllobothrium* sp. relative to mainland sites (Fig. 3a,b; Fisher's test $P<0·001$, 0·01, respectively). The fecal prevalence of taeniid and *Sarcocystis* sp. infections was similar across mainland and island sites (Fig. 3c,d; Fisher's test $P=1·00$, 0·14, respectively).

DISCUSSION

Ecology and diversity of parasites detected in wolf feces

The most common parasites in feces relate to coastal wolves' diet of terrestrial and marine prey. *Sarcocystis* sp. sporocysts and taeniid eggs had the highest fecal prevalence, reflecting wolves' overall annual diet of >80% deer across the population (Dubey and Odening, 2001; Jones and Pybus, 2001; Darimont *et al*. 2008*a*). The high fecal prevalence of taeniid eggs (23·9%) is similar to a metaprevalence of 28·2% reported in Alberta and southern Alaska and >19% worldwide (Craig and Craig, 2005).

Genetic analyses provide further support that taeniids are passed to wolves via deer and suggest that these parasites may be maintained in a sylvatic wolf-deer cycle. *E. canadensis* is thought to cycle primarily between wolves and large cervids such as moose (*Alces alces*) (Messier *et al*. 1989; Jenkins *et al*. 2005; Thompson *et al*. 2006; Foreyt *et al*. 2009). Moose have expanded into parts of the study area within the past 100 years but remain absent on most islands (Darimont *et al*. 2005). It is possible that moose introduced *E. canadensis* to the area; however, several samples came from sites where moose do not occur, suggesting that the cycle can be maintained even where Sitka black-tailed deer is the only cervid.

Despite limited genetic analyses, *E. canadensis* is a significant finding because the G8 and G10 (cervid) strains have zoonotic potential (Jenkins *et al*. 2005). Case-based evidence suggests that these strains are less pathogenic to humans than are strains of *Echinococcus* from domestic animals (Pinch and Wilson, 1973). A human case in Alaska, however, revealed that the G8 strain—which we detected in 5 of 6 samples—can cause severe disease (McManus *et al*. 2002). Moreover, these results provide further support that the G8 and G10 strains co-occur and should be considered the same species as proposed by Thompson (2008).

Our finding of *T. ovis krabbei* in one sample is consistent with morphologic identification of adult worms from wolf carcasses collected on nearby Vancouver Island (H. Bryan, *unpublished data*). *T. ovis krabbei* had a metaprevalence of 25% in wolves from southern Alaska and Alberta and has been linked with a diet of cervids (Craig and Craig, 2005). We found no evidence of other *Taenia* spp. commonly reported in wolves, notably *T. hydatigena*, which may reflect the absence of large cervids (e.g., moose, elk [*Cervus elaphus canadensis*], and caribou [*Rangifer tarandus*]) and lagomorphs in the area and low prevalence of rodents in the diet of coastal wolves

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Table 3. PCR and sequencing of partial NAD1 sequences of taeniid or *Diphyllobothrium* sp. eggs isolated from 12 wolf feces

(Feces were collected from 8 wolf packs on islands or mainland on the central and north coasts of British Columbia, Canada, in 2008.)

Sample (Pack)	Habitat	Nearest neighbour based on DNA% identity over sequence[a]	DNA% identity over nucleotides
A (Ft)	Island	AB235848 *Echinococcus canadensis* G8	99% over 488
B (23)	Island	AB235848 *Echinococcus canadensis* G8[c]	99% over 488
C (23)	Island	AB235848 *Echinococcus canadensis* G8	99% over 488
D (45)	Island	EF420138 *Diphyllobothrium nihonkaiense*	99% over 488
E (38)	Island	EF420138 *Diphyllobothrium nihonkaiense*	99% over 488
F (Ms)	Mainland		No data
G (22)	Island	AB235848 *Echinococcus canadensis* G8	99% over 428
H (37)	Mainland		No data
I (37)	Mainland	EU544628 *Taenia ovis krabbei* TkSv4	100% over 390
J (37)	Mainland		No data[b]
K (23)	Island	AB235848 *Echinococcus canadensis* G8	100% over 139
L (BC)	Mainland	AF525297 *Echinococcus canadensis* G10	99% over 434

[a] Primer sequences were removed prior to analysis.
[b] Sequence was consistent with mixed template.
[c] Sequences identical over available nucleotides.

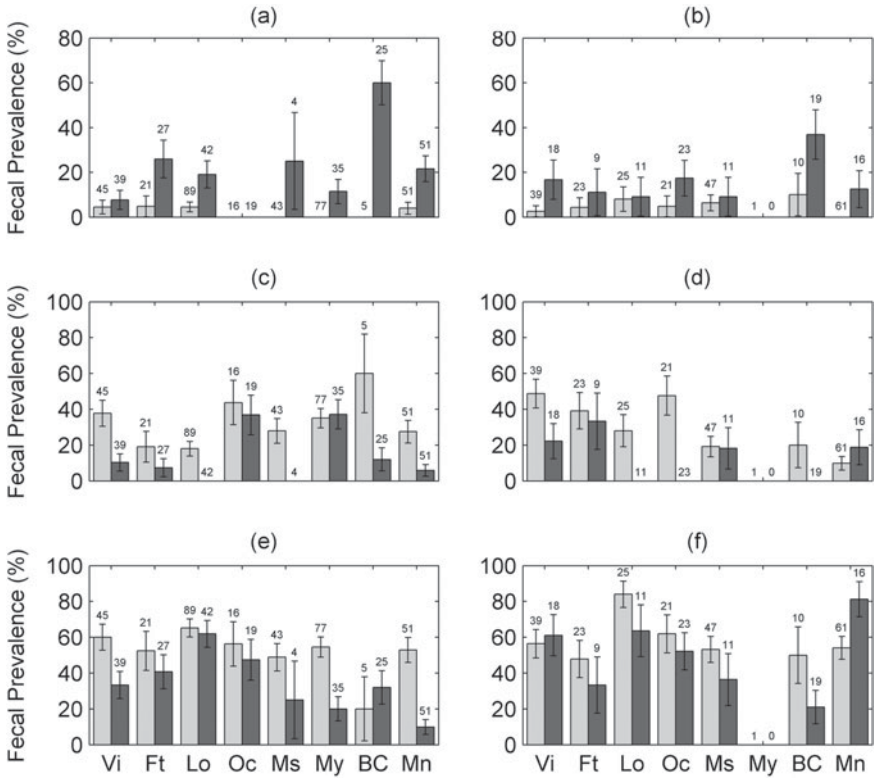

Fig. 2. Seasonal patterns in fecal prevalence of *Diphyllobothrium* sp. (a) 2007 and (b) 2008, Taeniidae (c) 2007 and (d) 2008, *Sarcocystis* sp. (e) 2007 and (f) 2008 in 8 wolf packs. Feces were collected from the central and north coasts of British Columbia in spring (May–June, grey bars) and autumn (September–October, black bars). The first 4 packs (Vi, Ft, Lo, Oc) occupy islands and the rest (Ms, My, BC, Mn) are from mainland areas. *Giardia* was not plotted because of low fecal prevalence per pack. Error bars represent standard error of prevalence estimates for each pack. Sample sizes are plotted above each bar.

(Darimont *et al*. 2004). We likely did not detect the full diversity of taeniids due to the small number of samples subject to genetic analysis and limited temporal sampling.

Compared with other reports in canids (Bagrade *et al*. 2009; Craig and Craig, 2005; Stien *et al*. 2010), we found a high—9·1% overall and 16·6% in fall—prevalence of *Diphyllobothrium* sp. This reflects

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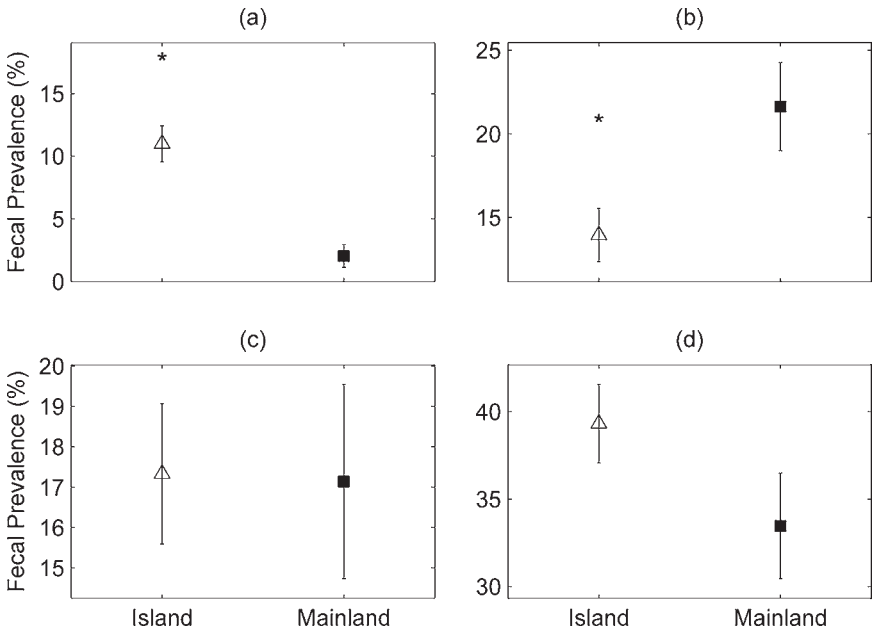

Fig. 3. Fecal prevalence and standard error of *Giardia* sp. (a), *Diphyllobothrium* sp. (b), Taeniidae (c), and *Sarcocystis* sp. (d) collected from islands (△; $n=473$) and mainland areas (■; $n=245$). Collectively, these 4 taxa represent 86% of all parasite detections and each occurred in >5% of fecal samples. Wolf feces were collected from 21 island wolf packs and 13 mainland packs on the central and north coasts of British Columbia in autumn 2005, 2007 and 2008. Prevalence was calculated as total parasite detections in feces collected from islands or mainland.

coastal wolves' dietary shift of up to 70% Pacific salmon in the fall. Sequences from *Diphyllobothrium* eggs matched *D. nihonkaiense*, a species that infects salmonids (*O. gorbuscha* and *O. keta*) commonly available to coastal wolves (Scholz *et al*. 2009).

Notably, we found no evidence of the trematode *Nanophyetus salmincola* that carries the causative agent of salmon poisoning disease in canids, *Neorickettsia helminthoeca* (Foreyt, 2001a). Salmon poisoning disease is transmitted by flukes that infect salmonids or other species of fish and is highly fatal in domestic and wild canids that consume raw fish (Foreyt, 2001a). It is possible that wolves in coastal BC have immune or behavioural mechanisms to avoid infection with *N. helminthoeca* (Darimont *et al*. 2003). Alternatively, *N. salmincola, Neo helminthoeca,* or its snail intermediate hosts may not occur in the study area (Booth *et al*. 1984; Foreyt, 2001*a*) or infected hosts may have died rapidly making collection of infected feces unlikely. Given the possible conservation implications of salmon poisoning disease for coastal wolves, combined with potential climate-driven changes in distribution of the intermediate host, regular monitoring for *N. helminthoeca* and *N. salmincola* in coastal BC would be a sound strategy.

In addition to parasites with indirect life cycles, we found at least 9 genera with direct life cycles. Of note are the protozoans *Giardia* sp. and *Cryptosporidium* sp., which include species with zoonotic potential. We found the fecal prevalence of *Cryptosporidium* sp. was similar to that reported in wolves from interior Canada (Stronen *et al*. 2011). In contrast, the prevalence of *Giardia* sp. (6·8%) was lower (21·9–46·7%; Stronen *et al*. 2011). Differences may relate to the immune status of wolves or prey availability, habitat and other ecological characteristics influencing transmission of *Giardia*. Notably, we detected only the zoonotic *Giardia* assemblages A and B and not the specific dog assemblages C and D (Thompson, 2004). This finding suggests that the zoonotic assemblages are dominant in wolves even in remote locations where current human population density is low.

Several parasitic taxa—*Soboliphyme, Parelaphostrongylus odocoilei,* and oocysts we identified as possibly *Eimeria*—are not known to infect wolves. One logical explanation for these occurrences is that wolves consumed the viscera or feces of an infected definitive host and excreted eggs and/or larvae in their feces (Bryan *et al*. 2010). Nematodes *Trichuris* sp., *Toxocara canis*, and *Toxascaris leonina,* which are potentially pathogenic to wolves, occurred rarely in fecal samples. Landscape or climatic conditions might limit transmission of these parasites to adult wolves. In pups (<6 months), prevalence might be higher because of age-related immunity. Alternatively or concomitantly, the low prevalence of these parasites may reflect the general good health of wolves and their relatively intact habitat.

Seasonal patterns in parasite occurrence

Strong seasonal differences in the fecal prevalence of *Diphyllobothrium* sp., taeniid eggs, and *Sarcocystis* sp.

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oocysts reflect coastal wolves' known dietary shift to salmon in fall. Similar seasonality in *Diphyllobothrium* sp. has been reported in black (*Ursus americanis*) and grizzly (*Ursus arctos*) bears (Frechette, 1978; Gau *et al*. 1999). Although the mechanism for these changes is unknown, it is possible that the worms have a long dormancy period between seasons, that most adults complete their life cycle and are expelled between seasons, or that a seasonal change in host immunity promotes expulsion of adults. Alternatively or concomitantly, *Diphyllobothrium* sp. could compete with taeniids in the gastrointestinal tract of their host (Read, 1951; Roberts, 2000; Bush and Lotz, 2000; Conlan *et al*. 2009). Notably, a lower prevalence of Taeniids and *Sarcocystis* sp. in fall may also be explained by decreased consumption of deer when salmon are available.

Seasonality may be adaptive for *Diphyllobothrium* sp. Intense egg-shedding when wolves or other definitive hosts are near salmon-spawning streams would maximize transmission of eggs to zooplankton intermediate hosts and subsequently, to anadramous Pacific salmon which may retain pleurocercoids for several years during their time at sea (Arizono *et al*. 2009; Scholz *et al*. 2009). Seasonal shifts could also benefit parasites if host immunity wanes between seasons and increases host susceptibility. A higher prevalence of *T. canis* eggs in fall may be due to pups (<6 months) that are susceptible to infection from their mothers or the environment and then clear the infection by the following spring. Seasonal changes in parasitic infections may also reflect overall population health. Most wolves in healthy populations should clear infections when no longer exposed to larval stages whereas immunocompromised populations might show increasing levels of parasites over time (Gentes *et al*. 2007).

Spatial patterns in parasite occurrence in wolf feces

Across the 60 000-km^2 area, fecal prevalence of *Diphyllobothrium* sp. and *Giardia* sp. differed between island and mainland sites separated on average by only 1·5 km. These differences over a small geographical scale within a wide-ranging wolf population (average home range size 200 km^2) mirror known variation in resource availability. For example, island areas host lower salmon spawning density (*unpublished data*), which might explain why the prevalence of *Diphyllobothrium* sp. in feces is lower on islands. Geographical factors may also play a role; differences in topography, precipitation, or water flow between islands and mainland areas may influence *Giardia* sp. prevalence (Biek and Real, 2010). Alternatively, hosts that could be a source of environmental contamination, such as seals or seabirds (Lasek-Nesselquist *et al*. 2008), might have a higher density on islands. These ecological discontinuities demonstrate potential effects of increasing habitat fragmentation and other landscape change on parasite-host relationships.

In conclusion, this study provides a comprehensive picture of gastrointestinal parasite stages in feces from a wolf population that is relatively undisturbed by recent, large-scale anthropogenic activities and is likely among the least human-influenced in the world. The survey could serve as a useful comparison with other studies of parasites in wolves and for monitoring future change that could affect ecosystem or wolf health.

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