THE OCCURRENCE OF *LEPEOPHTHEIRUS SALMONIS* AND *CALIGUS CLEMENSI* (COPEPODA: CALIGIDAE) ON THREE-SPINE STICKLEBACK *GASTEROSTEUS ACULEATUS* IN COASTAL BRITISH COLUMBIA

Simon R. M. Jones, Gina Prosperi-Porta, Eliah Kim, Paul Callow, and N. Brent Hargreaves
Pacific Biological Station, 3190 Hammond Bay Road, Nanaimo, British Columbia, V9T 6N7, Canada. *e-mail: joness@pac.dfo-mpo.gc.ca*

ABSTRACT: Infections with sea lice species belonging to *Lepeophtheirus* and *Caligus* are reported from examinations of 1,309 three-spine sticklebacks collected in coastal British Columbia. Over 97% of the 19,960 *Lepeophtheirus* specimens and nearly 96% of the 2,340 *Caligus* specimens were in the copepodid and chalimus developmental stages. The parasites were identified as *Lepeophtheirus salmonis* and *Caligus clemensi* based on morphology of adult stages. Between 1,763 and 1,766 base pairs (bp) of 18S rDNA from adult specimens collected from sticklebacks and salmon differed from the GenBank *L. salmonis* reference sequence by a single bp and were distinct from those of 2 other *Lepeophtheirus* species. A 530-bp region of 18S rDNA from chalimus stages of *Lepeophtheirus* obtained from sticklebacks and salmon was identical to that of the *L. salmonis* reference sequence. The three-spine stickleback is a new host record for *L. salmonis*. The prevalence of *L. salmonis* was 83.6% and that of *C. clemensi* was 42.8%. The intensities of these infections were 18.3 and 4.2, respectively. There was no significant relationship between sea lice abundance and stickleback condition factor. Significant spatial and temporal variations both in abundance of sea lice and surface seawater salinities were measured. The abundance of both sea lice species was lowest in zones in which surface seawater salinity was also lowest. Sticklebacks appear to serve as temporary hosts, suggesting a role of this host in the epizootiology of *L. salmonis*. The stickleback may be a useful sentinel species with which to monitor spatial and temporal changes in the abundance of *L. salmonis* and *C. clemensi*.

Copepods of the Caligidae (Siphonostomatoida: Copepoda), collectively referred to as sea lice, occur on the skin, fins, and gills, and in the buccal cavity of marine fishes. In coastal waters of British Columbia (BC), Canada, 11 species of *Lepeophtheirus* and 1 species of *Caligus* have exploited these niches on 44 host species (Margolis and Arthur, 1979; McDonald and Margolis, 1995). The development of caligids (Caligidae) includes 2 nonparasitic planktonic nauplii, a fish-infective planktonic copepodid, and 7 parasitic stages (copepodid, chalimus I–IV, pre-adult, and adult) that lead to sexual maturity on the fish host (Pike and Wadsworth, 1999). Species are distinguished from each other based mainly on morphological features of the adults (Kabata, 1988), although mitochondrial DNA sequences have also been used for species identification (Øines and Heuch, 2005).

Lepeophtheirus salmonis has a Holarctic distribution and occurs on anadromous salmonids in the Atlantic and Pacific oceans. The parasite is found on adult Pacific salmon (*Oncorhynchus* spp.) collected in the mid-Pacific Ocean (Nagasawa et al., 1993; Nagasawa, 2001), in BC coastal waters (Beamish et al., 2005), and on salmon that are farmed in BC (Johnson and Margolis, 1993). *Lepeophtheirus salmonis* has recently been reported on pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon fry shortly after migrating from their natal streams into BC coastal waters (Morton et al., 2004). The parasite is considered specific to salmonids (Salmonidae), although it has been reported on white sturgeon (*Acipenser transmontanus*), sand lance (*Ammodytes hexapterus*), and saithe (*Pollachius virens*) (Kabata, 1973; Bruno and Stone, 1990). *Caligus clemensi*, another sea louse, has been reported from 13 species of fish in BC, including juvenile coho salmon (*Oncorhynchus kisutch*), pink salmon, and chum salmon (Parker and Margolis, 1964). *Caligus clemensi* shows no evidence of host specificity and the latter authors suggested the parasite will infect any species of fish inhabiting surface waters.

Annual surveillance of juvenile pink and chum salmon in

coastal BC has been conducted by Fisheries and Oceans Canada since 2003 to monitor the abundance and distribution of sea lice (Jones and Nemec, 2004). In addition to the salmon, sea lice were observed on three-spine stickleback (*Gasterosteus aculeatus*) collected as a frequent by-catch. This report describes the spatial and temporal patterns of the sea lice infections on stickleback. Molecular evidence is used to assist in the identification of *Lepeophtheirus* spp. specimens. The three-spine stickleback is identified as an important host of *L. salmonis*.

MATERIALS AND METHODS

Collections of sticklebacks and sea lice identification

Fish were collected from sites in Knight Inlet, Tribune Channel, and Kingcome Inlet north of Vancouver Island, BC, Canada (Fig. 1). The study area was arbitrarily subdivided into 11 zones, designated A to K (Fig. 1), each containing approximately 10 sample sites. Whenever possible, both beach (46 m in length) and purse (180 m to 275 m in length) seines were used at each site to collect fish from depths of up to 3.7 m and 16 m, respectively (see Boldt and Haldorson, 2004). The effective stretched mesh size in the bunt of all nets was 6.4 mm. Attempts were made to collect fish from each site in each of the following 3 periods: 10–16 May 2004, 25 May–1 June 2004, and 8–13 June 2004. These periods shared the same dates as sea lice surveys of juvenile salmon and stickleback in 2003 (Jones and Nemec, 2004) and of juvenile salmon in 2004 to be reported elsewhere. Up to 30 fish from each catch were individually bagged directly from the net, labeled, and immediately stored at −20 C. Frozen specimens were transported to the Pacific Biological Station, Nanaimo, BC, where total length and wet weight were measured and condition factor calculated (CF = (weight/length^3) × 100). Each fish was examined under a dissecting microscope and all sea lice were counted. All lice were identified to stage and species (or to genus for nonadult specimens of *Lepeophtheirus*) using criteria adapted from Johnson and Albright (1991a) and Kabata (1972, 1988). All *Caligus* sp. specimens were assumed to be *C. clemensi* based on earlier observations (Parker and Margolis, 1964). Identified sea lice were stored in 10% buffered formalin or 95% ethanol. Prevalence, mean abundance, and mean intensity were calculated according to Bush et al. (1997). The significance of differences in the abundance of sea lice among weeks and zones was tested using the Kruskal–Wallis test. Differences were considered significant when $P \leq 0.05$.
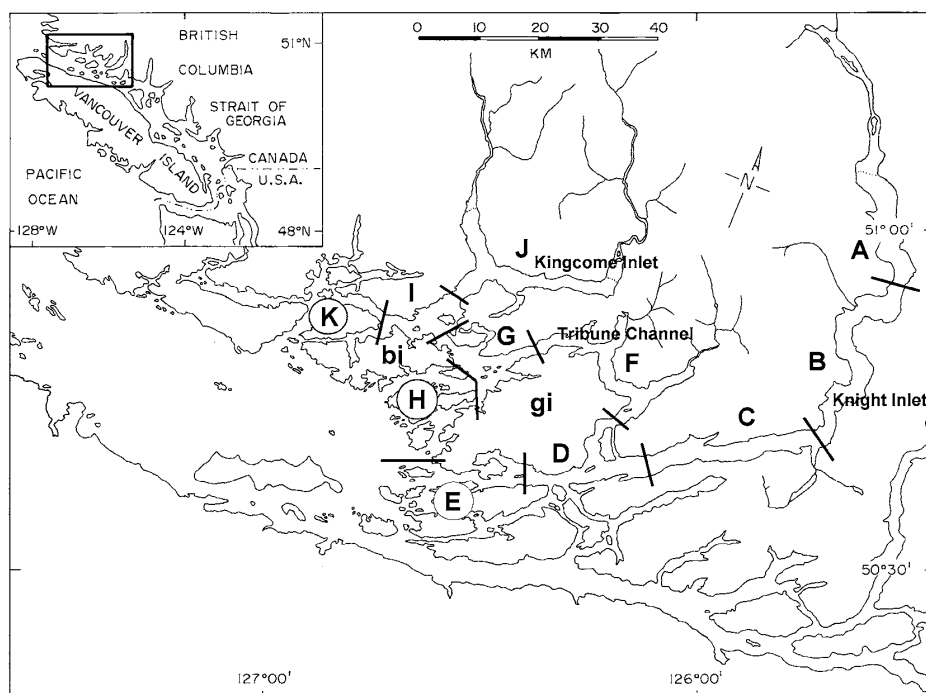
FIGURE 1.   Map of study area showing major water bodies and zone designations. bi, Broughton Island; gi, Gilford Island.

Seawater salinity

Surface seawater samples were collected coincidentally with fish throughout the study area. The salinity was calculated from the conductivity by using a Portasal® salinometer. The significance of differences in mean salinity was determined by ANOVA and Bonferroni-adjusted multiple comparison tests. Differences were considered significant when $P \leq 0.05$.

Molecular identification of sea lice

DNA was extracted from adult and preadult stages of *C. clemensi* collected from chum salmon and from *Lepeophtheirus* spp. collected from pink salmon, quillback rockfish (*Sebastes maliger*), starry flounder (*Platichthys stellatus*), and three-spine stickleback. The salmon and the

TABLE I.   Sequences and locations of polymerase chain reaction primers used to amplify 18S rDNA from *Lepeophtheirus* spp. and *Caligus clemensi*.

Primer	Location*	Nucleotide sequence (5′–3′)
9F	1	CCTGGTTCCTGCCAGTAGTC
7F	317	ATAGACGCCCACAGTGGTTT
9R	612	GCTTTTTGACCGCAACAACT
5F	866	GACAGTCGGGGGCATTAGTA
7R	897	CGGTCCAAGAATTTCACCTC
1F	1038	GCGATCCGCAGTTGTTTATT
3F	1192	AACACGGGAAATCTCACCAG
5R	1461	GCAGCCCAGAACATCTAAGG
1R	1633	GTACAAAGGGCAGGGACGTA
3R	1797	GATCCTTCCGCAGGTTCAC

* Location relative to *Lepeoptheirus salmonis* reference sequence (GenBank accession number AF208263).

flounder were collected coincidentally with the sticklebacks whereas the rockfish were collected in an unrelated study in the same study area in February 2005. Adult parasites were identified using the taxonomic keys of Kabata (1988). In addition, DNA was extracted from *Lepeophtheirus* sp. chalimus III or IV collected from chum salmon and stickleback. DNA was extracted from individual ethanol-fixed lice using DNeasy tissue kits following overnight digestion in protease K according to manufacturer's recommendations (Qiagen, Mississauga, Ontario, Canada). Complete and partial segments of the 18S ribosomal RNA gene (rDNA) were amplified by polymerase chain reaction (PCR) using primers 1F and 1R, 3F and 3R, 5F and 5R, 7F and 7R, and 9F and 9R (Table I) derived from the *L. salmonis* complete rDNA sequence (GenBank accession number AF208263). The conditions for all PCR amplifications were as follows: initial denaturation at 94 C for 2 min, 40 subsequent cycles of 94 C for 1 min, 56 C for 1 min, and 72 C for 2 min, followed by a final extension at 72 C for 10 min. Sequencing reactions on all purified (QIAquick, Qiagen) PCR products were performed using Big Dye™ Terminator V3.1 (Applied Biosystems, Foster City, California). Reaction products were purified using DyeEx 2.0 (Qiagen) and sequences were obtained from a 48-capillary DNA analyzer (Applied Biosystems). Sequence data were assembled and edited using Sequencher 4.1.4 (Gene Codes Corporation, Ann Arbor, Michigan) and BLASTn analyzed (http://www.ncbi.nlm.nih.gov/BLAST/). Consensus sequences obtained from each louse following bidirectional sequencing were aligned using ClustalW. Similarities among nucleotide sequences were estimated by using neighbor-joining (NJ) and maximum parsimony algorithms in MEGA 3 (Kumar et al., 2004). Bootstrap values, calculated as percentages over 1,000 replicates, provided an estimate of confidence in the branch nodes.

RESULTS

Stickleback surveillance

Sea lice were observed on a total of 1,103 (84.3%) of 1,309 sticklebacks from all zones with the exception of zone A (Table

TABLE II. Number and weight of three-spine sticklebacks (*Gasterosteus aculeatus*) among study zones in coastal British Columbia.

| | Collection period | | | | | |
| | 1 | | 2 | | 3 | |
Zone	Number	Weight (g)	Number	Weight (g)	Number	Weight (g)
A	0		21	4.8 ± 0.4*	0	
B	0		11	5.4 ± 0.5	0	
C	0		92	4.7 ± 0.3	0	
D	22	3.3 ± 0.2	24	3.0 ± 0.4	11	4.4 ± 0.5
E	4	2.8 ± 0.4	0		1	7.2
F	78	2.5 ± 0.2	67	2.6 ± 0.2	47	2.5 ± 0.1
G	73	2.5 ± 0.1	83	3.1 ± 0.1	33	2.9 ± 0.1
H	41	2.8 ± 0.2	61	2.7 ± 0.1	43	2.6 ± 0.1
I	86	4.0 ± 0.1	90	3.5 ± 0.1	76	4.1 ± 0.1
J	87	5.0 ± 0.2	64	3.7 ± 0.2	107	4.5 ± 0.1
K	14	3.1 ± 0.2	45	2.9 ± 0.1	28	2.9 ± 1.0

* Mean ± SEM.

II). Lice belonging to *Lepeophtheirus* sp. occurred on virtually all infected fish (83.6% prevalence) and approximately half of these fish were also infected with *C. clemensi* (42.8% prevalence). The overall intensities were 18.3 (range 1 to 290) and 4.2 (range 1 to 34), respectively. Among zones, the prevalence of *Lepeophtheirus* sp. ranged from 0% to 100% and the variance-to-mean ratio ranged from 3.0 to 124.2 (Table III). Similarly, the prevalence of *C. clemensi* ranged from 0% to 86.0% and the variance-to-mean ratio ranged from 1.4 to 28.9 (Table III). Throughout the study, the mean abundance of *Lepeophtheirus* sp. and *C. clemensi* ranged from 1.3 to 73.2 and from 0.02 to 5.9, respectively (Figs. 2, 3). For each of zones D, F, G, H, I, J, and K, differences in the abundance of *C. clemensi* and

Lepeophtheirus sp. over the 3 collection periods were significant (KW ≥ 2, P = 0). The abundance of *Lepeophtheirus* sp. increased in zones G, I, J, and K but showed no consistent trend elsewhere (Fig. 2). The abundance of *C. clemensi* increased in zones D and H, decreased in zones F and K, and showed no consistent temporal pattern elsewhere (Fig. 3). For each collection period, differences in the abundance of *C. clemensi* and *Lepeophtheirus* sp. among zones were significant (KW ≥ 2, P = 0). The abundance of *Lepeophtheirus* sp. was consistently greatest in zone K and least in zone J. The abundance of *C. clemensi* was consistently greatest in zones H and K, and least in zone J.

In total, 19,960 specimens of *Lepeophtheirus* sp. were ex-

TABLE III. *Lepeophtheirus salmonis* and *Caligus clemensi* on three-spine sticklebacks (*Gasterosteus aculeatus*) from coastal British Columbia.

| | *Lepeophtheirus salmonis* | | | | *Caligus clemensi* | | | |
Zone	P (%)*	S²/mean†	Lice‡	Cop (%)§	P (%)	S²/mean	Lice	Cop (%)
A	0	—	—	—	0	—	—	—
B	27.2	8.9	30	6.7	9.0	5.0	5	0
C	68.5	14.9	658	2.6	16.3	1.5	22	0
D	71.2	13.2	149.6	33.2	59.7	11.6	57.3	1.7
	(45.5–100.0)	(3.0–32.5)	(14–358)	(22.9–48.1)	(36.4–79.2)	(1.8–28.9)	(39–92)	(0–2.6)
E	100	4.5	16	37.5	50	4.0	8	0
F	95.9	23.9	1209.7	36.4	71.8	3.6	172.3	3.8
	(91.5–100.0)	(14.2–40.7)	(898–1331)	(31.5–43.9)	(57.4–83.3)	(1.4–4.8)	(56–297)	(1.8–6.0)
G	99.5	11.1	797.3	37.2	72.4	2.9	139.3	5.0
	(98.6–100.0)	(9.4–11.9)	(571–1065)	(27.8–43.0)	(68.7–75.8)	(2.3–3.8)	(72–203)	(4.2–6.3)
H	98.4	8.9	618.3	36.9	79.0	5.2	238.0	8.9
	(95.1–100.0)	(6.4–12.5)	(573–677)	(30.6–46.6)	(65.9–86.0)	(4.8–5.6)	(126–336)	(4.8–11.3)
I	93.4	32.6	2223.3	49.1	17.9	7.4	56.0	11.7
	(84.9–98.7)	(10.9–53.9)	(648–4239)	(39.9–61.6)	(8.1–31.1)	(3.3–13.2)	(22–121)	(0–19.0)
J	52.0	56.0	345.7	33.6	2.9	2.2	5.0	0
	(35.9–67.3)	(22.9–124.2)	(146–602)	(15.8–43.0)	(0.9–4.6)	(1.9–2.7)	(2–9)	
K	100.0	50.5	1404.7	44.1	69.4	7.3	94.3	16.5
	(45.5–59.6)	(219–2049)	(38.4–53.9)	(53.6–85.7)	(6.1–9.8)	(63–140)	(3.6–33.3)	

ᵃ Mean prevalence in 3 collections (range). Only 1 collection was made from zones A, B, and C. The data from zone E relate to collection 1 (see Table II). The single fish from collection 3 was infected with 14 *L. salmonis* and 18 *C. clemensi*.
† Variance-to-mean ratio (range).
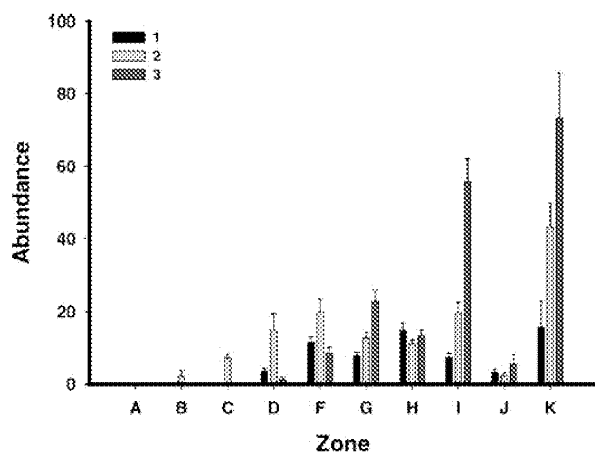‡ Mean number of lice (range).
§ Mean percent copepodids (range).

FIGURE 2.   Mean abundance (±SEM) of *Lepeophtheirus salmonis* on three-spine sticklebacks among zones (see Fig. 1 for locations of zones) during 3 collection periods, designated 1 (10–16 May 2004), 2 (25 May–1 June 2004), and 3 (8–13 June 2004). Zone E is omitted because of small sample size.

FIGURE 4.   Scatterplot showing for each stickleback, the correlation of condition factor (CF = (weight/length^3) × 100) and the number of *Lepeophtheirus salmonis* and *Caligus clemensi*. R^2 = 5 × 10^{-5}.

amined. Copepodids were most frequently observed, representing 40.4% of all specimens. The copepodid, all 4 chalimus and preadult stages together, included 99.9% of all specimens. Adult stages, representing 0.03% (n = 5) of all specimens, were identified as *L. salmonis*. Among zones, the proportion of copepodids ranged from 2.6% to 61.6% (Table III).

A total of 2,340 specimens of *C. clemensi* were examined. The first chalimus stage was most frequently observed (35.2%), whereas the copepodid represented 7.8% of all *C. clemensi* stages. Developmental stages of *C. clemensi* up to, and including, preadults, represented 97.1% and adults represented 2.9% of all specimens. The adult stages (n = 68) were confirmed to be *C. clemensi*. Among zones, the proportion of copepodids
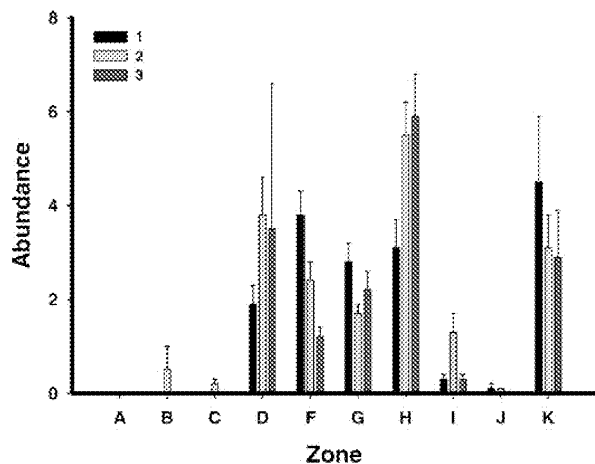
ranged from 0% to 33.3% (Table III). There was no significant relationship between the total number of sea lice and stickleback condition factor (R^2 = 5 × 10^{-5}) (Fig. 4).

Seawater salinity

Salinity was measured in a total of 307 water samples and on average, 9 measurements were obtained for each zone in each period. However, measurements were not made from zones A, B, and C in periods 1 and 3, and only 1 measurement was made from zone E in period 3. Throughout the study, salinity ranged from 0.48 to 30.0 parts per thousand (ppt), with a mean of 18.0 ppt. Significant differences in mean salinity were observed among zones in all sample periods. Mean salinity declined (P < 0.05) between the first and second period in all zones (Fig. 5). Between the second and third sample periods, mean salinity remained stable in zones D, E, and G (P ≥ 0.05)

FIGURE 3.   Mean abundance (±SEM) of *Caligus clemensi* on three-spine sticklebacks among zones (see Fig. 1 for locations of zones) during 3 collection periods (see Fig. 2 for dates). Zone E is omitted because of small sample size.
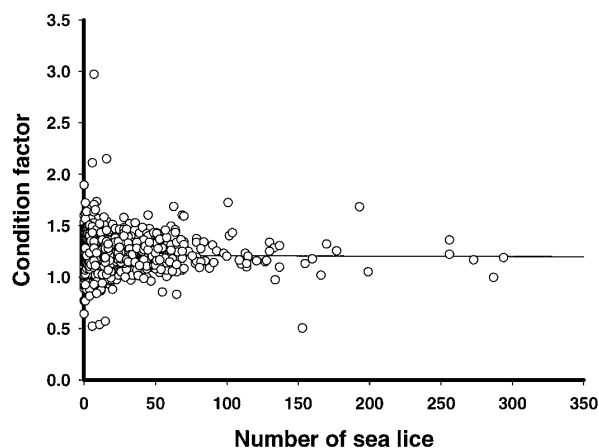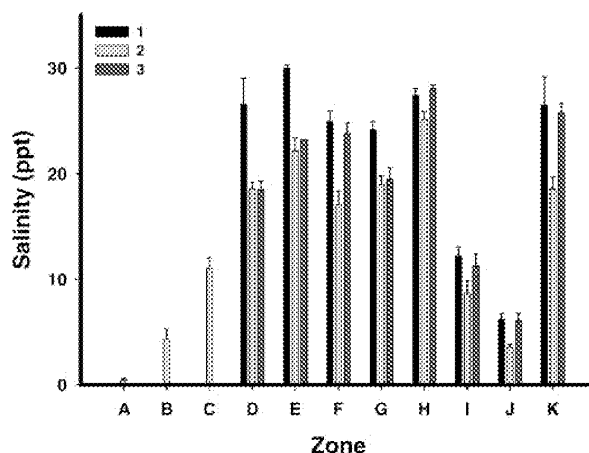

FIGURE 5.   Mean salinity (±SEM) of surface seawater among zones (see Fig. 1 for locations of zones) during 3 collection periods (see Fig. 2 for dates).
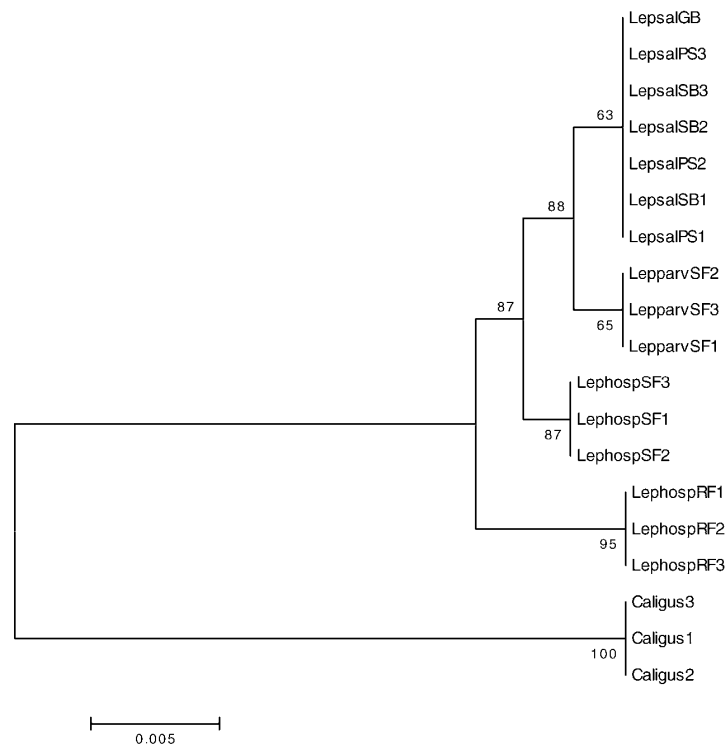
FIGURE 6.   Unrooted neighbor-joining phylogram showing relationships among 18S rDNA sequences of *Lepeophtheirus* spp. and *Caligus clemensi* collected from fish in the study area. Comparisons were based on 530 base pairs amplified using primers 1F and 1R identified in Table I. Bootstrap confidence levels are based on 1,000 resamplings. LepsalGB, *Lepeophtheirus salmonis* GenBank reference sequence (AF208263); LepsalSB, *L. salmonis* from stickleback (DQ123829); LepsalPS, *L. salmonis* from pink salmon (DQ123828); LephospSF, *Lepeophtheirus hospitalis* from starry flounder (DQ123831); LephospRF, *L. hospitalis* from quillback rockfish (DQ123832); LepparvSF, *Lepeophtheirus parvicruris* from starry flounder (DQ123830); Caligus, *Caligus clemensi* from chum salmon (DQ123833).

and rose ($P < 0.05$) in the other zones (Fig. 5). The highest salinity was associated with zone H and the lowest with zone A.

Molecular identification of sea lice

A region of 1,763 to 1,766 base pairs (bp) of the 18S rRNA gene was sequenced from 3 adult *L. salmonis* collected from each of pink salmon and sticklebacks and from 2 adult *C. clemensi* collected from chum salmon. Similarly, a region of 1,768 to 1,799 bp was sequenced from 3 adult *Lepeophtheirus parvicruris* collected from starry flounder and from 5 adult *Lepeophtheirus hospitalis*, 3 collected from starry flounder and 2 from quillback rockfish. A shorter segment was amplified using primers 1F and 1R from a third specimen from the rockfish. The BC *L. salmonis* consensus sequences, regardless of host, differed from the GenBank sequence (accession number AF208263) by the insertion of a cytosine at position 445. The *C. clemensi* consensus sequence, at 97.4% identity over 1,768 bp, was most similar to that of *Caligus elongatus* (GenBank accession number AY627020). The consensus sequence obtained from *L. parvicruris* was most similar to that of *L. salmonis* with an identity of 99.7% over 1,799 bp. Both *L. hospitalis* consensus sequences were most similar to *L. salmonis*; however, they differed from one another. The sequence identity with *L. salmonis* was 99.3% for specimens from rockfish and

99.6% for specimens from starry flounder. NJ analysis resolved the individual sequences obtained using primers 1F and 1R into 5 clusters corresponding to host–parasite groupings (Fig. 6). All *L. salmonis* sequences, whether derived from pink salmon, stickleback, or the GenBank reference, formed a single cluster that was most closely related to the *L. parvicruris* sequence. The *L. hospitalis* sequences from starry flounder clustered distinctly from those of *L. hospitalis* derived from the rockfish, with the latter more distantly related to the *L. salmonis*–*L. parvicruris* cluster. The 18S rDNA sequences from *C. clemensi* clustered distinctly from all *Lepeophtheirus* spp. sequences.

The 530-bp sequences obtained from 34 *Lepeophtheirus* sp. chalimus stages collected from salmon (n = 14) and sticklebacks (n = 20) using primers 1F and 1R shared 100% identity with the homologous GenBank *L. salmonis* sequence. NJ analysis of these sequences along with the homologous sequences obtained from adult *L. salmonis*, *L. hospitalis*, *L. parvicruris*, and *C. clemensi* produced a phylogram virtually identical to Figure 5 and confirmed that all chalimus sequences lay within the *L. salmonis* cluster. All rDNA sequences obtained from adult specimens have been deposited in GenBank (accession numbers as follows: *L. salmonis* [pink salmon], DQ123828; *L. salmonis* [stickleback], DQ123829; *L. parvicruris* [starry flounder], DQ123830; *L. hospitalis* [starry flounder], DQ123831; *L.*

hospitalis [quillback], DQ123832; and *C. clemensi* [chum salmon], DQ123833).

DISCUSSION

Three-spine sticklebacks occurring in a coastal BC marine ecosystem were frequent hosts of sea lice belonging to species of *Lepeophtheirus* and *Caligus*. The morphology of adult *Caligus* sp. specimens confirmed that they belonged to *C. clemensi*. The sea lice identified as *Lepeophtheirus* sp. were approximately 8.5 times more abundant than *C. clemensi*, but their specific identification was hampered by the observation that virtually all were in early developmental stages that are difficult to assign to species by using available morphological criteria. This is particularly important in coastal BC where 11 *Lepeophtheirus* spp. have been reported (Margolis and Arthur, 1975; McDonald and Margolis, 1995). Seven of these *Lepeophtheirus* spp. are described from more than 1 host species indicating a lack of host specificity. Controlled studies to examine the extent of host specificity of most *Lepeophtheirus* spp. have not been conducted and workers either assign immature stages only to genus (e.g., Kabata, 1988) or assume species identity based on host association (e.g., Morton et al., 2004). Molecular data were used to assist in the identification of the early *Lepeophtheirus* stages. Analysis of over 1,700 bp of the 18S rRNA gene (rDNA) from adult *L. salmonis* collected from sticklebacks and pink salmon showed that they differed from the GenBank *L. salmonis* reference by the insertion of a single base pair. This supported the observation that the adult stages collected from sticklebacks were morphologically identical to *L. salmonis*. Furthermore, rDNA sequences from *L. salmonis* and at least 2 other *Lepeophtheirus* spp., when subjected to a phylogenetic analysis, formed clusters that were consistent with the identifications based on morphology, thus validating the use of this sequence in species identification. Specimens identified as *L. hospitalis* from starry flounder and quillback rockfish were found to have distinct rDNA sequences. These distinct sequences may be evidence for 2 strains of *L. hospitalis*, possibly analogous to the occurrence of distinct strains of *Lepeophtheirus pectoralis* that occur on flounder (*Platichthys flesus*) and plaice (*Pleuronectes platessa*) (Boxshall, 1976). In the case of *L. salmonis*, rDNA sequences formed a single cluster regardless of host species. The 34 chalimus were, therefore, confirmed to be *L. salmonis* by the identity of 530 bp of their rDNA with the GenBank reference sequence and by phylogenetic analysis. Comparisons of 18S rDNA sequences have been used to distinguish species in several taxa of parasites (Andree et al., 1997; Whipps et al., 2004; Dykova et al., 2005). The cytochrome C oxidase 1 gene encoded in mitochondrial DNA has also been used for phylogenetic analyses among *Caligus* spp. (Øines and Heuch, 2005). In addition, the *L. salmonis* mitochondrial genome has recently been sequenced (Tjensvoll et al., 2005), providing further opportunities for the use of molecular analysis in the systematics of this genus. Morphologically indistinguishable early sea lice developmental stages are identifiable to species using genomic and, possibly, mitochondrial DNA sequences.

This, therefore, is the first evidence that *L. salmonis* is a frequent parasite of the three-spine stickleback in a coastal BC ecosystem. Previous reports of sea lice on sticklebacks in coastal BC include *C. clemensi* (Parker and Margolis, 1964; Lester,

1974) and chalimus stages of a *Lepeophtheirus* species (Kabata, 1988). Neither prevalence nor abundance was documented in the earlier work; thus, there is no historical reference to the extent of sea lice infections in stickleback populations. Although infected sticklebacks occurred throughout the study area, significant variations were evident in the abundance of both parasite species. Low abundances of *C. clemensi* were observed in zones A, B, C, I, and J, coincident with lower salinities. In contrast, the abundance of *L. salmonis*, although low in zones A, B, C, and J, was significantly elevated in zone I. Schram et al. (1998) suggested that *C. elongatus* is less tolerant to reduced salinity than *L. salmonis* and this may also be true of *C. clemensi*, perhaps explaining the differences in abundances observed in zone I. Laboratory studies have documented the adverse effects of reduced salinity on the hatching, development, and survival of *L. salmonis* (Berger, 1970; Hahnenkamp and Fyhn, 1985; Johnson and Albright, 1991b; Tucker et al., 2000). The few available field data tend to corroborate the laboratory studies. Pemberton (1976) concluded that reduced salinity played a role in the lower winter infestations of *L. salmonis* on sea trout (*Salmo trutta*) in Loch Etive, Scotland. Similarly, residency in low-salinity water was one of several factors to reduce *L. salmonis* infestation rates on sea trout in 2 fjords in northern Norway (Rikardsen, 2004). Low salinity in the Skagerrak was related to the reduced presence of *C. elongatus* on sea trout over 2 winters and of *L. salmonis* in 1 winter (Heuch et al., 2002), possibly by the exclusion of sea lice copepodids from surface-orientated salmon smolts (Heuch et al., 2005). Together, the laboratory and field observations indicate the importance of low salinity in limiting the abundance of *L. salmonis* and *Caligus* spp.

Despite the high prevalence of *L. salmonis* throughout much of the study area, infections consisted primarily of early, non-motile developmental stages. Similarly, early developmental stages of *C. clemensi* dominated. The relatively low abundance of adults of both sea lice species may be related to a loss of the motile stages during or preceding fish capture. Indeed, adult *Caligus* spp. stages display a tendency to swim from their hosts (Pike and Wadsworth, 1999) and this locomotory behavior has been described for *L. salmonis* (Kabata and Hewitt, 1971). Swimming from the host may be one of several mechanisms to explain the decline in the abundance of *L. salmonis* from experimentally infected salmon that has been repeatedly observed during louse development (Johnson, 1993; Fast et al., 2002). The effect is most pronounced on Pacific salmon (*Oncorhynchus* spp.) and may be related to a host response (Fast et al., 2002). Research is required to determine the mechanisms associated with the extremely low abundance of adult *L. salmonis* on sticklebacks. In contrast, the relatively high percentage of *L. salmonis* copepodids (and of *C. clemensi* chalimus I stages) indicated exposures to new infections were ongoing throughout the study. Considerable variation in the percentage of copepodids of both species occurred among study zones, although it is not clear whether this reflects differences in the abundance of planktonic copepodids and, therefore, infection pressure. The consistently low percentage of *C. clemensi* copepodids may be related to the shorter duration of this stage, as was reported for *C. elongatus* (Hogans and Trudeau, 1989) relative to *L. salmonis* (Johnson and Albright, 1991b). Variance-to-mean (V/M) ratios greater than 1 throughout the study indicated that *L. sal-*

monis and *C. clemensi* were overdispersed within the population (Poulin, 1993). The lack of a significant relationship between sea lice intensity and condition factor suggested that the observed infections did not adversely affect stickleback growth. Although the low proportion of the larger and potentially harmful motile stages of *L. salmonis* (Bjørn and Finstad, 1998) suggested any other impact was minimal, the consequences of sea lice infection on stickleback require further laboratory study.

This study was conducted from mid-May to mid-June and very little is known regarding sea lice on sticklebacks in this area at other times of the year. Populations of marine, anadromous, and freshwater three-spine stickleback occur in coastal BC (McPhail, 1994); however, the extent to which strictly marine and anadromous forms differ in their spawning behaviors is not clear and some authors have recognized only anadromous and freshwater forms (Withler and McPhail, 1985). The sticklebacks examined in the present study are considered anadromous, even though spawning may occur in brackish nearshore habitats (Picard et al., 1989). In contrast to Pacific salmon (*Oncorhynchus* spp.), which migrate through the area, the stickleback is assumed to be nonmigratory. Given its evident susceptibility, widespread distribution in the study area, apparent abundance, and assumed nonmigratory behavior, the stickleback may be a useful sentinel species with which to monitor spatial and temporal changes in the abundance of sea lice. The factors that regulate sea lice abundance in coastal BC are not well understood. A prominent oceanographic characteristic of the study area is an estuarine circulation driven by winter precipitation and summer melting of glacial snow and ice (Brooks, 2005). This circulation resulted in the reduced surface salinity, and correspondingly reduced sea lice abundances, associated with those bodies of water farthest from the open ocean described here. The highest abundances of sea lice were generally observed in the high-salinity estuarine outflows nearest the open ocean, consistent with the flushing of planktonic copepodids at several cm/sec into this area (Brooks, 2005). Several salmon farms are also located within the high-salinity outflows. In the northeast Atlantic Ocean, salmon farms are important in the epizootiology of *L. salmonis* (Heuch et al., 2005). It is evident that considerable research is required to better understand the distribution and abundance of sticklebacks in this marine ecosystem, the extent of seasonal changes in the abundance and distribution of sea lice on sticklebacks, and the relationship between the sea lice on sticklebacks and on sympatric salmonids. The relative roles played by local oceanography and farmed salmon in regulating the abundance of sea lice also require clarification.

ACKNOWLEDGMENTS

LITERATURE CITED

ANDREE, K. B., S. J. GRESOVIAC, AND R. P. HEDRICK. 1997. Small subunit ribosomal RNA sequences unite alternate actinosporean and my-

xosporean stages of *Myxobolus cerebralis* the causative agent of whirling disease in salmonid fish. Journal of Eukaryotic Microbiology **44:** 208–215.

BEAMISH, R. J., C. M. NEVILLE, R. SWEETING, AND N. AMBERS. 2005. Sea lice on adult Pacific salmon in the coastal waters of central British Columbia, Canada. Fisheries Science **76:** 198–208.

BERGER, V. Y. 1970. Influence of sea water of different salinities on *Lepeophtheirus salmonis* (Krøyer), a parasite of salmon. Parazitologiya **4:** 136–138.

BJØRN, P. A., AND B. FINSTAD. 1998. The development of salmon lice (*Lepeophtheirus salmonis*) on artificially infected post-smolts of sea trout (*Salmo trutta*). Canadian Journal of Zoology **76:** 970–977.

BOLDT, J. L., AND L. J. HALDORSON. 2004. Size and condition of wild and hatchery pink salmon juveniles in Prince William Sound, Alaska. Transactions of the American Fisheries Society **133:** 173–184.

BOXSHALL, G. A. 1976. The host specificity of *Lepeophtheirus pectoralis* (Müller, 1776) (Copepoda: Caligidae). Journal of Fish Biology **8:** 255–264.

BROOKS, K. M. 2005. The effects of water temperature, salinity, and currents on the survival and distribution of the infective copepodid stage of sea lice (*Lepeophtheirus salmonis*) originating on Atlantic salmon arms in the Broughton Archipelago of British Columbia, Canada. Reviews in Fisheries Science **13:** 177–204.

BRUNO, D. W., AND J. STONE. 1990. The role of saithe, *Pollachius virens* L. as a host for the sea lice, *Lepeophtheirus salmonis* Krøyer and *Caligus elongatus* Nordmann. Aquaculture **89:** 201–207.

BUSH, A. O., K. D. LAFFERTY, J. M. LOTZ, AND A. W. SHOSTAK. 1997. Parasitology meets ecology on its own terms: Margolis et al. revisited. Journal of Parasitology **83:** 575–583.

DYKOVA, I., B. F. NOWAK, P. B. CROSBIE, I. FIALA, H. PECKOVÁ, M. B. ADAMS, B. MACHÁČKOVÁ, AND H. DVOŘÁKOVÁ. 2005. *Neoparamoeba branchiphila* n. sp., and related species of the genus *Neoparamoeba* Page, 1987: Morphological and molecular characterization of selected strains. Journal of Fish Diseases **28:** 49–64.

FAST, M. D., N. W. ROSS, A. MUSTAFA, D. E. SIMS, S. C. JOHNSON, G. A. CONBOY, D. J. SPEARE, G. JOHNSON, AND J. F. BURKA. 2002. Susceptibility of rainbow trout *Oncorhynchus mykiss*, Atlantic salmon *Salmo salar* and coho salmon *Oncorhynchus kisutch* to experimental infection with sea lice *Lepeophtheirus salmonis*. Diseases of Aquatic Organisms **52:** 57–68.

HAHNENKAMP, L., AND H. J. FYHN. 1985. The osmotic response of salmon louse, *Lepeophtheirus salmonis* (Copepoda: Caligidae), during the transition from sea water to fresh water. Journal of Comparative Physiology **B155:** 357–365.

HEUCH, P. A., P. A. BJØRN, B. FINSTAD, J. C. HOLST, L. ASPLIN, AND F. NILSEN. 2005. A review of the Norwegian "National Action Plan Against Salmon Lice on Salmonids": The effect on wild salmonids. Aquaculture **246:** 79–92.

———, J. A. KNUTSEN, H. KNUTSEN, AND T. A. SCHRAM. 2002. Salinity and temperature effects on sea lice over-wintering on sea trout (*Salmo truttae*) in coastal areas of the Skagerrak. Journal of the Marine Biological Association of the United Kingdom **82:** 887–892.

HOGANS, W. E., AND D. J. TRUDEAU. 1989. Preliminary studies on the biology of sea lice, *Caligus elongatus*, *Caligus curtus* and *Lepeophtheirus salmonis* (Copepoda: Caligoida) parasitic on cage-cultured salmonids in the lower Bay of Fundy. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1715. 14 p.

JOHNSON, S. C. 1993. A comparison of development and growth rates of *Lepeophtheirus salmonis* (Copepoda: Caligidae) on naïve Atlantic (*Salmo salar*) and chinook (*Oncorhynchus tshawytscha*) salmon. *In* Pathogens of wild and farmed fish: Sea lice, G. A. Boxshall and D. Defaye (eds). Ellis Horwood, Chichester, U.K. p. 68–80.

———, AND L. ALBRIGHT. 1991a. The developmental stages of *Lepeophtheirus salmonis* (Krøyer, 1837) (Copepoda: Caligidae) under laboratory conditions. Canadian Journal of Zoology **69:** 929–950.

———, AND ———. 1991b. Development, growth and survival of *Lepeophtheirus salmonis* (Copepoda: Caligidae) under laboratory conditions. Journal of the Marine Biological Association of the United Kingdom **71:** 425–436.

———, AND L. MARGOLIS. 1993. The efficacy of ivermectin for the control of sea lice on sea-farmed Atlantic salmon. Diseases of Aquatic Organisms **17:** 107–112.

JONES, S. R. M., AND A. NEMEC. 2004. Pink salmon action plan: Sea

lice on juvenile salmon and some non-salmonid species in the Broughton Archipelago in 2003. Canadian Science Advisory Secretariat Research Document 2004/105, Fisheries and Oceans, Ottawa, Ontario, Canada, 87 p.

KABATA, Z. 1972. Developmental stages of *Caligus clemensi* (Copepoda: Caligidae) from fishes of British Columbia. Journal of the Fisheries Research Board of Canada **29:** 1571–1593.

———. 1973. The species of *Lepeophtheirus* (Copepoda: Caligidae) from fishes of British Columbia. Journal of the Fisheries Research Board of Canada **30:** 729–759.

———. 1988. Copepoda and Branchiura. *In* Guide to the parasites of fishes of Canada. Part II—Crustacea, L. Margolis and Z. Kabata (eds.). Canadian Special Publication of Fisheries and Aquatic Sciences 101, Ottawa, Ontario, Canada, p. 3–127.

———, AND G. C. HEWITT. 1971. Locomotory mechanisms in Caligidae (Crustacea: Copepoda). Journal of the Fisheries Research Board of Canada **28:** 1143–1151.

KUMAR, S., K. TAMURA, AND M. NEI. 2004. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Briefings in Bioinformatics **5:** 150–163.

LESTER, R. J. G. 1974. Parasites of *Gasterosteus aculeatus* near Vancouver, British Columbia. Syesis **7:** 195–200.

MARGOLIS, L., AND J. R. ARTHUR. 1979. Synopsis of the parasites of fishes of Canada. Bulletin of the Fisheries Research Board of Canada No. 199. Department of Fisheries and Oceans, Ottawa, Ontario, Canada, 269 p.

MCDONALD, T. E., AND L. MARGOLIS. 1995. Synopsis of the parasites of fishes of Canada: Supplement (1978–1993). Canadian Special Publication of Fisheries and Aquatic Sciences No. 122. National Research Council of Canada, Ottawa, Ontario, Canada, 265 p.

MCPHAIL, J. D. 1994. Speciation and the evolution of reproductive isolation in the sticklebacks (*Gasterosteus*) of south-western British Columbia. *In* Evolutionary biology of the three-spine stickleback, M. A. Bell and S. A. Foster (eds.). Oxford University Press, Oxford, U.K., p. 399–437.

MORTON, A., R. ROUTLEDGE, C. PEET, AND A. LADWIG. 2004. Sea lice (*Lepeophtheirus salmonis*) infection rates on juvenile pink (*Oncorhynchus gorbuscha*) and chum (*Oncorhynchus keta*) salmon in the nearshore marine environment of British Columbia, Canada. Canadian Journal of Fisheries and Aquatic Sciences **61:** 147–157.

NAGASAWA, K. 2001. Annual changes in the population size of the salmon louse *Lepeophtheirus salmonis* (Copepoda: Caligidae) on high-seas Pacific salmon (*Oncorhynchus* spp.), and relationship to host abundance. Hydrobiologia **453/454:** 411–416.

———, Y. ISHDA, M. OGURA, K. TADOKORO, AND K. HIRAMATSU. 1993. The abundance and distribution of *Lepeophtheirus salmonis* (Co-

pepoda: Caligidae) on six species of Pacific salmon in offshore waters of the North Pacific Ocean and Bering Sea. *In* Pathogens of wild and farmed fish: Sea lice, G. A. Boxshall and D. Defaye (eds.). Ellis Horwood, Chichester, U.K., p. 166–178.

ØINES, Ø., AND P. A. HEUCH. 2005. Identification of sea louse species of the genus *Caligus* using mtDNA. Journal of the Marine Biological Association of the United Kingdom **85:** 73–79.

PARKER, R. R., AND L. MARGOLIS. 1964. A new species of parasitic copepod, *Caligus clemensi* sp. nov. (Caligoida: Caligidae), from pelagic fishes in the coastal waters of British Columbia. Journal of the Fisheries Research Board of Canada **21:** 873–889.

PEMBERTON, R. 1976. Sea trout in North Argyle sea lochs, population, distribution and movements. Journal of Fish Biology **9:** 157–179.

PICARD, P., JR., J. J. DODSON, AND G. J. FITZGERALD. 1989. Habitat segregation among the age groups of *Gasterosteus aculeatus* (Pisces: Gasterosteidae) in the middle St. Lawrence estuary, Canada. Canadian Journal of Zoology **68:** 1202–1208.

PIKE, A. W., AND S. L. WADSWORTH. 1999. Sea lice on salmonids: Their biology and control. Advances in Parasitology **44:** 233–330.

POULIN, R. 1993. The disparity between observed and uniform distributions: A new look at parasite aggregation. International Journal for Parasitology **23:** 937–944.

RIKARDSEN, A. H. 2004. Seasonal occurrence of sea lice *Lepeophtheirus salmonis* on sea trout in two north Norwegian fjords. Journal of Fish Biology **65:** 711–722.

SCHRAM, T. A., J. A. KNUTSEN, P. A. HEUCH, AND T. A. MO. 1998. Seasonal occurrence of *Lepeophtheirus salmonis* and *Caligus elongatus* (Copepoda: Caligidae) on sea trout (*Salmo trutta*), off southern Norway. ICES Journal of Marine Science **55:** 163–175.

TJENSVOLL, K., K. HODNELAND, F. NILSEN, AND A. NYLUND. 2005. Genetic characterization of the mitochondrial DNA from *Lepeophtheirus salmonis* (Crustacea: Copepoda). A new gene organization revealed. Gene **353:** 218–230.

TUCKER, C. S., C. SOMMERVILLE, AND R. WOOTTEN. 2000. The effect of temperature and salinity on the settlement and survival of copepodids of *Lepeophtheirus salmonis* (Krøyer, 1837) on Atlantic salmon, *Salmo salar* L. Journal of Fish Diseases **23:** 309–320.

WHIPPS, C. M., G. GROSSEL, R. D. ADLARD, H. YOKOYAMA, M. S. BRYANT, B. L. MUNDAY, AND M. L. KENT. 2004. Phylogeny of the Multivalvulidae (Myxozoa: Myxosporea) based on comparative ribosomal DNA sequence analysis. Journal of Parasitology **90:** 618–622.

WITHLER, R. E., AND J. D. MCPHAIL. 1985. Genetic variability in freshwater and anadromous sticklebacks (*Gasterosteus aculeatus*) of southern British Columbia. Canadian Journal of Zoology **63:** 528–533.