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ABSTRACT: The prevalence, intensity, and abundance of sea lice belonging to *Lepeophtheirus* or *Caligus clemensi* are reported from threespine stickleback (*Gasterosteus aculeatus*) collected from the Broughton Archipelago region of coastal British Columbia, Canada, between 2005 and 2008. In total, 25,130 sea lice were collected from 7,684 sticklebacks. The prevalence of *Lepeophtheirus* ranged from 51% in 2005 to 11% in 2008 and that of *C. clemensi* from 56% in 2007 to 24% in 2008. Chalimus stages accounted for approximately 69% of all *Lepeophtheirus* and 88% of *Caligus* specimens. Cytochrome *c* oxidase subunit 1 (COI) gene sequences, useful in distinguishing reference specimens belonging to 8 species of *Lepeophtheirus*, *Caligus*, and *Bomolochus*, were used to identify the *Lepeophtheirus* specimens from stickleback as *L. salmonis* (71%) and *L. cuneifer* (29%). A COI phylogenetic analysis confirmed a monophylogenetic origin of *Lepeophtheirus* but not of *Caligus*. Two genotypes were resolved in *L. cuneifer*, i.e., genotype A occurred twice as often as genotype B. Virtually all immature *Lepeophtheirus* specimens from juvenile salmon were *L. salmonis*. The results emphasized the need to accurately identify immature sea lice as a prerequisite to understanding sea lice ecology. The threespine stickleback may be a useful sentinel species for the abundance and diversity of the sea lice that are also parasites of wild and farmed salmon in coastal ecosystems in British Columbia.

Sea lice belonging to *Lepeophtheirus* (Copepoda: Caligidae) are ectoparasites of marine fishes, and 11 species have been reported from the Pacific Ocean of western Canada (Kabata, 1973, 1988). Although species descriptions are based on the morphology of adult specimens, there are no descriptions of the immature developmental stages (nauplius I and II, copepodid, chalimus I–IV, and preadult I and II) for most species. Another caligid copepod, *Caligus clemensi*, is also a common parasite in this region (Parker and Margolis, 1964).

Some coastal ecosystems in British Columbia, Canada, are shared by several species of Pacific salmon (*Oncorhynchus* spp.) and salmon aquaculture, in which Atlantic salmon (*Salmo salar*) are reared in open net pens. In the spring, juvenile Pacific salmon migrate through this coastal ecosystem between nursery streams and the open ocean; in the summer and autumn, adult salmon return to coastal waters to spawn in the nursery streams. These times of relative proximity present increased opportunities for the transmission of sea lice between captive and migrating salmon. It has been hypothesized that infections with sea lice on juvenile Pacific salmon, elevated as a result of transmission from infections on farmed Atlantic salmon, increase mortality rates and cause reductions in Pacific salmon populations (Krkošek et al., 2007). Annual monitoring of juvenile Pacific salmon began in 2003 to better understand sea lice dynamics in the Broughton Archipelago coastal ecosystem. Marine threespine sticklebacks, *Gasterosteus aculeatus*, a frequent by-catch with the juvenile salmon, were found to have relatively heavy sea lice infections. In 2004, the prevalence and intensity of the infections on sticklebacks exceeded those on the salmon by as much as 10 times (Jones and Nemec, 2004; Jones, Prosperi-Porta et al., 2006). Sea lice on sticklebacks were virtually all immatures, and analysis of a small subunit ribosomal RNA gene sequence showed that they belonged to *Lepeophtheirus salmonis*, the salmon louse or to *C. clemensi* (Jones, Prosperi-Porta et al., 2006). The present study further documented the occurrence of sea lice on sticklebacks in this ecosystem from 2005 to 2008 and used a mitochondrial gene sequence to test the hypothesis that sticklebacks are infected solely with *L. salmonis*.

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MATERIALS AND METHODS

Collection and processing of fish and sea lice

Details on the study design, including the collection of juvenile salmon, sticklebacks, and sea lice, have been reported previously (Jones and Nemec, 2004; Jones, Prosperi-Porta et al., 2006; Jones and Hargreaves, 2007). In brief, from each catch, up to 30 fish belonging to each species were sampled from the net, placed individually into a labeled bag, and stored at −20 C for laboratory processing. Fish identity was confirmed immediately after thawing, and length and weight data were collected. Fish were examined under a binocular dissecting microscope, and sea lice were removed by using fine forceps and stored in 95% ethyl alcohol.

Adult reference *Lepeophtheirus* specimens were obtained from fish belonging to the following species collected from Tribune Channel, British Columbia, Canada: *L. parviventris* from Irish Lord, *Hemilepidotus hemilepidotus*; *L. bifidus* from starry flounder, *Platichthys stellatus*; *L. oblitus* from kelp greenling, *Hexagrammos decagrammus*; and *L. hospitalis* from *P. stellatus*. Adult *L. cuneifer* and *L. salmonis* were obtained from farmed Atlantic salmon during routine sea lice monitoring in the Broughton Archipelago. In addition, adult and chalimus stages of *Caligus clemensi* and adult specimens of *Bomolochus cuneatus* and *L. cuneifer* were obtained from Pacific herring (*Clupea pallasi*) captured in the Gulf Islands region of coastal British Columbia and in Cordova Bay, Alaska. Freshly collected specimens were preserved in 95% ethyl alcohol. Adult sea lice were identified to species and gender by using morphological keys (Kabata, 1973, 1974, 1988), whereas immature specimens were identified to genus and developmental stage (Kabata, 1972; Johnson and Albright, 1991a).

Extraction and amplification of mitochondrial DNA

Total DNA was extracted from individual, alcohol-fixed specimens by DNeasy kits according to the manufacturer's extraction protocol for animal tissues (QIAGEN Inc., Mississauga, Ontario, Canada). DNA was quantified using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Nepean, Ontario, Canada) on a subset of all samples and for all samples where no polymerase chain reaction (PCR) amplification resulted. A mitochondrial cytochrome *c* oxidase I (COI) gene sequence was amplified by PCR using published primers (Folmer et al., 1994; Yazawa et al., 2008) or those designed in the present study (Table I). The PCR conditions were as published or were determined empirically for novel primer pairs. PCR products were visualized and photographed in a GelDoc-It imaging system (UVP, Uplands, California) after electrophoresis in 1.5% agarose containing SYBR Safe DNA gel stain (Invitrogen Canada Inc., Toronto, Ontario, Canada). Sequencing reactions of purified PCR products (ExoSAP-IT reagent, GE Healthcare, Quebec City,

TABLE I. Oligonucleotide primers used to amplify partial COI gene sequences from reference copepods.

Name	5′-to-3′ sequence	Reference
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer et al., 1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer et al., 1994
LsPcCOI-F	TCTGGATTAGTGGGTTTAGC	Yazawa et al., 2008
LsPcCOI-R	TCGATGCACCTTCCTCTATC	Yazawa et al., 2008
LcunCOI-F	TGGGCTGGTCGGTCTTGGGA	This study
LcunCOI-R	CCGGCCCCTCTCTCCACCAA	This study
LepG-R	AGAAGAAATTCCCGCCAAAT	This study
LcunCOI-1R	AGCCCCGGCTAAAACAGGTAAAGA	This study
LepG2F	GGGAGCCCCAGATATAGCAT	This study

Quebec, Canada) were performed with the same primers used for amplification and Big Dye Terminator version 3.1 (Applied Biosystems, Foster City, California). Reaction products were purified using Dye-Ex 2.0 kits (QIAGEN Inc.), and sequences were obtained from a 16 capillary 3130xl Genetic Analyzer (Applied Biosystems). The sequences were assembled in Sequencher 4.9 (Gene Codes Corporation, Ann Arbor, Michigan), edited, and deposited in GenBank (Table II). The following COI sequences were obtained from GenBank: *Caligus centrodonti* (AY861370), *C. curtus* (AY861366), *C. elongatus* genotype 1 (AY861371), *C. elongatus* genotype 2 (AY861365), *C. gurnardi* (AY861369), *Lepeophtheirus hippoglossi* (AY861362), *L. pectoralis* (AY861364), *L. salmonis* Atlantic type (AY602596), *L. thompsoni* (EF065617), and *Lepeophtheirus* sp. (listed in GenBank as *L. hospitalis*, AM235888).

A multiple alignment of the contigs was obtained by using the ClustalW algorithm in MEGA 4.1 (Tamura et al., 2007). Pairwise genetic distances among reference sequences were calculated using the Kimura 2-parameter method, and the SE associated with each distance was estimated by a 1,000-fold bootstrapping procedure. Phylogenetic relationships, also conducted in MEGA 4.1, were estimated using neighbor-joining analysis in which evolutionary distances were computed using the Kimura 2-parameter method. A gamma distribution (shape parameter = 1) was used to model the rate of variation among sites. All codon positions were included and alignment gaps, and missing data were eliminated by pairwise sequence comparisons.

TABLE II. Size and GenBank accessions of COI sequences amplified from adult reference parasitic copepods belonging to the genera *Lepeophtheirus*, *Caligus*, and *Bomolochus*.

Species (no.)	Amplicon size (position)*	GenBank accession
B. cuneatus (1)	660 (40)	HM582237
C. clemensi (1)	660 (40)	HM582236
L. bifidus (1)	651 (40)	HM582234
L. cuneifer type A (5)	660 (40)	HM582230, HM800829, HM800830, HM800831, HM800832
L. cuneifer type B (5)	660 (40)	HM800824, HM800825, HM800826, HM800827, HM800828
L. hospitalis (3)	660 (40)	HM582235, HM800843, HM800844
L. oblitus (3)	426 (40)	HM582233, HM800841, HM800842
L. parviventris (9)	537 (73)	HM582232, HM800833, HM800834, HM800835, HM800836, HM800837, HM800838, HM800839, HM800840
L. salmonis Pacific (3)	660 (40)	HM582238, HM800845, HM800846

* Position of 5′ end of product relative to the *L. salmonis* COI cDNA-determining sequence (EU288200).

Statistical analyses

Prevalence, mean abundance, and intensity were defined according to Bush et al. (1997). Mean prevalence (±95% confidence intervals) was calculated using Quantitative Parasitology 3.0 (Rozsa et al., 2000). Annual and monthly variations in the abundance and prevalence of sea lice infections on sticklebacks were compared by using Kruskal–Wallis and chi-square analysis, respectively. Differences in the significance of fish lengths were tested by using Bonferroni-adjusted 2-sample *t*-tests. Statistical tests were conducted in Systat II (Systat Software, Inc., Chicago, Illinois), and all differences resulting in P values ≤ 0.05 were considered statistically significant.

RESULTS

In total, 7,684 sticklebacks were sampled between March and July in 2005–2008 (Table III). The fish ranged from 57.5 to 69.2 mm in length and weighed 2.3–4.0 g (Table III). In all years, the mean length of sticklebacks collected at the earliest sampling was less than that of those collected at the last sampling ($P < 0.001$). Sea lice belonging to *Lepeophtheirus* and to *C. clemensi* occurred in all monthly collections in all years. The annual mean abundance of *Lepeophtheirus* ranged from 2.54 ± 0.09 fish^{-1} in 2005 to 0.15 ± 0.01 fish^{-1} in 2008 ($P < 0.001$; Table III). Similarly, the annual mean abundance of *C. clemensi* ranged from 2.82 ± 0.09 fish^{-1} in 2007 to 0.58 ± 0.03 fish^{-1} in 2008 ($P < 0.001$). The mean abundance of *Lepeophtheirus* between the last 2 sample months decreased in 2005 and in 2007 ($P < 0.01$) and increased in 2006 ($P < 0.05$). There was no change in the mean abundance between May and June in 2008 ($P = 0.11$; Table III). The mean abundance of *C. clemensi* decreased between the last 2 sample months in 2005, 2007, and 2008 ($P < 0.01$) and increased during this interval in 2006 ($P < 0.01$; Table III). The annual mean intensities (maximum value) of *Lepeophtheirus* were 5.0 (57), 1.9 (12), 2.1 (15), and 1.4 (7), and those of *C. clemensi* were 5.0 (105), 3.4 (85), 5.1 (42), and 2.5 (10) in 2005, 2006, 2007, and 2008, respectively. The annual mean prevalence of *Lepeophtheirus* ranged from 50.5% in 2005 to 11.0% in 2008 and that of *C. clemensi* from 55.5% in 2007 to 23.6% in 2008 (Table III).

Of the 25,130 sea louse specimens collected from sticklebacks, 10,128 belonged to *Lepeophtheirus* and 15,002 to *C. clemensi*. Each developmental stage occurred as a relatively similar percentage of the total in most years (Fig. 1). Chalimus stages accounted collectively for 69.2% of all specimens of *Lepeophtheirus* and 88.0% of *C. clemensi*. Adults, preadults, and copepodids constituted 1, 12, and 18%, of *Lepeophtheirus* specimens, respectively. Copepodid and preadult stages of *C.*

TABLE III. Number and size of threespine *Gasterosteus aculeatus* collected from the Broughton Archipelago, Canada, showing abundance and prevalence of sea lice.

		Stickleback (mean ± SE)			Abundance (mean ± SE)		Prevalence (95% confidence interval)	
Yr	Mo	No.	Length (mm)	Wt (g)	*Lepeophtheirus*	*Caligus*	*Lepeophtheirus*	*Caligus*
2005	March	0	—	—	—	—	—	—
	April	719	61.7 ± 0.5	3.1 ± 0.10	1.06 ± 0.09	2.16 ± 0.12	38.4, 34.8–42.0	52.3, 48.6–56.0
	May	1032	63.7 ± 0.3	3.3 ± 0.04	4.15 ± 0.21	4.34 ± 0.18	63.0, 60.0–66.9	71.6, 68.8–74.3
	June	735	68.0 ± 0.3	4.0 ± 0.05	3.28 ± 0.17	1.23 ± 0.17	63.5, 60.0–66.9	27.6, 24.5–31.0
	July	563	69.2 ± 0.3	3.9 ± 0.05	0.52 ± 0.06	0.87 ± 0.08	25.9, 22.5–29.7	31.1, 27.3–35.1
	Annual	3049	65.3 ± 0.2	3.5 ± 0.03	2.54 ± 0.09	2.44 ± 0.08	50.5, 48.7–52.2	49.0, 47.2–50.7
2006	March	0	—	—	—	—	—	—
	April	188	66.6 ± 0.6	3.7 ± 0.10	0.66 ± 0.08	2.22 ± 0.22	41.5, 34.5–48.7	68.1, 60.9–74.5
	May	571	63.2 ± 0.4	3.7 ± 0.07	0.40 ± 0.03	1.03 ± 0.09	27.0, 23.5–30.8	37.1, 33.2–41.2
	June	229	66.3 ± 0.5	3.7 ± 0.08	0.75 ± 0.10	0.96 ± 0.48	32.3, 26.4–38.6	12.2, 8.4–17.2
	July	260	68.9 ± 0.5	3.4 ± 0.07	1.04 ± 0.11	1.22 ± 0.18	41.2, 35.2–47.3	32.3, 26.8–38.3
	Annual	1248	65.5 ± 0.2	3.5 ± 0.04	0.64 ± 0.04	1.23 ± 0.11	33.1, 30.5–35.7	36.2, 33.6–38.9
2007	March	0	—	—	—	—	—	—
	April	787	59.1 ± 0.4	2.3 ± 0.04	0.84 ± 0.05	2.56 ± 0.11	41.2, 37.7–44.7	63.7, 60.2–67.0
	May	474	61.4 ± 0.4	2.9 ± 0.06	1.30 ± 0.08	5.50 ± 0.25	57.2, 52.6–61.6	79.3, 75.4–82.8
	June	623	62.9 ± 0.3	3.0 ± 0.04	0.23 ± 0.03	1.10 ± 0.11	14.8, 12.2–17.8	27.0, 23.6–30.6
	July	0	—	—	—	—	—	—
	Annual	1884	60.9 ± 0.2	2.7 ± 0.03	0.75 ± 0.03	2.82 ± 0.09	36.5, 34.3–38.7	55.5, 53.2–57.7
2008	March	245	57.5 ± 0.8	2.4 ± 0.08	0.15 ± 0.03	0.55 ± 0.07	11.4, 7.9–16.1	28.6, 23.2–34.7
	April	213	63.7 ± 0.8	3.2 ± 0.11	0.21 ± 0.03	1.84 ± 0.14	18.3, 13.6–24.1	62.0, 55.2–68.3
	May	617	58.6 ± 0.4	2.6 ± 0.07	0.13 ± 0.02	0.46 ± 0.05	8.3, 6.3–10.7	17.5, 14.6–20.7
	June	428	64.6 ± 0.4	3.3 ± 0.07	0.16 ± 0.03	0.16 ± 0.03	11.2, 8.5–14.6	10.5, 7.9–13.8
	July	0	—	—	—	—	—	—
	Annual	1503	60.9 ± 0.3	2.9 ± 0.04	0.15 ± 0.01	0.58 ± 0.03	11.0, 9.5–12.7	23.6, 21.5–25.8

clemensi were similar, sharing 5% of the overall total, whereas adults were observed least frequently, at <2% of the total (Fig. 1).

COI gene sequences, amplified from adult reference specimens belonging to 8 morphologically distinct species of copepods, ranged in length from 426 to 660 base pairs (bp; Table II). Intraspecific pairwise genetic distances ranged from 0.002 to 0.016, and the greatest distances occurred among specimens of *L. cuneifer* (n = 10). Intraspecific variation was less marked in other species: *L. salmonis*, 0.009 (n = 3); *L. parviventris*, 0.002 (n = 9); *L. oblitus*, 0.010 (n = 3); and *L. hospitalis*, 0.002 (n = 3). There was no variation in the 660 bp sequence among individual *C. clemensi* (n = 13). A pairwise genetic distance of 0.023 was measured between consensus sequences of *L. cuneifer* genotypes A and B (Table IV). Interspecific pairwise genetic distances among species of *Lepeophtheirus* ranged from 0.082 to 0.233, and this increased to maxima of 0.251 and 0.420 when comparisons included *C. clemensi* and *Bomolochus cuneatus*, respectively (Table IV).

Amplification of a COI sequence from 25 chalimus IV and preadult specimens of *Lepeophtheirus* collected from sticklebacks in each of 2005–2008 confirmed the presence of *L. salmonis* and *L. cuneifer* in all years except 2005, when only *L. salmonis* was found (Table V). Overall, *L. salmonis* was approximately 2.4 times more abundant than *L. cuneifer*, although in 2008, *L. cuneifer* was 3.5 times more abundant. The *L. cuneifer* genotype A was observed twice as frequently on sticklebacks as genotype B. The 2 specimens of *L. cuneifer* obtained from British Columbia herring belonged to genotypes A and B, and the specimen from Alaska herring belonged to genotype A. Of the 733 PCR products obtained from chalimus IV and preadult specimens collected from pink (*Oncorhynchus gorbuscha*) and chum salmon (*Oncorhynchus keta*) between 2004 and 2008, 732 were identified as *L. salmonis* and 1, from a pink salmon, as *L. parviventris*.

Analysis of the neighbor-joining tree derived from an alignment of new and existing COI sequences from species of *Caligus* and *Lepeophtheirus*, and from *Bomolochus cuneatus*, resolved species or clusters of species but failed to provide convincing bootstrap support for higher taxonomic groupings (Fig. 2). All sequences from specimens belonging to 12 species of *Lepeophtheirus* from the Pacific and Atlantic oceans clustered together in a single clade, which was a sister group to a cluster of sequences from *C. centrodonti* and *C. curtus*. Within the *Lepeophtheirus* clade, there is moderate bootstrap support for a cluster of sequences from species originating from the Pacific Ocean (*L. cuneifer*, *L. parviventris*, *L. bifidus*, and *L. hospitalis*). Sequences from the 3 remaining species of *Caligus* clustered in clades unrelated to *C. centrodonti* and *C. curtus*, and the sequence from *B. cuneatus* was basal to all others in the analysis (Fig. 2). Two COI genotypes resolved within *L. cuneifer*, *L. salmonis*, and *C. elongatus*.

DISCUSSION

Infections with sea lice were frequently observed on marine sticklebacks sampled in the springtime from the Broughton Archipelago. Confirming previous observations, these infections included sea lice belonging to *C. clemensi* and *Lepeophtheirus*. The percentage of sticklebacks infected with sea lice ranged from approximately 28% in 2008 to >68% in 2005. In the 2 yr preceding this study, 61.3% (2003) and 84.3% (2004) of sticklebacks were infected with sea lice. Of the latter, 83.6% were

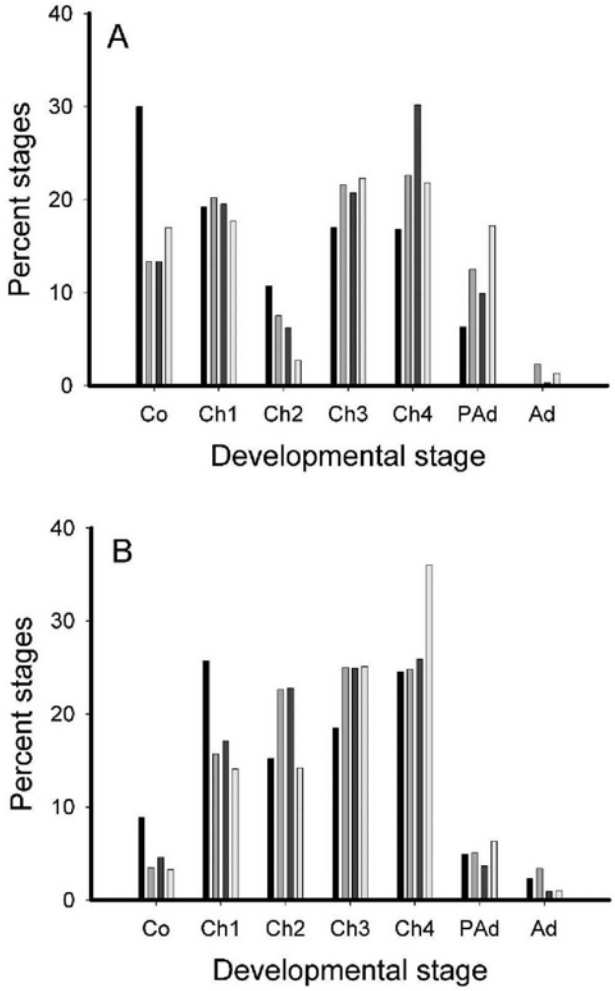

FIGURE 1. Sea lice developmental stages on threespine sticklebacks (*Gasterosteus aculeatus*) from the Broughton Archipelago, Canada. (**A**) *Lepeophtheirus*. (**B**) *Caligus clemensi*. Mean percentage of developmental stages from monthly counts (Co, copepodid; Ch1, chalimus I; Ch2, chalimus II; Ch3, chalimus III; Ch4, chalimus IV; PAd, preadult; and Ad, adult). Bars represent 2005, 2006, 2007, and 2008, respectively.

infected with specimens belonging to *Lepeophtheirus* (Jones and Nemec, 2004; Jones, Prosperi-Porta et al., 2006). Thus, since 2004, when *Lepeophtheirus* accounted for virtually all sea lice infecting sticklebacks (Jones, Prosperi-Porta et al., 2006), the prevalence of copepods in this genus declined more or less continuously until 2008. The mean intensity of *Lepeophtheirus* in 2005 was >3 times lower than that measured in 2004 and continued to decline, so that by 2008, the mean intensity was >13 times lower than in 2004. The maximum intensity on fish with mean weights of 2.3–4.0 g ranged from 7 in 2008 to 290 in 2004. A concurrent and similar declining trend was reported for *L. salmonis* on juvenile pink and chum salmon in the same area (Jones and Hargreaves, 2007, 2009), and Jones and Hargreaves (2009) suggested changing management practices on nearby salmon farms may have played a role in this decline. The prevalence of *C. clemensi* underwent considerable year-to-year variation, with greatest (56%) and least (24%) values observed in 2007 and 2008, respectively. Between 2005 and 2008, the mean intensity of *C. clemensi* infections on

TABLE IV. Pairwise genetic distances of COI (relative to positions 115–465 of *L. salmonis* reference sequence EU288200) sequences among parasitic copepods from British Columbia, Canada. Consensus sequences are used for species with more than one individual (see Table II). SEM is below the diagonal. See text for methods.

	Species*									
	1	2	3	4	5	6	7	8	9	10
1		0.233	0.230	0.213	0.214	0.218	0.198	0.197	0.226	0.387
2	0.032		0.023	0.082	0.187	0.201	0.186	0.202	0.220	0.379
3	0.032	0.008		0.089	0.180	0.194	0.190	0.202	0.221	0.396
4	0.030	0.017	0.018		0.175	0.233	0.182	0.205	0.232	0.420
5	0.030	0.028	0.027	0.027		0.231	0.161	0.203	0.248	0.355
6	0.031	0.028	0.027	0.032	0.033		0.197	0.167	0.215	0.364
7	0.027	0.028	0.028	0.027	0.024	0.028		0.160	0.251	0.382
8	0.028	0.028	0.028	0.030	0.029	0.025	0.025		0.192	0.340
9	0.030	0.030	0.029	0.031	0.034	0.029	0.033	0.026		0.321
10	0.043	0.042	0.043	0.046	0.040	0.041	0.043	0.037	0.036	

* 1, *Lepeophtheirus salmonis*; 2, *L. cuneifer* type A; 3, *L. cuneifer* type B; 4, *L. parviventris*; 5, *L. bifidus*; 6, *L. oblitus*; 7, *L. hospitalis*; 8, *Lepeophtheirus* sp. (AM235888); 9, *Caligus clemensi*; and 10, *Bomolochus cuneatus*.

sticklebacks seemed not to follow a consistent trend; although they oscillated, the maximum values declined from 105 in 2005 to 10 in 2008. Variations in the mean intensities of *Lepeophtheirus* and *C. clemensi* paralleled those of prevalence, similar to the trends reported for *L. salmonis* on farmed Atlantic salmon (Baillie et al., 2009). The declining mean abundance of *Lepeophtheirus* and *C. clemensi* observed between the latter 2 sample months in some years has been observed previously (Jones, Prosperi-Porta et al., 2006; Jones and Hargreaves, 2007) and may be related to the tendency of the salinity of surface sea water to become more diluted as a result of an estuarine flow, which is strongest in the spring and summer in this area (Foreman et al., 2006). Other factors, such as migration and maturation of fish hosts, are likely to influence seasonal trends in the abundance of *Lepeophtheirus* and *C. clemensi*.

The majority of sea lice observed on sticklebacks, regardless of species, were chalimus stages. It is not known why the chalimus II stage of *Lepeophtheirus* occurred relatively infrequently. No differences in developmental rates among stages of *L. salmonis* chalimi were reported on Atlantic salmon (Johnson and Albright, 1991a). However, the rate of development may differ on other host species, because fewer chalimus I and II stages were recovered from sticklebacks during laboratory exposures to *L. salmonis* (Jones, Kim, and Dawe, 2006). Thus, the consistently lower frequency of chalimus II on sticklebacks may reflect a briefer duration for this stage relative to that of other chalimus

TABLE V. Occurrence of *Lepeophtheirus salmonis* and *Lepeophtheirus cuneifer* on threespine sticklebacks (*Gasterosteus aculeatus*), based on COI sequences from chalimus IV or preadult specimens.

Yr	*L. salmonis*	*L. cuneifer* type A	*L. cuneifer* type B	Unreadable sequences	Total sequences
2005	22	0	0	3	25
2006	13	8	2	2	25
2007	21	0	1	3	25
2008	4	9	5	7	25
Total	60	17	8	15	100

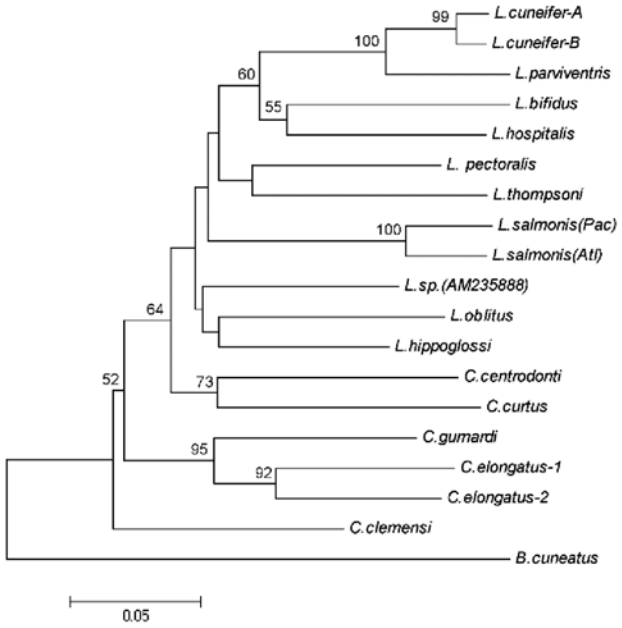

FIGURE 2. Unrooted neighbor-joining phylogram showing relatedness among COI gene sequences of parasitic copepod reference specimens with GenBank accessions listed in text and in Table II. Based on ClustalW alignment of 351 nucleotides (positions 115–465 of *L. salmonis* reference sequence EU288200) in all specimens. Bootstrap confidence levels are based on 1,000 re-samplings; levels ≥50 are shown. Included in the analysis is the homologous sequence from a copepod, *L.* sp., reported previously as *Lepeophtheirus hospitalis* (see text). The scale bar indicates the number of base substitutions per site.

stages. In contrast to the pattern on salmon, adults of *Lepeophtheirus* and *C. clemensi* on sticklebacks were the least abundant stages, supporting previous field and laboratory observations (Jones, Kim, and Dawe, 2006; Jones, Prosperi-Porta et al., 2006; Jones and Hargreaves, 2007). This consistently reduced frequency suggested a tendency for the more mature lice to leave sticklebacks. The highly motile behavior of *C. clemensi* preadults and adults may have further contributed to the reduced frequency of these stages. Although differences in the frequency of copepodids may reflect differences in the rate of acquisition of new infections, the copepodid stage is relatively short lived in *C. elongatus* (Hogans and Trudeau, 1989). A relatively brief period of copepodid development in *C. clemensi* also would contribute to a reduced frequency of this stage, as suggested previously (Jones, Prosperi-Porta et al., 2006).

A method for species identification that was independent of specimen anatomy was required because of the high proportion of chalimus stages combined with an absence of morphological information for the early developmental stages of most species of *Lepeophtheirus*. Previously, a partial small subunit ribosomal gene (18S rDNA) sequence used to differentiate among 5 reference species of caligid copepods showed that the specimens infecting marine sticklebacks in British Columbia were *L. salmonis* (Jones, Prosperi-Porta et al., 2006). In the present study, adult specimens belonging to 8 morphologically distinct species of parasitic copepods were used as sources of reference genetic information. Heterogeneity among mitochondrial COI sequences demonstrated the utility of the COI sequence as a taxonomic tool for recognizing species, as discussed previously (Waugh, 2007). Over 4 yr, approximately 71% of the specimens from stickleback were identified as *L. salmonis* and 29% as *L. cuneifer*; with *L. cuneifer* genotype A occurring twice as often as genotype B. Both genotypes were evident among the 3 specimens of *L. cuneifer* collected from Pacific herring in British Columbia and Alaska. In contrast, *L. cuneifer* was absent on juvenile pink and chum salmon collected concurrently with the sticklebacks from the Broughton Archipelago. *Lepeophtheirus cuneifer* was originally described from big skate (*Raja binoculata*) and rock greenling (*Hexagrammos lagocephalus*) from southeastern Alaska and Prince William Sound (Kabata, 1974) and subsequently from rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon farmed in British Columbia (Johnson and Albright, 1991b). The occurrences of *L. cuneifer* on threespine stickleback and Pacific herring represent new host records. With the exception of the present study, there are no prevalence data for *L. cuneifer* from any of its known hosts. The ability to detect *L. cuneifer* in relatively small subsamples of chalimus stages from sticklebacks seems to depend on the abundance of *L. salmonis*, which is driven by relatively large salmon populations in the area. In a previous study (Jones, Prosperi-Porta et al., 2006), all 20 juvenile specimens from sticklebacks collected in 2004 were identified as *L. salmonis* by using 18S rDNA sequence, and this was confirmed by COI sequence analysis from the same specimens (data not shown). In the present study, there was no evidence of *L. cuneifer* among the specimens examined from sticklebacks in 2005. Thus, the high prevalence and intensity of infections with *L. salmonis* observed in 2004 and 2005 may have reduced the likelihood of detecting *L. cuneifer* in those years because they occurred as a relatively small proportion of the total *Lepeophtheirus* population.

The *L. salmonis* mitochondrial genome was characterized by Tjensvoll et al. (2005), and COI nucleotide sequences have been used to understand phylogenetic relationships among caligid copepods (Øines and Heuch, 2005; Øines and Schram, 2008). In the present study, COI sequences derived from several species of *Lepeophtheirus*, regardless of ocean of origin, were monophyletic, as had been reported previously for specimens from the Atlantic Ocean (Øines and Heuch, 2005; Øines and Schram, 2008). However, unlike the sequences from species of *Lepeophtheirus*, those from 5 species of *Caligus* clustered within unrelated clades and were therefore paraphyletic, also in agreement with the previous reports (Øines and Heuch, 2005; Øines and Schram, 2008). The sequence derived from *C. clemensi* from the Pacific Ocean was only distantly related to the 5 congeneric sequences obtained from the Atlantic Ocean. Genetic divergence among and within *Lepeophtheirus* spp., measured as pairwise genetic distances, were similar to those reported previously for COI or 16S rRNA sequences from caligid copepods, crustaceans, and other animals (Hebert et al., 2003; Øines and Heuch, 2005; Lefébure et al., 2006). The current analysis confirmed the occurrence of genetic subtypes within *L. salmonis* and *C. elongatus* and recognized a similar phenomenon within *L. cuneifer*. Yazawa et al. (2008) reported that the *L. salmonis* COI gene differed by 6.1% between isolates from the Atlantic and Pacific oceans. In the present study, the pairwise genetic distance between the *L. salmonis* ocean types was 0.058 (data not shown). Similarly, there is a genetic distance of 0.12 between COI genotypes of *C. elongatus* (Øines and Heuch, 2005). In comparison, the genetic distance between the *L. cuneifer* genotypes was 0.022. The absence

of recent gene flow accounts for the divergence observed between *L. salmonis* from the Atlantic and Pacific oceans, whereas no barrier to gene flow has been identified that would explain the persistence of genotypes in *C. elongatus* and *L. cuneifer*. Morphological differences, in addition to the relative magnitude of the genetic distances between the *C. elongatus* genotypes, suggest that these represent sibling species (Øines and Schram, 2008). However, further research is required to determine the extent of morphological variation between the genotypes of *L. salmonis* and *L. cuneifer*. In a previous study, we concluded that genetically distinct, but morphologically correct, specimens of *L. hospitalis* occurred on starry flounder (*Platichthys stellatus*) and quillback rockfish (*Sebastes maliger*), respectively, in British Columbia. A COI genetic distance of 0.185 demonstrated that the specimens of *Lepeophtheirus* from starry flounder and quillback rockfish belong to distinct species. We assigned the specimens from starry flounder to *L. hospitalis* based on morphological identity and the previous recognition of the starry flounder as a host to this copepod (Kabata, 1988). A closer examination of additional *Lepeophtheirus* specimens from quillback rockfish is necessary and may result in the amendment of existing morphological keys.

Infections with *Lepeophtheirus* on the juvenile salmon consisted almost entirely of *L. salmonis*. In contrast, the single infection with *L. parviventris* on a juvenile pink salmon, although a new host record, seems to be extremely rare. In conclusion, we rejected the hypothesis that sticklebacks in the Broughton Archipelago are infected solely with *L. salmonis*, thus emphasizing the need to accurately identify immature sea lice as a prerequisite to understanding sea lice ecology in this region. The threespine stickleback may be a useful sentinel species for the abundance and diversity of the sea lice, which are also parasites of wild and farmed salmon in coastal ecosystems in British Columbia.

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