Effects of water temperature, diet, and bivalve size on the ingestion of sea lice (*Lepeophtheirus salmonis*) larvae by various filter-feeding shellfish

by

Janis Louise Webb
B.Sc., University of Guelph, 1979
B.Ed., University of Alberta, 1984

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Geography

Supervisory Committee

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Abstract

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The sea louse (*Lepeophtheirus salmonis*), whose larvae are planktonic and disseminated in the water column, is an economically important parasite of Atlantic salmon (*Salmo Salar*). The effect of temperature (5, 10, 15°C), diet (larvae alone, larvae plus phytoplankton), and bivalve size (small, medium, large) on the amount of *L. salmonis* larvae ingested by various species of filter-feeding bivalves (Pacific oysters, Pacific scallops, blue/Gallo's mussel hybrids, basket cockles) was examined in a series of laboratory experiments. Four separate temperature/diet experiments were conducted (one for each species) in which large bivalves were individually placed in 2-L containers holding 750 ml of aerated, filtered seawater and fed one of three treatment diets: (1) phytoplankton: ~7.1 x 10^4 cells ml^{-1} of *Isochrysis* sp. (Tahitian strain, TISO); (2) sea lice larvae: ~431 larvae (mostly nauplii); and (3) phytoplankton and larvae (at the levels mentioned above). There was also a control treatment of phytoplankton and larvae, but no bivalve. After feeding for 1 h, the bivalve soft tissues were excised and preserved, the digestive system was dissected, and sea lice larvae were removed and counted to provide direct evidence of ingestion. The larvae remaining free swimming in the container were

preserved and counted. The proportion missing from the container was used to estimate ingested larvae in statistical analyses. Two additional experiments investigating the effect of bivalve size (small, medium, large) on the ingestion of sea lice larvae were conducted with Pacific oysters and Pacific scallops. The heights for oysters (anterior-posterior axes) were 19.2, 44.2, and 84.0 mm, and scallops (dorsal hinges to ventral margins) were 40.3, 64.1, 102.7 mm. The methodology for the size experiments was as previously described for the temperature/diet experiments with the following changes: (1) the diet of larvae alone was not used; (2) the mean number of larvae in each container was ~498; (3) the mean concentration of TISO added to each container was ~7.8 $x10^4$ cells ml^{-1}, and (4) the mean water temperature was 10.4°C. The data for the four temperature/diet experiments indicate that all four bivalve species ingested sea lice larvae, whether their diet included phytoplankton or not, and that temperature had no significant effect. The data for the two size experiments indicated that all three sizes of oysters and scallops ingested sea lice larvae and that there was a significant size effect. Large shellfish consumed a significantly greater proportion of the sea lice larvae than the small shellfish. Bivalves grown at salmon net pens as part of an IMTA (Integrated Multi-Trophic Aquaculture) system may be able to reduce the number of sea lice larvae as well as being an additional crop of market value. Future research, conducted at a commercial scale at a salmon farm, is warranted in order to determine if bivalves can serve in this role.

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Abbreviations

General
BC—British Columbia, Canada
DFO—Department of Fisheries and Oceans Canada
IMTA—Integrated Multi-trophic Aquaculture
MHC—Marine Harvest Canada
NB—New Brunswick, Canada
PBS—Pacific Biological Station
Temp.—temperature
Chl—chlorophyll

Diet components
B—bivalve
C—copepodids
L—larvae, when used in the context of a component of a diet
N—nauplii
P—phytoplankton; TISO algae
TISO—*Isochrysis* sp., Tahitian strain, golden/brown flagellate

Diet combinations
BL—diet of larvae alone in presence of a bivalve
BP—diet of phytoplankton alone in presence of a bivalve
BPL—diet of phytoplankton and larvae in presence of a bivalve
PL—control diet of phytoplankton and larvae, without bivalve

Statistics
ANOVA—Analysis of Variance
LSN—least significant number
Pcons—proportion of larvae consumed by bivalve
SE—standard error of means
SD—standard deviation

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Dedication

To my parents:

- Douglas C. Webb, B. Sc., P. Eng., WWII Lancaster pilot, Canadian diplomat, and dad,
- Helen M. (Simonson) Webb, the intrepid establisher of family homes on three continents, driver in Tehran, and mom.

Chapter 1—Introduction

1.1. Summary

Bivalves have been successfully co-cultured with fish (Sarà et al., 2009). The bivalves can bolster the farm's income (Whitmarsh et al., 2006), while consuming a portion of the farm's organic waste material (*i.e.* faeces and uneaten food) (Barrington et al., 2009). Blue mussels (*Mytilus edulis*) can capture and absorb fish feed particulates as well as fish faeces (Reid et al., 2010). Different species of bivalves can consume significant quantities of mesozooplankton (length: 0.2–2 mm) (Horsted, et al., 1988; Kimmerer, et al., 1994; Lehane and Davenport, 2002; Maar et al., 2008) while *M. edulis* has recently been shown to ingest larvae of sea lice (*Lepeophtheirus salmonis*) in laboratory trials (Molloy et al., 2011). It is this latter point about bivalves consuming mesozooplankton that is at the centre of this research and which could be of interest to salmon farmers.

The salmon farming industry is economically important globally (FAO, 2010), to Canada, and to British Columbia (BC) (Statistics Canada, 2009). The sea louse, *L. salmonis*, is one of several species of sea lice found in nature that are external parasites on salmon, causing stress and creating skin lesions (Pike and Wadsworth, 1999). The cost of damage to fish and treatment to rid the fish of lice, more than US $480 million in one year, is expensive for the global salmon farming industry (Costello, 2009b). Sea lice from salmon farms are blamed for infesting wild salmon stocks with lice (Krkošek et al., 2007; Costello, 2009a) and may be vectors for fish diseases (Barker et al., 2009). Salmon farmers can employ a variety of husbandry methods to control sea lice numbers and, when an outbreak occurs, a chemotherapeutant can be used that kills the lice on the

salmon (Brooks, 2009). Sea lice can develop a resistance to the drug, however, as has been shown in New Brunswick (Barrington et al., 2009).

If sea lice could be controlled, without the use of medication, salmon farmers would be relieved of an expensive problem at the same time that public concern over sea lice might be reduced. Individual blue mussels (*M. edulis*) consumed up to 25 *L. salmonis* larvae within 1-h in laboratory trials (Molloy et al., 2011). Since various species of bivalves have been shown to consume mesozooplankton and sea lice larvae (length: 0.5–0.7 mm) are in the mesozooplankton size range, various shellfish species may be able to consume sea lice larvae.

This thesis tests the hypothesis that various bivalve species can consume the larvae of sea lice (*L. salmonis*) under controlled laboratory conditions and specifically examines the effects of shellfish size, seawater temperature, and the presence of phytoplankton on the ingestion rates. If commercially valuable bivalve species of different sizes consume larvae under conditions that exist at salmon farms, bivalves may be considered for year-round sea lice control at salmon farms, an additional role in integrated multi-trophic aquaculture (IMTA).

1.2. Salmon Aquaculture

Consumers' demand for fish is increasing while wild fish stocks are being depleted (FAO, 2010). Salmon aquaculture may relieve pressure on wild salmon stocks, help meet the market demands for fish protein, provide needed jobs in rural coastal communities, and contribute to the local economy. Salmon aquaculture is a large industry worldwide; in 2009, 1,440,725 tonnes of farmed Atlantic salmon (*Salmo salar*) were produced, worth more than US $6.4 billion (FAO, 2011).

Canada is the fourth largest producer of farmed salmon in the world, producing over 100,000 tonnes worth nearly CA $600 million in 2009—$46 million, $159 million, and $394 million being generated in Nova Scotia, New Brunswick (NB), and British Columbia, respectively (Statistics Canada, 2010). In BC, Atlantic salmon was the most significant seafood commodity in 2008, worth CA $455.5 million wholesale (compared to $135.2 million for all species of wild salmon) in a provincial seafood industry worth $1,216.3 million (BC Ministry of Environment, 2010). Most farmed salmon produced in Canada are exported to the United States (USA) with salmon exports to the USA in 2009 being valued at CA $503.8 million (Statistics Canada, 2010).

1.3. Organic Particulates at Salmon Farms

Farmed salmon are grown in relatively dense groupings within net pens (also called sea cages) in coastal areas. The net pens are commonly 30 m x 30 m in surface area and up to 30 m deep. They can hold 35,000–50,000 salmon. The openings in the netting allow water to flow through the pen, carrying fish faeces and feed particles away as the water is refreshed inside the pen. More dense particles drop down through the net pen, and finer particles settle out around the fish farm. The impacts are generally localized at net pens, but they can range from 15 to 205 m downstream (Brooks et al., 2002).

Feed formulation has improved over the years so that the fish digests a greater proportion of the feed resulting in fewer faeces produced. However, feed remains the largest expense to the production of salmon and underwater cameras monitor feeding behaviour. The addition of feed is terminated when the fish stop feeding and less feed is lost through the net pens. Approximately 5% of the feed goes uneaten while 4.25% goes into faecal production (Brooks and Mahnken, 2003). The organic particles of fish feed

and fish faeces can accumulate on the sea floor around fish farms. This results in increased organic matter, reduced oxygen, stimulation of sulphur-reducing bacteria, and leads to the production of ammonia or hydrogen sulphide gas leading to substantial changes in the infaunal community (Brooks and Mahnken, 2003). When organic loading is at its peak, the infaunal community consists of animals that can tolerate both high organic and sulphide levels (Brooks et al., 2002). The community may return to a more natural state, however, when the farm is left to fallow (Macleod et al., 2006). During a study in which salmon farms in the Broughton Archipelago (BC) were harvested and the effect of the waste on the benthos was followed over time, researchers found a succession of benthic infaunal communities. Twenty months after the harvest of the fish, the communities resembled a reference site without farmed fish, except for the presence of some rare species at the reference site not present at the farm site (Brooks and Mahnken, 2003).

To meet the requirements of the Canadian Environmental Assessment Act, salmon farms in Canada undergo an environmental assessment before being approved. The farms must also meet the requirements of the federal Fisheries Act. By their licensing, farms must comply with numerous requirements (Fisheries and Oceans Canada, 2010), including to: not exceed a specified peak mass of fish, comply with a benthic monitoring program, not exceed a set mean concentration of sulphide (soft substrate) or *Beggiatoa* sp. bacteria or organic particulate material (hard substrate), and conduct sediment sampling or video surveys.

1.4. Integrated Multi-Trophic Aquaculture

Salmon farmers are motivated to reduce organic accumulation at their farm sites in order to meet regulatory conditions. Blue mussels (*M. edulis*), native to the east coast of Canada, have the ability to extract organic waste particles originating from fish farm operations that could otherwise affect the immediate area around a farm (Reid et al., 2009). They can filter and absorb both fish feed particulates and fish faeces (Reid et al., 2010) and, in the Bay of Fundy (NB), showed increased feeding activity (MacDonald et al., 2011) and growth (Lander, 2006) when grown adjacent to salmon aquaculture sites, compared to mussels at reference sites a few hundred metres away. Pacific oysters (*Crassostrea gigas*), which are a west coast species, grown at a Chinook salmon (*Oncorhynchus tshawytscha*) farm in BC's Jervis Inlet from June to October 1989, had greater growth inside than immediately outside a net pen, which in turn was greater than growth for test oysters grown a few hundred metres distant from the farm (Jones and Iwana, 1991). Growth was the least at control sites kilometres away from the salmon farm, although it equalled typical cultivated oyster growth. Similarly, in the Mediterranean, when Gallo's mussels (*Mytilus galloprovincialis*) were co-cultured in suspension with cages of seabass (*Dicentrarchus labrax*) and seabream (*Sparus aurata*) for one year, the mussels grew larger than those at reference sites 1,000 m upstream from the farm (Sarà et al., 2009). In addition to environmental improvement, there can be an economic benefit when the bivalves are sold (Whitmarsh et al., 2006).

Mussels (*M. edulis*) growing at salmon farms in BC were shown to have a bactericidal effect in clearing water of suspended cells of the agent responsible for bacterial kidney

disease in salmon (*Renibacterium salmoninarum*) and killing most (Paclibare et al., 1994). Mussels at salmon farms may have a role in disease control.

Co-culturing fish with bivalves has been expanded to include multiple species at multiple trophic feeding levels in the ecological approach to fish farming called integrated multi-trophic aquaculture (IMTA). The concept behind IMTA is that organisms belonging to one trophic level can make use of nutrients in the aquaculture system that another trophic level cannot, *i.e.* the waste of one species is food for another The fish, at the highest trophic level, are the fed component in the system (Barrington et al., 2009).

While suspended bivalves may feed on the less dense particles transported to them in the water column, other components are needed in the IMTA system to uptake the more dense feed and fecal particles that drop down through the net pens and the inorganic molecules resulting from fish excretions. More advanced IMTA systems may help mitigate the accumulation of organic waste directly below the fish net pens by incorporating various deposit-feeding invertebrates, such as sea urchins, sea cucumbers, and polychaete worms. Similar to the aforementioned suspended bivalves, these invertebrates would feed on and sequester this organic material, thus reducing organic accumulation directly below the pens (Barrington et al., 2009).

To extract excess inorganic molecules, such as nitrogen and phosphorus, seaweeds can be incorporated into the IMTA system. The seaweeds intercept inorganic molecules transported downstream in the water and incorporate these nutrients as they grow (Chopin et al., 2001). Kelp (*Saccharina latissima*) grown near sablefish (*Anoplopoma fimbria*) net pens on Canada's west coast grew significantly longer than kelp grown at a

reference site located away from the farm (E. Prussin, 2011, personal communication). Chopin et al. (2004) reported that seaweed production was 46% greater when the algae was grown in proximity to a salmon farm compared to a reference site 1250 m away. By removing excess organic and inorganic material around the finfish operation, these invertebrate and algal components of an IMTA system help to create an improved, more environmentally sustainable, fish farm site. Bivalves, sea cucumbers, sea urchins, polychaetes, and seaweeds can all be value-added products for those fish farms that practice IMTA, and can reduce economic risk through product diversification (Ridler et al., 2007).

1.5. Life History of Sea Lice

The sea lice *L. salmonis* and *Caligus clemensi* belong to the phylum Arthropoda, sub-phylum Crustacea, class Maxillopoda, subclass Copepoda, order Siphonostomatoida, and family Caligidae. Members of the family Caligidae are responsible for the majority of problem outbreaks of sea lice in aquaculture (Johnson et al., 2004). *C. clemensi* are found on salmonids and a number of other marine fish species including Pacific herring (Parker and Margolis, 1964). *Caligus* have lunules (sucker-like features) on their frontal plates, which help distinguish them from *Lepeophtheirus*. *L. salmonis,* also known as salmon lice, are salmonid specialists and, in the northeast Pacific, infect wild and farmed Pacific salmon (*Oncorhynchus* spp.), farmed Atlantic salmon (*S. salar*), and trout (Pike and Wadsworth, 1999).

Lepeophtheirus cuneifer is also found in the northeast Pacific and is a generalist species that has also been observed on the salmonids *Oncorhynchus mykiss* and *S. salar*.

Adults may be distinguished from *L. salmonis* by differences in the genital complex, and abdomen, which is much shorter (Johnson and Albright, 1991a).

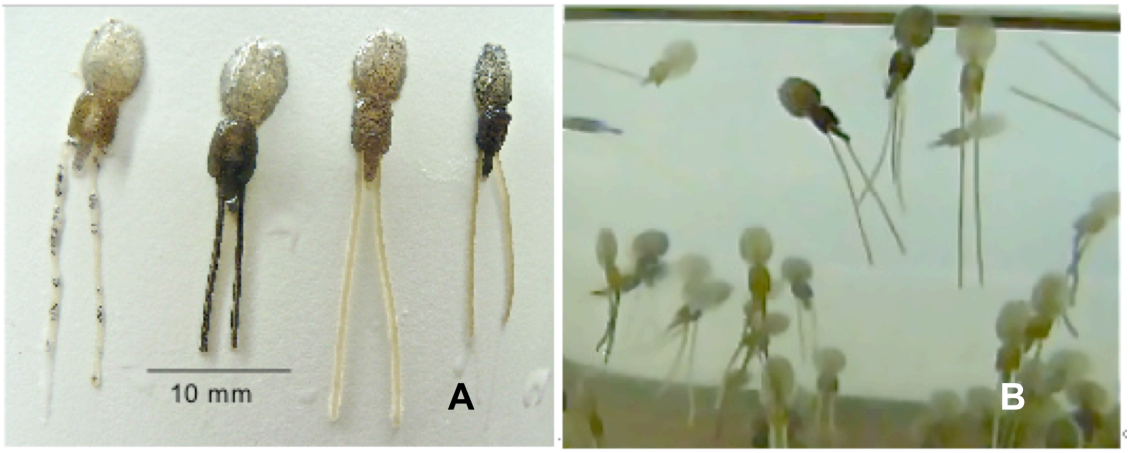

Figure 1. Photos of (A) gravid sea lice (*Lepeophtheirus salmonis*) with egg strings at various stages of development and (B) gravid sea lice swimming

Adult gravid female sea lice (length: ~10 mm) carry two egg strings (Figure 1) that can hold 55–704 fertilized eggs string^{-1} (4–25% non-viable). Adult females survived a maximum of 191 days on salmon held at 7.2ºC in the laboratory and produced up to 11 pairs of egg strings. Between 4 and 25% of eggs were non-viable. The first pair of egg strings was typically shorter and held fewer eggs. (Heuch et al., 2000). The number of eggs depends on factors such as louse size, egg string length, season, species of salmon, and whether the salmon has previously been treated with a chemotherapeutant (Pike and Wadsworth, 1999). The average development rate of eggs is 8.6 days at 10°C (Johnson and Albright, 1991b).

Figure 2. Photos of *Lepeophtheirus salmonis* (A) a hatching nauplius, (B) an adult louse surrounded by larvae, and (C) a copepodid

There are three larval stages in the life cycle of the salmon louse: nauplius 1, nauplius 2, and copepodid (Figures 2, 3 and 4). The nauplius 1 hatches into the seawater as a live, free-swimming larva. Usually, the egg string is still attached to the female louse on a salmon during hatching. The nauplius 1 stage is about 500–580 µm long. Zooplankton within the 200 µm to 2000 µm size range can be classed as mesozooplankton. The nauplius 1 moults into the nauplius 2 stage, which is similar in size. After another moult, the larva enters its third planktonic stage, the copepodid, which is about 700 µm long (Johnson and Albright, 1991c). The amount of time between moults varies with water temperature (Johnson and Albright, 1991b). All three planktonic stages of sea lice larvae are in the middle of the mesozooplankton size class.

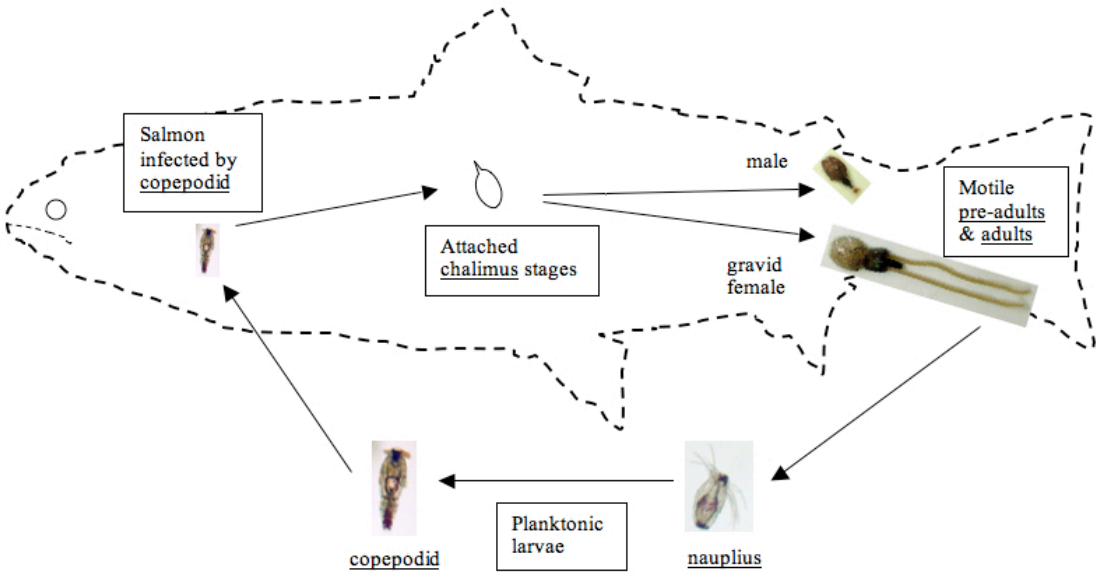

Figure 3. Simplified life cycle of *Lepeophtheirus salmonis*

The copepodid is the infective stage and does not remain planktonic if it can find a suitable fish host on which to hook. At temperatures of 8–10ºC, copepodids can survive approximately 7 d (Johnson and Albright, 1991b). Upon encountering a fish, the copepodid clings to the animal using its clawed antennae and its maxillipeds, and begins its first feeding on the fish mucus and skin. It then moults to the chalimus 1 stage and attaches itself to a fish scale or other hard structure by a frontal filament. *L. salmonis* have three additional chalimus stages, all attached, followed by two pre-adult stages, which have no frontal filament and are motile. After another moult, the larger and still motile adult is ready to mate. Adult females have W-shaped genital segments and can produce the aforementioned egg strings. Adult males have U-shaped genital segments, and are approximately half the length of the females (Pike and Wadsworth, 1999).

In the northeast Pacific, salmon lice have been found on non-salmonid marine fish. In the Broughton Archipelago of BC in spring, threespine sticklebacks (*Gasterosteus*

aculeatus) were frequently observed carrying sea lice, mostly *L. salmonis*, but some *C. clemensi* at the chalimus stage. Very few lice were adults and laboratory studies showed that salmon lice do not complete their life cycle on fish other than salmonids (Jones and Prosperi-Porta, 2011).

Pacific and Atlantic strains of *L. salmonis* have some genetic differences (Yazawa et al., 2008). Infestations of the Pacific strain of salmon lice on farmed Atlantic salmon in BC seem to be lower, and less pathogenic, than infestations of the Atlantic strain of salmon lice on farmed Atlantic salmon of eastern Canada and Europe (Saksida et al., 2007).

1.6. Sea Lice Larvae in the Field

When gravid females were present at salmon farms in a Scottish loch, sea lice larvae were found at river mouths in the intertidal zone at peak spring densities of 33–143 larvae m^{-3} (McKibben and Hay, 2004). Water sampled adjacent to salmon farms that were infected with sea lice contained mostly nauplii, whereas copepodids were widely dispersed (Costelloe et al., 1996; Penston et al., 2008), likely due to localized currents (Pahl et al., 1999). In another study, densities of copepodids were significantly correlated with the numbers of gravid salmon lice on the farmed salmon (Penston and Davies, 2009).

The larvae of salmon lice are positively phototactic and tend to be found at greatest densities in the top few metres of the water column during the day. Copepodids are even more responsive to light than nauplii and demonstrate diel vertical migrations, gathering near the surface during the day (Heuch, 1995). The copepodids sink to deeper water at night and it is thought that as salmon swim through them to reach surface water to feed at

night, the fish may come into contact with the infective copepodids. In experiments with long bags suspended in the sea containing sea lice larvae, copepodid mean depths ranged from 1.95 m (day) to 3.63 m (night) in 6 m bags and 2.82 m (day) to 6.63 m (night) in 12 m bags, indicating that larval depth may be somewhat relative to the depth of the water column. Nauplii in the 12 m bag were found at mean depths of 7.5 m (day) to 9.4 m (night) (*i.e.* deeper than copepodids) (Heuch et al., 1995).

Novales Flamarique et al. (2010) used an LED-based light to monitor and capture sea lice. In a tank, the light trap was most successful at capturing planktonic larval stages (70% of individuals removed), but it also captured adult lice from Chinook salmon (8%). In the open water, it caught 21 sea lice of two species and at several life stages when none were caught in a plankton tow (Novales Flamarique et al., 2010). Halogen light traps were previously tested in a salmon net pen during darkness in Maine, USA in 1997 (Pahl et al., 1999). They were reported to be successful in attracting the larvae of *L. salmonis* and reducing sea lice counts on farmed salmon. However, the light also attracted the larvae of other, desirable, species such as the American lobster, although it was thought that the trap could be modified to be more specific in the size of larvae retained (Pahl et al., 1999). If water is stratified in salinity, sea lice larvae will gather at strong haloclines and avoid low-salinity surface water, even though there is the attraction of daylight (Heuch, 1995). Copepodids will change their swimming behaviour to avoid salinities lower than 27. Copepodid survival decreased gradually between 29 and 16 and rapidly below 12 (Bricknell et al., 2006).

Sea lice have chemical receptors and alter their swimming behaviour based on sensing a salmon odour. It has been suggested that inter-specific semiochemicals could be

investigated and developed so that sea lice are repelled from a salmon cage while they are attracted by another kairomone to an odour trap (Mordue and Birkett, 2009).

1.7. The Cost of Sea Lice to the Industry

At salmon farms, where fish are maintained in net pens at relatively high densities and are readily available hosts for the parasite, *L. salmonis* are present year-round and have been responsible for epizootics (Boxaspen, 2006). The abundance of lice at salmon farms rises during the autumn in BC. This is likely due to a transfer of sea lice from wild salmon returning from the open ocean to farmed salmon as the wild fish migrate past salmon farms to their natal rivers (Saksida et al., 2007; Marty et al., 2010).

Sea lice are an economic burden to the salmon culture industry with estimated global costs in 2006 of US $480 million plus 6% of the product value (Costello, 2009b). The purchase of medications and equipment and the value of staff time account for the largest part of this cost. Reduced fish growth rate and feed conversion efficiency as well as market downgrading due to damage caused by lice account for additional significant costs of sea lice at salmon farms. As profit margins become narrower, the cost of sea lice control remains a significant limitation to the farm operation.

Sea lice are a concern wherever salmon farming is practiced. *L. salmonis*, as a salmonid specialist, affects all the key salmon farming areas in the northern Pacific and Atlantic including Canada (both BC and NB), Scotland, and Norway. *Caligus elongatus* is a generalist sea lice species and found on more than 80 species in the Atlantic including farmed salmon. In Chile, most sea lice infestations are caused by *Caligus rogercresseyi* (Bravo et al., 2009), although *Caligus teres* has also been a problem species for the salmon farming industry there (Revie et al., 2009).

Adult sea lice can eat through fish skin to tissues below causing lesions and stress. Erosion of the epidermis may create osmoregulatory complications for infected fish (Pike and Wadsworth, 1999). Sea lice carry bacteria that, if transferable, are potentially pathogenic to fish. Several bacterial species have been isolated from external and stomach samples of *L. salmonis*, *e.g. Tenacibaculum maritimum* that may be implicated in infectious gill disease (Barker et al., 2009).

1.8. Control of Sea Lice

It is important that salmon farms monitor and control sea lice numbers, not only for the health of the salmon, to avoid the cost of treating an outbreak with chemotherapeutants, or harvesting at an inopportune time, but also to ensure low sea lice numbers so wild salmon are not potentially infected from farm sources. In BC, it is stated in the finfish aquaculture license that salmon farms must initiate action to reduce mean sea lice numbers to less than three motile *L. salmonis* per Atlantic salmon (Fisheries and Oceans Canada, 2010). From March through June, decisive action is required if sea lice numbers exceed this limit, because it is in spring that wild juvenile salmon exit rivers into coastal waters on their migration to the open ocean.

Sea lice on farmed salmon can be controlled most of the time by utilizing a variety of husbandry techniques, as practiced in BC and other regions. A single-year class is grown at a farm site and, ideally, this is synchronized at all farms in the same area. When salmon are harvested, the site or area should be left fallow between production cycles depriving lice of salmon to feed on. Biofouling build-up on net pens should be removed

so water can easily flow through and sea lice larvae are not retained within the pens to re-infect salmon (Brooks, 2009).

When sea lice outbreaks occur, the salmon may be harvested as appropriate, or treated with a medication that kills the sea lice on the fish. The current chemotherapeutant of choice is emamectin benzoate (SLICE®), which is provided to the fish in medicated feed. During a 2003–2005 study of sea lice at salmon farms in the Broughton Archipelago (BC) sea lice medication was used relatively infrequently, on average 1.6 times per production cycle (Saksida et al., 2007).

Emamectin benzoate could be considered a very successful method for controlling outbreaks of sea lice at salmon farms. It is quite effective in BC. However, in a multi-year Scottish experiment, the medication was showing signs of reduced efficacy due to sea lice becoming less sensitive to the chemical (Lees et al., 2008). In the Bay of Fundy (NB) emamectin benzoate has lost its effectiveness for the treatment of sea lice on farmed salmon indicating that lice may have become resistant to the drug (Burridge et al., 2010). Instead of emamectin benzoate, New Brunswick salmon farms were permitted to use, on a limited basis, AlphaMax™ (deltamethrin), Salmosan® (azamethiphos), and Interox® Peramove® 50 (hydrogen peroxide). By 2011, enough well boats were in place that the hydrogen peroxide treatment could be administered with improved timing and effectiveness, and sea lice were kept under control by this means after the typical spring rise in lice numbers (Atlantic Canada Fish Farmers Association, 2011).

An effective non-chemical method, or several non-chemical methods, of reducing sea lice at farms would be highly beneficial, as there would be no question of drug resistance. Native to the Atlantic, several small cleaner-fish species in the wrasse family (Labridae)

have been shown to feed on sea lice on the salmon. These small fish are being grown in Norwegian net pens along with salmon to help control sea lice by biological means (Costello, 2006). The species of wrasse used in salmon farming are not a Pacific species.

To reduce sea lice larvae by non-chemical means, the previously mentioned light trap is another potential non-chemical method and this has been tested in the Atlantic and Pacific, including British Columbia (Pahl et al., 1999; Novales Flamarique et al., 2010). Similarly, growing bivalves at salmon farm sites has the potential to remove larvae from the water column. In the current research, bivalves were investigated as a potential biological control of sea lice larvae. If the number of planktonic larvae can be reduced then the number of infections of sea lice on salmon (both cultured and wild) might also be reduced.

1.9. Potential for Bivalves to Consume Sea Lice

More than 20 years ago, bivalve ingestion of zooplankton in estuarine environments was described. Horsted et al. (1988) showed that blue mussels (*M. edulis)* preyed on smaller zooplankton while fish consumed larger zooplankton. A few years later, a small, introduced clam was identified as the likely cause of substantial declines of three species of copepods in upper San Francisco Bay, USA (Kimmerer et al., 1994). During that study, a clam was observed ingesting copepod nauplii. More recently, research on the significance of bivalves preying on mesozooplankton has become more topical. The blue mussel was reported to be a “significant consumer and destroyer of mesozooplankton” (Davenport et al., 2000). Suspended blue mussels of various size classes (mean shell lengths: 2.0, 3.5, and 5.3 cm) consumed mesozooplankton of a mean size of 450–600 μm,

and three different species of bivalves were shown to prey upon such zooplankton (Lehane and Davenport, 2002). In another study, a wide size range of zooplankton were found in the stomachs of blue mussels, including amphipods 1000–6000 µm, harpacticoid copepods 231–1281 µm, and crustacean nauplii 373–588 µm (Lehane and Davenport, 2006). More recently, zooplankton were shown to be depleted due to raft-cultured Gallo's mussels and associated epifauna in an aquaculture operation in Spain. Depletion of phytoplankton, represented by chlorophyll (chl) *a*, was greater than for zooplankton (Maar et al., 2008).

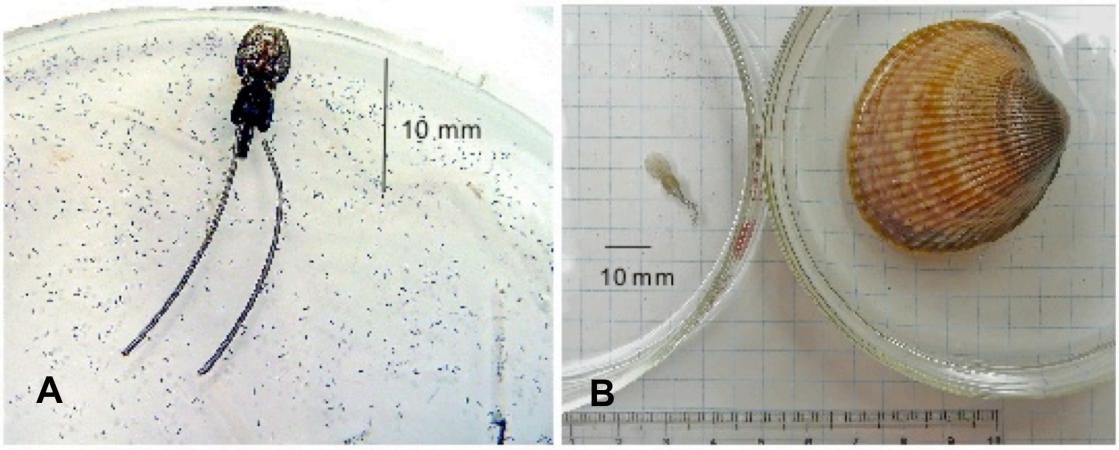

Figure 4. Photos showing (A) relative sizes of gravid sea louse (*Lepeophtheirus salmonis)* and larvae, and (B) gravid sea louse and adult cockle (*Clinocardium nuttallii*) (height (dorsal hinge to ventral margin): ~43 mm)

In another study, sea lice copepodids were fed to 10 individual blue mussels for up to 1 h, and the mussels were then dissected. Between one and 15 copepodids were retrieved from the guts of five of the mussels after dissection, and copepodids were observed on the foot, gill, mantle, and in the buccal cavity of four others. Only one individual did not ingest a copepodid (Molloy et al., 2011). If bivalves of a species preferred by the farmer

can consume a significant quantity of sea lice larvae at salmon farms, bivalves could prove to be a non-chemical method of controlling sea lice.

1.10. Factors Affecting Bivalve Filtration Rates and Ingestion

To ingest sea lice larvae, bivalves must capture larvae as they filter seawater and select the larvae to continue into the digestive system. Different current speeds permit maximum clearance rates for different bivalve species (Cranford et al., 2011). Significantly more zooplankton, including copepodites 300–600 μm, were filtered by mussels and epifauna of a *M. galloprovincialis* raft culture at greater current speeds (4–5 cm s^{-1}) than at lower current speeds (Maar et al., 2008). One explanation for this is that, unlike phytoplankton, mesozooplankton may be able to escape a bivalve either by their sensitivity in detecting the bivalve's inhalant flow early enough to escape or by using a short burst of speed as an escape jump (Green et al., 2003). Mesozooplankton swimming speeds tend to be in the range of 1 to 2 cm s^{-1}. *M. edulis* (length: 30–50 mm) created a flow rate that was sufficient to capture larvae of both *Artemia* sp. and *Tigriopus brevicornis* once they were in the inhalant stream (Davenport et al., 2000). In an experiment by Heuch and Karlsen (1997), when stimulated by vibrations at 3 Hz, copepodids with a normal swimming speed of ~2 cm s^{-1} were induced to swim 9 cm in the first second.

Significant ingestion of mesozooplankton by the blue mussel *M. edulis* has been shown (Davenport et al., 2000). Ingestion of mesozooplankton has been reported in mussels, cockles and scallops (*M. edulis*, *Cerastoderma edule* and *Aequipecten opercularis*) (Lehane and Davenport, 2002). Bivalve ingestion of mesozooplankton (such as copepod larvae) was reported in three size classes of suspended and two size classes of benthic

blue mussels, and the mean number of zooplankton ingested increased with bivalve size (Lehane and Davenport, 2002).

Few studies consider the combined effects of phytoplankton and mesozooplankton—such as sea lice larvae—on bivalve ingestion. Kamiyama (2011) showed that when phytoplankton concentration is low, microzooplankton become a more important component in the diet of the oyster, *C. gigas*. In a microcosm study, Wong and Levinton (2004) demonstrated that *M. edulis* could grow on diets of zooplankton (rotifers *Brachionus plicatilis*; mean length 255.8 μm) alone, phytoplankton (*Tetraselmis* sp.) alone, or a mixture of the two.

If bivalves could be effective in controlling sea lice by ingesting their larvae, one concern is that bivalves should be able to filter mesozooplankton during winter when water temperatures are cold and phytoplankton can be at low concentration. If low phytoplankton levels or low temperature were to significantly influence whether bivalves open their valves to ingest sea lice larvae in the laboratory, then this could be an indication of seasonality such that bivalves cultivated at salmon farms may not ingest sea lice larvae effectively during the winter. Maximum feeding rates may be affected by temperature when bivalves are being tested for maximum filtration under ideal conditions, such as with the provision of high quality algae in the laboratory (Cranford et al., 2011). In the field, temperature has not been shown to be an important control of filtration rate of mussels or scallops. *Placopecten magellanicus*, for instance, had variable filtration at 3°C, which at times reached maximum filtration (Cranford et al., 2005). Seston (*i.e.* phytoplankton, flagellates, ciliates, zooplankton, detritus) concentration, however, does influence bivalve clearance rate. Bivalve ingestion of lower quality seston

is less than that shown for a diet of higher quality algae. At low seston there was an initial peak in clearance of chl *a* for both *M. edulis* and *Pecten maximus*. As chl *a* concentration increased, the clearance rate was largely maintained by *M. edulis* while it gradually decreased with *P. maximus* (Strohmeier et al, 2009). Strohmeier et al, (2009) also demonstrated large individual bivalve variation. Factors that affect bivalve filtration rate include fluid dynamics, salinity, gut capacity, digestion time, and composition and nutritional value of food (Cranford, 1995; Cranford et al., 2011).

1.11. Research Questions

On the way to determining if bivalves can negatively impact sea lice numbers in the field at a salmon farm, there are some questions not yet answered in the literature that should be explored first in the laboratory. We know that various species of bivalves can consume mesozooplankton (Lehane and Davenport, 2002) and more specifically that blue mussels (*M. edulis*) can consume copepodids of *L. salmonis* (Molloy et al., 2011) but we do not know if other species of bivalves—that could be potentially grown at an IMTA facility—can also ingest sea lice larvae.

Objective 1: To determine if different species of bivalves of commercial interest are capable of ingesting larval *L. salmonis*.

H_{O1}: Different species of bivalves are not capable of ingesting larval *L. salmonis.*

While it has been shown that *M. edulis* can consume sea lice copepodids at 12ºC in laboratory trials (Molloy et al., 2011), the effect of water temperature on the ingestion rate of sea lice larvae by various species of bivalves has not been investigated. Temperature effects have been detected in laboratory trials investigating maximum

feeding by bivalves on phytoplankton (Kittner and Riisgård, 2005). It is unknown if sea lice larvae might induce maximum clearance rates in bivalves, possibly making them subject to temperature effects.

Objective 2: To determine the effect of temperature on the ingestion rate of larval *L. salmonis* by various species of bivalves.

H_{O2}: Temperature will not significantly affect the ingestion rate of larval *L. salmonis* by various species of bivalves.

While it has been reported that bivalves can ingest sea lice larvae in the presence of phytoplankton (Molloy et al., 2011), the ingestion of sea lice larvae by bivalves when no phytoplankton is present has not been reported. Phytoplankton levels vary seasonally, with relatively low concentrations being present during the winter months (Cebrián and Valiela, 1999). If presence or absence of phytoplankton significantly affect ingestion rate of sea lice larvae in the laboratory, then this could have large repercussions in the field.

Objective 3: To determine the effect of phytoplankton (presence/absence) on the ingestion rate of larval *L. salmonis* by various species of bivalves.

H_{O3}: Phytoplankton presence/absence will not significantly affect ingestion rate of larval *L. salmonis* by various species of bivalves.

While blue mussels of a similar but unknown size can ingest sea lice larvae (Molloy et al., 2011) and bivalve size can significantly affect filtration rates (Gerdes, 1983), the effect of size on the ability of bivalves to ingest sea lice larvae has not been investigated.

Objective 4: To determine the effect of bivalve size on the ingestion rate of larval *L. salmonis* by various species of bivalves.

H_{O4}: Bivalve size will not significantly affect ingestion rate of larval *L. salmonis* by various species of bivalves.

Chapter 2—Materials and Methods

2.1 Sea Lice: Collection and Larval Production

2.1.1. Sea Lice Collection

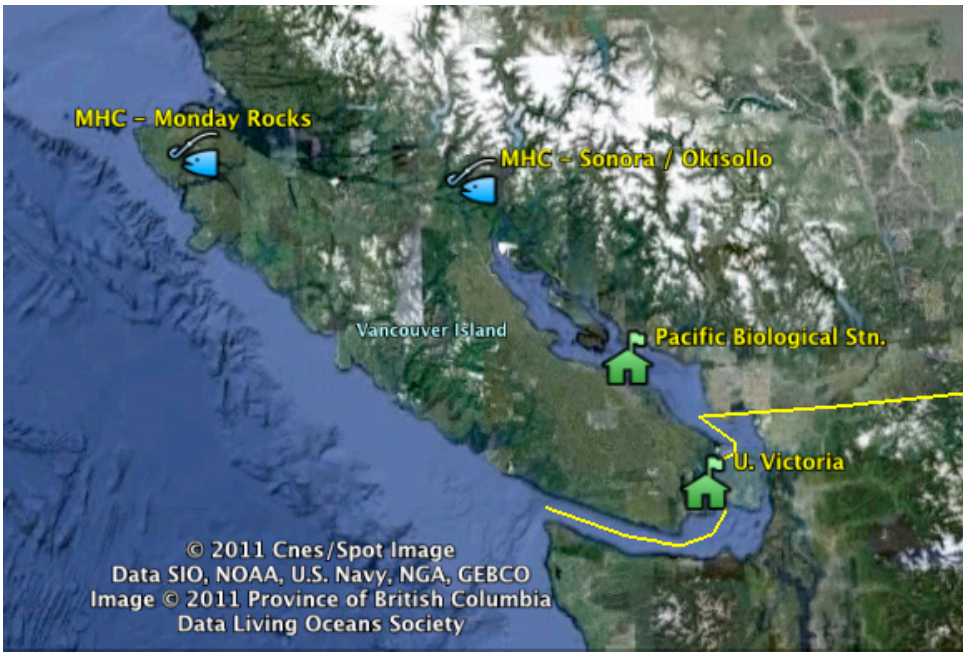

Figure 5. Map showing sea lice (*Lepeophtheirus salmonis*) collection sites (Google© 2011). MHC=Marine Harvest Canada

For each experiment, 900 gravid *L. salmonis* were collected from salmon farms experiencing sea lice outbreaks and that were in the process of harvesting the fish. Marine Harvest Canada Ltd. provided access and transportation to their Sonora/ Okisollo (50° 18’ 34.83” N; 125° 18’ 56.34” W) and Monday Rocks (50° 29’ 8.88” N; 127° 52’ 32.19” W) farm sites (Figure 5) on 8 and 19 November 2010 and 26–27 January 2011, respectively.

2.1.2. Sea Lice Larval Production

The day after collection, egg strings were separated from the adult body and placed into 2-L glass beakers of seawater. All seawater used for larval culturing and subsequent experiments was 0.2-µm cartridge filtered, had salinity ≥30, and was aerated at ~2400 ml min^{-1}. A temperature of 8.8 ±0.4°C (SE) for hatching eggs and larval rearing was used. Daily samples of larvae were removed by siphon, while filtering out any egg strings, into a new 2-L beaker of seawater. Sixty percent of the seawater in each culture beaker was replaced with fresh seawater daily. Fluorescent light was provided for 12 h d^{-1}.

In November and December 2010, for the temperature/diet experiments, ≤4% of the larvae developed to the copepodid stage. In February 2011, for the size experiments, ~15% of the larvae developed to the copepodid stage.

2.2. Temperature/Diet Experiments

Four bivalve species were used in these experiments: basket cockle (*Clinocardium nuttallii*), Pacific oyster (*Crassostrea gigas*), Pacific scallop (*Mizuhopecten yessoensis x Patinopecten caurinus*), and mussel (*Mytilus* spp.; a mix of *M. edulis*, *M. galloprovincialis,* and their hybrid) (Figure 6).

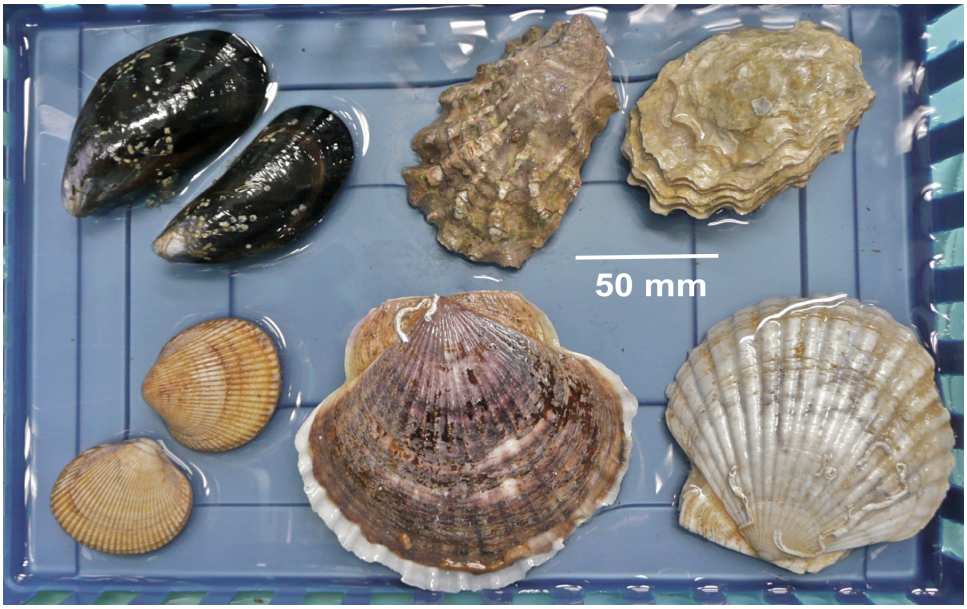

Figure 6. Photo of (clockwise from upper left) pairs of large mussels (*Mytilus* spp.), Pacific oysters (*Crassostrea gigas*), Pacific scallops (*Mizuhopecten yessoensis x Patinopecten caurinus*), and basket cockles (*Clinocardium nuttallii*) used in temperature/diet experiments

These particular bivalve species were selected using the following criteria: (1) commercial importance in Canada (as is preferred for IMTA species), (2) able to be cultivated in suspension, (3) of different families, and (4) readily available as adults (*i.e.* market-size) and juveniles. The bivalves were obtained from commercial suppliers near Vancouver Island: oysters from Mac's Oysters Ltd., Fanny Bay, BC, April 2010; scallops from Island Scallops Ltd., Qualicum Beach, BC, 23 November 2010; mussels from Cortez Island, Island Sea Farms, Saltspring Island, BC, 23 November 2010; and basket cockles from Vancouver Island University, Deep Bay/Departure Bay, 22 September 2010. Table 1 shows mean bivalve shell heights (dorsal hinges to ventral margins for cockles and scallops; anterior-posterior axes for oysters) or lengths (anterior-posterior

axes for mussels) (Seed, 1968; Quayle and Newkirk, 1989; Jacobson et al., 2010) and whole wet weights with standard errors of the mean as well as ranges.

Table 1. Bivalve mean shell heights (cockle *Clinocardium nuttallii*, oyster *Crossostrea gigas*, scallop *Mizuhopecten yessoensis x Patinopecten caurinus*) or length (mussel *Mytilus* spp.) and whole wet weights and their ranges for temperature/diet experiments. *n*=54 for each mean

Species (*n*=54)	Mean shell height or length ±SE (range) (mm)	Mean whole wet weight ±SE (range) (g)
Cockle	43.2 ±0.2 (39.3–47.5)	29.5 ±0.4 (23.1–36.2)
Oyster	87.3 ±0.7 (74.0–98.1)	116.0 ±1.1 (102.3–134.7)
Mussel	79.1 ±1.8 (78.2–83.8)	47.9 ±1.1 (39.6–55.0)
Scallop	100.4 ±0.5 (93.9–114.0)	134.1 ±1.7 (116.5–171.3)

Upon receipt, each bivalve was manually scrubbed to remove any epifauna, measured (whole wet weight, shell height, length, depth), and maintained in species-specific flow-through seawater tanks at 10.5 ±0.2°C (SE). Twice each week, they were fed algae, typically *Isochrysis* sp. (Tahitian strain, TISO). Four days before an experiment, algae were no longer provided to allow the bivalve digestive systems to clear of any food. Two days before the experiment, bivalves were brought to the experimental temperature by adjusting temperature by 1ºC h^{-1} and one day before the experiment the bivalves were moved into 2-L lidded experimental containers (diameter: 15 cm, height: 13.5 cm), holding 750 ml of static seawater that was aerated at 600 ml min^{-1}. The aeration created water movement. The volume was based on a bivalve filtration rate of 1.58 L h^{-1} for blue mussels (shell length: ~60 mm) at 4°C (Comeau et al., 2008), such that under experimental conditions, the bivalves would be capable of filtering through the contents in the container more than once within 1 h (experimental duration). Water was exchanged

three times d^{-1} up to 1 h before the start of an experiment. Fluorescent lighting intensity in the wet laboratory was ~72 lx and the timing mimicked seasonal daylight hours. The three temperatures, and standard errors of the mean, used for the experiments were 5.3 ±0.08°C, 10.0 ±0.004°C, and 14.5 ±0.01°C, which were generally representative of seasonal variation in seawater temperature in BC.

Four diet groups were established: (1) BPL: bivalve with both phytoplankton and larvae; (2) BL: bivalve with larvae only; (3) BP: bivalve with phytoplankton only; and (4) PL: phytoplankton and larvae with no bivalve (control)—to assess the fate of phytoplankton and larvae in the absence of bivalves.

When the experimental diet called for larvae, a mean of 431 ±5.8 (SE, *n*=72) larvae (≤4% copepodids, >96% nauplii) were added into each experimental container in a 50-ml aliquot of water from a stock flask of larvae at an appropriate density. The density of larvae was determined by counting the number of individuals, using a Borogov zooplankton counter under a dissecting microscope, in at least five 5-ml samples taken from the stock flask, which had been swirled. The larval density in each experimental container at the beginning of a trial was ~575 larvae L^{-1}. Preliminary trials confirmed that at a density of ~600 larvae L^{-1}, the stomachs of large mussels and oysters contained a considerable number of sea lice larvae within 1 h, as desired for this research.

When the experimental diet called for phytoplankton, TISO was added to provide a mean concentration of 7.1×10^4 cells ml^{-1} $\pm 1.9 \times 10^3$ (SE, *n*=72) in each experimental container. Algal cell concentration was determined using a haemocytometer and a compound microscope. This density was similar to that of 10^5 cells ml^{-1} used by Molloy et al. (2011) in their sea lice trials. The algae were grown semi-continuously in 4-L flasks

at a temperature of 18.7 ±0.1ºC (mean ±SD, *n*=588) under full-spectrum fluorescent bulbs. Seawater for algal culturing was filtered to 0.2 μm, sterilized with sodium hypochlorite, neutralized with sodium thiosulfate, and fertilized with a Harrison's formula (Harrison et al., 1980) modified by the partial substitution of organic phosphates with inorganic phosphates.

The experimental duration for each trial was 1 h to allow sufficient time for larval ingestion, although not enough time for thorough digestion. For each of the four separate temperature/diet experiments (*i.e.* one for each bivalve species), there were 72 containers: three temperature treatments (5, 10, 15ºC) crossed with four diet treatments (BPL, BL, BP, PL) with six replicates of each treatment combination in a randomized block design. Bivalves were randomly assigned to containers with one individual per unit. The shellfish were placed on the base of the containers which were held in raised mesh trays in seawater tables (length x width x height: 122.0 x 91.5 x 30.5 cm) filled to 14 cm deep with flowing, filtered seawater at the appropriate experimental temperature. A single table could accommodate four trays of four containers (Figures 7 and 8).

Figure 7. Photo of seawater table with 16 aerated containers for temperature/diet experiments (12 for experiments and 4 as part of wet/dry weight comparisons)

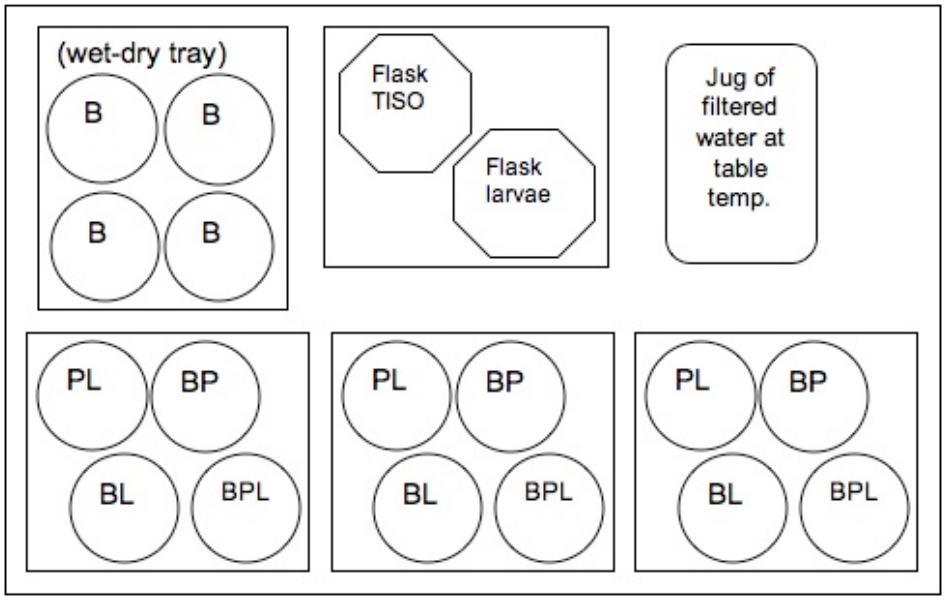

Figure 8. Schematic of a seawater table in a temperature/diet experiment showing three trays of containers with four diet treatments, and one tray of bivalves for wet/dry weight comparison, with additional space for flasks of TISO and sea lice larvae, and refill water

The experimental design was a randomized block. One experimental container of each of the four diets (BPL, BL, BP, PL) was placed into three trays within a seawater table

running at one of the experimental temperatures. The table also held a fourth tray of containers with four bivalves destined for a wet/dry weight comparison. Logistically, only one seawater table at one temperature could be run at a time. Three tables (5, 10, 15ºC) were run each day, which comprised all treatment combinations, and three of the six replicates of each treatment. The additional three replicates were completed for all treatments the subsequent day using the same method. The order of the experiments ran from 15 to 10 to 5ºC to allow for gradual drops in temperatures of flasks of TISO and sea lice larvae toward table temperature as the experiments progressed. Before measuring the designated quantity of either larvae or TISO into the experimental container at the start of bivalve feeding during the experiment, stock flasks were swirled so that the contents were well distributed. The basket cockle, Pacific oyster, Pacific scallop, and mussel experiments were run on 19–20 November, 3–4 December, 6–7 December, and 9–10 December 2010, respectively. During all experiments dissolved oxygen levels ranged between 8.3–8.6 ppm at 15ºC, 8.9–9.6 ppm at 10ºC, and 10.4–10.6 ppm at 5ºC.

It was theorized that placement of bivalves on the container bases with a relatively low aeration rate (*i.e.* 600 ml min^{-1}) might give a larval feeding advantage to bivalve species that siphon water from near the base of the container. This possibility was tested with two experiments in which bivalves were placed either on the bases of the containers or raised 2 cm above the bases on a mesh with 5 mm^2 openings, each position with two rates of aeration: 600 or 2400 ml min^{-1} (two factors fully crossed to give four treatments with four replicates for each treatment). Analysis of variance (ANOVA) of the data for oysters and scallops indicated that position, aeration rate, or their interaction did not significantly affect larval ingestions (all $P>0.4$).

At the end of the trials (1 h), the following samples were collected:

1) Algae for later cell counts as an indication of whether the bivalve fed during the experiment. A 10-ml sample of water was taken from the estimated centre of each replicate container (being mixed by aeration) and preserved with one drop of Lugol's iodine. Algal cells were later counted under a compound microscope using a haemocytometer.
2) Various bivalve weight measurements and preservation of digestive system for later dissection and confirmation of larval ingestion. After rinsing off any larvae from the bivalve exterior into its container, the bivalve's whole wet weight was measured. The bivalve soft tissue was then excised and soft tissue and shell wet weights were each measured. The shell was discarded and the soft tissue was placed in a 50-ml vial pre-filled with 5 ml of 37% formaldehyde and up to 40 ml of filtered seawater. Final formaldehyde concentration ranged from approximately 4–10% depending on soft tissue volume.
    a) The digestive system within the soft tissues of bivalves that were fed the BPL diet was later dissected and sea lice larvae retrieved from within were counted as direct evidence of ingestion. The larvae in the digestive system were categorized as being retrieved from the stomach (mouth through stomach), crystalline style, or intestines. Larvae were retrieved and counted as nauplii or copepodids. Bivalves fed diets of larvae alone and phytoplankton alone were not dissected.
    b) The wet weights of experimental bivalves—along with the bivalves for wet/dry comparison that were shucked, weighed, dried at 60ºC to constant weight and re-weighed—provided the means to estimate a dry weight for each experimental

bivalve and thereby standardize larval ingestion rates on a bivalve dry weight basis. There were 24 bivalves for wet/dry weight comparison. The wet and dry weights of these bivalves were compared using statistical regression. The resulting equation from the line of best fit was used to estimate dry weights for the bivalves in the temperature/diet experiments.

3) Container contents collected on a mesh were saved for later sea lice larval counts as indirect evidence of ingestion. The remaining contents of the container were poured through a 125 μm^2 opening mesh fabric and preserved for later counts in a 50-ml vial pre-filled with 5 ml of 37% formaldehyde and 40 ml of filtered seawater (~4% formaldehyde). The remaining, free-swimming larvae were not ingested. When compared with the larvae remaining in the control containers without bivalves, a proportion of larvae consumed (Pcons) could be estimated. Larvae missing from the container were assumed to have been consumed.

2.3 Bivalve Size Experiments

Size experiments were conducted using the same methodology as the temperature/diet experiments with the following differences. Two species (Pacific oysters and Pacific scallops) were tested in two separate experiments. Bivalve groupings for the size experiments were small, medium, and large as related to their own species (Table 2; Figure 9). Table 2 shows mean bivalve shell heights and whole wet weights and their ranges. When received, bivalves were graded into groupings with as narrow a shell size as available, before being randomly assigned to their containers.

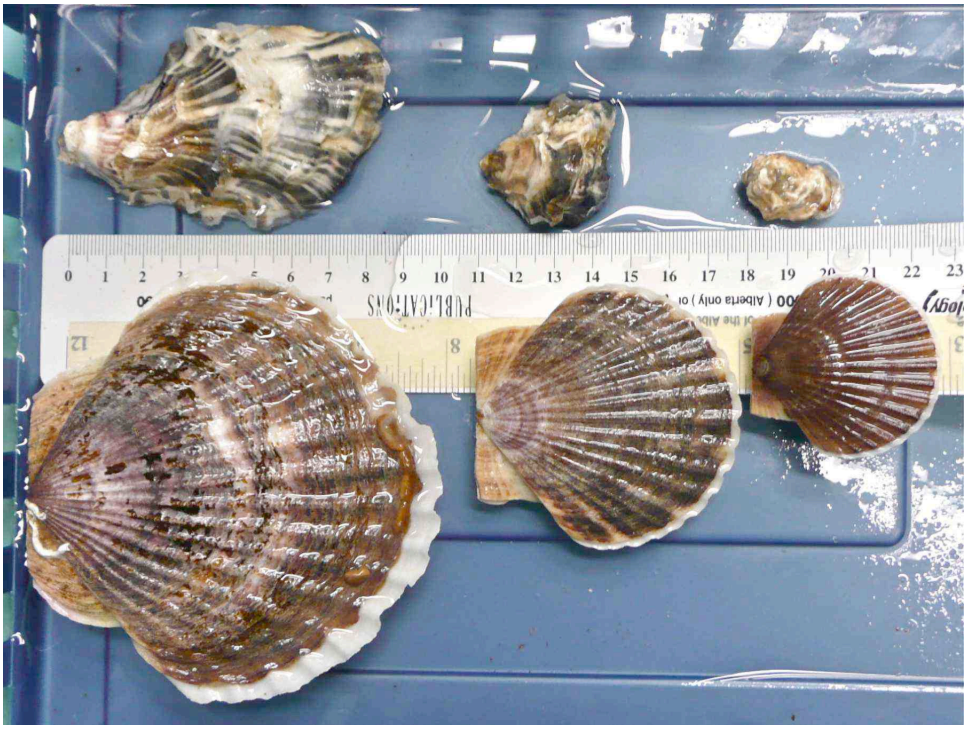

Figure 9. Photo of (left to right) large, medium, and small Pacific oysters (*Crassostrea gigas)* (top row) and Pacific scallops (*Mizuhopecten yessoensis x Patinopecten caurinus*) (bottom row)

Table 2. Bivalve mean heights (anterior-posterior axes for oysters *Crossostrea gigas*; dorsal hinges to ventral margins for scallops *Mizuhopecten yessoensis x Patinopecten caurinus*) and whole wet weights and their ranges for size experiments. *n*=12 for each mean

Species (*n*=12)	Size	Mean shell height ±SE (range) (mm)	Mean whole wet weight ±SE (range) (g)
Oyster	Small	19.2 ±0.7 (15.8–23.0)	0.68 ±0.06 (0.48–1.09)
	Medium	44.2 ±1.3 (34.2–49.7)	10.3 ±0.5 (7.9–12.6)
	Large	84.0 ±1.5 (77.1–95.1)	44.4 ±1.9 (34.5–56.9)
Scallop	Small	40.3 ±0.7 (36.3–44.8)	6.8 ±0.4 (5.0–9.7)
	Medium	64.1 ±1.2 (57.7–68.9)	32.6 ±2.3 (21.5–43.0)
	Large	102.7 ±1.3 (97.0–109.0)	166.1 ±6.1 (130.1–204.3)

The mean temperature for all size experiments was 10.4 ±0.1°C (SE). There were 54 containers: three sizes crossed with three diets with six replicates per treatment involving 36 bivalves, with 18 control containers of PL with no bivalve. Each seawater table held nine experimental containers with one replicate of each treatment. There was no replication within a block (*i.e.* seawater table). No BL diet was fed. For wet/dry weight comparisons, 12 bivalves of each size group were used. The larval component of the experimental diet included a mean of 498 ±10.3 (SE, n=36) larvae with ~15% copepodids in 750 ml of water. Phytoplankton was added so that the mean concentration of TISO was 7.8×10^4 cells ml^{-1} $\pm 2.6 \times 10^3$ (SE, n=36) in each container. The Pacific oyster and Pacific scallop experiments were synchronized to run on the same days, 3–6 February 2011.

2.4. Method of Statistical Analyses

For all analyses, the significance level is α=0.05.

Statistical analyses were run on the proportion of larvae consumed (Pcons) during the 1-h trials.

This proportion was calculated by subtracting the number of free-swimming larvae remaining at the end of the trial in each container from the mean number of larvae in the PL treatments (no bivalve) in the block and expressing this as a proportion of the mean number of larvae in the PL treatment. The same procedure was followed for phytoplankton.

The number of larvae consumed in the BPL, BL, and PL treatments and the number of phytoplankton consumed in the BPL, BP, and PL treatments were analyzed with nonparametric tests (*i.e.* Wilcoxon), since Shapiro–Wilk values were <0.0001 and the

data could not be normalized by any standard transformations. A shadowgram was generated and checked to be sure that the data for the nonparametric tests met the assumption of symmetrical distribution.

For the temperature/diet experiments, ANOVA was used on a subset of the data comprising the BPL and BL treatments to determine the effects of temperature, diet, and their interaction on the proportion of larvae consumed. Separate three-factor ANOVAs were used for each bivalve species with temperature (fixed factor, three levels), diet (fixed factor, two levels), and block (random factor, two levels). Data sets from all four bivalve species were non-normal before transformation (*P*-value range: 0.0004–0.007). Cockle and scallop data sets were normalized with modified Arcsine (Zar, 1999, Zar equation 13.8) and Box-Cox (JMP® 9, SAS Institute Inc., 2010) transformations, respectively. The oyster and mussel data sets could not be normalized by any standard transformations. Since ANOVA is robust to departures from normality, ANOVAs were run on data sets using the transformation that made the data the least non-normal (Arcsine for oysters, P=0.004, and Box-Cox for mussels, P=0.024). Nonparametric tests (Wilcoxon) were used to verify ANOVA results for oysters and mussels. Levene's tests indicated that all data sets used in the statistical analyses were homogeneous with respect to variances. Power analysis for temperature/diet experiments, which involved 36 individuals fed BPL and BL diets at three temperatures, were conducted after the experiments to determine the LSN (least significant number). In the experiment with scallops, LSNs for temperature, diet, and temperature crossed with diet were 15, 13 and 36 respectively (*i.e.* there were sufficient numbers of bivalves used in the experiment).

Power analyses for the other three bivalve species are discussed in the section on sources of error.

For the size experiments, ANOVA was used on the BPL subset of the data to determine the effect of size on the proportion of larvae consumed. Separate two-factor ANOVAs were used for both bivalve species with size (fixed factor, three levels) and block (random factor, six levels). Data sets from both bivalve species were non-normal before transformation (Shapiro–Wilk *P*-values for oysters and scallops, respectively: 0.006 and 0.016). Oyster and scallop data sets were both normalized with Arcsine transformations (Shapiro–Wilk *P*-values 0.157 and 0.192). Levene's tests indicated that all data sets used in the statistical analyses were homogenous with respect to variances.

When an ANOVA indicated that there was a significant treatment effect, post-hoc analysis was conducted using Tukey-Kramer HSD tests to compare all pairs of treatment means. All statistical analyses were conducted with JMP® 9 (SAS Institute Inc., 2010).

Power analyses were conducted after the experiments and indicated that for both size experiments, which involved 18 individuals of three sizes, the LSN of individuals to detect differences at $P \leq 0.05$ was available (*i.e.* LSN=15 for oyster (Power=0.75) and 13 for scallops (Power=0.87)).

2.5. Sources of Error

2.5.1 Experimental Design and Methodology

1) The phrase "proportion of larvae consumed" used in the statistical analyses is imprecise since it depends on a measured volume of larval culture water that added an approximate number of larvae to each container.

2) Larvae counted in the digestive system on average accounted for ~40% of the larvae missing from the container, and varied, so this count does not accurately quantify the number of larvae consumed. However, it does provide direct evidence of larval ingestion.
3) The method used to add larvae to the containers was inaccurate and imprecise. The flask of larvae, which was prepared each day to ideally contain a range within 10% of 431 or 498 larvae (for temperature/diet and size experiments, respectively) in each 50-ml aliquot actually varied more than this, *e.g.* up to a difference of 18% in one block of oysters in the size experiment. In addition, the quantity of larvae removed from the flask in each aliquot varied, as seen by the PL controls. Either the larvae were not distributed homogenously by the swirling technique for uptake into the pipette, or the larvae's natural ability to swim and possibly react to the current created by the suction of the pipette caused variation (Titelman, 2001). So, there was variation between blocks due to the number of larvae added.
4) Larvae, particularly nauplii, develop quickly so some larvae at the end of the day may not be at the same developmental stage as larvae at the beginning of the day. These can create differences between blocks. In addition, a different batch of larvae was used each day, again creating larval differences between blocks.
5) Additional replications would have provided results that were more precise for all experiments. Cranford et al. (2011) points out that 12 replicates are advisable to achieve a precision of 10–15% in clearance rate estimates. For the temperature/diet experiments, which involved 36 individuals fed BPL and BL diets at three temperatures, the LSN of mussel individuals to detect significant differences in main

effects/interactions ranged from 120–134. For cockles and oysters, these values ranged from 52–254, other than LSN of 4697 for the oyster temperature. As previously discussed, the least significant number of scallops was available in that experiment.

6) For the temperature/diet experiments, normality was not achieved for the oyster and mussel data. Since ANOVAs are robust with respect to normality, the ANOVAs were run. The concern in using non-normal data is the potential for a false positive result. In the present case, neither oyster nor mussel data came out positive (significant) for temperature and diet. Nonparametric testing (Wilcoxon) was also used and indicated the same results as the ANOVA.
7) Counts of larvae remaining free-swimming in the container may have been lower than actual because some larvae could have been lost during four transfers of larvae (from the container to vial of formaldehyde to 1st vial of ethanol to 2nd vial of ethanol to vial of water).
8) There was variable re-filtration of the water in the static containers such that bivalves that filtered a greater volume of water during 1 h like the large (adult) scallop, drew down the quantity of phytoplankton and larvae more quickly, so that by the end of the experiment, had access to fewer larvae and algal cells and may not have been feeding *ad libitum* as a result. This likely resulted in a lower rate of ingestion reported for larger bivalves. This same effect would have been minimal for the smallest bivalves such as the small oysters.
9) The bivalves in these laboratory experiments were fed TISO as the non-larval diet component, which is a higher value food than the seston they would encounter in the

field. Bivalves fed algae in laboratory experiments tend to filter-feed closer to a maximal rate compared to bivalves feeding on seston. The bivalves in this trial may therefore have been filter feeding at higher rates than would be expected *in situ*.

10) The relatively small container size in comparison to the size of large oysters and scallops could affect how the results translate to the field (see Green et al., 2003).
11) Inexperience in soft-tissue dissections likely resulted in a lower larval count from digestive systems of the first block of experiment 1 (cockles) in particular, and of the temperature/diet experiments in general, compared to the size experiments. While many practice dissections had been done during pre-trials, there was a continued learning curve until improved care in investigating various folds and cavities when removing material from the digestive system, and picking apart clumps of TISO to a very fine degree were achieved. Dissections could have been done on a randomized basis to distribute the error across species, blocks, and treatments.

2.5.2 Bivalves and Sea Lice

1) Bivalve filtration varies among individuals. If two or more bivalves out of six within a treatment group did not open to feed during the experiment, this could significantly affect the results.
2) Sea lice larvae may be able to escape from the flow of bivalve filtration with different success depending on their larval development (Titelman, 2001)
3) Differences among bivalve species are great (although species are generally not compared in the present research as each species was run as a different experiment)
    a) Mature bivalves of the different species used in the research are naturally quite different in size. As a result, the large scallop, for instance, did not have much

free water volume around it, while the cockle did. The size difference could affect currents developed by the aeration and the bivalve's filtration and, since sea lice larvae swim and can elicit an escape jump, affect the level of difficulty for the bivalve to filter the larvae.

b) Bivalves of different species behave differently and react differently. Oysters, for instance, were particularly sensitive to activity around them and might close for a period even when only slightly disturbed. Lids were used on the containers during acclimation and the experiment to help alleviate this effect.

c) Every species and size is unique in various aspects of its filtration. Cockles, for instance, have siphons that can extend relatively far from the shell, and that tend to point upward into the water column. This could create differences in success in filtering-in the larvae.

Chapter 3—Results

3.1. Temperature/Diet Experiments

3.1.1. Larvae Consumed

ANOVAs revealed that there were no significant effects of temperature, diet [(larvae with phytoplankton (BPL), or without (BL)], or the interaction between these two factors on the proportion of larvae ingested by any of the four tested bivalve species (Table 3, Figure 10). Additional quantitative analysis of numbers of larvae consumed and percentage of bivalves that consumed >100 and >200 each is found in Table 4. Both nauplii and copepodids were ingested. The number of larvae ingested was standardized per unit dry weight of bivalve (Table 5, Figure 11). Although no statistical comparison is made among species from different experiments, scallops appear to consume a greater proportion of larvae per individual than the other three species, whereas cockles appear to consume more larvae per unit dry weight than the other three species (Figures 10 and 11).

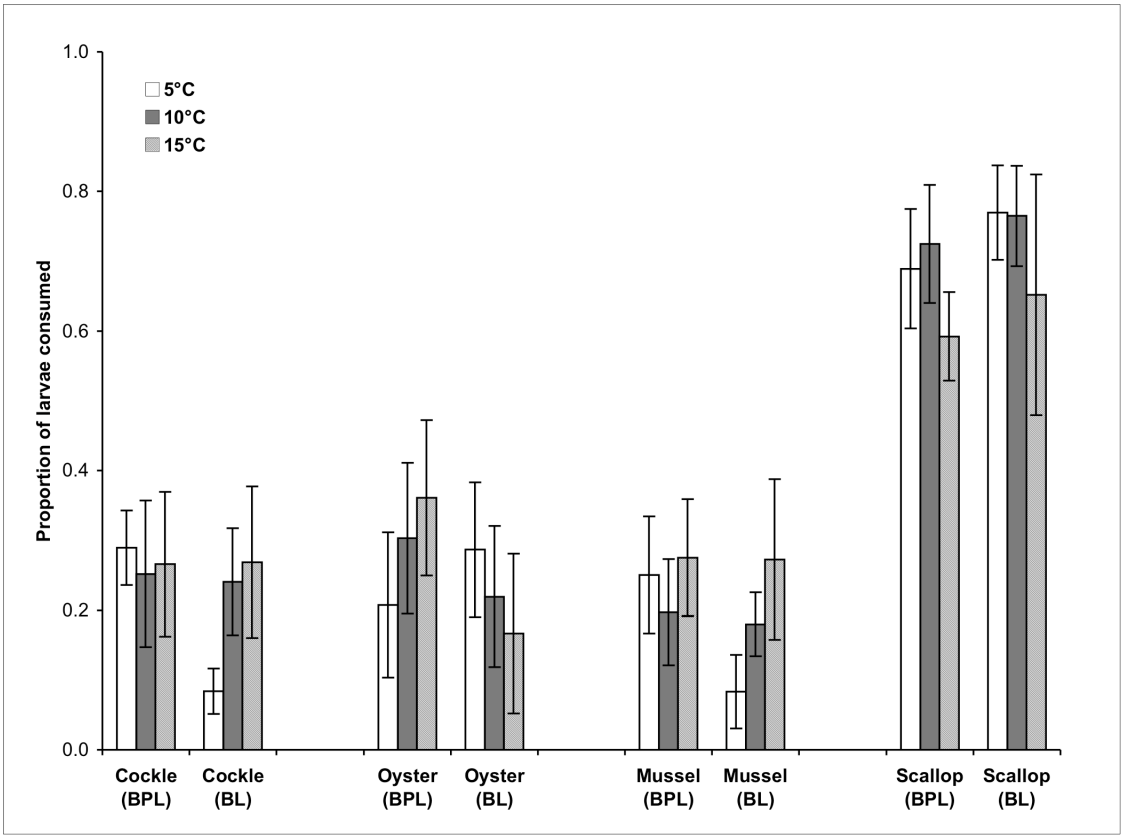

Figure 10. Mean (±SE, *n*=6) proportion of sea lice (*Lepeophtheirus salmonis*) larvae consumed by four species of bivalves held at three different temperatures (5, 10, 15ºC) and given two different diets (BPL: bivalve, phytoplankton, larvae; BL: bivalve, larvae)

Table 3. ANOVAs on the proportion of sea lice (*Lepeophtheirus salmonis*) larvae consumed by four bivalve species during temperature/diet experiments. *P*-values in bold are <0.05.

Cockle	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	0.043	MS_T/MS_{TB}	0.097	0.912
Diet (D)	1	0.072	MS_D/MS_{DB}	111.365	0.060
T x D	2	0.225	MS_{TD}/MS_{TDB}	2.501	0.286
Block (B)	1	0.719	MS_B/MS_E	14.274	**0.001**
T x B	2	0.447	MS_{TB}/MS_E	4.436	**0.023**
D x B	1	0.001	MS_{DB}/MS_E	0.0128	0.911
T x D x B	2	0.090	MS_{TDB}/MS_E	0.893	0.422
Error (E)	24	1.209			
Total	35	2.805			

Oyster	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	0.005	MS_T/MS_{TB}	0.044	0.958
Diet (D)	1	0.090	MS_D/MS_{DB}	0.987	0.502
T x D	2	0.221	MS_{TD}/MS_{TDB}	1.725	0.367
Block (B)	1	0.956	MS_B/MS_E	8.623	**0.007**
T x B	2	0.116	MS_{TB}/MS_E	0.522	0.600
D x B	1	0.091	MS_{DB}/MS_E	0.821	0.374
T x D x B	2	0.128	MS_{TDB}/MS_E	0.578	0.568
Error (E)	24	2.660			
Total	35	4.267			
Mussel	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	0.058	MS_T/MS_{TB}	0.604	0.623
Diet (D)	1	0.032	MS_D/MS_{DB}	4.235	0.288
T x D	2	0.052	MS_{TD}/MS_{TDB}	1.210	0.452
Block (B)	1	0.001	MS_B/MS_E	0.028	0.869
T x B	2	0.096	MS_{TB}/MS_E	1.525	0.238
D x B	1	0.008	MS_{DB}/MS_E	0.243	0.626
T x D x B	2	0.043	MS_{TDB}/MS_E	0.679	0.517
Error (E)	24	0.758			
Total	35	1.048			
Scallop	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	0.064	MS_T/MS_{TB}	0.603	0.624
Diet (D)	1	0.062	MS_D/MS_{DB}	1.172	0.475
T x D	2	0.012	MS_{TD}/MS_{TDB}	0.101	0.908
Block (B)	1	0.178	MS_B/MS_E	3.916	0.059
T x B	2	0.107	MS_{TB}/MS_E	1.173	0.327
D x B	1	0.053	MS_{DB}/MS_E	1.163	0.292
T x D x B	2	0.123	MS_{TDB}/MS_E	1.350	0.278
Error (E)	24	1.09			
Total	35	1.69			

Table 4. Quantities of sea lice larvae consumed by the 36 bivalves fed BPL (bivalve with phytoplankton and larvae) and BL (bivalve and larvae) during temperature/diet experiments (*n*=36 per row), and percentages of the population that ingested >100 and >200 sea lice (*Lepeophtheirus salmonis*) larvae, as calculated based on counts of larvae remaining swimming in the container

Species	Size	Greatest # larvae consumed by 1 bivalve	Least # larvae consumed by 1 bivalve	% (of 36) that consumed >200 larvae	% (of 36) that consumed >100 larvae
Cockle	Large	316	0	19	50
Oyster	Large	351	0	25	47
Mussel	Large	433	0	6	39
Scallop	Large	472	0	81	97

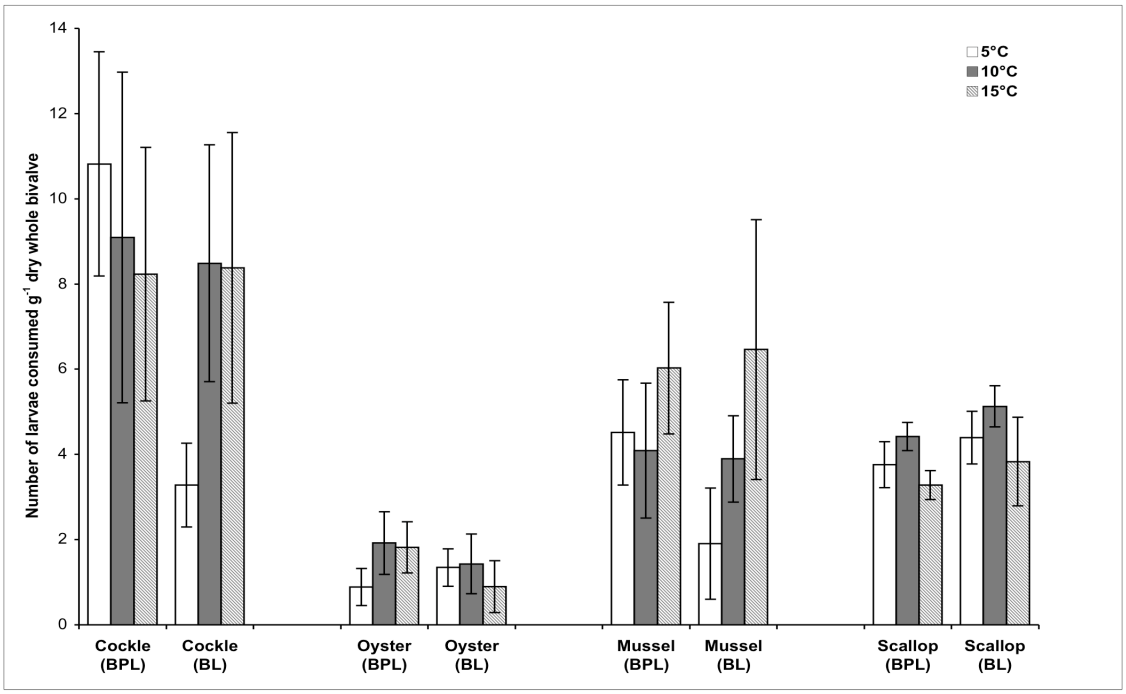

Figure 11. Mean (±SE, *n*=6) number of sea lice (*Lepeophtheirus salmonis*) larvae consumed per unit dry weight by four species of bivalves held at three different temperatures (5, 10, 15ºC) and given two different diets (BPL: bivalve with phytoplankton and larvae; BL: bivalve with larvae)

Table 5. ANOVAs on the number of sea lice (*Lepeophtheirus salmonis*) larvae consumed g^{-1} whole dry bivalve weight for four bivalve species during temperature/diet experiments. *P*-values in bold are <0.05

Cockle	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	1.968	MS_T/MS_{TB}	0.044	0.957
Diet (D)	1	6.631	MS_D/MS_{DB}	102.015	0.063
T x D	2	17.249	MS_{TD}/MS_{TDB}	1.918	0.343
Block (B)	1	35.940	MS_B/MS_E	11.126	**0.003**
T x B	2	44.266	MS_{TB}/MS_E	6.852	**0.004**
D x B	1	0.065	MS_{DB}/MS_E	0.020	0.888
T x D x B	2	8.993	MS_{TDB}/MS_E	1.392	0.268
Error (E)	24	77.526			
Total	35	192.638			
Oyster	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	0.832	MS_T/MS_{TB}	0.539	0.650
Diet (D)	1	0.022	MS_D/MS_{DB}	1.000	0.500
T x D	2	0.222	MS_{TD}/MS_{TDB}	0.294	0.773
Block (B)	1	0.902	MS_B/MS_E	3.640	0.068
T x B	2	1.545	MS_{TB}/MS_E	3.118	0.063
D x B	1	0.022	MS_{DB}/MS_E	0.089	0.768
T x D x B	2	0.755	MS_{TDB}/MS_E	1.523	0.238
Error (E)	24	5.947			
Total	35	10.247			
Mussel	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	2.407	MS_T/MS_{TB}	1.091	0.478
Diet (D)	1	0.531	MS_D/MS_{DB}	8.297	0.213
T x D	2	1.062	MS_{TD}/MS_{TDB}	1.348	0.426
Block (B)	1	0.030	MS_B/MS_E	0.034	0.855
T x B	2	2.206	MS_{TB}/MS_E	1.257	0.303
D x B	1	0.064	MS_{DB}/MS_E	0.073	0.789
T x D x B	2	0.788	MS_{TDB}/MS_E	0.449	0.643
Error (E)	24	21.057			
Total	35	28.144			

Scallop	df	SS	*F* ratio	*F* value	*P* value
Temp. (T)	2	9.077	MS_T/MS_{TB}	1.664	0.375
Diet (D)	1	3.738	MS_D/MS_{DB}	0.935	0.511
T x D	2	0.051	MS_{TD}/MS_{TDB}	0.008	0.500
Block (B)	1	4.694	MS_B/MS_E	2.475	0.129
T x B	2	5.454	MS_{TB}/MS_E	1.438	0.257
D x B	1	4.000	MS_{DB}/MS_E	2.109	0.159
T x D x B	2	6.552	MS_{TDB}/MS_E	1.727	0.199
Error (E)	24	45.520			
Total	35	79.086			

3.1.2. Phytoplankton Consumed

The PL treatment was the control diet of phytoplankton and larvae with no bivalve. With no shellfish present, the PL samples at the end of the trials contained near the original concentration of TISO initially added in the diet, with algal means across temperatures ranging from 6.9–7.7 $x10^4$ cells ml^{-1} for all 4 experiments (Figure 12). At the end of 1 h in the presence of a bivalve, the mean concentrations of TISO remaining in the containers with BPL (bivalve, phytoplankton, larvae) and BP (bivalve, phytoplankton) were dramatically reduced, ranging from 0.1–3.1 $x10^4$ cells ml^{-1} (Figure 12). While all bivalves removed TISO from the containers during 1 h of feeding, scallops left a lower concentration of TISO remaining compared to the other three bivalve species (Figure 12). For all four bivalve species, using a nonparametric method (Wilcoxon), statistical results show that the PL diet was significantly different from both the BPL and BP diets (all $P<0.0001$), and the BP and BPL diets were not significantly different (both $P\geq0.600$) in the concentration of TISO remaining in the container. There was no significant difference shown in reduction of algal concentration detected at the three temperatures (all $P\geq0.071$).

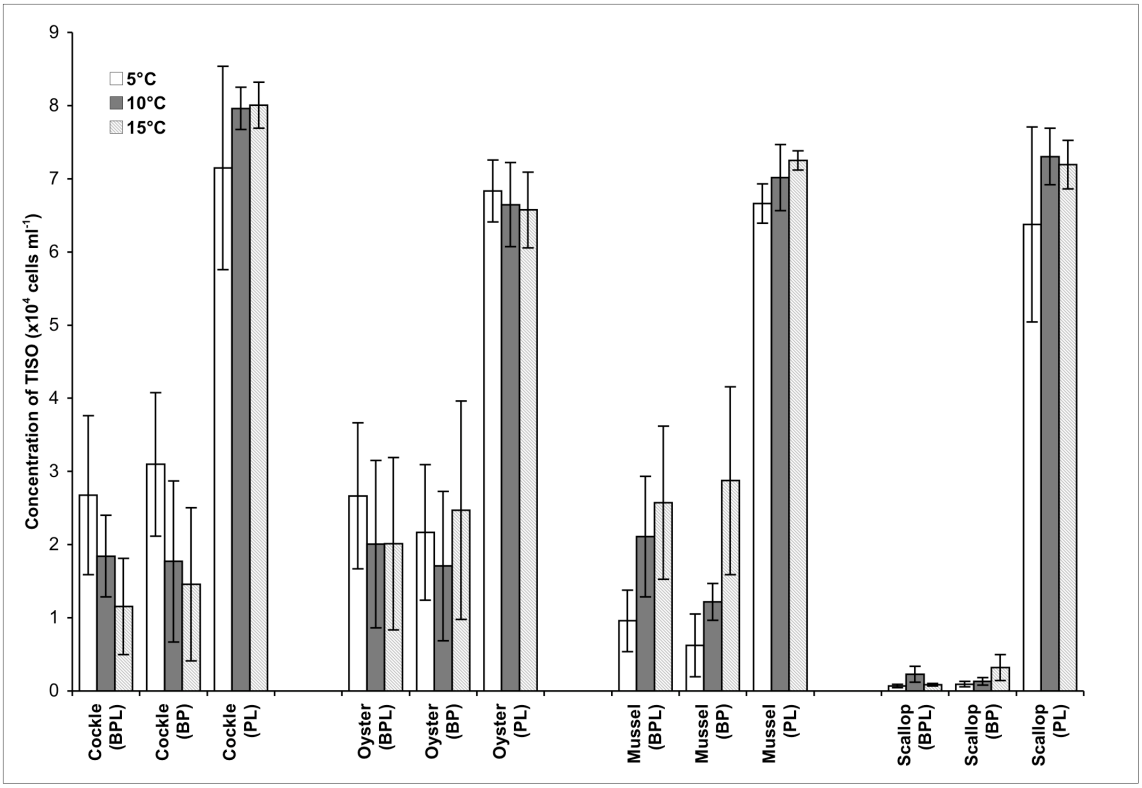

Figure 12. Mean (±SE, *n*=6) concentration of TISO in containers held at three different temperatures (5, 10, 15°C) with three different diet treatments (BPL: bivalve with phytoplankton and larvae; BP: bivalve with phytoplankton; PL: phytoplankton with larvae) provided to four bivalve species

3.1.3. Digestive System Dissections

The 18 BPL bivalves in each experiment were dissected. Most larvae were located in the stomach region (including the mouth through the esophagus). The greatest number of larvae retrieved during a dissection of a bivalve stomach from the temperature/diet experiments was 123 individuals from a scallop. Additional information from quantitative analysis is found in Table 6 and Figures 13–14. Copepodids as well as nauplii were retrieved from the digestive systems of all four species.

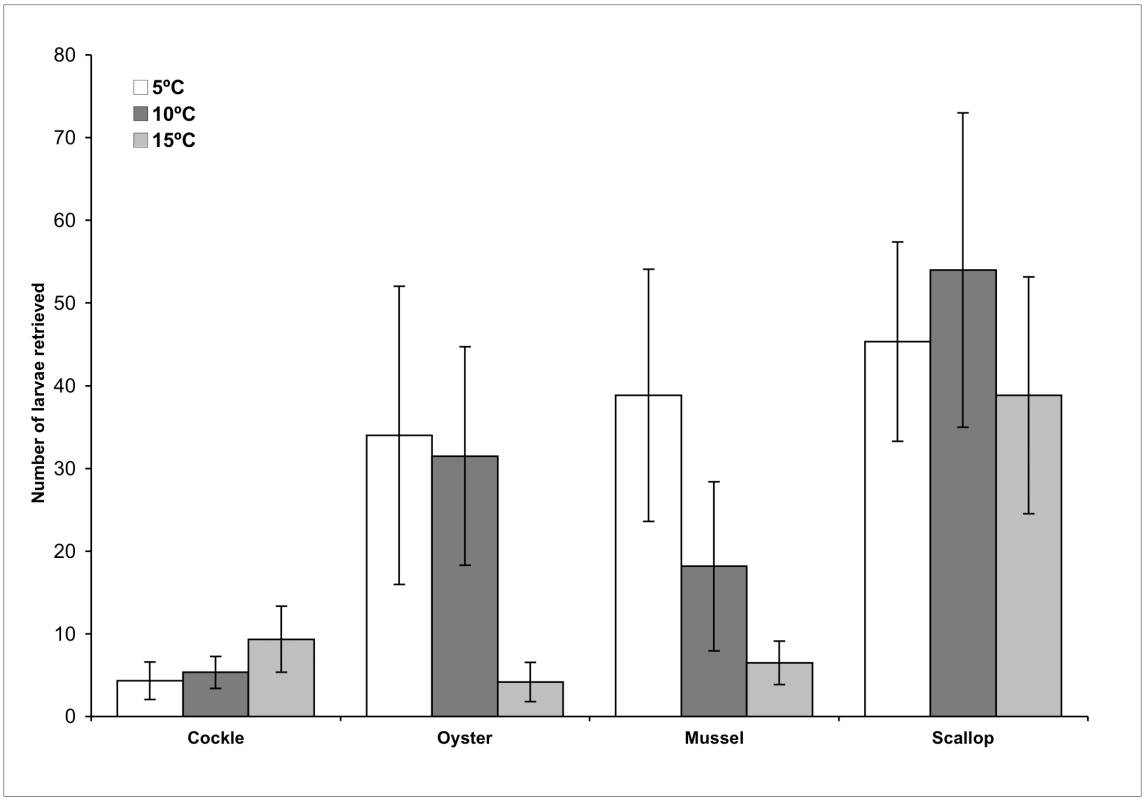

Figure 13. Mean (±SE, *n*=6) number of sea lice (*Lepeophtheirus salmonis*) larvae retrieved from digestive systems of four species of bivalves (given the BPL diet: bivalve with phytoplankton and larvae) held at three different temperatures (5, 10, 15ºC)

Table 6. Comparison of number of sea lice (*Lepeophtheirus salmonis*) larvae retrieved by dissection from 18 bivalves of four bivalve species given the BPL diet (bivalve with phytoplankton and larvae), and the number of larvae estimated consumed during temperature/diet experiments

Species	Range of number of larvae retrieved from whole digestive systems	Total # larvae retrieved in 18 stomachs (A) [Mean (SE)]	Total # larvae retrieved in 18 styles & intestines (B) [Mean (SE)]	Total # larvae estimated consumed by same 18 bivalves (container counts) (C)	% of larvae consumed, that were retrieved by dissection (A+B/C=D)
Cockle	0–27	101 [5.6 (2.7)]	13 [0.7 (0.9)]	2 209	5.2
Oyster	0–119	338 [18.8 (10.5)]	80 [4.4 (3.8)]	2 231	18.7
Mussel	0–108	372 [20.7 (11.2)]	9 [0.5 (0.4)]	1 692	22.5
Scallop	0–130	699 [41.1 (13.8)]	76 [4.2 (2.2)]	5 007	15.5
Experiment Total		1 510	178	11 139	15.1

Figure 14. Photo of preserved cockle (*Clinocardium nuttallii*) with crystalline style (arrow) exposed. (Photo: B. Pirie)

3.2. Size Experiments

3.2.1. Larvae Consumed

Statistical analysis was run for bivalves that were fed the BPL diet. Significant differences in the proportion of larvae consumed were found when comparing different sizes of oysters (P=0.021) and scallops (P=0.008) (Table 7; Figure 15). Post–hoc comparisons for all pairs using Tukey-Kramer HSD shows that large oysters were significantly different from small oysters (P=0.020) while medium oysters were not significantly different from large (P=0.093) or small (P=0.621) oysters in proportion of larvae consumed. Large scallops were significantly different from both small (P=0.027) and medium (P=0.010) scallops while small and medium scallops were not significantly different (P=0.811). Additional quantitative analyses of numbers of larvae consumed and percentage of bivalves that consumed >100 and >200 each are in Table 8. Both nauplii and copepodids were ingested by bivalves in proportions similar to the proportion initially added to the container (≤8% difference). On a unit dry weight basis, size was not significant for oysters (P=0.218) whereas it was significant for scallops (P=0.003) (Table 9; Figure 16). Post-hoc analysis indicates that small scallops are significantly different from large (P=0.008) and medium scallops (P=0.008), while large and medium scallops are not significantly different (P=0.218) in the number of larvae consumed gram^{-1} whole dry bivalve. Note that while mean heights were within 3 mm, the mean whole weights of large oysters in the size experiments was considerably less than that for large oysters in the temperature/diet experiments.

Statistical comparisons are not made between species in different experiments, however scallops in general, and large scallops in particular, appear to consume a greater proportion of sea lice larvae from the experimental containers than oysters, whereas on a dry weight basis, it appears that the two species consume roughly similar numbers of larvae.

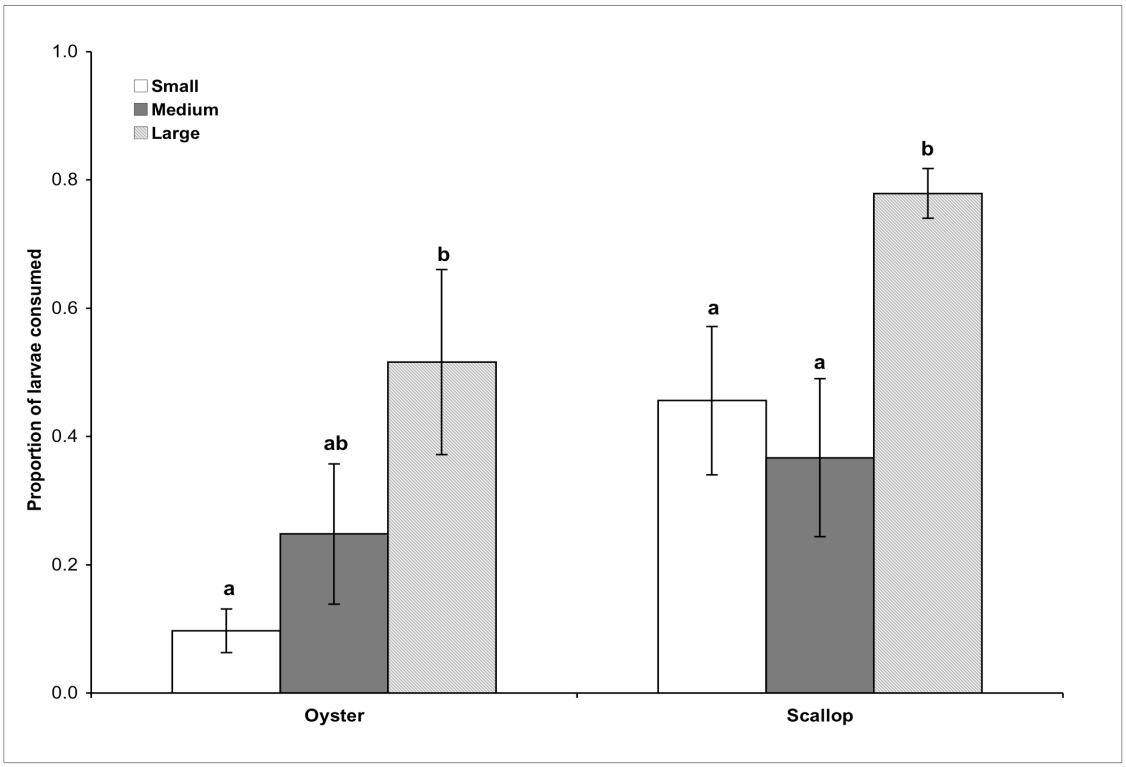

Figure 15. Mean (±SE, *n*=6) proportion of sea lice (*Lepeophtheirus salmonis*) larvae consumed by two species of bivalves of three sizes (small, medium, large) (provided BPL diet: bivalve with phytoplankton and larvae)

Table 7. ANOVAs on the proportion of sea lice (*Lepeophtheirus salmonis*) larvae consumed by two bivalve species during size experiments. *P* values in bold are <0.05

Oyster	df	SS	*F* ratio	*F* value	*P* value
Size (S)	2	0.858	MSs/MS_R	5.812	**0.021**
Block (B)	5	1.035	MS_B/MS_R	2.803	0.077

S x B	~	~	no test	~	~
Remainder (R)	10	0.738			
Total	17	2.631			
Scallop	df	SS	*F* ratio	*F* value	*P* value
Size (S)	2	0.896	MS_S/MS_R	7.986	**0.008**
Block (B)	5	0.638	MS_B/MS_R	2.276	0.126
S x B	~	~	no test		
Remainder (R)	10	0.561			
Total	17	2.095			

Table 8. Quantities of sea lice larvae consumed by the 6 bivalves of various species and sizes that were fed BPL (bivalve with phytoplankton and larvae) during size experiments (*n*=6 per row), and percentages of the population that ingested >100 and >200 sea lice (*Lepeophtheirus salmonis*) larvae, as calculated based on counts of larvae remaining swimming in the container

Species	Size	Greatest # larvae consumed by 1 bivalve	Least # larvae consumed by 1 bivalve	% (of 6) that consumed >200 larvae	% (of 6) that consumed >100 larvae
Oyster	Large	398	31	50	83
	Medium	316	0	17	50
	Small	92	0	0	0
Scallop	Large	458	269	100	100
	Medium	347	16	50	50
	Small	354	8	67	83

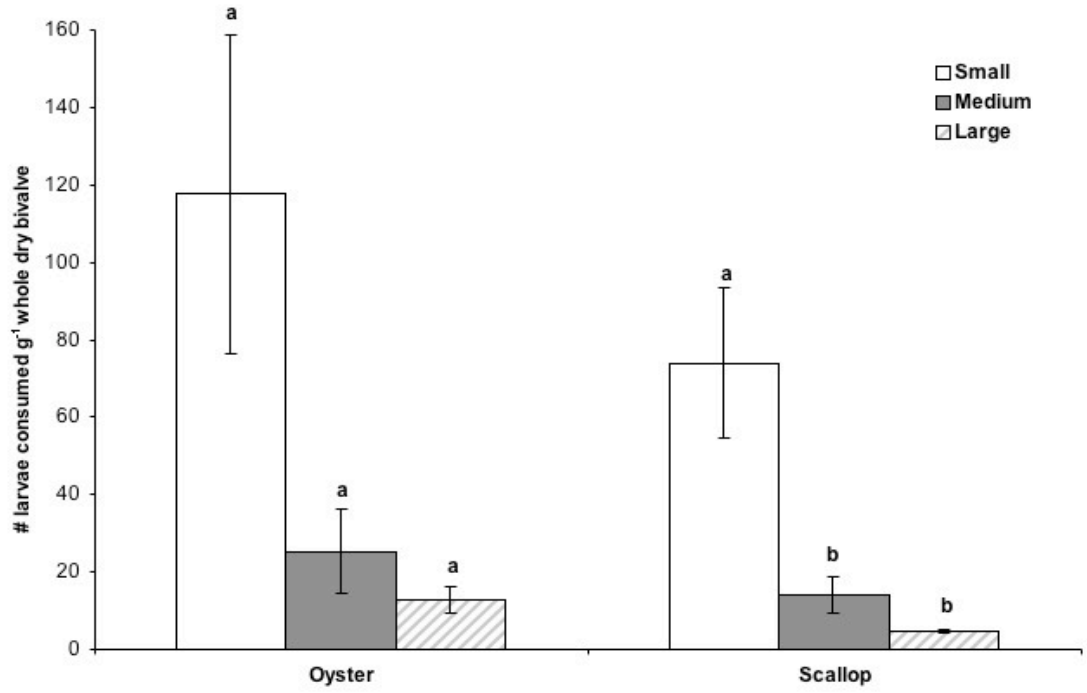

Figure 16. Mean (±SE, *n*=6) number of sea lice (*Lepeophtheirus salmonis*) larvae consumed per unit dry weight by three sizes (small, medium, large) of two species of bivalves (provided BPL diet: bivalve with phytoplankton and larvae)

Table 9. ANOVAs on the number of sea lice (*Lepeophtheirus salmonis*) larvae consumed g^{-1} whole dry bivalve weight for two bivalve species during size experiments. *P* values in bold are <0.05

Oyster	df	SS	*F* ratio	*F* value	*P* value
Size (S)	2	1.802	MSs/MSrem	1.783	0.218
Block (B)	5	3.192	MSb/MSrem	1.263	0.351
S x B	~	~	no test	~	~
Remainder (R)	10	5.053			
Total	17	10.047			
Scallop	df	SS	*F* ratio	*F* value	*P* value
Size (S)	2	16.335	MSs/MSrem	11.279	**0.003**
Block (B)	5	13.599	MSb/MSrem	3.756	**0.036**
S x B	~		no test		
Remainder (R)	10	7.241			
Total	17	37.176			

3.2.2. Phytoplankton Consumed

The control diet, PL, had a mean TISO concentration of 7.6–8.0 x10^4 cells ml^{-1}. The range in the concentration of TISO remaining in the containers of BPL (bivalve, phytoplankton, larvae) and BP (bivalve, phytoplankton only) was 0.3–6.9 x10^4 cells ml^{-1} (Figure 17). For both scallops and oysters, using a nonparametric method (Wilcoxon), the results show that the PL diet was significantly different from both the BPL and BP diets (both $P \leq 0.027$), and the BP and BPL diets were not significantly different (both $P \geq 0.624$) in the concentration of TISO remaining in the container. Using the nonparametric method, there was no significant difference shown in reduction of algal concentration detected by the three sizes of scallops; scallops of all three sizes considerably reduced the concentration of algal cells. Large oysters reduced the concentration of algal cells significantly more than small or medium oysters (both $P \leq 0.011$).

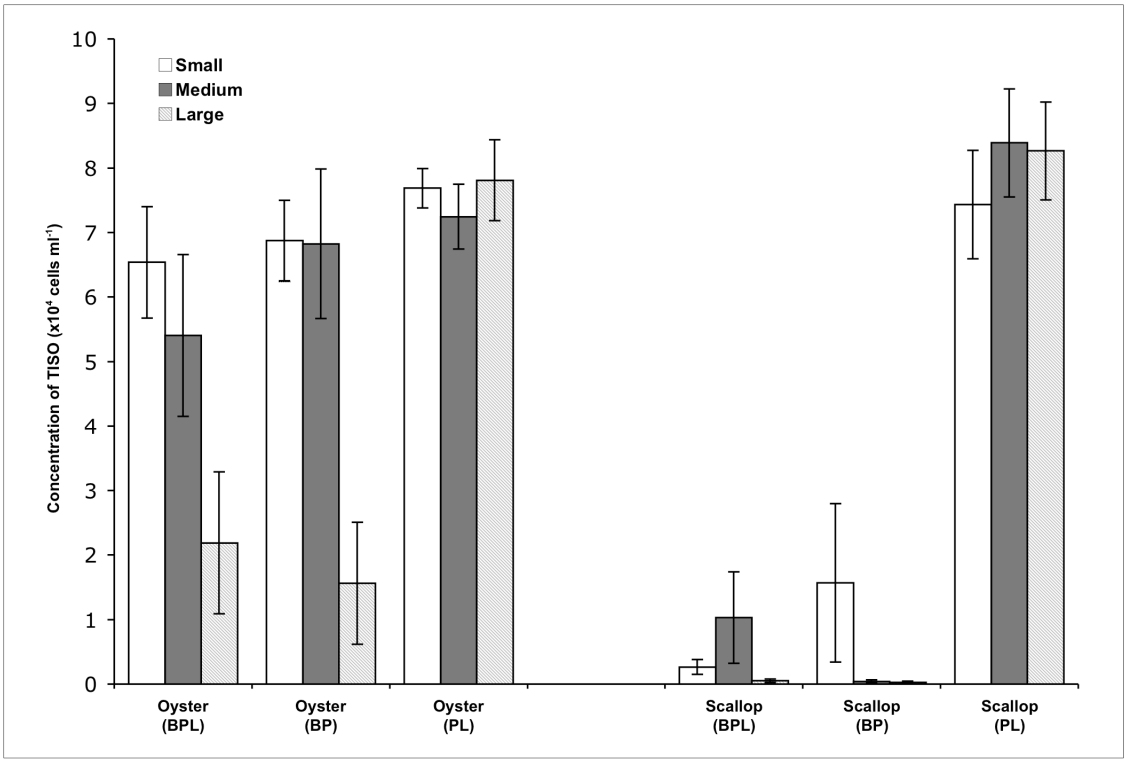

Figure 17. Mean (±SE, *n*=6) concentration of TISO remaining in containers when three different sizes (small, medium, large) of two bivalve species were provided TISO as part of the diet in three different treatments (BPL: bivalve with phytoplankton and larvae; BP: bivalve with phytoplankton; PL (control): phytoplankton with larvae but no bivalve)

3.2.3. Digestive System Dissections

The 18 BPL bivalves in each experiment were dissected. The greatest number of larvae retrieved by dissection in one stomach was 347 in a large oyster with 28 additional larvae found along the crystalline style to account for 375 larvae in its whole digestive system. Quantitative analysis indicates that there was variation in the total number of sea lice larvae retrieved from the digestive systems based on bivalve size, with the greatest number of larvae found in large oysters and small scallops (Table 10; Figure 18). On average, 43% of the sea lice larvae that were consumed were retrieved. With the

exception of small oysters, copepodids as well as nauplii were retrieved from the digestive systems of all sizes of both species.

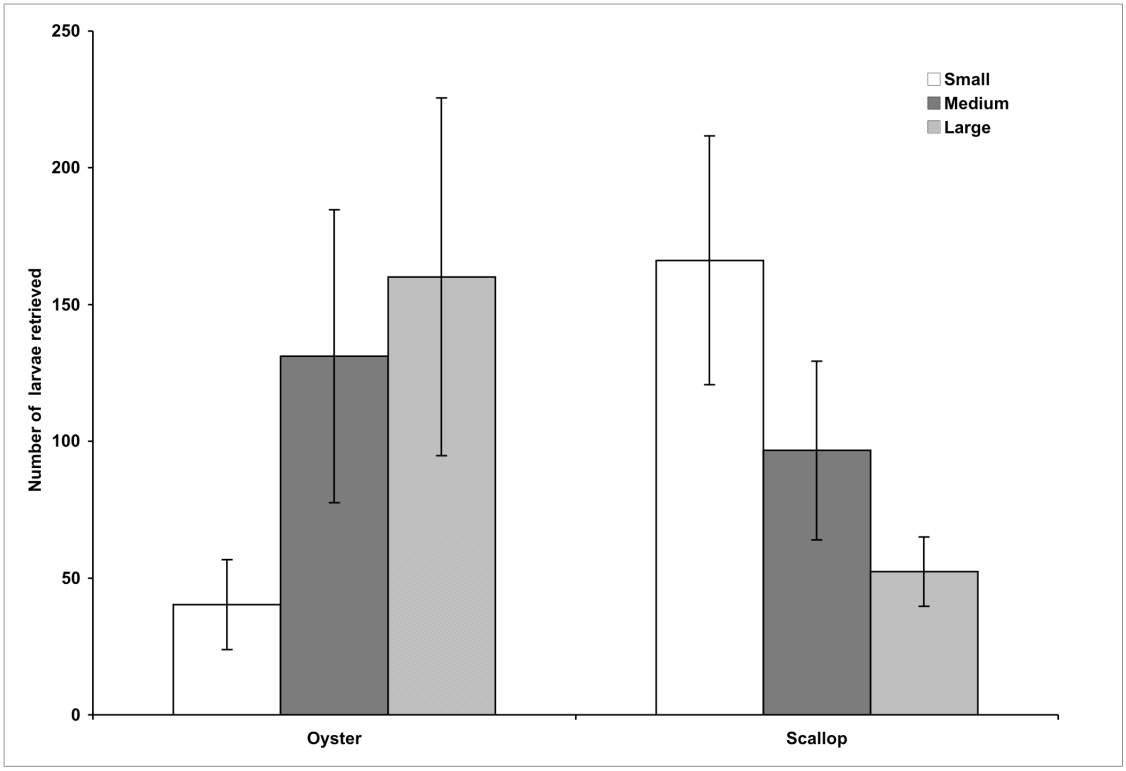

Figure 18. Mean (±SE, *n*=6) number of sea lice (*Lepeophtheirus salmonis*) larvae retrieved from digestive systems of two species of bivalves of three sizes (small, medium, large) (given the BPL diet: bivalve with phytoplankton and larvae)

Table 10. Comparison of number of sea lice (*Lepeophtheirus salmonis*) larvae retrieved by dissection from six bivalves of each of three sizes (small, medium, large) of two bivalve species given the BPL diet (bivalve with phytoplankton and larvae), and the number of larvae estimated consumed during size experiments

Species–Size	Range of number of larvae retrieved from a complete digestive system	Total # larvae retrieved in 6 stomachs (A) [Mean (SE)]	Total # larvae retrieved in 6 styles & intestines (B) [Mean (SE)]	Total # larvae estimated consumed by same 6 bivalves (container counts) (C)	% of larvae consumed, that were retrieved by dissection (A+B/C=D)
Oyster–Small	0–16	14 [3.5 (2.9)]	2 [0.3 (0.3)]	281	5.7
Oyster–Medium	0–134	138 [23 (17.6)]	96 [16.0 (11.5)]	730	32.1
Oyster–Large	0–375	907 [151.2 (66.1)]	52 [8.7 (4.4)]	1 501	63.9
Scallop–Small	8–263	861 [143.5 (38.9)]	136 [22.7 (9.1)]	1 339	74.5
Scallop–Medium	2–200	576 [96.0 (32.4)]	4 [0.7 (0.5)]	1 092	53.1
Scallop–Large	17–86	286 [47.7 (10.5)]	28 [4.7 (2.4)]	2 266	13.9
Experiment Total		2782	318	7209	43.0

Chapter 4—Discussion

4.1. Bivalve Species

All four species of bivalves tested in the present study were shown to ingest larvae of *L. salmonis*. This is not surprising, as a number of studies have reported that bivalves can ingest various species of mesozooplankton. Horsted et al. (1988) showed that blue mussels (*M. edulis*) preyed on smaller zooplankton. A small clam was identified as the likely cause of substantial declines of three species of copepods (Kimmerer et al., 1994). More recently, research on the significance of bivalves preying on mesozooplankton has become more topical. Davenport et al. (2000) suggested that post-spawning, energy-depleted blue mussels (*M. edulis*) may be able to improve in condition on a diet of *Artemia* sp. alone. A field study from February (winter) through mid-April involved the investigation of stomach contents of 100 *M. edulis*. Eleven stomachs contained solely whole-animal material, which occurred mostly in late winter, while another 55 stomachs contained both plant and animal matter, so 66% of stomachs included identifiable animals. The authors note that digestion of mesozooplankton can occur in <40 min in *M. edulis* (Davenport et al., 2000). Lehane and Davenport (2002) reported ingestion of mesozooplankton by mussels, cockles and scallops (*M. edulis, C. edule,* and *A. opercularis)*. They found that the nauplii of crustaceans were well represented among the numerous types of mesozooplankton ingested during 24 h by *M. edulis* in cages either suspended or on the benthos (Lehane and Davenport, 2002). Zeldis et al. (2004) showed that greenshell mussels (*Perna canaliculus*) consume various sizes of zooplankton including mesozooplankton such as adult copepods (length: 430 μm). Wong and

Levinton (2004) conducted a microcosm study that showed that *M. edulis* could consume and grow on a diet of zooplankton alone, as well as phytoplankton alone and a mixture of the two. Prato et al. (2010) used fatty acids as biomarkers to investigate feeding and trophic relationships of *M. galloprovincialis*. More than one ratio (18:1w9/18:1w7 and 20:1w9) indicated animal dietary inputs, with one fatty acids trophic marker relatively high at 4–5.8%, suggesting that the bivalve feeds, in part, on zooplankton. The zooplankton biomarkers did not significantly vary throughout the year, even though zooplankton abundance increased during the summer (Prato et al., 2010). Microzooplankton were shown to be an important component in the diet of *C. gigas,* particularly when other food sources (*i.e.* phytoplankton) were less abundant (Kamiyama, 2011). In addition, copepod nauplii were commonly found in the oyster gut contents. While microzooplankton levels in the gut were significantly correlated with their abundance in the water column, a similar relationship was not shown for metazooplankton such as copepod nauplii (Kamiyama, 2011). Copepodids of *L. salmonis* were ingested by *M. edulis* in a laboratory study by Molloy et al. (2011). It is apparent that various species and sizes of bivalves ingest mesozooplankton, including during the winter when phytoplankton or seston are at low concentrations and the water is cold.

All species of bivalves used in this study were successful in ingesting sea lice larvae although individual variation in ingestion rates within species was high. Individual variation in clearance rate was reported in bivalve experiments involving blue mussels and the great scallop (Strohmeier et al., 2009). In the temperature/diet experiments the percentage of individuals from each species that consumed >200 and >100 larvae (Table 4) provided an indication of how successful bivalves under these laboratory conditions

can be in consuming sea lice larvae. Individual variation during these experiments was evident, and is shown in the table (Table 4) by the range of greatest to least number of larvae consumed by an individual. The individual mussel and scallop that it was calculated consumed 433 and 472 larvae respectively were in sub-groups that received ~530 larvae in their container. They had therefore cleared 81 and 89% of the sea lice larvae in 1 h respectively. Figure 19 shows the stomach contents of the large oyster in which the most sea lice larvae were found during dissections (347 in the stomach, 375 in the whole digestive system).

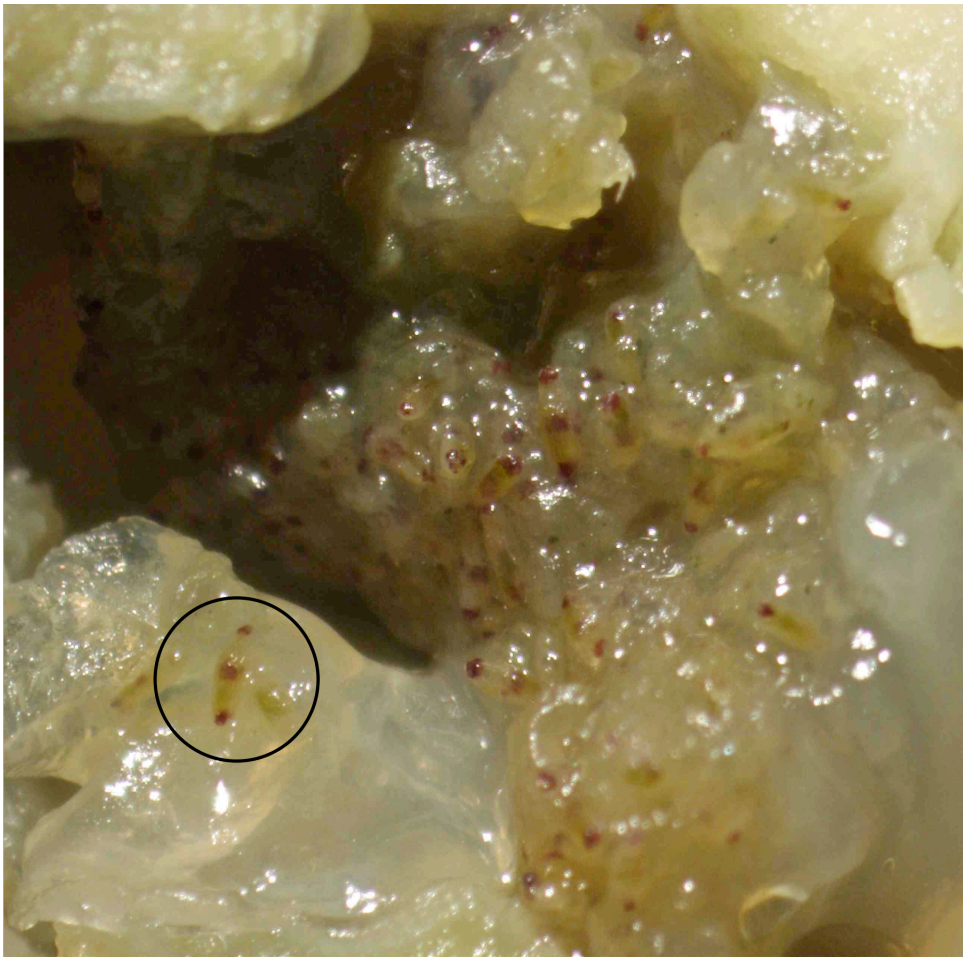

Figure 19. Photo of hundreds of sea lice (*Lepeophtheirus salmonis*) larvae in the stomach of a dissected large oyster (*Crassostrea gigas*) in the size experiment. There were 347 larvae in this stomach (375 in the whole digestive system). Encircled is one larva immediately to the left of a mass of ingested larvae and TISO. (Photo: B. Pirie)

For the size experiments, 18 bivalves received larvae in a BPL treatment of larvae and phytoplankton. Both species and all sizes had individuals within their treatment group that ingested more than 300 larvae within 1 h, with the exception of small oysters (Table 8).

All bivalves in this study were tested in the laboratory with very high densities of larvae, ~575 and ~664 larvae L^{-1} for the two experiments. The nauplii of *Artemia* sp.

were used at a density of 333 larvae L^{-1} in research by Davenport et al. (2000) with *M. edulis*. Similar densities were used by Wong and Levinton (2004) during their microcosm study in which *M. edulis* received *Brachionus plicatilis* rotifers at a density of 480 inds L^{-1} when fed zooplankton alone, and half that density when also fed with phytoplankton at $1.55x10^6$ cells L^{-1}. Molloy et al. (2011) showed that mussels (*M. edulis*) ingested sea lice larvae at a density of 200 copepodids L^{-1}. In contrast, maximum densities of sea lice larvae retrieved during plankton tows in water bodies associated with salmon farms were 0.143 larvae L^{-1} (McKibben and Hay, 2004) and 0.5 larvae L^{-1} (Penston et al., 2004). Since sea lice larvae are not known to exist in densities nearly as great as those used in the laboratory, it cannot be expected that bivalves used at a salmon farm would ingest the numbers of sea lice larvae shown to be ingested during this study within an hour. Over longer periods of time in the field, bivalves may be exposed to the numbers of sea lice larvae experienced in this study, and indications are that the bivalves would be able to consume them.

4.2. Bivalve Size

The present study showed that ingestion rates of sea lice larvae by both Pacific oysters and Pacific scallops were significantly affected by size, larger bivalves consuming more larvae than smaller bivalves on a per individual basis, with a reserved trend when standardizing per unit dry weight of bivalve. Unit dry weight results were significant for scallops, with small scallops ingesting a greater proportion of the larvae in the container than medium or large scallops. This effect of size agrees with feeding results shown by Gerdes (1983) when *C. gigas* were provided algae, the filtration rate increased with

increased body size. Lehane and Davenport (2002) conducted a study of zooplankton ingestion by three species of bivalves. Only the *M. edulis* were tested in different size classes: three sizes for suspended mussels and two sizes for benthic ones. Zooplankton was found in the stomachs of all sizes of mussels. For suspended *M. edulis*, size affected the number of zooplankton ingested and a significant difference was evident between all three size classes. Larger *M. edulis* (mean shell length: ~54 mm) ingested approximately five times the number of zooplankton compared to small mussels (mean length: ~21 mm) (Lehane and Davenport, 2002). No significant difference was shown between the two benthic size classes.

If a salmon farm were growing bivalves on site for the purpose of consuming sea lice larvae, then the best results would come from using large individuals of a species, particularly large scallops. For practicality, it would seem that a mixture of sizes of bivalves would likely be grown at the same time, so as the largest were harvested, there were medium sized bivalves on site growing toward the larger size and smaller spat would be brought on site to start growing. If deploying the lowest biomass of shellfish possible was the main concern, then based on a unit dry weight basis, a greater number of small sized bivalves might be similarly effective compared to fewer larger bivalves.

4.3. Temperature

The present study determined that there was no significant effect of temperature (5, 10, 15ºC) on the ingestion of sea lice larvae by the four bivalve species tested. The temperatures selected in this study were representative of the range of surface ocean temperatures in a salmon farming area on the BC coast which, during a 2003–2005 study,

was 6–13.2ºC in the Broughton Archipelago (Saksida et al., 2007). No temperature effect is expected with bivalves in the field. Cranford et al. (2011) indicates that temperature is not an important feeding control. It is difficult to compare this experiment with maximum filtration studies in the laboratory that have shown a correlation with temperature as those experiments did not involve mesozooplankton in the diet as these experiments did. For the temperature/diet experiments, there was no significant temperature effect shown for any of the four bivalve species. Scallops provided the most reliable results of the four species tested for a temperature effect (see Methods of Statistical Analyses and Sources of Error sections).

Literature on bivalve ingestion of mesozooplankton at different temperatures is non-existent. Davenport et al. (2000) dissected *M. edulis* that had fed in the field during winter and early spring and found that 66% of stomachs included identifiable animal content. The 11% of stomachs that contained only animal material were from the late winter, a cold time of year in Scotland, suggesting that ingestion of mesozooplankton can occur at lower temperatures. All species of bivalves in the current study consumed sea lice larvae at all three test temperatures.

If temperature is not a significant factor, then bivalves that have access to sea lice larvae in the field may be able to consume them at any time of year. In BC, sea lice numbers fluctuate annually, being low from March through June and rising through the autumn and often into the winter. Lice carrying wild salmon returning from the open ocean that migrate past fish farms are the likely source of the autumn rise (Saksida, 2007). It would be best if bivalves used for sea lice control were able to consume sea lice

larvae both in the heat of summer as well as the cold of winter. The results from this study suggest that this may be the case since temperature was not significant.

4.4. Phytoplankton

The present study showed that the presence or absence of phytoplankton did not significantly affect the ingestion of sea lice larvae by the four bivalve species tested for diet. There was also no significant difference detected in the amount of TISO ingested whether larvae were present or not. TISO is commonly fed as part of the diet during broodstock conditioning for a variety of bivalve species (including cockles, mussels, oysters, and scallops) (Navarro et al., 2000, Chávez-Villalba et al., 2002; Liu et al., 2008; Nevejan et al., 20008). The current result with TISO is similar to that of another study using *Tetraselmis* sp., which showed that *M. edulis* consumed zooplankton and grew whether phytoplankton was present or not, though growth of bivalves was significantly greater on the diet with both phytoplankton and zooplankton (Wong and Levinton, 2004).

No significant difference was detected in the proportion of sea lice larvae ingested between bivalves fed larvae in the presence and absence of TISO at ~7.1×10^4 cells ml^{-1}, and no significant difference was found in the amount of TISO ingested whether larvae were present or not. Scallops provided the most reliable results of the four species tested for a diet effect (see Methods of Statistical Analyses and Sources of Error sections).

It appears that the presence of phytoplankton may not be required for the bivalves to ingest sea lice larvae—at least when larvae were present in the very high densities provided in these laboratory experiments. If bivalves are used in the role of sea lice control at salmon farms, it would likely be a year-round farm operation, including times

of higher or variable phytoplankton concentration in the water column (spring through fall) as well as times of low phytoplankton concentration (winter). If there is no significant effect of the presence or absence of phytoplankton in the water column on bivalve ingestion of sea lice larvae, then bivalves may be effective consumers of sea lice larvae independent of the presence of phytoplankton, year round, including winter. It would be worthwhile testing whether various concentrations of phytoplankton affect larval sea lice ingestion by bivalves.

TISO is a higher quality food than bivalves would normal experience in the seston that they consume in the field, so the bivalves may have been feeding at higher rates than they might normally in the field.

TISO, being passively inhaled by bivalves, was a good indicator of the filtration of the bivalves in these experiments. If larvae were as passive and well distributed as phytoplankton in the water in the container, they might have been cleared in the same proportion as phytoplankton. This was not the case. A greater proportion of phytoplankton was consumed when both phytoplankton and larvae were present in the diets. One possible explanation is that the larvae were not as well distributed throughout the container, for instance if they settled on the bottom of the container. Another possible explanation is that the larvae could have been filtered in but rejected by the bivalve as pseudofeces. Pseudofeces were not observed during these experiments. (If they had been, it is theorized that copepod larvae in pseudofeces would not escape the mucus since their exoskeletons have no cilia to handle mucus (Davenport et al., 2000)). A third possible explanation for the greater intake of phytoplankton is that larvae can elicit a swimming escape response when they detect a bivalve's current (Green et al., 2003), at least when

the current is not too strong. While the current in the experimental containers was not measured, it was a combination of a current due to mild aeration and the current generated by the bivalve itself, and was likely small. In the Maar et al. (2008) study of a mussel raft culture, when sampling was performed at times of low current, the bivalves and epifauna were not able to deplete the zooplankton as effectively as when the current was greater. This was attributed to zooplankton escape response being effective at low current.

4.5. Potential Application in Integrated Multi-Trophic Aquaculture

The current research showed that various bivalve species (basket cockle, Pacific oyster, blue/Gallo's mussel hybrids, and Pacific scallop) consumed the larvae of the sea louse, *L. salmonis*, including the infectious copepodid stage, and were often capable of consuming >200, and up to 472 sea lice larvae during the 1-h experiment. These results show that bivalves have the capability of ingesting a considerable quantity of sea lice larvae and, thus, may have the potential to reduce sea lice populations at salmon farms.

If deployed at a salmon farm, it would make sense that bivalves at various stages of growth would make up the population. Farms would likely have a broad range of bivalve sizes, as they would want to sell product on a continual basis. At least a portion of the bivalves should be large, for instance within one year of market size, as large bivalves consumed significantly more sea lice larvae than smaller ones. Small bivalves can also ingest sea lice larvae, and on a unit dry weight basis, can consume even more larvae than large bivalves, such as found for the scallop. The shellfish can consume larvae at year-round sea surface temperatures of 5, 10, and 15ºC. Phytoplankton was not a necessary

component in the water for bivalves to ingest sea lice larvae. Phytoplankton at ~7.1 x10^4 cells ml^{-1} had no significant effect on the consumption of sea lice larvae, at least when sea lice larvae were present at a high density, such as the ~575 larvae L^{-1} used in the temperature/diet experiments. This further supports the idea that the bivalves could consume sea lice larvae year round, even during the autumn and winter when temperatures drop and phytoplankton concentration becomes low, but sea lice numbers at salmon farms are on the rise.

Future research should be conducted at IMTA salmon farms (or salmon farms now interested in how bivalves may benefit their operation), where bivalve ingestion of sea lice larvae is studied along with any effect on the sea lice larval population and infection of salmon.

Bivalves could also serve as an indicator of larval population in the water column. Novales Flamarique et al. (2010) suggested that their sea lice light trap could be used in sea lice monitoring. Just as sea lice counts on salmon are made on a regular basis at salmon farms, gut contents of bivalves grown at the net pens could be regularly inspected for sea lice larvae as an indication of the larval population. If bivalves are used for this purpose, it is important that their digestive process be stopped immediately upon removal from the water column. Davenport et al. (2000) found that larvae could be fully digested within 40 minutes. In this research, bivalve soft tissues were immediately excised and preserved in formaldehyde (ethanol may be an alternative to formaldehyde for this purpose).

4.6. Considerations

If bivalves are to be used to help control sea lice larval numbers at salmon farms, a key question is whether, in the field, bivalves will encounter the number of sea lice larvae that they are capable of ingesting. If bivalves could be suspended, where the sea lice larvae are known to aggregate or if larvae could be concentrated where the bivalves are suspended, then suspended bivalves could conceivably consume the considerable quantity of sea lice larvae of which they have been shown capable during this research.

If there are any regions near a salmon farm where sea lice larvae densities are high, then bivalves could be positioned to take advantage of those locations. Sea lice copepodids aggregate at salinity gradients (Heuch, 1995). Using plankton tows, McKibben and Hay (2004) found the greatest density of larvae at river mouths in the intertidal zone at times when gravid females were present at salmon farms. During daylight, larvae are more likely to have been attracted toward the surface by the light.

The siting of bivalves to ingest sea lice larvae at a salmon farm may be different from siting for bivalves used for organic particulate reduction. This would have to be investigated. Costelloe et al. (1996) found more than ten times the density of sea lice larvae inside a single net pen (66.1 m^{-3}) than outside. There was a significant inverse relationship between distance from the net pen and larval numbers. The ratio of copepodids to nauplii increased with distance to the farm. They also noted that densities of larvae were relatively high at a position 10 m outside the net pen on the lee side (densities so high they were considered outliers and not used) due to the effect of the net pen as it created concentrating eddies, although net biofouling was likely also implicated.

Sea lice larvae react to certain semiochemicals. If a sea lice larva attractant could be developed (Mordue and Birkett, 2009), then the larvae might be attracted and concentrated where bivalves are positioned.

The artificial use of light to concentrate sea lice larvae (Pahl et al., 1999; Novales Flamarique et al., 2010) uses the natural phototaxis of sea lice larvae to attract them to the light, and into a trap. Bivalves could be the trap. Unlike mechanical traps, bivalves require no filter changes and have the potential to provide an additional source of revenue for the farm if sold to market. Ideally, salmon would be kept separated, possibly by depth, from where sea lice larvae collect, whether that is due to artificial light or natural daylight. In a Norwegian study, salmon kept within the top 4 m of the surface, where larvae migrate during the day due to phototaxis, had up to 40 times more lice per salmon than salmon held deeper in the water column (Hevrøy et al., 2003).

Bivalves can cause localized negative impacts. Kimmerer et al. (1994) reported that a clam introduced in a shallow bay removed significant quantities of phytoplankton and three species of marine copepods. It was estimated that these clams could remove more than 8 percent of the nauplii d^{-1}. The presence of the bivalves was continuing to suppress the zooplankton community, which was an uninvited effect from the invasive clam. Pahl et al. (1999) reported that their light trap caught desirable American lobster larvae. The team thought that by using a series of filters, a narrower size range of mesozooplankton could possibly be trapped. With bivalves, there is no such filter; bivalves ingest mesozooplankton of a great variety of species and in a wide size range. This, however, may not be very different from bivalve cultivation not associated with salmon farming,

except that if the bivalves consume sea lice larvae in the field, they may have the intended benefit of a reduced sea lice larval population.

Another question that could be investigated in the field is whether bivalves could provide a prophylactic benefit to the salmon farm by being positioned either fully surrounding a farm if possible, or on the upstream sides of the net pen as determined by the direction of prevailing currents and ebb and flood tides. Since farmed salmon are hatched and initially raised in fresh water, they arrive at a marine farm site without sea lice. If bivalves could intercept a significant proportion of sea lice larvae including the infectious copepodids, before they reach the farm, then initial sea lice infections of salmon could be reduced. It would be important to study the hydrodynamics of the area as well as winds to know prevalent currents that may bring sea lice toward or away from the farm. As the fish require refreshed water brought by water movement through the net pens, any bivalves deployed close to the net pens to control sea lice larvae must not hinder this.

Salmon farms have gone though great expense and trouble to keep sea lice numbers low, from the cost of chemotherapeutants and the purchase of well boats to added staff time. The growth of bivalves could require additional buoys and lines to manoeuvre around in the vicinity of the net pens and personnel responsible for their cultivation. Bivalves produce feces and there is potential for added organic build-up around the farm site. Water flow and quality would need to be assured for the salmon. Bivalves also have potential positive effects of clearing the water of and making use of nutrients from salmon feed and waste while providing an additional crop for market and helping to diversifying the farm's income.

Chapter 5—Conclusions

Under laboratory conditions, four species of bivalves of commercial interest, basket cockle (*C. nuttallii*), Pacific oyster (*C. gigas*), mussel (*M. edulis/M. galloprovincialis* hybrid), and Pacific oyster (*M. yessoensis x P. caurinus*), were provided ~431–498 sea lice (*L. salmonis*) larvae in 750-ml aerated, filtered seawater. All species and sizes of bivalves were capable of consuming more than 100 larvae in 1 h, with the exception of small oysters (height: ~19 mm). It was calculated that individual market-sized cockles, oysters, mussels and scallops consumed >300 larvae (and up to 472 larvae in the case of one large scallop) in 1 h. As direct evidence of sea lice larval ingestion, more than 100, 200, and 300 larvae were retrieved from the digestive systems of mussels, scallops and oysters, respectively, with 375 found inside the digestive system of a large oyster.

Size (small, medium, large) was significant for oysters and scallops, which were the two species tested for this effect, with large bivalves consuming a significantly greater proportion of sea lice larvae in the experimental container within 1 h compared to small bivalves. There was no significant temperature effect (5, 10, 15ºC) or effect of the presence/absence of phytoplankton (TISO provided at ~7.1 $x10^4$ cells ml^{-1}) detected in the proportion of larvae ingested by scallops. Neither effect was detected with cockles, oysters, or mussels, although there may have been insufficient numbers of bivalves involved in this study to detect these effects, if they existed, for those species. All four of the bivalve species did ingest a considerable quantity of sea lice larvae at all three test temperatures and whether there was phytoplankton or not.

Various commercial bivalve species, particularly those of medium to large size, grown at salmon farms as part of an integrated multi-trophic aquaculture (IMTA) system have the potential to be effective in a new role: sea lice control. Bivalves may be capable of ingesting considerable quantities of sea lice larvae, year-round, including when water temperatures and phytoplankton counts are low, where sea lice larvae are plentiful.

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