Assessment of Sea Lice Infestations on Wild Fishes of Cobscook Bay

Alexander Jensen

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ASSESSMENT OF SEA LICE INFESTATIONS
ON WILD FISHES OF COBSCOOK BAY

by

Alexander Jensen

A Thesis Submitted in Partial Fulfillment
of the Requirements for a Degree with Honors
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Advisory Committee:
Gayle B. Zydlewski, Ph.D, Research Associate Professor in Marine Science
Sarah Barker, Ph.D, Research Associate at the Aquaculture Research Institute
Mimi Killinger, Ph. D., Rezendes Preceptor for the Arts
Paul Rawson, Ph. D., Associate Professor in Marine Science
Jeffrey Vieser, Graduate Student in Marine Science
Abstract

Sea lice are ectoparasitic copepods on fishes and can negatively impact aquaculture operations. Little work on sea lice, specifically *Lepeophtheirus salmonis* and *Caligus elongatus*, has occurred in the northwest Atlantic. This project characterized sea lice infestations on wild fishes in Cobscook Bay during 2012. Trawling, seine netting, and fyke netting occurred from March to November. Netting sites were selected to sample the bay’s three regions: Outer, Central, and Inner Bay. Visual examinations of fish were used to identify wild hosts and characterize sea lice life stage abundances, attachment locations, and infection prevalence and intensity. DNA sequencing was used to identify sea lice species. *Caligus elongatus* was the only identified sea lice species, and was found on 12 fish species. Threespine sticklebacks (*Gasterosteus aculeatus*), blackspotted sticklebacks (*Gasterosteus wheatlandi*), and winter flounder (*Pseudopleuronectes americanus*) were prominent hosts with the most infestations (n = 204, n = 32, n = 9). Over 95% of sea lice were in the non-motile chalimus stages, which were predominantly attached to the fish fins. Infection intensity and prevalence on threespine sticklebacks varied significantly between months, reaching maximal values during June. Infection prevalence on threespine and blackspotted sticklebacks differed spatially, with lower levels in Inner Bay than in Central and Outer Bay. Infection prevalence and intensity differed among threespine sticklebacks (12.26%), blackspotted sticklebacks (1.98%), and winter flounder (2.07%), indicating differences in host suitability and importance. These results establish a baseline for sea lice dynamics in Cobscook Bay and inform future sea lice surveys.
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**Introduction**

Sea lice, parasitic copepods on marine and freshwater fishes, are found in wild fish communities throughout the world, and can pose a significant problem for fish aquaculture operations by reaching high abundances and damaging fish. An abundance of research has focused on the potential for sea lice transfer from farmed to wild fish and the harm that inflated sea lice levels may cause to wild fish populations, especially to already threatened salmonid species (Frazer, 2008; Marty et al., 2010; Krkošek et al., 2012). The opposite case, in which wild fish naturally harbor and transfer sea lice to the farming operations, however, should receive equal consideration in order to gain a more complete understanding of sea lice transfer between farmed and wild fish. A fundamental understanding of the distribution of sea lice among wild fish communities is necessary to objectively assess any sea lice transfers, either to or from wild fishes. Furthermore, research on sea lice distribution within an entire fish community will better characterize the influence these parasites may have on wild fish communities.

*Sea Lice Biology*

Sea lice belong to the family Caligidae, a taxonomic group of only parasitic copepods. Sea lice are ectoparasitic, meaning that they attach and feed on the external surface of their hosts. They attach to the skin and fins of fish, and feed on the hosts’ mucus, skin, and tissue with rasping, piston-like mouthparts (Kabata, 1979; Costello, 2006).

The sea lice life cycle is split into several distinct stages (Fig. 1). First, two stages of nauplii, the non-infectious, planktonic larval stages, develop from fertilized eggs
produced by adult females and reside in the water column for anywhere from 5 to 15 days (Costello, 2006). Sea lice then molt from nauplii to an infectious, planktonic copepodid stage (Boxaspen, 2006). The copepodids find a host, settle on its surface, and attach themselves using their second antennae (Treasurer and Wadsworth, 2004; Bailey et al., 2006). They remain attached to the host and develop into non-motile chalimus stages (I through IV), secured to the host via a frontal filament. After the chalimus stages, some species of sea lice go through two motile pre-adult stages. At this stage, the sea lice can freely move around the surface of the host or detach and find a new host (Boxaspen, 2006). Finally, they reach the reproductive adult stage that all species of sea lice share (Boxaspen, 2006). The total generation time for sea lice, specifically the species *Lepeophtheirus salmonis* (Krøyer, 1837), and *Caligus elongatus* (Nordmann, 1832), is between approximately 40 and 50 days at 10°C, and varies with temperature and host suitability (Costello, 2006).

The two dominant species of sea lice in the Gulf of Maine, *L. salmonis* and *C. elongatus*, differ in geographic distribution, size, feeding style, life cycle, body structure, and host specificity. *L. salmonis* has a circumpolar distribution within temperate to sub-arctic latitudes in the northern hemisphere, while *C. elongatus* is restricted to warmer, more temperate latitudes in both hemispheres (Boxaspen, 2006). *L. salmonis* reaches a larger size and exhibits more aggressive feeding than *C. elongatus* (Fig. 2; Westcott et al., 2004). *L. salmonis* possesses two pre-adult stages preceding the adult stage while *C. elongatus* lacks the pre-adult stages entirely (Boxaspen, 2006). *C. elongatus* adults also possess lunules, cups on the anterior body used for adhesion, which *L. salmonis* adults lack (Kabata, 1979). *L. salmonis* has narrow host specificity, predominantly settling on
salmonid species, compared to *C. elongatus*, which is parasitic to over 80 species of fishes, including fishes from the families Actinopterygii, Clupeidea Gadidae, Gasterosteiformes, Pleuronectidae, and Salmonidae (Boxaspen, 2006). Finally, *C. elongatus* populations possess two distinct genotypes, genotype 1 and genotype 2, which have been demonstrated to vary in host preference (Øines et al., 2006).

Together, *L. salmonis* and *C. elongatus* parasitize a diversity of fish species. *L. salmonis*, in addition to parasitizing all species of Pacific salmon and all species of the genus *Salmo* in the Atlantic Ocean (Tully and Nolan, 2002), has also been found on threespine sticklebacks (*Gasterosteus aculeatus*; Jones et al., 2006), Atlantic pollock (*Pollachius virens*; Bruno and Stone, 1990), sand lance (*Ammodytes hexapterus*; Jones et al., 2006), white sturgeon (*Acipenser transmontanus*; Jones et al., 2006), and sea bass (*Dicentrarchus labrax* L.; Pert et al., 2012). Pert et al. (2012) further demonstrated that Atlantic cod (*Gadus morhua*), though not confirmed as a host for *L. salmonis*, could serve as a suitable secondary host for the adult stage. *C. elongatus* is known to parasitize a greater diversity of species, including Atlantic salmon (*Salmo salar*), Atlantic pollock, pollack (*Pollachius pollachius*), Atlantic herring (*Clupea harengus*), haddock (*Melanogrammus aeglefinus*), European flounder (*Platichthys flesus*), Atlantic mackerel (*Scomber scombrus*), lumpfish (*Cyclopterus lumpus*), sea trout (*Salmo trutta*), and Atlantic cod (Kabata, 1979).

Not all observed sea lice hosts, particularly those for *L. salmonis*, appear to be suitable for all sea lice life stages. Most observations of *L. salmonis* on non-salmonids consist of the non-motile chalimus stages (Costello, 2006). For example, less than 1% of *L. salmonis* found on threespine sticklebacks collected off British Columbia were adult
stages (Jones et al., 2006; Jones and Prosperi-Porta, 2011). Experimental studies have successfully demonstrated that threespine sticklebacks are suitable hosts for the chalimus stages of *L. salmonis* (Jones et al., 2006). When experimentally exposed to pre-adults and adults, however, the sticklebacks were observed actively predating upon the sea lice and were not infested by them (Pert et al., 2012). These findings reveal stage-specific differences in the suitability of a wild fish species as a host for *L. salmonis*.

The attachment and feeding sites of chalimus, pre-adult, and adult sea lice life stages on fish seem to follow a consistent pattern. The chalimus stages of both *C. elongatus* and *L. salmonis* are predominantly found on the dorsal, caudal, and pectoral fins of their hosts (Bjorn and Finstad, 1998; Treasurer and Wadsworth, 2004). These locations reflect the attachment point of the copepodids (Genna et al., 2005). Fins may be the primary attachment point for copepodids due to a more suitable epidermis composition or increased protection from water currents (Dawson et al., 1997; Genna et al., 2005). Experimental trials have also demonstrated settlement and attachment of *L. salmonis* on the gills (Genna et al., 2005). Unlike chalimus stages, pre-adult and adult stages of *L. salmonis* and *C. elongatus* are typically concentrated on the dorsal surface, ventral surface, and head region (Dawson et al., 1997; Treasurer and Wadsworth, 2004).

Sea lice may play an important role in wild fish communities by altering ecosystem energy flow, trophic interactions, and competition. This influence is often excluded or underestimated from food webs due to the complexity they introduce and the tendency to study more macroscopic species and processes (Lafferty et al., 2008). Parasites in general can redirect energy flow through an ecosystem by altering competitive interactions (Hudson et al., 2006), diverting energy from hosts through
feeding, and causing direct mortality of their hosts (Lafferty et al., 2008). Sea lice, as macroparasites that cause an intensity-dependent effect on their hosts, potentially play a similar role in energy flow redirection within marine ecosystems (Lafferty et al., 2008).

**Sea Lice and Aquaculture**

Numerous studies on wild fish hosts of sea lice have been conducted in the northeast Pacific, southwest Pacific, and northeast Atlantic, but little is known for the northwest Atlantic. Hayward et al. (2011) examined wild fishes near southern bluefin tuna aquaculture pens off the coast of South Australia for sea lice load, Heuch et al. (2007) surveyed the infestations of coastal and oceanic fishes caught in the North Sea, and Jones and Prosperi-Porta (2011) investigated sea lice infestations of threespine sticklebacks in coastal British Columbia. These studies were largely conducted to examine the potential for sea lice transfer from wild to farmed fish. Apart from known sea lice hosts in the northeast Atlantic and Pacific oceans, little is known about infestations in the northwest Atlantic. In one of the few studies examining sea lice infestations of wild fishes, adult Atlantic salmon in the Penobscot River estuary in Maine were found to host *L. salmonis* (Powell et al., 1999). This study was only conducted to identify the harmful implications of sea lice on returning salmon, so no other fish species were examined for sea lice (Powell et al., 1999).

Atlantic salmon aquaculture is a major industry in Maine, especially in estuaries such as Cobscook Bay. Commercial salmon aquaculture operations worldwide experience an estimated annual loss due to sea lice of approximately $480 million (Costello, 2009). Before this problem can be fully addressed, a greater understanding of
sea lice dynamics is needed. In the northwest Atlantic, further research on the wild hosts of sea lice will aid in identifying potential reservoir hosts and increase the global understanding of interactions among sea lice, farmed fish, and wild fish.

*L. salmonis* and *C. elongatus* cause the majority of sea lice-associated economic losses to salmonid aquaculture in the northern Atlantic Ocean (Mordue and Birkett, 2009). If enough sea lice are present on a fish, they can lower its respiratory and osmoregulatory capacity, increase vulnerability to bacterial and viral infection through damage to epidermis and immune suppression, and decrease swimming and cardiac performance (Costello, 2006; Wagner et al., 2008). Negative effects associated with sea lice are typically noticeable once the parasite reaches the more detrimental pre-adult or adult stages (Wells et al., 2006). Sea lice are an issue for Atlantic salmon farms around the world, with increased costs associated with treatment, reduced fish growth, reduced feed conversion efficiency, and reduced market value due to disfigurement (Sinnott, 1998; Mustafa et al., 2001). According to Costello (2009), they are the most detrimental parasite to the salmonid farming industries in Europe and the Americas, and cost the Maine salmon farming industry over $1 million in damages in 2006 alone.

The cost of sea lice infestations to the Atlantic salmon aquaculture industry has prompted extensive research into new and innovative methods to treat, control, and eliminate sea lice from salmon pens. Chemicals such as emamectin benzoate (the active ingredient of Slice®, an in-feed chemotherapeutant), have been extensively used by salmon farmers in the Gulf of Maine (Westcott et al., 2004). However, due to the emergence of resistance to chemotherapeutants, alternative treatments are being sought. Fallowing (the complete removal of the salmon stock for a set period of time) is now
widely used, with mixed results (Bron et al., 1993). This technique operates on the principal of removing the source population of sea lice. If wild fish serve as reservoirs of sea lice, however, this precludes all extant lice treatments from being useful for long-term removal of sea lice (Costello, 2009). Little prior research has examined the existence or importance of such reservoirs.

Sticklebacks (family Gasterosteidae) have been studied extensively as a potential sea lice reservoir for farmed salmon. They are found in marine, brackish, and fresh waters of the temperate and sub-polar zones in the northern hemisphere (Wootton, 1976). The threespine stickleback’s range in particular extends along the margins of both the northern Atlantic and Pacific Oceans. On North America’s east coast, threespine sticklebacks occur from Hudson Strait, Baffin Island, to as far south as Chesapeake Bay or Cape Hatteras (Wootton, 1976). Threespine sticklebacks are well established hosts of both Caligus spp. and L. salmonis in the northeast Pacific (Jones et al., 2006). Y-tube experiments have demonstrated that pink salmon (Oncorhynchus goruscha) and threespine stickleback water-cues increase L. salmonis activity, suggesting that both species are important hosts for L. salmonis (Losos, 2008). Jones et al. (2006) even suggested that threespine sticklebacks could be used as a sentinel species (for monitoring purposes) for sea lice abundance. This no longer appears to be a viable use for sticklebacks, as Losos (2008) found that L. salmonis infected salmon 2.5 times faster than sticklebacks, and experienced higher survival on their salmonid hosts. Sticklebacks therefore represent a lower quality host for L. salmonis, and Losos (2008) suggested they may be unlikely to serve as an infection source to farmed salmon operations. Despite these findings on relative host suitability, the fact that threespine sticklebacks host both
Caligus spp. and L. salmonis suggests that they are potential reservoirs of sea lice during aquaculture fallow periods, as well as periods when wild salmonids are not located within the coastal zone. To date, no studies have examined the relationship between sea lice and threespine sticklebacks in the northwest Atlantic.

**Project Objectives**

This study sought to characterize the infestation of wild fishes by sea lice in Cobscook Bay. To achieve this goal, the following were examined: (a) the species of sea lice found on wild fishes, (b) the different host species of sea lice, (c) the relative proportions of the different sea lice life stages on these species, (d) patterns in the settlement locations (host body location) of chalimus stages, (e) spatial and temporal trends in sea lice infection intensity (number of sea lice per fish), and (f) spatial and temporal trends in infection prevalence (proportion of fish infested). The results of this work establish a baseline for sea lice dynamics in Cobscook Bay, comparable to similar studies in the northeast Pacific and Atlantic Oceans. Furthermore, this project has identified potential wild reservoirs of sea lice for farmed salmon, providing some indication of the effectiveness of fallowing as a means of parasite control.
Materials and Methods

Fish Sampling in Cobscook Bay

Fish sampling in Cobscook Bay, ME, was conducted in March, April, May, June, August, September, and November of 2012 using seine, fyke, pelagic trawl, and benthic trawl nets. Only seine netting was used in March (8th, 9th, and 10th), April (13th and 14th), and November (2nd). Seine, fyke, and trawl nets were used in the remaining months on the following dates: 25-30 May, 23-28 June, 25-31 August, and 22-28 September. All sampling was part of a larger project, led by Jeff Vieser and Dr. Gayle Zydlewski, to characterize the fish community of Cobscook Bay.

Cobscook Bay is a boreal, macrotidal estuary at the mouth of the Bay of Fundy, experiencing semidiurnal tides with a mean range of 5.7 meters (Larsen, 2004a). Cobscook Bay’s convoluted shoreline divides it into three distinct regions: Inner, Central, and Outer Bay (Fig. 3). Within the bay, salinities are usually greater than 30, temperatures vary seasonally between 0°C and 12°C, and turbidity is generally low (Larsen, 2004a). Cobscook Bay also has an average depth of 10 meters, and receives only a small freshwater input (Larsen, 2004a). The bay exhibits very high primary productivity and ecological richness due to the intense tidal mixing (Larsen, 2004b). Once noted for abundant alewives, herring, pollock, cod, and other fish species, Cobscook Bay now primarily supports a rich benthic community of mussels, clams, scallops, and macroalgae (Brooks, 2004).

Seine and fyke net sampling sites (Fig. 3) within Cobscook Bay were selected for balanced effort between Inner, Central, and Outer Bays (Table 1). Waterfront access and land cover precluded a random site selection process. The netting sites were chosen for
gradually sloping intertidal zones with mudflat, cobble, eelgrass, or rockweed habitats. The Deep Cove, Broad Cove, Pennamaquon River, Burnt Cove, and South Bay sites were sampled using seine nets only. Both seine and fyke netting were carried out at Carryingplace Cove, East Bay, and Dennys Bay. South Bay, Deep Cove, and Broad Cove sites were the only sites near salmon net pens that were active in 2012.

Seine nets were 30.48 m x 1.83 m with 0.64 cm diamond mesh. The net was deployed parallel to shore, in water approximately 2 m deep, and pulled to shore. Each tow lasted approximately 2 min, and all tows were performed on the ebb tide. In March, April, and November, seining only occurred at the Deep Cove, Broad Cove, Carryingplace Cove, East Bay, Pennamaquon River, and Dennys Bay sites. The fyke net had 9.14 m wings, 1.22 m-square hoops leading to the cod end, and 3.81 cm stretch mesh. Two nets were deployed at each site at low tide. For each sampling period, fyke nets fished the front half of one tide (approximately 3 hours). Both seine and fyke nets sampled the intertidal zone.

Pelagic trawling was conducted at five sites: Shackford Head, East Bay, South Bay, Whiting Bay, and Dennys Bay (Fig. 3, Table 2). With the exception of Shackford Head, which was replaced by a site near Treat Island, benthic trawling occurred at the same sites (Fig. 3, Table 3). Sampling sites were selected to survey Inner, Central, and Outer Bays. The pelagic trawl net had 12.19 m headrope, footrope, and breastlines, while the benthic trawl net had a 13.72 m headrope, 10.67 m headrope, and no breastlines. The stretch mesh size of the pelagic trawl net was 10.16 cm in the belly, square, and side panels, 5.08 cm in the tapers, and 2.54 cm in the extensions and cod end. The benthic trawl net had stretch mesh sizes of 5.08 cm and 2.54 cm in the net body and cod end,
respectively. For each sample, the net was towed for approximately 20 minutes in the mid-water column or along the bottom substrate for the pelagic and benthic trawls, respectively, to sample the subtidal zone in Cobscook Bay.

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 MS222 (Argent Chemical Laboratories), stored on ice, and transported to the field house for measurements. Additional fish with obvious sea lice were often isolated, euthanized, and stored as “unmeasured” fish in 95% (v/v) ethanol for later examination under a dissecting microscope. Remaining fish were released unharmed.

**Examination of Fish for Sea Lice**

Fish brought back to the field house (“measured” fish) were examined for sea lice infestations after being measured for length and weight. Examination for sea lice relied upon the naked eye, and consisted of a thorough scan of the pectoral, caudal, anal, and dorsal fins as well as the body surface. For each fish, the examiner recorded the number of observed sea lice (irrespective of sea lice species).

All fish with observed sea lice infestations were then separated by species and tow, and stored in a sealable container with 95% (v/v) ethanol. Subsamples consisting of the first five sticklebacks, flounder, and lumpfish measured and observed to not have sea lice were also stored in ethanol. These individuals were used to assess the accuracy of identifying infestations while in the field (at the field house).

When returned to the laboratory, all unmeasured and measured fish with recorded sea lice infestations, in addition to subsamples of the stored fish possessing no apparent
infestations, were examined for sea lice under a dissecting microscope. The left, right, dorsal, and ventral surfaces were carefully examined, as well as the pectoral, dorsal, pelvic, anal, and caudal fins. Magnifications between 10x and 15x were used during fish examination. Additionally, approximately 10% of fish in each sample were checked for gill infestations by removing and carefully examining the gill structures.

The life stage, position, and genus of each observed sea louse was recorded on a fish diagram data sheet (Appendix 1) and in an Excel spreadsheet. Life stage identification was limited to the following stages: copepodid (CO), chalimus (CH), adult (AD), unknown (?). Location was characterized as generalized positions on the fish (Fig. 4): pectoral fins (PCF), pelvic fins and spines (PVF), anal fin (ANF), dorsal fin (DF), caudal fin (CF), caudal peduncle (CP), dorsal surface (DS), ventral surface (VS), right side (RS), left side (LS), and head surface (H). Sea lice identification was restricted to genus (Caligus, Lepeophtheirus, other) using morphological features. Frontal filament structure, head shape, and eye structure characteristics, as well as presence/absence of lunules in the fourth chalimus stage, were utilized to identify the chalimus stages (Johnson, 2004). Adult Caligus and Lepeophtheirus were distinguished by total size, eye structure characteristics, and the presence/absence of lunules. After detailed observations, each sea louse was removed from the fish and stored in 95% (v/v) ethanol at 4°C for species identification by PCR and DNA sequencing.

**PCR-Based Species Identification**

PCR and DNA sequencing were used to identify sea lice to the species level and verify visual identifications. Genomic DNA (gDNA) was extracted from all sea lice
visually identified as either *Lepeophtheirus* spp. or undetermined, as well as a subsample of up to 10 individuals (per sample site and month) visually identified as *Caligus* spp., 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 2000c spectrophotometer (Thermo Scientific) to determine DNA concentration.

Polymerase chain reaction (PCR) was employed to amplify the mitochondrial cytochrome c oxidase I (COI) gene sequence using the universal primers LCF and LCR (Jones and Prosperi-Porta, 2011), which target the COI gene of both *L. salmonis* and *C. elongatus*. The LCR and LCF primers, also known as LCO1490 and HCO2198, respectively, had the following 5’-to-3’ nucleotide sequences: GGTCAACAAATCATAAAGATATTGG and TAAACTTCAGGGTGACCAAAAAATCA, respectively (Folmer et al., 1994). PCR was performed in a 50 µl master mix using the GoTaq® Flexi DNA Polymerase (Promega) following the manufacturers’ instructions. Briefly, 400 ng gDNA template extracted from individual sea lice was mixed with 0.5µM LCF primer, 0.5µM LCR primer, and 1x 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.

PCR products were resolved on a 1% agarose gel with 100 ng/ml ethidium bromide (Fisher) run at approximately 85V for 60 min in 1x TAE buffer. Five microliters of PCR product were mixed with 1 µl 6x loading dye (NEB) and loaded onto the agarose gel. Molecular weights of PCR products were calibrated using a 1 kb DNA
ladder (NEB). Gels were imaged under ultraviolet illumination to verify the success of the PCR process for each sample.

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 analyzed with the NanoDrop spectrophotometer for DNA concentration. Finally, the PCR products were direct sequenced at the University of Maine DNA Sequencing Facility using primer LCR (Table 4).

Sequences were edited using the software Geneious 4.7.6. A blast search was carried out on all COI sequences using the NCBI BLASTn program (National Center for Biotechnology Information Basic Local Alignment Search Tool) to determine which species-specific COI sequences they were most closely homologous to. Multiple sequence alignments were also performed using CLUSTALW (cluster analysis of the pairwise alignments) in order to compare the COI sequences of the sea lice sampled in this project to reference COI nucleotide sequences for C. elongatus and L. salmonis published in the NCBI database.

Data Analysis

Infection intensity was calculated as the average number of sea lice per fish among infected fish only, and infection prevalence was calculated as the proportion of fish with sea lice in a given group. Sea lice examination data from the field house were used for infection prevalence analyses. To avoid selection bias, sea lice counts observed with the dissecting microscope on only measured fish were used to analyze infection intensities and relative sea lice life stage abundances. Data from both measured and
unmeasured fish were used to identify both sea lice species and wild fish hosts, and to characterize the attachment locations of the chalimus stages.

Due to inconsistent examination techniques and low sample sizes from fish collected in both March and April, sea lice counts from these fish were excluded from infection intensity and prevalence analyses. Sea lice found floating in jars containing multiple fish were also excluded from intensity calculations.

The examination of subsamples of stored (measured) fish that were identified as sea lice-free from May and June (20% and 10% subsamples, respectively; n = 50, n = 23, respectively) revealed that 18% and 43.48%, respectively, of these fish did in fact have sea lice infestations. Additionally, 55 of 268 (20.52%) fish stored as samples with sea lice infestations were found to not host sea lice when examined under a dissecting microscope. Most of these misidentified samples (33 of 55, or 60%) were fish collected and examined during May. May prevalence data was therefore excluded from analyses because there were multiple, previously untrained examiners checking for sea lice over the course of the sampling week. June prevalence data was included because there was one consistent, experienced person responsible for identifying infections. Examinations of 10% of fish collected in August, September, and November that were identified as sea lice-free (n = 29, 27, and 6, respectively), however, revealed no infestations. Fish collected in these months, along with those collected in June, were included in the prevalence analyses.

Because all analyses on intensity failed the Shapiro-Wilk test for normality (p < 0.05), spatial and temporal trends in species-specific infection intensity among fish
species, months, sites, and sub-bays were examined using ANOVA on ranks and Dunn’s pairwise test.

Spatial and temporal trends in infection prevalence, using sea lice count data collected at the field house, were examined using a Fisher Exact Test by Monte Carlo simulation. A total of 5,000 replicates were used in each Monte Carlo test, and each test was run five times. As p-values are only simulated values with the Monte Carlo simulation, the range of p-values is listed for each analysis. The results of each analysis were considered significant only if all simulated p-values fell below the significance level of 0.05.
Results

Fish Hosts, Sea Lice Species, and Sea Lice Life Stages

Among all gears and months, 6329 individual fish of 34 different species were caught and examined for sea lice in the field house (Table 4). Threespine sticklebacks were the most abundant species (n=1996), followed by blackspotted sticklebacks, winter flounder, and mummichogs (n=882, 690, 587 respectively). A total of 342 measured fish and 131 unmeasured fish (Table 5) were examined under a dissecting microscope to determine infection intensity, sea lice life stage, and sea lice species. The number of examined fish was determined by the number of fish with sea lice that were stored in ethanol.

All sea lice (n = 679) observed microscopically were identified as Caligus sp. or unknown based on morphological features. Sea lice were identified as unknown if they had unusual morphological characteristics, were degraded, or were in the copepodid stage (stage at which it is extremely difficult to differentiate below the genus level). No individuals were identified as Lepeophtheirus sp..

DNA was successfully extracted from 80.93% (208 of 257) of sea lice selected for DNA analysis, and DNA amplification with PCR had an 85.10% (177 of 208) success rate. Gel electrophoresis confirmed the success of PCR and verified that the size of the amplified gene was the expected size of approximately 700 bp (Fig. 5).

Of the 177 sequenced sea lice samples, 175 matched the sequence of C. elongatus genotype 1. The extent of alignment of the COI gene sequence from an isolated sea louse, collected in this study on a winter flounder, with published C. elongatus genotype 1, C. elongatus genotype 2, and L. salmonis COI gene sequences is demonstrated by the
CLUSKALW-generated sequence comparison (Fig. 6). The remaining two samples were contaminated during processing, and were similar to COI sequences from *Homo sapiens* and *Hygrobates longipalpis* (a water mite).

Twelve fish species were identified as hosts for sea lice. The 12 species, in order of most to least numbers of individuals with sea lice, are as follows: 1) threespine sticklebacks, 2) blackspotted sticklebacks, 3) winter flounder, 4) longhorn sculpin, 5) lumpfish, mummichogs, red hake, tomcod, rainbow smelt, ninespine stickleback, Atlantic herring, and blueback herring (Table 6). Lumpfish (n = 1) possessed the highest infection prevalence and intensity at 100% and 22 sea lice per fish, respectively. All other species, excluding the threespine sticklebacks, blackspotted sticklebacks, winter flounder, and longhorn sculpin, had just one infected individual. Among species with more than one infected individual, the threespine sticklebacks had the greatest infection prevalence and median intensity at 12.26% and 2 sea lice per fish, respectively. The sea lice infestations on blueback herring and Atlantic herring are uncertain because they may be artifacts of the sampling (all retrieved fish were allowed to mix freely before being counted and examined). The observed sea lice on these fish were adults that may have been transferred to them after their initial capture in the seine net.

From the 515 sea lice associated with measured fish, 95.92% were chalimii (n = 494), 2.72% were adults (n = 14), 0.39% were copepodids (n = 2), and 0.97% were unidentifiable due to distorted or degraded morphology (n = 5).

*Location of Chalimii on Hosts*
The locations of 656 chalimii on all measured and unmeasured fish examined under the dissecting microscope were recorded and characterized. Among chalimii attached to threespine sticklebacks, blackspotted sticklebacks, and winter flounder (n = 556, 39, 25 respectively), the majority (86.15%, 92.31%, and 100% respectively) were attached to the fins (Fig. 7). The same was true for the pooled group of all analyzed fish, as 84.76% of chalimii were attached to the fins (n = 656). The caudal fins and pectoral fins were the primary attachment points for sea lice: 27.74% and 25.30% of chalimii, among all fish, were attached to these fins, respectively. The lumpfish was the notable exception to this trend; 15 of 22 observed chalimii were found on the body surface.

**Infection Intensity**

There was a significant difference in infection intensity on threespine sticklebacks, blackspotted sticklebacks, and winter flounder collected in June (p = 0.009). In particular, blackspotted sticklebacks had lower infection intensity than threespine sticklebacks (Dunn’s post-hoc test, p < 0.05; Fig. 8). Threespine sticklebacks had the highest median intensity at 2 sea lice fish$^{-1}$, followed by winter flounder and blackspotted sticklebacks at 1 sea louse fish$^{-1}$.

Threespine sticklebacks had the greatest number of measured infested fish from May to November (n = 202; Table 6). This species was used to examine spatial and temporal trends in infection intensity. To avoid the confounding effect of month, infection intensity data for threespine sticklebacks collected just in June (n = 117) were used to analyze spatial trends. All infected threespine sticklebacks were collected by seine netting. There were no significant differences in intensity among sites or sub-bays
(p = 0.727 and 0.505, respectively). Since infection intensity did not significantly differ by site, threespine sticklebacks were pooled across sample sites to examine temporal trends. There was a significant difference in intensity among months (p < 0.001, n = 202; Fig. 9). Threespine sticklebacks collected in June had the highest median intensity of 2 sea lice fish\(^{-1}\), while fish collected in the remaining months had median intensities of 1 sea louse fish\(^{-1}\). There were significant differences between two pairs of months, June and August and June and May (p < 0.05 for both, Dunn’s post-hoc test), with fish collected in June always having the higher infection intensity.

There were no spatial or temporal differences in infection intensity for blackspotted sticklebacks. Blackspotted sticklebacks collected in June (n = 17) from different sites or sub-bays did not have significantly different infection intensities (p = 0.581 and p = 0.581, respectively). When fish were pooled across sites, there were no significant difference in infection intensity among months (p = 0.657). Sample sizes for all other host species were too low for detailed analyses of spatial and temporal trends in infection intensity (n \(\leq\) 9).

**Infection Prevalence**

Winter flounder, threespine sticklebacks, and blackspotted sticklebacks had different infection prevalences in June, August, and September (p < 0.001 for all simulations, 0.002 \(\leq\) p \(\leq\) 0.003, 0.002 \(\leq\) p \(\leq\) 0.003, respectively; Fig. 10). Threespine sticklebacks had the highest infection prevalence in June, August, and September, with values of 27.16%, 4.96%, and 6.13%, respectively. Blackspotted sticklebacks had the next highest prevalences and winter flounder had the lowest prevalences (Fig. 10).
Infection prevalence varied temporally for threespine sticklebacks, blackspotted sticklebacks, and winter flounder. For threespine sticklebacks (n = 1636), there were significantly different infection prevalences between fish collected in June, August, September, and November (p < 0.001 for all simulations). Threespine sticklebacks collected in June possessed the highest prevalence at 27.16%, followed by those from November, September, and August at 9.26%, 6.13%, and 4.96% respectively. Infection prevalence varied by month for blackspotted sticklebacks and winter flounder (n = 631, n = 479, respectively; p < 0.001 for all simulations, 0.008 ≤ p ≤ 0.004, respectively). Infection prevalence peaked in June for both blackspotted sticklebacks and winter flounder, at 13.16% and 5.22%, respectively.

Infection prevalences on both threespine and blackspotted sticklebacks were significantly different among fish collected in the three sub-bays. When threespine sticklebacks and blackspotted sticklebacks were each pooled from August, September, and November, there were significant differences in infection prevalence among sub-bays (n = 1123, n = 517, respectively; 0.001 ≤ p ≤ 0.004, 0.001 ≤ p ≤ 0.002, respectively; Fig. 11). Infection prevalence on threespine sticklebacks from Inner Bay was just 2.6%, compared to 8.82% from Central Bay and 7.62% from Outer Bay. Infection prevalences on blackspotted sticklebacks from Inner, Central, and Outer Bays were 0.40%, 3.47%, and 7.94%, respectively. Pooling fish from August, September, and November increased sample size and was justified by the lack of significant differences in prevalence among those months for either threespine or blackspotted sticklebacks (0.2897 ≤ p ≤ 0.3065, 0.1009 ≤ p ≤ 0.1239, respectively).
Temporal differences may be confounded by the detected spatial differences. Therefore, differences in infection prevalence among months were analyzed separately for threespine sticklebacks collected from each sub-bay. Infection prevalences were significantly different between months for fish from Inner, Central, and Outer Bays (p < 0.0002 for all simulations and for all sub-bays; Fig. 12). Threespine sticklebacks sampled in June had the highest prevalence at all three locations, with prevalence values of 26.09%, 25.29%, and 30.08% for Inner, Central, and Outer Bay, respectively.
Discussion

Is Cobscook Bay a Caligus elongatus Monoculture?

*C. elongatus* infested 12 different fish species in Cobscook Bay, and was observed on fish collected from all sampling sites, months, and gear types. All observed sea lice infections of sampled wild fish were by a single species and genotype of lice, *C. elongatus* genotype 1. The observed *C. elongatus* were predominantly in the chalimus stage, and were located on the fins of their hosts. *C. elongatus* infection intensity and prevalence varied significantly among fish species with multiple infestations (*n* ≥ 2). Among fish with multiple infestations, threespine sticklebacks had the highest infection intensity and prevalence, and these parameters varied significantly over time (with peak values in June) and space (fish from Inner Bay possessed lower infection intensities than those from Central and Outer Bay).

The species uniformity of observed sea lice on the fish sampled in Cobscook Bay is unique amongst surveys of wild fishes for sea lice from other regions of the world. Heuch *et al.* (2007) and Hayward *et al.* (2011) observed at least two *Caligus* species in their respective study sites in the North Sea and off the south coast of Australia. Jones *et al.* (2006) and Jones and Prosperi-Porta (2011) identified *C. clemensi*, a species found only in the Pacific Ocean, and numerous *Lepeophtheirus* spp. in an examination of lice infestations on threespine sticklebacks off the coast of British Columbia. It is possible that our sampling techniques could have missed other species of sea lice present within the bay system, especially if they were present at low abundances, as our sample sizes (*n* = 6329 and *n* = 473 for fish examined in the field and under a dissecting microscope, respectively) and sea lice sample sizes (*n* = 679 and *n* = 177 for individuals visually
examined and sequenced, respectively) were smaller than those in similar surveys. Jones and Prosperi-Porta (2011) reported that over 25,000 sea lice were examined from their survey of 7,684 threespine sticklebacks, and Heuch et al. (2007) examined 4,427 fish under magnification.

Low genetic diversity among sequenced sea lice was surprising as well, as all sea lice were identified as genotype 1. Research performed off the southeast coast of Norway, in the North Sea, identified two dominant C. elongatus genotypes, genotype 1 and 2 (Heuch et al., 2007). Øines et al. (2006) reported that genotype 1 is the most dominant genotype on salmon farms in Norway and Scotland and observed that C. elongatus genotype 1 preferred lumpfish and Atlantic cod over all other hosts, including Atlantic pollock, plaice, and sea trout. However, they did not include any species of sticklebacks in their host preference experiment. As all observed sea lice from Cobscook Bay appeared to be exclusively C. elongatus, genotype 1, genotype-specific trends in host preference may exist throughout the bay.

The lack of L. salmonis infestations among sampled wild fish was particularly unexpected, especially considering the presence of active salmon net pens in close proximity to three of the sampling sites (South Bay, Deep Cove, Broad Cove). A study on threespine stickleback infestations off the coast of British Columbia, for example, reported prevalences of Lepeophtheirus spp. infections on threespine sticklebacks as high as 83.6% (Jones et al., 2006). There are many possible reasons for the lack of L. salmonis among the sampled wild fish. The population of L. salmonis in Cobscook Bay may simply possess different host preferences due to underlying genetic differences, or the resident wild fish may be more resistant to infection by this sea lice species. The
overall sea lice infectious pressure in other study sites, like coastal British Columbia, may also be higher than in Cobscook Bay, due to higher abundances of either farmed or wild salmonids acting as ideal hosts for *L. salmonis* population growth. *L. salmonis* possesses a narrow host range, usually infecting only salmonids from the genera *Salmo, Salvelinus,* and *Oncorhynchus,* and has been shown to have a greater preference for salmonid hosts than other reported species, such as Atlantic and Atlantic cod (Pert *et al.*, 2012). No salmonids were captured in any type of gear, indicating a low abundance of wild salmonids in the region. The expected low abundance of wild salmonids is supported by the U.S. Atlantic Salmon Assessment Committee (2009) salmon survey, which recorded that only eight returning Atlantic salmon were caught in the weir trap on Denny’s River, the primary Atlantic salmon running river in Cobscook Bay, in 2008. Finally, the fallowing of all farmed salmon pens in Cobscook Bay in 2012, from February to April for a total of 90 days, may have decreased *L. salmonis* infectious pressure in the bay, resulting in the lack of detectable *L. salmonis* infestations on the wild fish sampled.

**C. elongatus Lives Up to its Reputation of Ubiquity**

The large number of fish species found to be infected by *C. elongatus* supports the notion of *C. elongatus* as a ubiquitous parasite among wild fishes. This species of sea lice was found on 12 out of 34 fish species examined in the present study, representing infestations on just over one-third of observed species (35.29%). For comparison, Heuch *et al.* (2007) determined that 21 of their 40 examined species of wild fish were infested (52.5%). Hayward *et al.* (2011) identified *Caligus* sp. infestations on 2 of 7 examined wild species of fish, yielding a 28.57% infestation rate among species. The variability in
infection rate between the study systems indicates a high degree of variability in either fish infections or sampling methods between locations. Potential variability in fish infections might simply be a result of different fish assemblages among the study sites. The lower proportion of infested species in Cobscook Bay, compared to that off the coast of Norway, may also simply be the result of lower infection pressure. Alternatively, some actual hosts in Cobscook Bay may not have been identified due to the low sample size of some species, as 12 of the 22 fish species not identified as hosts were sampled only rarely (n ≤ 10 over the entire sampling period). Further sampling in Cobscook Bay may identify additional hosts.

The results of the study detected 10 previously unrecognized hosts for *C. elongatus*: threespine sticklebacks (*Gasterosteus aculeatus*), ninespine sticklebacks (*Pungitius pungitius*), blackspotted sticklebacks (*G. wheatlandi*), Atlantic tomcod (*Microgadus tomcod*), mummichogs (*Fundulus heteroclitus*), red hake (*Urophycis chuss*), longhorn sculpin (*Myoxocephalus octodecemspinosus*), blueback herring (*Alosa aestivalis*), rainbow smelt (*Osmerus mordax*), and winter flounder (*Pseudopleuronectes americanus*). While most of these species are only found in eastern North American waters, where large-scale sea lice studies are conspicuously absent, threespine sticklebacks, ninespine sticklebacks, and rainbow smelts are also found in European waters (Fishbase, 2013). Rainbow smelts in particular have a circumpolar distribution (Fishbase, 2013). Though ninespine sticklebacks and rainbow smelt, two widely distributed species, were not sampled by Heuch *et al.* (2007), the potential for them to act as hosts in the North Sea and other European waters deserves further investigation. Heuch *et al.* (2007) sampled a small number (n = 20) of threespine sticklebacks in the
North Sea, but observed no sea lice infestations. Either threespine sticklebacks do not act as significant *C. elongatus* hosts in the North Sea, or the sample size was too small to detect any infestations.

The identification of the two remaining fish species, specifically lumpfish and Atlantic herring, as hosts of *C. elongatus* is well supported by past studies. Heuch *et al.* (2007) confirmed that lumpfish and Atlantic herring are important hosts for *C. elongatus* in the North Sea, with infection prevalences of 83.6% and 20.1%, respectively. Kabata (1979) also identified lumpfish and Atlantic herring as known hosts for *C. elongatus*.

There were recognized *C. elongatus* host species examined in Cobscook Bay that were not observed to be hosts of *C. elongatus*. Heuch *et al.* (2007) found that Atlantic pollock, Atlantic cod, and Atlantic mackerel were infested by *C. elongatus* at infection prevalences of 19.8%, 12.7%, and 4.4%, respectively. Few individuals from these three species, however, were sampled in Cobscook Bay (n = 5, 11, 6 for Atlantic pollock, Atlantic cod, and Atlantic mackerel, respectively), limiting the ability to successfully identify these as host species. Data collected from rarely sampled fish species, therefore, must be interpreted with caution.

Finally, there were several known host species that may play a role in sea lice dynamics in Cobscook Bay, but that were simply not sampled in 2012. Sea trout and Atlantic salmon are known hosts of both *C. elongatus* and *L. salmonis* that were not collected by any of the gear types utilized in the present study (Kabata, 1979; Bruno and Stone, 1990).

*C. elongatus* Life stage Abundances and Attachment Locations on Hosts
The relative abundances of sea lice life stages observed on all measured fish varied widely compared to studies done elsewhere. The high relative abundance of *C. elongatus* chalimii (95.92%) is similar to the relative abundances of observed *Lepeophtheirus* spp. and *C. clemensi* chalimii (62.9% and 88%, respectively) on three-spine sticklebacks reported by Jones and Prosperi-Porta (2011). Furthermore, they indicated that less than 2% of observed *C. clemensi* stages were motile adults. In contrast, Heuch *et al.* (2007) found that 75 to 100% of observed *C. elongatus* on 13 fish species sampled in the North Sea were in the adult stage. The researchers, however, did not observe any sea lice on three-spine sticklebacks, a major host species in Cobscook Bay.

The observed dominance of the chalimii in the present study may reflect the actual levels on fish, or it may be an artifact of sampling. Netting has the potential to knock the motile stages off the sampled fish, biasing the proportion of sessile chalimii upwards as they are securely attached to the fish via a frontal filament. Jones *et al.* (2006) also indicated that loss of adults and copepodids during fish capture may cause the observed dominance of chalimus stages. Additionally, it is important to consider that these proportions do not measure actual life stage abundances in the wild. Most copepodids and adults likely spend significant time in the water column, searching for or switching between hosts. For example, Heuch *et al.* (2007) reports that *C. elongatus* adults are relatively strong swimmers compared to *L. salmonis*, and may spend significant time among the plankton while switching hosts. By only measuring abundances of sea lice attached to fish, there is already a bias towards inflating the measured abundance of chalimus stages.
The observed distribution of *C. elongatus* attachment points on fish hosts is similar to the results of previous sampling and experiment-based research. The majority of non-motile chalimus stages were attached to the fins, and most often the caudal and pectoral fins. No *C. elongatus* chalimii were found on the gills. To date, the distribution of chalimus attachment locations on wild fish of either *L. salmonis* or *C. elongatus* has not been reported in the literature. In the closest resemblance to a survey of wild fish, Treasurer and Wadsworth (2004) reported that the caudal and pectoral fins were the most important attachment points for *C. elongatus* among randomly sampled farmed salmon. They also state that *C. elongatus* does not attach to the gills of salmon. Treasurer and Wadsworth’s (2004) results agree with the findings from this study, suggesting that the overall settlement pattern of *C. elongatus* may be similar between farmed and wild fish hosts.

There were noticeable differences in *C. elongatus* chalimii attachment sites between threespine sticklebacks, blackspotted sticklebacks, and winter flounder, suggesting that there may be species-specific differences in attachment locations. No chalimii were found on the body surface of winter flounder (n = 25), while small proportions of chalimii (13.85% and 7.69%) were attached to the body for threespine and blackspotted sticklebacks, respectively (n = 556 and n = 39, respectively). The differences observed between host species may have been due to low sample sizes, especially for winter flounder. However, living near or on benthic substrate, winter flounder have a drastically different lifestyle than the more pelagic sticklebacks. Sea lice attached to the body surface of winter flounder could be scoured off by contact with the
substrate, whereas copepodids and chalimii on sticklebacks would for the most part only have to deal with water flow along the body surface.

**Possible Explanations for Observed Trends in Infection Pressure**

The significant temporal trends in infection intensity and prevalence of *C. elongatus* on threespine sticklebacks suggest the possibility of varying infection pressure among months. Peak infection intensities and prevalences occurred in June. A peak in *C. elongatus* abundance in June was the probable cause of the observed intensity and prevalence trends. Higher abundances of *C. elongatus* would increase infection pressure on all species of fish, throughout Cobscook Bay. The significant differences in infection prevalence among months for blackspotted sticklebacks and winter flounder support the notion that overall infection pressure was highest in June for all fish species. Finally, as 43.48% of examined fish from June that were initially observed to be lice-free did in fact host lice infections, actual infection prevalences among fish in June were much higher than indicated by reported prevalence values.

The observed peak in *C. elongatus* infection intensity and prevalence in June may be the result of several possible factors, including naturally increasing numbers after farmed salmon were returned to Cobscook Bay, a regularly occurring seasonal trend, or an infrequent episodic population explosion. The fallowing of Cobscook Bay’s salmon farms in the early spring of 2012, in an attempt to control primarily *L. salmonis* infestations (Pietrak, personal communication), may have reduced *C. elongatus* abundances throughout the bay, which then rebounded subsequently in June. According to Pietrak, Cobscook Bay salmon farms do experience infestations of both *C. elongatus*
and *L. salmonis*. Though the lack of data on *C. elongatus* infections from these salmon pens precludes any definitive conclusions, there may in fact be some interaction in *C. elongatus* infestations between wild fish and salmon in pens. The *C. elongatus* population abundances may also follow a previously unobserved seasonal trend. According to Pietrak, *L. salmonis* counts on salmon farms follow a seasonal trend in which levels peak in August, September, and October due to peaking water temperatures in late summer and fall. Sea lice in general experience decreased generation times with increasing temperature, facilitating increasing population growth (Costello, 2006). *C. elongatus* abundances on wild fishes may simply follow a different seasonal trend compared to *L. salmonis* on farmed salmon, with a peak in early summer rather than late summer. A final alternate explanation is that the sampling effort happened to capture an infrequent population explosion of *C. elongatus*. The timing of the peak in June does not correspond to the maximal population growth expected in later months, when water temperatures are greatest. Additionally, Cobscook Bay’s salmon farms experience infrequent peaks in *C. elongatus* infections throughout the summer (Pietrak, personal communication), suggesting that the *C. elongatus* population undergoes episodic population explosions. The measured peak in intensity and prevalence of *C. elongatus* in June 2012 could therefore be a result of sampling efforts by chance capturing one of these infrequent population explosions. As these data were collected on only a few species over a single year, however, further data collection on a wider variety of hosts, and spanning several years, is needed to resolve the current uncertainties regarding the observed temporal trends and characterize any interannual variability.
The significant spatial trend in prevalence of *C. elongatus* on threespine and blackspotted sticklebacks may have been caused by varying physical conditions throughout Cobscook Bay’s different sub-bays or the exclusive presence of active salmon pens in both Central and Outer Bay. There were significant differences in *C. elongatus* prevalences between sub-bays for threespine and blackspotted sticklebacks, with prevalences on these species from both Central and Outer Bay noticeably higher than those from Inner Bay. The spatial differences may be due to varying salinity regimes. Temperature and salinity are known to affect the survival and incidence of both *L. salmonis* and *C. elongatus*. For *L. salmonis* specifically, salinities below 30 ppt reduce the survival and development of copepodids and decrease overall fecundity (Brooks, 2005; Mordue and Birkett, 2009). The same trends likely apply to *C. elongatus*. Whiting Bay and Dennys Bay, both located in Inner Bay, have the lowest salinities in Cobscook Bay (Phinney et al. 2004). Alternatively, the distribution of salmon pens may have influenced the difference in *C. elongatus* prevalence between sub-bays. Active salmon pens in 2012 were only found in South, Deep, and Broad Cove, which are all located in Central and Outer Bay. The absence of active salmon pens, and potential sources of *C. elongatus*, could have produced the lower prevalence values in Inner Bay. However, the lack of data on *C. elongatus* infections on salmon in pens, in addition to possible differences in the abundances of “preferred” host species of *C. elongatus* between bays, makes it difficult to verify this hypothesis.

The lack of any spatial trend in *C. elongatus* intensity on threespine sticklebacks, despite the clear trend in prevalence, was likely due to the skewed distribution of intensity values. Over half of the sampled fish had just one louse, making detection of
significance between months, sub-bays, or species difficult. The lack of significant spatial differences may also be due to biased measures of infection intensity. Measured *C. elongatus* intensity is likely biased on the low side, as some sea lice (n = 17) were found floating in jars of ethanol containing multiple fish, and therefore could not be attributed to a single fish. These sea lice were excluded from intensity measurements.

*Wild Fish as Reservoirs of Sea Lice*

Threespine sticklebacks were the most heavily parasitized fish among species with multiple infestations (n ≥ 3), and may serve as reservoirs of *C. elongatus* to farmed fishes within Cobscook Bay, ME. Threespine sticklebacks had the greatest number of infestations, making them the most common, and possibly preferred, hosts for *C. elongatus* in Cobscook Bay. The widespread distribution of this species throughout the bay, in addition to its importance as a host, makes threespine sticklebacks the most likely reservoir host of *C. elongatus* to other wild fish, and possibly even farmed fish. The presence of adult sea lice on threespine sticklebacks also confirms that they are not simply transient hosts for the non-motile stages. Lumpfish may also play an equally important role in sea lice transmission, as the one collected individual in 2012 was infested by over 20 chalimii. Additionally, lumpfish in the North Sea had a median infection intensity of eight lice, second only to plaice (*Pleuronectes platessa*) among fish sampled by Heuch *et al.* (2007). Future sampling efforts are necessary to collect more lumpfish and truly quantify their importance to *C. elongatus* dynamics within Cobscook Bay. Based on current data, though, threespine sticklebacks appear to be Cobscook Bay’s most important hosts and likely reservoirs of *C. elongatus*. The presence of *C.
*elongatus* infestations on wild fish throughout Cobscook Bay and throughout all months, including the fallow period, suggests that wild fish are likely reservoirs of *C. elongatus* to salmon farming operations. Fallowing may therefore be ineffective in regulating *C. elongatus* infestations on salmon farms in Cobscook Bay.

The wild fishes sampled during this study do not appear to serve as reservoirs of *L. salmonis* in Cobscook Bay. No *L. salmonis* individuals, of any life stage, were detected on wild fish sampled during the seven month survey. Though abundant fish species in Cobscook Bay are known to hosts *L. salmonis* in other parts of the world, they did not appear to play the same role in Cobscook Bay in 2012. Genetic differences in host preference and resistance to *L. salmonis* infestations by wild fishes between basins may explain this finding. Additionally, lower numbers of salmonids in Cobscook Bay relative to other systems may decrease infection pressure of *L. salmonis*. If wild fishes in the region do not carry *L. salmonis* infestations, fallowing may be an effective means of reducing the short-term infectious pressure of *L. salmonis* on the salmon farming operations. The failure to observe wild reservoirs of *L. salmonis* in this study indicates that no readily apparent sources of *L. salmonis* will be present to immediately re-infect the salmon post-fallowing. However, the fact that post-fallowing cultured salmon are re-infected by *L. salmonis* does not support the hypothesis that wild fish reservoirs of *L. salmonis* are completely absent from the region. Salmonids like Atlantic salmon and sea trout may be important hosts to *L. salmonis* in Cobscook Bay.

The results of this work inform and can be used to improve future sampling efforts in Cobscook Bay, which are necessary to increase understanding of sea lice dynamics. Future sampling has the potential to identify any as of yet unrecognized sea
lice hosts. Another year of sampling may resolve uncertainties regarding the causative factors of observed temporal trends in infection pressure. Additionally, the capture and examination of more lumpfish will better elucidate the role these fish play in hosting and potentially transferring sea lice. As important hosts of *C. elongatus* and observed hosts of the tapeworm *Schistocephalus solidus*, the infestations of threespine sticklebacks by multiple species of parasites can also be explored more fully. There may be yet unidentified relationships between stresses induced by both endoparasites and ectoparasites on threespine sticklebacks which affect their susceptibility to infection. Furthermore, all sampling in 2012 occurred after a 90 day fallowing period for farmed salmon from February to April, which may have had an influence on sea lice dynamics, and especially that of *L. salmonis*. Because fallowing only occurs once every three years, sampling in 2013 and 2014 will identify infestation trends in years when farmed salmon are present year-round. Finally, continued sampling is critical to confirm the distribution and role of sea lice in the wild fish community of Cobscook Bay as a whole.
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### Tables

Table 1. Seine and fyke net sampling sites in Cobscook Bay, Maine, in 2012.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Sub-Bay Position</th>
<th>Approximate Seine Tows Per Month</th>
<th>Approximate Fyke Sets Per Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deep Cove</td>
<td>44° 54.507’’</td>
<td>67° 1.113’’</td>
<td>Outer</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Broad Cove</td>
<td>44° 54.080’’</td>
<td>67° 0.084’’</td>
<td>Outer</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Carryingplace</td>
<td>44° 55.432’’</td>
<td>67° 0.941’’</td>
<td>Outer</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Cove</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East Bay</td>
<td>44° 56.435’’</td>
<td>67° 7.472’’</td>
<td>Middle</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Pennamaquon</td>
<td>44° 55.990’’</td>
<td>67° 8.277’’</td>
<td>Middle</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>River</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South Bay</td>
<td>44° 50.142’’</td>
<td>67° 2.891’’</td>
<td>Middle</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Burnt Cove</td>
<td>44° 50.380’’</td>
<td>67° 8.901’’</td>
<td>Inner</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Dennys Bay</td>
<td>44° 54.371’’</td>
<td>67° 9.356’’</td>
<td>Inner</td>
<td>11</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2. Pelagic trawl sampling sites in Cobscook Bay, Maine. Coordinates represent typical start locations in May 2012.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Sub-Bay Position</th>
<th>Tows per Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shackford Head</td>
<td>44° 53.543’</td>
<td>67° 0.968’</td>
<td>Outer</td>
<td>4</td>
</tr>
<tr>
<td>East Bay</td>
<td>44° 55.025’</td>
<td>67° 5.773’</td>
<td>Middle</td>
<td>2</td>
</tr>
<tr>
<td>South Bay</td>
<td>44° 53.744’</td>
<td>67° 4.827’</td>
<td>Middle</td>
<td>2</td>
</tr>
<tr>
<td>Whiting Bay</td>
<td>44° 52.483’</td>
<td>67° 8.739’</td>
<td>Inner</td>
<td>1</td>
</tr>
<tr>
<td>Dennys Bay</td>
<td>44° 53.388’</td>
<td>67° 9.843’</td>
<td>Inner</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 3. Benthic trawl sampling sites in Cobscook Bay, Maine. Coordinates represent typical start locations in May 2012.

<table>
<thead>
<tr>
<th>Site Name</th>
<th>Latitude (N)</th>
<th>Longitude (W)</th>
<th>Sub-Bay</th>
<th>Tows per Month</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treat Island</td>
<td>44° 53.107’</td>
<td>67° 0.467’</td>
<td>Outer</td>
<td>4</td>
</tr>
<tr>
<td>East Bay</td>
<td>44° 55.450’</td>
<td>67° 6.223’</td>
<td>Middle</td>
<td>2</td>
</tr>
<tr>
<td>South Bay</td>
<td>44° 52.754’</td>
<td>67° 4.045’</td>
<td>Middle</td>
<td>2</td>
</tr>
<tr>
<td>Whiting Bay</td>
<td>44° 51.104’</td>
<td>67° 8.602’</td>
<td>Inner</td>
<td>1</td>
</tr>
<tr>
<td>Dennys Bay</td>
<td>44° 52.899’</td>
<td>67° 8.966’</td>
<td>Inner</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 4. Fish species collected and examined in Cobscook Bay during 2012, with the scientific and common names, number examined, month(s) and sub-bay(s) of collection, and the type of gear(s) used to collect each species included. The * indicates the presence of sea lice infections.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common Name</th>
<th>Number Sampled</th>
<th>Months Sampled</th>
<th>Sub-Bays Present</th>
<th>Successful Gear</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alosa aestivalis</td>
<td>Blueback herring</td>
<td>7</td>
<td>6* 8,9</td>
<td>Outer, Central, Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Alosa pseudoharengus</td>
<td>Alewife</td>
<td>271</td>
<td>6,8,9,11</td>
<td>Outer, Central, Inner</td>
<td>Seine, FYKE, Pelagic</td>
</tr>
<tr>
<td>Apeltes quadracus</td>
<td>Fourspine stickleback</td>
<td>37</td>
<td>8,9</td>
<td>Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Clupea harengus</td>
<td>Atlantic herring</td>
<td>553</td>
<td>5,6* 9</td>
<td>Outer, Central, Inner</td>
<td>Seine, Benthic, Pelagic</td>
</tr>
<tr>
<td>Cyclopterus lumpus</td>
<td>Lumpfish</td>
<td>1</td>
<td>6*</td>
<td>Outer</td>
<td>Benthic</td>
</tr>
<tr>
<td>Enchelyopus cimbrius</td>
<td>Fourbeard rocking</td>
<td>1</td>
<td>5</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Fundulus heteroclitus</td>
<td>Mummichog</td>
<td>587</td>
<td>5,6* 8,9,11</td>
<td>Outer, Central, Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Gadus morhua</td>
<td>Atlantic cod</td>
<td>11</td>
<td>5,6</td>
<td>Outer</td>
<td>Benthic</td>
</tr>
<tr>
<td>Gasterosteus aculeatus</td>
<td>Three-spine stickleback</td>
<td>1996</td>
<td>3* 4* 5* 6* 8* 9* 11*</td>
<td>Outer, Central, Inner</td>
<td>Seine, FYKE, Pelagic</td>
</tr>
<tr>
<td>Gasterosteus wheatlandi</td>
<td>Blackspotted stickleback</td>
<td>882</td>
<td>4* 5* 6* 8* 9* 11*</td>
<td>Outer, Central, Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Hemitripterus americanus</td>
<td>Sea raven</td>
<td>15</td>
<td>5,6,8</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Hippoglossus hippoglossus</td>
<td>Atlantic halibut</td>
<td>10</td>
<td>5,6,8,9</td>
<td>Outer, Central, Inner</td>
<td>Benthic</td>
</tr>
<tr>
<td>Leucoraja ocellata</td>
<td>Winter skate</td>
<td>2</td>
<td>6</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Lophius americanus</td>
<td>Goosefish</td>
<td>1</td>
<td>6</td>
<td>Outer</td>
<td>Pelagic</td>
</tr>
<tr>
<td>Lumpenus lumpretaeformis</td>
<td>Snakeblenny</td>
<td>21</td>
<td>5,6</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Menidia menidia</td>
<td>Atlantic silverside</td>
<td>382</td>
<td>4,5,6,8,9,11</td>
<td>Outer, Central, Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Merluccius bilinearis</td>
<td>Silver hake</td>
<td>173</td>
<td>5,6,8,9</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Microgadus tomcod</td>
<td>Tomcod</td>
<td>76</td>
<td>5,6* 8,9</td>
<td>Outer, Central, Inner</td>
<td>Seine, FYKE</td>
</tr>
<tr>
<td>Myoxocephalus aeneus</td>
<td>Grubby</td>
<td>114</td>
<td>5,6,8,9</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Myoxocephalus octodecemspinus</td>
<td>Longhorn sculpin</td>
<td>177</td>
<td>5* 6* 8,9</td>
<td>Outer, Central, Inner</td>
<td>Benthic</td>
</tr>
<tr>
<td>Myoxocephalus scorpius</td>
<td>Shorthorn sculpin</td>
<td>3</td>
<td>6</td>
<td>Outer</td>
<td>Benthic</td>
</tr>
<tr>
<td>Osmerus mordax</td>
<td>Rainbow smelt</td>
<td>140</td>
<td>5,6* 8,9</td>
<td>Outer, Central, Inner</td>
<td>Seine, FYKE, Pelagic</td>
</tr>
<tr>
<td>Pholis gunnellus</td>
<td>Rock gunnel</td>
<td>1</td>
<td>6</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Pollachius viridis</td>
<td>Atlantic pollock</td>
<td>5</td>
<td>9</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Poronotus triacanthus</td>
<td>Butterfish</td>
<td>65</td>
<td>6,8,9</td>
<td>Outer, Central, Inner</td>
<td>Benthic</td>
</tr>
<tr>
<td>Pseudopleuronectes americanus</td>
<td>Winter Flounder</td>
<td>690</td>
<td>5,6* 8,9</td>
<td>Outer, Central, Inner</td>
<td>FYKE, Benthic</td>
</tr>
<tr>
<td>Pungitius pungitius</td>
<td>Ninespine stickleback</td>
<td>14</td>
<td>8* 9</td>
<td>Central, Inner</td>
<td>Seine</td>
</tr>
<tr>
<td>Raja eglanteria</td>
<td>Clearnose skate</td>
<td>1</td>
<td>9</td>
<td>Outer</td>
<td>Benthic</td>
</tr>
<tr>
<td>Raja erinacea</td>
<td>Little skate</td>
<td>1</td>
<td>6</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Raja senta</td>
<td>Smooth skate</td>
<td>2</td>
<td>5</td>
<td>Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Scomber scombrus</td>
<td>Atlantic mackerel</td>
<td>6</td>
<td>8,9</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
<tr>
<td>Scophthalmus aquosus</td>
<td>Windowpane</td>
<td>1</td>
<td>6</td>
<td>Outer</td>
<td>Benthic</td>
</tr>
<tr>
<td>Urophycis chuss</td>
<td>Red hake</td>
<td>31</td>
<td>5,6* 8,9</td>
<td>Outer, Central</td>
<td>Seine, Benthic</td>
</tr>
<tr>
<td>Urophycis tenuis</td>
<td>White hake</td>
<td>54</td>
<td>6,8,9</td>
<td>Outer, Central</td>
<td>Benthic</td>
</tr>
</tbody>
</table>
Table 5. Measured and unmeasured fish examined under a dissecting microscope.

<table>
<thead>
<tr>
<th>Species</th>
<th>Measured Fish</th>
<th>Unmeasured Fish</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alewife</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Atlantic cod</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Atlantic halibut</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Atlantic herring</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Blackspotted stickleback</td>
<td>50</td>
<td>1</td>
</tr>
<tr>
<td>Blueback herring</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Longhorn sculpin</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Lumpfish</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Mummichog</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ninespine stickleback</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Rainbow smelt</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Red hake</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Threespine sticklebacks</td>
<td>258</td>
<td>118</td>
</tr>
<tr>
<td>Tomcod</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Winter flounder</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Undetermined*</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>342</strong></td>
<td><strong>131</strong></td>
</tr>
</tbody>
</table>

*Did not differentiate between threespine and blackspotted sticklebacks
Table 6. Fish species found to host sea lice in Cobscook Bay. The number of infected fish is indicated for each species, as well as the overall infection intensity and prevalence and the months and sub-bays with infected fish.

<table>
<thead>
<tr>
<th>Species</th>
<th>Infected Fish</th>
<th>Median Intensity</th>
<th>Intensity IQR</th>
<th>Prevalence (%)</th>
<th>Months with Infections</th>
<th>Sub-Bays with Infestations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threespine stickleback</td>
<td>204</td>
<td>2</td>
<td>1</td>
<td>12.26</td>
<td>3,4,5,6,8,9,11</td>
<td>Inner, Central, Outer</td>
</tr>
<tr>
<td>Blackspotted stickleback</td>
<td>32</td>
<td>1</td>
<td>0</td>
<td>1.98</td>
<td>4,5,6,8,9,11</td>
<td>Inner, Central, Outer</td>
</tr>
<tr>
<td>Winter flounder</td>
<td>9</td>
<td>1</td>
<td>1</td>
<td>2.07</td>
<td>6</td>
<td>Inner, Central, Outer</td>
</tr>
<tr>
<td>Longhorn sculpin</td>
<td>2</td>
<td>1</td>
<td>N/A</td>
<td>1.72</td>
<td>5,6</td>
<td>Outer</td>
</tr>
<tr>
<td>Lumpfish</td>
<td>1</td>
<td>22</td>
<td>N/A</td>
<td>100</td>
<td>6</td>
<td>Outer</td>
</tr>
<tr>
<td>Mummichog</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0.17</td>
<td>6</td>
<td>Outer</td>
</tr>
<tr>
<td>Ninespine stickleback</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>7.69</td>
<td>8</td>
<td>Outer</td>
</tr>
<tr>
<td>Red Hake</td>
<td>1</td>
<td>5</td>
<td>N/A</td>
<td>3.33</td>
<td>6</td>
<td>Central</td>
</tr>
<tr>
<td>Rainbow smelt</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>1.45</td>
<td>6</td>
<td>Central</td>
</tr>
<tr>
<td>Tomcod</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>5.56</td>
<td>6</td>
<td>Central</td>
</tr>
<tr>
<td>Blueback herring*</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0*</td>
<td>6</td>
<td>Outer</td>
</tr>
<tr>
<td>Atlantic herring</td>
<td>1</td>
<td>1</td>
<td>N/A</td>
<td>0*</td>
<td>6</td>
<td>Inner</td>
</tr>
</tbody>
</table>

*Tentative infection event. Each fish had a solitary, unattached adult sea louse. Both fish were unmeasured and therefore not included in the prevalence analysis.
Figures

Figure 1. Sea lice life history diagram, adopted from Tully and Nolan (2002). Not all species of sea lice possess the preadult stages.
Figure 2. Image of *Lepeophtheirus salmonis* (top) and *Caligus elongatus* (bottom) adult females, with attached egg strings. Image courtesy of Mike Pietrak, ARI Umaine.
Figure 3. Map of Cobscook Bay. Seine sites are indicated by black circles and approximate trawl locations are marked by the black lines. Trawl locations are not exact.
Figure 4. Illustration of sea lice attachment positions on a threespine stickleback, the most commonly examined species. Because this is a side profile, the dorsal, ventral, and right side body surfaces are not shown.
Figure 5. Agarose gel images such as this were used to verify PCR success. Molecular weight markers were run on each gel, and were used in this image to define molecular weights along both sides of the image.
Figure 6. Multiple sequence alignment of COI gene sequence from various sea lice, comparing COI gene sequence of lice specimen 83 (Sample83COI) to that of the following published reference sequences: *C. elongatus* genotype 1, genotype 2, and *L. salmonis*. Highlighted nucleotides do not align with that of lice specimen 83.
Figure 7. Stacked bar graph of relative proportions of chalimii attached to the different locations on their hosts. n = 656, 556, 39, and 25 for fish of all species, *G. aculeatus*, *G. wheatlandi*, and *P. americanus* (threespine sticklebacks, blackspotted sticklebacks, and winter flounder), respectively. *Any chalimii attached to the stickleback pelvic spines were included in the pelvic fin category.*
Figure 8. Box plot of infection intensities of *G. aculeatus*, *G. wheatlandi*, and *P. americanus* (threespine sticklebacks, blackspotted sticklebacks, and winter flounder) from June (*n* = 117, 17, and 9, respectively). Edges of boxes are 25th and 75th percentiles, center lines are medians, whiskers are 5th and 95th percentiles, and dots are outliers. Significant differences between pairs are indicated by numbers below the boxplot.
Figure 9. Box plot of infection intensities of threespine sticklebacks. $n = 24, 117, 24, 32$, and $5$ for May, June, August, September, and November, respectively. Edges of boxes are 25$^{th}$ and 75$^{th}$ percentiles, center lines are medians, whiskers are 5$^{th}$ and 95$^{th}$ percentiles, and dots are outliers. Significant differences between pairs are indicated by numbers below the boxplot.
Figure 10. Bar graph of infection prevalence for *G. aculeatus*, *G. wheatlandi*, and *P. americanus* (threespine sticklebacks, blackspotted sticklebacks, and winter flounder, respectively) in June, August, and September. Prevalence calculations were based on the following sample sizes for *G. aculeatus*: \( n = 394, 666, \) and 522 for June, August, and September, respectively. For *G. wheatlandi*, \( n = 114, 250, \) and 238 for June, August, and September, respectively. For *P. americanus*, \( n = 268, 84, \) and 127 for June, August, and September, respectively.
Figure 11. Bar graph of infection prevalence for *G. aculeatus* and *G. wheatlandi* (threespine and blackspotted sticklebacks, respectively) from Inner, Central, and Outer Bay. Prevalence calculations were based on the following sample sizes for *G. aculeatus*: n = 384, 306, and 433 for Inner, Central, and Outer Bays, respectively. For *G. wheatlandi*, n = 252, 202, and 63 for Inner, Central, and Outer Bays, respectively.
Figure 12. Bar graph of infection prevalence for threespine sticklebacks collected in different sub-bays and months. Prevalence calculations were based on the following sample sizes for Inner bay: n = 184, 183, 187, and 9 for June, August, September, and November, respectively. For Central Bay, n = 87, 291, 134, and 0 for June, August, September, and November, respectively. For Outer Bay, n = 123, 192, 196, and 45 for June, August, September, and November, respectively.
Author’s Biography

Hailing from landlocked Granton, WI, Alex Jensen traveled to Orono, ME, to pursue a major in marine sciences and a minor in fisheries. He became immersed in the expansive field of fisheries science after getting involved in research during the fall of his freshman year, and has since been fortunate enough to participate in fisheries research throughout the state of Maine and as far south as Cocodrie, Louisiana. His venture into the realm of parasitology through this thesis project opened his eyes to the wide array of interactions between the environment and biological populations, and established a strong interest in pursuing this line of research in the future. Outside of science, Alex enjoys a healthy dose of reading, hiking, and playing any sport requiring a racquet. After graduation, Alex plans to attend graduate school and continue to pursue his passion for fisheries science.