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ARTICLE

Sea Lice Infestations of a Wild Fish Assemblage in the Northwest Atlantic Ocean

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Abstract
Little research on wild fish and sea lice interactions has occurred in the Northwest Atlantic Ocean, particularly for the sea lice species *Caligus elongatus*. This project quantified infestations of sea lice on wild fishes of Cobscook Bay, Maine. Field examinations of 6,334 fish during March–November 2012 were used to determine host species and infestation prevalence. Of these, 253 fish were examined under magnification to identify sea lice genus, infestation intensity, sea lice life cycle stages, and attachment locations of chalimus stages. We used DNA sequencing on 175 (34%) individual lice to confirm sea lice species. *Caligus elongatus* was the only observed sea lice species and was found on 10 fish species, including eight newly identified hosts. Nonmotile chalimus were the predominant life stages observed, accounting for 95.87% of sea lice, and were predominantly attached to hosts' fins. Overall infestation prevalence and intensity among all sampled fish were low—less than 5% and just one sea louse per fish, respectively. Among the three most common host species, Threespine Sticklebacks *Gasterosteus aculeatus* had the highest infestation prevalence and intensity. These results are the first reported data for sea lice presence on a wild fish assemblage in the Northwest Atlantic Ocean. Furthermore, the identification of new hosts for *C. elongatus* and the lack of observed infestations by the salmon louse *Lepeophtheirus salmonis* will be used to inform sea lice management efforts in aquaculture operations.

Caligus elongatus is a ubiquitous, ectoparasitic copepod of wild fishes in the North Atlantic Ocean, known to infest over 80 species (Kabata 1979). This species, in addition to the salmon louse *Lepeophtheirus salmonis*, are the dominant representatives of the family Caligidae in this region (Costello 2006). Sea lice of the family Caligidae attach to the skin and fins to feed on the hosts' mucus, skin, and tissue (Kabata 1979; Costello 2006). *Caligus elongatus* host species include salmonids (e.g., Atlantic Salmon *Salmo salar* and Brown Trout *S. trutta*), gadids (e.g., Atlantic Cod *Gadus morhua* and Atlantic Tomcod *Microgadus tomcod*), and many others, including Lumpfish *Cyclopterus lumpus* and Atlantic Herring *Clupea harengus* (Kabata 1979; Margolis and Arthur 1979). In contrast, *L. salmonis* predominantly infests only salmonids (Kabata 1979), though it has been observed on other species, including Threespine Sticklebacks *Gasterosteus aculeatus* in the Pacific Ocean (Jones et al. 2006). While *L. salmonis* infests fish in both the North

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Atlantic and Pacific oceans, *C. elongatus* is found only in the North Atlantic Ocean.

These two species differ in additional respects, most notably in size and life cycle. *Caligus elongatus* is smaller and exhibits a less aggressive feeding behavior on host fish than *L. salmonis* (Westcott et al. 2004). *Caligus elongatus* lacks the two motile preadult stages which precede the motile, reproductive adult stage of *L. salmonis* (Tully and Nolan 2002; Boxaspen 2006). While *C. elongatus* possesses four nonmotile chalimus stages, only two are present in *L. salmonis* (Hamre et al. 2013).

Little prior research has examined sea lice infestations on wild fish assemblages. In the North Atlantic Ocean, such surveys have been restricted to the North Sea (Heuch et al. 2007). Most research on wild fish interactions with sea lice has focused instead on infestations of single fish species, including Atlantic Salmon (Powell et al. 1999; Todd et al. 2000), Sea Trout (MacKenzie et al. 1998; Urquhart et al. 2008; Urquhart et al. 2010), Atlantic Cod (Heuch et al. 2011; Mehrdana et al. 2014), and Threespine Sticklebacks (Jones et al. 2006; Jones and Prosperi-Porta 2011; Eaves et al. 2014). Powell et al. (1999) and Eaves et al. (2014) are the only known sea lice surveys conducted on wild fish in the Northwest Atlantic Ocean, and both observed a predominance of copepods belonging to families other than Caligidae. Much of this research on wild fish sea lice infestations was conducted, at least in part, to assess potential transfers of sea lice between wild and cultured fish (Heuch et al 2007; Urquhart et al. 2010; Eaves et al. 2014).

Severe sea lice infestations in fish aquaculture operations pose an economic problem due to fish mortality, treatment costs, reduced fish growth, reduced feed conversion efficiency, and reduced market value from grazing lesions (Sinnott 1998; Mustafa et al. 2001). Commercial salmon aquaculture operations worldwide experience an estimated annual loss due to sea lice of approximately US$480 million (Costello 2009), and outbreaks of *C. elongatus* and *L. salmonis* in the North Atlantic have killed thousands of sea cage-raised Atlantic Salmon (Hogans and Trudeau 1989a). In addition, aquaculture of nonsalmonid species, including Atlantic Cod, has raised concerns about sea lice issues similar to those experienced by salmonid farmers (Bricknell et al. 2006). Infestations of *C. elongatus* are a potential problem because of the difficulty in controlling parasite species with a broad range of available wild hosts (Johnson et al. 2004). *Caligus elongatus* infestations of hatchery-reared Atlantic Cod juveniles have already been reported in Norwegian coastal waters (Karlsbakk et al. 2001).

Though the attachment and feeding sites of most sea lice life stages on cultured and experimentally infested fish are well documented, the distribution of chalimus stages on wild fish has not been reported in detail for either *C. elongatus* or *L. salmonis*. Pre-adult and adult stages of *C. elongatus* and *L. salmonis* were found to be concentrated on the body surfaces of wild Sea Trout (Urquhart et al. 2008) and cultured and experimentally infested Atlantic Salmon (Dawson et al. 1997; Treasurer and Wadsworth 2004). On cultured and experimentally infested Atlantic Salmon, the chalimus stages of both *C. elongatus* and *L. salmonis* were found predominantly on the dorsal, caudal, and pectoral fins (Bjørn and Finstad 1998; Treasurer and Wadsworth 2004). MacKenzie et al. (1998) briefly reported that most chalimus stages on wild Sea Trout were located at the base of the dorsal fin. However, they analyzed only the locations of pre-adult and adult stages in detail. To the extent of our knowledge, no prior studies have further described the attachment locations of the chalimus stages on wild fish hosts; therefore, this information has the potential to inform future sea lice surveys on wild fish assemblages.

This study characterized the infestation of wild fishes by sea lice in Cobscook Bay, Maine, a representative bay of the Northwest Atlantic Ocean. The study contributes to the limited knowledge of assemblage-wide sea lice infestations. The species of sea lice found on wild fishes, the host fish species, sea lice life stage proportions, chalimus attachment locations, and infestation intensity and prevalence were quantified. The results of this work establish a first report of sea lice presence on wild fish in Cobscook Bay and expand the already broad host range of *C. elongatus*.

METHODS

Study site.—Cobscook Bay is a boreal, macrotidal estuary at the mouth of the Bay of Fundy (Figure 1), experiencing semidiurnal tides with a mean range of 5.7 m (Larsen 2004a). Cobscook Bay's convoluted shoreline divides it into three distinct regions: Inner, Central, and Outer bays (Figure 1). Within the bay, salinities are usually greater than 30‰, temperatures vary seasonally between 0°C and 12°C in most years, and turbidity is generally low (Larsen 2004a). Cobscook Bay also has an average depth of 10 m and receives minimal freshwater input (Larsen 2004a). The bay exhibits very high primary productivity and ecological richness due to the intense tidal mixing (Larsen 2004b). For finfish specifically, the intertidal and subtidal assemblages are made up of 46 different species that exhibit annual consistency in the most abundant species but distinct spatial differences in their relative abundance (Vieser 2014).

Fish sampling and examinations.—Fish sampling in Cobscook Bay, Maine, was conducted monthly between March and November (with the exception of July) in 2012 using beach seine, fyke, pelagic trawl, and benthic trawl nets. Only seine netting was used March 8–10, April 13 and 14, and November 2. Seine, fyke, and trawl nets were used in the remaining months on the following dates: May 25–30, June 23–28, August 25–31, and September 22–28, 2012.

All sampling sites in Cobscook Bay were selected with the intended purpose of balanced effort between Inner, Central, and Outer bays (Figure 1; Table 1), and beach seine and fyke netting sites were chosen for gradually sloping intertidal zones with mudflat, cobble, eelgrass, and rockweed habitats. Beach seine nets were 30.48 × 1.83 m with 0.64-cm diamond mesh.

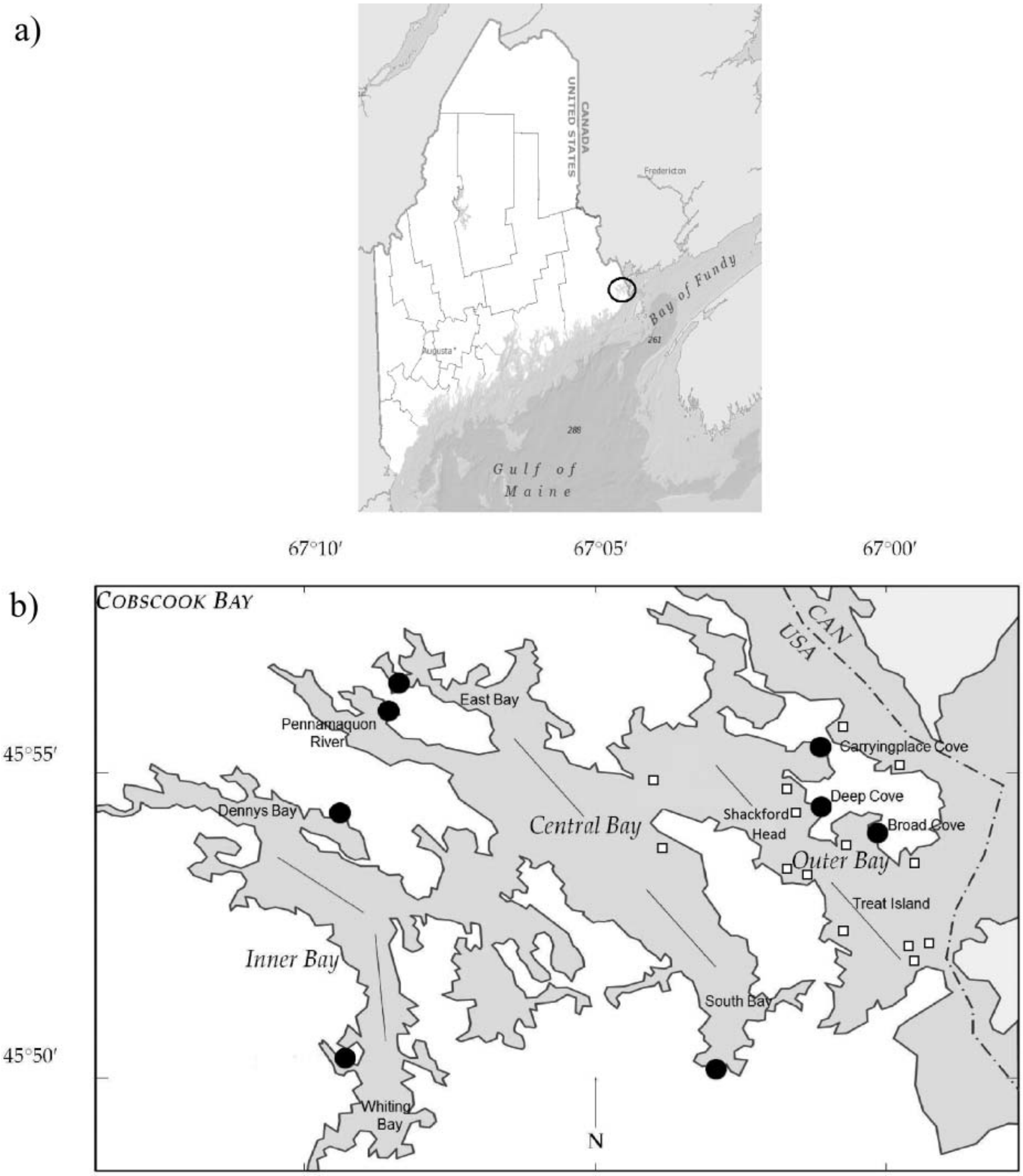

FIGURE 1. **(a)** Location (obtained on November 20, 2014, from the Maine Office of GIS, State of Maine: http://maine.maps.arcgis.com/home/webmap/viewer.html?useExisting = 1) and **(b)** detailed map of Cobscook Bay, Maine (modified from Vieser 2014). The location of Cobscook Bay is indicated by the black ellipse in (a). In (b), seine sites are indicated by black circles, approximate trawl locations are marked by black lines, and approximate locations of Atlantic Salmon aquaculture pens (Maine Department of Marine Resources 2012) are marked by open squares.

The fyke nets had 9.14-m wings, 1.22-m square hoops leading to the cod end, and 3.81-cm stretch mesh, and fished the beginning half of one tide with each sample. The pelagic trawl net had 12.19-m headrope, footrope, and breast lines, while the benthic trawl net had a 13.72-m headrope, 10.67-m footrope, and no breast lines. Both trawl nets were towed for approximately 20 min with each sample.

From each tow for all gear types, subsamples of up to 30 individuals of each fish species caught were euthanized with an overdose of 250 mg/L MS-222 (tricaine methanesulfonate;

TABLE 1. Beach seine, fyke, benthic trawl, and pelagic trawl net sampling effort in Cobscook Bay, Maine, in 2012. Effort indicates the approximate number of net tows or sets per month, which varied slightly by month due to differences in sampling schedules and fish processing time.

Site name	Sub-bay	Beach seine	Fyke net	Benthic trawl	Pelagic trawl
Deep Cove	Outer	3			
Broad Cove	Outer	3			
Carryingplace Cove	Outer	7	2		
Shackford Head	Outer			4	
Treat Island	Outer				4
East Bay	Central	13	2	2	2
Pennamaquon River	Central	5			
South Bay	Central	3[a]		2	2
Whiting Bay	Inner	4[a]		1	1
Dennys Bay	Inner	11	2	1	1

[a]Sites not sampled in March, April, and November due to difficulty of access.

Argent Chemical Laboratories), measured for length (TL) and mass, and examined for sea lice. These examinations relied upon the naked eye and consisted of a thorough scan of the body surface and fins. All fish with sea lice infestations were recorded as infested for the prevalence analysis, separated by species and tow, and stored in a 95% (v/v) solution of ethanol for later detailed examination under a dissecting microscope. Under magnifications between 10× and 15×, the left, right, dorsal, and ventral body surfaces of the stored fish were carefully examined as well as all fins. Approximately 10% of the fish in each sample were checked for gill infestations by removing and carefully examining the gill structures under similar magnifications. For each observed sea louse, the life stage, position on the fish, and genus were recorded. Life stage identification was limited to the following stages: copepodid, chalimus, and motile (preadult or adult). Similar to the classification system used by Bjørn and Finstad (1998), location was characterized as one of the following generalized positions on the fish: pectoral fins, pelvic fins and spines, anal fin, dorsal fin, caudal fin, caudal peduncle, dorsal surface, ventral surface, right side, left side, and head surface. Sea lice identification was restricted to genus (e.g., *Caligus, Lepeophtheirus*, undetermined) using morphological features. After detailed observations, each sea louse was removed from the fish and stored in 95% (v/v) ethanol at 4°C for species identification.

Sea lice species identification.—We used DNA sequencing to identify sea lice to the species level and to verify visual identifications. Genomic DNA (gDNA) was extracted from all sea lice visually identified as either *Lepeophtheirus* sp. or undetermined as well as a subsample of up to 10 individuals per sample site and month visually identified as *Caligus* sp. (resulting in proportional subsamples ranging from 15.6% to 100%), with a DNeasy Blood and Tissue Kit (Qiagen) following the manufacturer's protocol optimized for insects. Sea lice gDNA was then analyzed with a NanoDrop 1000c spectrophotometer (Thermo Scientific) to determine DNA concentration.

We employed PCR to amplify the mitochondrial cytochrome c oxidase I (COI) gene sequence using the universal primers LCO1490 and HCO2198, which target the COI gene of both *L. salmonis* and *C. elongatus* (Folmer et al. 1994). The PCR was performed in a 50 μL master mix using the GoTaq Flexi DNA Polymerase (Promega) following the manufacturer's instructions. Briefly, 400 ng gDNA template extracted from individual sea lice was mixed with 0.5 μM LCO1490 primer, 0.5 μM HCO2198 primer, and 1× PCR mix, and made up to 50-μL total volume with nuclease-free water (IDT). The PCR was performed on a Biometra thermocycler with an initial denaturation of 95°C for 2 min, followed by 40 cycles of 95°C for 1 min, 44°C for 1 min, and 72°C for 1 min, and a single final elongation cycle step of 72°C for 5 min.

The PCR products were then purified using the QIAquick PCR purification kit (Qiagen) following manufacturer's instructions into nuclease-free water. The purified PCR products were subsequently direct sequenced at the University of Maine DNA Sequencing Facility using the HCO2198 primer. Sequences were edited using the software Geneious 4.7.6. A blast search was carried out on all COI sequences using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI BLASTn) to determine to which species-specific COI sequences they were most closely homologous.

Data analysis.—Infestation intensity was calculated as the mean number of sea lice per fish among infested fish only, and infestation prevalence was calculated as the proportion of fish with sea lice among all examined fish. Intensity sample sizes did not always match those calculated from prevalence values and sample sizes because (1) some fish samples were lost prior to examination by dissecting microscope, and (2) fish caught in Deep and Broad coves in June were not examined for prevalence in the field due to time constraints. Because infestations of several fish species were only observed in June, only fish collected from this month were used in the infestation intensity and prevalence analyses, in order to eliminate the influence of infestation pressure variability among months. We also standardized intensity values by the average length among all fish sampled for each considered species in June. In the absence of established length or weight-to-surface area relationships for the examined species, average length roughly accounted for the effect of fish size on observed infestation intensity. Increasing host size, independent of fish species, is expected to result in increased infestation intensities due to increased surface area for parasite attachment (Dogiel et al. 1958). Differences in both unstandardized and standardized infestation intensity among fish species were examined using the Kruskal–Wallis test and Dunn's pairwise test due to a lack of normality among the residuals generated from ANOVA (Shapiro–Wilk test of normality: $w = 0.583, 0.589$; df = 142, 142; $P < 0.001$, 0.001, respectively). Differences in prevalence among species were examined using Pearson's chi-square test.

RESULTS

Among all gears and months, 6,334 individual fish of 35 different species were caught and examined for sea lice in the field. Sample size and infestation information are summarized for the more common species ($n \geq 10$) as well as for all species with observed sea lice infestations (Table 2). A total of 13 sampled but rare species were excluded from Table 2. Threespine Sticklebacks were the most abundant captured species ($n = 1,996$), followed by Blackspotted Sticklebacks ($n = 882$), Winter Flounder ($n = 690$), and Mummichogs ($n = 587$). A total of 253 infested host fish were examined under a

TABLE 2. Summarized list of fish species examined in Cobscook Bay, Maine, in 2012. Sample size and infestation information are provided for the more common species ($n \geq 10$) as well as all species with observed sea lice infestations. For fishing gear, letters represent the gear that successfully sampled each species (S = beach seine, F = fyke, B = benthic trawl, and P = pelagic trawl net). Terms in bold italics indicate the presence of *C. elongatus* infestations among fish sampled in a given month or by a given gear type.

Species	Prevalence sample size	Prevalence (%)	Intensity sample size	Median intensity	Months sampled	Fishing gear
Threespine Stickleback *Gasterosteus aculeatus*	1,996	12.26	204	2	***3***, ***4***, ***5***, ***6***, ***8***, ***9***, ***11***	***S***, F, P
Blackspotted Stickleback *Gasterosteus wheatlandi*	882	1.98	32	1	***4***, ***5***, ***6***, ***8***, ***9***, ***11***	***S***
Winter Flounder *Pseudopleuronectes americanus*	690	2.07	9	2	5, ***6***, 8, 9	F, ***B***
Mummichog *Fundulus heteroclitus*	587	0.17	1	1	5, ***6***, 8, 9, 11	***S***
Atlantic Herring *Clupea harengus*	553	0			5, 6, 9	S, B, P
Atlantic Silverside *Menidia menidia*	382	0			4, 5, 6, 8, 9, 11	S
Alewife *Alosa pseudoharengus*	271	0			6, 8, 9, 11	S, F, B, P
Longhorn Sculpin *Myoxocephalus octodecemspinosus*	177	1.72	2	1	***5***, ***6***, 8, 9	***B***
Silver Hake *Merluccius bilinearis*	173	0			5, 6, 8, 9	B, P
Rainbow Smelt *Osmerus mordax*	140	1.45	1	1	5, ***6***, 8, 9	S, F, B, ***P***
Grubby *Myoxocephalus aenaeus*	114	0			5, 6, 8, 9	B
Atlantic Tomcod *Microgadus tomcod*	76	5.56	1	1	5, ***6***, 8, 9	S, ***F***
Butterfish *Peprilus triacanthus*	65	0			6, 8, 9	B, P
White Hake *Urophycis tenuis*	54	0			6, 8, 9	B
Fourspine Stickleback *Apeltes quadracus*	37	0			8, 9	S
Red Hake *Urophycis chuss*	31	3.33	1	5	5, ***6***, 8, 9	***S***, B
Snakeblenny *Lumpenus lampretaeformis*	21	0			5,6	B
Sea Raven *Hemitripterus americanus*	15	0			5,6,8	B
Ninespine Stickleback *Pungitius pungitius*	14	7.69	1	1	***8***,9	***S***
Atlantic Cod *Gadus morhua*	11	0			5,6	S, F, P
Atlantic Halibut *Hippoglossus hippoglossus*	10	0			5,6,8,9	B
Lumpfish *Cyclopterus lumpus*	1	100	1	22	***6***	***B***

dissecting microscope to determine sea lice species, infestation intensity, sea lice life stages, and chalimus attachment locations.

All sea lice ($n = 508$) observed microscopically were identified as *Caligus* sp. or unknown based on morphological features. Sea lice were identified as unknown if they experienced severe degradation or were in the copepodid stage, where it is extremely difficult to differentiate to the genus level. No individuals were identified as *Lepeophtheirus* sp. For all 175 successfully sequenced sea lice samples, the COI sequences most closely matched the COI sequence of *C. elongatus* genotype 1, with >90% homology in all cases. All examined sea lice were therefore considered *C. elongatus*.

The following 10 fish species were identified as hosts for *C. elongatus*: Threespine Sticklebacks, Blackspotted Sticklebacks, Winter Flounder, Longhorn Sculpin, Lumpfish, Mummichogs, Red Hake, Atlantic Tomcod, Rainbow Smelt, and Ninespine Sticklebacks (Table 2). Of these species, only Lumpfish and Atlantic Tomcod have been previously recorded as hosts of *C. elongatus* (Kabata 1979; Margolis and Arthur 1979). The solitary captured Lumpfish had an infestation intensity of 22 *C. elongatus* per fish. Among species with more than one infested individual, however, Threespine Sticklebacks had the greatest infestation prevalence and median unstandardized intensity at 12.26% and two sea lice per fish, respectively. The overall infestation prevalence and unstandardized intensity, among all sampled fish, were 4.60% and one sea louse per fish, respectively.

Of the 508 *C. elongatus* individuals, 2 were copepodids (0.39%), 487 were in the chalimus stages (95.87%), 15 were adults (2.95%), and 4 individuals (0.79%) were unidentifiable due to distorted or degraded morphology. Because of the relatively high number of unattached adults ($n = 12$), likely due to detachment during fish preservation, and low sample sizes of the remaining stages, we characterized the attachment locations of the chalimus stages only. Twenty of the 487 examined chalimus *C. elongatus* were also unattached from their host fish and subsequently excluded from the location analysis. Of the remaining 467 chalimus individuals, the vast majority (83.30%) were attached to the fins of their wild hosts (Figure 2), and over half were found specifically on caudal and pectoral fins (26.77% and 23.34%, respectively). No *C. elongatus* were observed on the gills. These trends in chalimus attachment locations among all wild fish hosts were primarily driven by chalimus locations on Threespine Sticklebacks, which contributed 380 of the 467 attached chalimus. On Threespine Sticklebacks specifically, 85.53% of chalimus were attached to the fins, with 26.84% and 22.63% attached to the caudal and pectoral fins, respectively (Figure 2). The lone Lumpfish was the notable exception to this attachment location tendency among the chalimus stages; 16 of 22 observed nonmotile stages were found on the body surface.

The unstandardized *C. elongatus* infestation intensity differed significantly between Threespine Sticklebacks, Blackspotted Sticklebacks, and Winter Flounder, the three species with more than two infested individuals, collected in June (Kruskal–Wallis test: $\lambda^2 = 9.4522$, df $= 2$, $P = 0.0089$). The only significant pairwise difference was between Blackspotted Sticklebacks and Threespine Sticklebacks (Dunn's test: $z = -2.959$, df $= 140$, $P = 0.0015$; and $z = -0.929$, 1.113; df $= 140$, 140; $P = 0.1765$, 01328, for comparisons between Blackspotted Sticklebacks and Winter Flounder and between Winter Flounder and Threespine Sticklebacks, respectively). Threespine Sticklebacks and Winter Flounder had the highest median intensity at two *C. elongatus* per fish, followed by Blackspotted Sticklebacks at one *C. elongatus* per fish. When infestation intensities were standardized by the average fish length for each species in June, Threespine Sticklebacks still had the highest median intensity of 0.0308 *C. elongatus* $\times$ mm^{-1} $\times$ fish^{-1}, followed this time by Blackspotted Sticklebacks then Winter Flounder, with median intensities of 0.02294 and 0.0161 *C. elongatus* $\times$ mm^{-1} $\times$ fish^{-1}, respectively. Again, there was a significant difference in intensity among the three species (Kruskal–Wallis test: $\lambda^2 = 9.7776$, df $= 2$, $P = 0.0075$), and there were significant pairwise differences between Blackspotted Sticklebacks and both of the remaining species (Dunn's test: $z = -3.122$, 2.329; df $= 140$, 140; $P < 0.001$, $P = 0.010$ for Threespine Sticklebacks and Winter Flounder, respectively; $z = -0.463$, df $= 140$, $P = 0.3218$ for the comparison between Threespine Sticklebacks and Winter Flounder).

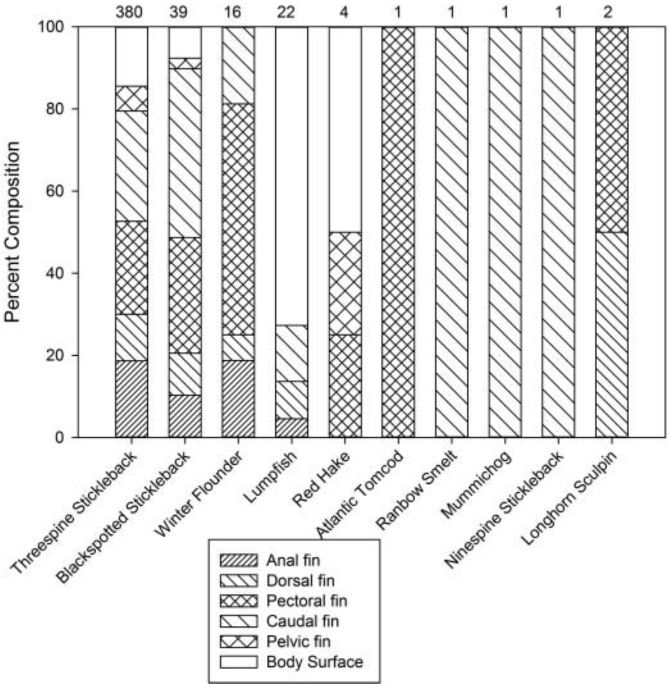

FIGURE 2. Stacked bar graph of *C. elongatus* chalimus stages' attachment locations on their hosts. Numbers across the top indicate the total number of sea lice observed on the associated hosts; any chalimus stages attached to the stickleback pelvic spines were included in the pelvic fin category.

TABLE 3. Infestation prevalence and unstandardized intensity across months and sub-bays for Threespine Sticklebacks, with sample sizes indicated in parentheses.

Sub-bay	Mar	Apr	May	Jun	Aug	Sep	Nov
Infestation prevalence (%)							
Inner	(0)	(0)	14.7 (217)	26.1 (184)	1.6 (183)	3.1 (192)	11.1 (9)
Central	0 (1)	0 (1)	6.5 (93)	25.3 (87)	5.5 (291)	8.2 (134)	(0)
Outer	25 (4)	12.5 (8)	2.8 (36)	30.1 (123)	7.3 (192)	7.7 (196)	8.9 (45)
Median infestation intensity (number of sea lice per fish)							
Inner	(0)	(0)	1 (15)	2 (49)	1 (3)	1 (6)	1 (1)
Central	(0)	(0)	1 (7)	2 (13)	1 (11)	1 (11)	(0)
Outer	1 (1)	1 (1)	1.5 (2)	2 (55)	1 (10)	1 (15)	1.5 (4)

There were significant differences in *C. elongatus* infestation prevalence between Threespine Sticklebacks, Blackspotted Sticklebacks, and Winter Flounder collected in June (Pearson's chi-square test: $\lambda^2 = 14.0619$, df = 2, $P < 0.001$). Threespine Sticklebacks had the highest prevalence at 27.16%, followed by Blackspotted Sticklebacks and Winter Flounder at 13.16% and 5.22%, respectively.

Because Threespine Sticklebacks were the most abundant species and showed significantly higher infestation intensity and prevalence than other common hosts, this species was selected for an exploratory assessment of any temporal or spatial trends in infestation pressure. Threespine Sticklebacks exhibited higher infestation prevalence and intensity values in June than in other months. Spatially, there were no clear differences in infestation pressure among sub-bays (Table 3).

DISCUSSION

The observed presence of exclusively *C. elongatus* among Cobscook Bay's wild fish assemblage allows a unique examination of this species' host range, as this species is often overlooked in favor of *L. salmonis*. In 2012, *C. elongatus* parasitized 10 fish species, including eight newly reported species, among a total of 35 examined species, fulfilling its reputation as a pervasive parasite among wild fish. Five of these species—specifically Blackspotted Sticklebacks, Winter Flounder, Red Hake, Longhorn Sculpin, and Mummichogs—are found only in the Northwest Atlantic Ocean, where previous large-scale sea lice studies on wild fish assemblages are conspicuously absent. The remaining three species—Threespine Sticklebacks, Ninespine Sticklebacks, and Rainbow Smelt— are found on both sides of the North Atlantic Ocean (Froese and Pauly 2014). Heuch et al. (2007) successfully collected only Threespine Sticklebacks among these three species in the North Sea but did not observe *C. elongatus* infestations on any of the 20 examined individuals.

Among the sampled wild fish in Cobscook Bay, Threespine Sticklebacks were clearly the dominant host for *C. elongatus*. They were successfully sampled and observed to carry *C. elongatus* infestations during all seven sample months and throughout all three sub-bays (Table 3), and possessed the highest infestation intensity and prevalence among fish with multiple cases of sea lice infestations. Although sea lice retention on fish can vary by differing fishing gear (Nagasawa 1985), the observed host importance of Threespine Sticklebacks was unlikely to have been driven by any such differences in retention. Krkošek et al. (2007) observed no difference in sea lice abundance on fish sampled simultaneously using trawls and dip nets. We have no relevant data to inform sea lice retention among our gears, as the vast majority of any given species was sampled using a single gear type and only one gear type for any given species captured infested individuals used in the intensity analyses (Table 2). Regardless, these results for Threespine Sticklebacks from the Northwest Atlantic Ocean correlate closely with the findings of a study conducted in the Northeast Pacific Ocean off of British Columbia. The study found that Threespine Sticklebacks may be used as sentinel species for *Caligus* spp. and *L. salmonis* infestations due to their observed high infestation prevalence (Jones et al. 2006). The high infestation pressure of *C. elongatus* on Threespine Sticklebacks, relative to the other sampled fish, in Cobscook Bay suggest that it may also serve as a host for *C. elongatus* in the Northeast Atlantic Ocean, where it has not been identified previously as a host.

The ubiquitous presence of Threespine Sticklebacks as a host for *C. elongatus* also allows for a preliminary assessment of spatial and temporal trends in infestation pressure in Cobscook Bay. Although no differences were observed among sub-bays, both infestation intensity and prevalence on Threespine Sticklebacks indicated a spike in infestation pressure in June (Table 3). A similar trend was observed when looking at all species of wild fish caught. Twenty-eight species of fish were sampled in June, compared with 18, 22, and 23 in May, August, and September, respectively. This higher fish diversity was associated with greater host diversity in June, as 10 species hosted *C. elongatus* in that month, compared with just three, four, and two for May, August, and September, respectively. These results contrast with the findings of Hogans and Trudeau (1989b), who observed peak infestation levels of *C. elongatus* on farmed Atlantic Salmon in the Bay of Fundy in late summer and fall, during a time of peak temperature. An ongoing sentinel cage study in Cobscook Bay with Atlantic Salmon also observed high levels of infestation by *L. salmonis* in late summer and fall during peak water temperatures (I. R. Bricknell, University of Maine, personal communication). Additional sampling is required to resolve the uncertainty regarding the temporal trends in infestation pressure and to determine if the observed spike in infestation pressure is more representative of a normal seasonal dynamic for *C. elongatus* on wild fish in this area or an anomaly.

If the June peak in infestation pressure represents an anomaly, the observed spike might be explained by a stochastic population boom for *C. elongatus* or the appearance of a new external source of *C. elongatus* to Cobscook Bay via transport on wild fish or dispersal with water currents. There are several factors to suggest that Lumpfish specifically could serve as a key vector for *C. elongatus* transmission: (1) their observed exclusive presence in samples during June, (2) their propensity to host *C. elongatus* (Heuch et al. 2007), and (3) their tendency to spawn in shallower sublittoral waters in the spring and early summer (Collins 1976). *Caligus elongatus* planktonic and motile stages could also disperse into Cobscook Bay from Passamaquoddy Bay or the Bay of Fundy via the connecting Western Passage and Head Harbor Passage. There are at least 19 marine finfish aquaculture lease sites in Passamaquoddy Bay, in addition to numerous others in the Western Passage connecting Cobscook and Passamaquoddy bays (Agriculture, Aquaculture, and Fisheries 2015). While many of these sites were fallow or inactive during the study period, aquaculture farms could serve as way stations for dispersing *C. elongatus* populations within the region. We were unable to obtain information on sea lice infestation levels on aquaculture farms in the study area, but infestation by *L. salmonis*, and to a lesser extent *C. elongatus*, is known to occur frequently (D. Bouchard, University of Maine, personal communication).

While Threespine Sticklebacks were the major host identified in this study, there are several indications they may not be the ideal host for *C. elongatus* in Cobscook Bay. The vast majority of observed sea lice on these wild fish were in the chalimus stages, not the reproductive adult stage. While it is possible that our netting techniques dislodged the mobile adults and skewed the observed sea lice count towards the chalimus stages (Jones et al. 2006), the fact that over 95% of all observed *C. elongatus* were in the chalimus stages suggests that observed hosts in Cobscook Bay may be transitional, or opportunistic, rather than preferred. The overall infestation pressure of *C. elongatus* among the Cobscook Bay fish assemblage was also low in comparison with other studies. Heuch et al. (2007) observed an overall *C. elongatus* prevalence of 15% among all sampled wild fishes off the coast of Norway, a value noticeably higher than the 4.60% assemblage-wide prevalence from this study. Our study's overall median unstandardized intensity was also low, at just one louse per fish.

The low overall *C. elongatus* infestation pressure may be due to low numbers of preferred hosts. Several previously recognized *C. elongatus* hosts were collected at only very small sample sizes in Cobscook Bay. Specifically, Pollack *Pollachius virens*, Atlantic Cod, and Atlantic Mackerel *Scomber scombrus* were collected and examined at sample sizes of just 5, 11, and 6, respectively. Heuch et al. (2007) found that these three species were infested by *C. elongatus* at prevalence levels between 4.4% and 41% in the North Sea. Conversely, the *C. elongatus* population sampled in Cobscook Bay may simply possess different infestation dynamics and host preferences than populations present in the Northeast Atlantic Ocean. For example, Heuch et al. (2007) reported an infestation prevalence of 20.1% for Atlantic Herring in the North Sea. In our study, 553 Atlantic Herring were examined for sea lice in the field, and none were infested by *C. elongatus*.

Our finding that over half of the nonmotile *C. elongatus* were attached to the pectoral and caudal fins of their hosts complements past research and will facilitate future surveys of wild fish sea lice infestations. The results correspond well with Treasurer and Wadsworth's (2004) report that the caudal and pectoral fins were the most important attachment points for *C. elongatus* chalimus stages on farmed Atlantic Salmon. This similarity suggests that the distribution of chalimus stages is not significantly different between farmed and wild fish hosts. Fins may be the primary attachment point for the nonmotile *C. elongatus* stages due to more suitable epidermis composition or increased protection from water currents (Dawson et al. 1997; Genna et al. 2005). These findings can expedite examination protocols in future wild fish surveys.

The results of 2012 sampling are also notable for the exclusive presence of a single sea lice species in Cobscook Bay, which represents a rare occurrence among the few surveys of wild fishes. Heuch et al. (2007) and Hayward et al. (2011) each observed at least two *Caligus* spp. in the North Sea and off the coast of South Australia, respectively. Similarly, Urquhart et al. (2010) identified both *L. salmonis* and *C. elongatus* on wild Sea Trout collected off the coast of Scotland. All sequenced sea lice samples from this study, which was the first to genotype *C. elongatus* from the Western Atlantic Ocean, were also identified specifically as genotype 1, the genotype found to be more dominant on Atlantic Salmon farms in Norway and Scotland (Øines et al. 2006). The absence of *L. salmonis* infestations could be due to a low abundance of preferred wild hosts, specifically salmonids, in the region. Of the 10 observed *C. elongatus* host species in Cobscook Bay, only Threespine Sticklebacks have been previously reported as hosts for *L. salmonis* (Jones and Prosperi-Porta 2011). However, this species is likely not an ideal host for all *L. salmonis* life stages. When Threespine Sticklebacks were exposed to *L. salmonis* adult females in a laboratory setting, no females survived due to active predation from the fish (Losos et al. 2010; Pert et al. 2012). Additionally, all three of the *Lepeophtheirus* spp. specimens observed on Threespine Sticklebacks by Eaves et al. (2014) were in the nonreproductive chalimus stages. No salmonids, the preferred host species for *L. salmonis*, were captured during sampling in 2012, and the U.S. Atlantic Salmon Assessment Committee (2013) caught zero returning Atlantic Salmon in their weir trap on the Dennys River, the primary Atlantic Salmon running river in Cobscook Bay, in 2012.

This infestation uniformity of sea lice species in Cobscook Bay is somewhat surprising because the bay is home to several Atlantic Salmon aquaculture pens. As of July 2012, there were 14 finfish aquaculture lease sites in the Cobscook Bay region; however, the vast majority of these sites were not under production at the time of this study (Maine Department of Marine Resources 2012). The lack of observed *L. salmonis* infestations on wild fish in this study is possibly explained by the baywide fallow period of at least 30 d that occurred in the spring of 2012. By removing all cultured Atlantic Salmon from the bay, the fallowing may have disturbed *L. salmonis* dynamics associated with the pens, with the cultured fish subsequently having minimal influence on infestations among the wild fish assemblage. Conversely, the absence of *L. salmonis* on wild fish provides tentative support for the effectiveness of fallowing as a control tactic for this sea lice species, as no wild fish were observed to act as potential reservoirs throughout sampling in 2012. Finally, although Hogans (1995) noted that *L. salmonis* quickly became the bigger nuisance species soon after the establishment of Atlantic Salmon aquaculture, salmonid aquaculture in the Northwest Atlantic Ocean has been observed to exhibit vulnerability to *C. elongatus* infestations (Hogans and Trudeau 1989a). Furthermore, Bricknell et al. (2006) suggested that *C. elongatus* could pose a major potential threat to gadoid aquaculture due to its wide host range and direct life cycle. The increased understanding of the host range and use of wild host assemblages by *C. elongatus* and *L. salmonis* in the Northwest Atlantic Ocean therefore has the potential to improve sea lice management efforts in this region by improving the understanding of interactions among parasites, farmed fish, and wild fish.

This study successfully characterized the presence and distribution of sea lice on wild fish in Cobscook Bay, Maine. Both the observed lack of *L. salmonis* infestations and host range expansion for *C. elongatus* have the potential to inform aquaculture management and the improved understanding of *C. elongatus*' distribution among wild fish hosts establishes a baseline for the distribution of sea lice among a wild fish assemblage in the Northwest Atlantic Ocean. Further sampling is needed to expand the data set and to allow for more detailed temporal and spatial analyses of sea lice dynamics in the Cobscook Bay system.

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