THE USE OF MODELLING APPROACHES TO EXPLORE INTERACTIONS IN TWO AQUATIC HOST-PEST SYSTEMS

BY

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We, the undersigned, certify that **Thitiwan Patanasatienkul PhD**, candidate for the degree of **Doctor of Philosophy**, has presented his/her thesis with the following title: **"The use of modelling approaches to explore interactions in two aquatic host-pest systems"** and that the thesis is acceptable in form and content, and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate through an oral examination held on February 10, 2015.

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ABSTRACT

Modelling is a useful tool that has been applied in both human and animal epidemiological research. A model is a simplified system that represents a much more complex phenomenon. Various types of models are available. They are generally used for the purposes of explaining phenomena, making predictions, or exploring different scenarios. Several challenges have been encountered during the construction of models in aquatic animal health and are discussed in the dissertation. The research documented in this dissertation aimed to demonstrate the application of modelling to address specific health and production issues associated with two aquatic animal species (blue mussels and wild Pacific salmon).

The first problem dealt with sea lice infestations in wild Pacific salmon populations on the west coast of British Columbia, Canada. The levels of sea lice infestations on wild chum and pink salmon were described and factors associated with inter-annual variation of the infestations were identified using a multivariable logistic regression model. This model included site information as a random effect, to account for spatial aggregation, which provided further details on the degree of clustering at the site level and suggested that the infestation levels depended on the location of fish. This raised the question as to where the risks were and, as a result, a spatial cluster analysis technique (i.e. spatial scan statistics) was used to identify when and where the clusters (of elevated sea lice infestation levels) occurred. The results from clustering analysis can facilitate the hypothesis-generating process for future studies.

The second issue was the problem of mussel loss due to biofouling by tunicates (*Ciona intestinalis*) on Prince Edward Island mussel farms, which was assessed through the use of a mathematical model to describe the dynamics of *C. intestinalis* populations over the growing season. The model incorporated temperature dependencies, which allowed for the assessment of population dynamics under different temperatures, and was then used to evaluate the effectiveness of different mitigation strategies, using fewer resources than would be required if field trials were undertaken.

The research documented in this dissertation demonstrates the use of modelling to address production and health issues in the context of aquatic animals. In addition to the use of field-based trials the research also suggests that modelling can be used as an alternative method to investigate various scenarios and facilitate management planning with advantages in time and cost savings.

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Chapter 1

Introduction and Objectives

1.1. Introduction

1.1.1. World aquaculture and wild fisheries

Global fish production has grown steadily, with an average annual growth rate of 3% over the past five decades (Food and Agriculture Organization of the United Nations, 2014). In 2012, close to 160 million tonnes of fish were produced around the world, about half of which came from aquaculture. World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (Food and Agriculture Organization of the United Nations, 2014). To keep up with the increasing global demand of fish protein, the aquaculture industry has expanded production by introducing new species that give high yields and developing new culture systems and practices, which can influence the spread of pathogens, resulting in disease outbreaks (Murray and Peeler, 2005).

The shift from small to large scale aquaculture production has resulted in the need to improve management and culture systems (Muench et al., 1986). In the past, research and development within the aquaculture sector focused mainly on production enhancement to achieve economic viability (McSweeny, 1986). However, as is the case in any business, the ideal aquaculture farm is one that can not only maximize production but can minimize losses, which are commonly a result of disease or poor husbandry practices.

1.1.2. Production and health issues in aquatic animals

Health issues in aquatic animals may relate to environmental stress factors, such as sea water temperature, salinity, or pollution in water from organic or chemical compounds (Bly et al., 1997). The problem may also be associated with infection by disease organisms, which may cause morbidity and/or mortality in the aquatic animals.

This dissertation focuses mainly on the use of different modelling approaches in addressing the health and production problems in two aquatic animals. These are, wild Pacific salmon on the coast of British Columbia, Canada and the Prince Edward Island (PEI) blue mussel (*Mytilus edulis* Linnaeus, 1758), which are used as case studies to explore a range of research questions, such as risk factor analysis and simulation of disease transmission to assess the effect of interventions. The following section provides an overview of the disease context, as well as the current research methods and challenges associated with each case study.

I) ***Wild Pacific salmon and sea lice infestation***

The presence of wild Pacific salmon in Canadian water systems can be traced back four to six million years, as a result of their ability to move between fresh and salt water (anadromous), which has allowed them to survive through at least five major ice ages (Agricultural and Agri-Food Canada, 2002). Chum (*Oncorhynchus keta* Walbaum, 1792) and pink salmon (*O. gorbuscha* Walbaum, 1792) are the two most abundant wild Pacific salmon species found in the North Pacific Ocean (Noakes and Beamish, 2011). Juveniles belonging to these species are similar in that they enter estuarine habitats soon after they hatch, at mean weights of <1 g. These juvenile salmon disperse into deeper coastal waters following an initial period of acclimation to saltwater that occurs in nearshore habitats (Heard, 1991; Salo, 1991) where there tends to be an increased risk of exposure to sea lice (Morton and Williams, 2003; Jones and Hargreaves, 2007; Gottesfeld et al., 2009).

Sea lice are parasitic copepods of the family Caligidae, which infest both wild and farmed salmonids (Boxaspen, 2006). The most commonly reported sea lice species on wild salmonids on the west coast of Canada are *Lepeophtheirus salmonis*, Krøyer, 1837, and *Caligus clemensi*, Parker & Margolis, 1964 (Morton and Williams, 2003; Jones and Nemec, 2004; Krkošek et al., 2005b). These salmon parasites feed on the hosts' skin, mucus, and blood, which can cause skin erosion, facilitating secondary infections with opportunistic bacteria (Mustafa, 1997), and increase susceptibility to and risk of viral infection (Petterson et al., 2009; Jakob et al., 2011). Because the parasite's attachment to the host causes a generalized stress response mediated through cortisol release, resulting in immune function suppression (Wagner et al., 2008), large numbers of mobile *L. salmonis* can result in host morbidity and mortality (Pike and Wadsworth, 1999).

In the past, the salmon supply in Canada relied on wild capture; however, due to the increasing demands, salmon farming in British Columbia was first started in the early 1970s (Coastal Alliance for Aquaculture Reform (CAAR)). Concerns about the impact of fish farming on the ocean and on communities have been raised by fishermen and environmentalists; and disease agent exchange between wild and farmed salmon, including sea lice, has been one of these concerns (Krkošek et al., 2005a, Marty et al., 2010).

Indeed, the issue of sea lice infestation in wild Pacific salmon populations has been a public focus of attention over the past decade (Morton and Williams, 2003), and several surveys and monitoring programs were developed to evaluate sea lice infestations in wild salmon at various locations on the west coast of Canada (Jones and Nemec, 2004; Beamish et al., 2005; Krkošek et al., 2005b; Jones and Hargreaves, 2007). The impact of sea lice on wild Pacific salmon has been the focus of a number of studies (Jones and Hargreaves, 2007; Krkošek and Hilborn, 2011). Some have addressed the problem of high sea lice infestation levels in wild Pacific salmon populations associated with the open sea-cage aquaculture (Morton et al., 2005; Krkošek et al., 2007a; Price et al., 2010). Different modelling approaches have been applied in these sea lice studies. Several models have been developed to assess the transmission of sea lice between wild and farmed salmon (Krkošek et al., 2005a; Krkošek et al., 2006). The ecological and economic impact of sea lice from salmon farms on wild salmon populations has been evaluated using an age-structured disease model coupled with an economic model (Liu et al., 2011). Additionally, circulation models have been developed to study the impact of hydrodynamics, for example in the Broughton Archipelago (Foreman et al., 2006; Foreman et al., 2009), which aid in understanding the dispersal of sea lice (Stucchi et al., 2011).

Jones & Nemec (2004) suggested that spatial aggregation may be an important dimension in understanding the impact of sea lice infestations on wild salmon. However, a significant degree of inter-annual variability has been reported in several studies (Jones et al., 2006; Jones and Hargreaves, 2007; Krkošek et al., 2007b; Saksida et al., 2011) that needs to be taken into consideration.

These spatial and temporal infestation patterns and the impact of sea lice on wild salmon at the population level are still not well understood. The debate around the effect of salmon farming on the populations of wild Pacific salmon remains (see, for example, Marty et al, 2010, and Krkošek et al, 2011). In order to better understand the impact on sea lice infestations in wild salmon, the effect of factors related to study designs and the disease itself need to be separated and studied. For instance, the time at which sampling takes place, and the differences in sampling protocols (e.g., seining techniques and sea lice evaluation using lethal and non-lethal sampling methods) need to be understood. In addition, how much of the difference is associated with the natural inter-annual variability of sea lice infestation levels on salmon, and the spatial aggregation of sea lice infestations in wild salmon. These questions led to the establishment of a research collaboration between several interested organizations.

In 2010, a collaborative research program, the Broughton Archipelago Monitoring Program (BAMP), was developed with involvement of the salmon farming companies operating in the Broughton Archipelago, Fisheries and Oceans Canada (DFO), university researchers, and the Coastal Alliance for Aquaculture Reform (CAAR). A key objective of BAMP was to gain a better understanding of sea lice levels and their dynamics on juvenile wild pink and chum salmon in the Broughton Archipelago. As part of the BAMP initiative (www.bamp.ca), historical data (from 2003 to 2009) were pooled to create a unified database, while a standard sampling protocol was also developed and implemented, starting in 2010.

With the aim of summarizing this large dataset in order to gain a better understanding of the various factors that may be associated with variations of sea lice infestation levels in wild salmon populations, the use of statistical modelling, which allows for the evaluation of the associations between an outcome and multiple variables to explain the occurrences of sea lice infestations, is explored in Chapter 2. In addition, a spatial cluster modelling technique was used to evaluate the spatial aggregation of sea lice infestations, and is described in Chapter 3.

*II) **PEI blue mussel and biofouling by invasive tunicate species***

In 2012, mussels accounted for 68% of total Canadian shellfish production (41,301 tonnes), with an estimated market value of CAD$44.5 million (Statistics Canada, 2013). The PEI blue mussel industry produced approximately 78% of all mussels cultured in Canada (Statistics Canada, 2013). In PEI, mussels are cultivated using the suspended method with a longline system at the sea surface (Figure 1.1). After retrieving the mussel seeds from the wild, mussels are packed in sleeves (or socks), which are tied to a backline (i.e. a long rope attached to several buoys to prevent the rope from sinking). The socks are then suspended vertically under the sea water for 18-24 months until the mussels reach market size (approximately 5.5-6 cm) (Fisheries and Oceans Canada, 2006).

Over the past 15 years, the industry has encountered increasing challenges related to aquatic invasive species, especially tunicates. These biofouling species compete for food and space, reduce water flow rates from species overgrowth, and jeopardize mussel health and yield. This can cause significant economic losses to both mussel farmers and processors, as a consequence of the costs associated with controlling tunicate growth and the additional labour required during the mussel cleaning process at processing plants (Carver et al., 2006; Fisheries and Oceans Canada, 2006; Daigle and Herbinger, 2009).

Four species of invasive tunicates are found in PEI (MacNair, 2005; Fisheries and Oceans Canada, 2006): clubbed tunicate (*Styela clava* Herdman, 1881), vase tunicate (*Ciona intestinalis* Linnaeus, 1767), golden star tunicate (*Botryllus schlosseri* Pallas, 1766), and violet tunicate (*Botrylloides violaceus* Oka, 1927). Of these, the vase tunicate is considered to be the greatest threat for PEI aquaculture. Two years after the first identification of *C. intestinalis* in the Montague River, PEI, in the autumn of 2004, it became the dominant fouling species, causing severe problems for the PEI mussel industry (Carver et al., 2006; Ramsay et al., 2008).

C. intestinalis is a fast growing, solitary tunicate, with a short-lived planktonic stage, which becomes a sessile filter feeder after settling and metamorphosis (Carver et al., 2006). Because of the rapid growth of the *C. intestinalis* population, a mussel sock can be infested with a heavy tunicate biomass in a short time, compromising the mussel attachment to the socking material, and resulting in mussel loss due to fall-off when socks are lifted (Figure 1.1) (Gill et al., 2007).

A number of mitigation techniques are used to remove tunicates from mussel socks and aquaculture gear; including mechanical, chemical, and natural methods (e.g., calcium hydroxide (hydrated lime) for *S. clava*, 4% acetic acid treatment for *C. intestinalis*, and mechanical methods (high-pressure washing with water) for *C. intestinalis* (Carver et al., 2003; Carver et al., 2006; Ramsay, 2008)). The use of rock crab and green crab predation to control tunicate populations on infested mussel socks has also been explored (Carver et al., 2003; Gill et al., 2007), but high-pressure washing is the mitigation method used most often by farmers in PEI to control *C. intestinalis* populations (Paetzold et al., 2012). This method can knock up to 100% of *C. intestinalis* (Carver et al., 2003); however, the effect does not last long, as new tunicate larvae quickly settle on the mussel socks, especially during the warm months when larval abundance and recruitment levels are at their peaks (Ramsay, 2008; Ramsay et al., 2009).

Time of treatment is an important factor that should be taken into account, in order to achieve a treatment that can effectively control the biomass of tunicates fouling the mussel socks (Gill et al., 2007; Davidson et al., 2009; Arens et al., 2011). A conventional approach, involving field trials, has been conducted for colonial tunicates (Arens et al., 2011; Paetzold et al., 2012) and *C. intestinalis* (Davidson et al., 2009) to carry out a comparison of effectiveness between different mitigation strategies in terms of treatment timing and frequency. Biologically, it appears that the more frequently treatments are applied, the lower the *C. intestinalis* biomass. Investigating a variety of possible scenarios to determine cost-benefit trade-offs in the field is difficult, since these trials require considerable time to execute and are both cost- and labour- intensive. As a result, the use of computer-based modelling, which allows for an evaluation of the likely impact of changes in treatments prior to implementation, is explored in Chapters 4 and 5 of this dissertation.

1.1.3. Modelling

Modelling is a tool that has been applied in both human and animal epidemiological research. A model is a simplified system, representing a much more complex phenomenon (Leung, 1986; Schichl, 2004; Vynnycky and White, 2010), and the construction of a model involves putting together dominant elements that best explain the behaviour of the system in question. Because it is impossible to build a perfect model that can completely describe a real world event, the process of model building usually incorporates only that set of variables which contribute to the explanation of a particular question and, hence, the usefulness of each model is restricted to its scope of application (Schichl, 2004). There are three important properties that have to be balanced when constructing a model: accuracy, transparency, and flexibility (Keeling and Rohani, 2008). The appropriate trade-offs between these elements depend on the goal of the model.

Models have different functions. They are generally used for the purposes of explaining phenomena, making predictions, or exploring different scenarios.

a) Models that aim to *explain phenomena* in the real world have been applied in many areas of study, for example: predator-prey models in ecology – e.g., a model that explores the response of ascidian as a predator on the change in prey density levels (Whitlatch and Osman, 2009); epidemiological models that use statistical methods to find relationships between various factors and disease outcomes – e.g., a logistic regression model to find risk factors associated with Toxoplasmosis in sea otters (Miller et al., 2002); or the use of spatial cluster modelling to detect spatiotemporal clustering patterns of Rift Valley Fever outbreaks in Tanzania (Sindato et al., 2014).

b) After models have been built to explain phenomena, they can often be used further to *make predictions* about real world events. Models that are built for this purpose require a higher degree of accuracy, especially when the results are used to help policy makers in the decision-making process (Schichl, 2004; Keeling and Rohani, 2008). The UK foot-and-mouth epidemic in 2001 is an example where modelling has been widely used to predict disease events, and to develop strategies for disease outbreak control (Morris et al., 2001; Keeling et al., 2003).

c) Models also allow for an *exploration* around an event of interest, such as a disease outbreak, and the effectiveness of different control strategies under a range of scenarios by simulating large numbers of virtual 'what-if' experiments associated with the event. This becomes very useful when such experiments are not practical in the real world due to ethical, economic, or logistic limitations (Keeling and Rohani, 2008; Vynnycky and White, 2010).

In epidemiology, models are constructed in an attempt to understand factors related to disease occurrence, to predict the patterns of an outbreak, and to explore the outcomes of the model when various alternative control strategies are adopted. The use of models can benefit policy makers in that they provide guidance for choosing the most effective disease mitigation strategies and can increase understanding of the life-cycles of infectious agents.

1.1.4. Applications of modelling in aquatic epidemiological research

Disease transmission in aquatic environments typically differs from that seen in the context of terrestrial animals, and as such existing models are often not readily adaptable. Some methods can simply be adopted from those used in terrestrial systems (e.g., risk factor analysis and statistical models), while others require more effort to modify in order to take into account the differences in transmission mechanisms and environmental settings. Two modelling approaches are discussed in this section:

statistical and mathematical models. Examples of these types of models and their applications to animal health in aquatic environments are presented in Tables 1.1 and 1.2.

I) ***Statistical models***

Statistical models predict future trends from observed or historic data, using statistical techniques to describe a relationship that is believed to exist between the outcome and a predictor (for a simple regression model) or multiple predictors (multivariable regression model) (Dohoo et al., 2009, pp. 323-364). In epidemiological research, disease status and health event are the outcomes that epidemiologists are often interested in, while predictors are linked to a range of exposures, such as demographic characteristics, environmental factors, location of an individual (i.e. spatial factors), as well as time.

Statistical models are relatively common in the research of aquatic animal diseases. They have been used to describe parasite loads in fish populations, for example, a generalized linear model was used to identify risk factors associated with sea lice levels on farmed Atlantic salmon (Revie et al., 2003), or a multivariable, two-part random effects model was used to determine the source of sea lice on salmon farms in Chile (Kristoffersen et al., 2013). Additionally, multilevel models were used to determine the degree of spatial aggregation of renal myxosporidiosis in wild brown trout populations (Peeler et al., 2008), and space-time aggregations of infectious pancreatic necrosis virus in Scottish Atlantic salmon farms (Murray, 2003). Some studies have applied regression models to obtain parameter estimates used in their mathematical models (Revie et al., 2005; Taylor et al., 2011; Groner et al., 2013), while others have used regression models to analyze the output simulated from the model or to make comparisons between observed and modelled data (Krkošek et al., 2005a; Murray and Raynard, 2006).

Additionally, statistical models have been applied to evaluate spatial elements of disease or events of interest in epidemiological studies. Various methods have been developed to assess the spatial aggregation of disease or events of interest, which help to elucidate spatial patterns of disease and can be useful in disease surveillance and the hypothesis-generating process. The methods for analyzing clusters can be classified as ‘global’ and ‘local’, based on their properties (Pfeiffer et al., 2008). Global methods only assess whether clustering exists in the study region, while local methods are used to identify where in that study region disease clusters are located. One of the most common methods used for cluster detection is the spatial scan statistic (Kulldorff, 1997). Examples that use of this technique include a determination of the clustering of salmonids with antibodies against viral haemorrhagic septicaemia virus in Switzerland (Knuesel et al., 2003), or the identification of areas with low or high risk of *Toxoplasma gondii* infections in southern sea otters (Miller et al., 2002), and the detection of space-time clusters of infectious salmon anemia virus cases in Chilean Atlantic salmon farms (Godoy et al., 2013).

II) ***Mathematical models***

Mathematical models use equations to explain the change(s) in a system from one state to another and can be analyzed in a precise way by means of mathematical theory and algorithms (Schichl, 2004). Such models can be used in the design and analysis of large, complex, and dynamic systems, since the cost of deriving knowledge from the model is generally much lower than acquiring such information in the real world system (Leung, 1986). Moreover, mathematical models are adaptable and can be manipulated by digital computers, and thus the approach is adopted to use in many fields of study, including human and animal epidemiological research. Examples of mathematical models include the compartmental model (e.g., SIR: susceptible-infectious-recovered model), population dynamic models, and agent-based models.

Compartmental and population dynamic models divide the population into different compartments, and models how the population in each compartment changes over time. The compartments are categorized, based on the individual’s disease status for the compartmental model, and characteristics of the individual (e.g., age, size, developmental life stage, etc.) in the population dynamic model. These models function under the assumption that each compartment is homogeneous, while an agent-based model offers more flexibility to incorporate individual level heterogeneities such as demographic characteristics and level of susceptibility to an infection.

Population dynamic models concentrate on population growth and use the rate-based approach to model the dynamics within a population. This type of model has long been used in aquaculture engineering to understand the ecology and improve the culture systems of aquatic food animals (Polovina and Brown, 1978; Chamberlain et al., 2006; Sadykova et al., 2009; Rosland et al., 2011). In terms of diseases, population dynamic models have been coupled with the natural history of the disease to the study of macro-parasite populations with the intention of mitigating the impacts on the host species. Revie et al. (2005) modelled the growth of sea lice populations on farmed Atlantic salmon in Scotland and used the model to evaluate treatment strategies to control these parasite population levels (Robbins et al., 2010). Population dynamic models may also be used together with compartmental models (e.g., SIR model) to describe transmission of a disease in an age-structured population (Schenzle, 1984). One example of this type of age-structured model was applied to whirling disease in a population of salmonids in order to describe disease status (e.g., susceptible and infected stages) in three developmental stages of salmonids (Turner et al., 2014).

1.1.5. Challenges for modelling diseases in aquatic environments

Various challenges will likely be encountered in any effort to model diseases in aquatic environments. Some of these issues may also be found in modelling diseases in terrestrial and wild animals. Examples include missing data, parameter estimation, ecological or environmental-dependent factors, clustering, and distance measurement. Some of these issues are introduced below.

Missing data can be a challenge in many types of study, including those involving modelling. In the case where the missing data do not occur at random, this will likely cause bias, for example, in the association between two factors. Data imputation, a process of replacing missing values with values predicted from the available data, may be used to mitigate the missing data problem to an extent, assuming that enough data are available (Dohoo et al., 2009, pp.365-394).

Parameter estimation is a key step in the model-building process. This whole process of model construction can be challenging when there is limited information to carry out such estimation. Additional laboratory work can sometimes be carried out to obtain more information regarding parameters; however, this will not be possible in many studies due to limited time and/or budget. An alternative approach to dealing with this challenge is to perform sensitivity analysis to explore the extent to which the modelled output is sensitive to changes in values of a particular parameter (Smith et al., 2008). This may help in justifying additional laboratory or other research that may be required to better estimate the parameter.

Models of diseases in aquatic animals are often found to involve environmental-dependent parameters, such as temperature- and salinity- dependent parameters, which can add more complexity to the model. This has also been seen in studies of vector-borne diseases (Karim et al., 2012; Ogden et al., 2014). One study normalized the environmental-forcing parameters before including them in the model (Jian et al., 2014), while other studies use the approach of 'degree-day', which measures numbers of days required to reach a certain cumulative temperature, to drive the temperature-dependent parameters in a model (Dobson et al., 2011; Mweya et al., 2014).

Clustering occurs when the observations in a dataset share some common features (Dohoo et al., 2009, pp. 529-552). For example, salmon from the same cage (i.e. sharing the same environment) may be exposed to a similar level of sea lice infestation, as compared to those located in other cages (Revie et al., 2007). Clustering can cause the violation to the assumption of independence in many statistical models, and can lead to bias of the estimate and an incorrect estimate of standard error if the analysis is carried out without appropriate adjustment. Several methods can be used to deal with clustering, depending on the type of data; including mixed, or random effects models, generalized estimation equations (GEE), and robust variance (Dohoo et al., 2009, pp. 529-552).

The dispersal of organisms in an aquatic system can be affected by a range of factors associated with the hydrodynamics of the water body. The distance that an organism can travel may depend on intrinsic factors (e.g., animal size, accumulated energy, etc.), as well as extrinsic factors, such as light, tide and water current speeds. Euclidean distance is the common method by which to measure the distance from one place to another (Knuesel et al., 2003; Godoy et al., 2013). However, this measuring system is often unsuitable for use in the study of disease transmission in aquatic environments, which may involve coastal geography. In such cases, a measurement involving some non-Euclidean metric (such as sea-way distance) should be considered to better represent the realistic distances that an organism may travel.

1.2. Objective

1.2.1. Overall objective

This dissertation focuses on the application of modelling to better understand and address health and production issues of aquatic animals. The main objective of the research documented in this dissertation is to illustrate the use of statistical and mathematical models in aquatic epidemiological research to solve problems that occur in the aquatic environmental setting, focusing on two aquatic host-parasite systems: sea lice infestations on wild Pacific salmon in British Columbia, and invasive tunicate species on PEI blue mussels.

1.2.2. Specific hypotheses

The research in the various chapters of this dissertation attempted to address the following hypotheses:

i. That the use of multivariable logistic regression is appropriate for the purpose of examining the temporal patterns (inter-annual trends) of sea lice infestations on wild

Pacific salmon populations and the factors, such as fish species, fish length, month, and gear type used for sampling, that are associated with such infestations (Chapter 2).

ii. That space-time cluster modelling is a sufficient approach to describe the spatio-temporal patterns of elevated sea lice infestations on wild Pacific salmon populations by identifying geographic areas and times that fish infested with sea lice appear to be clustered, and allows for a comparison of the spatial-temporal distribution of different sea lice (*C. clemensi* and *L. salmonis*) and fish (chum and pink salmon) species (Chapter 3).

iii. That the space-time cluster modelling approach facilitates the hypothesis-generation process, by providing additional information concerning factors whose association with sea lice infestation should be investigated at the spatial level (Chapter 3).

iv. That spatial aggregation of sea lice infestation can be assessed by different modelling methods, depending on the goal of the analysis (Chapters 2 and 3).

v. That a mathematical model using a rate-based system dynamics approach is an adequate framework to mimic the population dynamics of *C. intestinalis* for the purpose of investigating its likely infestation characteristics on a mussel farm (Chapter 4).

vi. That a mathematical model using rate-based system dynamics can effectively serve as a tool to explore different mitigation strategies, in terms of combining treatment timing and frequency, to control *C. intestinalis* populations in areas with mussel production (Chapter 5).

vii. That the dynamics of *C. intestinalis* populations are significantly influenced by sea water temperatures and that this impact can be assessed by means of a mathematical model that adopts a rate-based system dynamics approach (Chapters 4 and 5).

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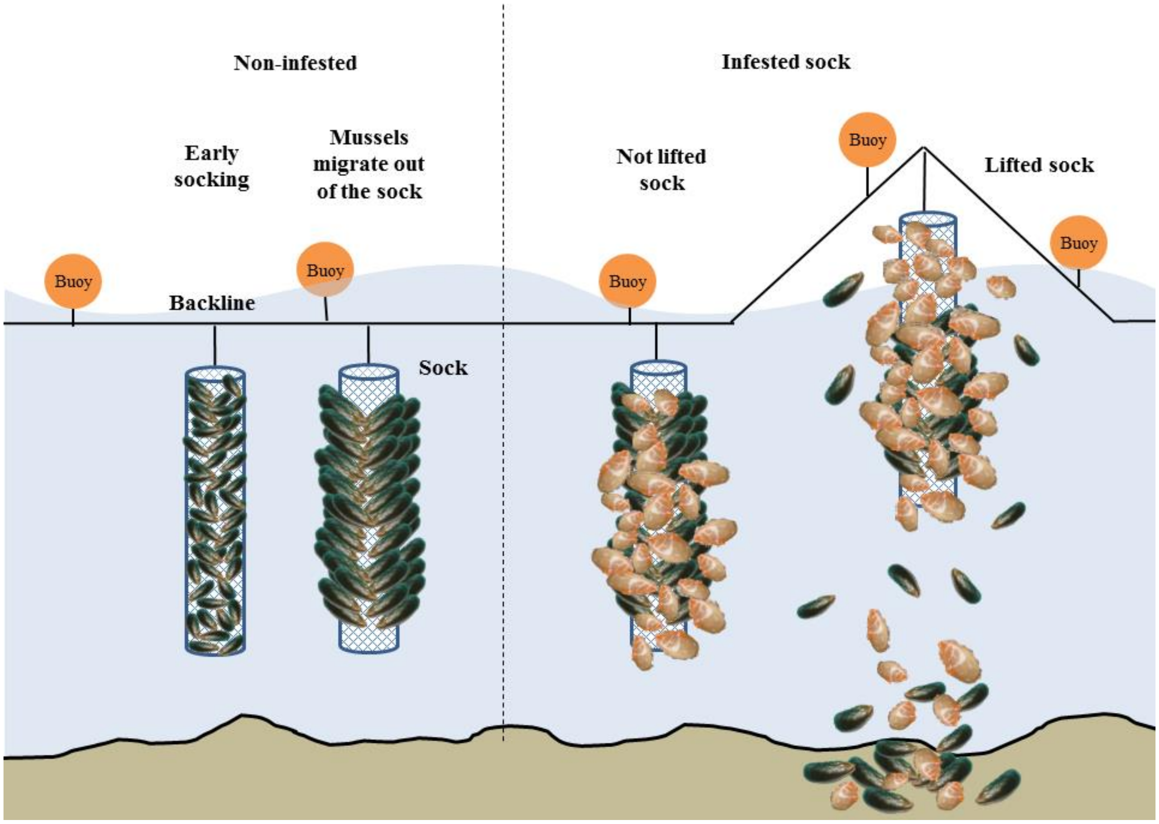

Figure 1.1 Diagram of mussel cultivation in Prince Edward Island using the suspended method with a longline system, for the non-infested socks (left) and *Ciona intestinalis* infested socks (right).

Table 1.1 Examples of statistical modelling approaches used to study production and health issues associated with animals in an aquatic environmental setting.

Approach	Subject of assessment/ purpose	Animal species	Pathogen	Brief description	References
Statistical model					
Regression analysis	Risk factors	Atlantic salmon	Sea lice	Use of GLM to identify factors affecting sea lice abundance on salmon	(Revie et al., 2003)
	Risk factors	Atlantic salmon	Sea lice	Using multivariable, two-part random effects model was used to determine the source of pathogens	(Kristoffersen et al., 2013).
	Disease surveillance	Wild brown trout	Myxosporidia	Estimation of prevalence variances to determine degree of spatial aggregation of disease	(Peeler et al., 2008)
	Disease surveillance	Atlantic salmon	IPNV[1]	Determining the space-time aggregation of disease	(Murray, 2003)
	Parameter estimation	Atlantic salmon	Sea lice	Data fitting to estimate parameters for a mathematical model	(Revie et al., 2005)
	Parameter estimation	Carp	KHV[2]	Estimating transmission probability	(Taylor et al., 2011)
Spatial analysis	Disease surveillance	Sea otter	*Toxoplasma gondii*	Identifying areas with low or high risk of the infections	(Miller et al., 2002)
	Disease surveillance	Salmonids	VHS[3]	Determining the clustering of infected salmonids in Switzerland	(Knuesel et al., 2003)
	Disease surveillance	Atlantic salmon	ISAV[4]	Detecting space-time clusters of cases in Chilean farmed salmon	(Godoy et al., 2013)

[1] Infectious pancreatic necrotic virus

[2] Koi herpesvirus

[3] Viral hemorrhagic septicaemia virus

[4] Infectious salmon anemia virus

Table 1.2 Examples of mathematical modelling approaches used to study production and health issues associated with animals in an aquatic environmental setting.

Approach	**Subject of assessment/ purpose**	**Animal species**	**Pathogen**	**Brief description**	**References**
Deterministic compartmental model	Disease spread	Salmonids	Sea lice	Exploring the emergence of treatment-resistance sea lice under difference scenarios	(Murray, 2011)
	Disease spread	Salmonids	Hypothetical pathogen	Determining viral transmission in aquaculture settings	(Murray, 2013)
	Disease control	Salmonids	BKD[1]	Evaluating management strategies to control disease	(Murray et al., 2011)
	Disease transmission	Salmonids	Sea lice	Assessing parasite transmission between populations	(Krkošek et al., 2005a)
Stochastic compartmental model	Disease spread	Catfish	Hypothetical pathogen	Modelling the dispersal of emerging infectious disease within and between farmed populations	(Zagmutt et al., 2013)
Population dynamic models	Population control, Treatment evaluation	Atlantic salmon	Sea lice	Modelling the growth of sea lice populations on farmed salmon and evaluating treatment strategies to control the sea lice populations	(Revie et al., 2005)
Age-structure, population model	Disease control, Treatment evaluation	Salmonids	*Myxobolus cerebralis*	Determining disease status in three developmental stages of salmonids and evaluating intervention strategies	(Turner et al., 2014)
Agent-based model	Population control, Treatment evaluation	Atlantic salmon	Sea lice	Simulation of sea lice infestation pattern under different control strategies using wrasse	(Groner et al., 2013)
	Disease spread	Manila clams	*Vibrio tapetis*	Simulation of brown ring disease spread	(Paillard et al., 2014)
Hydrodynamic model	Disease spread	-	Sea lice	Modelling the dispersal of copepodid stage of sea lice	(Amundrud and Murray, 2009)
Population matrix model	Pathogen dispersal	-	*Ciona intestinalis*	Modelling the larval dispersal pattern of vase tunicates to evaluate the invasion feasibility in PEI water	(Kanary et al., 2011)
	Impact of temperature on population	-	Sea lice	Modelling the impact of temperature change on sea lice populations	(Groner et al., 2014)

[1] Bacterial kidney disease

Chapter 2

Sea lice infestations on juvenile chum and pink salmon in the Broughton Archipelago, Canada from 2003 to 2012*

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*Patanasatienkul, T., Sanchez, J., Rees, E.E., Krkošek, M., Jones, S.R., Revie, C.W., 2013. Sea lice infestations on juvenile chum and pink salmon in the Broughton Archipelago, Canada, from 2003 to 2012. Dis. Aquat. Org. 105, 149-161.

2.1. Abstract

Juvenile pink salmon (*Oncorhynchus gorbuscha*) and chum salmon (*O. keta*) were sampled by beach or purse seine to assess levels of sea lice infestation in the Knight Inlet and Broughton Archipelago regions of coastal British Columbia, Canada during the months of March to July from 2003 to 2012. Beach seine data were analyzed for sea lice infestation that was described in terms of prevalence, abundance, intensity, and intensity per unit length. The median annual prevalence for chum was 30%, ranging from 14% (in 2008 and 2009) to 73% (in 2004), while for pink the median was 27% and ranged from 10% (in 2011) to 68% (in 2004). Annual abundance varied from 0.2 to 5 sea lice with a median of 0.47 for chum and from 0.1 to 3 lice (median 0.42) for pink salmon. Annual infestation followed broadly similar trends for both chum and pink salmon. However, the abundance and intensity of *Lepeophtheirus salmonis* and *Caligus clemensi*, the two main sea lice species of interest, were significantly greater on chum than on pink salmon in around half of the years studied. Logistic regression with random effect was used to model prevalence of sea lice infestation for the combined beach and purse seine data. The model suggested that there was inter-annual variation as well as a spatial clustering effect on the prevalence of sea lice infestation in both chum and pink salmon. Fish length had an effect on prevalence, though the nature of this effect differed according to host species.

Keywords: Sea lice, *Lepeophtheirus salmonis*, *Caligus clemensi*, Chum salmon, Pink salmon, Broughton Archipelago, Epidemiology

2.2. Introduction

Pink and chum salmon are the most abundant species of anadromous salmon in the North Pacific Ocean (Noakes and Beamish, 2011). Juveniles belonging to these species are similar in that they enter estuarine habitats soon after emergence at mean weights of less than one gram. Juvenile pink and chum salmon disperse into deeper coastal waters following the initial period of adaptation to saltwater that occurs in nearshore or estuarine habitats; see reviews by Heard (1991) and Salo (1991). Subsequent rapid growth in the presence of suitable forage in the marine environment is typical for juvenile pink and chum salmon (Heard, 1991; Moss et al., 2009). The tendency of these early juvenile salmon to occupy relatively shallow nearshore habitat has been associated with an increased risk of exposure to sea lice (Morton and Williams, 2003; Jones and Hargreaves, 2007; Gottesfeld et al., 2009).

Sea lice are parasitic copepods of the family Caligidae which infest both wild and farmed salmonids (Boxaspen, 2006; Wagner et al., 2008). *Lepeophtheirus salmonis* and *Caligus* spp. are the most commonly reported sea lice species (Morton and Williams, 2003; Wertheimer et al., 2003; Jones and Nemec, 2004; Beamish et al., 2005; Krkošek et al., 2005b; Boxaspen, 2006; Rolston and Proctor, 2009; Price et al., 2010, 2011) on salmonids. Sea lice feed on the host skin, mucus, and blood which can cause skin erosion and induce secondary infection (Mustafa, 1997). Large numbers of mobile *L. salmonis* can cause host morbidity and mortality (Pike and Wadsworth, 1999; Tully and Nolan, 2002; Johnson and Fast, 2004). *L. salmonis* has been associated with decreases in swimming performance and post-swim body ion concentrations that are particularly evident in the smallest juvenile pink salmon (Nendick et al., 2011). Lice attachment also causes a generalized stress response mediated through cortisol release resulting in immune function suppression (Wagner et al., 2008). Sea lice infestation has been a problem for aquaculture producers in many countries including Norway, Chile, Scotland, Ireland and Canada (Heuch et al., 2003; Jones and Nemec, 2004; Christopher et al., 2012; Jackson et al., 2012). The issue of sea lice infestation in Pacific wild salmon populations has been a focus of attention over the past decade; several surveys and monitoring programs have been developed to evaluate sea lice infestation on wild salmon at various locations on the west coast of Canada (Jones and Nemec, 2004; Beamish et al., 2005; Krkošek et al., 2005b; Jones et al., 2006; Krkošek et al., 2006; Jones and Hargreaves, 2007). Some studies have addressed the issue of louse infestation in wild salmon associated with the open sea-cage aquaculture (Jacobsen and Gaard, 1997; Krkošek et al., 2005a, 2007a, 2011b; Morton et al., 2005, 2008; Marty et al., 2010; Price et al., 2010, 2011; Saksida et al., 2011). Jones and Nemec (2004) suggested that spatial aggregation may be an important dimension in understanding the impact on sea lice infestations of wild salmon. Some inter-annual variability has been reported in previous studies (Jones et al., 2006; Jones and Hargreaves, 2007, 2009; Krkošek et al., 2007b; Saksida et al., 2011); however, none have reported these trends over a full decade.

Two different sampling protocols were used in the major studies carried out in the Broughton Archipelago; live (Krkošek et al., 2005a, 2005b, 2006, 2007a, 2011b) and lethal (Jones and Nemec, 2004; Morton et al., 2005; Jones et al., 2006; Jones and Hargreaves, 2009; Saksida et al., 2011) methods. The live sampling protocol was unable to identify the species or developmental stage for sea lice in early life stages, while with the lethal method the fish were collected and sent to a laboratory, allowing for a detailed evaluation of any lice present. All of the samples examined under the live protocol were caught by beach seine, whereas those assessed using the lethal protocol were caught either by beach or purse seine. Patterns of lice infestation reported from these studies in the past appeared to be somewhat different from one another and it was assumed that variation in sampling protocols and geographic regions targeted were at least partially responsible for this variability. In 2010 a collaborative research program, the Broughton Archipelago Monitoring Program (BAMP), was developed with involvement of the salmon farming companies operating in the Broughton Archipelago, Fisheries and Oceans Canada (DFO), university researchers, and the Coastal Alliance for Aquaculture Reform (CAAR), with the objective of better understanding sea lice levels and their dynamics on juvenile wild pink and chum salmon in the Broughton Archipelago. As part of the BAMP initiative (http://www.bamp.ca) historical data were pooled to create a unified database; while a standard sampling protocol was also developed and used starting in 2010 through to the present.

The objectives of this study were to describe the prevalence, abundance, and intensity of sea lice infestation on out-migrating juvenile pink and chum salmon in the Broughton Archipelago during sampling months (March to July) from 2003 to 2012 using beach seine data and to model factors, such as fish species, fish length, month, and gear type used for sampling, that may be associated with the inter-annual trends in prevalence of sea lice infestation using the combined beach and purse seine data.

2.3. Materials and methods

2.3.1. Study area

The study area is located in the Knight Inlet and Broughton Archipelago regions of coastal British Columbia, Canada. There are 160 sampling sites which were repeatedly sampled by the Krkošek or DFO teams during 2003 to 2009 (Figure 2.1). The sample design for the Krkošek team was designed to intensively study infestations on juvenile salmon as they migrate through Knight Inlet and Tribune Channel. The sample design for DFO provided a broader representation of locations and habitats across the study area. In 2010 and 2011, the BAMP initiative reduced the number of sites to 98, and further to 79 sites in 2012, by progressively eliminating sites at which few, or no, fish had been caught in previous years.

2.3.2. Sampling

Sampling was carried out using one of three different protocols: the non-lethal sampling method described in (Krkošek et al., 2005b), the lethal sampling method used by DFO (Jones and Nemec, 2004; Jones et al., 2006), and the BAMP protocol (http://www.bamp.ca), which merges the spatial distribution of sampling effort between the two previous programs and which uses lethal lab-based analysis of lice on fish. Data collection took place weekly, biweekly, or monthly during the sampling period March to July over the years 2003 to 2012. Table 2.1 describes the sampling intervals for each type of collection by year. Two fishing gear types, beach and purse seines, were used to collect samples. Purse seine was used only by DFO from 2003 to 2009. The geographic coordinates for every sampling site were collected. Typically, at each site, a maximum of 100 specimens of each species (Krkošek team) or 30 of each species (DFO and BAMP) were randomly selected from the sample. Each fish was measured for fork length (mm) and wet weight (g). Weight was not recorded by the Krkošek team due to difficulty weighing live fish. Lice count, developmental stage (copepodite, chalimus, pre-adult, or adult), species (*Caligus clemensi*, *Lepeophtheirus salmonis*, or not identified to species), and gender (motile stages) data were also collected when the lethal sampling protocol was used. In the case of the non-lethal sampling method, lice were categorised to broad developmental stage but only motile lice were identified to species.

2.3.3. Statistical analyses

A total of 166,316 fish were available for sea lice infestation analysis over the study period. Data quality checking for missing or biologically implausible fish lengths was carried out. All lice were categorised as “non-motile” (copepodite or chalimus stages) or “motile” (preadult or adult stages[1]). A more detailed assessment of specific developmental stages was not possible given that some lice were not classified to this level. Lice that were not identified by species were classified as “not identified to species”.

Means and 95% confidence intervals for fork length and weight of chum and pink salmon were calculated. The total numbers of salmon used to calculate mean weights differed from those used in the estimation of mean length as weight values were missing for 43,750 chum and 40,428 pink salmon respectively. Fish with at least one sea louse of any species and any developmental stage were classified as infested. Louse infestation of each fish species was expressed in terms of prevalence (proportion of fish

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[1] From a biological perspective this may not be entirely correct, in that the copedodites are mobile until they fully attach, while the adult female stages tend to be mostly stationary on the fish. However, these broad categories, i.e. non-motile and motile are widely adopted in the scientific literature around sea lice.

infested), mean louse abundance (lice per fish), and mean louse intensity (lice per infested fish) after Bush et al. (1997), and mean lice intensity per fish length (lice per mm of infested fish). We used a robust standard error to adjust for clustering of sampling sites when estimating the 95% confidence intervals associated with lice infestation (for details on robust standard error see Dohoo et al., 2009, pp. 547-548).

To determine whether to combine beach and purse seine data for descriptive statistics, a comparison of fish length between samples caught using beach and purse nets was made using a paired t-test for all locations at which both gear types were used on the same day. As fish size between the two gear types was different (see Results), and there were more data across the years from beach seine sampling, we restricted our analyses concerning lice infestation to these data.

The difference in proportion of *L. salmonis* and *C. clemensi* was computed with the total number of lice which were identified to species in a given year as a denominator. A zero difference represents an equal proportion of lice from each species. A positive value represents a higher proportion of *L. salmonis*, while a negative value represents to higher proportion of *C. clemensi*. The proportion of all lice that were identified to species in a given year was also calculated. The results are presented using a modified forest plot with weightings derived from the proportions of lice identified to species.

Multivariable logistic regression analysis was used to assess factors affecting levels of lice infestation. Both beach and purse seine data were included in the model. The presence or absence of sea lice (all species) on fish was used as the outcome variable. Year, month, fish length, fish species, and gear type were included as fixed effect predictors, and sampling site as a random effect. The assumptions for logistic regression model were assessed (Dohoo et al., 2009, pp. 399-400). As the assumption for linearity between fish length and the outcome variable is not met, the quadratic term of this variable was added and checked for statistical significance of the term. An interaction between fish species and fish length was also included in the model to account for potential differences in growth characteristics between species (Moss et al., 2009). Akaike's Information Criterion (AIC) was used to assess the fit of alternate models (Burnham, 2002).

2.4. Results

2.4.1. Sampling instances and sampled fish

A total of 7,396 sampling instances were carried out in the Broughton Archipelago area from 2003 to 2012 during mostly monthly surveys (Table 2.1), providing a total of 166,194 sampled fish (122 fish were excluded from the analyses due to missing data) from the combined beach and purse seine data. Typically four to five monthly samples were collected between March and July with some variation among years (Table 2.2). The number of sampling instances per year varied from 236 to 1,514 (Table 2.2). Over the course of the whole study the average number of fish assessed per sampling instance was 11 chum (SD 19.1) and 12 pink (SD 19.4) salmon. In some years (e.g. 2004) chum salmon dominated the samples, while in other years, especially from 2008 onwards, pink salmon were the dominant species sampled.

A summary of the physical characteristics of the salmon sampled can be found in Table S2.1. A paired comparison indicated that fish caught using a purse net were significantly longer than those caught by a beach seine at the same time/location, with mean differences in length of 15.4 mm, 95% CI [13.8, 17.0]) and 7.8 mm, 95% CI [6.6, 9.1]) for chum (n=356) and pink (n=265) salmon, respectively. In the case of beach seined fish, there were far more data reported for length than weight (Table S2.1). As a consequence we assessed only length as a size metric in our analyses. The breakdown of length estimates by year shown in Figure 2.2 indicates a similar finding with additional information on annual variation. As a result of the significant association of gear type and fish size, all the analyses for lice infestation include only the beach seined fish with the exception for multivariable model that includes both beach and purse seine data.

A similar pattern of apparent growth in fish size over the season is shown for chum and pink salmon in Figures 2.3a and 2.3b respectively. For pink salmon (Figure 2.3b) there appear to be few clear differences among the years. In the case of chum salmon (Figure 2.3a) it is interesting to note that in one year (2009) apparent growth was consistently slower while in another (2005) fish exhibited much faster growth.

2.4.2. Sea lice infestation

Table 2.3 provides a summary of the overall sea lice infestation in terms of mean annual prevalence, abundance and intensity for chum and pink salmon caught by beach seine. Annual sea lice abundance varied from as low as 0.17 (2009) to as high as 5.05 (2004) for chum, and from 0.12 (2011) to 3.05 (2004) for pink salmon. It should however be noted that 2004 appears to be very much the outlier in this set of observations, with only one other annual average (chum in 2005) being over one louse per fish. Approximately half of the years assessed have a mean abundance less than 0.5 lice per chum or pink. There are no data from 2010 to 2012 in the purse seine samples (Table S2.2) due to the modification in sampling protocol. Sea lice abundance for the fish sampled by purse net ranged from 0.07 (2008) to 5.78 (2004) for chum, and from 0.17 (2009) to 0.69 (2004) for pink salmon.

The annual prevalence estimates and their 95% CIs are illustrated in Figure 2.4. The median prevalence was around 30% though in many of the latter years this value has fallen to below 20%, with 2004 once again proving the exception with a prevalence of around 70%.The graphical summary indicates that the prevalence of sea lice on both salmon species tend to show similar trends over time. Indeed the confidence intervals for the prevalence estimates of sea lice on chum and pink salmon overlap in every year apart from 2012 suggesting that significant differences are not common. In contrast it can be seen from Table 2.3 that when considering either the abundance or intensity estimates for sea lice on chum, these are significantly higher than those on pink salmon in around half the years (i.e. 95% confidence intervals show no overlap).

2.4.3. Sea lice species

A total of 150,060 sea lice on beach and purse seined fish were assessed over the course of the study. Around 38% were identified as *L. salmonis*, 9% as *C. clemensi*, while the remainder were not identified to species. Variation in the proportion of lice species observed and the percent classified for beach seine data are detailed per year in Table S2.3. From this point the analyses focused on beach seine data. Figure 2.5 summarizes the difference in proportions of lice identified as either *L. salmonis* or *C. clemensi* from all lice which were identified to species in a given year. In the early years, pre 2009, *L. salmonis* was the dominant species with between 48% and 84% more lice than those recorded as *C. clemensi*. The exception was 2003 where the proportions were closer with difference values of just over 15%. However, these were based on a small sample set (only 13% of the 11,133 reported in that year) so the result should be treated with some caution. The sizes of the solid squares in Figure 2.5 are proportional to the numbers of infested fish on which all lice present were identified to species in each of the years. It is interesting to note that in only one year (2011) was there clear evidence of *C. clemensi* being the dominant species observed on the salmon sampled. As can be seen, in all years, the results indicate that similar differences in the proportion of *L. salmonis* compared to *C. clemensi* were found to be present on both chum and pink salmon.

Within the annual summaries, as well as the multivariable model which follows, we have chosen to adopt prevalence as the key measure of infestation. Figures 2.6a and 2.6b illustrate the strong linear relationship between prevalence and abundance when prevalence was less than 60%. As expected, this relationship ceases to hold when sea lice prevalence levels reach 65% or more, as was the case in 2004. There also appears to be an outlier in the pink monthly data (in March of 2005) which has an abundance value over twice the level that might be expected given a prevalence of 26%.

As can be seen from Table 2.3, louse intensity ranged from 1.26 (2009) to 6.90 (2004) for chum and from 1.18 (2009) to 4.48 (2004) for pink salmon. Intensities broken down by month are illustrated in Figure 2.7. As was noted above, 2004 exhibited a high level of prevalence (Figure 2.4) which leads to qualitatively distinct patterns of infestation (Figures 2.6a/2.6b). The inclusion of these heavily infested fish would thus have a disproportionate impact on mean intensity and for this reason data from 2004 were excluded from the estimate of intensity per unit length. Figure 2.7 shows that for all infested fish, although both chum and pink start the season exhibiting no difference in lice intensity the chum exhibit a modest increase in intensity as the season develops, while the intensity of lice on pink salmon remains largely constant, at around 1.5 lice per fish. Normalizing intensity by fish length shows the decreasing trend for both fish species.

2.4.4. Non-motile and motile lice

The development of sea lice coincides with growth of the juvenile salmon during their residence in the study area. Sea louse development was assessed in terms of proportion of motile lice in samples taken over time. Figure 2.8 demonstrates the monthly average proportion of motile lice in comparison to all lice sampled. In general, this proportion increased as the season progressed for both fish species. However, the proportion of motile lice on pink salmon was significantly higher than for chum salmon from May onwards.

The analyses above have purposely not taken into account the species of lice which were identified as being present on the fish. As has been noted, many of the lice observed, particularly those in the non-motile stages, were not labelled according to species. The proportion of lice that were identified to species ranged from 13% in 2003 to 100% of the samples (under the BAMP protocol from 2010). However, prevalence trend analyses incorporating sea lice species, where available, resulted in similar patterns and trends (Figure S2.1).

2.4.5. Multivariable logistic regression model

A summary of results from the logistic model of factors associated with the presence of sea lice is given in Table 2.4. Year, month, gear type, fish species, and fish length were all significant factors, as was the interaction between fish species and fish length. The model shows a better fit when interaction terms were included. Diagnostic analysis provided no indication of heteroscedasticity and modelled residuals were normally distributed. In 2004, the odds of finding a fish with a louse were around 4 times greater than in 2003. The likelihood of lice infestation was lowest in 2009, with an odds ratio of 0.16 in comparison to 2003, or roughly 25 times lower than that seen in 2004. The likelihood of finding fish with a louse increased from March to May, which had the highest odds ratio of 2.77, after which the likelihood decreased in the final two months sampled and by July was no different from the odds of having an infestation in March. Fish sampled by purse net had reduced odds of 0.6 of having lice present on them when compared to those sampled by beach seine. A significant interaction between fish species and fish length was found to be present. In general a quadratic relationship was found to hold between length and sea lice prevalence in that both smaller and larger fish had a reduced likelihood of infestation, while mid-sized fish were most likely to be infested. The interaction was due to the fact that this ‘convex’ relationship was more pronounced in chum than was the case for pink salmon.

Site variance was significantly different from zero, suggesting that there was a spatial clustering effect. The model estimated that the proportion of the variance was 38% at the site level (Table 2.4). The site median odds ratio (MOR_{site}) for two fish with identical risk factors from two randomly selected sites is 3.9, suggesting that if a fish moves from one site to another site with a higher risk, its likelihood of being an infested fish will increase 3.9 times and thus that inter-site variation has a strong impact on the likelihood of infestations for individual fish.

2.5. Discussion

This research summarises findings from the longest continuous surveillance effort for sea lice in populations of juvenile wild salmon. The temporal trends in sea lice infestations were generally mirrored in the two fish species, though at a higher magnitude in chum. The lice were identified to species and stage-classified only when the lethal protocol was used, as a result of which most of the analysis was carried out at the coarse level by combining the two lice species (*L. salmonis* and *C. clemensi*) and aggregating lice stages to motile or non-motile lice.

Incorporating beach and purse seines provided an opportunity to sample juvenile salmon over a broader range of sizes than was possible with beach seines alone. This reflects the tendency of smaller fish to remain in near-shore waters and are thus more likely to be caught by beach seine which is suitable for shallow water (Hahn et al., 2007). The association between gear type and fish size reflects this behaviour and agrees with the findings of several other studies (Johnsen and Sims, 1973; Sims and Johnsen, 1974; Dawley et al., 1986). Although the study indicated that chum caught by purse net were larger than the purse seined pink salmon, no such species effect was present in the case where a beach seine was used (Figure 2.2).

The patterns of fish body growth we observed were similar to those found in earlier studies (Jones and Nemec, 2004; Jones et al., 2006; Jones and Hargreaves, 2009). However, the detailed trends reported here provide a better understanding of factors influencing apparent fish size. For example, the apparently greater length of pink salmon in 2004 (shown in Figure 2.2), appears to be an artefact of sampling effort as there were no fish samples from March (i.e. the smallest pink salmon) in 2004. Both fish species have similar patterns of apparent growth throughout the season, however chum appear to grow faster later in the year, consistent with the observations of the pink and chum population in Kamchatka (Karpenko and Koval, 2012) and in the Northern Bering and Chukchi Seas (Moss et al., 2009). There was little inter-annual variation in these growth patterns for either species over the study period. One exception was for 2005, with which there was higher apparent growth for chum; though not an outlier year in pink, growth was also higher in 2005 than most of the other observed years. Inter-annual variation in fish growth may be attributed to inter-annual variation in ocean hydrological factors affecting available food resources and fish metabolism (i.e. ectotherms grow faster in warmer temperatures) (Gillooly et al., 2001; Atwood et al., 2012).

The inter-annual variation of sea lice infestation on juvenile chum and pink salmon was seen to be similar to those which have been observed in previous studies (Jones et al., 2006; Jones and Hargreaves, 2007, 2009; Saksida et al., 2011). With the exception of one year (2004) the prevalence of infestation appears to vary around the range 15% to 35% for both species. Both the descriptive summaries and the logistic model indicate a slightly higher level of infestation on chum than on pink salmon. However, fish size should also be taken into consideration as pink salmon of 0.5 to 1.0 g are at greater risk of physiological compromise or mortality resulting from infestations with *L. salmonis* (Jones and Hargreaves, 2009). Jones and Hargreaves accounted for fish size using weight and found a decreasing monthly trend of *L. salmonis* density (lice$\cdot$g^{-1}) in pink salmon which is similar to the result from this study using fish body length rather than weight.

The results also suggest that relative variation in prevalence and abundance are similar. At low to moderate prevalence levels it can be shown theoretically that a linear relationship is likely to hold between prevalence and abundance due to the fact that a negative binomial distribution best describes the pattern of infestation (Shaw et al., 1998; Baillie et al., 2009). In our study this linear relationship was clearly exhibited in all months other than those for which the prevalence was higher than 60%. This has previously shown to be the case in empirical data from Scottish farms (Baillie et al., 2009) as well as for a much smaller sub-set of the data currently being analysed, which looked only at infestation levels on wild pink salmon over a four year period (Heuch et al., 2011).

Although our analytical approach could not account for sea lice species for most analyses given the lack of these data across the study, the relative proportions of the two major lice species on sampled fish was explored by computing the difference in proportion of *L. salmonis* and *C. clemensi* to the total number of lice that were identified to species. We found no evidence of a difference in the distribution of lice species across the two host species. In the early years of the study, where *L. salmonis* was the dominant species, its higher relative proportion was seen on both host species. Likewise in later years as the trend shifted towards a more equal occurrence of both sea lice species or a predominance of *C. clemensi* (in 2011). Laboratory studies (Jones et al., 2007) suggest juvenile chum salmon maintain higher burdens of *L. salmonis* compared to size-matched pink salmon. The data presented here on natural infestations do not contradict this finding; however, in a number of years and particularly early in the migration season lice infestation levels appear to be similar across fish species.

A multivariable logistic regression helps increase our understanding of sea lice infestation on wild salmon over the past ten years in the Broughton Archipelago by accounting for the possible confounding factors. The model supports the observation that temporal variation and spatial clustering exist in sea lice infestation of wild chum and pink juvenile salmon made in several studies (Jones and Nemec, 2004; Jones et al., 2006; Saksida et al., 2011). Moreover, the model indicated that the probability of lice infestation depends on fish length and that this risk factor differs between the two fish species studied. The probability followed a 'quadratic' pattern with likelihood of infestation increasing as fish grow until they reach a certain size, after which the likelihood of infestation decreases. The 'convex' pattern observed is one of a number of age-intensity relationships that have been proposed to exist (Hudson and Dobson, 1995; Raffel et al., 2011) and would be consistent with a mechanism such as acquired immunity following prolonged exposure (Yang and Yang, 1998). Given that infestation is acquired after fish enter seawater the initial increase in probability is not surprising. A number of mechanisms may explain the subsequent declining trend: (i) larger fish tend to be infested with older motile stage lice and experimental data indicate few lice survive to reach motile stage (Jones et al., 2007; Krkošek et al., 2009); (ii) larger fish may swim faster and have better developed scales, making it more difficult for copepodids to successfully make contact, attach, and survive (Tucker et al., 2002); or (iii) infested fish may have been killed by direct and indirect effects of lice (Krkošek et al., 2011a). Additionally larger fish tend to stay in deeper water which has lower concentrations of copepodids than the shallow water near the shore (Costelloe et al., 1995; McKibben and Hay, 2004; Costello, 2006). The finding that fish caught by purse seine have a lower likelihood of lice infestation may be confounded by the depth of the water since purse seine is often used to catch fish in deep water, whereas beach seine is suitable for shallow water (Hahn et al., 2007). There was clear evidence as the season progressed and fish became larger that the sea lice became more mature. It is not known, however, why lice infestations on pink salmon later in the season were more likely in the form of motile lice, than those found on chum salmon.

This study provides a descriptive and comprehensive 10 year overview that merges for the first time data from two large monitoring programs of sea lice infestation on wild juvenile Pacific salmon in the Broughton Archipelago. These trends are consistent with trends observed over a shorter time frame from data limited to a more localized area (Tribune Channel) of the Broughton Archipelago (Peacock et al., 2013). There was inter-annual as well as inter-month variation around the prevalence of lice infestation on wild chum and pink juveniles. While there were some difference between infestation levels on the two host species the overall prevalence followed similar trends and this was true for both sea lice species, to the extent that this could be assessed. Further modelling will be conducted to find biotic and abiotic factors which can better explain some of this variation; in particular the reasons that 2004 appears to show such marked difference from the rest of the decade. The model also indicated that around one third of the variation in lice infestation was associated with unmeasured factors at the site level. This suggested that strong spatial clustering of sea lice infestation occurred in this area, consistent with localized sources of infestation and/or spatial aggregation of infested fish. Future studies will focus more directly on this spatial dimension as well as the environmental and aquaculture management factors which likely play an important role in sea lice infestation.

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Table 2.1 Sampling intervals by collector and year. (W=Weekly, BW=Biweekly, and M=Monthly).

Collector	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012
Krkošek team	M	M		M	M	M	M	-	-	-
DFO	W	BW	M	M	M	M	M	-	-	-
BAMP	-	-	-	-	-	-	-	M	M	M

Table 2.2 Number of sampling instances, and number of wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach or purse seine assessed per year by sampling month in the Broughton Archipelago from 2003 to 2012.

		Chum assessed					Pink assessed				
Year	Sampling instances	Mar	Apr	May	Jun	Jul	Mar	Apr	May	Jun	Jul
2003	1,514	2,146	5,140	4,287	1,618	-	1,351	3,547	3,823	1,131	-
2004	832	-	4,048	9,409	1,569	987	-	41	1,201	1,080	385
2005	679	149	655	741	459	312	200	970	1,292	858	562
2006	813	143	3,184	5,333	703	559	185	3,792	5,510	440	120
2007	812	290	4,756	7,965	1,456	-	134	4,785	5,686	876	-
2008	870	212	2,073	4,993	3,847	-	332	4,659	7,173	5,739	-
2009	823	236	1,789	5,113	3,124	154	243	2,477	6,823	4,235	107
2010	409	37	469	688	191	-	324	1,313	1,784	1,172	-
2011	408	237	579	481	252	-	833	977	1,559	1,005	-
2012	236	-	1,113	1,341	997	-	-	1,131	1,362	1,264	-

Table 2.3 Mean and 95% confidence intervals adjusted for site clustering for prevalence of lice infestation (*Lepeophtheirus salmonis* and *Caligus clemensi*), lice abundance and lice intensity in wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago from 2003 to 2012.

	Chum				Pink			
Year	n	Prevalence	Abundance	Intensity	n	Prevalence	Abundance	Intensity
2003	10,127	0.32 [0.26, 0.39]	0.70 [0.52, 0.89]	2.18 [1.96, 2.40]	7,518	0.32 [0.27, 0.38]	0.53 [0.43, 0.64]	1.65 [1.54, 1.76]
2004	13,086	0.73 [0.66, 0.80]	5.05 [4.14, 5.96]	6.90 [6.02, 7.78]	1,308	0.68 [0.64, 0.72]	3.05 [2.40, 3.69]	4.48 [3.69, 5.26]
2005	1,299	0.38 [0.29, 0.47]	1.15 [0.76, 1.54]	3.03 [2.57, 3.50]	2,723	0.32 [0.27, 0.37]	0.64 [0.51, 0.78]	2.01 [1.81, 2.22]
2006	8,701	0.27 [0.23, 0.32]	0.41 [0.33, 0.49]	1.50 [1.42, 1.59]	8,994	0.31 [0.26, 0.37]	0.53 [0.41, 0.65]	1.68 [1.56, 1.81]
2007	13,229	0.41 [0.37, 0.45]	0.81 [0.69, 0.93]	1.99 [1.87, 2.10]	10,711	0.34 [0.30, 0.37]	0.58 [0.50, 0.65]	1.72 [1.66, 1.78]
2008	9,530	0.14 [0.11, 0.17]	0.20 [0.14, 0.27]	1.44 [1.22, 1.65]	16,390	0.13 [0.10, 0.16]	0.17 [0.12, 0.23]	1.35 [1.22, 1.47]
2009	9,262	0.14 [0.12, 0.16]	0.17 [0.14, 0.21]	1.26 [1.19, 1.34]	12,170	0.12 [0.10, 0.14]	0.14 [0.12, 0.17]	1.18 [1.13, 1.23]
2010	1,384	0.30 [0.25, 0.35]	0.46 [0.37, 0.56]	1.53 [1.40, 1.66]	4,591	0.23 [0.21, 0.26]	0.32 [0.28, 0.36]	1.38 [1.33, 1.42]
2011	1,548	0.16 [0.12, 0.20]	0.23 [0.16, 0.31]	1.46 [1.34, 1.58]	4,374	0.10 [0.08, 0.12]	0.12 [0.10, 0.15]	1.22 [1.18, 1.27]
2012	3,450	0.30 [0.26, 0.34]	0.48 [0.40, 0.55]	1.58 [1.46, 1.70]	3,755	0.18 [0.16, 0.20]	0.24 [0.20, 0.28]	1.33 [1.27, 1.39]

Table 2.4 Random effects logistic model for factors associated with the presence of sea lice (*Lepeophtheirus salmonis* and *Caligus clemensi*) on wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach or purse seine in the Broughton Archipelago.

Parameters	Coefficients (β)	95% CI of β	Odds ratios
Year*			
• 2003	Reference		
• 2004	1.46	[1.40, 1.51]	4.30
• 2005	0.06	[-0.02, 0.13]	1.06
• 2006	-0.48	[-0.54, -0.43]	0.62
• 2007	-0.11	[-0.16, -0.07]	0.89
• 2008	-1.64	[-1.69, -1.58]	0.19
• 2009	-1.80	[-1.86, -1.75]	0.16
• 2010	-0.78	[-0.86, -0.71]	0.46
• 2011	-1.64	[-1.73, -1.54]	0.19
• 2012	-1.01	[-1.08, -0.94]	0.36
Month*			
• March	Reference		
• April	0.72	[0.63, 0.82]	2.06
• May	1.02	[0.92, 1.12]	2.77
• June	0.40	[0.29, 0.51]	1.49
• July	0.13	[-0.03, 0.29]	1.14
Gear type*			
• Beach seine	Reference		
• Purse seine	-0.48	[-0.54, -0.43]	0.62
Fish species*			
• Chum	Reference		
• Pink	-0.37	[-0.401, -0.338]	
Length*	0.037	[0.035, 0.038]	
Length^{2}*	-0.00058	[-0.00062, -0.00055]	
Interaction terms**			
• Fish species x Length*	-0.016	[-0.018, -0.014]	
• Fish species x Length^{2}*	0.00024	[0.00019, 0.00030]	
Intercept*	-1.46	[-1.71, -1.21]	
Random effect			
Site*: variance (with SE)	2.04 (0.27)		
Site-median odds-ratio, MOR_{site}	3.90	[4.50, 12.27]	
Intraclass Correlation Coefficient, ICC	0.38		

* indicates the statistical significance at <0.01

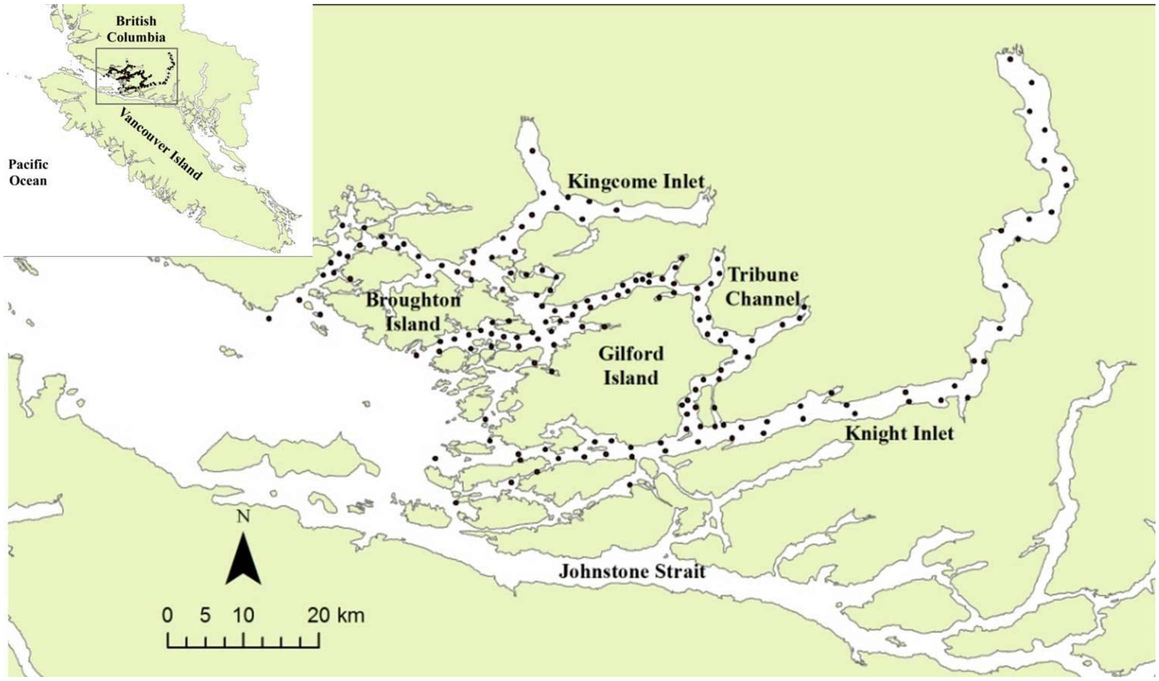

Figure 2.1 Map of the Broughton Archipelago showing the study area and median coordinates of sampling sites collected during 2003 and 2012.

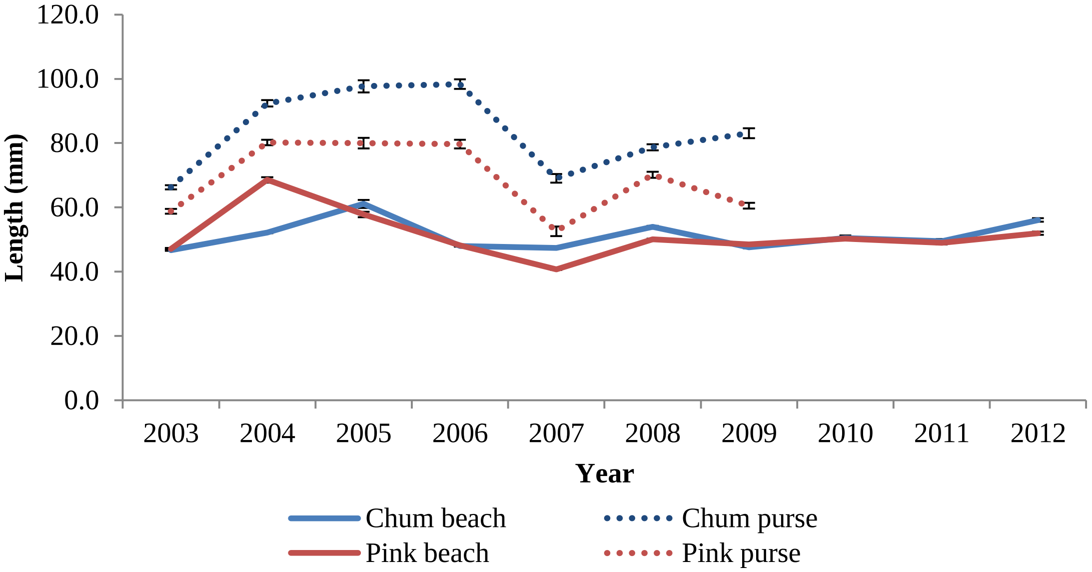

Figure 2.2 Average length with 95% confidence intervals for wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon in the Broughton Archipelago assessed during the sampling months (March to July) from 2003 to 2012 by gear type.

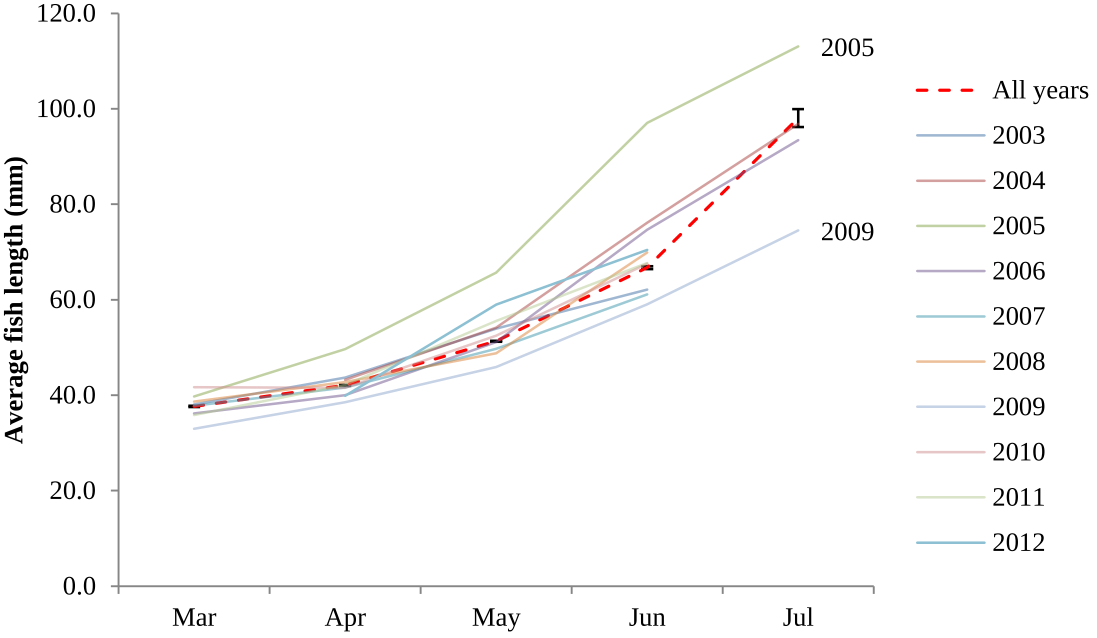

Figure 2.3a Monthly average length of wild chum salmon (*Oncorhynchus keta*) in the Broughton Archipelago sampled by beach seine from 2003 to 2012.

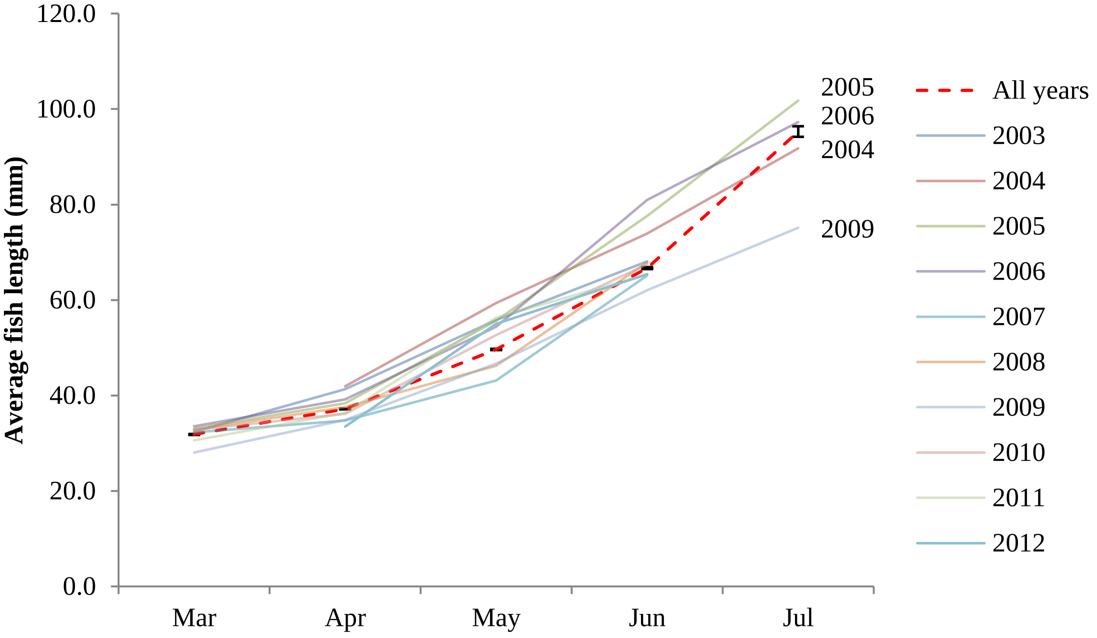

Figure 2.3b Monthly average length of wild pink salmon (*Oncorhynchus gorbuscha*) in the Broughton Archipelago sampled by beach seine from 2003 to 2012.

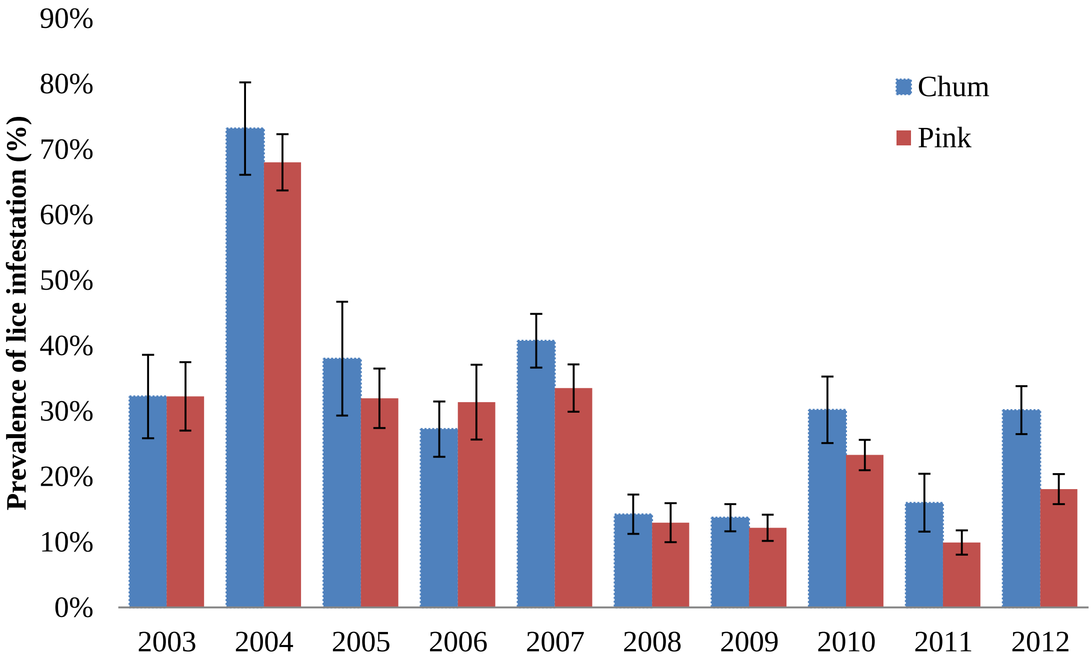

Figure 2.4 Yearly prevalence and 95% confidence intervals adjusted for site clustering of lice infestation in wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago from 2003 to 2012.

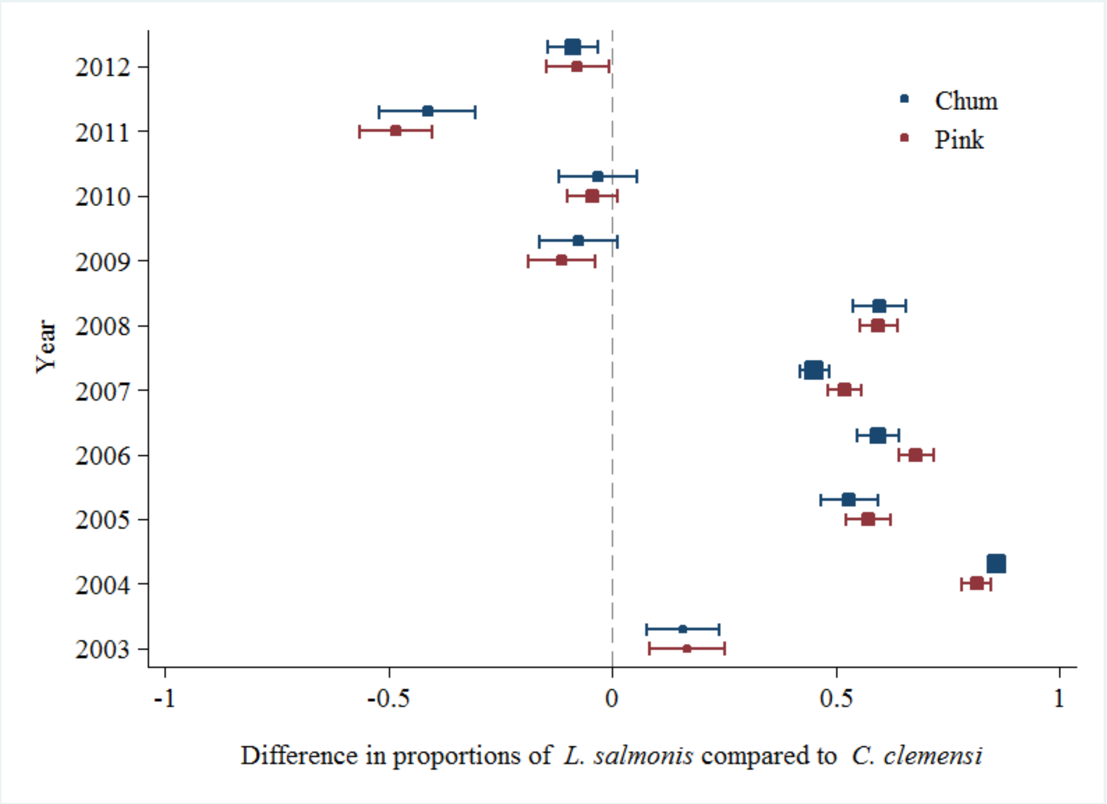

Figure 2.5 Average difference in proportion of *Lepeophtheirus salmonis* compared to *Caligus clemensi* for all lice that were identified to species, on wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago, assessed from 2003 to 2012. The area of the box is proportional to the number of infested fish where all lice were identified to species. Negative values refer to a higher proportion of *C. clemensi* while positive values indicate a higher proportion of *L. salmonis*.

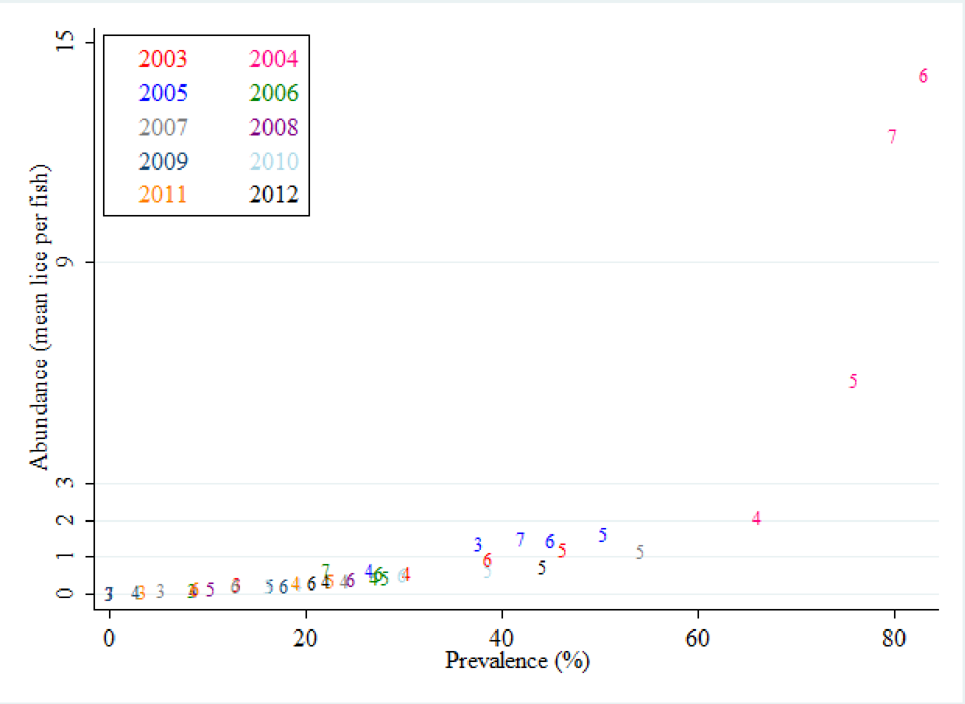

Figure 2.6a Scatter plot of lice infestation prevalence and lice abundance for chum salmon (*Oncorhynchus keta*) sampled by beach seine in the Broughton Archipelago assessed from 2003 to 2012 with the numbers representing sampling month from March (3) to July (7).

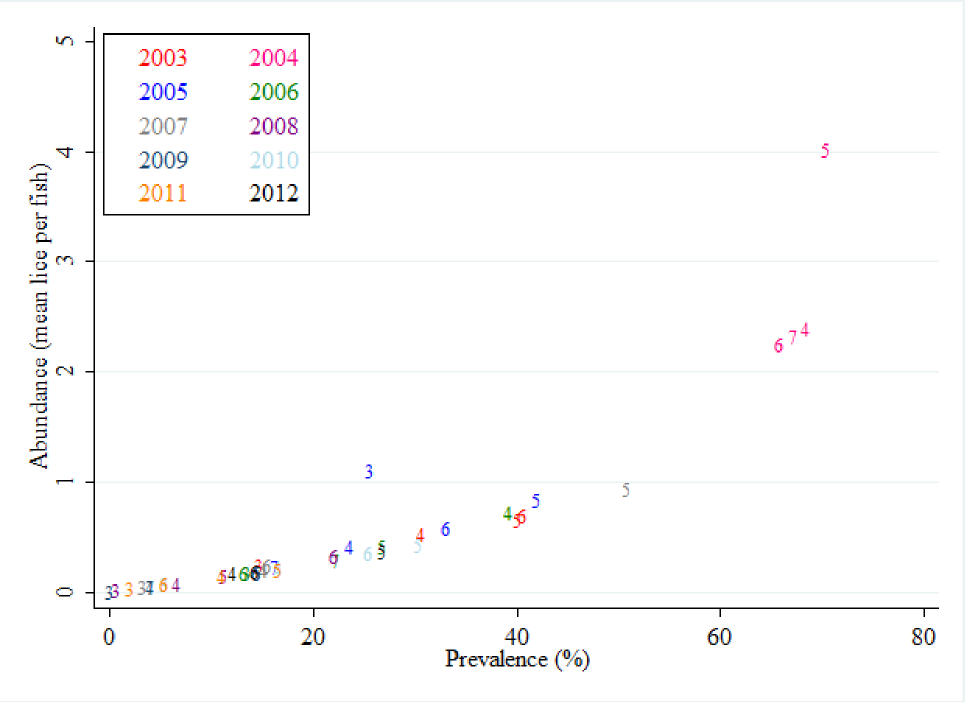

Figure 2.6b Scatter plot of lice infestation prevalence and lice abundance for pink salmon (*Oncorhynchus gorbuscha*) sampled by beach seine in the Broughton Archipelago assessed from 2003 to 2012 with the numbers representing sampling month from March (3) to July (7).

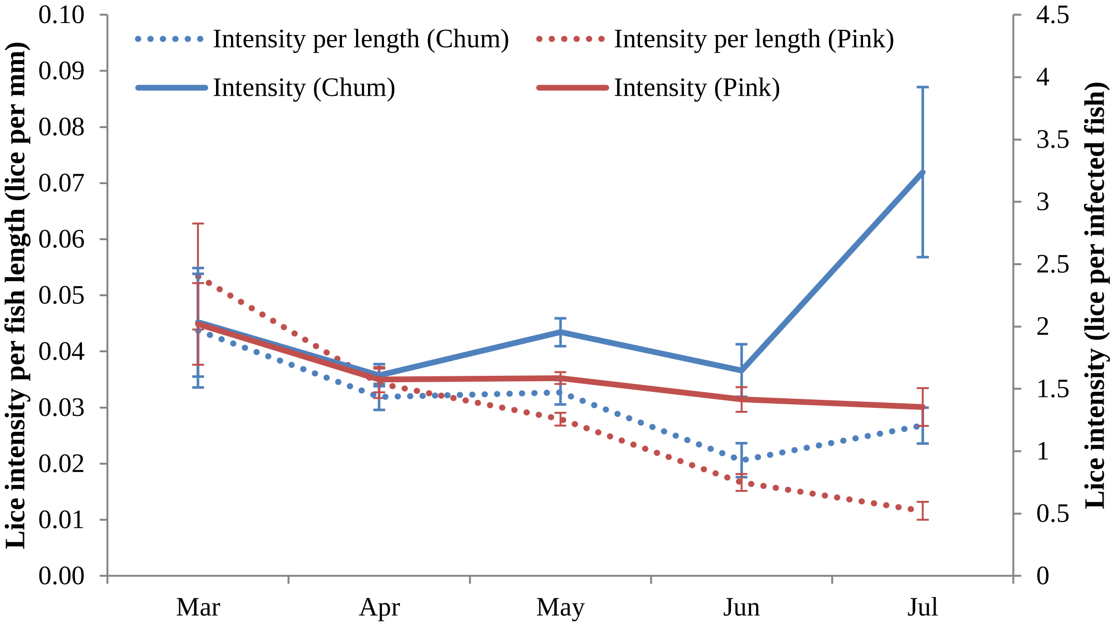

Figure 2.7 Monthly lice intensity and lice intensity per fish length (means and 95% confidence intervals) adjusted for site clustering for wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago assessed in 2003 and from 2005 to 2012.

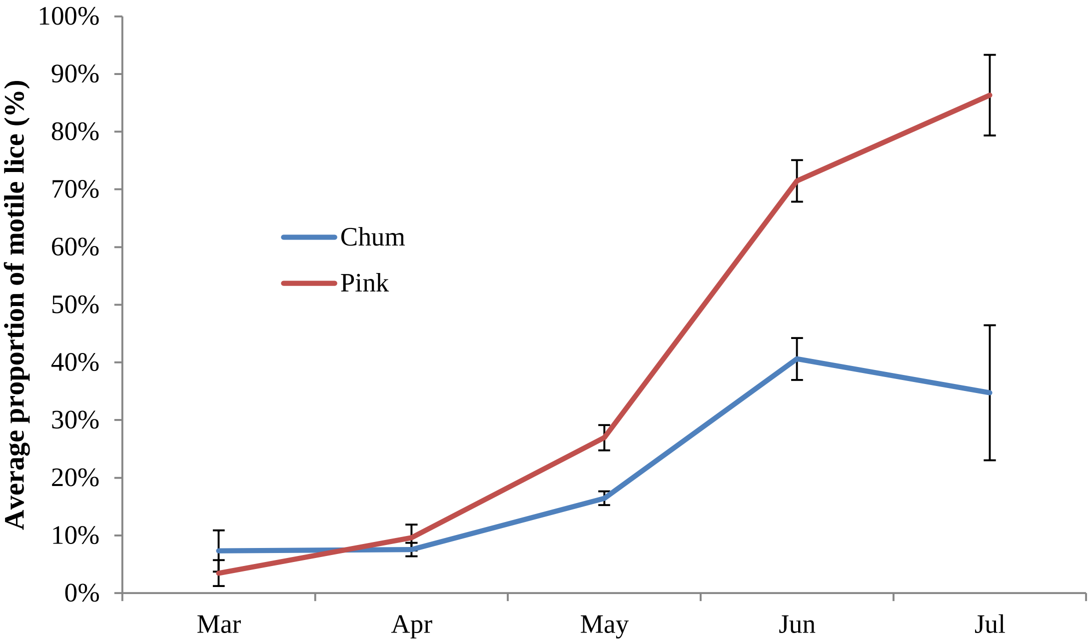

Figure 2.8 Average proportion of motile lice to all lice per fish and 95% confidence intervals adjusted for site clustering on wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago assessed from 2003 to 2012.

2.7. Supplementary materials for Chapter 2

Table S2.1 Number of fish assessed, with mean and 95% confidence intervals for length and weight of chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon by gear type.

		Beach		Purse	
Parameters	Species	n	Mean [95% CI]	n	Mean [95% CI]
Length (mm)	Chum	71,616	49.9 [49.8, 50.0]	12,174	81.9 [81.4, 82.3]
	Pink	72,534	48.6 [48.5, 48.7]	9,870	68.1 [67.6, 68.5]
Weight (g)	Chum	27,866	1.81 [1.78, 1.84]	12,174	8.68 [8.52, 8.85]
	Pink	32,106	1.65 [1.63, 1.67]	9,870	4.56 [4.47, 4.64]

Table S2.2 Mean and 95% confidence intervals adjusted for site clustering for prevalence of lice infestation (*Lepeophtheirus salmonis* and *Caligus clemensi*), lice abundance and lice intensity in wild chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by purse seine in the Broughton Archipelago from 2003 to 2009.

	Chum				Pink			
Year	n	Prevalence	Abundance	Intensity	n	Prevalence	Abundance	Intensity
2003	3,039	0.23 [0.16, 0.31]	0.48 [0.27, 0.68]	2.03 [1.73, 2.32]	2,279	0.16 [0.11, 0.22]	0.23 [0.14, 0.32]	1.44 [1.31, 1.58]
2004	2,921	0.61 [0.53, 0.68]	5.78 [4.44, 7.13]	9.55 [8.09, 11.01]	1,399	0.65 [0.60, 0.70]	2.69 [2.07, 3.31]	4.12 [3.32, 4.92]
2005	1,017	0.27 [0.18, 0.36]	0.72 [0.43, 1.02]	2.69 [2.32, 3.06]	1,159	0.29 [0.24, 0.35]	0.49 [0.37, 0.61]	1.67 [1.46, 1.87]
2006	1,216	0.22 [0.16, 0.28]	0.47 [0.29, 0.64]	2.12 [1.81, 2.43]	1,048	0.18 [0.15, 0.22]	0.26 [0.19, 0.32]	1.42 [1.27, 1.56]
2007	1,237	0.18 [0.13, 0.24]	0.38 [0.23, 0.54]	2.07 [1.51, 2.62]	769	0.16 [0.12, 0.21]	0.24 [0.15, 0.32]	1.48 [1.26, 1.70]
2008	1,593	0.06 [0.03, 0.09]	0.07 [0.04, 0.11]	1.28 [1.14, 1.42]	1,509	0.12 [0.07, 0.18]	0.20 [0.09, 0.31]	1.64 [1.34, 1.94]
2009	1,151	0.18 [0.13, 0.22]	0.43 [0.25, 0.62]	2.47 [1.74, 3.20]	1,707	0.11 [0.09, 0.14]	0.17 [0.12, 0.21]	1.46 [1.29, 1.62]

Table S2.3 Total lice on chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine in the Broughton Archipelago assessed from 2003 to 2012.

	Chum			Pink		
Year	*L. salmonis*	*C. clemensi*	Not identified to species	*L. salmonis*	*C. clemensi*	Not identified to species
2003	461	311	6,348	378	266	3,369
2004	16,655	997	48,443	3,228	260	496
2005	1,141	358	-	1,432	321	-
2006	1,105	314	2,150	1,423	292	3,038
2007	2,690	1,066	6,960	1,713	580	3,892
2008	788	167	998	1,317	291	1,252
2009	238	291	1,081	319	428	1,005
2010	300	340	-	712	761	-
2011	99	263	-	133	398	-
2012	728	917	-	420	485	-

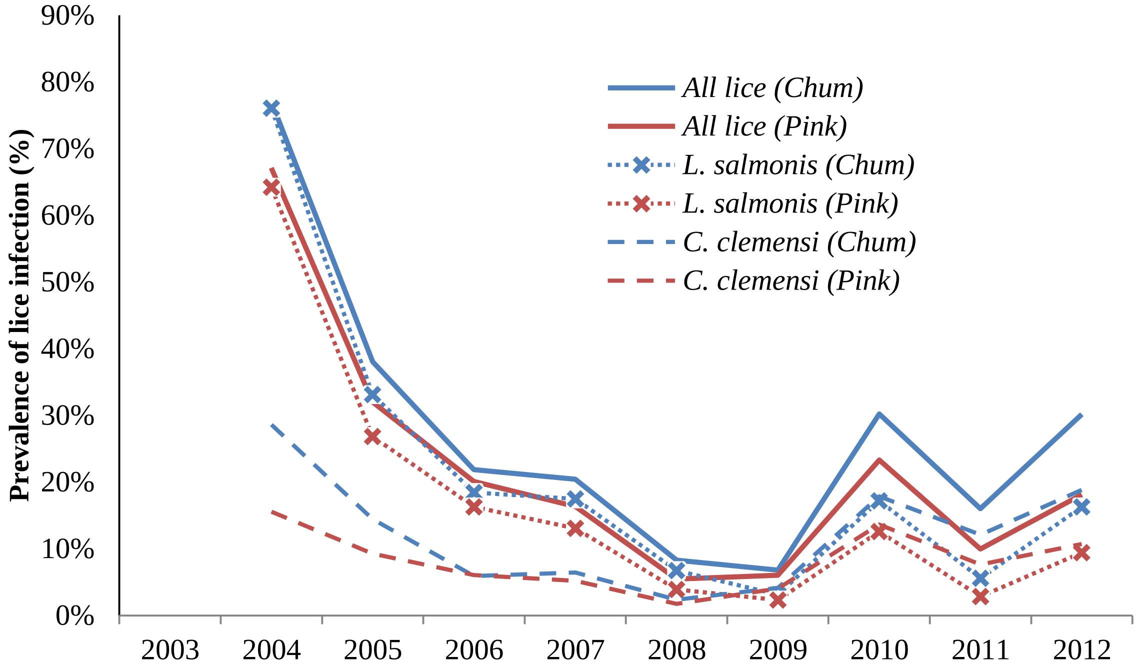

Figure S2.1 Annual prevalence of lice infestation by lice species on chum (*Oncorhynchus keta*) and pink (*O. gorbuscha*) salmon sampled by beach seine with lethal protocol in the Broughton Archipelago assessed from 2004 to 2012.

Chapter 3

Space-time cluster analysis of sea lice infestation (*Caligus clemensi* and *Lepeophtheirus salmonis*) on wild juvenile Pacific salmon in the Broughton Archipelago of Canada[*]

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[*] Patanasatienkul, T., Sanchez, J., Rees, E.E., Pfeiffer, D., Revie, C.W., 2015. Space-time cluster analysis of sea lice infestation (*Caligus clemensi* and *Lepeophtheirus salmonis*) on wild juvenile Pacific salmon in the Broughton Archipelago of Canada. Prev. Vet. Med. Advance online publication. doi:10.1016/j.prevetmed.2015.03.006

3.1. Abstract

Sea lice infestation levels on wild chum and pink salmon in the Broughton Archipelago region are known to vary spatially and temporally; however, the locations of areas associated with a high infestation levels have yet to be investigated. In the present study, the multivariate spatial scan statistic based on a Poisson model was used to assess spatial clustering of elevated sea lice (*C. clemensi* and *L. salmonis*) infestation levels on wild chum and pink salmon sampled between March and July of 2004 to 2012 in the Broughton Archipelago and Knight Inlet regions of British Columbia, Canada. Three covariates, seine type (beach and purse seining), fish size, and year effect, were used to provide adjustment within the analyses. The analyses were carried out across the five months and between two fish species to assess the consistency of the identified clusters. Sea lice stages were explored separately for the early life stages (non-motile) and the late life stages of sea lice (motile). Spatial patterns in fish migration were also explored using monthly plots showing the average number of each fish species captured per sampling site. The results revealed three clusters for non-motile *C. clemensi*, two clusters for non-motile *L. salmonis*, and one cluster for the motile stage in each of the sea lice species. In general, the location and timing of clusters detected for both fish species were similar. Early in the season, the clusters of elevated sea lice infestation levels on wild fish are detected in areas closer to the rivers, with decreasing relative risks as the season progresses. Clusters were detected further from the estuaries later in the season, accompanied by increasing relative risks. In addition, the plots for fish migration exhibit similar patterns for both fish species in that, as expected, the juveniles move from the rivers towards the open ocean as the season progresses. The identification of space-time

clustering of infestation on wild fish from this study can help in targeting investigations of factors associated with these infestations and thereby support the development of more effective sea lice control measures.

Keywords: Sea lice, wild salmon, *Caligus clemensi*, *Lepeophtheirus salmonis,* Cluster analysis, Multivariate spatial scan statistic

3.2. Introduction

Chum (*Oncorhynchus keta*) and pink salmon (*O. gorbuscha*), the most abundant wild salmonid species in the North Pacific Ocean (Noakes and Beamish, 2011), hatch from their natal streams and out-migrate into coastal marine waters during the spring and early summer for a saltwater acclimation period in near-shore or estuarine habitats, before heading out to the open ocean later on in the autumn (Heard, 1991; Salo, 1991). During this staging event, these fish can become exposed to sea lice (Morton and Williams, 2003; Jones and Hargreaves, 2007; Gottesfeld et al., 2009).

Sea lice are copepods of the family Caligidae, infesting both farmed and wild salmonids (Boxaspen, 2006; Wagner et al., 2008). The main sea lice species on wild salmon reported on the west coast of Canada are *Caligus* spp. and *Lepeophtheirus salmonis* (Morton and Williams, 2003; Rolston and Proctor, 2003; Wertheimer et al., 2003; Jones and Nemec, 2004; Beamish et al., 2005; Krkošek et al., 2005b; Boxaspen, 2006; Price et al., 2010). Transmission of sea lice occurs when the infective copepodids settle on a host, which is the beginning of the non-motile stage. The sea lice then undergo a moulting process, developing into pre-adult and adult stages (motile stage) that can move freely on the host (Fast, 2014). Through their feeding behaviour, these salmon parasites can cause skin erosion, facilitating secondary infections with opportunistic bacteria of the host (Mustafa, 1997), and increase the susceptibility to and/or risk of viral infection (Petterson et al., 2009; Jakob et al., 2011). Sea lice infestation on chum and pink salmon in the Broughton Archipelago have been shown to be associated with fish length, and to have spatial and temporal variation (Jones and Nemec, 2004; Saksida et al., 2011). In addition, fish captured using different sampling protocols, e.g. seining technique, and live versus lethal sampling have different infestation levels (Chapter 2; Patanasatienkul et al., 2013; Rees et al., 2015).

Survival of sea lice depends on many factors, including the sea water temperature and salinity. The infective ability and survival of *L. salmonis* copepodids are compromised at salinity levels below 29-30 Practical Salinity Unit (PSU) (Bricknell et al., 2006; Brooks and Stucchi, 2006; Connors et al., 2008). These copepodids may survive at a salinity of 25 PSU; however, the percentage of sea lice that developed into copepodids is very low (Johnson and Albright, 1991). Sea-water is progressively diluted when the river freshet occurs during the spring resulting in the reduced salinity along the surface in areas proximate to the river outflows (Foreman et al., 2009). This creates spatial variation in near-coast salinity levels, possibly causing spatial variation of sea lice, which can be assessed by means of the spatial cluster analysis.

Spatial clustering is a term used to describe the spatial aggregation of some disease, or other event of interest, in a manner that differs from what would be expected simply due to chance. Disease clustering can occur for many reasons such as the infectious spread of disease, the occurrence of disease vectors in a specific location, the clustering of risk factors and the existence of potential health hazards (Pfeiffer et al., 2008). The interplay of the hosts, pathogens together with the compatible environmental condition that could either increase host susceptibility or enhance pathogen infectivity drives the occurrence of a disease (Engering et al., 2013) and their spatial clustering have the impact on disease transmission and the disease control measurement (Tildesley et al., 2009).

Clustering of a disease in space and time can be evaluated using cluster analysis techniques. These analyses can be classified into global and local clustering methods (Pfeiffer et al., 2008). Global clustering methods assess whether clustering exists throughout the study and they measure the degree of spatial clustering without providing the spatial and/or temporal location of the cluster. In contrast, local clustering methods detect the locations of clusters and their extent. Due to the focus of the study in identifying the location of clusters of fish with sea lice infestation, a local method of cluster detection using the scan statistic technique developed by Kulldorff (1997) was applied to this study. The space-time scan statistic is a commonly used method for detection of clusters of disease or some other event of interest in space and time. The analysis can be performed through freely available software: SaTScan^{TM} (Kulldorff, 2011).

The scan statistic technique has been applied in various areas of study, including human and animal epidemiological research (Carpenter, 2001). The scan statistic has been widely used in the human health, for examples, to detect cluster areas with high breast cancer mortality in the United States (Kulldorff et al., 1997), to study spatiotemporal clusters of tuberculosis in Portugal (Nunes, 2007), and to identify hot-spots of malaria transmission and predict future infection (Mosha et al., 2014). In animal health, this method has been applied mostly in disease surveillance of terrestrial animal populations. Norström et al. (2000) detected clusters of acute respiratory disease in cattle using the space-time scan statistic. The space-time permutation model was applied to assess spatial and temporal pattern of Rift Valley fever outbreaks in humans and domestic ruminants (Sindato et al., 2014). Although this method has become familiar to animal health researchers, there are still very few applications of the scan statistic technique in an aquatic context. It has been used to assess clustering of salmonids that had antibodies against viral haemorrhagic septicaemia virus in Switzerland (Knuesel et al., 2003), to detect low/ high risk areas of *Toxoplasma gondii* infections in southern sea otters (Miller et al., 2002), and to detect space-time clusters of infectious salmon anemia virus cases in Chilean Atlantic salmon farms (Godoy et al., 2013; Vanderstichel et al., 2015). All of these studies assumed Euclidean distances between locations and, therefore, may have misrepresented true distances. The mechanisms of disease transmission in aquatic environments typically differ from those in terrestrial animals. In an aquatic environment, transmission between any two locations usually occurs through the water body and the seaway distance is an appropriate measure for quantifying the distance. In river networks or complex coastal inlet geographic situations, non- Euclidean distances will have to be used, which can be accommodated in SaTScan^{TM} when defining the size of the scanning window.

Analysing surveillance data can be a challenging task for a number of reasons, including missing data and the need to use multiple datasets. The univariate scan statistic tests whether there is an increased or decreased risk of an event of interest within, compared to outside, a typically circular area in a single dataset (Kulldorff, 1997). To be able to allow combined analysis of multiple datasets with each representing different host populations or time periods, Kulldorff et al. (2007) developed the multivariate version of the scan statistic. This technique provides the ability to evaluate the consistency of the identified cluster across all input datasets, by testing for clustering within and across datasets, without compromising the power of detecting the statistically significant clusters. Missing data may result in the false positive detection of clusters using the spatial scan statistic, e.g. detection of significant low rate clusters where there is missing data, or detection of significant high rate clusters in other locations that do not have missing data (Kulldorff, 2014). This potential bias can be adjusted for by a calculation technique adjusting for known relative risk, suggested by Kulldorff et al. (1998). The adjustment defines the location and time combinations for which the data are missing, and assigns a relative risk of zero to those location/time combinations, which will then be multiplied with the expected counts to ensure these location/ time combinations do not contribute to the expected count.

Although in a previous study which utilised the same datasets (Chapter 2; Patanasatienkul et al., 2013) there was no evidence of a difference in the temporal distribution of the two main sea lice species between chum and pink salmon (e.g., in the years when *L. salmonis* were dominant species in the chum population, a similar trend was also observed in the pink population), a number of questions around their spatial distribution in the two salmonid species remain. For example, it is not known whether that lack of temporal separation was a real effect or was a result of the different out-migrating locations of the two fish species. Describing fish migration patterns over the season, in addition to an investigation as to whether any clusters identified for the two lice species were similar, would help address these questions. Furthermore, identification of space-time clustering of sea lice infestation on wild fish could inform targeted investigation of the factors associated with these infestations and support the development of more effective sea lice control measures. The objectives of this study were to illustrate the use of the multivariate scan statistic to: 1) identify geographic areas and times of elevated sea lice (*C. clemensi* and *L. salmonis*) infestation on out-migrating wild juvenile Pacific (chum and pink) salmon in the Broughton Archipelago region to facilitate the formulation of hypotheses for further investigation of factors associated with such infestation at an appropriate spatial resolution, 2) compare the identified clusters between the two sea lice species in terms of location and time, 3) compare the identified clusters between the two wild host species. In addition, the migration patterns of out-migrating juvenile chum and pink salmon were assessed.

3.3. Materials and methods

3.3.1. Study area

The study area covered the Knight Inlet and the Broughton Archipelago regions, which are located on the west coast of British Columbia, Canada (50°42′N 126°27′W) . The area included 164 sites at which wild juvenile salmon were sampled and 20 active Atlantic salmon farms during the time of this study (2003 to 2012) (www.bamp.ca) (Figure 3.1).

Sampling at each of the 164 sites did not always occur at the exact same location. A median centre algorithm, which is less influenced by data outliers, was therefore used to determine a central point that minimizes the accumulated Euclidean distance to all other sampling points at the same site (Kulin and Kuenne, 1962; Burt and

Barber, 1996; ESRI, 2010b). The study area was then sub-divided into Thiessen polygons (ESRI, 2010a), using these 164 median centre points.

3.3.2. Sampling

Sampling was carried out using 3 protocols: The non-lethal sampling approach (Krkošek et al., 2005b), the mainly lethal sampling approach (Jones and Nemec, 2004; Jones et al., 2006), and, from 2010 to 2012, a standardised approach that resulted from a multi-stakeholder research initiative: the Broughton Archipelago Monitoring Plan (BAMP; www.bamp.ca). As samples collected using the non-lethal protocol (i.e. all samples from 2003 and the Krkošek datasets) could not provide detailed information on sea lice species and stages, these data were excluded from the current study. For lethal sampling, data collection took place biweekly or monthly during the sampling period, March to July, over the years 2004 to 2012. The pre-2010 samples were obtained using beach or purse seining techniques, while samples from 2010-2012 (the BAMP protocol) used only the beach seining method. The geographic coordinates of each sampling site were recorded, and for each sampling event, a maximum of 30 specimens per fish species (chum and pink salmon) were randomly selected and euthanized. All fish were subsequently examined for sea lice in a laboratory setting as described by Jones and Nemec (2004). Collected data included the number of fish caught and the number of fish assessed per sampling event for each fish species. In addition, the fish length and the lice counts on each sampled individual were recorded, in terms of sea lice species (*C. clemensi* and *L. salmonis*) and stage (non-motile and motile).

3.3.3. Cluster detection method

For the analysis, data were aggregated at the Thiessen polygon site level by month and year. Based on the finding that the relationship between sea lice infestation levels on chum and pink salmon and fish length appear to follow a quadratic pattern (Chapter 2; Patanasatienkul et al., 2013; Rees et al., 2015), fish samples were grouped into three size categories (small, medium, and large) based on fish length, as a proxy of exposure to sea lice in the marine environment, given that larger fish are assumed to be older and more likely to have emerged from their natal streams earlier than smaller fish. Values used to define size categories were determined based on the relationship between sea lice intensity and fish length reported by Rees et al. (2015). The lower cut-points were associated with the point where lice intensity reached its peak and the higher values were set at the point from which the intensity appeared to become stable. Break-point values for length categories for chum were 45 and 90 mm, while for pink the values used were 35 and 70 mm. The size categorization was defined differently for each fish species because growth rates differ for chum and pink salmon (Moss et al., 2009). Data from each of the five months (March to July) and for each of the two fish species were treated as separate datasets, giving a total of ten datasets.

The SaTScan^{TM} software version 9.1.1 (Kulldorff, 2011) was used to perform the space-time cluster analyses. The retrospective spatial scan statistic with the multivariate analysis option (Kulldorff et al., 2007) was used to assess the presence of statistically significant clustering of elevated numbers of fish with sea lice infestation (high cluster) and to identify the potential locations of these clusters across ten datasets. Any fish sampled with at least one sea louse was defined as a case. The analysis was based on models assuming a Poisson distribution of the number of cases at each site, under the null hypothesis that the expected number of cases in each area would be proportional to the number of fish examined in that area (Kulldorff, 1997). Cluster analyses were run separately for each sea lice species. Due to the associations between the infestation levels and sampling years, seine type (beach, purse), and fish size (small, medium, large), these covariates were used to provide adjustment within the analyses using the indirect standardisation method described by Kulldorff et al. (1997). Because the analysis algorithm conditions on the total number of cases observed, any input dataset with zero cases could not be analysed and was removed from the analysis, including the datasets with information on motile *C. clemensi* of both chum and pink salmon that were sampled in March.

To define the scan window size, non-Euclidian seaway distances among all sites were calculated using ‘gdistance’ package in R (Etten, 2012). The maximum scanning window size was set to 20-kilometre-seaway distance to cover the infective copepodids dispersal range (Gillibrand and Willis, 2007) or a maximum of 50% of the population at risk to ensure higher power as recommended in SaTScan^{TM} (Kulldorff, 2011). Missing data due to non-sampling were accounted for by defining sites/ times combinations for which the data were missing. The expected counts for those sites/ times combinations were then multiplied by an adjusting-relative risk (RR_a) of zero, while the other sites/ times were multiplied by RR_a of one (Kulldorff, 2014). Clusters were tested for statistical significance using Monte Carlo hypothesis testing (Dwass, 1957). A cluster was considered statistically significant at a p-value of less than 0.05, calculated from 999 simulated Monte Carlo replications.

3.3.4. Spatial distribution of chum and pink salmon

Fish capture data were aggregated across locations within each Thiessen polygon. Monthly average numbers of fish captured and their coefficients of variation were calculated by fish species. The mean number of fish captured were shown as choropleth maps, for each fish species, according to six categories: no fish captured, 1-10 fish, 11-30 fish, 31-100 fish, greater than 100 fish captured and missing data (i.e. no sampling event occurred). Coefficients of variation by month for each fish species were also plotted to show the dispersion of the data. The maps included five categories: missing values due to no variation (i.e. only one sampling event occurred), missing data due to non-sampling, and the other three categories with break point values at 25th (1.41 %), 50th (1 .83 %), and 75th percentiles (2.28 %)

3.4. Results

The mean numbers of fish captured per month and within each polygon associated with sampling sites were estimated, and the outcomes are presented separately for chum and pink salmon from March to July in Figure 3.2. The grey areas, indicating no sampling, are seen at several sampling sites in the early (March) and late (July) sampling months. The dispersion of the data measured in terms of coefficients of variation can be seen in Figure S3.1. The plots of mean number of fish captured indicate that the fish were out-migrating from the rivers towards the open ocean as the season progressed, and that similar patterns were observed for both chum and pink salmon. Early in the season, both chum and pink can be seen to dominate in the more northerly areas of the Broughton. As the season progresses, a clear shift can be seen, with the dominant densities of both chum and pink being in the mid zones in May, while by June and, particularly, July the areas with the highest mean number of fish tend to be in the outer (sea-ward) areas of the Broughton Archipelago.

A total of 28,775 chum and 34,547 pink salmon were examined. The number of fish by month, seine type, fish size, and fish species are presented in Table 3.1. Almost no large fish were captured in March or April, while in July very few small fish (three chum and no pink) were collected. It can be seen in Figure S3.2 that when considering fish size the proportions shift markedly from small to large for both fish species as the season progresses. The shift towards larger fish can be seen most clearly in the purse seine samples (Figure S3.2(B)), a method by which fewer fish were sampled, but in which those that were tended to be larger. Details on the number of cases (i.e. fish infested with *C. clemensi* : *L. salmonis*) can be found in Tables S3.2 (non-motile sea lice) and S3.3 (motile sea lice). Figures 3.3 and 3.4 summarize the proportion of sea lice infested fish ('case' fish) amongst total fish examined per dataset, where samplings were carried out using the beach seine method, for non-motile and motile sea lice, respectively. Similar details for samples collected using the purse seine method are shown in Figures S3.3 and S3.4. Overall, the temporal patterns of infestation over the season appear similar for both lice species (Figures 3 and 4). When looking at infestation by lice stage, small fish tend to pick up non-motile lice early in the season, while the mid-class and the larger fish obtained their infestations later in May and July (Figure 3.3). The proportion of fish infested with motile sea lice tended to be lower than non-motile infestations, and the proportion of motile infestations progressively increased with time, for all size classes and fish species (Figure 3.4). Fairly similar trends were also observed amongst the fish caught using the purse seine method (Figures S3.3 and S3.4).

Three significant clusters for chum and pink salmon infested with non-motile *C. clemensi* (Table 3.2a) and two significant clusters for salmon infested with non-motile *L. salmonis* (Table 3.2b) were identified. Only one cluster was detected for each of the lice species when considering the motile stage (Tables 3a and 3b). The geographic extent of each of the clusters identified from each run/model can be seen in Figure 3.5 for infestations relating to non-motile sea lice and in Figure 3.6 for motile sea lice. Clusters of elevated prevalence levels of sea lice infestation for each fish species are shown as arrows. Right arrows represent locations where clusters linked to chum salmon were found, while left arrows are associated with pink salmon clusters. It is suggested that Figure 3.5 and Table 3.2 be considered together, and Figure 3.6 and Table 3.3 be read together. For example, the most likely cluster is coded in red for both chum and pink salmon. When a cluster associated with both fish species is detected at the same location, that location appears with a right arrow, attached to a left arrow, which form a rectangle shape. Comparisons are only carried out amongst the results from the same model/run, which means that the most likely clusters for *L. salmonis* may not be located in the same area as those observed for *C. clemensi*. A comparison can be performed across the five months for both fish species within the same lice species. A cluster may be seen in one month but not in another if the analysis does not find a significant cluster in that month (as noted in Tables 3.2 and 3.3). For example, Figure 3.5(B) indicates two clusters of fish infested with non-motile *C. clemensi* in April. The results indicate that the most likely cluster involves both chum and pink salmon (right and left red arrows), but that the 3rd likely cluster is only associated with pink salmon (left blue arrows only). The 2nd most likely cluster, which would have been shown by yellow arrows, was not detected for this month for either fish species.

The area along the northwest edge of Gilford Island, south into the Fife Sound, was detected as the most likely cluster (i.e. the area most likely to have an increased number of infested fish) for non-motile stages of both sea lice species and for both fish species, and was consistent through the season from March to June; the cluster was also detected in July for chum salmon (Figure 3.5(J)). A cluster in this area was also detected for motile sea lice from April to July (Figure 3.6). The 2nd most likely clusters for non-motile lice of both species were detected along Wells Passage located on the northwest side of North Broughton Island, starting from April for *L. salmonis* and May for *C. clemensi* (though the data were quite sparse, particularly for pink salmon). A 3rd likely cluster for non-motile *C. clemensi* appeared in the area around Crease Island and the channel along the north of Harbledown Island and remained in that location throughout the season for pink salmon (Figure 3.5(A) - 5(E)). This 3rd cluster was not detected in March or April for chum, nor did it appear in the analysis of non-motile *L. salmonis* for either fish species.

The relative risks (RR), comparing between the risks within and outside the identified clusters for each dataset (i.e. five months for each of the two fish species) and combined log likelihood ratios associated with the identified clusters, are presented in Tables 3.2 and 3.3. RR over the sampled months exhibited a similar trend for chum and pink salmon. The RR of the most likely cluster for non-motile sea lice on both host species (Table 3.2) and motile *L. salmonis* (Table 3.3b) decreased as the season progressed. In contrast, in the case of the motile stage of *C. clemensi*, the RR increased over time (Table 3.3a). The RR for the next likely cluster tended to decrease as the season progressed for all lice species with the exception of this cluster for non-motile *C. clemensi* on pink salmon; however there were only two months with data, so the trend should not be over-interpreted.

3.5. Discussion

The use of the multivariate scan statistic for the detection of space-time clustering of parasites in an aquatic environment is illustrated in the present study. Although spatial and temporal variations have been noted for sea lice infestation levels on wild juvenile Pacific salmon in the Broughton Archipelago region (Jones and Nemec, 2004; Chapter 2; Patanasatienkul et al., 2013), the infestation patterns have yet to be described. This study identified space-time clusters of sea lice infestation on wild chum and pink salmon in the Broughton area. The results indicated that infestation patterns appeared to be similar for the two host species (chum and pink salmon) and for the sea lice species (*C. clemensi* and *L. salmonis*), after accounting for the spatial distribution of the hosts.

The migration patterns of chum and pink salmon appeared to be similar, both spatially and temporally. Both fish species can be seen in greater densities close to the rivers in the north of the Broughton Archipelago from which the juveniles emerge early in the season. This is more clearly seen in April than March, but it is primarily due to limited sampling in the earlier month. They then migrate seaward as the season progresses, and by the end of the season most fish tend to be in the outer areas of the Broughton Archipelago. This finding is in agreement with previous studies that chum and pink salmon migrate downstream around early spring to mid-summer (Heard, 1991; Salo, 1991).

The results from this study show that there was spatiotemporal clustering of elevated sea lice infestation on chum and pink salmon in the Broughton Archipelago region, which is consistent with previous analyses of the same dataset (Chapter 2; Patanasatienkul et al., 2013; Rees et al, 2015). The study did not find any evidence that the infestation patterns were different between chum and pink salmon, after taking spatial distribution of these hosts into account. In general, the locations of the space-time clusters of elevated sea lice infestation detected for both fish species were similar. Early in the season, the clusters of fish infested with non-motile sea lice are detected closer to the river estuaries, and then as the season progresses, clusters are detected closer to the open ocean. This pattern is arguably clearer for *C. clemensi* than is the case for *L. salmonis*. It is not surprising that the cluster of motile sea lice infested fish did not appear early in the season, as the fish had only just entered the area, exposing them to infectious stage lice (i.e. copepodid) which require time to develop to the adult stage (Pike and Wadsworth, 1999). A similar pattern of infection (i.e. high proportion of motile sea lice on the high-seas captured fish) has also been reported in sea trout (Tingley et al., 1997). There are a number of mechanisms which may explain these clusters:

(1) *Distribution of population at risk*: This might be the case for the cluster areas that are closer to the ocean (i.e. 2nd and 3rd most likely clusters). Early in the season (March-April) most fish are beginning their out-migration and are still residing in areas near the river estuaries; fewer fish are seen in areas further from the estuaries. The clusters located in the open water areas do not appear until later in the season, which is consistent with the fish migration pattern shown in Figure 3.2.

(2) *Occurrence of disease vectors in specific locations*: Given that sea lice can infest a range of host species (Pike and Wadsworth, 1999; Jones et al., 2006; Beamish et al., 2009; Fast, 2014), it is possible that other fish species such as Pacific herring (*Clupea pallasii* Valenciennes in Cuvier and Valenciennes, 1847), coho salmon (*Oncorhynchus kisutch* Walbaum, 1792), Atlantic salmon (*Salmo salar* Linnaeus, 1758) or three-spine sticklebacks (*Gasteroteus aculeatus* Linnaeus, 1758) residing within the identified cluster areas may have acted as a vector for sea lice (Price et al., 2011)

(3) *Clustering of particular risk factors or combination of risk factors*: Although the analyses have accounted for several confounding factors such as seine type, fish size, and year effect, several factors that may have influenced the emergence of clusters have not been adjusted for. For example, salinity and 'salmon farm in vicinity' are two factors that may have contributed to the elevated number of fish infested with sea lice within the cluster areas. Salinity plays an important role in the development of sea lice and their survival (Brooks and Stucchi, 2006; Connor et al., 2008); low salinity levels (<28 PSU) compromises sea lice survival (Johnson and Albright 1991; Bricknell et al., 2006). Salinity levels greater than 28 PSU, found to be suitable for sea lice development (Uu-a-thluk, 2009), were observed in the areas at times when the clusters were identified (illustrative values for salinity in two of the sampling months can be seen in Figure S3.5), which might cause the spatial clustering. Furthermore, these cluster areas are located in the vicinity of salmon farms, which several studies have reported as a factor positively associated with sea lice infestation of wild salmon (Krkošek et al., 2005a; Morton et al., 2008; Marty et al., 2010; Price et al., 2010; Price et al., 2011; Rees et al., 2015).

Several interesting patterns emerge when the identified clusters were investigated in more detail. The clusters around Wells Passage, located to the northwest side of North Broughton Island (2nd likely cluster), and Crease Island and the channel along the north shore of Harbledown Island (3rd likely cluster) showed an increased relative risk (RR) throughout the season for both fish species. This is likely associated with the distribution of the fish as discussed above. Conversely, the RRs of the most likely clusters of both lice species, located along the North West edge of Gilford Island, south into the Fife Sound, showed a decreasing trend over time (the opposite trend was observed for motile *L. salmonis*). A possible explanation for the decreasing trend of RR is that fish may have developed physiological and/ or biological defense mechanisms, e.g. changes in swimming behaviour, development of immunity against infestation (Tucker et al., 2002; Boxaspen, 2006; Fast, 2014) and, therefore, be able to clear off the attached sea lice. Alternatively, the infested fish could have died due to the direct or indirect effect of sea lice (Krkošek et al., 2011; Peacock et al., 2014), resulting in a reduced number of cases and, consequently, reduced RR.

One of the objectives of this study was to assess similarity of clustering patterns of sea lice infestation between the two host species across the five month study period. The host- specific datasets could have been analyzed either separately or simultaneously, using the univariate or multivariate scan statistic, respectively. The latter had the advantage that it was possible to statistically and robustly investigate clustering within and between fish species. It also allows for a comparison of the risk estimates across input datasets. The study took inter-annual variation into account, and treated monthly data as separate datasets to be able to assess whether the cluster appears consistently throughout the season.

SaTScan^{TM} has the advantage of being able to identify the locations of clusters; however, dealing with missing data is still a challenge. The software provides an option to adjust for missing data in the discrete Poisson model, using the "known relative risk" feature to prevent the location/time combinations associated with missing data from contributing to the analysis (Kulldorff, 2014); however, a limitation exists for a dataset with a long time series. The datasets used in this study contain data from five months (March to July) and nine consecutive years (2004 to 2012). In the attempt to simplify the analysis by using generic settings, and aggregating the nine-year data into five months, adjusting for missing data was constrained by the input file format required by the software, and analysis of the data using the generic setting approach could not be performed.

The aquatic setting of the sea lice infestation and transmission was accounted for by using the Non-Euclidean distance to perform the scan. The spatial scanning window was set to the maximum of 20 km-seaway distance, which could cover the sea lice larval dispersal range (Krkošek et al., 2005a; Gillibrand and Willis, 2007). Different distances (5-30 km) were also assessed to test the robustness of the size of scanning window chosen in this study. The clusters were detected in similar areas for all the tested distances. The 20 km-seaway distance provides the most reasonable spatial aggregation, and thereby was used for the analyses. The study did not use salmon dispersal range estimates to decide on the maximum scanning window size, as these fish can travel significant distances in a short period of time. If the scan window size had been based on the swimming range of fish, the scan window would have covered a large area (perhaps even the whole region) and the ecological zones controlled by landscape factors (e.g. water salinity) would have been crossed by the area within that distance.

In summary, this study has found that juvenile chum and pink salmon tended to have similar out-migration patterns. The study has also identified space-time clusters of sea lice infestation on wild salmon in the Broughton Archipelago region during the out-migrating period of March to July, which can provide insight for hypothesis generation for further, targeted investigation of factors that may be associated with these clusters. This study also suggests that *C. clemensi* and *L. salmonis* have similar habitat, as the spatiotemporal clusters of elevated numbers of fish infested with sea lice are not different between the two sea lice species. Furthermore, the study suggests that the clusters of fish infested with sea lice are similar for chum and pink salmon, both spatially and temporally.

3.6. References

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Table 3.1 Number of fish assessed per dataset (chum and pink salmon from March to July, 2004 to 2012) by seine type (beach or purse) and fish size as classified small, medium, or large using length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon.

Dataset	Species	Month	Number of fish assessed					
			Beach			Purse		
			Small	Medium	Large	Small	Medium	Large
1	Chum	March	1,198	42	0	62	2	0
2	Chum	April	4,689	1,192	0	320	227	0
3	Chum	May	2,510	5,644	82	119	2,354	226
4	Chum	June	231	3,344	396	7	1,922	2,196
5	Chum	July	1	89	220	2	114	1,586
6	Pink	March	1,992	228	0	28	3	0
7	Pink	April	4,122	3,130	1	447	460	1
8	Pink	May	718	9,161	805	158	1,902	744
9	Pink	June	14	3,707	2,595	1	443	2,713
10	Pink	July	0	18	464	0	1	691

Table 3.2 Cluster analyses of chum and pink salmon infested with non-motile (a) *C. clemensi* and (b) *L. salmonis*. Relative risks (RR) for each month and fish species are shown. A hyphen (-) indicates that the identified cluster was not statistically significant for that dataset.

(a) Non-motile *C. clemensi*

	Most likely cluster (1st)		Second likely cluster (2nd)		Third likely cluster (3rd)	
Month	Chum	Pink	Chum	Pink	Chum	Pink
March	2.65	2.72	-	-	-	1.53
April	2.71	2.00	-	-	-	1.31
May	1.78	1.78	1.53	-	1.73	1.19
June	1.06	1.08	2.13	4.79	1.23	1.71
July	-	-	2.33	2.37	2.20	2.82
Log Likelihood Ratio	196.6		61.5		60.7	

(b) Non-motile *L. salmonis*

	Most likely cluster (1st)		Second likely cluster (2nd)	
Month	Chum	Pink	Chum	Pink
March	2.42	2.54	-	-
April	2.35	1.64	1.28	-
May	1.43	1.42	1.17	1.14
June	1.45	1.42	1.43	1.61
July	1.26	-	1.47	2.32
Log Likelihood Ratio	192.1		35.4	

Table 3.3 Cluster analyses of chum and pink salmon infested with motile (a) *C. clemensi* and (b) *L. salmonis*. Relative risks (RR) for each month and fish species are shown. A hyphen (-) indicates that the identified cluster was not statistically significant for that dataset. Data for fish sampled in March (showing as N/A) were not included in the analysis.

	(a) Motile *C. clemensi*		**(b) Motile *L. salmonis***	
	Most likely cluster (1st)		Most likely cluster (1st)	
Month	Chum	Pink	Chum	Pink
March	N/A		-	-
April	1.90	5.89	2.75	2.04
May	2.00	1.62	1.82	1.47
June	2.50	1.68	1.49	1.43
July	3.78	2.67	1.31	-
Log Likelihood Ratio	146.3		144.1	

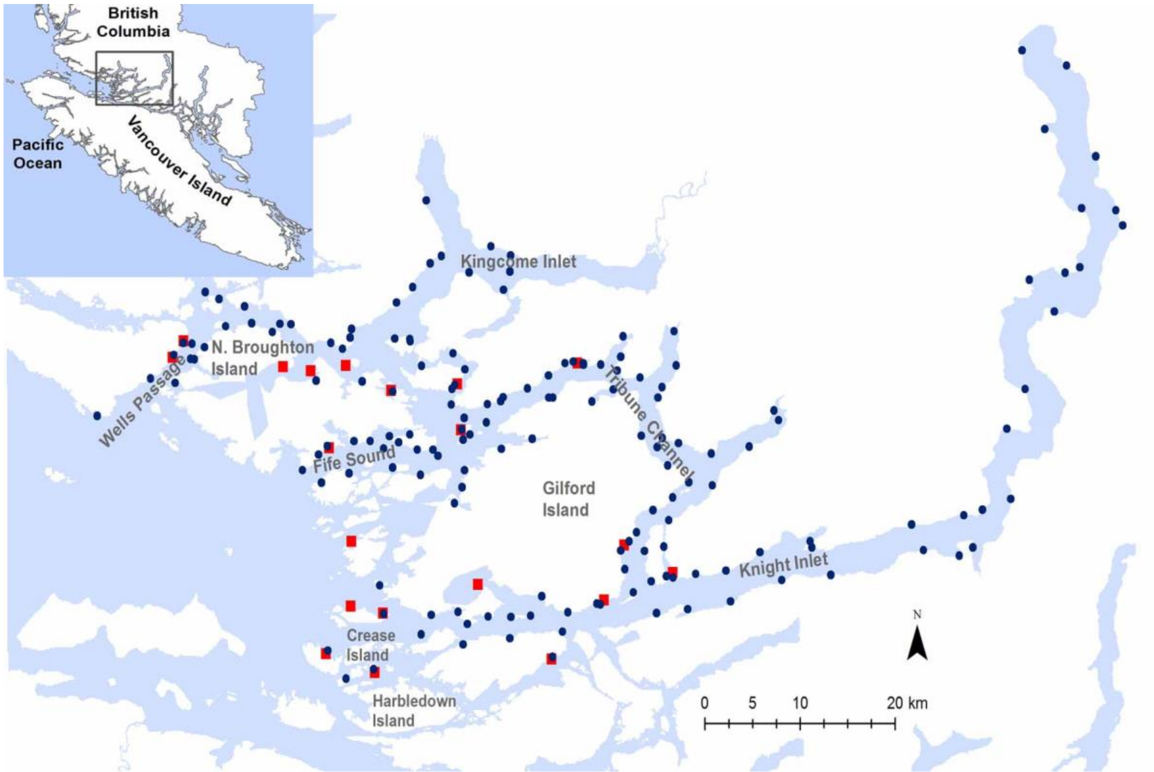


Figure 3.1 The study area, Broughton Archipelago, showing median coordinates of sampling sites (blue circles) and 20 locations of Atlantic salmon farms (red rectangles)

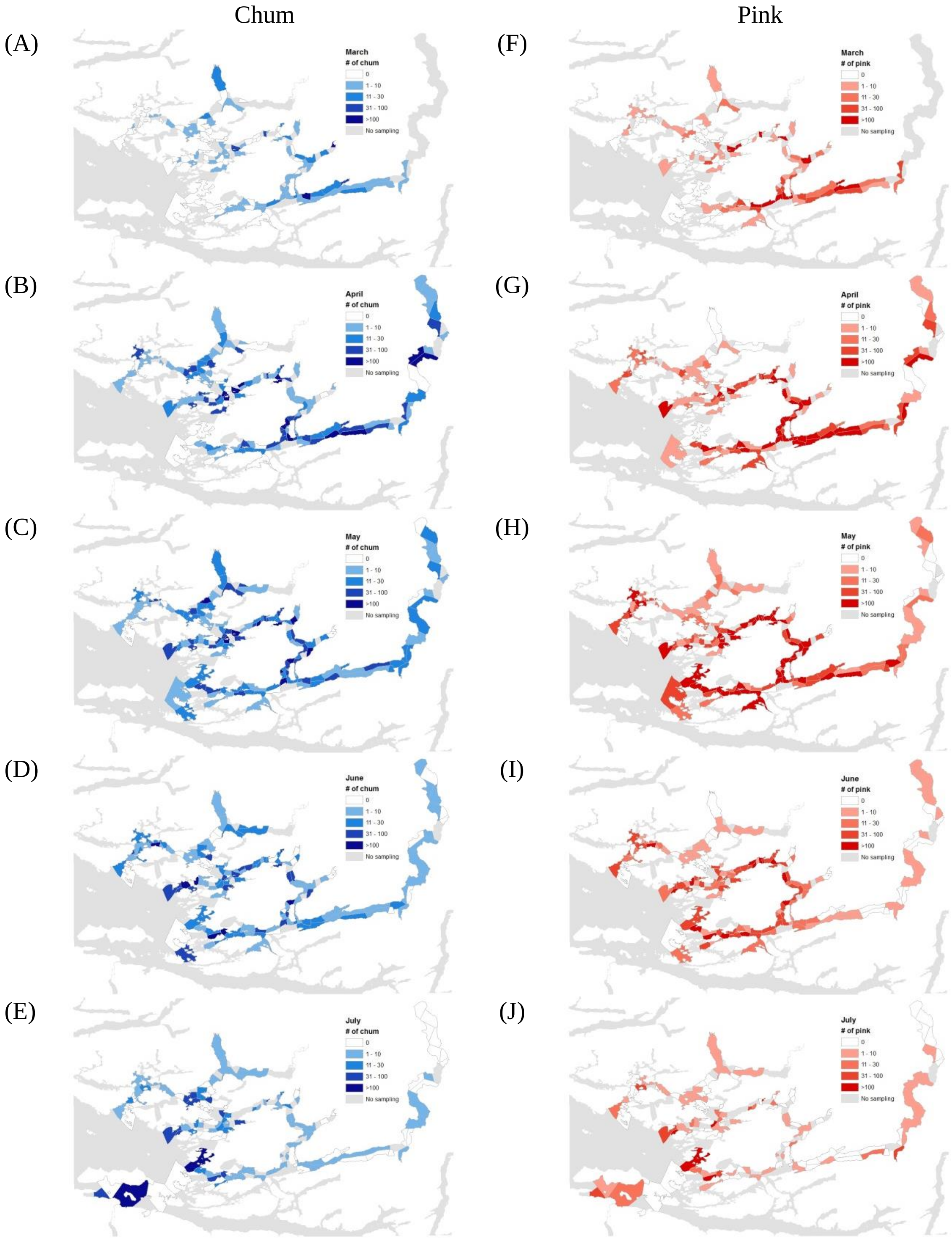


Figure 3.2 Spatial distribution of chum (blue) and pink (red) salmon, described as monthly average numbers of fish caught. Plots for chum are shown in the left column from (A) March to (E) July, while plots for pink are presented in the right column from (F) to (J).

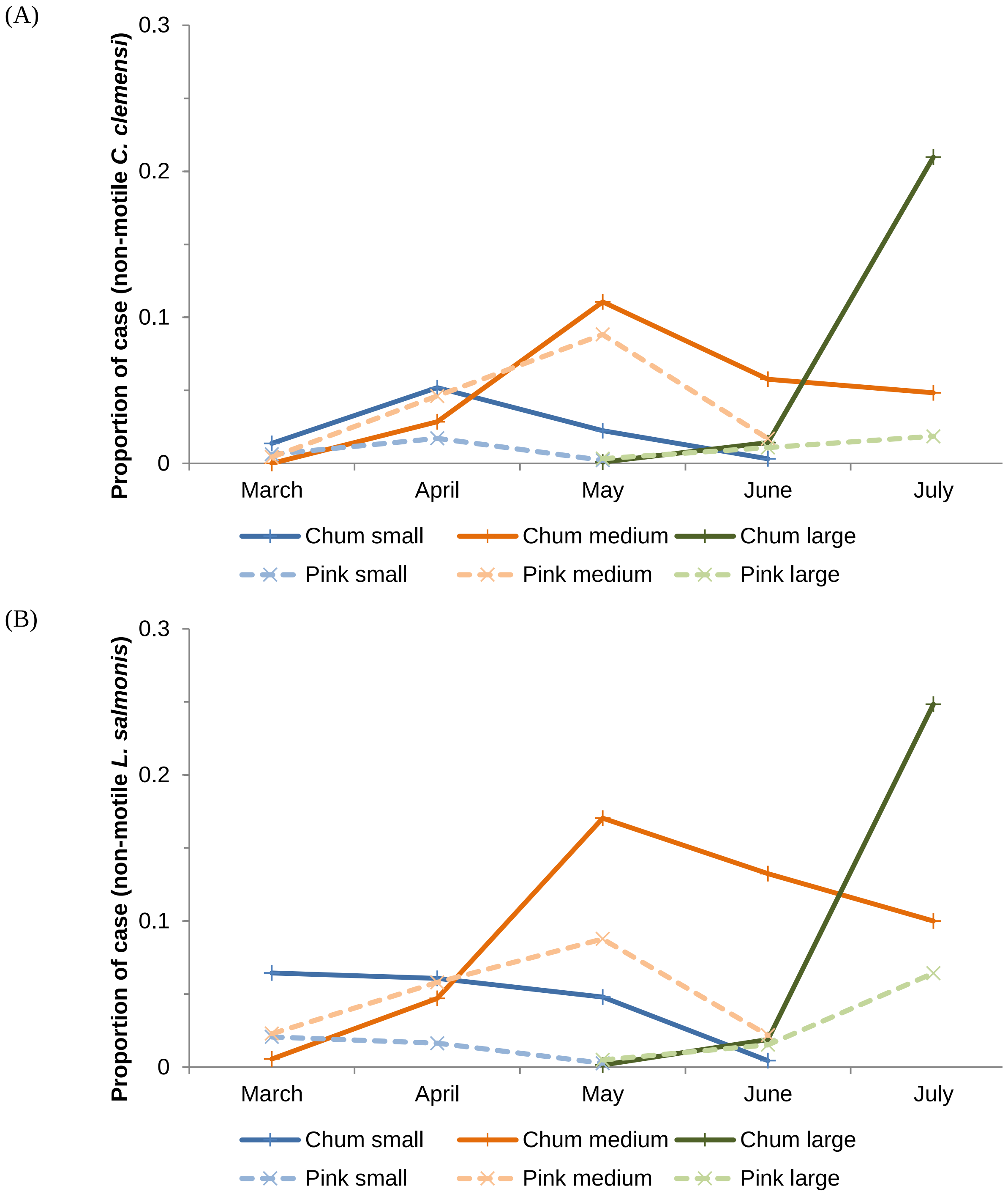


Figure 3.3 Proportion of fish infested with non-motile (A) *C. clemensi* and (B) *L. salmonis*, sampled by beach seine during each sampling month (March to July) from 2004 to 2012 by fish species and size categories (classified as small, medium, and large using fish length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). Data points with number of fish sampled less than 20 fish were not included in the calculation.

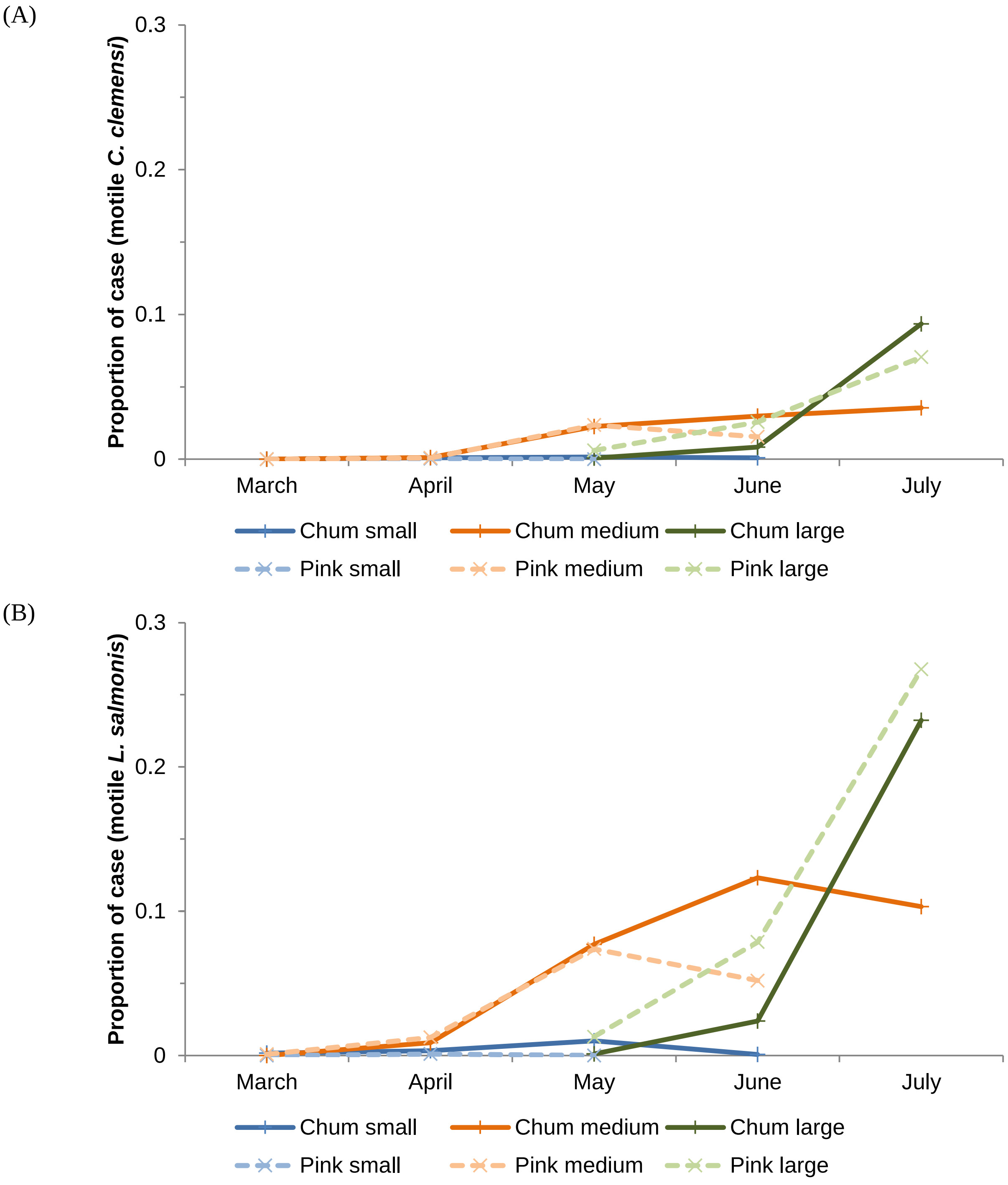


Figure 3.4 Proportion of fish infested with motile (A) *C. clemensi* and (B) *L. salmonis*, sampled by beach seine during each sampling month (March to July) from 2004 to 2012 by fish species and size categories (classified as small, medium, and large using fish length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). Data points with number of fish sampled less than 20 fish were not included in the calculation.

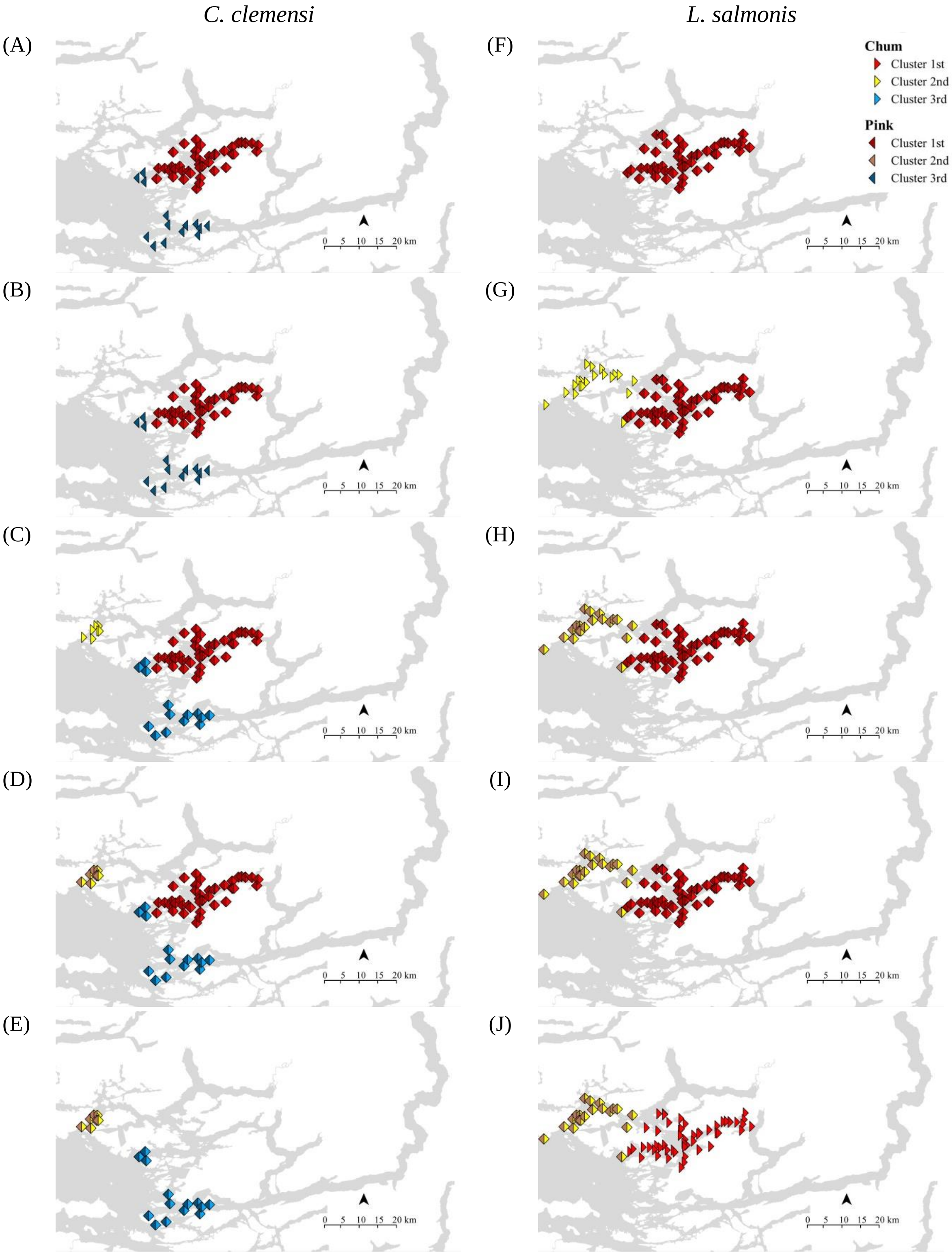


Figure 3.5 Cluster analyses of chum and pink salmon infested with non-motile sea lice. Clusters for *C. clemensi* are illustrated in the left-hand panels from (A) March to (E) July, while the right-hand panels show clusters for *L. salmonis* (F) to (J). Right arrows represent clusters of chum and left arrows indicate clusters of pink salmon. The color codes of red, yellow and blue indicate locations associated with the most, the second, and the third most likely clusters, respectively.

Figure 3.6 Cluster analyses of chum and pink salmon infested with motile sea lice. Clusters for *C. clemensi* are illustrated in the left-hand panels from (A) March to (E) July, while the right-hand panels show clusters for *L. salmonis* (F) to (J). Right arrows represent clusters of chum and left arrows indicate clusters of pink salmon. The color code of red indicates locations associated with the most likely cluster. Data for fish sampled in (A) March were not included in the analysis.

3.7. Supplementary materials for Chapter 3

Table S3.1 Number of cases (fish infested with non-motile *C. clemensi* : *L. salmonis*) per dataset (chum and pink salmon from March to July) by seine type and fish size (fish were classified as small, medium, or large using length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). A hyphen (-) denotes that there is no fish assessed in that data category.

Dataset	Species	Month	Number of fish infested with non-motile sea lice (*C. clemensi*: *L. salmonis*)					
			Beach			Purse		
			Small	Medium	Large	Small	Medium	Large
1	Chum	March	17: 80	0: 7	-	0: 0	0: 0	-
2	Chum	April	305: 357	168: 277	-	8: 27	23: 49	-
3	Chum	May	185: 396	912: 1,403	8: 13	6: 8	278: 731	45: 75
4	Chum	June	13: 18	229: 526	57: 75	0: 1	114: 301	342: 368
5	Chum	July	0: 0	15: 31	65: 77	0: 0	2: 4	177: 229
6	Pink	March	14: 46	10: 51	-	0: 0	0: 0	-
7	Pink	April	125: 119	335: 421	0: 0	8: 19	19: 54	-
8	Pink	May	24: 29	944: 938	36: 53	6: 3	126: 310	24: 76
9	Pink	June	0: 3	108: 138	69: 97	0: 0	9: 16	60: 107
10	Pink	July	-	0: 0	9: 31	-	0: 0	21: 32

Table S3.2 Number of cases (fish infested with motile *C. clemensi* : *L. salmonis*) per dataset (chum and pink salmon from March to July) by seine type and fish size (fish were classified as small, medium, or large using length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). A hyphen (-) denotes that there is no fish assessed in that data category.

Dataset	Species	Month	Number of fish infested with motile sea lice (*C. clemensi*: *L. salmonis*)					
			Beach			Purse		
			Small	Medium	Large	Small	Medium	Large
1	Chum	March	0: 2	0: 0	-	0: 1	0: 0	-
2	Chum	April	6: 20	7: 53	-	0: 1	1: 16	-
3	Chum	May	12: 84	186: 635	7: 10	3: 2	90: 398	22: 26
4	Chum	June	4: 3	118: 489	33: 95	0: 0	69: 224	230: 339
5	Chum	July	0: 0	11: 32	29: 72	0: 0	5: 3	139: 186
6	Pink	March	0: 0	0: 2	-	0: 0	0: 0	-
7	Pink	April	3: 8	7: 91	0: 0	1: 1	4: 24	0: 0
8	Pink	May	0: 1	253: 789	65: 139	0: 0	56: 263	41: 133
9	Pink	June	0: 0	98: 328	162: 497	0: 0	22: 40	218: 636
10	Pink	July	-	0: 0	34: 129	-	0: 0	78: 205

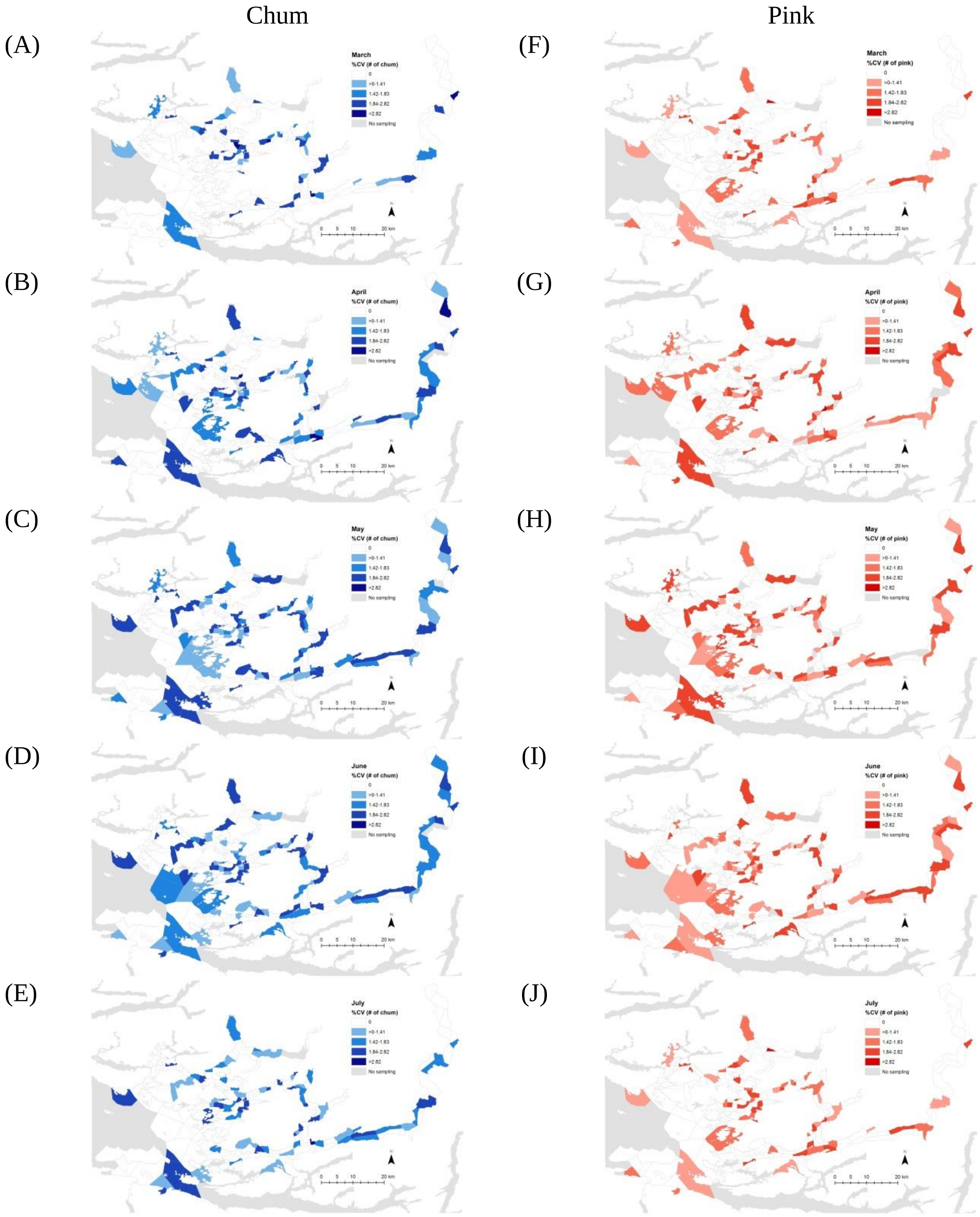


Figure S3.1 Coefficients of variations for the average numbers of fish captured by month for chum (blue) and pink (red) salmon. Plots for chum are shown in the left column from (A) March to (E) July, while plots for pink are presented in the right column from (F) to (J).

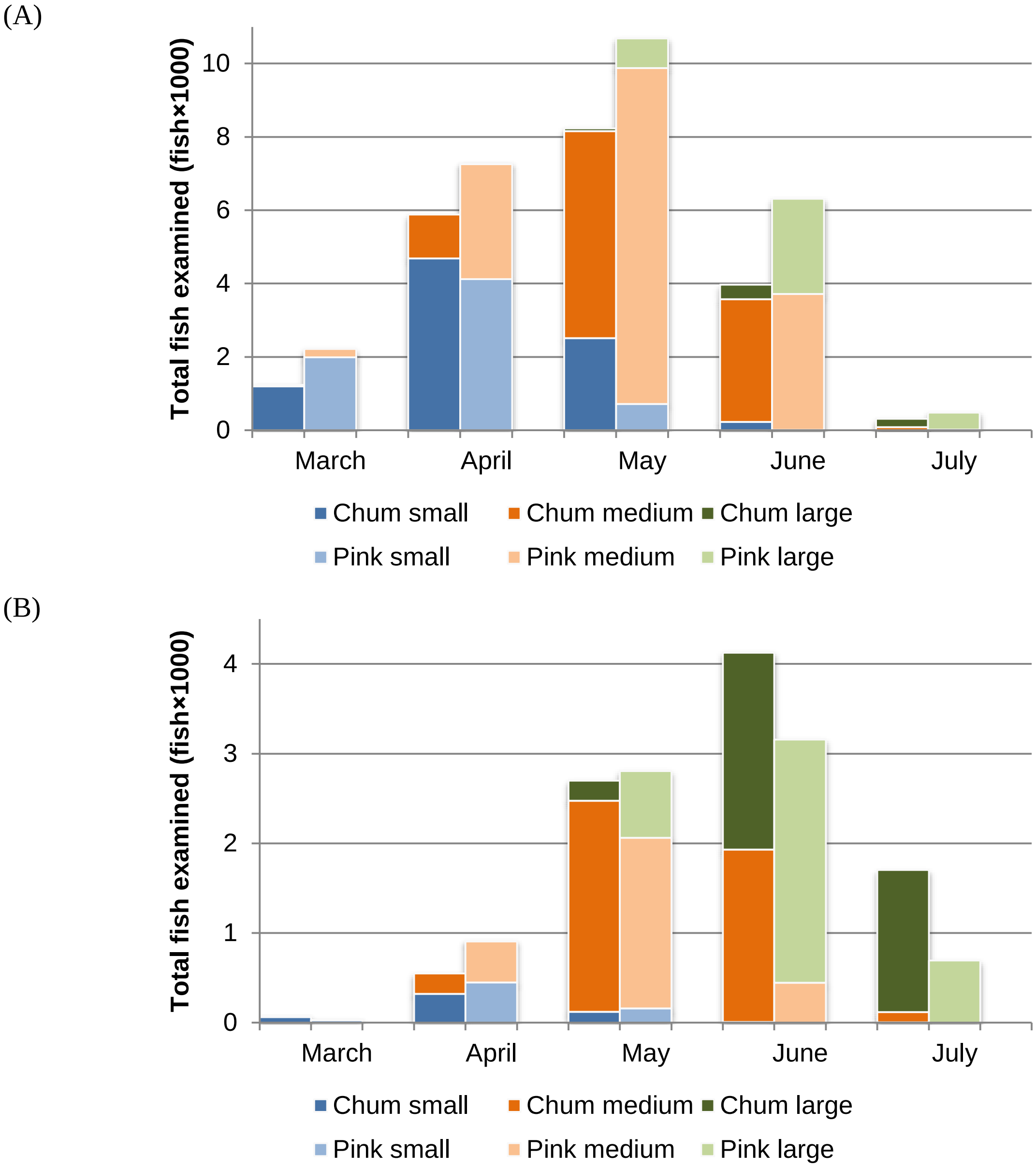


Figure S3.2 Total number of fish examined, sampled using either (A) beach seine or (B) purse seine during each sampling month (March to July) from 2004 to 2012 by fish species and size categories (classified as small, medium, and large using fish length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon)

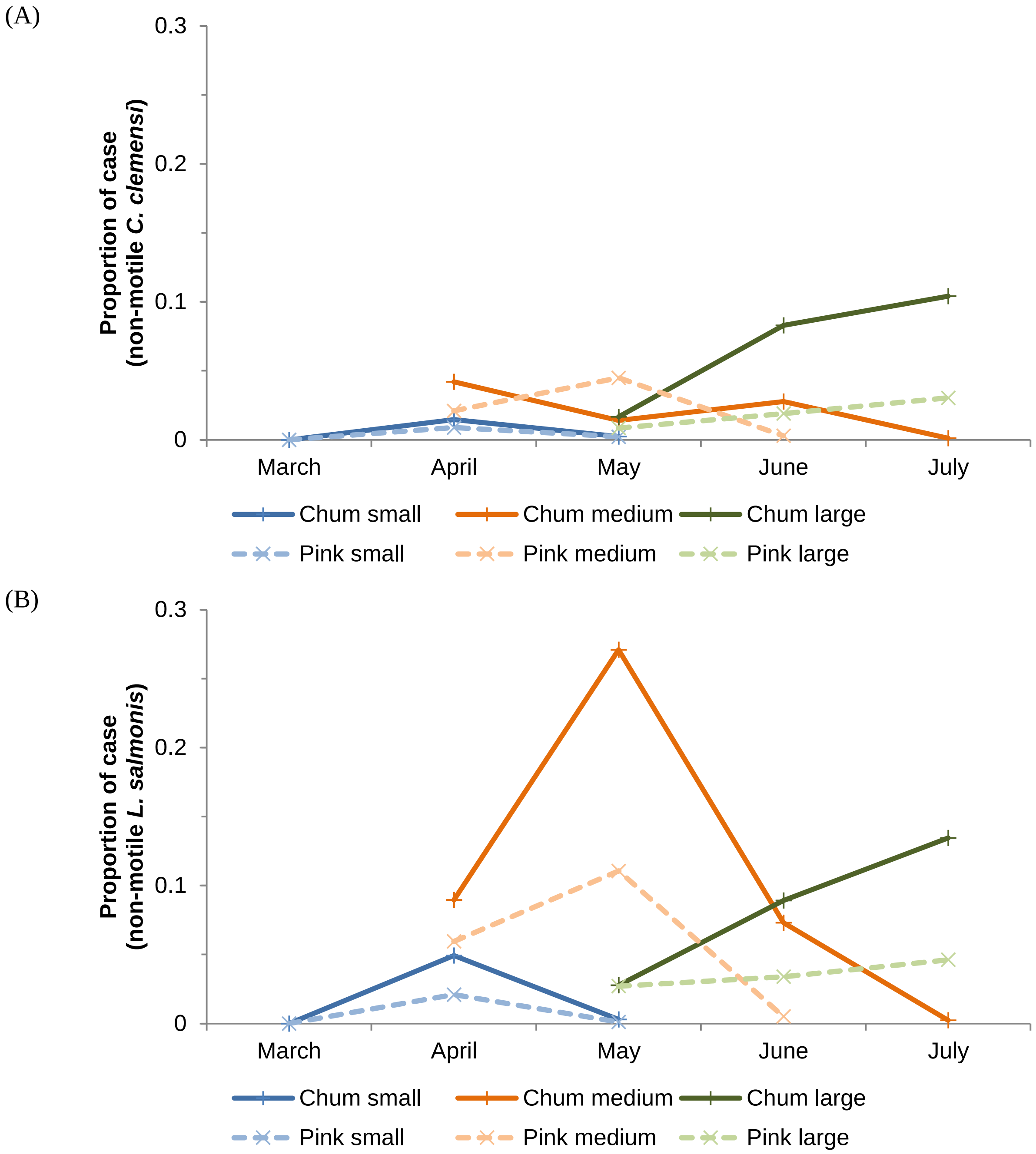


Figure S3.3 Proportion of fish infested with non-motile (A) *C. clemensi* and (B) *L. salmonis*, sampled by purse seine during each sampling month (March to July) from 2004 to 2012 by fish species and size categories (classified as small, medium, and large using fish length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). Data points with number of fish sampled less than 20 fish were not included in the calculation.

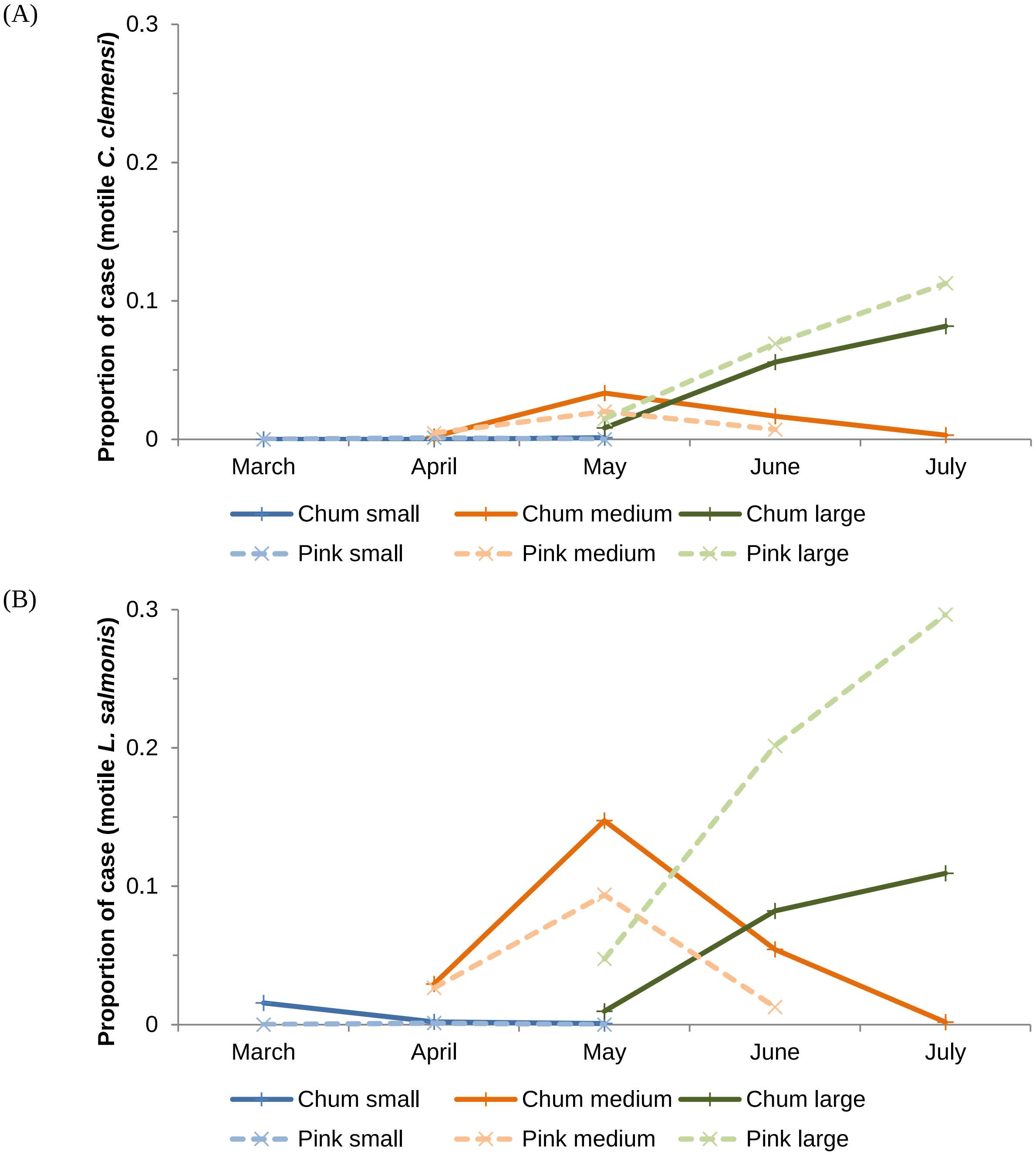
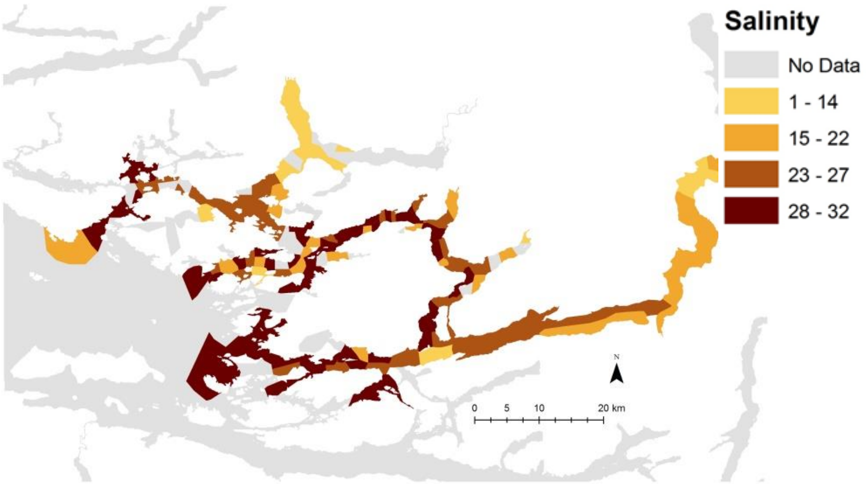


Figure S3.4 Proportion of fish infested with motile (A) *C. clemensi* and (B) *L. salmonis*, sampled by purse seine during each sampling month (March to July) from 2004 to 2012 by fish species and size categories (classified as small, medium, and large using fish length with break-point values of 45 and 90 mm for chum, and 35 and 70 mm for pink salmon). Data points with number of fish sampled less than 20 fish were not included in the calculation.

(A) April



(B) May

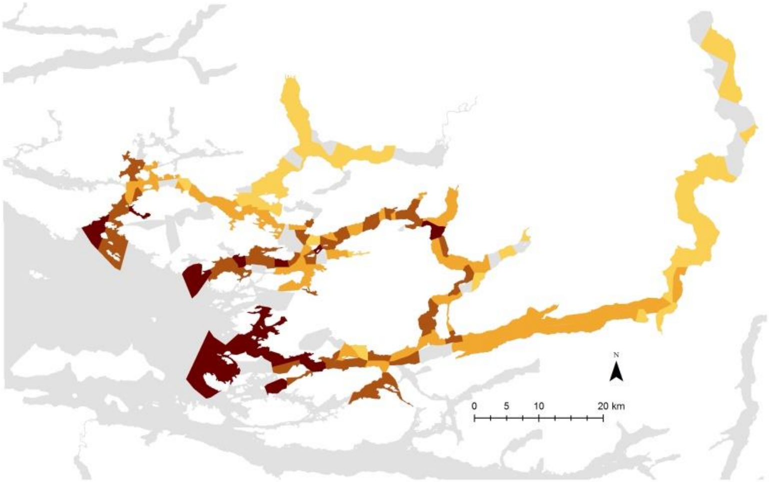


Figure S3.5 Average salinity in the Broughton Archipelago from (A) April and (B) May during the year 2004-2012.

The study by Rees et al. (2015)

To provide further discussion on additional factors that were not included in this study and may be associated with the spatial clustering of sea lice infestations on wild chum and pink salmon, details on the key findings from work reported in Rees et al. (2015) to which the current author was a contributor, are discussed in this section.

The objectives of the Rees et al. study were to: (1) identify locations in term of distance relative to Atlantic salmon farm where wild chum and pink juvenile salmon were associated with high abundances of non-motile sea lice, (2) determine the spatial scale over which there was an association between sea lice levels on farmed and wild juvenile salmon, and (3) compare how sea lice abundance on the wild juvenile salmon varied given different levels of farm infestation given conditions favourable to sea lice infestation on the wild salmon.

In common with Chapters 2 and 3 of this thesis, the study used sea lice count data from wild chum and pink salmon populations, sampled in the Broughton Archipelago regions during sampling month of March to July from 2003 to 2012. Factors hypothesized to influence sea lice infestation levels (non-motile life stage), including: sampling method, salmon species, fish length, sea water salinity, and infestation pressure from Atlantic salmon farm, were assessed using a multivariable two-part random effects model, while controlling for year and month of sampling.

Results suggested that the abundance of non-motile sea lice on wild juvenile chum and pink salmon was associated with sampling method, salmon species, fish length, sea water salinity, and the infestation levels on farms located within a sea-way distance of 30 km. The predicted abundance of non-motile sea lice on wild juvenile salmon increased with an increase in water salinity. These findings complement the results report in Chapter 3 regarding the locations of identified clusters of sea lice infestation on wild chum and pink salmon.

Chapter 4

Mathematical model describing the population dynamics of *Ciona intestinalis*, a biofouling tunicate on mussel farms in Prince Edward Island, Canada[*]

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[*] Patanasatienkul, T., Revie, C.W., Davidson, J., Sanchez, J., 2014. Mathematical model describing the population dynamics of *Ciona intestinalis*, a biofouling tunicate on mussel farms in Prince Edward Island, Canada. Manag. Biol. Invasions 5, 39-54.

4.1. Abstract

A mathematical model was used to describe the population of the aquatic invasive species, *Ciona intestinalis* in the presence of cultured mussel production. A differential equation model was developed to represent the key life stages: egg, larva, recruit, juvenile and adult. Stage transition rates were calculated from time spent in a stage and transition probabilities. Because surface availability for the settlement phase is a key determinant of population growth, dead juvenile and dead adult stages were also modelled, together with their drop-off rates. This model incorporated temperature dependencies and an environmental carrying capacity. Model validation was carried out against field data collected from Georgetown Harbour, in 2008. Relative sensitivity indices were calculated to determine the most influential factors in the model. The results indicated that the modelled outputs were broadly in agreement with the observed data. Under baseline conditions the number of *C. intestinalis* increased from early September to late October, after which they reached a plateau at an abundance of approximately five individuals per cm^2. Sensitivity analyses revealed that a reduction in spawning interval or the development time of larva accelerated *C. intestinalis* population growth. In contrast, decreasing either carrying capacity or the percentage drop-off of live juvenile and adult stages resulted in a decline in the population. This research provides the first detailed model describing population dynamics of *C. intestinalis* in mussel farms and will be valuable in exploring effective treatment strategies for this invasive species.

Keywords: Mathematical model, Population dynamics, *Ciona intestinalis*, Aquatic invasive species, Tunicates, Blue mussel

4.2. Introduction

Mussels accounted for 66% of total Canadian shellfish production (38,646 tonnes), with an estimated market value of CAD$39 million, in 2011 (Statistics Canada, 2012). The Prince Edward Island (PEI) blue mussel (*Mytilus edulis* Linnaeus, 1758) industry produces approximately 80% of all mussels cultured in Canada (Statistics Canada, 2012). Over the past 15 years the industry has encountered increasing challenges related to aquatic invasive species, especially tunicates. These biofouling species compete for food and space, reducing water flow rates from the species overgrowth, jeopardizing mussel health and yield, which can cause significant economic losses to mussel farmers and processors as a result of the costs associated with controlling their population growth as well as the additional labour costs during the mussel cleaning process at processing plants (Carver et al., 2006; Locke et al., 2009). Four species of invasive tunicates are found in PEI (MacNair, 2005; Fisheries and Oceans Canada, 2006): clubbed tunicate (*Styela clava* Herdman, 1881), vase tunicate (*Ciona intestinalis* Linnaeus, 1767), golden star tunicate (*Botryllus schlosseri* Pallas, 1766), and violet tunicate (*Botrylloides violaceus* Oka, 1927). Of these, the vase tunicate is considered to be the greatest threat for PEI aquaculture. Two years after the first identification of *C. intestinalis* in Montague River, PEI in the autumn of 2004, it became a dominant fouling species, causing severe problems for the PEI mussel industry (Carver et al., 2006; Ramsay et al., 2008).

C. intestinalis is a solitary tunicate, with a short-lived planktonic stage before settling on a suitable substrate during metamorphosis and becoming a sessile filter feeder (Carver et al., 2006). The growth and reproductive rates are strongly temperature dependent (Dybern, 1965; Carver et al., 2006); exhibiting rapid growth in the summer, before decreasing with declining temperature (Carver et al., 2006). A study of *C. intestinalis* populations on the Atlantic coast of Nova Scotia, Canada estimated 12,000 eggs were produced per a 100-mm long individual over a season (Carver et al., 2003). Another study in Japan gave an estimate of 100,000 eggs per individual (Yamaguchi, 1975). With its high fecundity and ability to reproduce rapidly (Carver et al., 2006), a mussel sock can be covered with *C. intestinalis* individuals in a short time; increasing the biomass on the mussel socks and resulting in mussel mortality through fall-off. To mitigate this, farmers remove tunicates from mussel socks by chemical and mechanical methods including 4% acetic acid treatment and high-pressure washing with water for *C. intestinalis* (Carver et al., 2003; Carver et al., 2006; Ramsay, 2008).

There is a need to compare the efficacy of treatments to find the best mitigation strategies in terms of time and frequency of treatment. A conventional approach involving field trials has been conducted for *C. intestinalis* (Davidson et al., 2009) and *B. violaceus* (Arens et al., 2011) to carry out such comparisons. However, these trials require considerable time to execute and are both cost and labour intensive. As an alternative, computer-based modelling can be used to mimic the population dynamics of a particular species (e.g. *C. intestinalis*) and subsequently to explore the likely effect of different control measures. While field-based experiments continue to provide a vital role, both in establishing the value of key parameters as specified within any model as well as in validating modelled outputs, a key advantage of such models is that they provide a mechanism to explore a range of possible intervention strategies in an inexpensive and timely manner.

Mathematical models are based on a set of equations with fixed parameters to describe a system. They have been applied to a wide range of problems associated with parasites and their control (Ebert et al., 2000; Jerwood and Saporu, 1988; Luis et al., 2010; White et al., 2011). They can be used to represent complex phenomena and interactions, including those found in aquatic contexts (Fenton et al., 2006; Ford et al., 1999; Kanary et al., 2011; Murray, 2011; Revie et al., 2005; Robbins et al., 2010; Thebault et al., 2007). These models typically predict the number of parasites/species of interest or the rates of change in the numbers of a species at a given time. Applying such approaches to model the population dynamics of *C. intestinalis* should provide the basis for a better understanding of population growth over time, as well as an ability to compare modelled results among various scenarios. The objective of this study was to develop a mathematical model that could describe the population dynamics of *C. intestinalis* in areas with mussel production, to better understand the growth of these populations under different temperature conditions.

4.3. Materials and methods

4.3.1. *C. intestinalis* population dynamics

The life cycle of *C. intestinalis* consists of egg, larva, recruit, juvenile, and adult stages. To capture the seasonal variation, six compartments representing the life stages of *C. intestinalis* were identified: egg (*E*), larva (*L*), recruit (*R*), juvenile (*J*), spring adult (A_{sp}), and autumn adult (A_{au}). Because surface area on which *C. intestinalis* can settle is a key determinant of population growth, two additional compartments to model dead stages were set up: dead juvenile (*DJ*) and dead adult (*DA*) (Figure 4.1). *Egg* refers to the *C. intestinalis* egg which has already been fertilized. *Larva* is the stage after the eggs hatch and become free-swimming larvae. *Recruit* refers to the tadpole that settles on a surface and develops through a process of metamorphosis. Those recruits that fail to metamorphose are assumed to detach from the surface. *Juvenile* is the stage at which the animal is completely metamorphosed but before it reaches sexual maturity. *Spring adult* refers to an animal that reaches its sexually mature size between May and September, and has the ability to reproduce. *Autumn adults* are animals that reach their adult stage between October and April. Two aggregate stages were also estimated: a surface-occupying stage (N_{SO}) and a visible surface-occupying stage (N_{VO}). N_{SO} comprises any individual that is attaching to the available surface, including R, J, A_{sp}, A_{au}, and the two dead stages, DJ and DA, which continue to occupy space regardless of their mortality status until they drop-off of the surface, releasing more space for recruits; while N_{VO}, which was only used for model validation purposes, represents the total number of individuals in a visible stage; which are those included in N_{SO} but excluding recruits. The dead others stage (DO) in Figure 4.1 was not captured in the model since individuals transferred to this stage do not result in any reduction in settling surface.

A sexually mature adult *C. intestinalis* spawns eggs repeatedly throughout its lifespan (L_{Asp} days for spring adult and L_{Aau} days for autumn adult). On average, α eggs are released per individual every G_{SI} days. The eggs are then fertilized with sperm externally at the rate of $F_f(T^{\circ})$. These fertilized eggs hatch at the rate of $F_h(T^{\circ})$ and develop to larvae in $G_E(T^{\circ})$ days. The egg is viable for up to L_E days before it is removed. After becoming a free-swimming larva, the tadpole turns into a recruit by finding a substrate to settle on at a percentage of $F_s(T^{\circ})$ which is capacity dependent and is thus adjusted by a capacity adjusting factor ($\gamma(a,t)$; details in a subsequent section). The larval phase can last for up to $G_L(T^\circ)$ days before settling and larva that do not settle in L_L days will die. The recruit stage takes G_R days to metamorphose to become a juvenile. The percentage of recruits that undergo the metamorphosis process is $F_m(T^\circ)$, whereas 1-$F_m(T^\circ)$ of recruits die in G_R days. Each individual in the juvenile stage takes $G_J(T^\circ)$ days to become a sexually mature adult and has a daily mortality rate of m_J. Space occupancy is released through a drop-off process, which occurs at the daily rates of μ_{DJ} and μ_{DA} for dead juveniles and dead adults, while juveniles and adults drop-off of mussel sock at the daily rates of μ_J and μ_A. A dichotomous variable, x, was used to control whether an adult can produce eggs with the cut-off temperature at 4˚C (*Eq. 1*). A similar approach was applied for spring and autumn adult compartments (*Eq. 5 and 6*). A dichotomous variable, y, was created to define whether the model was in spring (y=1) or autumn season (y=0). This allows the model to assign animals from juvenile stage to spring or autumn adult compartments depending on time of the model. The *C. intestinalis* life cycle can be described by the following set of differential equations:

$$\frac{dE(t)}{dt} = \frac{(x \times \alpha) \times F_f(T^\circ)}{G_{SI}} \times \left(A_{sp}(t) + A_{au}(t)\right) - \frac{F_h(T^\circ)}{G_E(T^\circ)} \times E(t) - \frac{E(t)}{L_E}\ ; x = \begin{cases} 0, & T^\circ < 4^\circ\text{C} \\ 1, & T^\circ \geq 4^\circ\text{C} \end{cases} \qquad \textit{Eq. 1}$$

$$\frac{dL(t)}{dt} = \frac{F_h(T^\circ)}{G_E(T^\circ)} \times E(t) - \frac{F_s(T^\circ)}{G_L(T^\circ)} \times \gamma(a,t) \times L(t) - \frac{L(t)}{L_L} \qquad \textit{Eq. 2}$$

$$\frac{dR(t)}{dt} = \frac{F_s(T^\circ)}{G_L(T^\circ)} \times \gamma(a,t) \times L(t) - \frac{F_m(T^\circ)}{G_R} \times R(t) - \frac{1-F_m(T^\circ)}{G_S} \times R(t) \qquad \textit{Eq. 3}$$

$$\frac{dJ(t)}{dt} = \frac{F_m(T^\circ)}{G_R} \times R(t) - \frac{J(t)}{G_J(T^\circ)} - m_J \times J(t) - \mu_J \times J(t) \qquad \textit{Eq. 4}$$

$$\frac{dA_{sp}(t)}{dt} = \frac{J(t)}{G_J(T^\circ)} \times y - \frac{A_{sp}(t)}{L_{A_{sp}}} - \mu_A \times A_{sp}(t)\ ; y = \begin{cases} 0, & t > 120 \\ 1, & t \leq 120 \end{cases} \qquad \textit{Eq. 5}$$

$$\frac{dA_{au}(t)}{dt} = \frac{J(t)}{G_J(T^\circ)} \times (1-y) - \frac{A_{au}(t)}{L_{A_{au}}} - \mu_A \times A_{au}(t)\ ; y = \begin{cases} 0, & t > 120 \\ 1, & t \leq 120 \end{cases} \qquad \textit{Eq. 6}$$

$$\frac{dDJ(t)}{dt} = m_J \times J(t) - \mu_{DJ} \times DJ(t) \qquad \textit{Eq. 7}$$

$$\frac{dDA(t)}{dt} = \frac{A_{sp}(t)}{L_{A_{sp}}} + \frac{A_{au}(t)}{L_{A_{au}}} - \mu_{DA} \times DA(t) \qquad \textit{Eq. 8}$$

The model was set to run for one calendar year, with Day 1 being the 1st of May until the termination of the model at Day 365 and was initialized with an initial autumn-adult presence of 0.002 individual·cm^{-2} (or approximately 12 adults per mussel sock) based on field observations made by the Atlantic Veterinary College (AVC) shellfish research group; all other life stages were initially set to zero. Spring was set to begin on the 1st of May and last for 120 days before switching to the autumn season. The model time step was set to 0.001 of a day.

4.3.2. Parameter estimation

A total of 20 parameters were identified, with seven of these being temperature dependent (see Table 4.1 and Figure 4.1 for details). Parameters were estimated based on values reported in the scientific literature. In cases where a range was reported (e.g. number of eggs laid per spawning (α) and larval lifespan (L_L)) values were randomly selected from a uniform (for α) or triangular (for L_L) distribution, while for parameters derived from more than one source, the average value based on these sources was estimated. Similarly, the average values of estimates at different temperatures were determined for the temperature-dependent parameters, i.e. development times and percentage of individuals successfully making the transition to a new phase for each stage (full details of the sources for these parameters are provided in Tables S4.1 and S4.2). All temperature-dependent parameters were linearly interpolated between reported values. Where no estimates could be found (e.g. temperatures less than 6 ˚C or greater than 24 ˚C in Figure 4.3) parameter values were assigned to the nearest reported value for %fertilization ($F_f(T^{\circ})$), %hatchability ($F_h(T^{\circ})$), %settlement ($F_s(T^{\circ})$) and %metamorphosis

($F_m(T^\circ)$) (Figure 4.3), while values for development time of each stage were linearly extrapolated (Figure 4.2).

Average daily sea water temperatures were assumed to broadly follow trends that could be modelled by a sine wave and were estimated using trigonometric regression (Beer, 2001; Cox, 1987). The dependent variable was daily average temperature, whereas the independent variable was day of the year expressed in terms of sine and cosine functions, $\sin(2j\pi t/p)$ and $\cos(2j\pi t/p)$, where j is an integer, representing the number of sine and cosine terms, t represents day of the year ranging from 1 to 365, and p is the period, which is assumed to be 365 days in this model (*Eq. 9*). The best fit temperature model was one comprising three sine/cosine terms (R-squared = 0.97). However for the sake of parsimony, a model with one sine and one cosine term was used (R-squared = 0.95). After acquiring the regression coefficients, the model was simplified to the simple sine function in *Eq. 10* to allow for a supple parameter. Amplitude (A) is computed as $\sqrt{\beta_{S_j}^2 + \beta_{C_j}^2}$ while the shift parameter (α) is $\tan^{-1}(\frac{\beta_C}{\beta_S})$, and the constant ($C$) is equal to β_0.

$$T^\circ = \beta_0 + \sum_{j=1}^{J} \beta_{s_j} \sin\left(\frac{2j\pi t}{p}\right) + \sum_{j=1}^{J} \beta_{c_j} \cos\left(\frac{2j\pi t}{p}\right) \qquad \textit{Eq. 9}$$

$$T^\circ = A \times \sin\left(\frac{2\pi t}{p} + \alpha\right) + C \qquad \textit{Eq. 10}$$

4.3.3. Environmental carrying capacity

The model assumes that the settling rate of larvae is density dependent, varying with the proportion of $N_{SO}(t)$ to environmental carrying capacity (K) of a surface area a, which is the maximum number of *C. intestinalis* that the system can accommodate per cm^2 multiplied by the total surface area (a cm^2) of mussel socks in a bay. The model used an estimate of 40 individuals per cm^2 (Ramsay et al., 2009) for K[2]. Mussel sock density in PEI was estimated to be 630 mussel socks per acre in the 500-acre bay of Georgetown Harbour (AVC shellfish research group pers. comm.). Each mussel sock was assumed to have a cylindrical shape with a length of 180 cm and a diameter of 10 cm. The capacity adjusting factor $\gamma(a,t)$ is the proportion of available surface area to the total surface area at time t. It is used to adjust the settling rate and is defined in *Eq. 11*.

$$\gamma(a,t) = 1 - \frac{N_{SO}(t)}{K \times a} \qquad \textit{Eq. 11}$$

4.3.4. Model validation

Model fit was assessed by comparing the modelled number of each *C. intestinalis* life stage to observed field data collected by the AVC shellfish research group during May to November in 2008 at Georgetown Harbour, PEI. Two datasets relating to larval concentration, and population development were used in the assessment. A third dataset, population recruitment, is provided in the supplementary materials.

For larval concentration, data collection was carried out using the larval sampling method described in Ramsay (2008). In brief, a 150-litre water sample in the water column between water surface and the first 2 metre depth was collected using bilge pump every two weeks from the study area and concentrated to 10 millilitres. This was then evaluated under a stereo microscope to identify and estimate the number of larvae.

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[2] It is possible that more than one fouling species live together, forming a “fouling community” (Sutherland and Karlson, 1997). However, *C. intestinalis* is the only species considered in the calculation of environmental carrying capacity in this model, since the reproduction cycle of this species occurs at a lower temperature (8ºC) than that which is observed in other species (e.g., *S. clava* at 12ºC). This provides a recruitment advantage for *C. intestinalis*, by inhibiting the settlement of other fouling species as a result of the early recruitment and growth of *C. intestinalis* populations (Ramsay et al., 2008).

Ramsay, A., Davidson, J., Landry, T., Arsenault, G., 2008. loc.cit.
Sutherland, J.P., Karlson, R.H., 1997. Development and stability of the fouling community at Beaufort, North Carolina. Ecol. Monographs. 47, 425–446.

The data thus provided a representation of larval concentrations across the season. Due to these differences in measurement scale, direct comparison between the modelled output and observed data was not carried out and validation was based on a comparison of temporal patterns.

For population development, the number of *C. intestinalis* accumulating during the reproductive season was investigated by deploying fifteen 100 cm^2 PVC plates at 2-3 metre depth below the water surface on May 1st, 2008. One plate was then randomly retrieved every two weeks to evaluate the numbers of *C. intestinalis* present at that time point. The samples were visually evaluated, so individuals smaller than 5 mm were not included in the count (for details see Ramsay et al., 2009). The data thus represented the total number of settled *C. intestinalis* per cm^2 over the season which was compared to the modelled N_{VO}. It was assumed that Based on the extensive field experience of one of the co-authors (Davidson) it was estimated that only around half of the juvenile population would be visually detectable in a field setting and that the dead recruit stages do not take up space since they detach from the surface after they die.

Population recruitment data were collected according to the method used by Ramsay et al. (2009). PVC plates measuring 100 cm^2 were left at 2-3 metre depth below the water and retrieved after a two-week period. The numbers of recruiting *C. intestinalis* were identified under a dissecting microscope. This procedure was repeated every two weeks over the study period (May to November, 2008). These data provide estimates of the numbers of early recruiting *C. intestinalis* over time, but can only sensibly be compared to the modelled numbers in the recruit stage for the initial two weeks when the recruitment occurred.

4.3.5. Sensitivity analysis

Sensitivity analysis was carried out to analyse the influence of each parameter on the N_{so} using a relative sensitivity function (S_p^F) which is the percentage of change in modelled output relative to a certain percent change in input. Each parameter (p) was increased or decreased by 20% of its default value one at a time, except for L_{Asp} and L_{Aau} that were changed simultaneously. S_p^F was calculated and averaged over time to obtain an average relative sensitivity index ($\bar{S}_p^F$) for each parameter (*Eq.12*). $F(t)$ denotes modelled N_{SO} at any time t when a parameter value is varied, while $F_b(t)$ refers to the modelled N_{SO} when all parameters were set to their default values.

$$\bar{S}_p^F = \frac{\overline{\%\ change\ in\ F(t)}}{\%\ change\ in\ p} = \frac{\sum_{t=0}^{T}\left[\frac{F(t)-F_b(t)}{F_b(t)}\right]/T}{\left|\frac{\Delta p}{p}\right|} \qquad Eq.\ 12$$

4.3.6. What-if scenarios

Four temperature conditions were modelled for what-if scenarios: baseline (replicating the temperature from Georgetown Harbour in 2008), cold year, long summer, and warm summer. Three parameters with high $\bar{S}_p^F$ values, together with environmental carrying capacity and the drop-off rates of live juvenile and adult stages were further evaluated for their influences on N_{SO}. These parameters were varied by a 20% increase or decrease on their default values and the outcomes assessed under the four temperature conditions.

4.4. Results

The observed and modelled average daily temperatures of Georgetown Harbour from May 2008 to May 2009 are illustrated in Figure 4.4. The modelled temperature was 3.3 ˚C at the start of the model with a mean of 7.1 ˚C, had a minimum of -2.7 ˚C and reached a maximum of 16.9 ˚C in late August.

The modelled total number of egg (eggs$\times 10^9$), larva (larvae$\times 10^9$), as well as the abundances per cm^2 of recruit, juvenile, adult, and visible occupying stages (N_{VO}) are illustrated in Figures 4.5-4.9. Each stage started to become active in early June when the temperature reached 8 ˚C, but the numbers were so low that they can hardly be detected in the summary plots. The modelled numbers of eggs rose from mid-August and reached a peak of 47×10^9 eggs in mid-October (Figure 4.5). The number of larvae began to increase just after the rise in egg abundance, as would be expected, reaching a peak of 4.8×10^9 larvae around mid-September at around the same time as the observed larval counts reached their maximum (Figure 4.6). The shapes of the observed and modelled larvae abundance over time are broadly similar though there is a limitation in comparing their magnitudes, as the two quantities are represented on quite different scales. Recruits followed a similar pattern to larvae, once again reaching a peak (4.5 $\text{recruit}\cdot\text{cm}^{-2}$) in mid-September (Figure 4.7). The abundance of juveniles began to rise in late August and reached its peak (4.3 $\text{juvenile}\cdot\text{cm}^{-2}$) in early October, while the abundance of adults increased from early September and reached the highest levels (1.9 $\text{adult}\cdot\text{cm}^{-2}$) in mid-December (Figure 4.8). A comparison between N_{VO} for the observed and modelled data is shown in Figure 4.9. The observed N_{VO} gradually increased from late July until mid-October with a rapid increase in late October. The modelled N_{VO} broadly followed the shape of the observed curve, reaching a plateau at an abundance of around 5 individuals·cm^{-2}. However, the modelled N_{VO} started to increase a month later than the observed data and showed a much more rapid rise after this initial increase than was the case for the observed data.

The modelled outputs using parameters fitted to the observed temperatures are presented in Figures S4.1-S4.5. The outputs of each stage appear to show an initial peak or early rise around late July (recruit and juvenile stages) to early August (egg, larval, adult, and N_{VO} stages). Additionally, the modelled outputs (bases observed temperature) result in values around 5-6 times higher than those seen in the outputs using a simple sine curve-based temperature model and as high as two orders of magnitude for egg and larval stages.

Figure 4.10 demonstrates the average relative sensitivity indices ($\bar{S}_p^F$) calculated from *Eq. 12* for the 20% increase/decrease models at the baseline temperature. It shows the impact of changes in parameter values on the modelled output. The further the value of $\bar{S}_p^F$ is from zero, the more influential a parameter is. The sign of $\bar{S}_p^F$ explains the direction of the modelled output with respect to changes in an input parameter. For instance, the $\bar{S}_p^F$ of 2.88 associated with a 20% decrease in spawning interval, the most influential parameter to the model (Figure 4.10), will generate on average a modelled output (N_{SO}) that is 57.6% higher than when the default parameters are used. In contrast, the effect of increasing the spawning interval by 20%, will generate a 28.8% decrease in the output. It can also be seen that the sensitivity in modelled output to changes of up to 20% in the mortality rate of juveniles and natural drop-off of dead juveniles and adults was very low, with $\bar{S}_p^F$ values close to zero (Figure 4.10). The effects of any two parameters can be compared by dividing $\bar{S}_p^F$ of one parameter by the other. For example, the effect of a 20% reduction in $G_L(T^{\circ})$ is 1.15 times greater than the effect of a 20% reduction in $G_E(T^{\circ})$. To show the temporal variation of relative sensitivity index (S_p^F), which cannot be seen in Figure 4.10, the plot of S_p^F for each parameter over time when parameters were decreased by 20% is also shown (Figure 4.11). It is not until Day 40 that the S_p^F values start to show an increase or decrease, and this change continued up to around Day 160, after which point they remained constant. Parameters with positive S_p^F such as those related to the development time appeared to have higher magnitudes when compared to those with negative values (Figure 4.11). Mapping S_p^F values over time for a 20% decrease in parameter values, indicates a similar and opposite trend to those found for the 20% increase model (data not shown).

The four temperature scenarios explored are shown in Figure 4.12. Figure 4.13 illustrates the N_{SO} stages for a baseline temperature scenario based on 20% variation in three parameters with high $\bar{S}_p^F$ values: spawning interval (G_{SI}), development time of larva ($G_L(T^{\circ})$), and number of laid eggs per spawning (α), as well as an increase to 1% and 2% of the drop-off of live juvenile and adult (μ_J, μ_A). As expected, increases in G_{SI} or $G_L(T^{\circ})$ caused a reduction in N_{SO}, while an increase in α increased the N_{SO} compared to default value (see Figure 4.13). However, when these variations were explored in a warm summer year (data not shown) the overall change in N_{SO} was marginal by comparison. Similarly changes in environmental carrying capacity (K) resulted in little or no change in N_{SO} stages for baseline and cold year scenarios. On the other hand there were more pronounced changes in the output for both long and particularly warm summer scenarios (Figure 4.14) when K was altered. The results also indicate that the model is highly sensitive to changes in temperature condition. Looking at the default values (dash lines in Figure 4.14), the modelled N_{SO} varied from only 0.004 individuals·cm^{-2} in a cold year (Figure 4.14B) to the maximum capacity of 40 individuals·cm^{-2} in a warm summer (Figure 4.14D). Figure 4.15 applied variation to the %drop-off of live juvenile and adult (μ_J and μ_A) to demonstrate its effect under different temperature conditions on the N_{SO} stages. When comparing %drop-off between the two temperature conditions, warm summer showed higher N_{SO} than long summer for every level of %drop-off. Additionally, the decrease in N_{SO} for a warm summer occurred later and with a larger relative change than was observed for the long summer scenario.

4.5. Discussion

The *C. intestinalis* populations' model has demonstrated a capacity to address a number of the objectives of this study. It is flexible and can be adapted to a range of different temperature conditions. The model, in general, provided similar outputs to the observed data based on a comparison of temporal patterns. Although differences in scale between the observed and modelled larval counts prevented any direct comparison, the model provided an accurate prediction as to the timing of both the growth and the decline of the larval stage.

For the recruit stage focussing on the first two weeks of recruitment period, the model using a simple sine curve to represent sea water temperature was unable to capture an initial moderate rise in mid-July. The likely explanation for this is that the temperature model did not capture the high temperatures seen during late June and July which would have affected the settling rate. When fitted to the observed temperature profile from Georgetown Harbour in 2008, as opposed to the simulated baseline (sine-wave model), the model was able to capture this initial rise in the recruit population (Figure S4.3).

A separate study of recruitment patterns of *C. intestinalis* took place in the Montague River, PEI in 2006 (Ramsay et al., 2009). The observed water temperature from the end of May to December in 2006 ranged from 6 ˚C to 18 ˚C which was quite similar to the modelled baseline temperature used in our study, though the model estimates were consistently around 1 ˚C lower. This field study reported that the first recruitment occurred in the second week of June when the temperature was nearly 9 ˚C and found one recruitment peak when the temperature was at its maximum (17.7 ˚C) in late August. Our model indicated a similar single recruitment peak pattern, though the peak was reached around one month later as the temperatures were not so high in the current study as compared to temperatures in the Montague River in 2006. This supports the argument that the model can adequately predict the recruitment timing of *C. intestinalis* given suitable temperature profiles.

As noted in Materials and Methods, comparing the observed and modelled recruitment data cannot strictly be justified, except during the first two weeks when recruitment occurs. This is due to the fact that in the observed data the surface on which recruits were counted was always based on a fresh plate, which would result in higher estimations of settling rates when compared to the modelled data. Furthermore the study design used to collect the observed recruitment data performed sampling every two weeks, which may not be frequent enough as the larval stage will last for only around 10 days at the temperatures involved (Jackson, 2008). In addition our model assumes that there was no external pressure affecting the modelled population, i.e. that all recruits derive from eggs and larvae produced by modelled adults in the population. However, within the observed data such external pressures are unknown and may therefore result in significant differences between the observed and modelled data.

The population development data (detailed in Materials and Methods) represented *C. intestinalis* stages that were attached on a surface and were of detectable size (larger than 5 mm) over the season. Due to the limitation of information on the life stages in the observed data (i.e. proportions of each stage were not identified) it was not easy to compare the modelled visible surface occupying stages to this dataset by simply adding up the numbers of juvenile, adult, dead juvenile and adult stages, since the modelled juveniles might have included individuals that were less than 5 mm in size. In validating the model against this population development data, it was assumed that only half of the juvenile stage individuals would be of detectable size. The model did not reflect the modest rise in N_{VO} seen early in the season in the observed data for the same reason that it could not simulate the initial rise of recruits, which are the source of juveniles, as discussed previously. The model also illustrated that the population remained relatively static after its peak in the middle of October. The abundance stopped increasing because the individual growth rate of *C. intestinalis* in cold temperatures is very low (Dybern 1965; Yamaguchi 1975), yet it did not significantly decrease because the drop-off of dead individuals is also low (AVC shellfish research group pers. comm.).

Temperature was modelled using a single sine term, though an equation utilising three sine terms provided a better fit. This was because the study aimed to create a model that explains how *C. intestinalis* populations behave under a range of temperature conditions, which requires a model that can be easily modified to different contexts and is not over-fitted to one specific set of temperatures. On the other hand, if the objective is to make specific predictions, real water temperature data from a given year or season may provide better results. The single sine term model provides the flexibility of changing parameter values in a sensible way, but in this case study failed to capture the higher temperatures that occurred during July and early August which will influence the modelled temperature-dependent flow rates. However, fitting the parameters to the observed temperatures resulted in an earlier increase in the population of each stage (when compared to those seen using the simple temperature model); indicating that the model can produce adequate outputs for different temperature scenarios.

The estimation of temperature-dependent parameters assumed linear interpolations when no values were reported between 6 ˚C and 26 ˚C. Where temperature was beyond this range, imputation, using either the nearest value or linear extrapolation, was carried out. As can be seen from Figure 4.2, a reasonable number of estimates exist at a range of temperatures for development times of egg, larval and juvenile stages which allowed for a sensible degree of interpolation, except at lower temperatures. However, there appeared to be more discontinuity when considering estimates of the percentage of individuals successfully making the transition to a new phase for various life stages (Figure 4.3) and therefore the interpolations adopted are inevitably more open to debate and refinement. In practice this was only a concern at lower temperature as sea water temperatures in the PEI coastal area rarely rise above 23 ˚C. Nonetheless, a reasonable amount of evidence that *C. intestinalis* do not develop at temperatures below 6 ˚C mitigates this as a serious concern (Dybern, 1965; Carver et al., 2003; Carver et al., 2006; Ramsay et al., 2009).

The plateau patterns observed in the modelled output (Figure 4.9 and 13-15) were a consequence of two factors: temperature and space availability. At high temperatures, the life stages grow quickly and rapidly reach maximum capacity. Although the population can still grow, there is no space available to accommodate the new recruit stages. In low to moderate temperatures (e.g. the modelled baseline and cold year scenarios), the population tends to grow more slowly. In these scenarios the maximum capacity is not reached prior to a time at which the temperature begins to decrease and thus limits the growth in the population. This strong relationship between growth rate and temperature is a characteristic of ectothermic organisms (Guarini et al., 2011), such as *C. intestinalis*. Under warm weather conditions such as the warm summer scenario presented in this study, the populations will grow faster and reach the maximum capacity very quickly (Gillooly et al., 2001). In contrast, when modelling the population under a cold weather scenario very few individuals successfully develop, indicating that the temperatures observed in this study represent values close to the lower thermal range limit for *C. intestinalis*. This is in agreement with Dybern (1965) who found no *Ciona* species in the sub-Arctic and Arctic regions, where temperature records are seldom higher than 3-4 ˚C

This study used a relative sensitivity function to evaluate the influence of changes in parameter values on the overall outcomes of the model. Although this method is known to have limitation, it is a relatively simple way to compare the effects of different parameters (Smith et al., 2008). Under the baseline temperature scenario, the model is particularly sensitive to development time and percentage of individuals successfully making the transition to the next life stages (i.e. egg and larva), as well as to spawning interval and number of eggs laid per spawning. As expected increasing spawning interval or development time slows down the growth of the *C. intestinalis* population, while increasing the number of eggs laid per spawning positively affects population growth. The sensitivity of the model to changes in the percentage drop-off of dead juvenile and dead adult stages was low; however, these drop-off rates only relate to the dead stages. A range of mitigation strategies to control *C. intestinalis* focusing on the removal of the occupying stages have been suggested elsewhere (Carver et al., 2003; Carver et al., 2006; Edwards and Leung, 2009; Gill et al., 2007). The predictions from this model suggest that changes in environmental carrying capacity have a larger impact on population growth under warm summer or long summer-like conditions as compared to what would be the case in cold years. Although drop-off of live *C. intestinalis* rarely occurs naturally (AVC shellfish research group pers. comm.), the model indicates that changes in this parameter can have major impacts on modelled outputs. The results suggest that increased drop-off of live *C. intestinalis*, as would be the case under certain mechanical treatments, can act to limit population growth and is worthy of further investigation in combination with space and time control.

The use of this model is currently limited to a one-year scenario. To use this model for multi-year scenarios, factors related to mortality during the winter would be required to adequately model the correct number of initial adults at the start of a new yearly cycle. The population may increase exponentially in a subsequent year if there is very low mortality during winter. On the other hand if high mortality of adults occurs there would be few initial adults to initiate the reproduction cycle, resulting in an outcome not dissimilar to the single year scenario modelling in this paper. Therefore, the effects of temperature on physiological rates and development stages of this species, particularly in the colder winter months, require further study.

Overall, this mathematical model provides reasonable predictions around the dynamics of *C. intestinalis* populations on mussel farms in PEI. This approach should prove useful for farm management and can be adapted to model populations in different regions or of other invasive species. Future studies will explore its application to an evaluation of the effectiveness of combining treatment and space management at different temperature profiles to develop mitigation strategies for the control of *C. intestinalis* populations and to improve bay management plans that might be implemented by mussel producers.

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Table 4.1 Parameter definitions, estimates, and parameter sources for *C. intestinalis* population dynamics model (Sources for temperature dependent parameters, which are marked with an asterisk, are shown in Tables S4.1 and S4.2.).

Parameter	Description	Value	Unit	Sources
$G_E(T^{\circ})$	Development time of egg*	0.51 – 2.63	day	*
$G_L(T^{\circ})$	Development time of larva*	0.31 - 10	day	*
G_R	Development time of recruit	12	day	Chiba et al., 2004
$G_J(T^{\circ})$	Development time of juvenile*	30 - 90	day	*
G_{SI}	Spawning interval	3	day	Yamaguchi, 1975; Carver et al., 2003
α	Number of eggs laid per spawning	1,000 – 1,500	egg	Carver et al., 2003
$F_f(T^{\circ})$	%Fertilization*	0 – 85	%	*
$F_h(T^{\circ})$	%Hatchability*	0 - 85	%	*
$F_s(T^{\circ})$	%Settlement*	0 - 65	%	*
$F_m(T^{\circ})$	%Metamorphosis*	0 – 80	%	*
L_E	Lifespan of egg	1.25	day	Svane and Havenhand, 1993
L_L	Lifespan of larva	0.25-1.5	day	Havenhand and Svane, 1991
L_{Asp}	Lifespan of Spring-Adult	150	day	Carver et al., 2006
L_{Aau}	Lifespan of Autumn-Adult	180	day	Yamaguchi, 1975
m_J	% Mortality of juvenile	0.11	%	Svane, 1984
μ_J	% daily drop-off of live juvenile	0	%	AVC shellfish research group pers. comm.
μ_A	% daily drop-off of live adult	0	%	AVC shellfish research group pers. comm.
μ_{DJ}	% daily drop-off of dead juvenile	0.05	%	AVC shellfish research group pers. comm.
μ_{DA}	% daily drop-off of dead adult	0.05	%	AVC shellfish research group pers. comm.
K	Environmental carrying capacity	40	individual·cm^{-2}	Ramsay et al., 2009

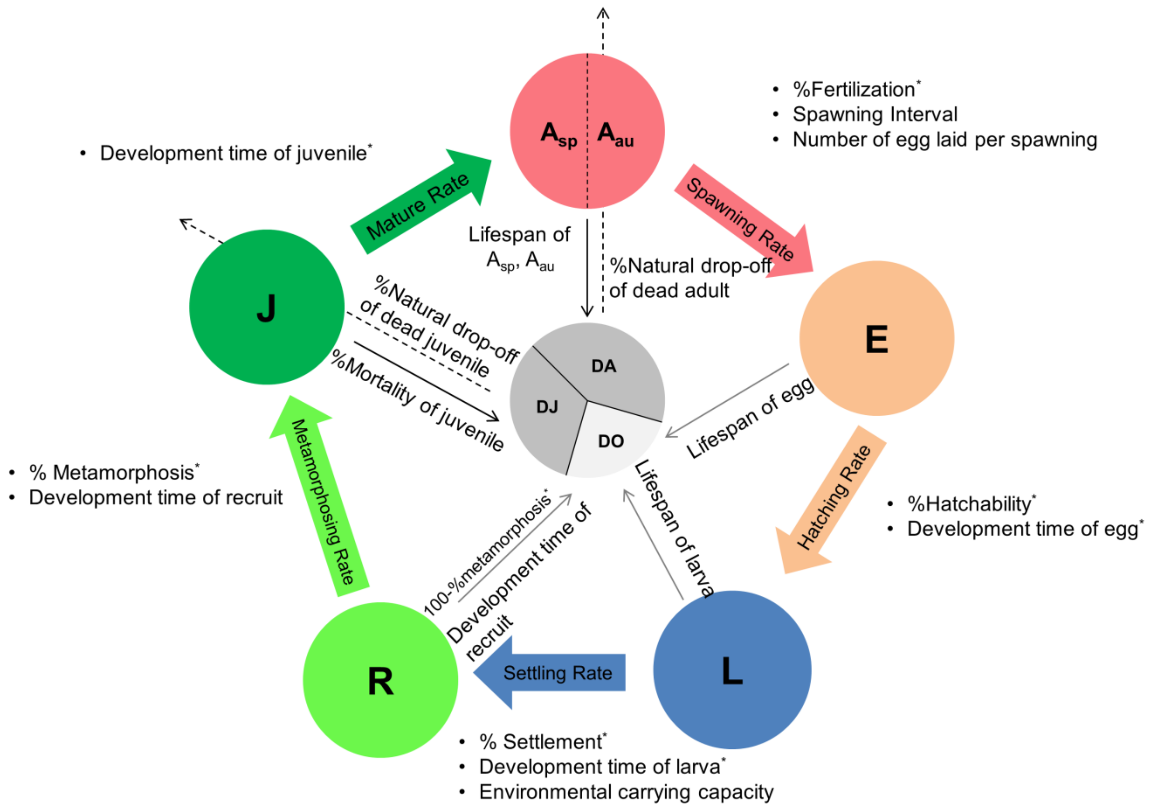


Figure 4.1 Diagram of *C. intestinalis* life cycle and parameters associating with flow rates. A_{sp}, A_{au}, *E*, *L*, *R*, *J*, *DA*, *DJ*, and *DO* represent adult (spring and autumn), egg, larva, recruit, juvenile, dead adult, dead juvenile, and dead other stages. Asterisks (*) denote temperature-dependent parameters.

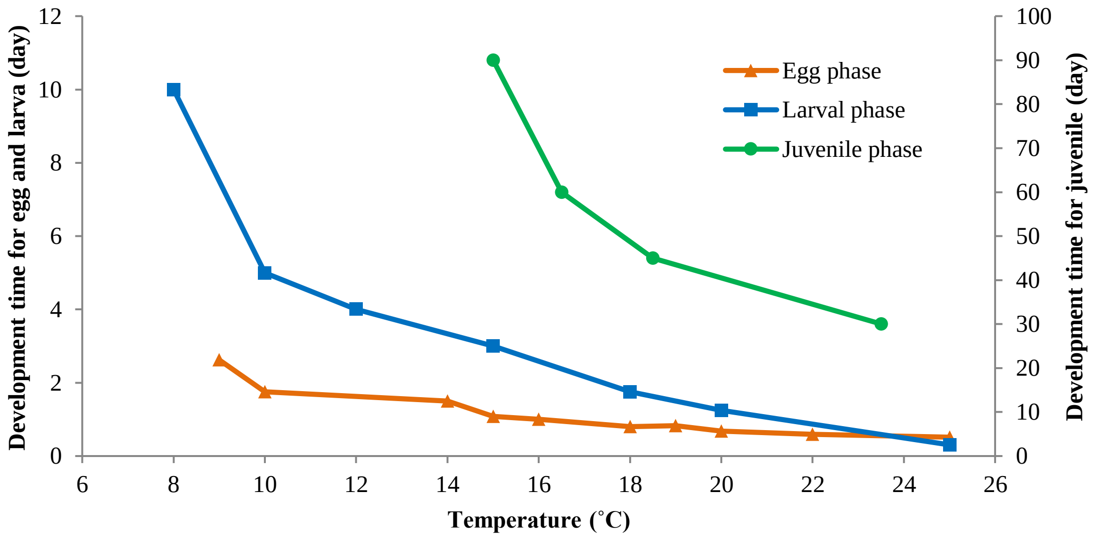


Figure 4.2 Reported values and linear interpolants of development time (day) at different temperatures for egg ($G_E(T^\circ)$), larval ($G_L(T^\circ)$), and juvenile ($G_J(T^\circ)$) phases.

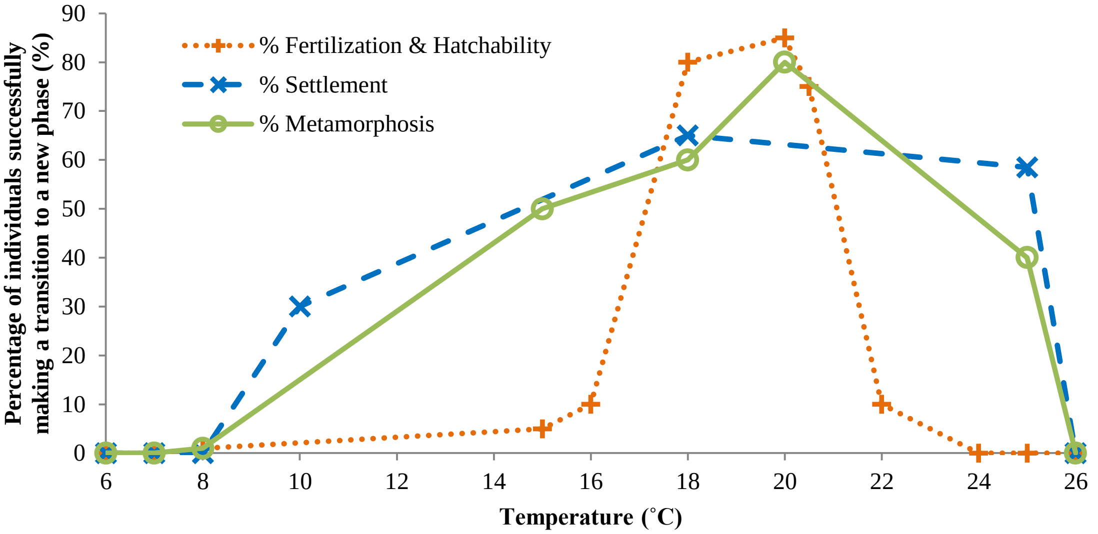


Figure 4.3 Reported values and linear interpolants for percentage of individuals successfully making a transition to a new phase: %fertilization ($F_f(T^\circ)$), %hatchability ($F_h(T^\circ)$), %settlement ($F_s(T^\circ)$), and %metamorphosis ($F_m(T^\circ)$) at different temperatures.

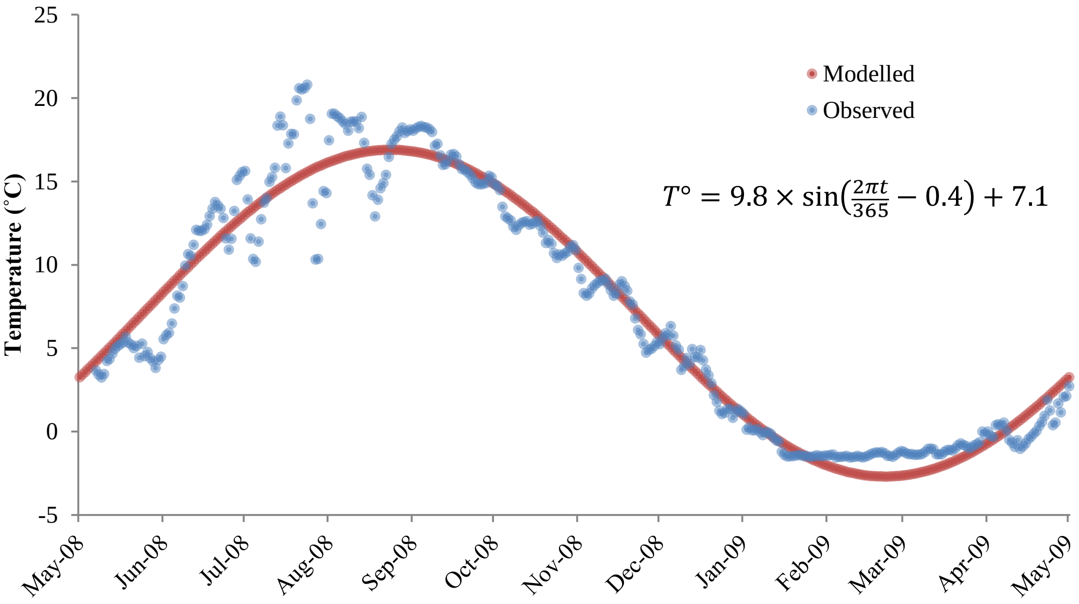


Figure 4.4 The observed and modelled average daily temperatures of Georgetown Harbour from May 2008 to May 2009.

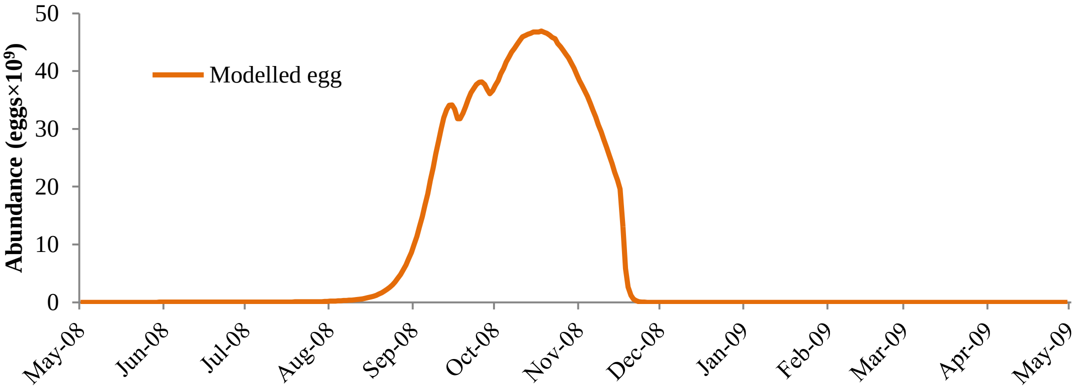


Figure 4.5 The modelled abundance of *C. intestinalis* at egg stage in Georgetown Harbour from May 2008 to May 2009.

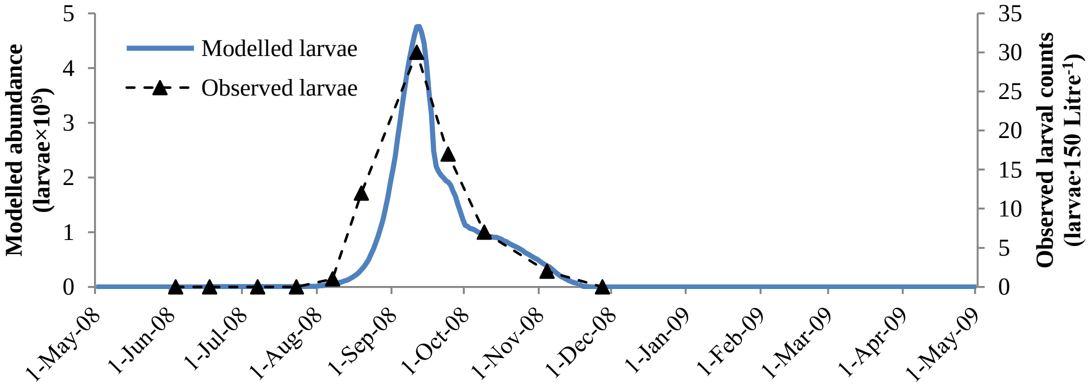


Figure 4.6 The modelled abundance of *C. intestinalis* at larval stage compared to observed larval counts (per 150 Litre) from Georgetown Harbour during May to November 2008.

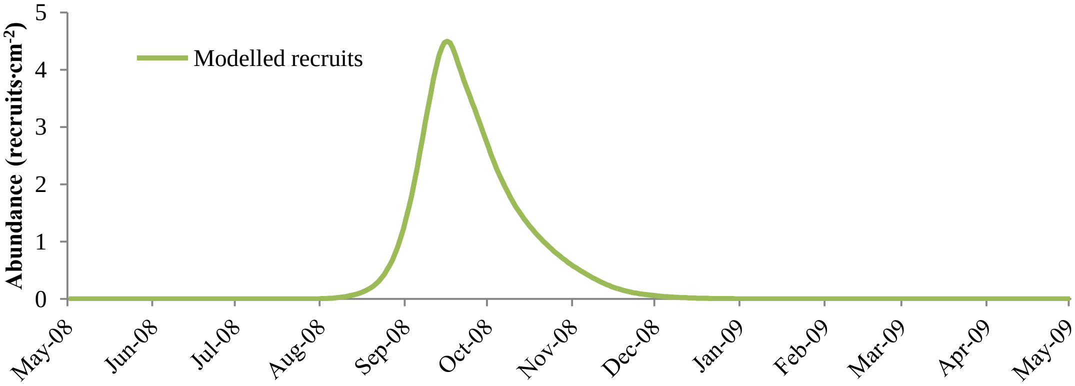


Figure 4.7 The modelled abundance of *C. intestinalis* at recruit stage from Georgetown Harbour during May 2008 to May 2009.

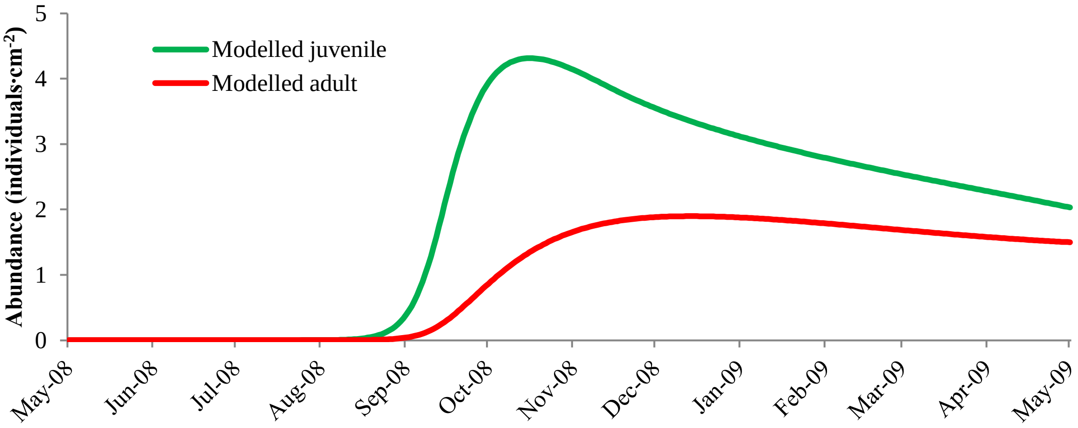


Figure 4.8 The modelled abundance of *C. intestinalis* at juvenile and adult stages in Georgetown Harbour from May 2008 to May 2009.

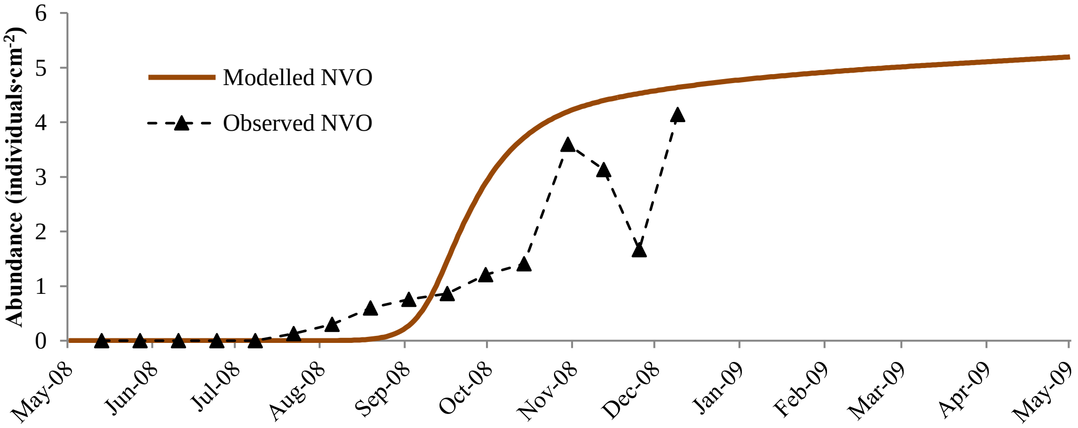


Figure 4.9 The modelled abundance of *C. intestinalis* at the aggregate visible occupying stage (N_{VO}) (half of juveniles, adults, and dead juveniles and adults) compared to the observed population developmental data collected from Georgetown Harbour during May to December 2008

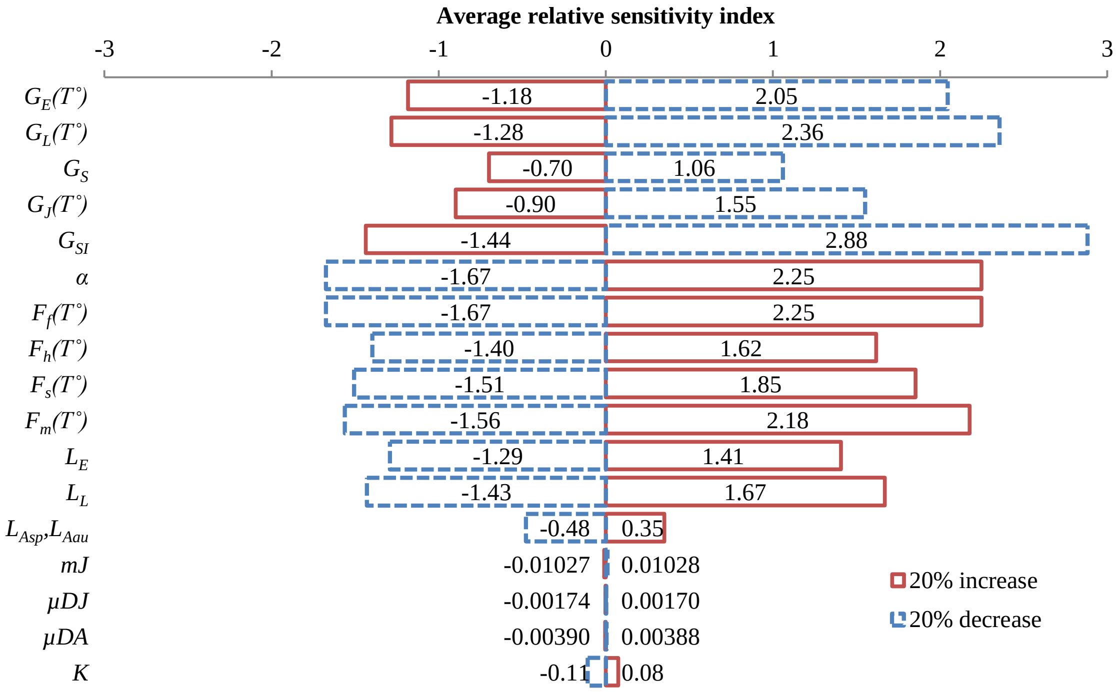


Figure 4.10 Average relative sensitivity index ($\bar{S}_p^F$) for 20% increase/decrease models (Acronyms are detailed in Table 4.1.).

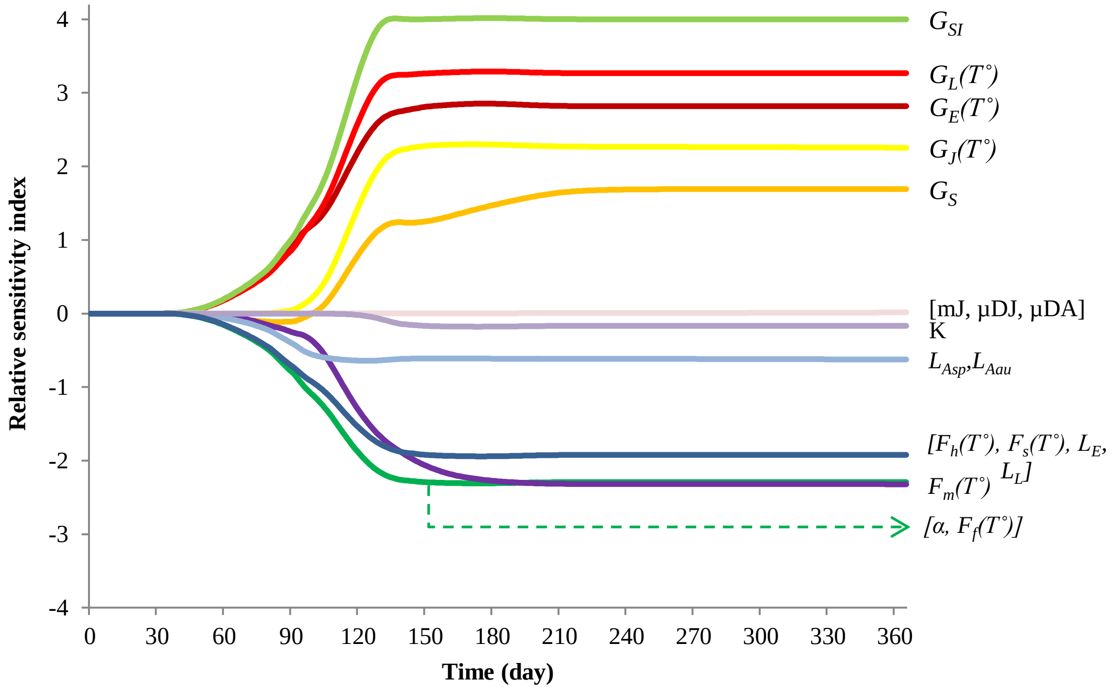


Figure 4.11 Relative sensitivity index [S_p^F] over time for variation (20% decrease) of different parameters of the *C. intestinalis* population dynamics model for Georgetown Harbour during May 2008 to May 2009 (Acronyms are detailed in Table 4.1.).

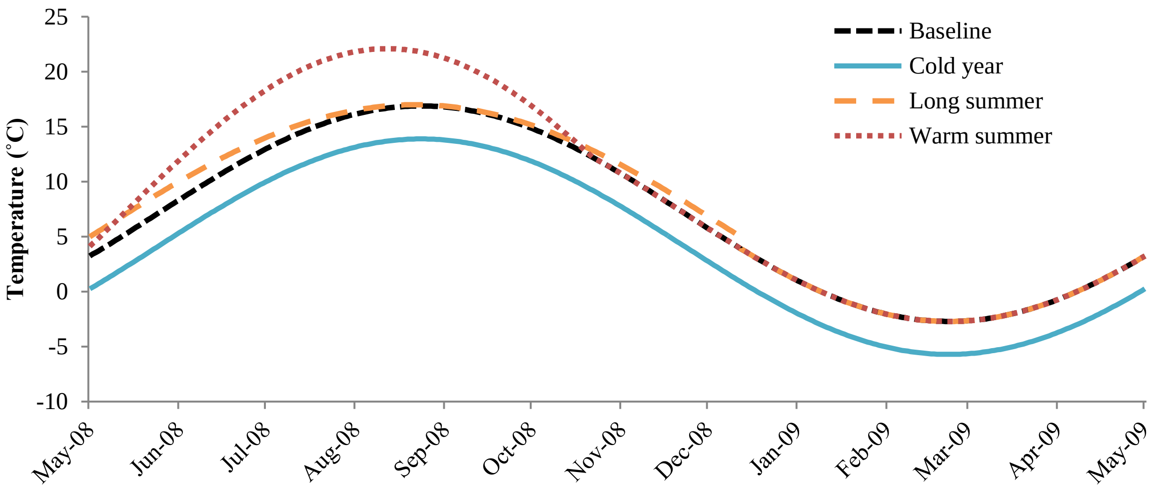
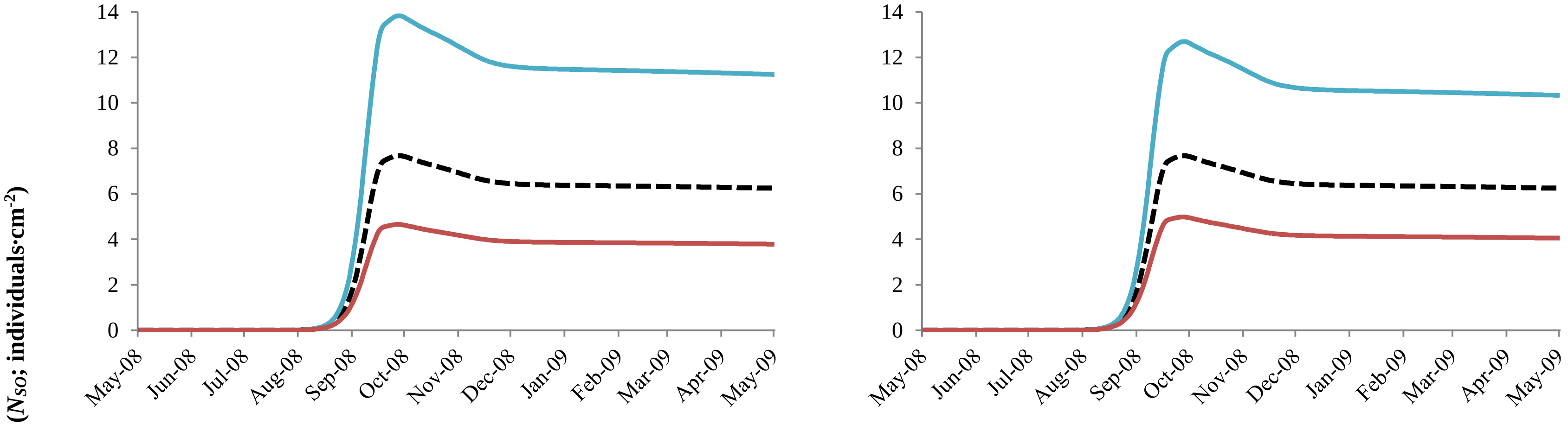
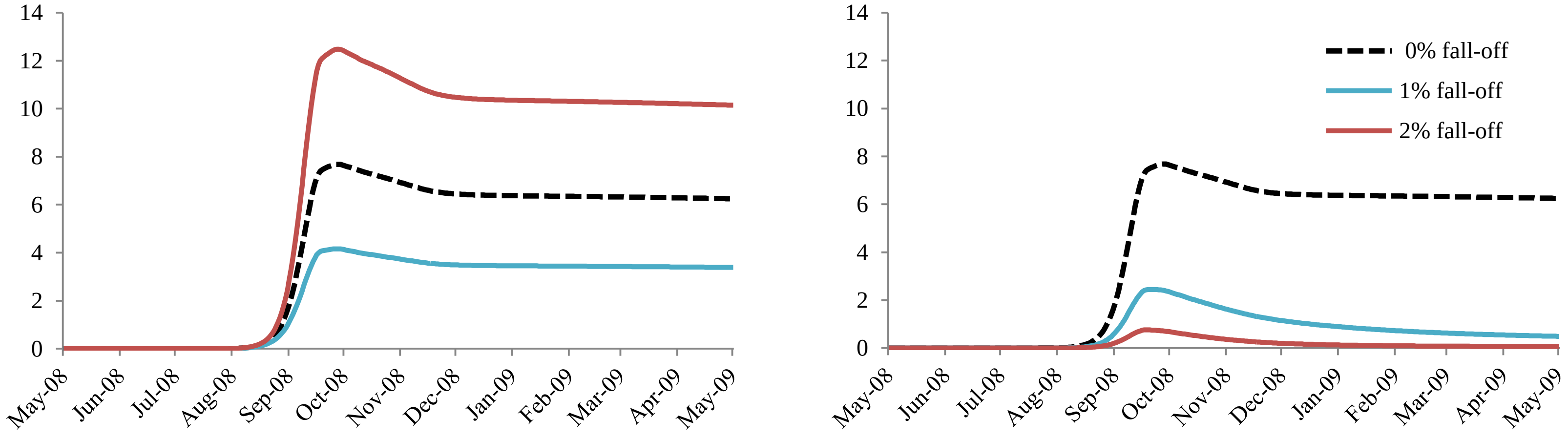


Figure 4.12 The modelled temperatures for 4 different conditions: baseline (replicating temperature from Georgetown Harbour in 2008), cold year, long summer, and warm summer.



(A) Spawning interval

(B) Development time of larva

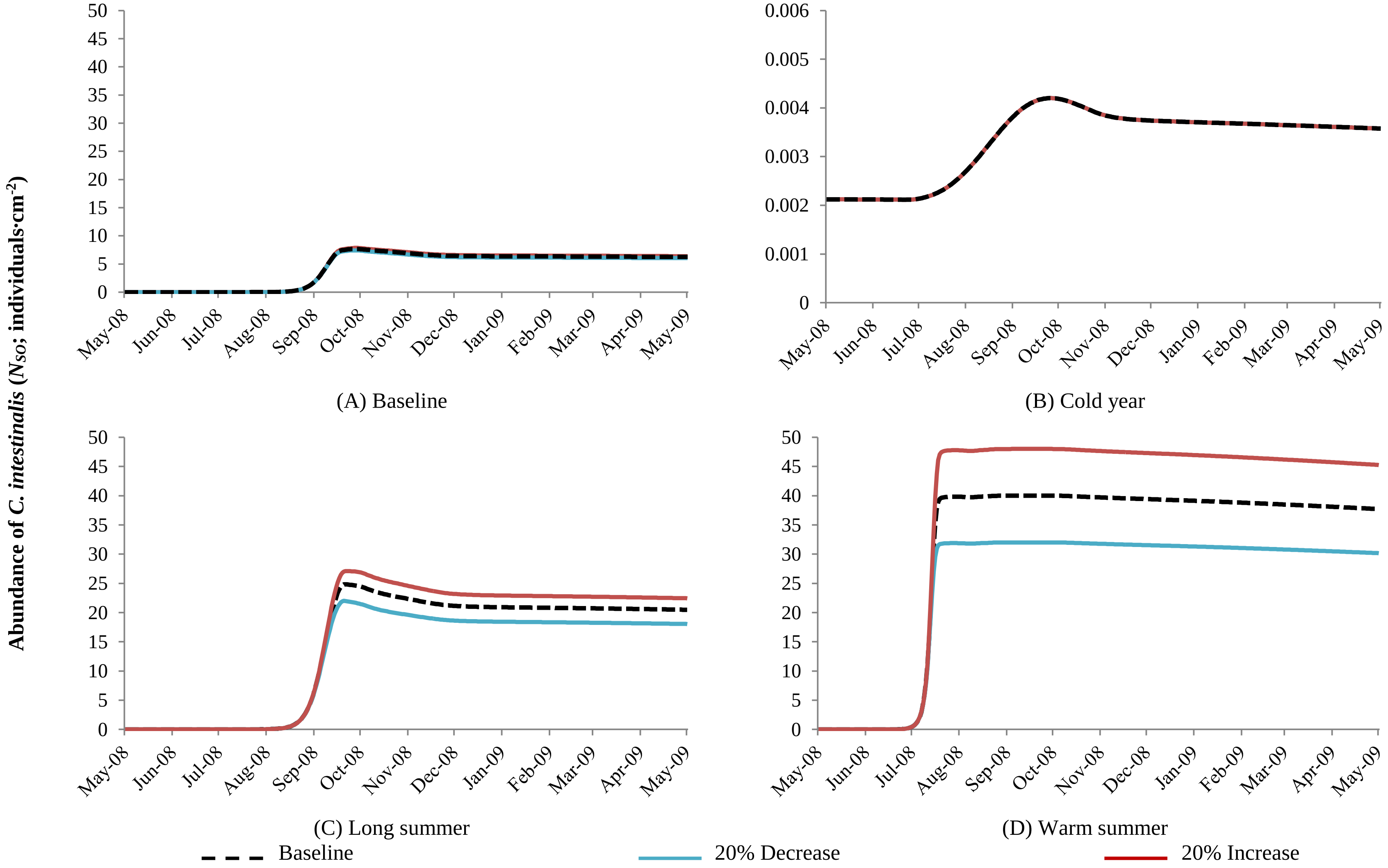


(C) Number of eggs laid per spawning

(D) %Drop-off of live juvenile and adult

Baseline 20% Decrease 20% Increase

Figure 4.13 Impact on main outcome (N_{SO}) of parameter variation in (A) spawning interval (G_{SI}), (B) development time of larva ($G_L(T^{\circ})$), (C) number of eggs laid per spawning (α), and (D) %drop-off of live juvenile and adult (μ_J and μ_A) at baseline temperature.



(A) Baseline

(B) Cold year

(C) Long summer

(D) Warm summer

Figure 4.14 Impact on main outcome (N_{SO}) of variation in environmental carrying capacity (K) at different temperature scenarios: (A) baseline, (B) cold year, (C) long summer, and (D) warm summer.

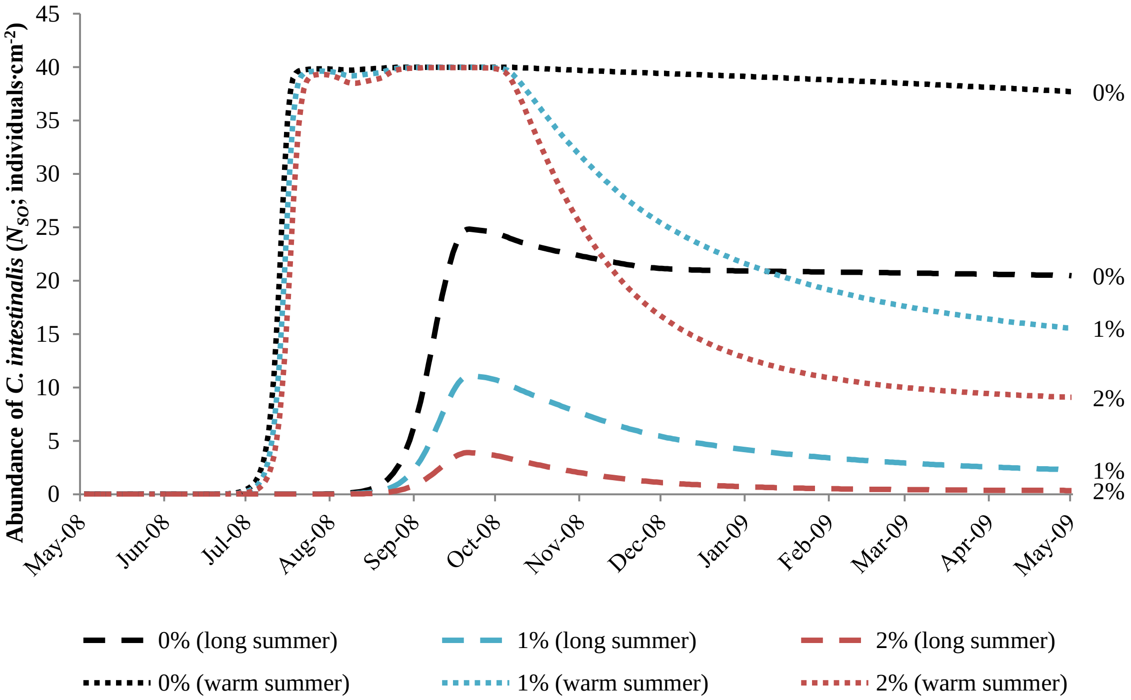


Figure 4.15 Impact on main outcome (N_{SO}) of variation in percentage drop-off of live juvenile and adult (μ_J and μ_A) (0%, 1% and 2%) in long summer and warm summer scenarios.

4.7. Supplementary materials for Chapter 4

Table S4.1 Development time for egg ($G_E(T^{\circ})$), larva ($G_L(T^{\circ})$), and juvenile ($G_J(T^{\circ})$) phases of *C. intestinalis* at different temperatures.

Temperature (˚C)	Egg phase (day)	Larval phase (day)	Juvenile phase (day)	Reference
8	-	10	-	Jackson, 2008
9	2.63	-	-	Svane and Havenhand, 1993
10	1.25	-	-	Liu, 2006
10	2	-	-	Wieczorek and Todd, 1997
10-12	2	4-5	-	Dybern, 1965
14	1.5	-	-	Szewzyk, 1991
14-19	-	-	60	Yamaguchi, 1975
15-18	0.92	-	60	Liu, 2006
15	1.25	-	-	Carver et al., 2003
15	-	-	90	Hendrickson et al., 2004
15	-	3	-	Havenhand and Svane, 1991
16	0.92	-	-	Hotta et al., 2007
16	1.08	-	-	Svane and Havenhand, 1993
16-20	1	-	-	Berrill, 1947
17-20	-	-	45	Carver et al., 2003
18	-	2	-	Zapata et al., 2007
18	0.73	-	-	Hotta et al., 2007
18	0.75	-	-	Baghdiguian et al., 2007; Swalla, 2004
18-20	0.75	-	-	Cirino et al., 2002
18-20	0.88	1-1.5	-	Dybern, 1965
18-24	0.88	-	-	Marshall and Bolton, 2007
20	0.58	1.5	-	Chiba et al., 2004
20	0.65	-	-	Hotta et al., 2007
20	0.67	-	-	Liu, 2006
20	0.83	-	-	Bellas et al., 2003
20-25	-	-	30	Yamaguchi, 1975
22	0.56	-	-	Hotta et al., 2007
22	0.63	-	-	Cirino et al., 2002
25	0.50	-	-	Cirino et al., 2002
25	0.53	-	-	Liu et al., 2006
25	-	0.21-0.42	-	Szewzyk, 1991

Table S4.2 Percentage of fertilization ($F_f(T^\circ)$)and hatchability ($F_h(T^\circ)$), settlement ($F_s(T^\circ)$), and metamorphosis ($F_m(T^\circ)$) of *C. intestinalis* at different temperatures.

Temperature (˚C)	**Fertilization & Hatchability (%)**	**Settlement (%)**	**Metamorphosis (%)**	**Reference**
Below 8	0	0	0	Dybern, 1965; Carver et al., 2003; Ramsay et al., 2009
8	1	-	1	Dybern, 1965; Bellas et al., 2003
8	-	0	-	Carver et al., 2003; Ramsay et al., 2009
10	-	30	-	Wieczorek and Todd, 1997
15	5	-	-	Bellas et al., 2003
15	-	-	50	Dybern, 1965
16	10	-	-	Bellas et al., 2003
18	-	-	60	Dybern, 1965
18	80	-	-	Zega et al., 2009
18	-	65	-	Zapata et al., 2007
18-23	75	-	-	Bellas et al., 2003
20	-	-	80	Dybern, 1965
20	85	-	-	Bellas et al., 2001
22	10	-	-	Dybern, 1965
24	0	-	-	Dybern, 1965; Bellas et al., 2003
25	0	-	40	Dybern, 1965
25	-	32	-	Holmstrom et al., 1992
25	-	70-90	-	Szewzyk, 1991
26	0	0	0	Dybern, 1965

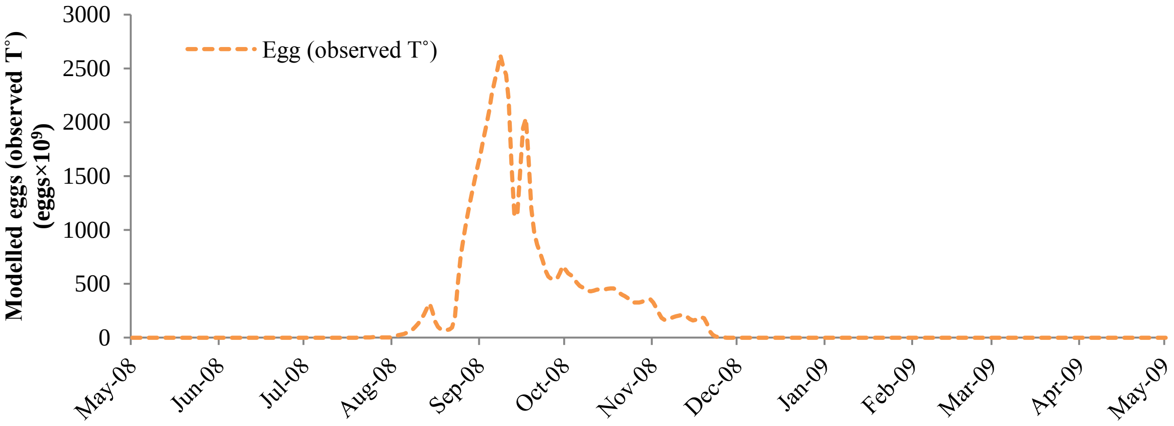


Figure S4.1 The modelled abundance of *C. intestinalis* at egg stage fitted with the observed temperatures in Georgetown Harbour from May to December 2008.

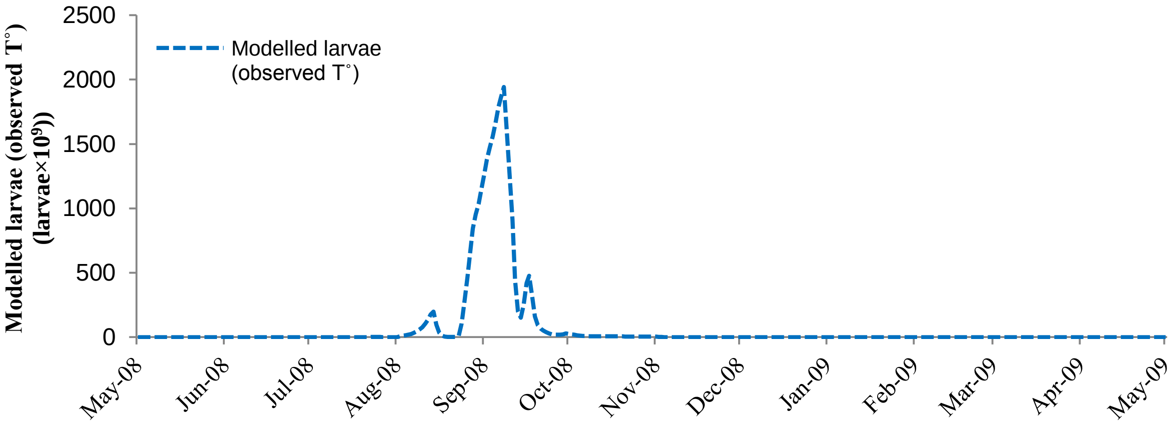


Figure S4.2 The modelled abundance of *C. intestinalis* at larval stage fitted with the observed temperatures in Georgetown Harbour from May to November 2008.

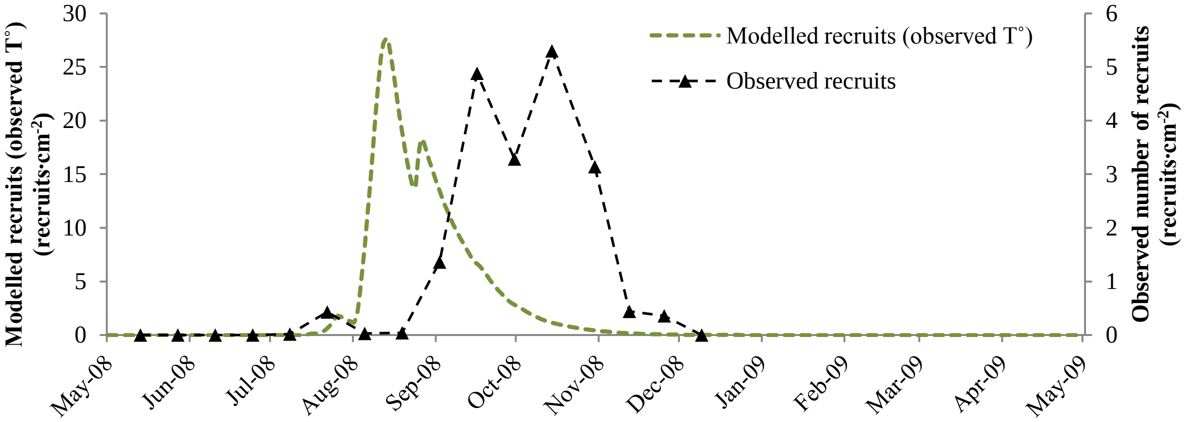


Figure S4.3 The modelled abundance of *C. intestinalis* at recruit stage fitted with the observed temperatures compared to observed recruitment data from Georgetown Harbour during May to December 2008.

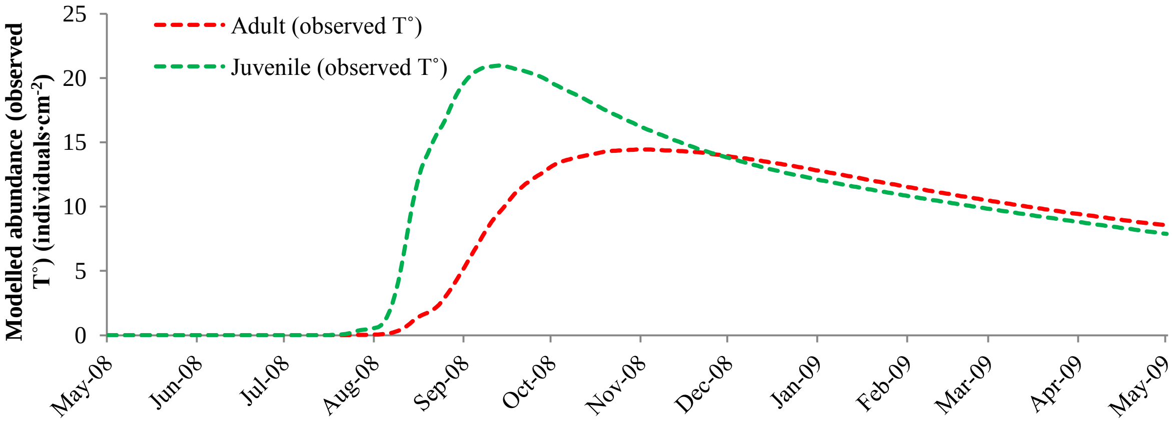


Figure S4.4 The modelled abundance of *C. intestinalis* at juvenile and adult stage fitted with the observed temperatures in Georgetown Harbour from May 2008 to May 2009.

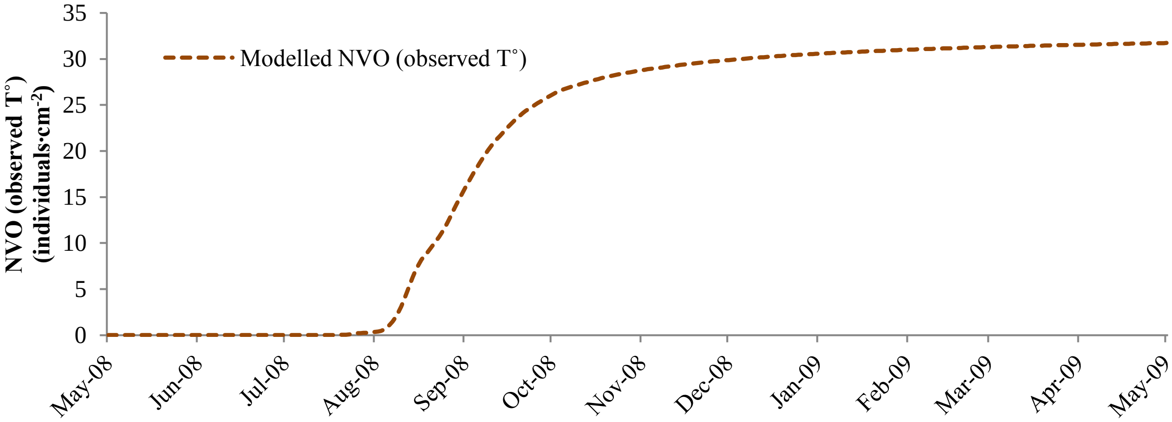


Figure S4.5 The modelled abundance of *C. intestinalis* at the aggregate visible occupying stage (N_{VO}) (half of juveniles, adults, and dead juveniles and adults) fitted with the observed temperatures from Georgetown Harbour during May 2008 to May 2009.

Chapter 5

The application of a mathematical model to evaluate the effectiveness of *Ciona intestinalis* population control strategies[*]

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5.1. Abstract

The Prince Edward Island (PEI) mussel industry has been challenged with the problem of invasive tunicate species over the past 15 years. Field experiments to find suitable mitigation strategies require considerable time and are resource intensive. This study demonstrates the application of a mathematical model to assess several control strategies against *Ciona intestinalis* populations under different temperature conditions in PEI. The model consists of eight compartments representing the key life stages; five live stages (egg, larva, recruit, juvenile, spring-, and autumn-adult), as well as two dead stages (juvenile and adult) to capture all individuals that typically occupy the surface of mussel socks. Stage transition rates were calculated based on the time spent in each stage, together with stage transition probabilities. Additionally, this model incorporated temperature dependencies and an environmental carrying capacity. The modelled output was measured in terms of the total abundance of surface-occupying stages. Treatment timing and frequency were explored to obtain the combination that maximized an objective value, which was the difference in modelled output between the control and the assessed treatment option. Treatment frequency was allowed to vary between one to four times over a given season. The model was assessed under baseline conditions, which mimicked water temperatures from Georgetown Harbour, PEI, in 2008, as well as under scenarios that reflected a prolonged summer or warm spring temperatures. Furthermore, the sensitivity of the model to variations in presumed treatment efficacy was evaluated. The results suggest that effective strategies should include one late timing treatment; and that treating more frequently will generally lead to better outcomes. The model also suggests that when multiple treatments are used, the first treatment should begin early in the season. Quadruple treatment, starting around the first week of July, was the most effective strategy, assuming the baseline temperature scenario. However, the effectiveness of treatments depended on temperature conditions. This research provides insights into the application of a mathematical model to explore the effectiveness of different mitigation strategies to control the abundance of *C. intestinalis* in an area with mussel production under different environmental conditions.

Keywords: Mathematical model, *Ciona intestinalis*, Tunicates, Treatment effectiveness, Population control, Optimization

5.2. Introduction

The blue mussel (*Mytilus edulis* Linnaeus, 1758) is a significant species in the Canadian aquaculture industry, accounting for over half of the total Canadian shellfish production value of $83 million (Statistics Canada, 2013). In 2012, PEI was the main producer, produced 78% of all mussel production in Canada, corresponding to 21,834 tonnes (Statistics Canada, 2013). Over the past 15 years, PEI mussel industry has been challenged with the infestation of aquatic invasive species, particularly the tunicates, which foul mussel socks and culture gear, causing significant economic losses to the industry due to the increased production costs of biofouling population control and labour costs during the initial processing at processing plants (Thompson and MacNair, 2004; Carver et al., 2006; Locke et al., 2009).

Several invasive tunicate species have been identified in PEI waters, including the vase tunicate (*Ciona intestinalis* Linnaeus, 1767), the species most impacting the PEI mussel production (Carver et al., 2006; Ramsay et al., 2008). *C. intestinalis* is a fast growing, solitary tunicate, with a short-lived planktonic stage, which becomes a sessile filter feeder after settling and metamorphosis (Carver et al., 2006). This biofouling species can grow very quickly when the sea water temperature is warm and the growth rate declines with the decreasing temperature (Dybern, 1965; Carver et al., 2006). Because of the rapid growth of *C. intestinalis* populations, a mussel sock can be infested with a heavy tunicate biomass in a short time, compromising the mussel attachment to the socking material, resulting in mussel loss due to the fall-off when socks are lifted (Gill et al., 2007).

A number of mitigation techniques are used to remove tunicates from mussel socks and aquaculture gear, including mechanical, chemical, and natural methods, e.g., calcium hydroxide (hydrated lime) for *Styela clava*, 4% acetic acid treatment for *C. intestinalis*, and mechanical methods (high-pressure washing with water) for *C. intestinalis* (Carver et al., 2003; Carver et al., 2006; Ramsay, 2008). The use of rock crab and green crab predation to provide biological control of tunicate populations on infested mussel socks has also been explored (Carver et al., 2003; Gill et al., 2007). High-pressure washing is the mitigation method used most often by farmers in PEI to control *C. intestinalis* populations (Paetzold et al., 2012). These methods can knock off up to 100% of *C. intestinalis* (Carver et al., 2003); however, the effect is not long lasting, as new tunicate larvae quickly settle on the mussel socks, especially, during the warm months when larval abundance and recruitment levels are at their peak (Ramsay, 2008; Ramsay et al., 2009).

A conventional approach involving field trials has been conducted for colonial tunicates (Arens et al., 2011; Paetzold et al., 2012) and *C. intestinalis* (Davidson et al.,

2009) to compare of effectiveness of different mitigation strategies in terms of treatment timing and frequency. However, investigating a variety of possible scenarios in the field is difficult, since these trials require considerable time to execute and are both cost- and labour- intensive. As a result, computer-based simulation modelling, which allows for an evaluation of the likely impact of changes in treatment prior to implementation, is considered a useful alternative approach.

In the context of computer-based simulation, optimization software (e.g., OptQuest® Engine (OptTek Systems, Inc. 2011a)) can provide effective algorithms to search for optimal solutions to complex problems (OptTek Systems, Inc. 2011b). This provides a method to search an extensive solution space to quickly determine the best combination of variables that will result in achieving a desired objective (in the case under consideration, maximizing the reduction of *C. intestinalis* abundance). The optimization approach adopted in this paper uses a ‘metaheuristics’ based tool that combines scatter search, genetic algorithms, simulated annealing and tabu search to guide the process (OptTek Systems, Inc. 2011b). This optimizer has no knowledge of the structure of the simulation model to find the optimal solution. Instead, it uses combinations of the decision variables that satisfy the objective function. The results are then stored and recombined by the optimizer into updated combinations that produce better solution using the above mentioned algorithms (Sorensen and Glover, 2013). Applying these techniques to a population dynamics model allows for an efficient exploration of the treatment intervention options.

A mathematical model, incorporating temperature-dependent growth and environmental carrying capacity, has been developed to describe the population dynamics of *C. intestinalis* in areas with mussel production (Chapter 4; Patanasatienkul et al., 2014). The basic structure of this model was explained in Chapter 4, together with its sensitivity to various parameter and temperature changes; however, the application of this model to evaluate mitigation strategies has not been explored. The objectives of this study were therefore: (1) to evaluate the use of a mathematical model in finding the best mitigation strategies in terms of a combination of treatment timing and frequency to control *C. intestinalis* populations in areas with mussel production; (2) to evaluate the effectiveness of the best strategies suggested by the model under different temperature conditions; and (3) to assess the sensitivity of the model to variations in presumed levels of treatment efficacy.

5.3. Materials and methods

5.3.1. *C. intestinalis* population dynamics model

A previously described population dynamics model of *C. intestinalis* (Chapter 4; Patanasatienkul et al., 2014) was used to model the abundance of *C. intestinalis* in cases where treatment occurs, so that these could be compared to the situation in which no treatment (control) was administered. Briefly, this model consists of six compartments to represent the live stages of *C. intestinalis*: egg (*E*), larva (*L*), recruit (*R*; the tadpole that settles on a surface and develops through a process of metamorphosis), juvenile (*J*; completely metamorphosed animal), spring adult (A_{sp}; the animal that reaches its sexually mature size between May and September), and autumn adult (A_{au}; the animal that reaches its sexually mature size between October and April). Two compartments are also set up to model the dead stages (dead juvenile (*DJ*) and dead adult (*DA*)), so that all the surface-occupying stages (N_{SO}) can be captured; which consist of these dead stages in addition to the *R*, *J*, A_{sp}, and A_{au} stages.

The adult *C. intestinalis* spawns eggs when the water temperature is suitable (>4˚C). These eggs are then fertilized and hatch into free-swimming larvae at water temperatures in the range from 8 to 26˚C. The larvae find a substrate to settle on, undergo a process of metamorphosis, and become juveniles. The reproductive system develops as the juvenile grows, until it reaches sexual maturity, transforming the *C. intestinalis* into an adult which can produce sperm and eggs throughout its lifespan. A set of differential equations, describing the rates of change for each *C. intestinalis* life stage, is shown in Table 5.1, while a description of the associated parameters is given in Table 5.2.

A dichotomous variable, *x*, was used to control whether (x=1) or not (x=0) an adult can produce eggs with the cut-off temperature at 4°C (*Eq. 1*; Table 5.1). A similar approach was applied for spring and autumn adult compartments (*Eq. 5* and *6*; Table 5.1). A dichotomous variable, *y*, was created to define whether the model was for the spring (y=1) or autumn season (y=0). This allowed the model to assign animals from the juvenile stage to spring or autumn adult compartments, depending on the time of year in the model. The model was set to run for 220 days, with Day 1 being the 1st of May, and was initialized with an initial juvenile presence of 0.1 juvenile *C. intestinalis* per mussel sock (or approximately 1 juvenile per 10 mussel socks); all other life stages were initially set to zero.

5.3.2. Space occupancy

Space was released through a drop-off process whereby dead juveniles and adults were removed from the model at rates of μ_{DJ} and $\mu_{DA,}$ respectively. Space was also released when treatments were applied. Each treatment was assumed to result in an instantaneously knock-down of ω for all the live and dead stages of juvenile and adult *C. intestinalis* from the treated mussel socks.

5.3.3. Parameter estimation

A total of 19 parameters relating to the life cycle of *C. intestinalis* were identified. The estimates were adopted from the previous study (Chapter 4; Patanasatienkul et al., 2014), which obtained many of these values from the scientific literature (Table 5.2). In cases where a range of values had been reported, estimates were selected from uniform (for α) or triangular (for L_L) distributions; while, for parameters derived from more than one source, the average value based on these sources was estimated. Similarly, the average values of estimates at different temperatures were determined for the temperature-dependent parameters.

The model assumes that the settlement of larvae is density dependent, varying with the proportion of $N_{SO}(t)$ to environmental carrying capacity (K). K for a given bay is estimated by multiplying the maximum number of *C. intestinalis* that the system can accommodate per cm^2 by the total surface area (a in cm^2) of all mussel socks in that bay. The model used a value of 40 individuals per cm^2 (Ramsay et al., 2009), while mussel sock density in PEI was estimated to be 630 mussel socks per acre in the 500-acre bay of Georgetown Harbour (AVC shellfish research group pers. comm.). Each mussel sock was assumed to have a cylindrical shape with a length of 180 cm and a diameter of 10 cm. A capacity adjusting factor $\gamma(a,t)$, representing the proportion of available surface area to the total surface area at time t, was used to adjust the settlement; as defined in *Eq. 9* (Table 5.1).

In addition, parameters related to treatment effect were defined (Figure 5.1). Treatment efficacy varies widely, depending on the method used; here, the model assumed an instantaneous knock-down of 80%, based on the efficacy of the high-pressure washing method reported by Arens et al. (2011). The treatment is less effective against the juvenile stages as the number of adults increases, as these protect the juvenile *C. intestinalis* from direct exposure to the treatment. Given that treatment efficacy for juveniles and dead juveniles depends on the proportion of live and dead adults to total abundance of juveniles and adults, this parameter was varied using an adjusting factor $\delta(t)$ (*Eq. 10* in Table 5.1).

5.3.4. Treatment strategies and optimization

The treatment optimization, which had the goal of maximizing the reduction in *C. intestinalis* populations, was carried out within the AnyLogic® software package (XJ Technologies 2014a), using the built-in OptQuest® Engine (OptTek Systems, Inc. 2011a). To find the optimal mitigation strategies, a range of treatment timings and frequencies were evaluated using the metaheuristic optimization approach. Based on current practicalities affecting PEI mussel farmers, the treatment time window was allowed to vary from Day 61 (1st of July) to Day 183 (31st of October). The optimization process (with varied treatment intervals) was carried out separately for each treatment frequency (one to four times) during the course of the season, with a minimum treatment interval of 14 days between any pair of treatments. The search began at a lower bound with increments specified as a minimum step-size, and could range up to an upper bound in no specific search order (XJ Technologies 2014b). We used a step size of 7 days (i.e. assumed that treatments that occur in the same calendar week are equally effective) for the optimization process to reduce the number of treatment combinations tested and the number of model runs, and to allow for the exploration of all possible treatment scenarios. The search stopped when all possible solution sets (i.e. treatment scenarios) were assessed for each treatment frequency. The number of model runs varied based on the treatment frequency; for example, the optimization for the single treatment group required a lower number of model runs than was the case for multiple treatment groups.

For each solution set, an objective value, used to measure the effectiveness of the intervention, was calculated as the sum of the differences in N_{SO} between the control (no treatment) and treatment scenarios across all modelled time points. Data were ranked in ascending order, based on this objective value (i.e. larger differences were given a higher rank), then percentiles of the objective value were computed. A treatment, in term of treatment timing, was considered to be among the “best mitigation strategies” when their objective value exceeded the 95th percentile for each of the four treatment groups, according to overall frequency of treatment (i.e. single, double, triple, and quadruple treatment groups).

5.3.5. What-if scenarios

Treatment optimization was explored under various scenarios to evaluate the impact of different treatment interval, temperature, and treatment efficacy on the treatment times that yield best results in controlling the populations of *C. intestinalis*.

I) Fixed treatment intervals

The advantage of treatment optimization with varied treatment intervals is that any combination of treatment times can be assessed; however, the optimal solutions suggested by the model may be unsuitable to put in practice, as the farm activities are usually scheduled in a periodic manner. Therefore, fixed-treatment intervals were explored in the 'what-if' scenarios.

The time of first treatment was varied between Day 61 (1st of July) and 183 (31st of October), and treatment frequency could range from one to four times, with fixed treatment intervals between each pair of treatments. Three treatment intervals (14, 28, and 56 days) were tested to assess their impact on the first treatment timing of treatment scenarios that were among the best mitigation strategies. Medians of treatment times within the best strategies were computed and used as a treatment combination for the evaluation of treatment effectiveness in the subsequent 'what-if' analyses.

II) Temperature conditions

Different sea water temperature conditions were used for two what-if scenarios: long summer, and warm spring. The best mitigation strategy from the treatment optimization process with a fixed treatment interval, using the median of first treatment time with fixed treatment interval as a treatment setting, was evaluated by comparing the modelled N_{SO} under these temperature conditions. Furthermore, treatment optimization with varied treatment intervals were carried out to find the optimal treatment strategy for each temperature condition.

III) Treatment efficacy

The influence of treatment efficacy assumptions on the modelled output (N_{SO}) was assessed using a parameter variation method. The median of the first treatment timing and interval for the best mitigation strategies from the treatment optimization process with a fixed treatment interval was used as a treatment setting. The efficacy was then varied from 10% to 100% under the baseline temperature conditions, and then the objective values were compared to the base case (80% treatment efficacy) scenario.

5.4. Results

5.4.1. Treatment optimization with varied treatment intervals

The objective value, the sum of the differences between N_{SO} in the control and each treatment scenario, ranged from 842 to 2,552 for treatment optimization with a 14 day minimum treatment interval setting. These objective values, broken down by treatment frequency grouping, are shown in Figure 5.2 together with the numbers of model runs associated with each grouping. An increasing trend in objective value can be seen as more treatments are included. The median values for each treatment group were 1302 (single treatment), 2037 (double treatment), 2332 (triple treatment) and 2465 (quadruple treatment).

A treatment was considered to be among the ‘best’ mitigation strategies when the objective value associated with that treatment was above the 95th percentile of the objective value for the particular treatment frequency group under consideration (single: 1772, double: 2254, triple: 2452 and quadruple: 2524). Figure 5.3 illustrates the variation of treatment times for the best mitigation strategies by treatment frequency group. The treatment time for single treatments varied from mid-July to late October; however, the objective value associated with these single treatments were low (median value of 1772) as compared to other treatments. The median of ‘best’ treatment times for the double treatment group were in mid-July (Day 75) and mid-September (Day 138). The triple treatment group had median treatment times of early July (Day 68), early August (Day 96), and late-September (Day 152); while treatments given twice in July (Days 68 and 89), once in early September (Day 124), and once in mid-October (Day 166) were the medians for the quadruple treatment group.

5.4.2. Treatment optimization with fixed treatment interval

A total of 687 treatment scenarios/ model runs were explored to find the optimal treatment strategies when time of first treatment could be varied and the treatment intervals between each pair of treatments were fixed at 14, 28, or 56 days. The objective values and number of model runs associated with the assessed treatment frequency and interval are presented in Figure 5.4. The median objective value of the single treatment (1300) was lower than the multiple treatment groups (around 2000 for double treatment; 2300 for triple treatment; and 2400 for quadruple treatment groups). When comparing the outputs of different treatment intervals within the same treatment frequency group, the objective values of the best treatment (the right tail) for each treatment group tended to increase as the treatment intervals decreased (Figure 5.4). The triple treatment with a 14-day interval and quadruple treatments with 14- and 28-day intervals were the only three treatment groups that generated objective values exceeding the 95th percentile (2500) when the objective values from all treatment groups were considered together. Among these three 'best' treatment groups, quadruple treatment with a 14-day interval tended to have the highest median objective values and, therefore, its median treatment times were used as the treatment setting for the best mitigation strategy in the subsequent what-if scenarios section. The median times of first treatment for each 'best' treatment group were Day 114 (23rd of August; triple treatment/14-day interval), 92 (1st of August; quadruple treatment/14-day interval), and 81 (21st of July; quadruple treatment/28-day interval).

5.4.3. Treatment effectiveness under different temperature conditions

The three temperature conditions explored are shown in Figure 5.5. The modelled temperature under the baseline condition was set to 3.3 ˚C at the start of the model with a mean of 7.1 ˚C. It peaked at a maximum of 16.9 ˚C in late August, reached 8 ˚C (the critical temperature for reproduction of *C. intestinalis*) at the end of May, and dropped below 8 ˚C again in mid-November. In the case of the warm spring scenario, the maximum temperature started at 4.5 ˚C and rose to around 5 ˚C higher than the baseline for much of the summer before converging to the baseline profile by mid-October; while for the long summer scenario the maximum temperature was around 2 ˚C higher from July, and remained so for around two months after the summer peak. Figure S5.1 illustrates the modelled N_{SO} of the control model under the three different temperature profiles. It can be seen that for these modest temperature changes, the growth of *C.*

intestinalis is a few orders of magnitude greater in the absence of any treatment. Figure 5.6 illustrates the N_{SO} when the 'best' (quadruple treatment/14 day interval, on Days 92 (1st of August), 106 (15th of August), 120 (29th of August), and 134 (12th of September) with treatment efficacy of 80%) mitigation strategy was carried out under the baseline, long summer, and warm spring temperature conditions. This 'optimal' treatment strategy was obviously much less effective, leaving a large number of N_{SO} by the end of the year, when implemented under the long summer or warm spring conditions.

Median treatment times for the best mitigation strategies (i.e. treatments with objective values that exceeded 95th percentiles), optimized under different temperature conditions, are presented in Table 5.3. The first treatment time of the multiple treatment groups (i.e. double, triple, and quadruple treatment) ranged from Day 68 to 75 (8th – 15th of July) for baseline temperature conditions, while the ranges were Day 75-121 (15th of July – 30th of August), and Day 82-124 (22nd of July – 2nd of September) for long summer, and warm spring conditions, respectively. The quadruple treatment group for the 'best' treatment strategies tended to show higher objective values than other treatment frequency groups, optimized under the same temperature conditions.

5.4.4. Sensitivity to treatment efficacy variation

Figure 5.7 illustrates the modelled N_{SO} when treatments were carried out at Day 92, 106, 120, and 134 (Quadruple treatment with 14-day interval, starting on 1st of August) and with treatment efficacy varying from 0% (control) to 80% (base case scenario) under the baseline temperature condition, while Figure 5.8 presents the changes in the objective values of different treatment efficacies relative to the base case scenario.

The actual objective values can be seen in Figure S5.2. The modelled N_{SO} for the control gradually increased from late July until late August with a rapid increase in early September, reaching a plateau at an abundance of over 30 individuals per 30 cm sock (Figure 5.7). When treatments were activated, the modelled N_{SO} broadly followed the output from the control scenario, with lower levels for the abundance of *C. intestinalis* and objective value. As the treatment efficacy increased, the modelled N_{SO} decreased, with the abundance that varied from 0.3 (for 100% efficacy) to 26 individuals per 30 cm sock (for 10% efficacy) (Figure 5.7). In contrast, the objective value increased considerably from 608 (for 10% efficacy) to 2,573 (for 100% efficacy), as the treatment efficacy increased (Figure S5.2). The variation of treatment efficacy between 10% and 60% caused a moderate change in objective value as compared to the outcome from the base case scenario, but the objective values did not vary much in the rest of the treatment efficacy range of 70% to 100% (Figures 5.8 and S5.2).

5.5. Discussion

5.5.1. Treatment optimization

The treatment optimization from the model suggests that the multiple treatment should start early in July, assuming baseline temperature conditions (Figure 5.3). This result agrees with a field trial study in PEI in 2008 (Davidson et al., 2009; PEI Aquaculture Alliance, 2009), which found that the best strategy to reduce tunicate fouling, regardless of mussel productivity, was to use 3 or 4 treatments starting in July. The model also suggested including one late treatment (> Day 135 or mid-September) in all strategies to achieve effective control of *C. intestinalis* populations (Figure 5.3 and Table 5.3), which is reasonable as this late treatment will clean up the mussel socks around the end of the season when *C. intestinalis* enters its slow reproduction period (Dybern, 1965), and provide limited time for the recruiting stage to re-settle on the socks.

The treatment optimization also suggests that the mitigation strategies with higher treatment frequency appear to be more effective than the less frequent strategies, especially, under the warm spring condition, which shows the highest objective value for the quadruple treatment group. This is in agreement with the results from a study (Paetzold et al., 2012) that evaluated the effectiveness of different treatment frequencies to control colonial tunicates. The result is also consistent with the result from a treatment trial (Gill et al., 2007), using vinegar and lime, which reported that double treatments resulted in larger reductions of *C. intestinalis* biomass than single treatments. Furthermore, when considering results from the optimization with fixed treatment intervals in Figure 5.4, the objective values among the best strategies (the right tails) of each treatment group showed an increasing trend as the time intervals between treatments decreased. This suggests that after taking into account the timing of first treatment, the mitigation strategy using multiple treatments may be more effective when the additional treatments are implemented shortly after the previous treatment.

5.5.2. Treatment effectiveness under different temperature conditions

When treatment timings based on the best mitigation strategies from the baseline temperature scenario were applied to the long summer and warm spring scenarios, their ability to control the population was poor, since the effectiveness of a treatment to control *C. intestinalis* populations depends greatly on when the treatment is implemented. If it is carried out during the warm months, when the temperature is suitable for the reproduction of *C. intestinalis* (Dybern, 1965; Ramsay et al., 2009), the treatment may not be so effective. This is because most treatments remove biofouling species from mussel sock surfaces (Carver et al., 2006), which indirectly facilitates the regrowth of the tunicate populations by increasing the surface availability for the larval stage to settle on the mussel socks. Therefore, a combination of treatment time and frequency that is considered the best mitigation strategy under one temperature condition may not perform well when implemented under different temperature conditions. A set of optimization exercises must therefore be carried out for various temperature profiles to gain a better understanding as to the most effective strategies for the control of *C. intestinalis* populations.

Given that the best mitigation strategies based on baseline conditions did not work well for other modelled temperature profiles, treatment optimizations were also attempted for the warmer temperature conditions (i.e. long summer and warm spring). The optimal time of the first treatment under these warmer conditions appeared to be 2-6 weeks later than was suggested for the baseline scenario (Table 5.3). As discussed above, temperature plays an important role in the development of *C. intestinalis* populations (Dybern, 1965; Yamaguchi, 1975) and should be taken into careful consideration when exploring the mitigation strategies to control this biofouling species. An alternative method of evaluating the temperature component may involve a modification of the model which replaced temperature-linked rates with degree-days to account for the strong influence of temperature in the population dynamics of *C. intestinalis*.

5.5.3. Sensitivity to treatment efficacy variation

As might be expected, an increase in treatment efficacy resulted in an increase in the objective value, which is after all an indication of successful treatment. The increased effectiveness of treatments rose substantially up to an efficacy level of approximately 70%. Thereafter, any increase in effectiveness of the treatment did not result in a significant improvement in terms of reduced abundance of *C. intestinalis*. This suggests that a minimum treatment efficacy of 70% should be sufficient to sustain control of *C. intestinalis* populations. However, this result should be interpreted with caution, as the objective value in this study is based on the abundance of aggregated surface-occupying stages, and has not accounted for the difference in the size/ weight of life stages (e.g., juvenile and adult), which is associated with sock attachment strength of mussels. Although biomass is an appropriate value to measure the effectiveness of a treatment, it was not applied to this study due to limited data from which to parameterize the model. Further research should explore the effectiveness of treatments, using biomass, before more conclusive statements regarding treatment efficacy are made.

In conclusion, this mathematical model performs reasonably well in terms of providing information on treatment optimization for the control of *C. intestinalis* populations, and can be used to assess the population under different temperature conditions. The model provides flexibility to explore the effectiveness of different treatment scenarios, e.g., varying the time of treatment, treatment frequency, and treatment efficacy. This model can therefore be used as a tool to develop better mitigation strategies to control populations of aquatic invasive species under different environmental conditions and to help improve bay management plans for the mussel industry. Future models should include information on *C. intestinalis* biomass, and cost effectiveness, to find the best mitigation strategies for controlling *C. intestinalis* populations without compromising mussel yield.

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Table 5.1 Mathematical equations for *Ciona intestinalis* population dynamics model used to evaluate the effectiveness of mitigation strategies.

Mathematical equations	**Number**
$\frac{dE(t)}{dt} = \frac{(x \times a) \times F_f(T^\circ)}{G_{SI}} \times \left(A_{sp}(t) + A_{au}(t)\right) - \frac{F_h(T^\circ)}{G_E(T^\circ)} \times E(t) - \frac{E(t)}{L_E}; x = \begin{cases} 0, T^\circ < 4°\mathrm{C} \\ 1, T^\circ \geq 4°\mathrm{C} \end{cases}$	*Eq. 1*
$\frac{dL(t)}{dt} = \frac{F_h(T^\circ)}{G_E(T^\circ)} \times E(t) - \frac{F_s(T^\circ)}{G_L(T^\circ)} \times \gamma(a,t) \times L(t) - \frac{L(t)}{L_L}$	*Eq. 2*
$\frac{dR(t)}{dt} = \frac{F_s(T^\circ)}{G_L(T^\circ)} \times \gamma(a,t) \times L(t) - \frac{F_m(T^\circ)}{G_R} \times R(t) - \frac{1-F_m(T^\circ)}{G_R} \times R(t)$	*Eq. 3*
$\frac{dJ(t)}{dt} = \frac{F_m(T^\circ)}{G_R} \times R(t) - \frac{J(t)}{G_J(T^\circ)} - m_J \times J(t) - \omega \times \delta(t) \times J(t)$	*Eq. 4*
$\frac{dA_{sp}(t)}{dt} = \frac{J(t)}{G_J(T^\circ)} \times y - \frac{A_{sp}(t)}{L_{A_{sp}}} - \omega \times A_{sp}(t); y = \begin{cases} 0, t > 120 \\ 1, t \leq 120 \end{cases}$	*Eq. 5*
$\frac{dA_{au}(t)}{dt} = \frac{J(t)}{G_J(T^\circ)} \times (1-y) - \frac{A_{au}(t)}{L_{A_{au}}} - \omega \times A_{au}(t); y = \begin{cases} 0, t > 120 \\ 1, t \leq 120 \end{cases}$	*Eq. 6*
$\frac{dDJ(t)}{dt} = m_J \times J(t) - (\mu_{DJ} + \omega \times \delta(t)) \times DJ(t)$	*Eq. 7*
$\frac{dDA(t)}{dt} = \frac{A_{sp}(t)}{L_{A_{sp}}} + \frac{A_{au}(t)}{L_{A_{au}}} - (\mu_{DA} + \omega) \times DA(t)$	*Eq. 8*
$\gamma(a,t) = 1 - \frac{N_{SO}(t)}{K \times a}$	*Eq. 9*
$\delta(t) = 1 - \frac{A_{sp}(t)+A_{au}(t)+DA(t)}{A_{sp}(t)+A_{au}(t)+DA(t)+J(t)+DJ(t)}$	*Eq. 10*

Table 5.2 Parameter definitions and estimates for *Ciona intestinalis* population dynamics model that includes a treatment effect. (Temperature-dependent parameters are marked with an asterisk).

Parameter	Description	Value	Unit
$G_E(T^{\circ})$	Development time of egg*	0.51 – 2.63	day
$G_L(T^{\circ})$	Development time of larva*	0.31 - 10	day
G_R	Development time of recruit	12	day
$G_J(T^{\circ})$	Development time of juvenile*	30 - 90	day
G_{SI}	Spawning interval	3	day
α	Number of eggs laid per spawning	1,000 – 1,500	egg
$F_f(T^{\circ})$	%Fertilization*	0 – 85	%
$F_h(T^{\circ})$	%Hatchability*	0 - 85	%
$F_s(T^{\circ})$	%Settlement*	0 - 65	%
$F_m(T^{\circ})$	%Metamorphosis*	0 – 80	%
L_E	Lifespan of egg	1.25	day
L_L	Lifespan of larva	0.25-1.5	day
L_{Asp}	Lifespan of spring-adult	150	day
L_{Aau}	Lifespan of autumn-adult	180	day
m_J	% Mortality of juvenile	0.11	%
μ_{DJ}	% daily drop-off of dead juvenile	0.05	%
μ_{DA}	% daily drop-off of dead adult	0.05	%
ω	Treatment efficacy#	80	%
K	Environmental carrying capacity	40	individual·cm^{-2}

* designates temperature dependent parameter.

This effect depends on the proportion of live and dead adult on the surface (see *Eq. 4, 7* and *10* in Table 5.1).

Table 5.3 The objective values (sum of the differences in surface-occupying stage between control and treatment; mean (standard deviation)), median of treatment time (D1-D4), and number of run (N) with the objective values, ranking above the 95th percentile days under baseline, long summer, and warm spring temperature conditions. Day 1 was set to the 1st of May.

Temperature condition	Treatment Frequency	N	Objective value	D1	D2	D3	D4
Baseline	1	8	1,772 (0)	145	-	-	-
	2	28	2,309 (24)	75	138	-	-
	3	113	2,464 (12)	68	96	152	-
	4	410	2,533 (7)	68	89	124	166
Long summer	1	8	947,132 (0)	163	-	-	-
	2	28	1,317,279 (29,064)	121	173	-	-
	3	113	1,565,439 (71,809)	96	145	173	-
	4	410	1,776,395 (51,952)	75	117	145	180
Warm spring	1	8	1,174,553 (0)	142	-	-	-
	2	28	1,592,415 (33,666)	124	173	-	-
	3	113	1,896,000 (51,479)	96	138	173	-
	4	410	2,167,043 (38,210)	82	117	145	173

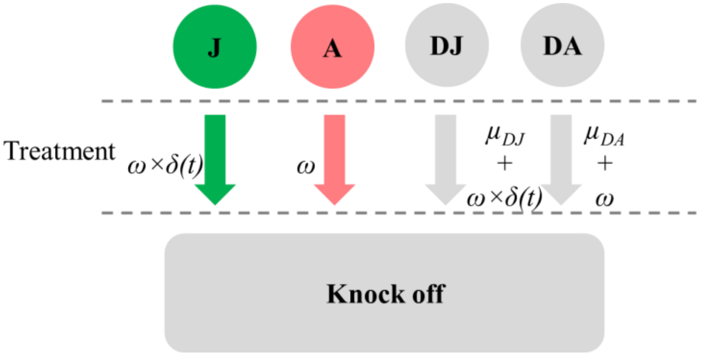

Figure 5.1 Diagram showing parameters related to drop-off rate from treatment effect (ω) on juvenile (J), adult (A), dead juvenile (DJ), and dead adult (DA) stages, the treatment effect adjustment factor for J and DJ ($\delta(t)$), and natural drop-off for dead stages (μ_{DJ}, and μ_{DA}).

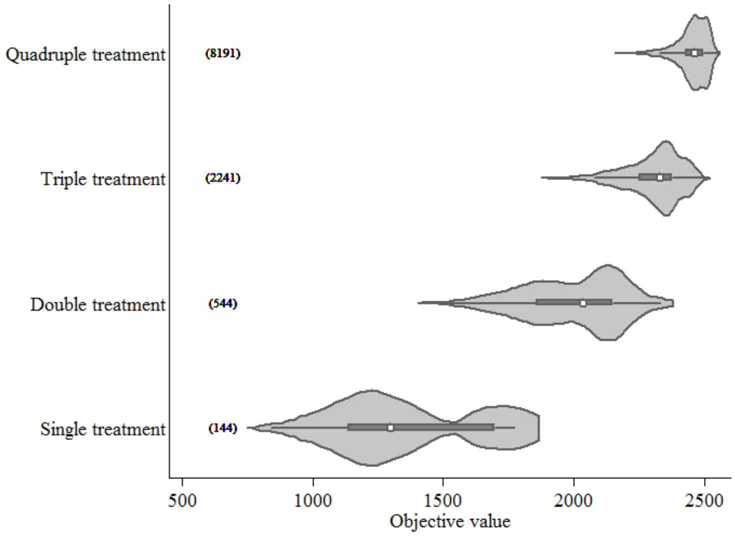

Figure 5.2 Violin plot of the objective values (sum of the differences in modelled surface occupying stages (N_{SO})) from *Ciona intestinalis* population dynamics model, and number of model runs (in brackets) associated with different treatment frequency groups.

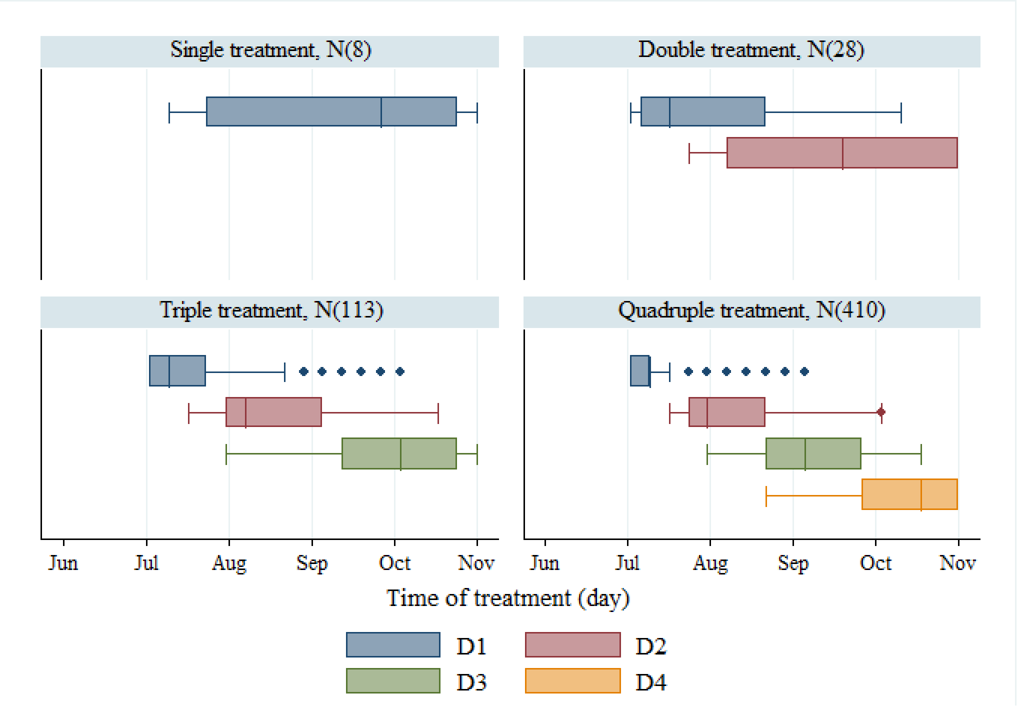

Figure 5.3 Treatment times of the first to fourth treatments (D1-D4) and number of observations (N) associated with the best mitigation strategies, which are the runs with objective values ranking above the 95th percentile of the objective values for each treatment frequency group.

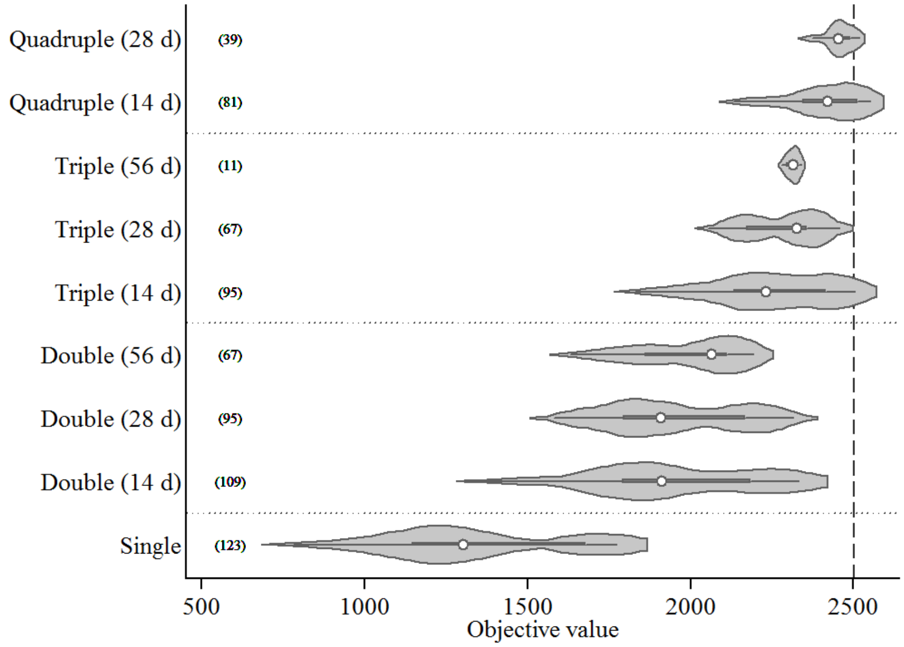

Figure 5.4 Violin plot of the objective values (sum of the differences in modelled surface occupying stages (N_{SO})) from *Ciona intestinalis* population dynamics model, and number of model runs (in bracket) for different treatment frequencies (one to four times) and fixed intervals (14, 28, and 56 days).

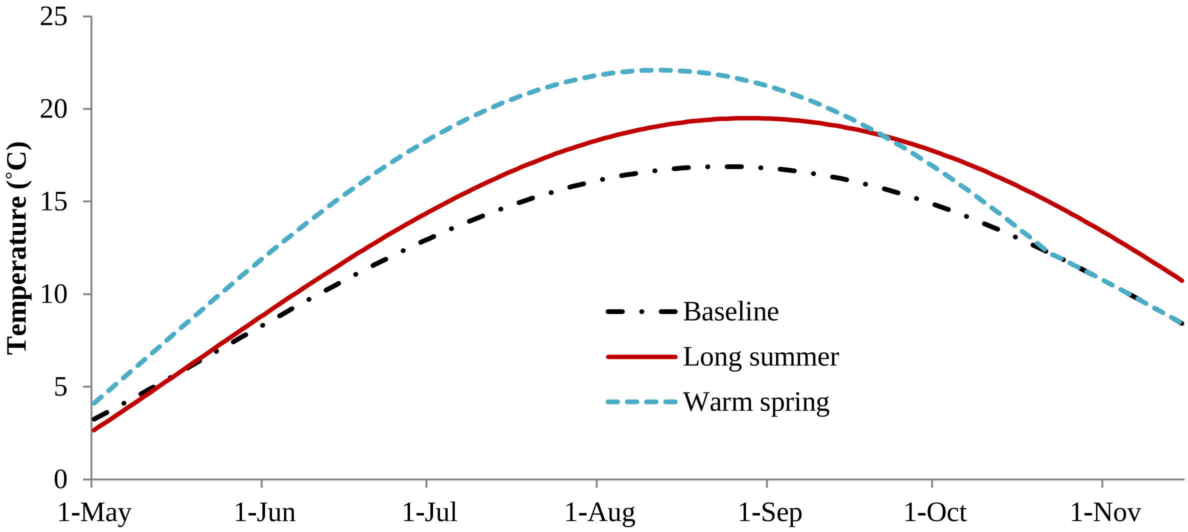

Figure 5.5 The temperatures for 3 different conditions: baseline (replicating temperature from Georgetown Harbour in 2008), long summer, and warm spring.

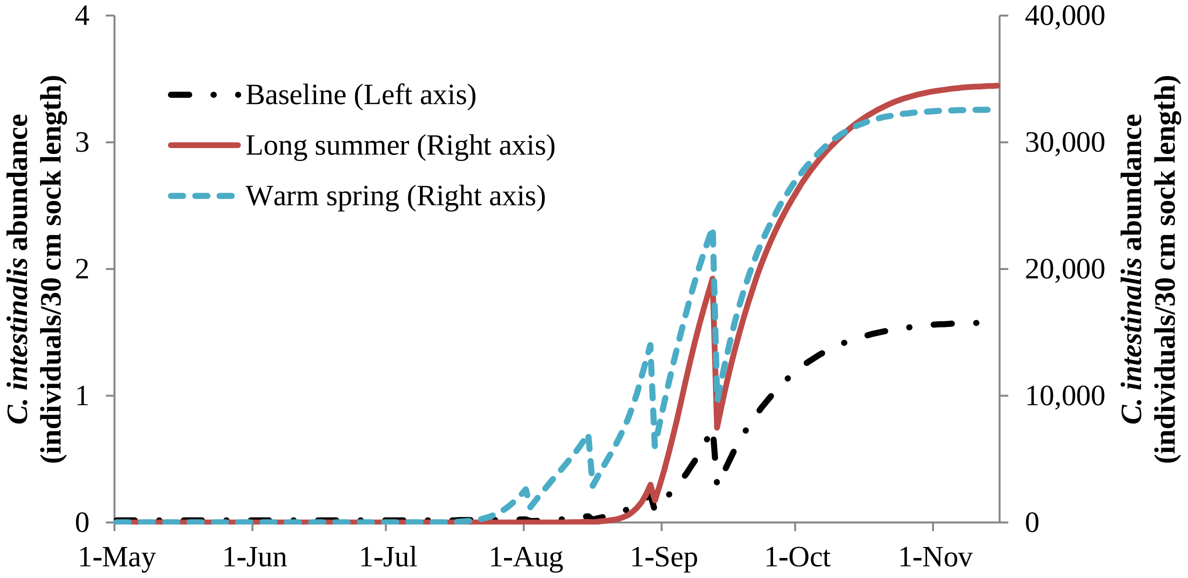

Figure 5.6 The modelled surface-occupying stages of *Ciona intestinalis* (N_{SO}) with the ‘best’ quadruple treatment (first treatment on Day 92 and repeated every 14 days) under baseline, long summer, and warm spring temperature conditions.

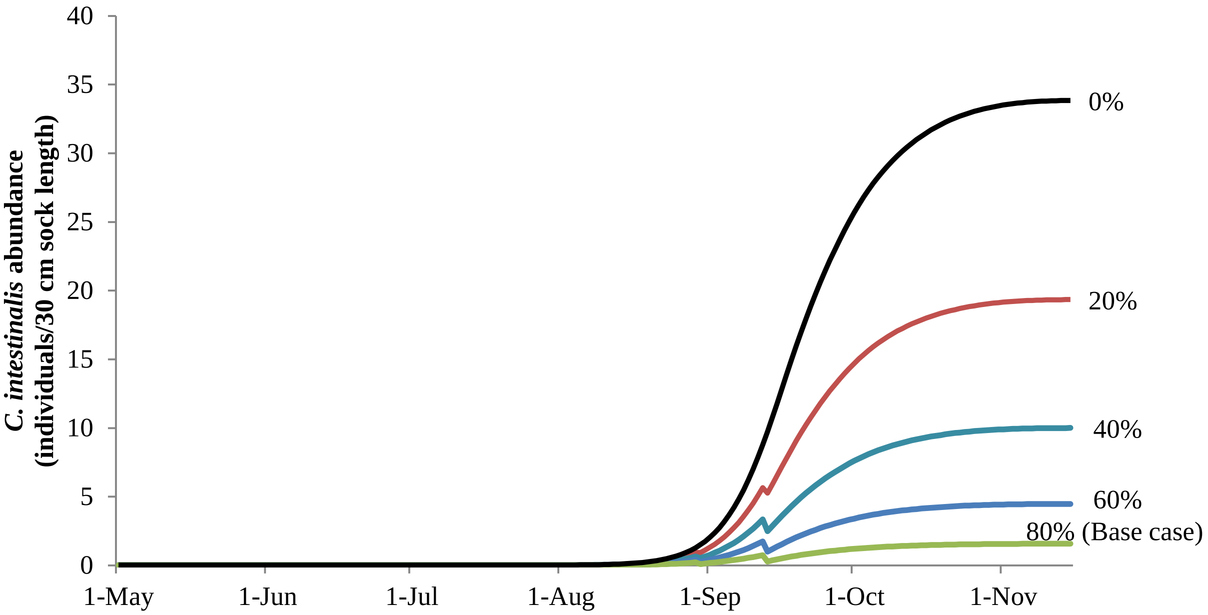

Figure 5.7 The modelled surface-occupying stages of *Ciona intestinalis* (N_{SO} (individuals per 30 cm sock length)) with the 'best' quadruple treatment (first treatment on Day 92 and repeated every 14 days) under different treatment efficacy assumptions with 80% treatment efficacy as the base case.

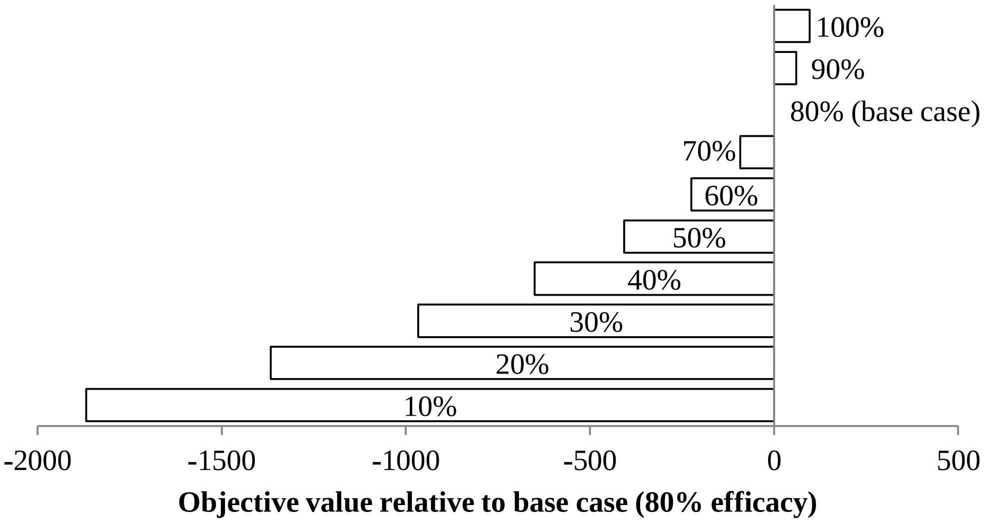

Figure 5.8 The changes in objective value (sum of the differences in surface-occupying stage between control and treatment) relative to base case (80% treatment efficacy) of *C. intestinalis* population dynamics under baseline temperature condition.

5.7. Supplementary materials for Chapter 5

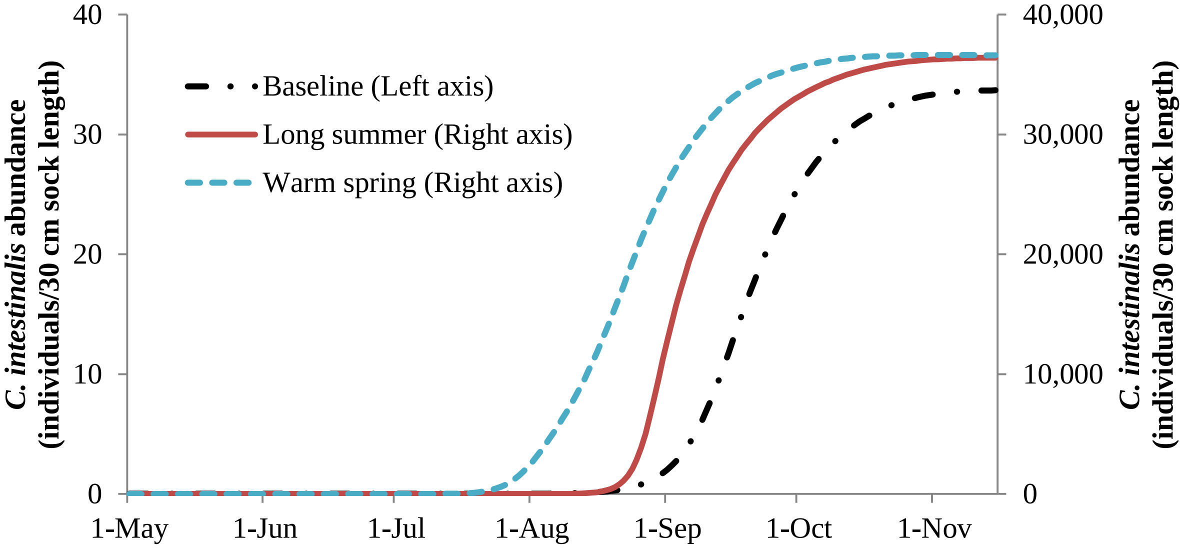

Figure S5.1 The modelled surface-occupying stages of *Ciona intestinalis* (N_{SO}) when no treatment was administered (control) under baseline, long summer, and warm spring temperature conditions.

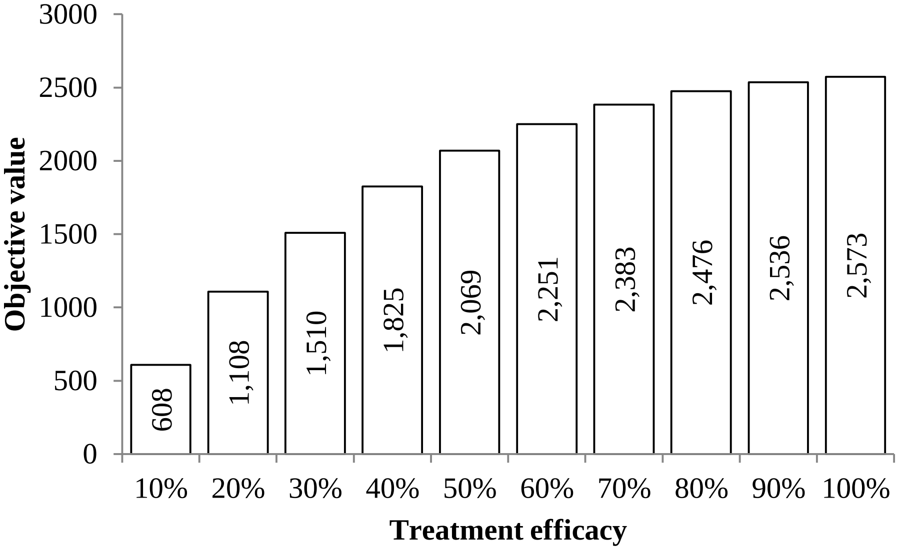

Figure S5.2 The objective values (sum of the difference in surface-occupying stage of *Ciona intestinalis* between control and treatment models) for the variation of treatment efficacy from 10% to 100%.

Chapter 6

General Discussion and Conclusion

6.1. General discussion

The use of modelling in two specific aquatic epidemiological contexts to better understand disease and the factors related to the health of aquatic animals has been demonstrated through the research documented in this dissertation. Two general classes of problems, risk factor analyses in the case of sea lice infestation in wild salmon populations and the use of mathematical simulation in modelling the infestation of tunicates on farmed mussels, were explored. The hypotheses associated with each research chapter were investigated and it was found that the use of the proposed models could adequately address the issues being studied.

Multivariable logistic regression modelling performed well for the purpose of determining the factors associated with inter-annual variation of sea lice infestations in wild Pacific salmon populations (Chapter 2). The use of this model aided our understanding of the varying infestation patterns on chum and pink salmon, while taking account of spatio-temporal correlations.

Space-time cluster modelling was also seen to be an adequate framework for the detection of cluster areas that had elevated numbers of fish infected with sea lice (Chapter 3). This research illustrated that this technique has the capacity to study disease in an aquatic context using a non-Euclidean distance merit to reflect the transmission mechanism through water of aquatic organisms. Furthermore, the use of this cluster modelling approach, spatial scan statistic with multivariable option, in particular, allows for an assessment of the consistency in detected clusters between different datasets (i.e. fish species), and the ability to compare risk estimates associated with the clusters across the fish species. The results from this model can facilitate hypothesis-generation, which should prove useful in future studies.

This research also illustrated how a research question can be answered by different modelling methods, each of which requires different inputs and resources. In this case, to assess the spatial aggregation of sea lice, two modelling approaches were used: multivariable logistic regression with random effects (Chapter 2) and the space-time scan statistic (Chapter 3). Both methods provided consistent results in confirming that spatial clustering of sea lice infestation on wild chum and pink salmon existed in the Broughton Archipelago. When there is a need to adjust for other factors in the analysis, the multivariable model has a flexibility to take account both categorical and continuous variables in the analysis, but it is not able to identify the location of clusters. On the other hand, spatial cluster analysis (assuming a Poisson distribution) can indicate where the clusters are located; however, the software used in the analysis described here (i.e. SaTScan^{TM}) can only adjust for categorical variables. These two methods have their own merits; the choice of the ‘best’ model depends on the questions of interest. In this dissertation, random effects-multivariable model was used for the explanatory purpose, while the spatial cluster analysis model was used for the exploratory purpose.

In addition, the application of mathematical modelling to describe the dynamics of tunicate populations that emerge on farmed mussel provided promising results (Chapter 4). The study suggested that this approach could be used not only to model parasite populations, but also to evaluate intervention strategies under different temperature scenarios (Chapter 5). The use of treatment optimization in Chapter 5 allows for the determination of the best treatment strategy to control *C. intestinalis* populations in terms of treatment timing and frequency. The optimization process for a large number of treatment combinations, especially in a complex model, can take a considerable amount of time to execute and, therefore, a good model building practice is to simplify the model and use powerful computers to minimize the issue.

6.2. Challenges in applying modelling to the study of aquatic diseases

When using modelling approaches to study diseases in an aquatic environment, details regarding the transmission of the disease or pathogen of interest through the water must be considered. Several challenges were encountered during the model building phase of the research documented in this dissertation and are discussed below. Some of these challenges have also been identified when modelling diseases of humans or terrestrial animals.

6.2.1. Parameter estimation

Parameter estimation is an essential step in the model building process, and is typically carried out after the model has been constructed. Three types of estimates (i.e. estimated values, referenced values, and assumed values) can be obtained respectively from, empirical data, where available, from scientific literature, and from expert opinion when there are no data available (Dorjee et al., 2013). However, this is rarely simple, especially when developing a system dynamic model (e.g. the *C. intestinalis* population dynamic model in Chapter 4) with parameters that depend on other parameter values, such as temperature-dependent parameters. This type of parameter appears to be common in biological modelling within aquatic environments (Filgueira et al., 2013, 2014) and is also a key feature in modelling vector-borne diseases such as Dengue (Andraud et al., 2012). Obtaining the estimates for these temperature-dependent parameters can be difficult due to the lack of information on the relationship between the parameters and temperatures, especially for relationships that are non-linear. Sensitivity analysis may help to clarify whether or not it is worth conducting experiments to collect data in order to obtain better estimates for these parameters.

6.2.2. Temperature dependencies

Because the population of *C. intestinalis* predicted from the model, in Chapter 4, depended so much on temperature, changes in one parameter (such as the environmental carrying capacity (*K*)) may have major impacts on the population growth under one temperature condition, but may have only a modest effect under another set of conditions. For example, results suggested that changes in *K* have a larger impact on population growth under warm summer or long summer-like conditions, as compared to what would be the case in cold years. However, if the impact of changes in *K* had only been assessed under cold year conditions, the conclusion might have been drawn that space management does not impact the population and so control plans could disregard this intervention, which is not true for warm temperature conditions. This suggests that extra care is required when making any conclusions where population growth is modelled based on temperature-dependent parameters, as small variations may have a large impact on optimal intervention plans.

6.2.3. Missing data

Missing data can be a challenge in modelling, as it may cause bias in the analysis if the remaining samples with complete data are not representative of the full data set. In the study of spatial clustering of sea lice infestation in Chapter 3, the dataset contained missing data due to non-sampling and the analysis was carried out with the adjustment of relative risks associated with that sites/times combinations which contained missing data to prevent false positive detection of clusters (Kulldorff, 2014).

6.2.4. Model validation

A final stage in model construction is model validation, which is a process that evaluates the reliability of the model in predicting outcomes, by checking modelled output against independent datasets (Vynnycky and White, 2010). One of the most common approaches to validate statistical models is ‘split-sample analysis’, which randomly divides data into two groups, builds a model based on one half of the data (“training set”), and then uses the other half validate the model (“test set”) (Dohoo et al., 2009, pp. 388-390); however, the results may vary widely depending on the division of the data. The resampling methods (e.g., random subsampling, k-fold cross validation, bootstrap, etc.) can be used to overcome this problem (Kohavi, 1995; Steyerberg et al., 2001).

For system modelling with mathematical equations, model validation can be a challenge when there is limited data to carry out the comparison between the modelled and empirical data. This has also been a challenge of disease modelling in terrestrial animals. For example, a direct comparison between the observed larval concentrations and the modelled abundance of *C. intestinalis* at the larval stage, in Chapter 4, could not be carried out due to the differences in measurement scales and, therefore, validation was based on a comparison of temporal patterns. Although the magnitudes of the modelled output and the empirical data can be different, the model still has the advantage when it can show a good comparison in terms of temporal patterns, depending on the purposes of the model.

6.2.5. Over-fitting of the model

Over-fitting is a term used to describe a model that was made to predict the training data too closely, such that it does not reflect the big picture in reality. An over-fitted model will generally have poor predictive performance due to its lack of flexibility. The temperature model that was used to model *C. intestinalis* populations was fitted to the observed data from Georgetown Harbour in 2008, using a trigonometric regression model. The best model fit consisted of three sine and cosine terms, which was too complex and was not easy enough to generalize to use in different contexts, and so the more parsimonious model (i.e. one using a single sine term) was selected to model temperatures, with a trade-off in terms of the prediction performance to the particular temperatures recorded in Georgetown Harbour in 2008.

6.2.6. Distance and disease transmission in an aquatic environment

For an infectious disease, one can possibly be infected if an animal has had contact with the causative agent. One element that plays a role in the transmission of disease is proximity to such infective agents, which in many cases will be measured in terms of Euclidean distance. For disease transmission in aquatic systems, which may involve complex river topology or coastal geography, the organism has to travel through the water body, and Euclidean distance is probably not suitable. In such situations, non-Euclidean distance metrics should be used. Hydrodynamic modelling may be a suitable approach to modelling disease transmission in an aquatic environment, as the model concentrates on the movement of particles and takes into account the characteristics of water, such as tides and water current. However, such models also tend to be highly complex and require significant amounts of computing resource and time. Typically they are constrained physical models that must be ‘forced’ to fit specific sets of environmental parameters and as such may suffer from the problems of over-fitting noted above. It may therefore be more appropriate to use some simpler distance measure to estimate transmission probabilities in aquatic environments; measures such as sea-way distance or a connectivity matrix between the sites of interest, are examples of such alternatives (Salama and Murray, 2013; Rees et al., 2015)

6.3. Future directions

Although the use of these types of modelling approaches has been increasing in the veterinary field, they have had limited application to the modelling of disease in aquatic animals. Choosing between the various types of models that are available to model disease in aquatic animals will depend on the purpose of the analysis.

Mathematical models are commonly used to predict the growth of animal populations and the transmission of disease. A combination of models may be used to help understand population growth and increased production of aquatic food animals. For example, using population dynamic modelling to model the growth of fish populations, and then evaluating the effects of an intervention to increase population growth with an economic model, such as cost-benefit analysis, to find an optimal solution that can maximize the production while the costs are kept to a minimum. Similar approaches may also be applied to the study of disease control in aquatic animals.

Such multi-method modelling is an alternative approach that may be used to model the complex system of host-pathogen-environment interactions. This may integrate system dynamic models with agent-based models to capture elements of heterogeneity at the individual level and then utilise this information in the system dynamic model.

Hydrodynamic modelling uses the environmental data such as water current to evaluate the flow of water and track particles. This approach can be used to model disease or pathogen dispersal. It may be used to determine the invasion feasibility of aquatic invasive species into a bay area. This may provide information to the decision maker to help create more robust management plans for the prevention and control of aquatic diseases.

The population dynamic model of *C. intestinalis* developed in this research was based on a static number of mussels. To help with management decisions within the mussel industry it would be important to include the dynamics of mussel populations that reflect the actual culture system. Thus in addition to modelling the abundance of *C. intestinalis* populations, the model should be able to predict output in term of biomass, which would provide a more meaningful measure of the *C. intestinalis* population from the farmer's point of view and enhance the model's ability to reflect the interaction between mussels and *C. intestinalis*; for instance, the level of mussel loss that might occur due to a given increase in *C. intestinalis* biomass resulting in mussel fall-off. At present, the use of the *C. intestinalis* model is limited to a one-year scenario. To use the model for multi-year scenarios, factors related to mortality during the winter would be required to adequately predict a realistic number of surviving adults at the start of each new yearly cycle. As such, the effects of a broader range of temperature profiles on physiological rates and development stages of this species, particularly in the colder winter months, would have to be studied in greater detail.

The sampling to estimate sea lice infestations on wild salmon in the Broughton Archipelago area required considerable labour and time. On several occasions the sampling failed to retrieve sufficient wild samples due to a timing mismatch between sampling effort and fish migration. Some of the findings from the current research, particular those linked to the spatial distribution of both the wild host and sea lice populations, could be used to more precisely plan sampling activities.

6.4. Conclusion

The studies included in this dissertation demonstrate the ability of modelling to address production and health issues in the context of aquatic animals. Modelling allows a broader set of scenarios to be investigated and management decisions formulated, at a distinct advantage in terms of time and cost savings when compared to field-based trials. The variety of models being applied to aquaculture can help it mature as a vibrant industry which successfully addresses the many challenges associated with its growth and sustainability.

6.5. References

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