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Evaluating transfers of harvested shellfish products, from the west to the east coast of Vancouver Island, as a potential vector for European Green Crab (*Carcinus maenas*) and other non-indigenous invertebrate species

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Canada

Foreword

This series documents the scientific basis for the evaluation of aquatic resources and ecosystems in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

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ABSTRACT

The spread of non-indigenous species (NIS), such as the European Green Crab (*Carcinus maenas*), through human-mediated vectors has become a global concern. The transfer of live seafood and aquaculture products has long been thought to be one of the primary vectors of many well established and notorious NIS around the world. However, there is little to no primary evidence of the potential of this particular vector to entrain and transport NIS to new areas. This has become particularly relevant given the arrival of the European Green Crab on Vancouver Island in the late 1990s as well as the recent transfer of regulatory authority of the aquaculture sector from the provincial government of British Columbia to the Pacific branch of the federal Department of Fisheries and Oceans (DFO Pacific) and the continued growth of shellfish aquaculture in British Columbia. In 2010, Aquaculture Management at DFO Pacific thus became concerned about this vector, particularly with regard to the European Green Crab, and developed Conditions of License as a precautionary approach to reduce the risk of NIS transfer to new areas. In 2011, Aquaculture Management requested scientific advice on these license conditions, which led to a multi-faceted project that investigated the NIS entrainment potential of shellfish transfers, and reviewed potential mitigation measures and current licensing conditions.

The potential for NIS entrainment on shellfish was investigated through a long-term experimental study carried out along the west coast of Vancouver Island in waters that were known to be infested with European Green Crab. Mid-way through the experimental study, a short-term observational study was also conducted using shellfish product as it arrived at processing facilities. The entrainment potential of six NIS on shellfish was investigated and was confirmed for the European Green Crab at two different life stages and four other NIS. Five NIS, not including the Green Crab, were also found on shellfish products that had been transported to processing facilities. They included three well known NIS tunicate species as well as two non-indigenous bryozoans. After an extensive review of the literature, we confirmed that none of the existing or experimentally tested mitigation methods to remove NIS from products was 100% effective at removing NIS prior to product transport. We also identified several areas of potential improvement of the current shellfish aquaculture license conditions which culminated in the development of a conceptual framework model to reduce the risk of spreading NIS at each stage of the shellfish transfer process.

Évaluation du transfert de produits conchylicoles récoltés de la côte ouest vers la côte est de l'île de Vancouver comme vecteur potentiel du crabe vert (*Carcinus maenas*) et d'autres espèces invertébrées non indigènes

RÉSUMÉ

La propagation d'espèces non indigènes (ENI), comme le crabe vert (*Carcinus maenas*), par l'intermédiaire de vecteurs d'origine anthropique est devenue source de préoccupation mondiale. Le transfert de fruits de mer et de produits aquacoles vivants a longtemps été considéré comme l'un des principaux vecteurs de nombreuses ENI bien établies et reconnues à travers le monde. Cependant, il n'y a aucune preuve du potentiel de ce vecteur particulier d'entraîner et de transporter des ENI vers de nouveaux secteurs. La question est devenue particulièrement pertinente après l'arrivée du crabe vert sur l'île de Vancouver vers la fin des années 1990, après le récent transfert de pouvoir réglementaire du secteur de l'aquaculture du gouvernement provincial de la Colombie-Britannique à la direction de la Région du Pacifique de Pêches et Océans Canada (MPO) et en raison de la croissance continue de la conchyliculture en Colombie-Britannique. En 2010, la Gestion de l'aquaculture de la Région du Pacifique du MPO a commencé à s'inquiéter de ce vecteur, surtout en ce qui concerne le crabe vert, et elle a élaboré des conditions de permis en tant qu'approche de précaution pour diminuer le risque de transfert d'ENI vers de nouveaux secteurs. En 2011, la Gestion de l'aquaculture a demandé un avis scientifique sur ces conditions de permis, ce qui a mené à un projet à volets multiples permettant d'enquêter sur le potentiel d'entraînement d'ENI lors du transfert de mollusques, et de passer en revue les mesures d'atténuation possibles et les conditions de permis actuelles.

Le potentiel d'entraînement des ENI associé aux mollusques a fait l'objet d'une étude expérimentale à long terme réalisée le long de la côte ouest de l'île de Vancouver, dans des eaux reconnues pour être infestées de crabes verts. Au milieu de l'étude expérimentale, une étude d'observation à court terme a aussi été réalisée sur les produits conchylicoles à leur arrivée aux installations de transformation. On a étudié le potentiel d'entraînement de six ENI; il a été confirmé chez le crabe vert (à deux différents stades biologiques) et quatre autres ENI. Cinq ENI, sans compter le crabe vert, ont aussi été trouvées sur des produits conchylicoles transportés aux installations de transformation. Il y avait entre autres trois espèces de tuniciers non indigènes bien connues, et deux bryozoaires non indigènes. Après un examen exhaustif de la documentation, nous avons confirmé qu'aucune méthode d'atténuation actuelle ou testée visant à supprimer les ENI des produits n'était entièrement efficace pour supprimer les ENI avant le transport des produits. Nous avons également défini plusieurs domaines d'améliorations potentielles des conditions actuelles des permis de conchyliculture qui ont mené à l'élaboration d'un modèle de cadre conceptuel visant à réduire le risque de propagation des ENI à chaque étape du processus de transfert des mollusques.

1. INTRODUCTION

The European Green Crab (*Carcinus maenas*) was first detected on the west coast of North America in 1989 and had reached western Canadian waters by 1999 (Cohen *et al.* 1995; Gillespie *et al.* 2007). Directed surveys have delineated the distribution of this invasive species along the west coast of Vancouver Island, including Sooke Harbour, and the central coast of British Columbia (BC) (Gillespie *et al.* 2007; G. Gillespie, Fisheries and Oceans Canada, Nanaimo, BC, personal observation), with no confirmed reports from the Strait of Georgia. In 2010, the Department of Fisheries and Oceans (DFO) Aquaculture Management Division made several stipulations on the transfer of shellfish aquaculture products from the west to the east coast of Vancouver Island, via conditions of license (Appendix 1; DFO 2013a), in an effort to prevent the movement of Green Crabs from the west to the east coast of the island. These conditions are highly relevant to such transfers since a processing facility for shellfish did not exist on the west coast of Vancouver Island at the time of this work; therefore most of the products were transferred to processors in the Strait of Georgia (Figure 1). These license conditions were created as a precautionary approach and DFO Science did not provide specific advice to inform the conditions at that time. In 2011, DFO Aquaculture Management formally requested advice from DFO Science on these license conditions. Specifically, they wanted to know the potential for the transport of non-indigenous species (NIS), especially the European Green Crab, on cultured shellfish products and if mitigation measures could be used to reduce such a risk.

At the same time, the Canadian Food Inspection Agency (CFIA) was also transferring shellfish from the west coast of the island, specifically wild mussels from Clayoquot Sound, to sites throughout the south coast of BC as part of its biotoxin monitoring program (Figure 1) and also requested advice concerning the risk of transfer of NIS. These transfers were subject to review by the Pacific Region Introductions and Transfers Committee (ITC). While the ITC included some conditions of transfer, the license permitted wet storage of the wild mussels. As such, DFO Science was also asked to provide advice on the potential for CFIA's biotoxin monitoring program to transport NIS, specifically Green Crabs, from the west to the east coast of Vancouver Island.

As a result, DFO Science conducted a research project through its Program for Aquaculture Regulatory Research (PARR) from July 2011 to October 2013 in an effort to address the potential issue of movement of NIS on shellfish products from the west to the east coast of Vancouver Island. The request for scientific advice from Aquaculture Management came in the form of five separate objectives, which were as follows:

1. Identification and review of the potential processes by which the transfer of a range of cultured shellfish products provide a mechanism by which non-target aquatic invertebrate invasive species may be relocated to new ecosystems using present aquaculture processes.
2. Description of the attributes of European Green Crabs that could influence their ability to establish populations in a receiving ecosystem.
3. Description of the range of transfer potential of European Green Crabs as measured through experimental research and as extrapolated to current and historical commercial shellfish transfers. Provision of considerations around areas of uncertainty and assumptions introduced during the experimental research and extrapolation process.
4. Evaluation of whether the information, data, and analysis presented for European Green Crabs can be used to provide advice on the potential for current bivalve harvest practices to be a vector for other non-commercially harvested aquatic invertebrate invasive species.

5. Advice on potential mitigation measures, including their efficacy, which may be utilized to reduce the potential for transfer of non-target aquatic invertebrate invasive species.

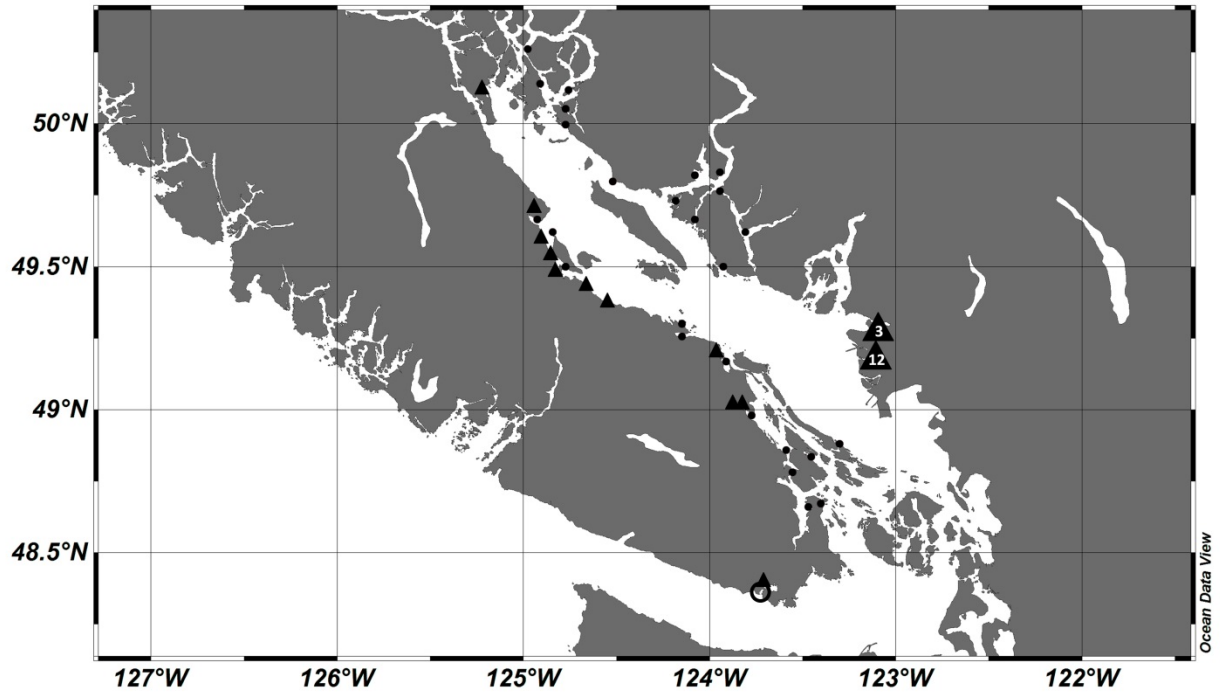


Figure 1. CFIA biotoxin monitoring program sites (filled black circles) and shellfish processing plants (black triangles) along the south coast of British Columbia. The open black circle marks Sooke Harbour, a longer-term storage site of Mytilus californianus that were harvested in Fisheries and Oceans Canada's Pacific Management Area 24 (Clayoquot Sound) and used in the CFIA biotoxin monitoring program.

This document forms the basis for scientific advice related to these specific objectives. It entails the results of experimental studies, historical data analyses, and observations to assess the potential for the transfer of NIS on cultured shellfish. Though the work focuses on the movement of cultured bivalves, the stages of the invasion process, study results, and mitigation measures discussed are relevant to the transfer of all shellfish whether it is cultured, used for monitoring programs, or part of a wild commercial fishery. The final advice based on this document and the Canadian Science Advisory Secretariat (CSAS) peer review process will be summarized in the Science Advisory Report (SAR). A brief outline of the present document, including where each objective will be addressed, is as follows:

Section 1: Introduction

Section 2: This section contains a scientific literature review that addresses the mechanisms and processes that facilitate the transfer of NIS on cultured shellfish, the known traits or attributes of successful NIS, and a review of governmental policy regarding mitigation measures employed to reduce the risk of NIS transfers on cultured shellfish in various regions (Objectives 1 and 2).

Section 3: This section outlines the methodology and results of experimental and observational studies and various pertinent observations. Based on this information, and known historical shellfish movements on Vancouver Island, we extrapolate our experimental findings to assess the overall potential for NIS transfer on cultured shellfish in BC (Objectives 3 and 4).

Section 4: This section includes a critical evaluation of current mitigation practices employed in BC (Shellfish Aquaculture License Conditions, Appendix 1), a review of scientific literature on known mitigation measures to prevent the spread of NIS, and a discussion of a model proposed to apply various mitigation measures given this critical evaluation and what is known about other mitigation practices (Objective 5).

Sections 5: This section summarizes the report conclusions and makes appropriate recommendations (Objective 5).

2. SHELLFISH AQUACULTURE AS A POTENTIAL VECTOR FOR TRANSFER OF NON-INDIGENOUS SPECIES

There are many potential vectors responsible for the re-distribution of marine organisms. In order to better understand the invasion process, Ruiz and Carlton (2003) proposed a framework whereby several steps or stages leading to an invasion can be assessed (Figure 2). In order for a successful invasion to occur, the potential invader must overcome several successive barriers. Once the invader becomes established in an area, the invasion process can be repeated at smaller spatial scales, thereby allowing the organism to spread.

Shellfish aquaculture practices have long been recognized as important vectors contributing to the introduction and spread of marine non-indigenous species (NIS) globally (see reviews by Ruesink *et al.* 2005; McKindsey *et al.* 2007; Forrest *et al.* 2009b). Oyster culture alone is believed to be responsible for the introduction of 78 NIS (including various species of macroalgae, invertebrates, and protozoa) in the developed world and 49% of the species' introductions to the west coast of the United States (Ruesink *et al.* 2005). In California, 57% of the recorded invasive species are known to exist within multiple estuaries, and Ruiz *et al.* (2011) suggested that this was attributable to their spread via vessels or oysters. While there are no estimates of the number of NIS in the Strait of Georgia (BC) that are directly attributable to shellfish aquaculture, Levings *et al.* (2002) concluded that there was a minimum of 65 invertebrate NIS present in its tidal waters. The focus of the present study is an examination of the potential for transferred shellfish product to act as a vector for the movement of NIS, predominantly Green Crabs, from the west coast of Vancouver Island (where the species currently exists) to the east coast of the island (where it has not yet been observed).

The stages of invasion conceptualized by Ruiz and Carlton (2003) have been adapted here to include sub-stages that are particularly relevant to the spread of NIS on cultured shellfish exported to non-invaded areas (*i.e.* the European Green Crab to the east coast of Vancouver Island). The European Green Crab has become well established on the west coast of Vancouver Island since its presence was first documented in the late 1990s (Gillespie *et al.* 2007). While these established populations may disperse naturally through prevailing currents to northern areas such as the Alaskan Panhandle, oceanographic current modelling does not suggest that the spread of the species will occur through natural larval dispersal to the Strait of Georgia (Therriault *et al.* 2008). These west-coast populations, however, have the potential to serve as sources for new invasions to the Strait of Georgia through human-mediated vectors. In order to be transported to new areas, individuals must first be entrained in an invasion vector. The transfer of cultured shellfish from the west to the east coast of Vancouver Island provides one such vector. For the invasion process to continue, entrained individuals must survive transport in the vector and subsequent discharge into the receiving environment. Should individual propagules be successfully introduced to new habitats they must possess environmental tolerances and life-history characteristics that will allow them to establish and reproduce. Whether or not the potential invader becomes established in this new location depends on many variables, notably propagule supply, environmental tolerances, and biotic

resistance (Lockwood *et al.* 2007). Since each of these depends on characteristics of the potential invader and specific conditions encountered in the receiving environment, an in-depth analysis and discussion of factors affecting establishment of Green Crabs and potential impacts is beyond the scope of the current exercise and will not be discussed here. However, many of these elements are considered in detail in previous risk assessments of the European Green Crab and some non-indigenous tunicates (Therriault and Herborg 2007; Therriault *et al.* 2008).

Given our interest in better understanding the entrainment processes for Green Crabs in BC, we have modified the general framework of Ruiz and Carlton (2003). In the present work entrainment is considered a two-fold process that involves both larval settlement/recruitment and microhabitat selection by juveniles and adults of cultured shellfish (termed microhabitat selection henceforth) (Figure 2: B_1 and B_2). Though these two sub-stages appear quite simple, there are several components or factors that lead to the successful arrival of NIS in new areas on cultured shellfish which are discussed below.

2.1. SHELLFISH CULTURE TYPES IN BRITISH COLUMBIA

Similar culture techniques are used for many different species of shellfish, but the specific culture conditions used are dependent on the intended end-use of the product and local site conditions. Shellfish culture techniques can be divided into two main categories: benthic and suspended. In the former, shellfish are grown on the benthos under predator netting, in mesh bags or cages, in racks, in plastic tubes, or sometimes without any form of predator protection. In the latter, shellfish are grown in the water column either in mesh trays or cages, on ropes or plastic tubes, or in mesh socks. These are suspended from rafts or long lines at the surface of the water. In BC, four of the most commonly cultured shellfish species are Pacific Oysters (*Crassostrea gigas*), Blue Mussels (*Mytilus edulis*), Gallo Mussels (*Mytilus galloprovincialis*), and Manila Clams (*Venerupis philippinarum*). Pacific Oysters may be grown in suspension (in trays, on plastic tubes, or on oyster shell woven into ropes) or on the benthos in the intertidal zone; Blue and Gallo Mussels are grown in long mesh socks in suspension; and Manila Clams are typically grown as small seed in suspended trays and are then transferred to the benthos in the intertidal zone, where they are placed under predator netting, as they get larger.

2.2. ENTRAINMENT OF NON-INDIGENOUS SPECIES ON CULTURED SHELLFISH

Entrainment involves NIS propagules being carried along with the cultured shellfish from the harvest site to another location with shellfish serving as the vector. Here, entrainment is the uptake of propagules onto cultured shellfish prior to harvest and their movement on the shellfish from the harvest site to the processing location. It is important to note that for species like the Green Crab, which has a planktonic larval phase, there are two main ways that the initial phase of entrainment can take place, larval recruitment (Figure 2: B_1) and microhabitat selection by juveniles and adults (Figure 2: B_2). For the purposes of this study, we define the term "recruitment" as encompassing both larval settlement and subsequent juvenile survival or recruitment (*sensu* Connell 1985).

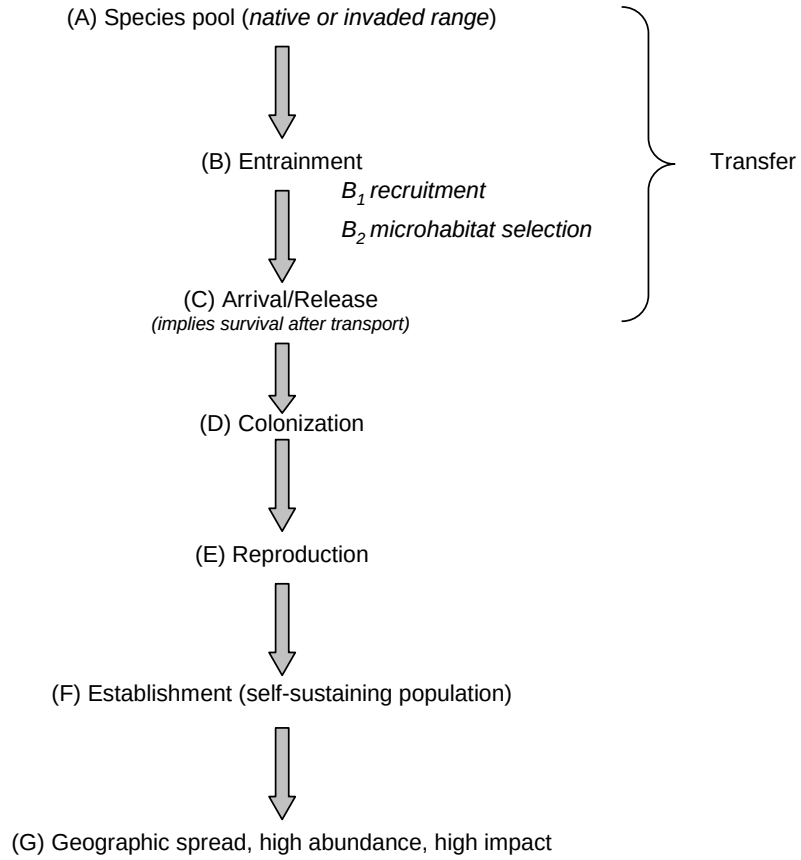


Figure 2. Stages of the invasion process with additional sub-stages specifically relevant to shellfish aquaculture as a non-indigenous species vector (in italics) (adapted from Figure 18.1 in Ruiz and Carlton 2003).

The other potential mechanism of entrainment involves the active selection of the microhabitat (*e.g.* cultured shellfish and gear) by juveniles and adults which we term "microhabitat selection". Although this selection is likely an active process by individuals, the inadvertent movement in conjunction with shellfish remains a passive mechanism. Juveniles and adults may actively choose cultured shellfish and other benthic microhabitats (*e.g.* eelgrass, macroalgae) due to the high structural complexity they provide, which may decrease predation, mitigate environmental stressors, and increase food availability (Thiel and Dernedde 1994; Moksnes 2002; Almeida *et al.* 2008). The differentiation between recruitment and microhabitat selection, as defined here, becomes important when assessing the differences between benthic and suspended shellfish culture with respect to entrainment potential and ultimately invasion risk. For example, juveniles and adults of benthic organisms that actively choose suspended culture through microhabitat selection are likely rare (*i.e.* low probability) compared to benthic culture where preference for shallow water and intertidal benthic microhabitats is more likely (*i.e.* higher probability) (Thiel and Dernedde 1994; Moksnes 2002; Almeida *et al.* 2008).

Like various natural microhabitats such as eelgrass beds, salt marshes, and oyster reefs, cultured shellfish and the associated gear (trays, cages, bags, ropes, *etc.*) are structurally complex (Dumbauld *et al.* 2009; Forrest *et al.* 2009b; Coen *et al.* 2011). Shellfish and the associated culture gear are known to have very diverse and abundant macrofaunal communities, much like many natural microhabitats (Dumbauld *et al.* 2009; Forrest *et al.* 2009b;

National Research Council (NRC) 2010; Coen *et al.* 2011). Increased structural complexity allows many macrofaunal species to flourish, including potential NIS (NRC 2010). This can also be equated to benthic and suspended shellfish culture where three-dimensionally complex structures are added to the benthos and water column, respectively. In addition, these foundation species facilitate the recruitment of other species by increasing the amount of living space and ameliorating predation pressure and environmental stressors (*e.g.* wave action, desiccation, temperature fluctuations) (Bruno and Bertness 2001).

The structural complexity associated with shellfish aquaculture derives from both the structure of the culture gear (trays, cages, bags, ropes, *etc.*) and the shellfish itself. Some shellfish species tend to be structurally complex naturally just by the shape of their shells, while others are not and it is the culturing practices themselves that increase the complexity. For instance, although mussels have relatively smooth shells, they create complex matrices among individuals with their byssal threads when grown together, while oysters have highly undulated and creviced shells and cluster together forming matrices. Regardless of the species, these matrices can be ideal places for animals such as crabs to avoid predation, locate food, and limit exposure to various environmental stressors (Thiel and Dernedde 1994; Cohen and Zabin 2009). Microhabitat structural complexity may facilitate the survival of not only native species, but in many cases NIS as well. Some studies suggest that the diversity and composition of the macrofaunal communities associated with cultured shellfish and culture gear are comparable to natural, structurally-complex habitats (Dumbauld *et al.* 2009; Forrest *et al.* 2009b; NRC 2010; Coen *et al.* 2011; Table 1). In the case of the Green Crab it is well known that it prefers the structural habitat provided by naturally occurring shellfish as settling megalopae, juveniles, and adults in their native range (Thiel and Dernedde 1994; Hedvall *et al.* 1998; Moksnes 2002, Norling and Kautsky 2007; Gestoso *et al.* 2013). Like many other species, they may actively choose this habitat for the reasons listed above, but also because shellfish, particularly mussels (*M. edulis*), are a key component of their natural diet (Ropes 1968; Menge 1983; Jensen and Jensen 1985; Lohrer and Whitlatch 2002; Norling and Kautsky 2007).

The aquaculture gear, regardless of shape and size, generally increases the structural complexity of the surrounding habitat and facilitates the growth of macrofaunal communities that can include NIS (Dumbauld *et al.* 2009; Forrest *et al.* 2009b; NRC 2010; Coen *et al.* 2011; Table 1). Regardless of how the structural complexity is created, either by the gear or shellfish themselves, when recruitment or microhabitat selection does occur, the main factor that can lead to differences in entrainment potential among these gear types is likely the mesh size of the culture equipment. For example, when a species recruits into the culture gear as a larva, if the conditions are adequate, it will grow within the gear and often get larger than the mesh size through which it entered (Grosholz *et al.* 2001). Similarly, small juveniles may migrate into the bags and live within the matrices of the shellfish (microhabitat selection) long enough to grow to a size where they cannot exit through the gear mesh. Alternatively, the animals may be able to escape through the mesh but simply remain in the culture gear due to the hospitable environment.

*Table 1. Review of shellfish aquaculture related studies that have found Brachyuran and Anomuran crabs entrained within gear or on the shellfish itself. NS: Not Specified; * CW: carapace width (notch); ** Natural habitat termed "clumps" in these studies. None of these studies, with the exception of Grosholz et al. (2001), occurred on the Pacific coast of North America.*

Shellfish	Culture type	Range (native/invasive)	Species found; size (CW mm)*	Reference
Mussel (*Mytilus edulis*)	Benthic	Native	*Carcinus maenas*; NS	Beadman *et al.* (2004)
Mussel (*M. galloprovincialis* and *Limnoperna securis*)	Benthic	Native	*C. maenas*; NS	Gestoso *et al.* (2013)
Mussel (*M. galloprovincialis*)	Benthic	Native	*C. maenas*; NS	Ysebaert *et al.* (2008)
Mussel (*M. edulis*)	Natural habitat**	Native	*C. maenas*; NS	Nizzoli *et al.* (2005)
Mussel (*M. edulis*)	Natural habitat**	Native	*C. maenas*; 10-60	Thiel and Dernedde (1994)
Oyster (*Crassostrea gigas*)	Benthic: beach	Native	*C. maenas*; NS	Dubois *et al.* (2007)
Oyster (*C. virginica*)	Benthic: modified rack, beach, natural	Invasive	*Callinectes sapidus*; molting adults and hard-shelled juveniles	Erbland and Ozbay (2008)
Oyster (*C. virginica*)	Benthic: cages	Invasive	*C. maenas*; NS *Hemigrapsus sanguineus*; NS	Dealteris *et al.* (2004)
Oyster (*C. virginica*)	Suspended: floating cages, live oysters, and float alone	Invasive	*C. sapidus*; <60 *Rhithropanopeus harrissii* (invasive); NS, not found on float alone	Marenghi and Ozbay (2010)
Oyster (*C. virginica*)	Benthic: rack and bag Natural: reed Suspended: floating cages	Native and invasive	*C. sapidus* (native); juveniles *R. harrissii* (invasive); NS, floating cages only *Hemigrapsus sanguineus* (invasive); NS, floating cages only	Marenghi *et al.* (2010)
Oyster (*C. gigas*)	Suspended: floating cages	Invasive	*C. maenas*; NS	Haupt *et al.* (2010)
Clam (*Venerupis philippinarum*)	Benthic: beach/bags	Invasive	*C. maenas*; 15-70	Grosholz *et al.* (2001)
Clam (*Mercenaria mercenaria*)	Benthic: beach/bags	Invasive	*C. sapidus*; NS	Powers *et al.* (2007)
Scallop (*Argopecten irradians*)	Benthic: spatfall bags	Invasive	*C. maenas*; <35	Goldberg *et al.* (2000)

2.2.1. Transport of NIS on Cultured Shellfish

The matrices created by the shellfish and the gear used to contain it during transport can hold residual water, sometimes in large quantities. Further, moisture is readily retained by macrophytes and other epibionts that also grow on the cultured product and by the transport bags (L. Curtis, personal observation). Even when the shellfish are removed from the gear and transported alone, they can harbour and protect fauna from harsher environmental conditions encountered during transport (*e.g.* desiccation, UV exposure, temperature fluctuations). Truck beds, trailers, and fish totes themselves may also act in the same way as transport bags to increase the probability that propagules could be transported to new locations in a viable condition. If water, specifically salt water, is held during transport, even the smallest amount could substantially enhance the survival of some species. Thus, the shellfish and methods used to contain them during transport may actually enhance the transport of live organisms to new locations (Minchin 2007). Further, it is in the best interest of the farmer or harvester to maintain conditions during transport that allow the shellfish to survive to market, allowing hitchhiking species to survive as well.

2.2.2. Entrainment of Biofouling NIS

While the primary focus of this study was on Green Crabs and we have centred the discussion thus far on that species, many of the same processes are involved in the entrainment of other significant NIS, such as tunicates and bryozoans, on shellfish. In particular, the processes involved in the recruitment and transport of mobile NIS (*e.g.* Green Crabs) on cultured shellfish discussed above are equally as relevant to the entrainment of sessile species. There is growing evidence that suggests that some non-indigenous tunicates, especially colonial species, are actually more abundant on human-made structures such as cultured shellfish and grow-out equipment (Ruesink *et al.* 2005; Tyrrell and Byers 2007; Crooks *et al.* 2011; Ruiz *et al.* 2011) and that these populations are likely self-recruiting. Essentially, due to the short lifespan of the larvae (minutes to days) and because the cue for settlement can be the colonies themselves, these populations are likely self-sustaining (Bates 2005; Lambert 2005; Bock *et al.* 2011; Ruiz *et al.* 2011). Combined, these factors likely lead to a high probability of entrainment of non-indigenous tunicates on cultured shellfish through continual recruitment when conditions are favourable. Further, many of the conditions created by the shellfish and the equipment used to transport it also likely favour the survival of sessile invertebrates such as tunicates and bryozoans (refer to Section 2.2.1 for further details). In contrast to mobile species such as the Green Crab, it is important to note that sessile species are generally more vulnerable to the unfavourable conditions that can occur during transport (*e.g.* desiccation, heat stress, exposure to freshwater) simply because they are not motile and cannot escape them. Further, many NIS vectors, including shellfish transfers, were highlighted as potential vectors in a risk assessment of several species of tunicates by Therriault and Herborg (2007). The level of knowledge about non-indigenous bryozoans is severely limited and the available information is summarized in Section 2.4.2.

2.3. OTHER VECTORS OF NIS

Shellfish transfers, both wild and cultured, for commercial sale at the regional level (10s to 1000s of kilometres) are not the only means of spreading NIS to new areas; there are many other vectors that can transport NIS. These include recreational boating (Darbyson *et al.* 2009; Davidson *et al.* 2010; Rothlisberger *et al.* 2010; Murray *et al.* 2011; Lacoursière-Roussel *et al.* 2012), commercial shipping [both ballast water (Carlton 1987; Carlton and Geller 1993; Wasson *et al.* 2001; Ruiz *et al.* 2011; Briski *et al.* 2012) and hull fouling (Ruiz *et al.* 2000; Davidson *et al.* 2010; Sylvester *et al.* 2011)], and live transport of marine species for bait, aquaria, and seafood (Chapman *et al.* 2003; Weigle *et al.* 2005; Keller and Lodge 2007). In addition to shellfish

transfers there are also other practices employed by the shellfish culture industry that are NIS vectors. These practices include de-fouling procedures (*e.g.* power washing) (Bock *et al.* 2011; Morris and Carman 2012) and spat collection (Darbyson *et al.* 2009).

2.4. NIS Biology and Attributes Influencing Invasion Success

2.4.1. Green Crab

Unfortunately there is no simple model to predict which species out of the potential species pool will become invasive. However, many studies have identified the importance of certain traits or attributes of a species as being key to invasion success (Briski *et al.* 2012; Chapple *et al.* 2012; Mata *et al.* 2013; Parker *et al.* 2013). Most invasive crustaceans, including the Green Crab, have several traits or qualities that have allowed them to successfully invade areas outside their native range (Weis 2010; Hänfling *et al.* 2011). For Green Crabs, these particular traits include high fecundity, high dispersal capability, robust feeding behaviour, broad preferred habitat and environmental tolerances, and behavioural and phenotypic plasticity (Roman and Palumbi 2004; Lockwood *et al.* 2007; Roman and Darling 2007; Tepolt *et al.* 2009; Weis 2010; Hänfling *et al.* 2011; Todd *et al.* 2012 and references therein).

2.4.1.1. Green Crab Life Cycle

The Green Crab has five larval stages. The first four are termed zoea, distinguished from other stages by their rostral spine (*i.e.* the spine on the carapace between their eyes) and plumose setae on thoracic appendages (*i.e.* the lower half of the body is very feathery) (Shanks 2001). The final larval stage is the megalopa, which appears morphologically more similar to an adult than the other larval stages (Shanks 2001). The megalopa settles and transforms into the first instar juvenile (J1) (Shanks 2001). In Barkley Sound, the zoeal stage is present from March through November, while the megalopal stage is present from August through November (Figure 3).

Determining the size at age of Green Crabs in the field is very difficult as growth is variable and affected by both environmental conditions (*e.g.* temperature) and diet (Klein Breteler 1976; Mohamedeen and Hartnoll 1989). But based on a synopsis of field and laboratory experiments throughout the Green Crab's current range, size after the first winter is somewhere between 10 and 30 mm carapace width (CW)[1] (Behrens Yamada *et al*. 2005). While Green Crabs in BC are thought to be larger than those in other parts of its range (McGaw *et al.* 2011), the size of females at sexual maturity is less variable, about 30 mm CW (Behrens Yamada *et al.* 2005). Given the lack of data on juveniles in BC, we have chosen to use the size of juvenile stages described in Silva *et al.* (2006) as a guide to differentiate new recruits and juveniles (Table 2). In general, the juvenile stages range from J1 through J12 with J13 being the first post-pubescent moult (Berrill 1982; Mohamedeen and Hartnoll 1989; Silva *et al.* 2006). Juveniles were present in Barkley Sound from March through November in the present study (Figure 3), though it is important to note that these data were not collected throughout the year. Based on the ratio of males to females caught at the head of Pipestem Inlet, DiBacco and Therriault (unpublished data) estimated that female Green Crabs brood their eggs from late fall (December) to early summer (July) (Figure 3).

[1] Carapace width measured as the distance between the notches immediately anterior of the fifth anteriolateral spine.

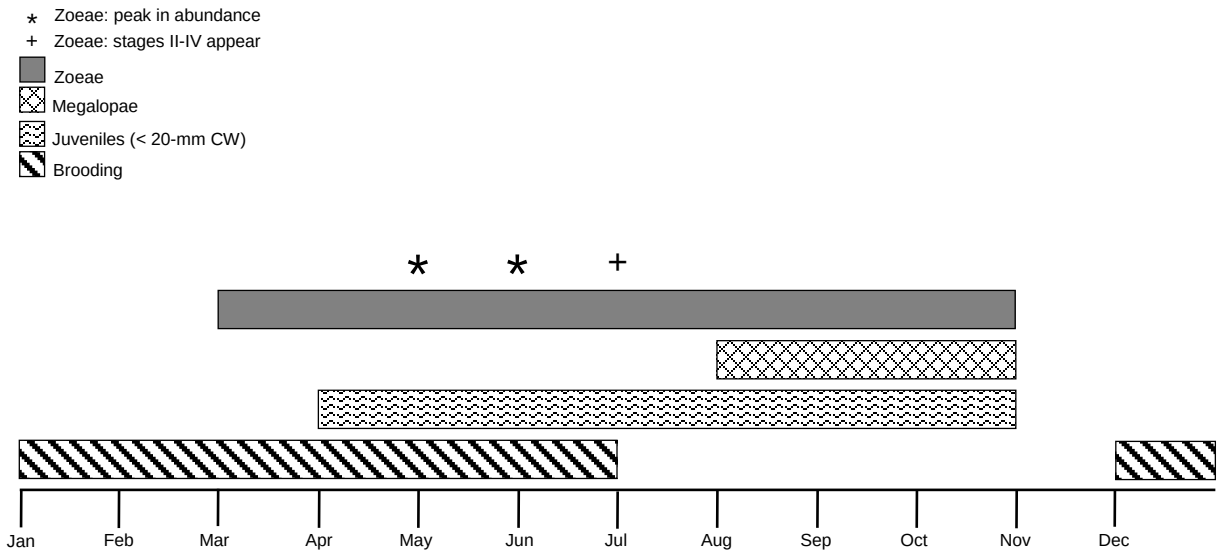


Figure 3. Seasonal presence of each pre-adult life stage and brooding females of the Green Crab (Carcinus maenas) in Barkley Sound based on the results of the present study and those of DiBacco, C. and Therriault, T. W. (unpublished data). Juvenile classification is based on Berrill (1982), Mohamedeen and Hartnoll (1989), and Silva et al. (2006). See Table 2 for further details on juvenile classification.

2.4.1.2. Green Crab Larval Dispersal Capabilities

Green Crab larvae are capable of travelling great distances through wind- and tidal-driven currents, particularly during the zoeal stages (Quieroga 1996). It is believed that the species spread to BC from the western United States as larvae drifting on oceanic currents during the 1998 El Niño event (Behrens Yamada *et al.* 2000; Behrens Yamada and Hunt 2000). Generally, zoeal stages of decapods are longer than the megalopal one(s). The length of the four zoeal stages (stages 1–4) in the Green Crab varies, depending on water temperature and diet (Klassen and Locke 2007 and the references therein), having been found to be approximately 25 days at 15^{o}C (Mohamedeen and Hartnoll 1989; Isle of Man) and 40 days at 12^{o}C (Dawirs *et al.* 1986; North Sea). The megalopal stage is much shorter, the duration also being dependent on temperature; approximately 14 days at 15^{o}C (Mohamedeen and Hartnoll 1989; Isle of Man) and about 23 days at 12^{o}C (Dawirs *et al.* 1986; North Sea).

The horizontal and vertical distribution of these stages also varies – the first and second zoeal stages are abundant near shore, in the upper part of the water column (top 30 m), while the later zoeal stages disperse to outer waters (Quieroga 1996; Quieroga *et al.* 1994, 2006). The megalopae are distributed throughout the water column (0–60 m) and actively change their position to take advantage of onshore advection and tidal currents to transport themselves back to nursery areas (Quieroga 1996; Quieroga *et al.* 2006). Due to these differences in length of each stage and vertical migration behaviour the later zoeal stages can travel greater distances than the megalopae as the zoeae tend to be transported off shore where they may be exposed to stronger, prevailing upwelling currents (Quieroga 1996; Quieroga *et al.* 1994, 2006). Unfortunately, there is little to no data on the vertical distribution or the dispersal of the larval stages in Barkley Sound as all the sampling done thus far employed oblique plankton tows, which sweep the entire water column. The findings reported here are all derived from European studies.

Table 2. Size at class of juvenile Green Crabs (Carcinus maenas) based on the findings of Silva et al. (2006).

Class	Size range (mm)	Stage
J1	0.5 - 1.5	Recruit
J2	1.5 - 3	Recruit
J3	2 - 3	Early juvenile
J4	3 - 7	Early juvenile
J5	5 - 8	Juvenile
J6	7 - 11	Juvenile
J7	8 - 11	Juvenile

2.4.1.3. Green Crab Feeding Behaviour

Like many other invasive crustaceans, Green Crabs also display a high degree of variability in their diet and are usually classified as omnivores (Weis 2010; Hänfling *et al.* 2011 and the references within both). This allows them to exploit a variety of resources, another characteristic of highly successful invasive species. Along the Pacific coast of North America, Green Crabs have been found to actively consume Purple Dwarf Venus (*Nutricola tantilla*) (Grosholz *et al.* 2000), and Manila Clams (*Venerupis philippinarum*) (Grosholz *et al.* 2001, 2011). They are voracious predators which can consume up to 28% of their body weight per day (Pihl 1985) and are known to affect the composition of benthic communities (Reise 1977). When Green Crab populations are high they may substantially influence the recruitment of a number of benthic invertebrate species including bivalves, gastropods, sea urchins, polychaetes, and barnacles (Kitching *et al.* 1959; Muntz *et al.* 1965; Reise 1977; Menge 1983; Jensen and Jensen 1985; Janke 1990; Tyrrell *et al.* 2006). The claw morphology of Green Crabs allows them to eat many different types of organisms, including most shellfish aquaculture species (Weis 2010 and the references therein). In BC, these aquaculture species include Pacific Oysters, Manila Clams, Varnish Clams (*Nuttallia obscurata*), Gallo Mussels (*Mytilus galloprovincialis*), and native Little Neck Clams (*Protothaca staminea*) (Curtis *et al.* 2012). Green Crabs are also capable of out-competing several native decapod species for food and shelter when paired with individuals that are approximately the same size (Weis 2010; Hänfling *et al.* 2011 and the references within both). Their ability to consume a wide variety of prey combined with their voracious appetite, aggressive behaviour, and ability to out-compete native crab species could have a substantial impact not only on shellfish aquaculture but also other fisheries such as the Dungeness Crab (*Metacarcinus magister*) one. These effects could also be extended to the entire ecosystem of the Strait of Georgia by altering faunal community structure.

2.4.1.4. Green Crab Environmental Tolerances and Behavioural Plasticity

Many invasive crustaceans are highly tolerant of environmental change. Green Crabs are able to live and thrive in salinities as low as 5 (Broekhuysen 1936, cited in Berrill 1982), temperatures from 0 to 26°C (Cohen *et al.* 1995), and oxygen concentrations as low as 3 kPa (Legeay and Massabuau 2000) with few ill-effects. The conditions within these ranges are physiologically stressful, or even lethal, for many native species and are often avoided by them (McGaw and McMahon 2003; Curtis *et al.* 2007; Curtis and McGaw 2012). Green Crabs are very efficient osmoregulators as well as being tolerant to stressful environmental conditions such as hypoxia (low oxygen) and temperature fluctuation (McGaw *et al.* 1999; Weis 2010 and the references therein; Hänfling *et al.* 2011 and the references therein; McGaw and Whiteley 2012). Their behaviour and aggressive nature, combined with their broad environmental tolerances, allow them to out-compete many native species for space (*e.g.* shelter protection) and different microhabitats (Jensen *et al.* 2007; Weis 2010; Hänfling *et al.* 2011). These particular traits also

likely facilitate their survival during transport to new areas and are key factors in their continued spread.

2.4.2. Other NIS

Many other NIS, including non-indigenous tunicates and bryozoans, have similar traits that facilitate their transport, establishment, and spread into new areas. We investigated five species of biofouling NIS – three species of tunicates and two bryozoans. The presence of three of the most common and notorious invasive tunicates in North America – the Violet Tunicate (*Botrylloides violaceus*), the Golden-star Tunicate (*Botryllus schlosseri*), and *Didemnum vexillum* – was examined during the present study. We also examined shellfish for the presence of two bryozoan NIS, *Schizoporella japonica* and *Cryptosula pallasiana*. With the exception of *C. pallasiana*, all these biofouling species are distributed along the coastal waters off both the eastern and western shores of Vancouver Island, unlike the Green Crab (Osburn 1952; Powell 1970; Dick and Ross 1985; Cohen and Carlton 1995; Sloan and Bartier 2004; Gartner 2007; Therriault and Herborg 2007). These organisms are all colonial ones and can reproduce both sexually and asexually by budding.

All three of the tunicate species investigated are capable of undergoing hibernation, budding, and fragmentation, as well as quickly recovering from physical and environmental stress (Therriault and Herborg 2007 and the references therein; Epelbaum *et al.* 2009). These properties are particularly relevant for invasions success. Once fragmented, it is possible that small pieces could travel great distances and establish new populations. For instance, *B. schlosseri* can survive as a colony fragment for up to 150 days (Rabinowitz and Rinkevich 2004) and is capable of hibernating at least eight weeks (Epelbaum *et al.* 2009). *Botryllus schlosseri* and *D*. *vexillum* are also capable of hibernating and recovering quickly (Therriault and Herborg 2007 and references therein). Furthermore, once fragments of either of these species lands on a hard substrate it does not take long for it to re-attach and begin growing asexually (Bullard *et al.* 2007b). Under experimental conditions, this process began within 24 hours (Epelbaum *et al.* 2009) and Bullard *et al.* (2007b) found that after 30 hours all three species were capable of firmly adhering to a hard substrate. These fragments can also release larvae once attached at the new location if fragmentation occurred during sexual reproduction (Bullard *et al.* 2007a).

Tunicate larvae and their dispersal potential are very different than that of Green Crab larvae. While the larval stage of the Green Crab can be up to several weeks (see Section 2.4.1.2), the larval stage of all three of these tunicates species is very short. In general, the larval lifespan of these tunicates can last anywhere from minutes to days depending on environmental conditions and species (Hiscock 2005; Osman and Whitlach 2007; Bock *et al*. 2011). The long-distance spread of these organisms through natural larval dispersal is highly unlikely given their relatively short larval period and the results of genetic studies (Lambert 2005; Therriault and Herborg 2007; Bock *et al.* 2011). Genetic studies are confirming that the spread of some of these species is likely due to aquaculture practices that induce fragmentation (*e.g.* power washing) and/or hull fouling (Bock *et al.* 2011). For a more detailed summary of their invasion risk in Canadian waters and the biological background of these three species, the reader is referred to Therriault and Herborg (2007), Carver *et al.* (2006), and Daniel and Therriault (2007).

Like the tunicate species referred to above, the larval stage of the bryozoans investigated is also short and is not a contributing factor in their dispersal from their native range (Watts *et al*. 1998). The most likely factor in their dispersal is their ability to foul hard substrates and their subsequent transport on these substrates to new ranges (*e.g.* by hull fouling) (Watts *et al*. 1998). Much like the tunicates investigated, these animals are also colonial and reproduce asexually through budding. Unlike these colonial tunicates, however, the survival of bryozoan colonies after fragmentation (off the substrate) appears to be unknown; dispersal does occur,

however, when the substrate and the bryozoan colony are carried to new areas (*e.g.* by hull fouling or rafting) (Watt *et al*. 1998). Although the two species of bryozoans investigated are known to be NIS along the coast of BC (Osburn 1952; Powell 1970; Dick and Ross 1985; Cohen and Carlton 1995; Fofonoff *et al.* 2003; Sloan and Bartier 2004; Gartner 2007), little research has been done on these species, their invasion, and their impacts post-invasion. Research that has been conducted has demonstrated that *C. pallasiana* is highly tolerant of common anti-biofouling agents such as copper sulphate and is a competitive dominant in the biofouling communities of San Francisco Bay (Crooks *et al*. 2011). Similarly, *S. japonica* has also been found to be a competitive dominant in experimental fouling communities (Needles and Wendt 2013).

2.5. MITIGATION POLICY REGARDING NIS TRANSFERS ON CULTURED SHELLFISH

For intentional introductions, Canada uses an introductions and transfers code of practice to reduce the risk of ecosystem harm related to aquaculture practices. This code considers the potential of introducing unwanted organisms such as disease or hitchhiking species and has resulted in most cultured shellfish product originating from certified hatcheries. It is also applied to many other aspects of transferring shellfish in Canada including the collection of culture brood stock from wild populations (DFO 2013c, d, e), the transfer of shellfish for biotoxin monitoring, and the application of licences for scientific research.

Most developed countries recognize that shellfish aquaculture can be a potential vector for NIS, contributing to their range expansion, but do not have any policies or legislation in place to restrict or enforce better practices to mitigate NIS movement on cultured shellfish (see Appendix II for references and details on each of these policies around the globe). Further, very few countries are pro-active about mitigating the risk of spreading Green Crabs; some of the pro-active countries (or states) include Canada, Australia, New Zealand, and the State of Washington (U.S.A.). These countries, except for New Zealand, have established populations of Green Crabs and NIS policies in place to mitigate the spread of the species, while other areas known to have Green Crab populations, such as South Africa and South America, do not. None of the top aquaculture (finfish and shellfish) producing countries (FAO 2013) apparently has legislation or policies in place that address shellfish aquaculture as a potential vector for NIS. Of those countries that do, the measures are largely preventative (Appendix II).

Each of the pro-active countries/states addresses the issue of the Green Crab as a pest species and most suggest preventative measures, though none are explicitly stated with the exception of the policies of the State of Washington and Canada. In these two jurisdictions there is a process that requires that a shellfish transfer plan must be submitted to an overseeing governing body. In Prince Edward Island, Canada, producers must apply for an introductions and transfer license if they wish to transfer shellfish out of a body of water that is known to be infested with invasive tunicates. Similarly, in the State of Washington, if growers wish to transfer product out of an area infested with Green Crabs they must submit a plan for review and approval to the Washington State Department of Fisheries and Wildlife. Shellfish product grown on the west coast of Vancouver Island (an area with known populations of Green Crab) that is transferred to non-infested areas, such as the Strait of Georgia, has restrictions placed on it. These restrictions are in the form of license conditions, which include a requirement for rinsing the product before transport and the prohibition of wet storage in the intertidal zone at the receiving location (see Appendix I, Section 7.1 for details of these conditions and Section 4.1 for a discussion on them). The policies in Australia and New Zealand imply mitigation methods, but details and their applications are not explicitly stated (exceptions included in Forrest *et al.* 2009a). Although there are some examples of well-defined policies with tangible

preventative measures, most of them either do not have a practical application for use on the ground or the literature supporting these policies was not readily available.

3. NIS SHELLFISH STUDIES

3.1. EXPERIMENTAL FIELD STUDY AND PROCESSOR STUDY

Two studies were conducted to determine if NIS entrainment occurred on cultured shellfish products exported from the west coast of Vancouver Island. The first was a field study conducted in Barkley Sound on the west coast of Vancouver Island. The project began in July 2011 with field work ending in November 2012. Three species of shellfish were out-planted with deployments mimicking some of the culture methods used by the shellfish aquaculture industry. Sampling events occurred every four weeks from August 15, 2011 through November 7, 2011 and from March 5, 2012 through December 10, 2012. During the winter of 2011–2012, sampling trips occurred approximately every eight weeks (*i.e.* sampling occurred the week of January 9, 2012). Following collection, shellfish samples were processed in the laboratory to determine if NIS were present.

The second NIS study investigated whether NIS were entrained on shellfish product from the west coast of Vancouver Island that was received by processors on the east coast of the island and product that was purchased directly from growers after harvest on the west coast of the island. Although there were two sources of product (processors and growers), this study will be referred to as the Processor Study from now on, for brevity. It was conducted from August through December 2012. The shellfish samples from both studies were collected and processed in the laboratory using the same methodologies. In addition to these two studies, we also estimated the potential number of Green Crabs that could be transferred on cultured shellfish based on the results of the experimental field study and historical transfers of shellfish from the west coast of Vancouver Island.

3.2. METHODOLOGY: EXPERIMENTAL FIELD STUDY

3.2.1. Study Sites

An experimental field study was carried out to establish the potential for Green Crabs to be transported from the west to the east coast of Vancouver Island along with cultured shellfish. The study was carried out at two sites in Barkley Sound, BC. These sites were selected based on a feasibility study that was carried out July 4 to 7, 2011. We focused this feasibility study within the north-eastern area of the sound as several studies and baseline data on Green Crab populations in the area were available (Gillespie *et al.* 2007; McGaw *et al.* 2011). Several conditions were needed at both sites in order to carry out this study including: the presence of an established population of Green Crabs, floats or a breakwater from which to hang aquaculture products, and a gradually-sloping beach that mimicked intertidal shellfish culture conditions or was an active Manila Clam lease. To minimize natural spatial variation these requirements had to be in relatively close proximity (*i.e.* less than an arbitrary 300 m apart). Other factors considered were the distance between sites and the feasibility of carrying out the study in sub-optimal weather conditions. Two sites met these criteria and will be referred to as ‘Refuge Island’ and ‘Sechart’ (Figure 4).

3.2.2. Determining the Presence of Green Crabs

In order for NIS entrainment to occur on aquaculture equipment or product, the species of concern must be present at (or prior to) the time of sampling. In the case of the Green Crab, it may be present and transferable at several stages of its life cycle: adult, juvenile, or larva.

Therefore, during each sampling event when shellfish were collected, several methods were employed in order to determine the presence of these various life stages.

3.2.2.1. Adults and Juveniles

During each sampling trip the presence of adults was determined through standardized trapping methods (Gillespie *et al.* 2007). Briefly, three strings of baited, Fukui, multi-species marine traps (12-mm mesh, Model FT 100, Fukui North America, Eganville, Ontario, Canada) were laid at each site and soaked for 18–24 hours. The strings were composed of six Fukui traps, laid on an anchored ground line, with 10 m spacing between traps. Each trap was baited with herring in a bait cup (Gillespie *et al.* 2007). Initially, all the traps were left high in the intertidal zone, but we could not continue to do so after August 2011 since the traps were damaged by bears. Subsequently, the traps were set as to not be exposed during diurnal low tides, therefore their minimum depth varied, based on daily tidal levels. The Fukui traps were set between -2.0 and 2.0 m and -2.0 and 1.0 m chart datum at Refuge Island and Sechart, respectively.

Due to size selectivity of the Fukui traps, the presence of smaller adults and juveniles was determined using modified minnow traps (Gee traps, ¼" or 7-mm mesh, 50-mm trap opening). Two strings of minnow traps, baited with herring, were laid in the same manner as described for the Fukui traps. The minnow traps were set between -2.0 and 2.0 m and -2.5 and 2.0 m chart datum at Refuge Island and Sechart, respectively.

In addition to trapping, 1-hour beach walks were conducted within 0.5 hour of a diurnal low tide to survey for the presence of Green Crabs potentially not vulnerable to either trapping method. Three people searched for small juveniles under rocks, cobble, and macrophytes parallel to and along the water line for the first 0.5 hour. During the second 0.5 hour, the search continued perpendicular to the water line up to the high tide line. The high tide line was delineated as the highest part of the *Fucus* bed or the wrack line (wrack is drifting, broken pieces of macrophytes such as eelgrass, *Fucus*, or kelp). All brachyuran crabs were identified to species and counted, the data from each Green Crab being recorded as per standard methodology described in Gillespie *et al.* (2007). During the January, October, and November 2012 sampling events, beach walks were not possible as there were no diurnal low tides during daylight hours. During the April 2012 sampling event, the duration of each portion of the beach walk was extended to 0.75 hour as it was conducted by only two people; this insured that the effort was consistent throughout the study. Small (<20-mm CW) individual Green Crabs were preserved in 95% ethanol in order to size them accurately through microscopy. All crabs larger than 20-mm CW caught or found were released alive following processing.

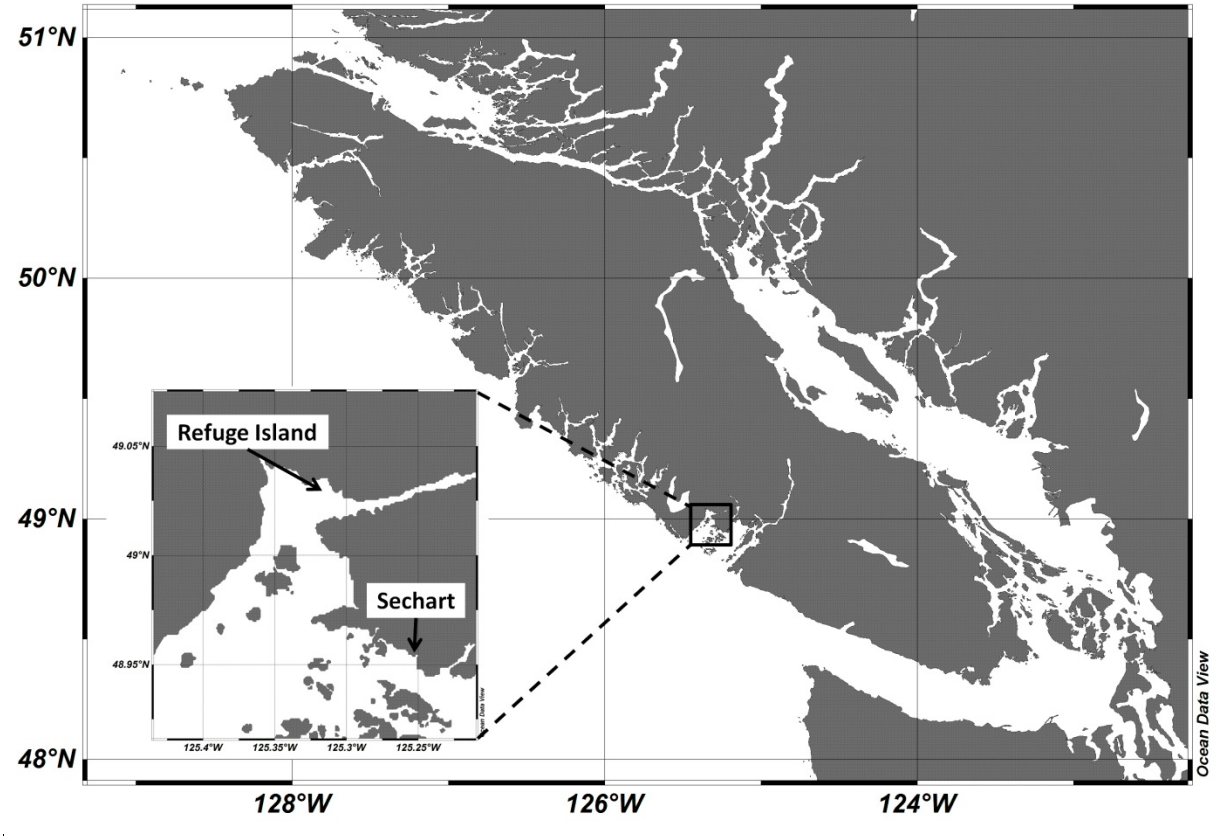


Figure 4. Experimental field sites at Refuge Island and Sechart located in the north-eastern portion of Barkley Sound on the west coast of Vancouver Island, British Columbia.

3.2.2.2. Larvae

During each sampling event, five oblique plankton tows were conducted at both sites during a diurnal flooding tide using a 333-µm mesh plankton net. At each tow, the net was lowered to within 5 m of the bottom and retrieved at a rate of 0.2 m s^{-1}. The stations were kept consistent at both sites throughout the study as they were geo-referenced using GPS during the feasibility study in early July 2011 (Table 3). These stations encompassed most of the potential, out-going waters surrounding both sites. After each tow, the net and cod-end were rinsed using a hand-pressurized sprayer, the contents of the cod-end being fixed in 3.7% seawater-buffered formalin.

Plankton samples were analyzed at the Pacific Biological Station (PBS) to document the presence or absence of larval Green Crab stages. Each sample was concentrated using a 333-µm sieve, rinsed with distilled water, and split into sub-samples using a 1831-F10 Folsom Plankton splitter (Wildco Inc., Florida, USA). Each sample was examined through a stereo-microscope in its entirety based on recommendations of the United States Environmental Protection Agency (2003) since Green Crab larvae were rare (< 60 individuals per sample). The samples were processed using a counting chamber similar to the Bogorov counting chamber (W x L x D: 7.3 x 15 x 1.3 cm; Volume: 100 mL). All decapod zoeae and megalopae present were counted and Green Crab larvae identified using taxonomic keys and studies (Hart 1935; Rice and Ingle 1975; Shanks 2001; Rice and Tsukimura 2007; Gonzales *et al.* 2009). In general, all brachyuran megalopae were identified to species, but the zoeae were not (due to the nature of the keys). All Green Crab zoeae and megalopae were separated and both the

Green Crab larval fraction and the rest of the sample were preserved in 70% ethanol for archiving.

Table 3. Locations of the plankton tow stations used throughout the experimental study.

Sampling site	Station number	Latitude	Longitude
Refuge Island	1	49° 01.399’	125° 18.182’
	2	49° 01.387’	125° 18.427’
	3	49° 01.332’	125° 18.809’
	4	49° 01.452’	125° 19.306’
	5	49° 01.634’	125° 19.371’
Sechart	6	48° 56.968’	125° 15.141’
	7	48° 57.001’	125° 15.332’
	8	48° 57.159’	125° 15.658’
	9	48° 57.274’	125° 15.325’
	10	48° 57.267’	125° 15.368’

3.2.3. Shellfish Study Organisms and Environmental Data

The potential for NIS entrainment on cultured shellfish was examined using three species of bivalves: Pacific Oyster, Manila Clam, and California Mussel (*Mytilus californianus*). The industry-standard, suspended-culture practices for the Pacific Oyster (tray) and California Mussel (bag/sock) were replicated, while the harvest practice associated with Manila Clams (bag storage in intertidal) was replicated for the experimental study. The California Mussel is not an aquaculture product *per se*, but is a sentinel species originally used in the CFIA’s biotoxin monitoring program. The mussels were transported from the west coast of Vancouver Island, where they were harvested, to monitoring sites along the south coast of British Columbia (Figure 1). Although the mussel species studied here is not the commonly cultured species in BC (*i.e. M. edulis/M. galloprovincialis*), the culture practices followed mimic those used by the industry and the matrix structure created between individuals by the byssal strands is also similar amongst all mussel species; therefore results reflect the potential of all mussels as an NIS vector. These study species will be referred to as oysters, clams, and mussels, respectively.

Shellfish sampling occurred every four weeks from August 15, 2011 through November 7, 2011 and from March 5, 2012 through December 10, 2012. During the winter of 2011–2012, sampling trips occurred approximately every eight weeks (*i.e.* sampling occurred the week of January 9, 2012). Oysters and mussels were out-planted in July 2011, November 2011, and June 2012 (Table 4). Due to space limitation, it was not possible to out-plant enough oysters and mussels for the entire study period. Because of the nature of the sequential sampling (*e.g.* every four weeks) of oysters and mussels, the samples were left in the field for a minimum of four weeks to a maximum of 32 weeks (Table 4). Clams were out-planted every sampling trip and remained on the shore for three to five days (see Section 3.2.3.3 for details).

Starting in October 2011, environmental data loggers (DST CT, Starr oddi, Iceland) were placed on a suspended oyster tray, a mussel bag, and a concrete block beside the clam drop-off location at each site; the loggers were replaced regularly throughout the study. The loggers recorded water temperature (°C) and conductivity (salinity, PSU) every 0.5 hour. Environmental data were collected in order to ensure that the field sites chosen and the conditions the shellfish were exposed to fell within the environmental tolerance range of the Green Crab during its entire lifecycle.

3.2.3.1. Oysters

Oysters were grown by, and purchased directly from, a commercial grower in Barkley Sound. They were 76–100 mm (3–4 inch) in shell height (commonly referred to as “smalls” by the industry). Twenty-five oysters were placed in commercial suspended oyster trays (L x W x H: 61 x 61 x 21 cm) which were hung in stacks of three at a depth of 3 m, mimicking commercial suspended tray culture for oysters. Prior to out-planting, each oyster was hand scrubbed and cleaned of all visible epibionts. During sampling, three replicate suspended oyster trays were collected from both sites. One tray was considered a replicate or sample of oysters from collection through to processing and analysis.

3.2.3.2. Mussels

The California Mussels were harvested from an area in Clayoquot Sound near Tofino (DFO Statistical Area 24) using the same contractor and methods as the CFIA used for their biotoxin monitoring program. Twenty mussels were placed in 20-mm mesh plastic (Vexar™) bags and stored in Clayoquot Sound until collection. We did not rinse or clean the experimental mussel samples prior to out-planting in the field study since we wanted to receive them in the same state as the CFIA would receive them in, given their ITC permit conditions and the use of the same contractor. When the mussel samples were collected from the contractor in Clayoquot Sound, five mussel bags were immediately bagged and placed in a fish tote for transport to the PBS during each out-planting event. This was done in order to determine what may have been associated with the mussels prior to out-planting at the experimental sites. The samples were processed, rinsed, and analyzed, as per methodology described in Section 3.2.5, after each out-planting event. Each of these sets of five mussel bags is termed “mussel controls” in the following text with the site description being “Tofino”. The rest of the mussel bags (samples) were hung at a depth of 1.5 m at both of the experimental sites in Barkley Sound during each out-planting event.

3.2.3.3. Clams

Clams were purchased from a local shellfish processor several days in advance of each sampling trip and were held in a flow-through seawater system at the PBS. These clams were cleaned prior to purchase: the seawater at the Station is sand-filtered and UV-sterilized and therefore it is highly unlikely they had any organisms on them before they were transported to the study sites. During each sampling event, five 13.6-kg (30-lb) bags of clams were laid in the mid-to-high intertidal zone at both sites. The bags, similar to onion sacs, were made of plastic mesh (mesh size ranged from 5 to 25 mm) and were similar to those used by commercial harvesters. The five bags were tied together with at least 3 m separating them. The locations where these bags were laid were geo-referenced using GPS during the first sampling trip in July 2011 and placed in the same spot (-2.5 to -1.0 m chart datum) during each subsequent sampling trip. The clams were left on the beach for a minimum of 60 to a maximum of 90 hours (3–5 days). Clam bags were collected at low tide whenever possible, each bag being in less than 0.75 m of water when collected with the exception of the November 2011 sampling event, when no daylight low tides occurred. Clams were out-planted during each sampling event except June 2012 since clams were not available from the processor or harvesters due to biotoxin closures. The wet-storage process was meant to mimic industry practices of both cultured and wild-harvested intertidal clams where dug clams are often stored in the intertidal zone for a period before enough are collected for transport to the processing plant. One 30-lb bag of clams was considered to be one replicate or sample from collection through to processing and analysis.

Table 4. Number of shellfish samples out-planted during the experimental study, as well as the minimum and maximum amount of time (weeks) samples were left in the field (extra samples were out-planted at Sechart to account for any potential sample losses).*

Outplant date		Oysters	Mussels
July 2011	No. samples	12	20
	Min. time (weeks)	4	4
	Max. time (weeks)	14	14
November 2011	No. samples	18	30
	Min. time (weeks)	8	8
	Max. time (weeks)	32	32
June 2012	No. samples	15	25*
	Min. time (weeks)	4	4
	Max. time (weeks)	22	22
Total no. samples per site		45	75

3.2.4. Shellfish Sample Collection

A total of 383 shellfish samples were collected, processed, and analyzed for the presence of Green Crabs and other NIS (Table 5). Several samples were lost from the Sechart site including one mussel sample from both of the July 2011 and June 2012 out-plant periods and two from the November 2011 out-plant period.

Table 5. Numbers of shellfish samples collected and processed after out-planting.

Shellfish	Refuge Island	Sechart	Total
Oysters	45	45	90
Mussels	75	69	144
Clams	75	74	149
Total	195	188	383

3.2.4.1. Mussel, Oyster, and Clam Collection

Each bag of mussels or tray of oysters was individually collected and immediately double or triple bagged in 0.08-mm (3-mil) thick, clear-plastic bags and tied closed (the oysters were individually handled and inspected for visible epibionts prior to packaging for transport, not simply poured into each bag from each tray). This prevented any leakage of water or loss of organisms during transport. The shellfish were then placed in a large fish tote for transport to the PBS and kept refrigerated at 4°C for up to four days before rinsing began. Throughout the study, most of the organisms associated with the shellfish that were visible to the naked eye were still alive when the samples were rinsed.

3.2.4.2. Tunicate Collection

When each mussel bag or suspended tray of oysters was hauled and packaged for transport it was inspected for tunicates. When large colonies (≥3 cm diameter) or solitary tunicates were found on the shellfish or mussel bags, a photograph of the colony or individual was taken, then a piece of the colony or the individual was removed and placed in a relaxant for 1–5 hours (Carlton 2007; H. Gartner, Royal BC Museum, Victoria, BC, personal communication). The relaxant solution consisted of magnesium sulphate in seawater (65–70 g L^{-1}) and was refreshed

as needed. After relaxation, the tunicates were fixed in cold, buffered 10% formalin (Carlton 2007; H. Gartner, personal communication). For *Didemnum* and *Tridemnum* spp. a second sample was fixed in 95% ethanol in order to preserve the spicules, which help with some species' identification (Carlton 2007; Gartner 2011). Whenever possible these samples were identified to species using taxonomic keys (Carlton 2007; Gartner 2011). After a tunicate sample was processed it was stored in 70% ethanol for archiving. Tunicate collection began in November 2011 and continued for the duration of the study.

3.2.5. Shellfish Sample Processing and Analysis

3.2.5.1. Shellfish Sample Processing

In the laboratory, each sample of shellfish was emptied onto a 7.5-mm sieve and rinsed with freshwater until all visible debris and epibionts were removed. The rinsate was then passed through a 0.5-mm sieve. Sieves were thoroughly rinsed between each sample to avoid cross contamination. All mobile fauna, large masses of tunicates (≥3 cm), and flora of interest (*e.g.* NIS) from the >7.5-mm fraction were retained and preserved (frozen) for later identification. The 7.5–0.5-mm fraction was fixed in either 3.7% buffered formalin or 95% ethanol.

3.2.5.2. Shellfish Sample Analysis

Each 7.5–0.5-mm shellfish rinsing fraction was inspected, in its entirety, using the same methods as described in plankton sample analysis (see Section 3.2.2.2). The search for known NIS was not exhaustive; the focus was on known NIS crab, tunicate, and bryozoan species. All brachuryan and anomuran crustaceans (larvae, juveniles, and adults) were separated and identified to the level of family, with the exception of hermit crabs (Families: Paguridae, Diogenidae, and Parapaguridae), using various taxonomic keys and references (Schmitt 1921; Hart 1935; Shanks 2001; Carlton 2007; Rice and Tsukimura 2007; Gonzales *et al.* 2009). If a specimen was suspected of being estuarine or from the genera *Cancer*, *Hemigrapsus*, or *Carcinus* it was identified to species whenever possible. Other organisms of interest that were also identified to species were NIS tunicates (*e.g. B. violaceus*, *B. schlosseri*, and *D. vexillum*) and bryozoans (*S. japonica*, *C. pallasiana*) as well as any easily identifiable non-indigenous algal species (*e.g. Sargassum muticum*) (Carlton 2007; Gartner 2011). The >7.5-mm shellfish rinsing samples were processed using the same species identification criteria as the 7.5–0.5-mm samples.

3.3. RESULTS: EXPERIMENTAL FIELD STUDY

3.3.1. Study Sites: Environmental Data

The range of salinity and water temperature at both field sites during much of the study period (Table 6) fell within the tolerance ranges of most of the Green Crab's lifecycle (refer to Section 2.4.1.4 for details on tolerance ranges). Exceptions were the ranges of both salinity and temperature that the clams were exposed to at both sites, particularly during the winter months. The clams at both sites were exposed to very low salinities and temperatures at low tide during winter months; the intertidal zone at both sites was likely inhospitable to juvenile and adult Green Crabs during this period. In general, the environmental conditions the clams were exposed to were beyond the extremes of both salinity and temperature tolerated by the zoeal stage. The low ends of the ranges that fall below the megalopa, juvenile, and adult Green Crab tolerance levels typically occur for very short periods of time during the late fall/winter (1–2 hours), with the exception of two large freshet events (November 27, 2011 and January 4–6, 2012); the data associated with these events are denoted with an asterisk in Table 6. The data associated with all three shellfish species at Refuge Island during the January 4–6, 2012 event reflect an exposure to a very large freshet over several days and, upon further inspection of the

data, it seems as though the oysters at Refuge Island were hung below the pycnocline while the mussels were not. The general trends of salinity and temperature exposure of each type of shellfish at the experimental field sites is described in the subsequent sections.

Table 6. Salinity and water temperature ranges at each of the experimental field sites from October 2011 through November 2012 (indicates that the low end of the range is attributable to a large winter freshet).*

		Site	
Environmental data	Shellfish	Refuge	Sechart
Salinity (PSU)	Clams	2–32	2–24
	Mussels	*2–30	*12–30
	Oysters	*7–30	15–32
Temperature (°C)	Clams	-2–26	-1–24
	Mussels	4–18	5–21
	Oysters	6–18	6–20

3.3.1.1. Mussels

Mussels were exposed to variable salinity during the study period. Mussels at Refuge Island were typically exposed to low salinity events more frequently than those at Sechart (Figure 5a). The level of low salinity exposure during these events was also more severe at Refuge Island, often approaching full freshwater. In contrast to differences in salinity exposure, the mussels at both sites were exposed to mostly similar water temperatures from October 2011 through June 2012 (Figure 6a). There was a slight seasonal difference in water temperature between the sites, mussels at Refuge Island being exposed to slightly higher and more variable water temperatures between the months of April and June 2012.

3.3.1.2. Oysters

Oysters were also exposed to low salinity conditions; however, they were never exposed to full freshwater (Figure 5b). Similar to the mussels at Refuge Island, the oysters there were typically exposed to lower salinities than those at Sechart. Oysters at both sites were exposed to mostly similar water temperatures (Figure 6b). Much like the mussels, the oysters at Refuge Island were exposed to more variable and slightly higher temperatures in the spring and summer compared to those at Sechart.

3.3.1.3. Clams

The sites where clams were out-planted were exposed to extreme variability of both salinity and water temperature (Figures 5c and 6c), typical of intertidal areas.

3.3.2. Presence of Green Crabs

3.3.2.1. Adults and Juveniles

Adult Green Crabs were found at both sites throughout the experimental period but were generally more abundant at Refuge Island (Figure 7 and Figure 9b). Both the Fukui and minnow traps consistently caught individuals greater than 30-mm CW, but the dominant size classes varied between trap types (Figure 8). Fukui traps had a two to ten fold higher CPUE than the minnow traps and were dominated by individuals that ranged between 41- and 70-mm CW, regardless of sex or site, whereas the minnow traps were dominated by individuals that ranged between 41- and 60-mm CW at both sites (Figure 8). Results from the beach walks also followed the same trend for smaller individuals (<30-mm CW), with greater abundances at Refuge Island than Sechart (Figure 7c and Figure 9a). The smaller individuals, however, were not consistently found at each sampling event or site. The beach walks at Refuge Island

showed a prevalence of Green Crabs between 11- and 50-mm CW, whereas at Sechart sightings during beach walks were less common (Figure 9a).

3.3.2.2. Larvae

Green Crab zoeal stages were found during a large part of the study period, particularly at Refuge Island where they were found during each sampling event except October 2011 and January, October, and November 2012 (Figure 10). They were detected only in very low densities at Sechart. They were present only during July 2011, early and late May, July, and August 2012 at this site. At Refuge Island, the density of zoeal stages peaked during early May 2012, secondary smaller peaks occurring in July 2011 and July 2012. The megalopal stage was far less abundant and appeared only in late summer and fall (Table 7). Megalopae were found in August 2011 and August 2012 at Refuge Island, while they were located at Sechart in August 2011 and September and October 2012.

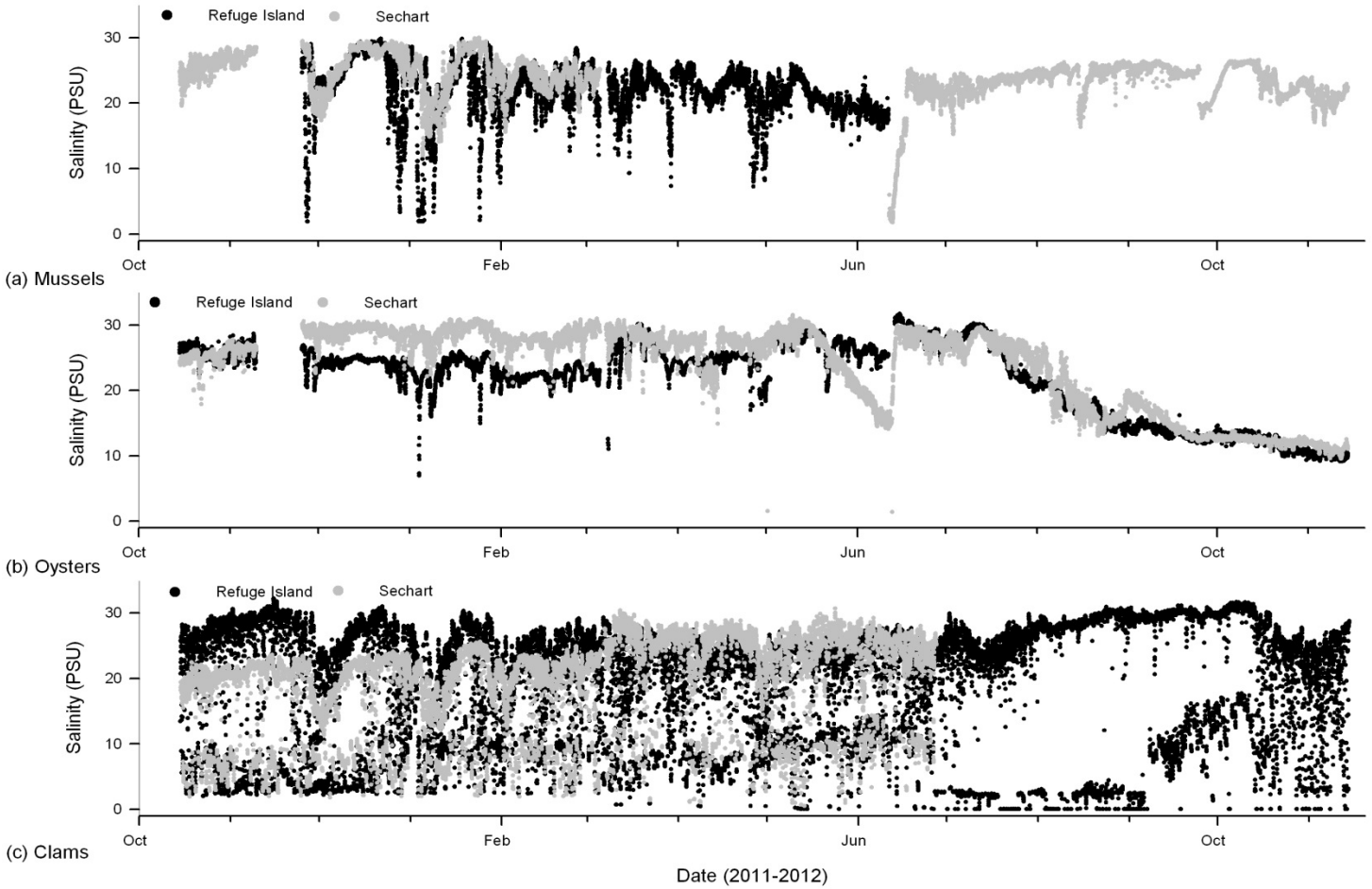


Figure 5. Seawater salinity exposure (PSU) of (a) mussels, (b) oysters, and (c) clams at the experimental field sites at Refuge Island (black) and Sechart (grey) from October 2011 through November 2012.

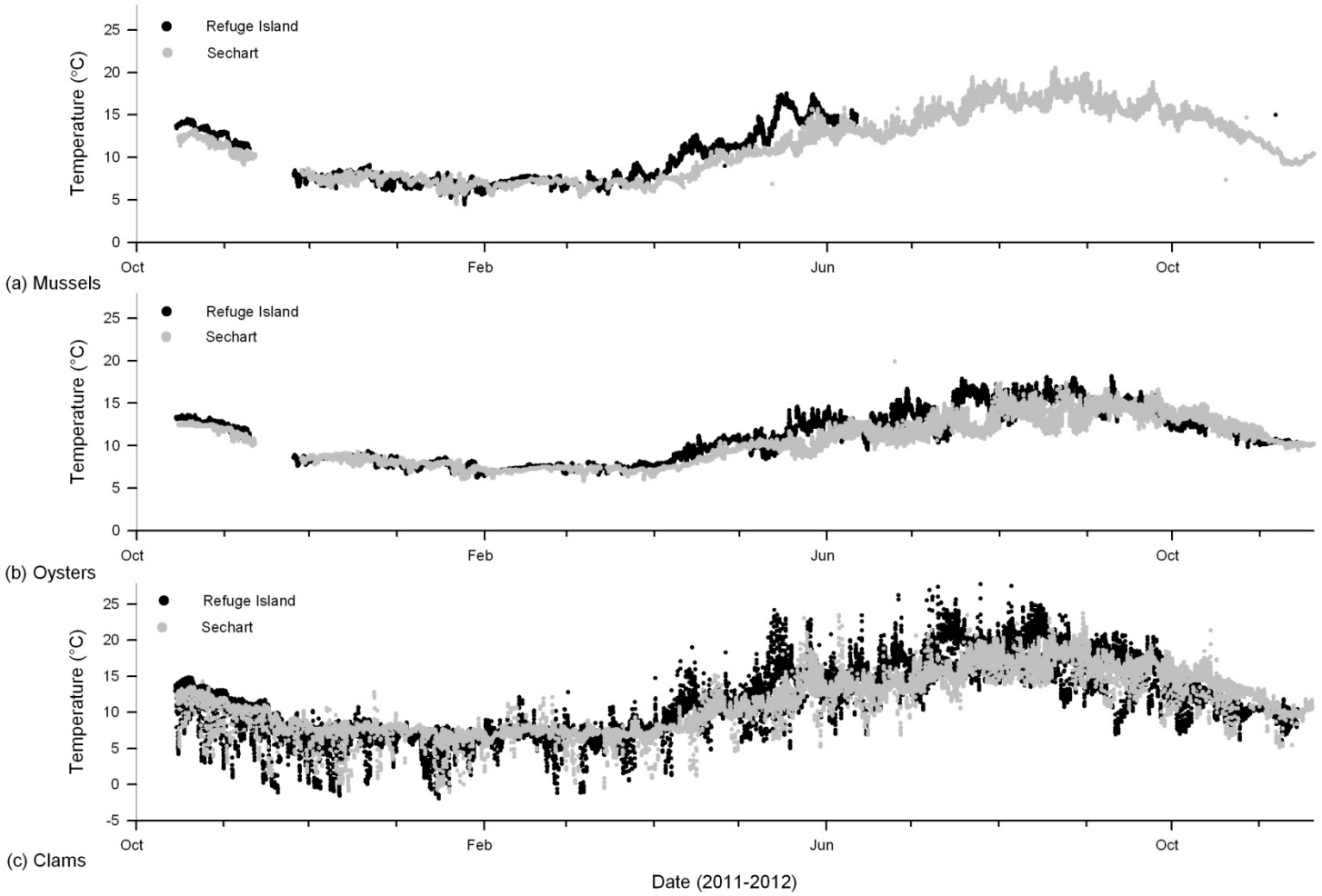


Figure 6. Seawater temperature exposure (ºC) of (a) mussels, (b) oysters, and (c) clams at the experimental field sites at Refuge Island (black) and Sechart (grey) from October 2011 through November 2012.

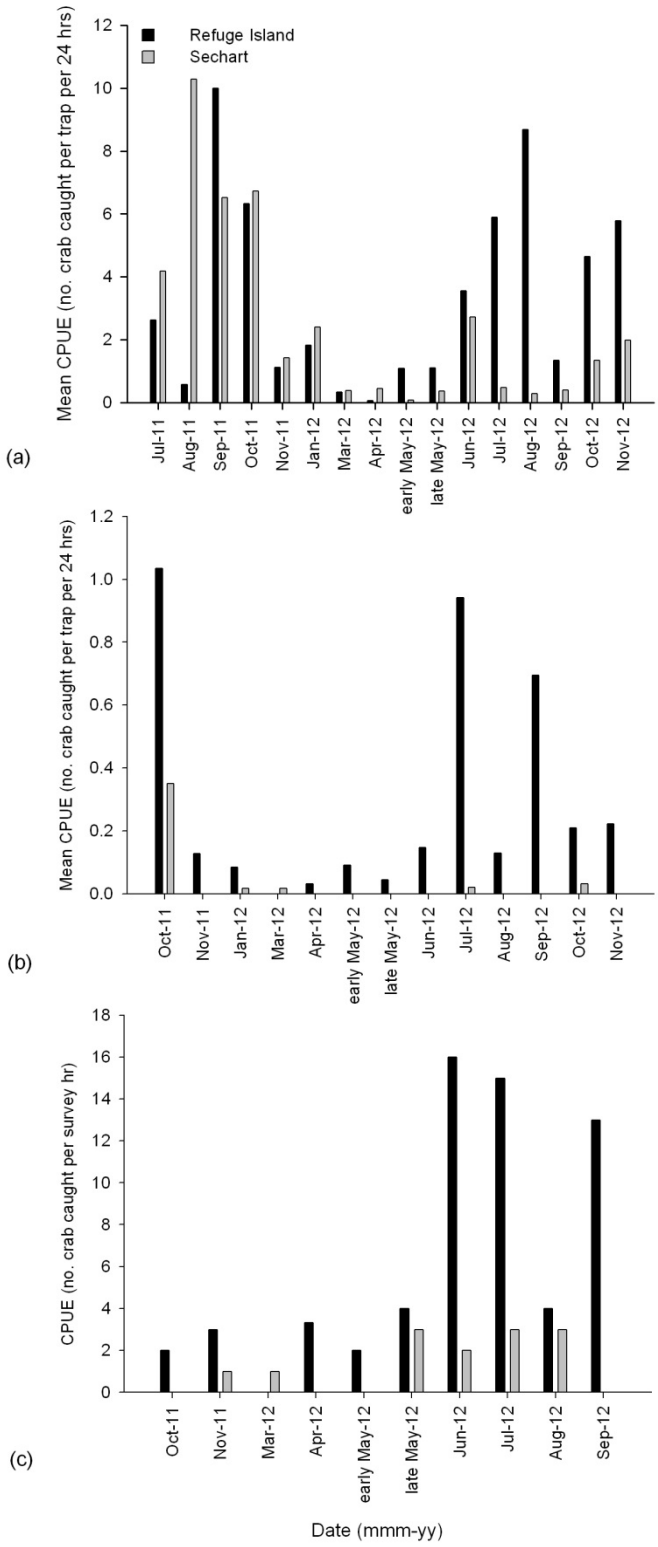


Figure 7. Mean catch per unit effort (CPUE) of Green Crabs (Carcinus maenas) at the experimental field sites at Refuge Island (black) and Sechart (grey) using (a) Fukui traps (n=2–18/event) and (b) minnow traps (n=10–12/event) as well as the (c) C. maenas CPUE of beach walk surveys at both sites.

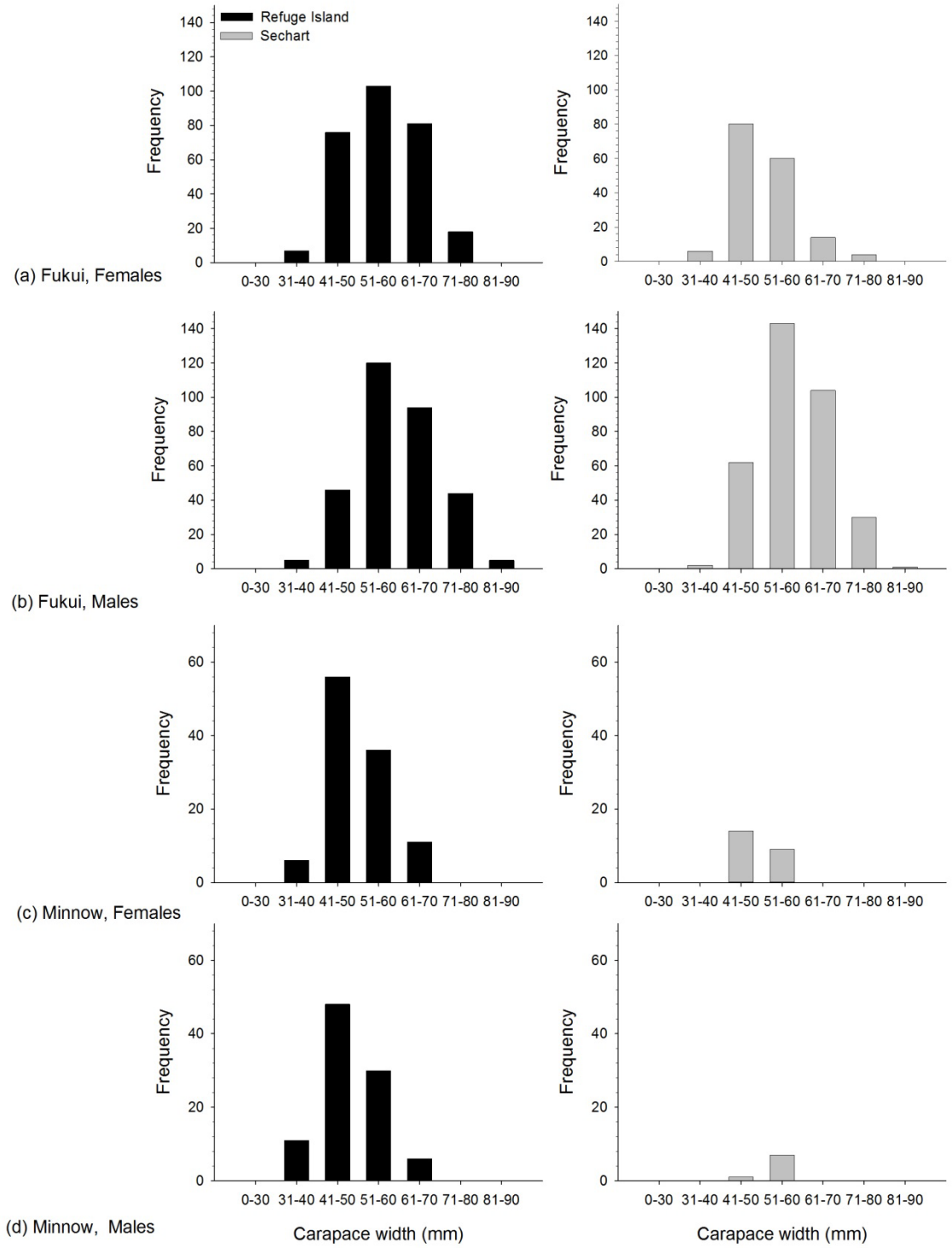


Figure 8. Size frequency distribution of male and female Green Crabs (Carcinus maenas) caught using (a,b) Fukui traps and (c,d) minnow traps at both experimental study sites, Refuge Island (black) and Sechart (grey).

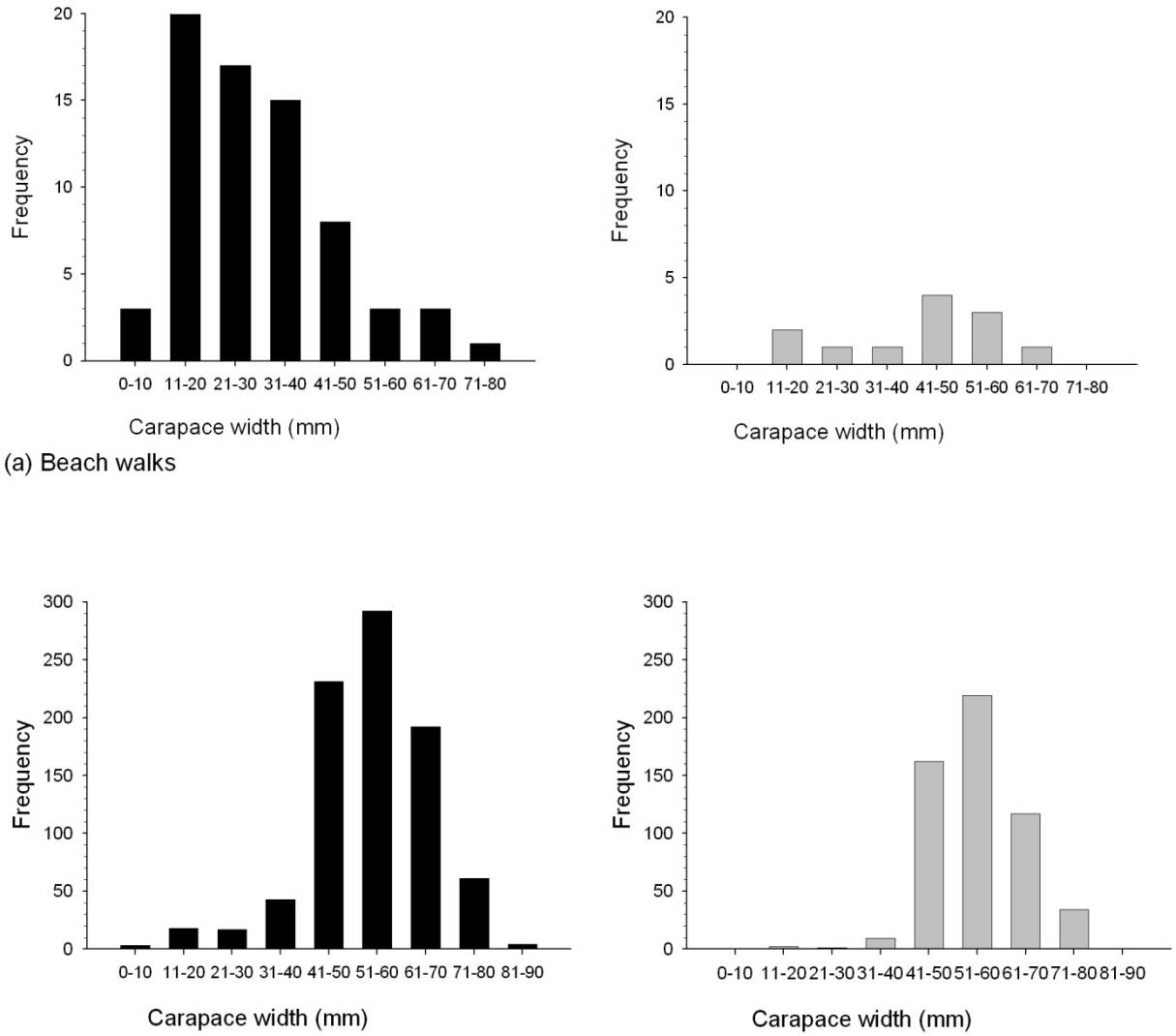


Figure 9. Size frequency distribution of Green Crabs (Carcinus maenas) at both experimental field sites, Refuge Island (black) and Sechart (grey), caught during (a) beach walk surveys and (b) the study total (i.e. all trapping and collection methods combined).

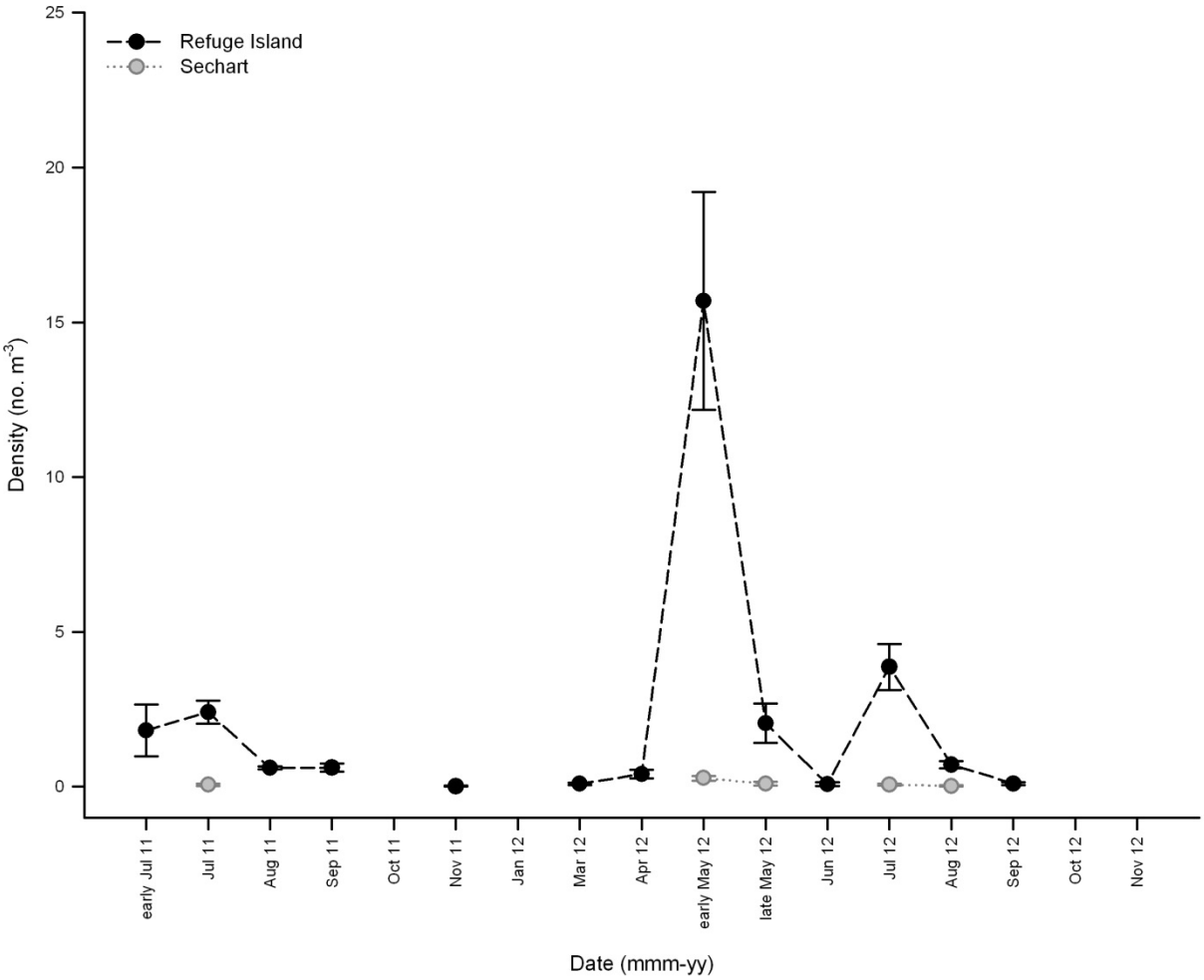


Figure 10. Mean density (± I S.E.) of Green Crab (Carcinus maenas) zoeae at both experimental field sites, Refuge Island (black) and Sechart (grey); n=5 per sampling event per site. For ease of interpretation, values of zero were removed from the data series, therefore dates without symbols represent a value of zero and not a lack of data.

Table 7. Number of Green Crab (Carcinus maenas) megalopae found in plankton tows at both experimental field sites, Refuge Island and Sechart, from July 2011 through November 2012.

	Site		
Date	Refuge Island	Sechart	Total
Jul-11	-	-	-
Aug-11	1	1	2
Sep-11	-	-	-
Oct-11	-	-	-
Nov-11	-	-	-
Jan-12	-	-	-
Mar-12	-	-	-
Apr-12	-	-	-
early May-12	-	-	-
late May-12	-	-	-
Jun-12	-	-	-
Jul-12	-	-	-
Aug-12	2	-	2
Sep-12	-	1	1
Oct-12	-	1	1
Nov-12	-	-	-
Total	3	3	6

3.3.3. Shellfish Sample Analysis

Juvenile and megalop stages of the Green Crab were found on all three species of shellfish sampled (Table 8). Megalopae were found in a mussel control (*i.e.* not out-planted in study) from June 2012 and a mussel sample collected from Sechart in September 2011. At Refuge Island, juvenile Green Crabs were found on all the shellfish species used in this study. One juvenile was found in an August 2012 mussel sample, while two were found in suspended oyster samples (one in August 2011, the other in August 2012). Juvenile Green Crabs were also found in clam samples at Refuge Island, two in October 2012 and one in November 2012. The juveniles found on the shellfish ranged in size from 1.5-mm to 13-mm CW. The presence of megalopal and juvenile Green Crabs (J1–J11, based on Mohamedeen and Hartnoll 1989; Silva *et al.* 2006) at Refuge Island throughout most of the study period was confirmed when the data from all the sampling methods were combined (Table 9). Megalopal and juvenile (J1–J11) Green Crabs were not found during July, September, and November 2011 and January and March 2012 sampling events, but were found during all other sample months. The presence of these stages was not as regular at Sechart, neither being detected for much of the study period (July 2011, October 2011, January through June 2012, and November 2012).

Non-indigenous tunicates and bryozoans were found, but associated only with the mussel and oyster samples. This is likely due to the fact that clams were cleaned prior to deployment and to the very short storage period (days) the clams were in the intertidal zone where tunicates are uncommon. *Botrylloides violaceus* was found in 43 mussel and nine oyster samples (Table 10). This species was relatively abundant at both study sites and was found in approximately 30% of the mussel samples and 4 and 15% of oyster samples at Refuge Island and Sechart, respectively. *Botryllus schlosseri* was found in three mussel samples and in no oyster samples (Table 10). It was found at both sites, representing 2.7 and 1.3% of the total mussel samples at Refuge Island and Sechart, respectively. The non-indigenous bryozoan *S. japonica* was found in 168 samples, of which 102 were mussel samples, 64 were oyster samples, and two were mussel controls (Table 11). This represented about 50% of both the mussel and oyster

samples at Refuge Island and 91% of the mussel and oyster samples at the Sechart site. Another non-indigenous bryozoan, *C. pallasiana*, was found on 48 samples at the Sechart site, of which 16 were mussels and 32 were oysters (Table 11). It was present in 36 and 46% of the mussel and oyster samples, respectively, at Sechart. Many other organisms, both mobile and sessile, also were found associated with the shellfish samples, particularly those of mussel and oyster. The search for NIS in these samples was not exhaustive and although many of these species were native the exact ratio of native versus NIS species in the 0.5–7.5-mm fraction is unknown.

*Table 8. Number, life stage, and size of Green Crabs (Carcinus maenas) found on the experimental shellfish samples: * the size classes (i.e. J1 and J2) are based on the relative size frequency distribution in Silva et al. (2006), J1–J2 being recruits and J3–J5 early juveniles.*

Sieve fraction (mm)	Collection date (dd-mmm-yy)	Sample	Site	No. found	Life stage	CW (mm)	Comment
>0.5 – 7.5	16-Sep-11	Mussels	Sechart	1	megalop	NA	
>0.5 – 7.5	11-Jun-12	Mussels	Control	1	megalop	NA	Not out-planted
>0.5 – 7.5	19-Aug-11	Oysters	Refuge	1	juvenile	2.5	J2*
>0.5 – 7.5	22-Aug-12	Oysters	Refuge	1	juvenile	1.5	J1*
>7.5	22-Aug-12	Mussels	Refuge	1	juvenile	13	
>7.5	18-Oct-12	Clams	Refuge	2	juvenile	7.6, 6.5	Found alive in two independent samples: J5/J6*
>7.5	16-Nov-12	Clams	Refuge	1	juvenile	5.1	Alive: J4*

Table 9. Presence of juvenile (<20-mm CW) and megalopal stages of the Green Crab (Carcinus maenas) found between July 2011 and November 2012 at Refuge Island and Sechart using all the experimental observation methods. The shaded areas represent dates on which neither stage was present using any of the methods (X = presence; - = none observed; NA = not applicable; NP = method not possible).

	Refuge Island			Sechart		
Date (mmm-yy)	Beach walk	Plankton	Shellfish	Beach walk	Plankton	Shellfish
Jul-11	NA	-	-	NA	-	-
Aug-11	NA	X	X	NA	X	-
Sep-11	NA	-	-	NA	-	X
Oct-11	X	-	-	-	-	-
Nov-11	-	-	-	X	-	-
Jan-12	NP	-	-	NP	-	-
Mar-12	-	-	-	-	-	-
Apr-12	X	-	-	-	-	-
early May-12	X	-	-	-	-	-
late May-12	X	-	-	-	-	-
Jun-12	X	-	-	-	-	-
Jul-12	X	-	-	X	-	-
Aug-12	X	X	X	X	-	-
Sep-12	X	-	-	-	X	-
Oct-12	NP	-	X	NP	X	-
Nov-12	NP	-	X	NP	-	-

Table 10. Number and percentage of experimental mussel and oyster samples with the invasive tunicates Botrylloides violaceus and Botryllus schlosseri found at the experimental sites at Refuge Island and Sechart from November 2011 through November 2012.

Shellfish	Collection date	*B. violaceus* Site			*B. schlosseri* Site		
	(mmm-yy)	Refuge Island	Sechart	Total	Refuge Island	Sechart	Total
Mussels	Nov-11	4	5	9	-	-	-
	Mar-12	-	-	-	-	-	-
	Apr-12	-	-	-	-	-	-
	early May-12	-	-	-	-	-	-
	late May-12	-	1	1	-	-	-
	Jun-12	-	-	-	-	-	-
	Jul-12	4	-	4	1	-	1
	Aug-12	4	-	4	-	-	-
	Sep-12	6	3	9	1	1	2
	Oct-12	5	4	9	-	-	-
	Nov-12	-	7	7	-	-	-
	Total	23	20	43	2	1	3
	% of total samples	30.7	29.0	29.9	2.7	1.3	2.1
Oysters	Nov-11	2	3	5	-	-	-
	Mar-12	-	-	-	-	-	-
	Apr-12	-	-	-	-	-	-
	early May-12	-	-	-	-	-	-
	late May-12	-	-	-	-	-	-
	Jun-12	-	-	-	-	-	-
	Jul-12	-	-	-	-	-	-
	Aug-12	-	3	3	-	-	-
	Sep-12	-	-	-	-	-	-
	Oct-12	-	-	-	-	-	-
	Nov-12	-	1	1	-	-	-
	Total	2	7	9	-	-	-
	% of total samples	4.4	15.6	10.0	-	-	-

Table 11. Number and percentage of experimental mussel and oyster samples with the non-indigenous bryozoans Schizoporella japonica and Cryptosula pallasiana found at the experimental sites at Refuge Island and Sechart and the control site at Tofino from November 2011 through November 2012.

Shellfish	Collection date	*S. japonica* Site			*C. pallasiana* Site		
	(mmm-yy)	Refuge Island	Sechart	Tofino	Refuge Island	Sechart	Tofino
Mussels	Aug-11	3	1	-	-	-	-
	Sep-11	3	4	-	-	-	-
	Oct-11	4	4	-	-	2	-
	Nov-11	1	5	-	-	1	-
	Jan-12	1	1	-	-	-	-
	Mar-12	3	4	-	-	1	-
	Apr-12	1	4	-	-	-	-
	early May-12	4	5	-	-	-	-
	late May-12	-	4	-	-	-	-
	Jun-12	1	5	-	-	2	-
	Jul-12	-	2	-	-	1	-
	Aug-12	4	5	-	-	-	-
	Sep-12	5	5	-	-	3	-
	Oct-12	5	6	-	-	3	-
	Nov-12	4	8	-	-	3	-
	Total	39	63	-	-	16	-
	% of total samples	52.0	91.3	-	-	35.6	-
Mussel control	Jul-11	-	-	-	-	-	-
	Nov-11	-	-	-	-	-	-
	Jun-12	-	-	2	-	-	-
	Total	-	-	2	-	-	-
	% of total samples	-	-	13.3	-	-	-
Oysters	Aug-11	2	2	-	-	-	-
	Sep-11	2	3	-	-	1	-
	Oct-11	3	3	-	-	4	-
	Nov-11	3	3	-	-	3	-
	Jan-12	-	3	-	-	-	-
	Mar-12	2	3	-	-	-	-
	Apr-12	-	1	-	-	-	-
	early May-12	2	3	-	-	-	-
	late May-12	2	3	-	-	3	-
	Jun-12	2	4	-	-	4	-
	Jul-12	-	1	-	-	1	-
	Aug-12	-	3	-	-	2	-
	Sep-12	1	3	-	-	1	-
	Oct-12	1	3	-	-	6	-
	Nov-12	3	3	-	-	7	-
	Total	23	41	-	-	32	-
	% of total samples	51.1	91.1	-	-	46.4	-

3.4. METHODOLOGY: PROCESSOR STUDY

3.4.1. Shellfish Sample Collection, Processing, and Analyses

In addition to the experimental field study, we were interested in examining shellfish collected from the west coast of Vancouver Island directly from harvesters and processors to determine if commercially harvested shellfish are potential vectors for Green Crabs or other NIS. These shellfish were harvested from sites within areas that encompass Effingham Inlet in Barkley Sound (DFO Statistical Area 23-6), Lemmens Inlet in Clayoqout Sound (DFO Statistical Area 24-9), and Bligh Island in Nootka Sound (DFO Statistical Area 25-6). Although Green Crabs were found in all these larger DFO Statistical Areas in the past (Gillespie *et al.* 2007), it was not known whether they were present at any of the harvest sites. We did not sample for any life stage of the Green Crab at these sites during the time of harvest, nor were there any other data collected during the time period of this project by other DFO field studies. A total of 35 clam and 75 oyster samples were purchased from either a harvester or processor between August and December 2012 (Table 12). Individual samples consisted of 100 oysters or 13.6-kg (30-lb) of clams. It is important to note that oysters collected for this part of the project were not cultured in suspended trays as in the experimental study, but rather on long lines or beach cultured.

The shellfish samples were processed and analyzed using the same rinsing methods described in Section 3.2.5. Any tunicates that were found that were well hydrated on the shellfish were fixed and preserved using the same methods described in Section 3.2.4.2. Although tunicates were found on many of the oysters cultured on long lines, they were often not well hydrated, making identification difficult. Attempts were made to re-hydrate them by refreshing them with the relaxant over several hours, but some of the tunicates did not recover from transport and were therefore not identifiable.

3.5. RESULTS: PROCESSOR STUDY

3.5.1. Shellfish Sample Analysis

Green Crabs were not found on any of the shellfish samples that were collected from the shellfish processing plants or growers. However, various non-indigenous tunicates and bryozoans were discovered. A total of 21 oyster samples (53% of total samples) in area 24-9 had the non-indigenous tunicate *B. violaceus* growing on them (Table 13). One of them was harvested in an area that encompasses Effingham Inlet in Barkley Sound (DFO Statistical Area 23-6) and was beach-cultured, while the rest were harvested from an area that encompasses Lemmens Inlet in Clayoquot Sound (DFO Statistical Area 24-9) and were long-line cultured. *Botryllus schlosseri* was also found on oysters that were long-line cultured in area 24-9 (Table 13); a total of three oyster samples had this invasive tunicate growing on them, which represents 7.5% of the samples collected. Unlike in the experimental study, we also found the invasive tunicate *Didemnum vexillum* growing on shellfish (Table 13). A total of 35 oyster samples harvested in area 24-9 had this species which represents 87.5% of all long-line cultured oysters sampled. The non-indigenous bryozoan *S. japonica* was also found on oysters (Table 14). Five beach-cultured samples (50.0% of total samples) from DFO Statistical Area 23-6 had this species, while 35 samples (87.5% of total samples) that were long-line cultured in DFO statistical 24-9 had this species. The non-indigenous bryozoan *C. pallasiana* was not found on any of the shellfish collected from processing plants or growers. None of the 35 clam samples examined contained any of the NIS biofouling species that we investigated.

Table 12. Number of clam and oyster samples processed and analyzed during the processer study, showing the DFO Statistical Area they were collected from and culture method used to grow them.

		Culture method and DFO Statistical Area			
		Beach culture		Suspended long line	
Shellfish species	Harvest date	23-6	25-6	24-9	Total
Clams	20-Oct-12	-	5	-	5
	4-Nov-12	-	5	-	5
	19-Nov-12	-	5	-	5
	27-Nov-12	5	-	-	5
	2-Dec-12	-	5	-	5
	10-Dec-12	5	-	-	5
	15-Dec-12	-	5	-	5
	Total	10	25	-	35
Oysters	21-Aug-12	-	-	5	5
	4-Sep-12	-	-	5	5
	20-Oct-12	-	5	-	5
	30-Oct-12	-	-	5	5
	4-Nov-12	-	5	-	5
	8-Nov-12	-	-	5	5
	18-Nov-12	-	-	5	5
	19-Nov-12	-	5	-	5
	26-Nov-12	-	-	5	5
	27-Nov-12	5	-	-	5
	2-Dec-12	-	5	5	10
	10-Dec-12	5	-	5	10
	15-Dec-12	-	5	-	5
	Total	10	25	40	75

Table 13. Number and percentage of long-line cultured oyster samples (from west coast of Vancouver Island and collected from processing plants and growers) with invasive tunicates (Botrylloides violaceous, Botryllus schlosseri, Didemnum vexillum); one beach culture sample contained B. violaceus in area 23-6 harvested 28-Nov-12.

		Tunicate species		
Harvest area	Collection date	*B. violaceus*	*B. schlosseri*	*D. vexillum*
24-9	21-Aug-12	4	-	5
	4-Sep-12	4	0	5
	31-Oct-12	3	-	5
	9-Nov-12	2	-	5
	19-Nov-12		1	4
	27-Nov-12	5	1	4
	3-Dec-12	-	-	2
	12-Dec-12	3	1	5
	Total	21	3	35
	% of total samples	52.5	7.5	87.5

Table 14. Number and percentage of oyster samples with S. japonica collected at processing plants along the east coast of Vancouver Island.

Harvest area	Collection date	Culture type	Total
23-6	28-Nov-12	Beach	2
	12-Dec-12	Beach	3
23-6 Total			5
% of total samples			50.0
24-9	21-Aug-12	Long line	5
	04-Sep-12	Long line	5
	31-Oct-2012	Long line	5
	09-Nov-2012	Long line	5
	19-Nov-2012	Long line	5
	27-Nov-2012	Long line	3
	03-Dec-2012	Long line	5
	12-Dec-2012	Long line	2
24-9 Total			35
% of total samples			87.5

3.6. METHODOLOGY: EXTRAPOLATION OF TRANSPORT

The potential number of Green Crabs transported with cultured shellfish per year was calculated by multiplying the number of Green Crabs found per unit shellfish weight in the experimental study by the annual mean weight of each species of shellfish commercially cultured on the west coast of Vancouver Island destined for processing plants on the east coast (Table 15), based on a historical database of commercial shellfish landings. The historical data used in the extrapolations was tabulated by the British Columbia Ministry of the Environment (Province of British Columbia Annual Aquaculture Statistical Report Surveys). It is important to note that due to privacy restrictions there were limited data for commercial mussel culture (*i.e.* primarily 2004 and 2005 landings). We combined available data for oysters reported in dozens (intended for the half-shell market) and those reported in gallons (shucked product) to represent potential NIS movement associated with both aspects of commercial oyster culture in BC. Our conversion factors for a dozen and a gallon of experimental oysters were 1.341 kg dozen^{-1} and 2.375 kg

gallon^{-1}, respectively. Generally, oysters sold in dozens are either grown through benthic culture (beach) or in trays in suspended culture, while “shuckers” are generally long-line cultured (suspended) and the entrainment potential could differ between the two, but there was no way to differentiate, based on our data. The weight of mussels used in the extrapolation was based on experimental mussels (*M. californianus*), not cultured mussels (*M. edulis* and/or *M. galloprovincialis*) as in the historical data. The mean weight of 20 experimental mussels was 3.23 kg.

Since the majority of Green Crabs found in the experimental study were from Refuge Island, two extrapolations were made; one based on the results from Refuge Island alone and the other from the entire study, to provide a range of potential outcomes. In addition, two historical means for the weight of shellfish landed, one from 1991 to 2010 and the other from 2000 to 2010, were used in the extrapolations since Green Crabs were first reported from the west coast of Vancouver Island in 1999 (Gillespie *et al.* 2007) and thus only available to this invasion vector after this time.

3.7. RESULTS: EXTRAPOLATION OF TRANSPORT

The weight of oysters and clams exported from the west coast of Vancouver Island steadily increased from 1991 to 2005, then declined until 2010 (Figure 11). Annual means of the two time periods, 1991–2010 and 2000–2010, are reported in Table 15 and reflect the increased harvest of shellfish in the 2000s. Currently, there is limited commercial culture of mussels on the west coast of Vancouver Island and the transport of mussels used in the CFIA biotoxin monitoring program is not considered here. Our results show that commercial oyster movements have the greatest potential to move Green Crabs from the west to the east coast of Vancouver Island (Table 16). However, commercial clam and mussel culture still have some potential to move Green Crabs (Table 16). It is important to note that should the relative volume of commercial shellfish movements change, the calculations should be re-done.

*Table 15. Yearly mean (± 95% CI) weight of various cultured shellfish species exported from the west coast of Vancouver Island (DFO Statistical Areas 20-27) (data source: Province of British Columbia Annual Aquaculture Statistical Report Surveys). * the mussel expansion data is based on a two-year average, as it was the only data available due to privacy concerns, and is not the actual data reflecting the movement of mussels in the CFIA biotoxin monitoring program.*

	Shellfish (kg)		
Year range	Clams	Oysters	Mussels*
1991–2010	60,873 ± 21,195	210,167 ± 39,385	309 ± 425
2000–2010	85,463 ± 9,248	264,204 ± 14,460	562 ± 227

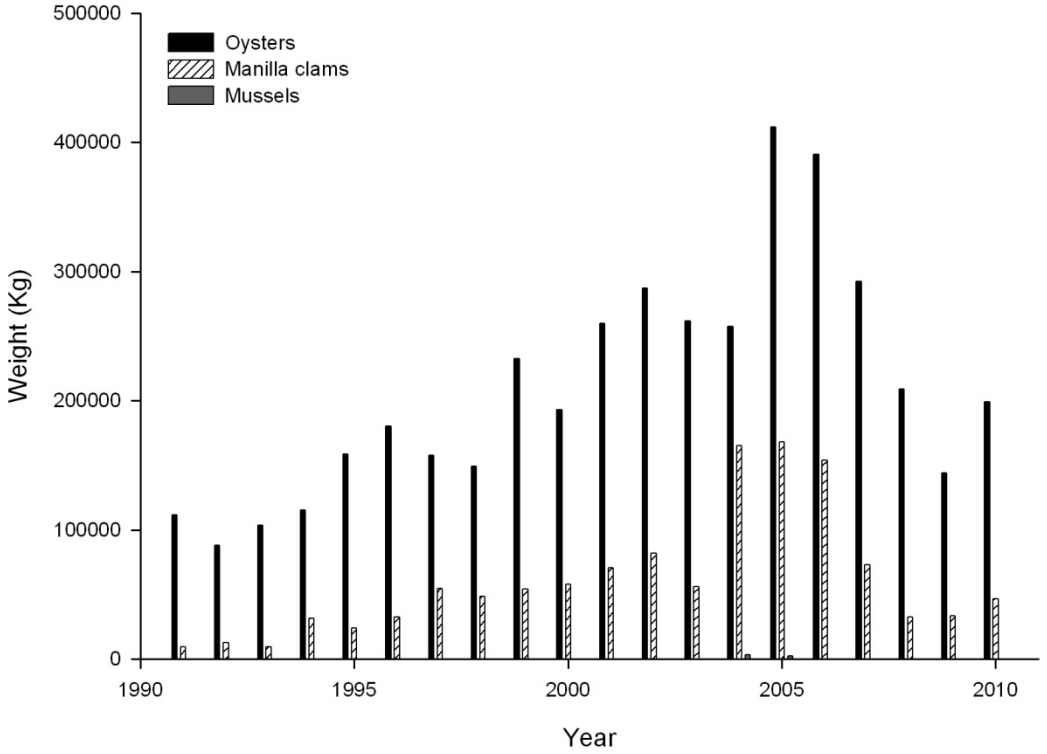


*Figure 11. Weight of various cultured shellfish species exported from the west coast of Vancouver Island (DFO Statistical Areas 20-27) from 1991 through 2010 (data source: Province of British Columbia Annual Aquaculture Statistical Report Surveys). * the mussel expansion data are based on a two-year average, as it was the only data available due to privacy concerns, and is not the actual data reflecting the movement of mussels in the CFIA biotoxin monitoring program.*

*Table 16. Potential number of entrained Green Crabs on various cultured shellfish species based on an algebraic expansion of the number found during the experimental study and the total mass of shellfish exported from the west coast of Vancouver Island between 2000 and 2010 (DFO Statistical Areas 20-27) (data source: Province of British Columbia Annual Aquaculture Statistical Report Surveys). * the mussel expansion data are based on a two-year average, as it was the only data available due to privacy concerns, and is not the actual data reflecting the movement of mussels in the CFIA biotoxin monitoring program.*

Source data	Shellfish	N	Weight of experimental samples (kg)	No. crabs found experimentally (inds kg^{-1})	No. of Green Crab potentially entrained
Refuge Island	Clams	75	1021	2.94E-3	3,000
	Oysters	45	126	1.59E-2	46,000
	Mussels*	75	242	4.13E-3	30
Refuge total					*49,000*
Whole Study	Clams	149	2028	1.48E-3	1,000
	Oysters	90	251	7.96E-3	23,000
	Mussels*	144	465	2.15E-3	10
Study total					*24,000*
Tofino	Mussel controls*	15	48	2.06E-2	6,000

3.8. DISCUSSION OF NIS STUDIES

3.8.1. Experimental and Processor Study

Our results demonstrate that several NIS, including European Green Crabs, can be entrained successfully with cultured shellfish. Green Crab megalopae or juveniles were found on all three of the shellfish species out-planted in the experimental study. However, Green Crabs were not found on any of the shellfish products collected from the processors or growers although other NIS were. This may be due to small sample sizes, the collection of shellfish from areas with potentially limited Green Crab populations, and/or effective rinsing measures employed by growers upon harvest. All the juvenile crabs found on the shellfish were under 30-mm CW (less than 1 year old; Berrill 1982; Mohamedeen and Hartnoll 1989; Silva *et al.* 2006). Since oysters and mussels were suspended in the water column, juveniles found on these species likely recruited as larvae while juveniles found on clams likely selected this habitat post-recruitment (microhabitat selection).

Green Crabs were not the only NIS found on cultured shellfish during this study. At least five different species of biofouling NIS were detected on the cultured shellfish, some of which were found in both the experimental and processor samples. The tunicates *B. schlosseri*, *B. violaceus*, and *D. vexillum* were all found on long-line cultured oysters purchased directly from processing plants, whereas only *B. schlosseri* and *B. violaceus* were noted on oysters and mussels in the experimental field study. The NIS bryozoan *S. japonica* was found on long-line cultured oysters purchased directly from processing plants, as well as on oysters and mussels in the experimental study. The bryozoan *C. palliasiana* was found only at the experimental Sechart site. Many other native organisms were also found associated with the shellfish; they included many species of seaweeds, as well as mobile and sessile fauna.

3.8.2. Entrainment of Green Crabs on Cultured Shellfish

In order for a species to become entrained, it must be present. The presence of adult Green Crabs (> 30-mm CW) throughout the experimental study was confirmed through trapping, since

adults were caught at each site throughout the study. However, the likelihood of either the recruitment or entrainment of the larval and juvenile stages on cultured shellfish varied over the experimental period. The zoeal stage was found during a large part of the experimental study, with highest abundances in early May and July at Refuge Island. The megalopal stage was not abundant or prevalent at any point during the experimental period (Table 7), which is in agreement with previous studies of Green Crab larval dynamics in Barkley Sound (Therriault and DiBacco, *unpublished data*), and is likely due to the short duration and increased swimming ability of the megalopal stage (Quieroga 1996; Quieroga *et al.* 2006). However, when the megalopal and juvenile (<20 mm CW) data from the entire experimental study were combined (Table 9), it is clear that these life-history stages are present for much of the year (particularly at the Refuge Island site); therefore, the entrainment potential on cultured shellfish also is probable throughout this period. Megalopae or small juveniles (<20 mm CW) were only absent from samples at Refuge Island on five dates: July 2011, September 2011, November 2011, January 2012, and March 2012. These data are similar to those derived from observations from the Green Crab's native range, where zoeal and megalopal stages are typically found from February to October (Queiroga *et al.* 1994, 2006; Queiroga 1996; Zeng *et al.* 1997; Moksnes 2002; Baeta *et al.* 2005; Paula *et al.* 2006). There are still seasonal peaks in the abundances of zoeae and peaks of megalopae between March and July, but unfortunately none of these studies, except Moksnes (2002) and Baeta *et al.* (2005), consider the period of August to November (Queiroga *et al.* 1994, 2006; Queiroga 1996; Zeng *et al.* 1997; Moksnes 2002; Paula *et al.* 2006).

In the processor study, Green Crabs were not detected in any of the samples analyzed. However, the presence of Green Crabs (the critical first step in transport) in the immediate harvest area cannot be confirmed, as trapping, plankton tows, and beach walks were not done at these culture locations (DFO Statistical Areas 26-6, 25-6, and 24-9). Although baseline studies of the extent of the invaded range of Green Crabs on the west coast of Vancouver Island have confirmed their presence in these areas, very little is known about their finer-scale distributions or population dynamics there (Gillespie *et al.* 2007). We cannot, therefore, say with certainty that the Green Crabs were present during the harvesting of shellfish there.

3.8.2.1. Recruitment

The presence of megalopae as well as first or second instars (juveniles < 5-mm CW) on the mussels and oysters in our study confirms that Green Crab larvae can settle and recruit on cultured shellfish. Other studies support our findings: Green Crabs (Grosholz *et al.* 2001) and other crab species such as *Cancer oregonensis* (Behrens Yamada *et al.* 1993 and the references therein)*, Pilumnus caribbaeus*, and *Mythrax forceps* (Freites *et al.* 2000 and the references therein) have been found to recruit on cultured clams, oysters, and scallops, respectively. Additional studies have found preferential selection and high recruitment rates of estuarine crab megalopae and first instars (newly molted juveniles, J1) on naturally occurring shellfish (Klein Breteler 1976; Fernandez *et al.* 1993; Thiel and Dernedde 1994; Eggleston and Armstrong 1995; Hedvall *et al.* 1998; Moksnes 2002; van Monfrans *et al.* 2003). Specifically, four of these studies demonstrated that juvenile Green Crabs are more abundant in natural mussel (*M. edulis*) habitat than on various macrophytes (filamentous green or brown algae) or in sand-flat microhabitats (Klein Breteler 1976; Thiel and Dernedde 1994; Hedvall *et al.* 1998, Moksnes 2002), while megalopae were generally more abundant on macrophytes (Hedvall *et al.* 1998; Moksnes 2002). Similar associations have been demonstrated for the estuarine crabs *Cancer magister* and *Callinectes sapidus* (Fernandez *et al.* 1993; Eggleston and Armstrong 1995; van Montfrans *at al.* 2003). *Cancer magister* megalopae settle in greater numbers on empty oyster shell compared to other estuarine microhabitats (Fernandez *et al.* 1993; Eggleston and Armstrong 1995), while *C. sapidus* megalopae and juveniles are more abundant on

eelgrass and oysters, respectively (van Montfrans *et al.* 2003). All the microhabitats described in these studies were estuarine and, although they state preference in recruitment based on higher abundances on certain substrata, both life-history stages were still present in all microhabitats evaluated (*i.e.* eelgrass, filamentous algae, mussels, oyster shell, sand flats, *etc.*), suggesting that Green Crabs and other estuarine crab species can and do settle on a wide variety of substrates, including naturally occurring shellfish. These findings agree with ours that Green Crabs recruit on cultured shellfish on the west coast of Vancouver Island, further confirming the potential for their transport to the island's east coast.

The potential transport of Green Crabs on shellfish was further confirmed by the fact that a Green Crab megalopa was found on a mussel control sample from the June 2012 out-planting event. During the collection, all the mussel control samples were bagged in Tofino and then transported and processed at PBS. This sample and all the mussel control samples were never hung at the experimental field sites (refer to Section 3.2.3.2 for further details on mussel control samples). The megalopa in the mussel control sample either recruited onto the mussels or into the mesh bag while it was hanging in Clayoquot Sound, then transported to the collection site (Tofino) by boat, and then to PBS. Due to the fixing process we cannot determine if this megalopa was alive upon arrival at PBS, we can only confirm that it was successfully transported from either the harvest location or storage location to the collection site in Clayoquot Sound and then finally to PBS. Subsequently, a juvenile Green Crab (13-mm CW) was found in one of the experimental mussel samples from that same sample batch, June 2012, after it was out-planted to the field site. Since a megalopa was found in a mussel control sample and the juvenile found from the same out-plant batch was 13-mm CW (10 weeks after out-plant), it is not possible to conclude where the latter came from. It could have been transported as a megalopa or a smaller juvenile from the harvest area or storage site in Clayoquot Sound, or it could have settled onto the mussels at the experimental site and grown to that size under favourable conditions in Barkley Sound (for range of potential size at age, see Klein Breteler 1976; Berrill 1982; Mohamedeen and Hartnoll 1989; Baeta *et al.* 2005). Unlike the Green Crabs that were present on the oysters, it remains uncertain whether those found on the mussels after the June 2012 out-planting recruited or were entrained onto them as there were two points between mussel harvesting and final sample rinsing where Green Crab could have been picked up. Regardless of when they were entrained, in each case they had the potential to be successfully transported to new locations.

3.8.2.2. Microhabitat Selection

Our results show that juvenile Green Crabs were found to be associated with each shellfish species and culture type tested. Similar results are available from the literature (Table 1) and suggest that, regardless of the species of shellfish or culture method used, if Green Crabs are present, they are likely to be associated with shellfish (natural or cultured). In fact, its native range the Green Crab shows a preference or is naturally abundant within natural shellfish beds, particularly mussel (*M. edulis*) beds (Thiel and Dernedde 1994; Norling and Kautsky 2007; Gestoso *et al.* 2013). This association with shellfish is usually attributed to the increased structural complexity associated with the shellfish themselves or with the gear. Some of these studies equate this complexity to other structurally complex natural habitats such as eelgrass beds, salt marshes, and oyster reefs (NRC 2010 and references therein).

Most of these investigations focussed on habitat value, rather than the mechanism by which Green Crabs became associated with cultured shellfish or gear (Thiel and Dernedde 1994; Beadman *et al.* 2004; Dealteris *et al.* 2004; Nizzoli *et al.* 2005; Dubois *et al.* 2007; Powers *et al.* 2007; Erbland and Ozbay 2008; Ysebaert *et al.* 2009; Marenghi and Ozbay 2010; Marenghi *et al.* 2010; Gestoso *et al.* 2013), and therefore rarely report the sizes of crabs found (for exceptions see: Grosholz *et al.* 2001; Dealteris *et al.* 2004). This makes it difficult to determine

if juvenile crabs recruited to the shellfish or gear as larvae and grew, or migrated to this habitat as juveniles. In the present study, similar uncertainty exists concerning the presence of juvenile Green Crabs on mussel samples that were placed experimentally using similar methods to those previously employed by the CFIA. The presence of larval Green Crabs in mussel control samples, which had never been placed in the water at either experimental site, suggests that juvenile Green Crabs found in the experimental mussel samples may have become entrained through larval recruitment or juvenile microhabitat selection. It is difficult to determine which of these mechanisms is responsible for the observed presence of juvenile Green Crabs in the samples as either process could have been involved. While it is unlikely that the juvenile Green Crabs found associated with the off-bottom cultured shellfish in our experimental study selected this microhabitat as juveniles, rather than having settled there as larvae, microhabitat selection cannot be ruled out. It is possible (though unlikely) that juvenile crabs swam, were swept up by storms, or travelled from other locations on floats or anchor lines to become associated with the suspended shellfish.

For the other culture methods/species investigated here, the mechanism by which Green Crabs became associated with the shellfish is clearer. For oysters, which were incubated off-bottom in trays and thoroughly scrubbed prior to out-plant, it is likely that the Green Crabs we detected recruited there as larvae, and metamorphosed, prior to being discovered as early juveniles (1.5 and 2.5 mm CW). Similarly, Haupt *et al.* (2010) reported the presence of Green Crabs in the off-bottom culture of *C. gigas*, further suggesting that recruitment is the primary means by which Green Crabs become associated with the off-bottom culture of oysters. This applies only if the suspended gear is not touching the bottom. For clams, the experimental method used here was meant to emulate local harvest conditions: clams are harvested, sometimes rinsed, and stored in mesh bags on the beach for some time prior to transport to a processor. In this case, the size of the juvenile Green Crabs discovered (5.1–7.6 mm CW), along with the short wet-storage period of previously rinsed clams, suggests that juvenile Green Crabs selected this microhabitat. Previous work has shown Green Crabs to be associated with clam grow-out bags and suggests that crabs entered the bags as megalopa or small juveniles, and grew to the point where they could no longer escape (Grosholz *et al.* 2001). In our study, given the short incubation period of the clam samples, it is likely that the crabs were able to escape the bags, but remained there despite disturbance during collection. This further emphasizes the findings reported in the studies in Table 1, which highlight the importance of the structurally complex microhabitats created by cultured shellfish. All the cases reported here, along with our experimental findings, suggest that Green Crabs are associated with cultured shellfish regardless of the species cultured or the methods employed, and that selection of shellfish as a microhabitat by juveniles is a possibility in all cases, but is more probable in benthic culture.

3.8.3. Presence of Biofouling NIS on Cultured Shellfish

Three common non-indigenous tunicate species – *B. violaceus*, *B. schlosseri*, and *D. vexillum* – which are known to have tremendous impacts on aquaculture on the east coast of Canada (Carver *et al.* 2006; Therriault and Herborg 2007; LeGresley *et al.* 2008), were among the fouling NIS found in both our experimental and processor/grower studies (Tables 10 and 13). *Botrylloides violaceus* was found on a large proportion of the experimental mussel samples as well as both experimental and processor oyster samples (Table 10 and 13). *Botryllus schlosseri* was not as prevalent in either the experimental or processor/grower samples. *Didemnum vexillum* was not noted in the experimental samples, but it was very abundant on the long-line cultured oysters (87%) sampled during the processor/grower study (Table 13). There are several possible reasons why *D. vexillum* was not present in the experimental samples, including absence at the experimental site, a mis-match between recruitment and out-planting, and slower initial growth in communities that are well established (McCarthy *et al.* 2007; Osman

and Whitlatch 2007). Two non-indigenous species of bryozoans were also found during this study, but only one (*S. japonica*) was present in both the experimental and processor studies (Tables 11 and 14). The other species, *C. pallasiana*, was found only at the Sechart site during the experimental study. Despite occurrences in California, the Strait of Georgia, Haida Gwaii, and Alaska (Osburn 1952; Powell 1970; Dick and Ross 1985; Cohen and Carlton 1995; Sloan and Bartier 2004), to our knowledge this is the first confirmed occurrence of it on the west coast of Vancouver Island.

3.8.4. The Transport of Mobile and Biofouling NIS on Cultured Shellfish

As mentioned previously, some of the juvenile Green Crabs found in the experimental study were alive when the samples were processed days later. Furthermore, many of the other organisms associated with both the experimental and processor study samples were alive when rinsing began (*i.e.* after transport, usually 3–6 days after harvest/collection). These organisms included many native species of fish, non-cultured shellfish, macrophytes (seaweeds, seagrasses, and algae), crabs, shrimp, amphipods, snails, and many more. The methods and containment in the transport of commercial shellfish products may vary greatly; our experimental study samples were kept cool (4°C), were packed in plastic bags, and consistently contained in a large fish tote. Due to these optimal transport conditions, the survival of macrofauna in our experimental samples may have been higher when compared to some commercially transported product. It is important to note that hypoxic conditions were likely created due to the packaging in the present study which could have resulted in increased mortality of some taxa. The survival of propagules during transport is an important factor in the invasion process, contributing to the introduction and spread of NIS.

There are many factors affecting the survival of NIS both within the vector and upon discharge to the receiving environment. For NIS hitchhiking on transported shellfish they include: species-specific behaviour and tolerances, environmental conditions during transport, and duration of transport. Further, the relative importance of each factor can vary depending on which part of the NIS's life cycle stage (*e.g.* larva, juvenile, adult,) or form (*e.g.* larva or colony fragment) is hitchhiking. For instance, the potential survivability of Green Crabs during transport increases with each subsequent developmental stage. Zoeal stages are the most prone to low salinity and desiccation, survival being extremely reduced at salinities below 20 (Bravo *et al.*, 2007). Megalopa and juvenile crab show an increased tolerance to low salinity and are able to osmoregulate in salinities as low as 10 and 5, respectively (Cieluch *et al.* 2004). Juveniles show a similar osmoregulatory capacity as adults, which are able to survive salinities as low as 4 ppt (Cohen and Carlton, 1995; McGaw *et al.* 1999). Following metamorphosis from megalop to juvenile, Green Crabs also display an increased tolerance to hypoxia (Reid *et al.* 1997) and desiccation, and in the adult stage are able to survive out of water for several days at high temperatures (24°C) (Darbyson *et al.* 2009). Their tolerance to environmental change combined with their behaviour [*e.g.* hiding in crevices and evaporative cooling (Ahsanullah and Newell 1977)] suggests that Green Crabs could easily survive transport on shellfish during over-land transfers to processors. Recall that often the conditions during transport are favourable for the shellfish, which is also favourable for many other species. It has been suggested that the initial transport of Green Crabs to San Francisco Bay from the eastern United States may have occurred with the transport of live oysters or bait worms (Cohen *et al.* 1995).

Given the high numbers of Green Crabs that could be transported on cultured shellfish (Table 16), the propagule pressure to the Strait of Georgia through this vector could be substantial. This, in turn, is likely to increase the risk of establishment and spread of Green Crabs to the Strait where environmental conditions are favourable. There is a strong positive relationship between propagule pressure and the likely establishment of non-native species (Lockwood *et al.* 2007). Further, the more this happens, the more likely introduced propagules will overcome

invasion barriers (Ruiz and Carlton 2003). Thus, even if a non-native population is extirpated the species can re-establish, provided continued presence of an invasion vector (Ruiz and Carlton 2003). Given the results of the experimental data and the extrapolation exercise, it seems very plausible that Green Crabs (propagules) could be transported in great numbers on cultured shellfish, are likely healthy after shellfish transport, and could be spread throughout the Strait of Georgia.

3.8.5. Study Uncertainties

The experimental design and methodology included some uncertainties. The major source of uncertainty in the methodology is how samples were rinsed in the experiment compared to rinsing methods employed in the field by industry (if any). Although our rinsing method removed much of the macrofauna, its efficacy is unknown and is not testable. There is no way to know for certain if 100% of all Green Crabs present on the samples were removed as we had no initial measure. This has important implications in that the number of Green Crabs found could have been underestimated which would confound extrapolations and underestimate the invasion risk. The samples in the experimental study were not rinsed prior to transport, therefore the number of Green Crabs documented may have been higher than if industry practices and license conditions were followed (see Section 7.1). In addition, there are uncertainties surrounding elements of the experimental design, including how well the study sites represent Green Crab populations along the west coast of Vancouver Island.

Population differences could contribute to variations in entrainment potential since a positive relationship between Green Crab population size and probability of entrainment in the vector was assumed (more Green Crabs = higher entrainment probability). Although both sites had all three of the components necessary for the successful conduct of this project, the population of Green Crabs was quite different between sites by the end of the study. Refuge Island and Sechart had similar CPUE values for Green Crabs >30-mm CW in 2011 (Fukui traps only; Figure 7a) but CPUE dropped at Sechart at the early-May 2012 sampling event and was no longer similar to that at Refuge Island for the rest of the study. The abundance of zoeal stages and juvenile Green Crabs also differed between these sites.

Admittedly, there are some differences between how shellfish farmers grow shellfish and how they were handled in our study which also could influence entrainment potential. Specifically, most shellfish are out-planted at an early stage and left largely uninterrupted for a couple of years. Thus, the actual entrainment potential is a function of exposure over at least two Green Crab reproductive periods prior to harvest. In contrast, our experimental design only allowed out-planting of shellfish material that would allow entrainment over a single growing season and in some cases our out-plantings did not coincide with the peak Green Crab reproductive period. Thus, entrainment potential indicated by our investigation could have been underestimated relative to that in commercial practices.

Potential uncertainties in the processor study included: rinsing issues, lack of knowledge surrounding Green Crab populations, potential changes in standard practices, and small sample sizes. The processor study samples should have been rinsed, but this was not confirmed and the method of rinsing was unknown. The distribution and population sizes of Green Crabs near shellfish culturing sites where the shellfish were sampled were also unknown. Although we have general information about the distribution of Green Crabs along the west coast of Vancouver Island (Gillespie *et al.* 2007), there is no information on site-specific distributions or population abundances. Growers and harvesters may have also changed their operating procedures, including the treatment of shellfish before transport, in an effort to show they comply with license conditions or to demonstrate that their industry does not pose a risk of

moving potential NIS. Lastly, relative to the experimental study, the sample sizes in this study were small and may not accurately reflect the potential of entrainment of mobile NIS.

4. POTENTIAL MITIGATION MEASURES AND THEIR EFFICACY

Several mitigation measures aimed at reducing the potential spread of Green Crabs from the west to the east coast of Vancouver Island on shellfish aquaculture products have been implemented via DFO Pacific Shellfish Aquaculture License Conditions (Section 11.3). While the CFIA has conditions placed on the transfer of shellfish used in the biotoxin monitoring program in its ITC permit, they are not as extensive as the aquaculture license conditions. Similarly, the collection and transfer of brood stock during the underwater harvest of wild Geoducks and Sea Cucumbers is subject to conditions in the ITC permits, but the actual transfer of harvested products (*i.e.* Geoducks, Red Sea Urchins, and Sea Cucumbers) is not subject to any conditions of transfer (DFO 2013c, d, e). Unlike these other wild fisheries, the harvest of wild intertidal clams is subject to the same license conditions as those of cultured clams – the DFO Pacific Shellfish Aquaculture License Conditions (Section 11.3) (DFO 2013f). The potential effectiveness of these conditions of licence at reducing the propagule pressure (hence invasion risk) associated with shellfish movements in BC is reviewed below. In addition, we explore other potential mitigation measures in an attempt to improve current practices to reduce the risk of NIS transfers on cultured shellfish.

4.1. DFO PACIFIC SHELLFISH AQUACULTURE LICENSE CONDITIONS

4.1.1. Examination and Cleaning of Cultured Shellfish

This section concerns license condition 11.3 (a), which states: “In Pacific Fishery Management Areas 23 to 27 the licence holder shall thoroughly examine harvested shellfish (oysters, clams, scallop, and mussels) for signs of European Green Crab, and rinse the harvested shellfish prior to being removed from the harvest area (DFO 2013a).” A thorough examination implies a visual inspection of the product before transport. However, this can be difficult, time consuming, and largely ineffective for some NIS species and life-history stages. Inspection may be difficult due to the structural complexity of the shellfish product, which has the potential to obscure NIS, especially small species and small early life-history stages. Prior to rinsing the experimental shellfish samples, each was carefully inspected as a whole, but none of the Green Crabs detected in this study were found during such initial visual inspection. It was only after samples were broken apart or rinsed on the sieve that juvenile crabs were detected. In the case of megalopae and very small juveniles (< 3-mm CW), which were barely visible to the naked eye, none were detected until the rinsate was examined under a stereo-microscope. For example, three of the Green Crabs (5–8 mm CW) found were only detected once the clam bags were opened and the clams poured onto the sieve in the laboratory and not upon initial visual inspection. Similarly, the largest Green Crab (13-mm CW) found in a mussel sample was only detected once the mussels and byssal threads were separated, not being visible on general inspection.

The inspection and rinsing process undertaken in the laboratory was very labour intensive and time consuming. Usually it took two individuals 15 to 19 hours to inspect and rinse roughly 136 kg (300 lbs) of clams, 2.8 kg (2.1 dozen) of oysters, and 3.2 kg of mussels during the study (the samples from a single sampling event). Each piece of shellfish was individually handled and rinsed until all visual biofouling and debris (*e.g.* seaweed and animals) were removed, which usually entailed rinsing the surface of each piece more than once. Given the length of time and labour it took to detect these crabs, it is unlikely that such a level of inspection and rinsing could be implemented by growers or harvesters.

The rinsing of the product before transport is not well described in this license condition. Specifically, it does not define what "rinsing" entails: does it involve simply dipping the product in the ocean, dipping or rinsing it in freshwater, or the common practice of power washing (presumably with seawater)? If rinsing or power washing, at what intensity? Without a clear definition of what rinsing entails, it is left up to the grower or harvester to interpret the intent of the condition. Our experimental samples were not rinsed before transport, but we assumed that the processor study samples were treated in a manner consistent with existing license conditions. However, it was unknown exactly how the processor samples were rinsed before transport. Since most of the juvenile crabs found in our study were either deeply embedded in a matrix of mussel byssal threads, the bag of shellfish itself (with associated biofouling), or within the shell micro-structures, it is possible that they would be protected during rinsing, even if a power-washer had been used.

Very little research has examined possible methods of removing mobile species, such as crabs, from shellfish. One study by Marenghi and Ozbay (2010) did test simple rinsing (using freshwater with a garden hose) and found species-specific differences in the abundance of crabs after rinsing; though the abundances varied depending on the species, individuals were still present after rinsing. The present project results, particularly those from the processor study, demonstrate that rinsing, as it may be currently employed, does not remove all NIS on shellfish before transport.

4.1.2. Examination and Cleaning of Culture Gear

The condition pertinent to culturing gear is contained in condition11.3 (b) and states: "Shellfish culture gear (trays, lines, etc.) shall be thoroughly examined and rinsed prior to removal from growing areas in Pacific Fishery Management Areas 23 to 27 for use in another area (DFO 2013a)." As with examining and cleaning cultured shellfish, there are issues around the ability to detect small species or individuals (<30 mm) and the definition of the term "rinsing". Gear in this license condition describes "trays, lines, *etc.*", however, it does not explicitly state the commonly used grow-out and anti-predation bags, commonly called onion sacs. The resultd from the experimental clam samples and beach-cultured oysters from the processors in the present study, as well as the work by Minchin (2007), suggest that when shellfish are contained within such bags they can be a vector for NIS. This is especially relevant for bags of shellfish that are left in the mid-to-high intertidal zone for holding or during the grow-out phase as there is a spatial overlap between where shellfish culturing practices occur and the preferred habitat of juvenile Green Crabs. In the present experimental study, small juvenile crabs were found in clam bags that were laid in the mid-to-high intertidal zone where small individuals (<20-mm CW) were also found during beach walks.

4.1.3. Wet Storage

The prevention of inappropriate wet storage is a key step to reducing the risk of accidentally transferring NIS to new locations as indicated in license condition 11.3(c): "Shellfish harvested from Pacific Fishery Management Areas 23 to 27 shall be wet stored or grown out only at approved licensed areas in Pacific Fishery Management Areas 23 to 27. Shellfish may be wet stored in tanks within licensed processing facilities where such activity is approved in the Quality Management Plan (DFO 2013a)." Results of the present study clearly demonstrate that NIS are entrained in cultured shellfish and can survive transport, and this preventative measure creates a barrier that should be maintained to reduce potential propagule pressure. However, the second component of this condition raises concerns over the treatment of holding-tank effluent as well as solid waste and effluent that occurs during processing (*e.g.* material sloughed off the shells, seawater carried during transport, liquid from inside the shells and innards, and liquid used to clean off product and processing area). While there are onshore wet storage guidelines

and processing building conditions [within the Canadian Shellfish Sanitation Program - Manual of Operations (Chapters 5 and 10 of CFIA 2013) and the Compliance and Assessment Guide for Schedule I and II of the Fish Inspection for Regulations Registered Establishments in the Quality Management Plan (Chapter 1.3 of CFIA 2013), respectively], the treatment of effluent for NIS is not specifically mentioned. The focus of the onshore wet storage guidelines is the disinfection or treatment of holding water that is contaminated with viruses, bacteria, or biotoxins, while the building regulations are concerned with back-flowing waters and vermin control in a building's drainage system. Neither of these shellfish processing regulations or guidelines addresses the treatment of effluent and solid waste from processing shellfish that are imported from non-local waters in order to prevent the transfer of NIS. Given the difficulty of visually detecting smaller species and individuals, the lack of treatment of the effluent produced from onshore (in-tank) wet storage and processing of non-local shellfish is a concern. Additional measures need to be developed to ensure that NIS are not inadvertently introduced because of such apparent gaps in regulation.

4.1.4. Disposal of Shell Waste

The regulations regarding the disposal of shell waste after processing are detailed in license condition 11.3(d) and state: "Shucked oyster shell from Pacific Fishery Management Areas 23 to 27 shall not be placed in or adjacent to the intertidal zone where it may be washed by the tide or where any entrapped crabs may reasonably travel to the shore until the shell refuse is sufficiently desiccated to kill any crab or crab larvae that may have accompanied the shipment (DFO 2013a)." European Green Crabs have the potential to move considerable distances overland (Darbyson *et al.* 2009; Perkins *et al.* 1967 in Cohen and Zabin 2009) but the "reasonable travel" distance from the high-tide line is not stated in license condition 11.3(d). Even for sessile species the specified disposal conditions could still allow some NIS to survive. Many of the processing plants in Baynes Sound are situated just above the high-tide line, but below the storm-surge line, and the disposal of oyster shell alongside the buildings appears to be common practice. Oyster shells themselves have been reported to act as an NIS vector even after processing when they are left in piles along the shoreline (Cohen and Zabin 2009). Even complete desiccation could allow some NIS propagules such as cysts or resting stages to survive. Admittedly it is not known how long the desiccation process takes and how effective it is at destroying sessile and mobile NIS. The full desiccation of shells may require a substantial amount of time and the only recommendations available are based on preventing the spread of the protozoan *Perkinsus marinus*. In this case, Bushek *et al.* (2004) suggest leaving shells out for 1–3 months, but their study does not address whether sessile species like tunicates or mobile species like crabs desiccate in the same time frame. The Washington Department of Fish and Wildlife requires that piles of oyster shell be left at least 200 feet (70 m) from any body of water and that the shell must not be transported to other sites until it has sat for at least 90 days (Cohen and Zabin 2009). Further, all oyster shell brought in from California must be baked in a propane oven before it is placed in local waters of Washington (Cohen and Zabin 2009) as a means to reduce the likelihood of spreading Green Crabs.

4.2. DISCUSSION

4.2.1. Literature Review of NIS mitigation Techniques Used in Shellfish Aquaculture

While the current shellfish license conditions concerning Green Crabs in BC exist largely as preventative policy measures, there are practical mechanisms currently employed by the shellfish culture industry in BC and other jurisdictions, or that have been experimentally studied, that could help prevent the proliferation of biofouling on aquaculture gear and cultured shellfish. Admittedly, the mechanisms to control general biofouling species do not fully represent an

attempt to reduce the risk of introducing potential NIS to new areas nor do they typically focus on mobile species such as crabs. The motivation for controlling biofouling in aquaculture settings is largely financial and the techniques used have been employed or experimentally tested to prevent or reduce the growth or spread of biofouling on aquaculture product or equipment (Dürr and Watson 2010; Fitridge *et al.* 2012). Even though the focus of many of these studies is not necessarily on NIS specifically, these methods likely decrease the growth and likelihood of transport of NIS (Lewis and Coutts 2010). The methods of removing or decreasing the growth of biofouling include mechanical removal (*e.g.* by hand, rinsing, pressure washing), chemical treatments (*e.g.* acetic acid, hydrated lime), biological control (*e.g.* use of predators such as sea urchins, whelks, and crabs), and coating applications (Dürr and Watson 2010; Fitridge *et al.* 2012). No experimental treatment has been demonstrated to fully eliminate NIS and although biomass can be reduced, it has never reached zero and coverage or abundance on the shellfish often increases soon after treatment (Adams *et al.* 2011; Arens *et al.* 2011; Switzer *et al.* 2011; Paetzold *et al.* 2012).

The most common method of biofouling removal in the United States is mechanical, either by hand or through pressurized seawater spray (pressure washing) (Adams *et al.* 2011). Mechanical removal is a very labour-intensive method that contributes significantly to the overall cost of culturing shellfish (Adams *et al.* 2011), but it is not necessarily an efficient method to reduce the growth or spread of biofouling NIS (Paetzold and Davidson 2010; Adams *et al.* 2011; Arens *et al.* 2011; Paetzold *et al.* 2012). This method of removing biofouling is generally an acute one used to clean product prior to transport or periodically during grow-out to prevent the potential smothering of shellfish caused by fouling species (Adams *et al.* 2011). Removal by either low-pressure washing (~40 psi) or hand scrubbing has been shown to be effective at reducing the biomass or coverage of invasive tunicates over a short period (months), but it was less clear how effective this method was over a longer time (Adams *et al.* 2011; Arens *et al.* 2011; Switzer *et al.* 2011; Paetzold *et al.* 2012). Hand rinsing of baskets, oyster clusters, and oyster shell with freshwater (with a simple garden hose), on a bi-weekly basis for four months, was assessed in a study by Marenghi and Ozbay (2010). The effect of freshwater rinsing treatment varied depending on treatment type and species of crab. The bi-weekly rinsings resulted in fewer Xanthid crabs (a family of crabs including the Harris Mud Crab, *R. harrisii*), but had no effect on the abundance of Blue Crab (*C. sapidu*s) (Marenghi and Ozbay 2010), a close relative of the Green Crab. Thus, drawing conclusions with respect to efficacy for other species should be done with caution as its effectiveness was not universal.

Among other forms of mechanical removal, low- and high-pressure power washing have been studied (notably with known invasive tunicates). For example, Arens *et al.* (2011) studied the effects of low-pressure (~40 psi) and high-pressure (~700 psi) washing techniques to control Botryllid tunicates growing on mussels (*M. edulis*). Although initial results confirmed species-specific responses to the treatments only *B. violaceus* in the high-pressure treatment showed reduced (not zero) re-growth by the end of the experiment while all other combinations contributed to a significant increase in tunicate coverage. None of the experimental treatments examined for tunicates or crabs fully eliminated them since mechanical removal treatments never reached zero biomass, coverage, or abundance on the shellfish and often increased soon after treatment (Adams *et al.* 2011; Arens *et al.* 2011; Switzer *et al.* 2011; Paetzold *et al.* 2012).

Much like the mechanical treatments, biological, chemical and equipment-coating treatments studied never fully eliminated non-indigenous tunicates or biofouling in general (Hidu *et al.* 1981; Ross *et al.* 2004; Switzer *et al.* 2011). Biological control using whelks, hermit crabs, *Cancer* crabs, and sea urchins has been studied and all tested species decreased the amount of fouling by varying degrees (Hidu *et al.* 1981; Ross *et al* 2004; Dürr and Watson 2010; Switzer *et al.* 2011) but never completely eliminated it. The chemical treatments for biofouling that have

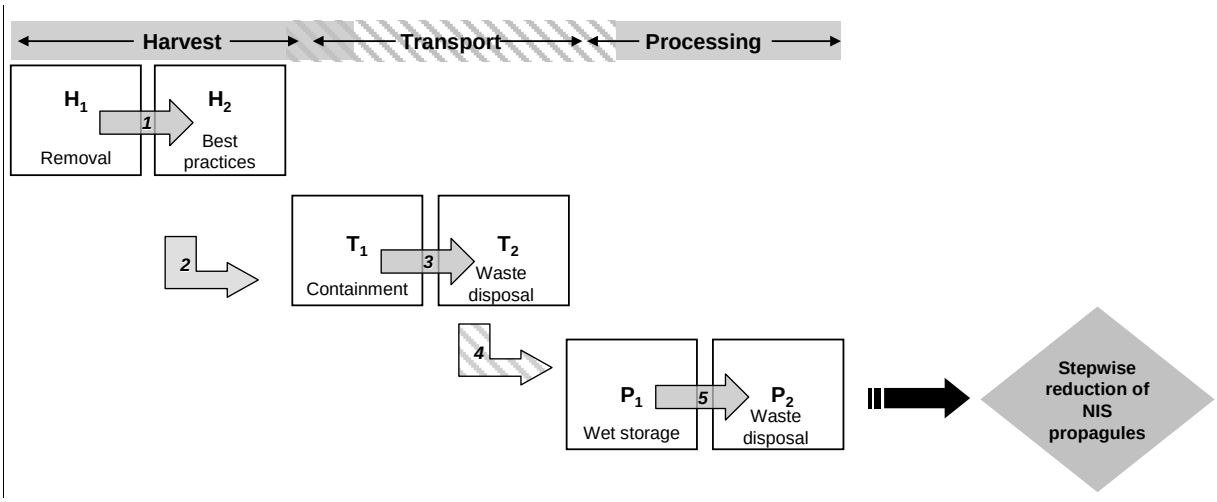
been experimentally tested include hydrated lime, acetic acid, heated brine, and chlorine (McKindsey *et al.* 2007 and the references therein; Switzer *et al.* 2011; Fitridge *et al.* 2012 and the references therein). Much like mechanical removal and biological control, most of these treatments do not entirely eliminate the biofouling and have differing effects depending on the target species (McKindsey *et al.* 2007 and the references therein; Switzer *et al.* 2011; Fitridge *et al.* 2012 and the references therein). Whether mechanical, biological, or chemical, the treatment of shellfish using such techniques, and their efficacy with respect to removing or killing NIS, is largely unknown. Many experimental studies report on the degree of reducing biofouling biomass or cover but none are 100% effective. Further, few look at long-term impacts to determine whether the NIS recover and grow back after treatment (but see Arens *et al.* 2011; Switzer *et al.* 2011). Many of the techniques are also known to be mechanisms for the spread of NIS (*e.g.* power washing of colonial tunicates), can indirectly enhance the growth of NIS, have deleterious effects on the shellfish itself, have unknown long-term environmental impacts, or are too costly or difficult to employ commercially (McKindsey *et al.* 2007 and the references therein).

4.3. CONCEPTUAL MITIGATION FRAMEWORK

To reiterate, the invasion cycle should be viewed as a series of steps with barriers between each (Figure 2). In order for propagules to successfully establish and spread in a new location they must first overcome barriers related to entrainment in the vector, survival during transport and discharge, as well as the environmental and biotic characteristics of the receiving system. Successful invasions require all of the barriers to be overcome. The invasion process can fail due to the disruption of the invasion cycle at a single point or the cumulative effects of differential mortality of propagules at multiple invasion stages. Thus one goal of management intervention with respect to NIS aims to invoke a barrier not otherwise present to reduce the likelihood of an invasion occurring (Ruiz and Carlton 2003). A conceptual framework can highlight steps in the invasion cycle where management intervention could be applied (similar to HACCP[2]). For the cultured shellfish vector this includes mapping the activities from the time of harvest until final disposal of processed shellfish (Figure 12). As long as propagules are taken up by an invasion vector (including shellfish movements), no method of mitigation should be assumed to be totally effective (see McKindsey *et al.* 2007). Thus, the most effective barrier to reduce invasion risk of Green Crabs would simply be to disallow movement of potentially infected shellfish product from the west to the east coasts of Vancouver Island, essentially establishing a quarantine area; however, this would heavily impact the commercial aquaculture industry on the west coast of the Island since there are no shellfish processing plants there.

The conceptual model also allows for the identification of additional places where control points might be applied with the goal of reducing the volume of propagules arriving in non-infested waters (and hence invasion risk). Potential mitigation measures should be designed to reduce the volume of propagules entrained in the invasion vector and to lower the survival of individuals within the vector during transport and/or release. Potential control points are discussed briefly below.

[2] "HACCP is a production control system for the food industry. It is a process that identifies where potential contamination can occur (the critical control points or CCPs) and strictly manages and monitors these points as a way of ensuring the process is in control and that the safest product possible is being produced (HACCPCanada 2014)."



Category	Definition
H_1	Removal of large proportion of the growth on shellfish (e.g. biofouling, seaweeds and mobile fauna) before transport: power washing, hand scrubbing and chemical dips can be used where appropriate
H_2	Best practices: store the product away from known AIS habitats until transport (e.g. avoid mid- to high intertidal for juvenile green crab), perform a thorough visual inspection of the product before transport
T_1	Contain the shellfish, water and any growth on the shellfish during transport
T_2	Dispose of water and any growth that has sloughed off the shellfish appropriately (i.e. not near any shoreline or sewage main)
P_1	Do not wet store any shellfish from non-local waters in local waters
P_2	Dispose of, or treat any effluent and solid waste produced during shellfish processing (i.e. sea water, shellfish innards, biofouling, shells) appropriately so that live organisms cannot enter any local waters
	Overlap in responsibility between the harvester and processor exists both at the front and back end of the transport phase of shellfish; transport of product is also done by both parties

Figure 12. Conceptual framework for considering cultured shellfish movements in relation to NIS which identifies where potential mitigation measures could be applied to reduce the risk of introducing NIS to non-infested areas. AIS refers to Aquatic Invasive Species.

4.3.1. Harvest

NIS have the potential to be entrained with harvested shellfish product, so any cleaning of shellfish at the farm site potentially lowers the number of propagules that are able to move from the site. As noted above, no technique is 100% effective at removing potential NIS but mitigation can reduce the propagule load eventually moved to non-infested areas (Figure 12 H_1). Hand removal may be an effective measure used to remove large (> 30-mm) mobile species such as sea stars and sea urchins or masses of biofouling such as colonial tunicates from the surface of shellfish products. Hand removal is likely to be less effective at removing smaller species or biofouling firmly attached to the shells (*e.g.* bryozoans, spionid polychaetes). High-pressure power washing (~700 psi as employed by Arens *et al.* 2011) is another form of removal that could be employed to dislodge a large proportion of epibionts (*e.g.* tunicates, seaweeds) attached to the shellfish. However, caution must be exercised as removal of biofouling can facilitate the re-growth of species, including NIS (Switzer *et al.* 2011), and can redistribute NIS propagules at the farm site (Paetzold and Davidson 2010).

Some shellfish does not immediately leave the farm site following harvest. Thus, applying best practices with respect to on-site storage of harvested product has the potential to reduce NIS

propagules entrained during this stage (Figure 12, H_2). Specifically, intertidal storage in Green Crab infested waters was shown in this study to allow recruitment of *C. maenas*. Three Green Crabs were found in bags of clams left for three to five days in the mid-to-high intertidal zone, a habitat preferred by juvenile Green Crabs (Warman *et al.* 1993; Thiel and Dernedde 1994). Removing the spatial overlap in storage and NIS habitats should lower the probability of entraining propagules at this stage.

4.3.2. Transport

Propagule pressure can also be reduced during the transport stage. In addition to any natural mortality that occurs during transport, management intervention can constrain propagules entrained with shellfish product during transport and reduce the volume of NIS propagules delivered to the receiving environment. To reduce the probability that NIS are inadvertently introduced to a new location, either via infested water or product, solid or liquid waste from the transport should be disposed of in a way that ensures NIS propagules are not released into the receiving environment in a viable condition.

4.3.3. Processing

Once transported shellfish reach processing plants there are additional mitigation measures that can be employed to reduce the probability of inadvertently introducing NIS propagules to new environments. They involve both the intentional and unintentional introduction of potentially infested shellfish product to non-infested waters. Given the confirmed presence of NIS, including both tunicates and Green Crabs, in the shellfish movement vector, potential propagules should not be directly deposited into non-infested waters. The direct deposit of any live shellfish in or near the intertidal zone has the potential to inadvertently introduce NIS to new locations. However, NIS propagules continue to have the potential to be introduced to new receiving environments indirectly via discharge of contaminated water, product, and solid waste from the processing plant. Processing water, product, and solid waste should thus be contained and treated to reduce the probability of NIS propagules being inadvertently introduced to new locations.

4.3.4. The Conceptual Mitigation Framework and Non-cultured Shellfish Transfers

Although the steps in the framework do not explicitly state that these measures can be applied to all types of shellfish transfers, they can (*i.e.* aquaculture, intertidal and underwater wild harvest, scientific licenses, biotoxin or contaminant monitoring). Some shellfish transfers are subject to conditions or policies placed on them, but when this project began none of them addressed all the steps in this framework. For instance, wet storage on the east coast of Vancouver Island of wild intertidal clams harvested on west coast of the island is prohibited (DFO 2013f) and the brood stock collection from the wild is subject to the Introduction and Transfers Committee review process. The off-loading and transfer of wild caught Geoducks, Sea Urchins, and Sea Cucumbers are not, however, subject to any mitigation measures to prevent the spread of NIS. In the latter case, for example, steps H_1, H_2, and P_1 may not be relevant to current practices, but all the other stages apply, as they could pose some risk of NIS transfer. The framework addresses gaps in the mitigation measures related not only to the transfer of cultured shellfish but all shellfish transfers from infested to non-infested waters.

5. SYNOPSIS

The only way to ensure NIS are not transported with shellfish is to not move the shellfish. Any shellfish transfer has the potential to transfer NIS to new locations and no mitigation method is 100% effective. Should transfer be necessary, the conceptual mitigation framework identifies

control points at which mitigation measures could be implemented. The purpose of the control points is to reduce the number of NIS propagules at different steps in the invasion cycle and so reduce the probability of a new invasion. Currently, some DFO Pacific Shellfish License Conditions are designed to reduce propagule pressure in accordance with this framework, but gaps still remain. A risk assessment could identify where potential mitigation measures might be most effective, but such an assessment is beyond the scope of the present exercise. Further, the conceptual model developed can be employed at different spatial scales (*e.g.* transfers along the west coast of Vancouver Island) and for different types of shellfish movements (*e.g.* biotoxin monitoring, shellfish grow-out, and wild fisheries). Some of these other movements are regulated, but reducing the risk of moving NIS is not the primary focus of these regulations. For example, current shellfish transfer zones restricting certain shellfish transfers are large (*i.e.* 100s-1000s of kilometres of coastline per zone; DFO 2013b) and, given that NIS have been shown to be entrained with shellfish, might not be effective at limiting the spread of NIS to new locations. In order to slow the spread of invasive tunicates in Prince Edward Island, DFO moved to bay-scale management, effectively creating infested and non-infested zones at smaller spatial scales then those currently employed on the Pacific coast. As mentioned in the Introduction (Section 1) the findings of this project are focused on cultured shellfish, but they are relevant to all movements of shellfish from infested to non-infested waters (*e.g.* biotoxin monitoring programs, wild shellfish harvest practices including intertidal and underwater). These other types of shellfish movements should also be subject to mitigation measures, such as those mentioned in the conceptual framework model, in addition to any other conditions or regulations currently in place.

5.1. RECOMMENDATIONS

1) If the management objective is to fully eliminate the risk of introducing potential NIS from infested to non-infested waters on transferred shellfish, the transfer of shellfish should be halted.
    a. For example, during the preliminary review of the project data, DFO contacted CFIA regarding the study findings in October 2012. After consultations with DFO, the CFIA quickly made adjustments to the biotoxin monitoring program and in November 2012 began sourcing cultured mussels grown in the Strait of Georgia (Shellfish Transfer Zone 4) in order to re-stock the biotoxin monitoring sites throughout the south coast of BC. The last CFIA transfer of wild mussels from the west to the east side of Vancouver Island occurred in April 2012.
2) If the management objective is to reduce the risk of introducing potential NIS from infested to non-infested waters on transferred shellfish, various mitigation measures included in the Conceptual Mitigation Framework (Section 4.3) should be invoked.
3) To facilitate further development of the conceptual framework for risk mitigation, a risk assessment needs to be undertaken to understand the relative reduction in propagule pressure at each step, under various scenarios, of the framework.
4) In order to determine the relative reduction in propagule pressure at each step of the conceptual framework, further experimental research is required.
    a. For instance, evaluating the efficiency of some of the discussed mitigations (*e.g.* power washing, chemical dips *etc.*) would help to measure the relative reduction of propagule pressure of "Stage H_1: Removal" in the conceptual mitigation framework model.

5.2. CONCLUSIONS

1) The transfer of shellfish (clams, oysters, and mussels) from NIS-infested areas is a vector for both mobile (e.g. Green Crabs) and sessile (e.g. tunicates/ bryozoans) NIS. This is true

regardless of the intended end-use of the shellfish (e.g. commercial culture, biotoxin monitoring programs, or wild harvest).
   a. Green Crabs were entrained on all three shellfish species examined.
   b. Three NIS tunicates and two NIS bryozoans were also entrained in most of the cultured shellfish species examined.
   c. Additional species were entrained including native fish, non-cultured shellfish, crabs, shrimp, macrophytes (seaweeds, seagrasses, and algae), and snails (among many others).
2) The potential propagule pressure due to the shellfish movement vector may be sufficient to overcome transfer barriers, based on historical industry production.
3) The only mechanism to ensure NIS are not inadvertently moved from infested to non-infested waters via this vector is to restrict all movement of shellfish (*i.e.* strict quarantine).
4) Based on a literature review, no mitigation measures to remove or destroy NIS on cultured shellfish are 100% effective, suggesting any transfer of shellfish poses some level of invasion risk. These methods and their efficacy are described briefly below (see Section 4.2.1 for details):
   a. Mechanical removal: hand removal and power washing. Both methods are very labour intensive and ineffective for smaller species or life stages of NIS that are not visible to the naked eye or hidden deep within the product. Power washing can also facilitate the spread from harvest sites and dominance of NIS growing at culturing/harvest sites.
   b. Chemical removal: use of lime, acetic acid, or brine dips. These treatments have been experimentally tested, the results with lime showing a high percentage of removal of biofouling NIS. None of the treatments, including lime, effectively removed 100% of the biofouling species of interest. These treatments have not been tested on mobile NIS such as the Green Crab.
   c. Biological removal: use of grazers and predator species such as sea urchins. Each species tested removed biofouling NIS to varying degrees, but none fully eliminated NIS growing on the shellfish. As with chemical removal, this work has largely been done on sessile NIS and its effect on mobile NIS is largely unknown.
5) Based on the results of the experimental and processor studies, the present conditions of licence do not eliminate NIS propagule pressure. Due to the gaps identified it is probable that the intended reduction in propagule pressure is not realized.
6) A conceptual framework was developed to identify control points where management intervention, such as application of license conditions, could lower propagule pressure and hence invasion risk. A full assessment of the relative effectiveness of each control point in the framework is beyond the scope of this project.

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7. APPENDIX I

7.1. DFO PACIFIC SHELLFISH AQUACULTURE LICENSE CONDITIONS: 11 .3 MEASURES TO PREVENT THE SPREAD OF EUROPEAN GREEN CRAB (DFO 2013A).

a) In Pacific Fishery Management Areas 23 to 27 the licence holder shall thoroughly examine harvested shellfish (oysters, clams, scallop, and mussels) for signs of European Green Crab, and rinse the harvested shellfish prior to being removed from the harvest area.
b) Shellfish culture gear (trays, lines, etc) shall be thoroughly examined and rinsed prior to removal from growing areas in Pacific Fishery Management Areas 23 to 27 for use in another area.
c) Shellfish harvested from Pacific Fishery Management Areas 23 to 27 shall be wet stored or grown out only at approved licensed areas in Pacific Fishery Management Areas 23 to 27. Shellfish may be wet stored in tanks within licensed processing facilities where such activity is approved in the Quality Management Plan.
d) Shucked oyster shell from Pacific Fishery Management Areas 23 to 27 shall not be placed in or adjacent to the intertidal zone where it may be washed by the tide or where any entrapped crabs may reasonably travel to the shore until the shell refuse is sufficiently desiccated to kill any crab or crab larvae that may have accompanied the shipment.
e) Transport water from Pacific Fishery Management Areas 23 to 27 discharged in other areas shall be disposed of in such a manner that the water does not run back to the intertidal shore or enter intertidal waters.
f) Transport containers from Pacific Fishery Management Areas 23 to 27 shall be rinsed in such a manner that the water does not run back to the intertidal shore or enter intertidal waters.

Note: Additional requirements for harvest by species may be found in Part C.

7.2. ACRONYMS

AIS – Aquatic Invasive Species

CFIA – Canadian Food Inspection Agency

CPUE – Catch Per Unit Effort

CSAS – Canadian Science Advisory Secretariat

CW – Carapace Width, notch width: measured as the distance between the notches

immediately anterior of the fifth anteriolateral spine.

DFO – Department of Fisheries and Oceans

ITC – Introductions and Transfers Committee

NIS – Non-Indigenous Species

PARR – Program for Aquaculture Regulatory Research

PBS – Pacific Biological Station

SAR – Science Advisory Report

8. APPENDIX II

8.1. INTERNATONAL AQUATIC NON-INDIGENOUS SPECIES MITIGATION POLICIES WITH THEIR OBJECTIVES AND PERTINENT DETAILS

Country	Document Type and Name	Objective	Pertinent Details of Document
International	Report Report of the Working Group on Marine Shellfish Culture (WGMASC)	-	-
	Report Report of the ICES Working Group on Introduction and Transfers of Marine Organisms (WGITMO)	-	-
European Union	Regulation Council Regulation (EC) No 708/2007 of 11 June 2007 Concerning Use of Alien and Locally Absent Species in Aquaculture	To establish a framework governing aquaculture practices concerning invasive, locally absent, and non-target species to assess and minimize the potential impact of these animals on aquatic environments.	Article 1 1. This regulation shall apply to the introduction of alien species and translocation of locally absent species for their use in aquaculture in the Community taking place after the date this Regulation becomes applicable by virtue of Article 25(1). 3. This regulation shall cover all aquaculture activities located within the jurisdiction of Member States irrespective of their size or characteristics. It shall cover all alien and locally absent aquatic organisms farmed. It shall cover aquaculture using any form of aquatic medium. Article 4: Measures for avoiding adverse effects. Member States shall ensure that all appropriate measures are taken to avoid adverse effects to biodiversity, and especially to species, habitats and ecosystem functions which may be expected to arise from the introduction or translocation of aquatic organisms and non-target species in aquaculture and from the spreading of these species into the wild. Article 6: Application for a permit 1. Aquaculture operators intending to undertake the introduction of an alien species or the translocation of a locally absent species not covered by Article 2(5) shall apply for a permit from the competent authority of the receiving Member State. Applications may be submitted for multiple movements to take place over a period not longer than seven years.
Australia	Control Plan Controlling the Northern Pacific Sea Star in Australia	To minimize the rate of spread of the Northern Pacific Sea Star, and reduce its impacts on Australia's marine biodiversity and shellfish industries.	This report strongly promotes a preventative approach towards a national management plan to minimize the spread and impacts of this pest species. It summarizes and assesses the risk of transport of the Northern Pacific Sea Star by human mediated vectors, ballast and other, including aquaculture stock and gear. This report found that marine-based aquaculture may be a high-risk vector for entraining Northern Pacific Sea Stars due to their tendency to congregate at farms. Due to cleaning practices (immersion of shellfish in freshwater) and limited gear and stock movement most shellfish species are not considered high-risk vectors for sea star entrainment. However, in Tasmania, such cleaning is not practised by shellfish farmers, and oysters from there were identified as the highest risk for entrainment of sea stars in Australia.

Country	Document Type and Name	Objective	Pertinent Details of Document
Australia	Control Plan National Control Plan for the Northern Pacific Seastar	To establish a coordinated national response and implementation strategy to the Northern Pacific Seastar, and provide guidance on the development of future strategies to minimize the impacts and spread of this species.	This control plan is the practical application of a preventative approach to control the Northern Pacific Sea Star in Australia. It considers human-mediated vectors, including non-ballast vectors, such as aquaculture, as potential high-risk vectors of Northern Pacific Sea Star entrainment. Oyster farming, in particular, is recognized as a high-risk vector for sea star introductions or transfers. Prevention strategies include: freshwater immersion of stock, additional pest prevention measures for transfers from infested waters to high value aquaculture areas, public communication and awareness campaigns, and removal of reproductive adults prior to spawning. A prevention strategy already in place is aimed at preventing translocation of Northern Pacific Sea Stars in high risk areas in Tasmania by providing a set of guidelines that outline cleaning procedures and instructions on how to store the captured pests. Another strategy considered is modifying the timing of commercial operations to minimise interaction between the sea star larvae and the stock/equipment.
	Control Plan National Control Plan for the European Green Shore Crab	To establish a coordinated national response and implementation strategy to the European Green Crab, and provide guidance on the development of future strategies to minimize the impacts and spread of this species.	This control plan provides an overarching framework necessary to manage the European Green Crab in Australia. It includes a pest prevention strategy, a contingency plan for new introductions and translocations, and an impact management strategy. The plan recognizes the aquaculture industry as a vector for Green Crab introductions and transfers. To address this issue pest prevention and impact management strategies are provided. Examples of pest prevention strategies include: reducing abundance and mitigating impacts of Green Crabs in high value areas (e.g. aquaculture regions), providing additional pest prevention measures for transfer of Green Crabs from high risk areas or infested areas to high value areas, establishing a public awareness campaign, and providing guidelines outlining cleaning procedures to prevent translocation of Green Crabs associated with aquaculture stock and gear movement. The impact mitigation strategies suggested for aquaculture would not only reduce predation on shellfish but potentially reduce the number of Green Crabs entrained in aquaculture gear and stock. These strategies include gear modifications, adjustment of shellfish out-plant timing, and construction of aquaculture barriers (e.g. fences) to minimize interactions between Green Crabs and aquaculture equipment and stock
Canada	Act Fisheries Act	To provide management, protection, and conservation of fisheries resources and habitat in Canada.	Fishery (General) Regulations made under this act.
	Regulation Fishery (General) Regulations	To consolidate common aspects of fisheries regulations that come under the Fisheries Act. Under these regulations are restrictions on the release and transfers of live fish.	Release or Transfer of Fish 55(1): Subject to subsection (2), no person shall, unless authorized to do so under a license, (a) release live fish into any fish habitat; or (b) transfer any live fish to any fish rearing facility (2) Subsection (1) does not apply in respect of fish that is immediately returned to the waters in which they were caught. License to Release or Transfer Fish 56: The Minister may issue a license if (a) the release or transfer of the fish would be in keeping with the proper management and control of fisheries; (b) the fish do not have any disease or disease agent that may be harmful to the protection and conservation of fish; and (c) the release or transfer of the fish will not have an adverse effect on the stock size of fish or the genetic characteristics of fish or fish stocks.

Country	Document Type and Name	Objective	Pertinent Details of Document
Canada	Code National Code on Introductions and Transfers of Aquatic Organisms	To establish a mechanism by which the movement of aquatic organisms from one body of water to another is assessed and that the process of assessing the potential impacts of intentional introductions and transfers of aquatic organisms is consistent across jurisdictions.	This code ensures that before introductions or transfers take place they are thoroughly evaluated using established criteria. Intentional or authorized transfers and introductions of aquatic organisms only.
	Action Plan A Canadian Action Plan to Address the Threat of Aquatic Invasive Species	To minimize or eliminate the introduction of aquatic invasive species and remediate the impact of those species already established in Canada. Covers unauthorized introductions.	This document is intended to provide governments with a fluid framework for monitoring and evaluating possible sources of invasion and assessing the magnitude of risk associated with other potential pathways, including the live foodfish pathway.
	Restriction Canadian Shellfish Sanitation program - Manual of Operations	To outline legislation and procedures pertaining to shellfish aquaculture.	2.3.4 Restricted Classification Restricted is the classification of shellfish area where the harvesting of shellfish is not permitted, except by license issued under Management of Contaminated Fisheries Regulations (DFO, 1990) due to contamination by faecal material, pathogenic micro-organisms, poisonous or deleterious substances, to the extent that consumption of the shellfish might be hazardous.
	Restriction Restricting a Body of Water Protocol	To reduce the risk of transferring invasive tunicate species to uninfected waters of PEI by establishing a protocol restricting bodies of water.	Restricted bodies of water resulting in the requirement for an Introductions and Transfers License (Section 55/56 of the Fishery (General) Regulations) for the movement of shellfish products.
	License Conditions Shellfish Aquaculture License Under the Pacific Aquaculture Regulations 2012	To prevent the accidental transfer of invasive species, specifically *Carcinus maenas*, from the west coast to the east coast of Vancouver Island, BC via shellfish aquaculture practices.	Applied changes to Shellfish Licensing Conditions 11.2 For the purposes of wet storage of market-sized bivalves, the licence holder shall not wet-store shellfish originating from other than the licensed area unless written approval has been received from the Canadian Food Inspection Agency. 11.3 Measures to Prevent the Spread of European Green Crab (a) In Areas 23 to 27 the licence holder shall thoroughly examine harvested shellfish (oysters, clams, scallop, and mussels) for signs of European Green Crab, and rinse the harvested shellfish prior to being removed from the harvest area.(b) Shellfish culture gear (trays, lines, etc.) shall be thoroughly examined and rinsed prior to removal from growing areas in Areas 23 to 27 for use in another area.(c) Shellfish harvested from Areas 23 to 27 shall be wet stored or grown out only at approved licensed areas in Areas 23 to 27. Shellfish may be wet stored in tanks within licensed processing facilities where such activity is approved in the Quality Management Plan.(d) Shucked oyster shell from Areas 23 to 27 shall not be placed in or adjacent to the intertidal zone where it may be washed by the tide or where any entrapped crabs may reasonably travel to the shore until the shell refuse is sufficiently desiccated to kill any crab or crab larvae that may have accompanied the shipment.(e) Transport water from Areas 23 to 27 discharged in other areas shall be disposed of in such a manner that the water does not run back to the intertidal shore or enter intertidal waters.(f) Transport containers from Areas 23 to 27 shall be rinsed in such a manner that the water does not run back to the intertidal shore or enter intertidal waters.

Country	Document Type and Name	Objective	Pertinent Details of Document
Finland	Act Animal Disease Act (55/1980)	To prevent the spread of animal disease to and within Finland.	Section 14a (408/2008) Fish, crayfish living in water and molluscs, and fertilized gametes of same, may be grown for human food, for sale or for transfer to further growing or stocking in the sea or in waterways only by operators duly authorized by the Finnish Food Safety Authority Evira.
Finland	Strategic Plan Finland's National Strategy on Invasive Alien Species	To address the problems associated with invasive species in Finland.	This strategy is only concerned with intentional transfers and introductions of alien aquaculture species.
Ireland	Code of Practice Marine Aquaculture Code of Practice (Draft)	To establish a framework governing aquaculture practices in relation to invasive and locally absent species.	The code of practice is a voluntary code promoting types of behaviour but compliance with the code of practice will prevent the introduction and spread of invasive species and ensure compliance with legislation as identified. *Didemnum*: thoroughly wash and dry all equipment that was near an infestation of this species. It is important to do this where the rinse will not return to the marine environment. Slipper Limpet: aquaculture managers and owners should avoid getting spat material from areas that are known to have Slipper Limpet present or nearby. Asian Rapa Whelk: never take oyster spat from an area known to have *Rapena* present and transfer to Ireland.
Japan	Act Invasive Alien Species Act (Law No. 78)	To prevent the adverse effects of invasive species on ecosystems by regulating actions such as raising, planting, storing, carrying and importing invasive species.	(Prohibition of Transfers) Article 8: Any actions relating transfers (hereinafter “Transfers”) of IAS are not allowed. Nevertheless, this does not apply to the case where persons, who perform or intend to perform Raising of IAS in conformity with the provisions of Article 4, Subparagraph 1, shall conduct Transfers of the IAS between them, or to the cases stipulated by the Ministerial Ordinance. (Prohibition of Releasing, Planting, or Sowing) Article 9: IAS regarding Raising, import, or Transfers must not be released, planted, or sowed outside the Special Raising Facility for the IAS. (Promotion of Public Understanding) Article 28: About mitigating IAS and other matters involved with Alien Species, the government must endeavor to deepen public understanding through measures such as educational activities and public relation activities.
	Policy Basic Policy for Preventing Adverse Effects on Ecosystems Caused by Invasive Alien Species	To supplement the Invasive Alien Species Act, providing the basic framework and principles regarding selection, handling, and mitigation of aquatic invasive species.	This policy was created for efficient implementation of the Invasive Alien Species Act. It addition to the basic framework of the regulation, this policy includes the principles concerning the selection, handling, and mitigation of aquatic invasive species, as well as other important issues regarding the prevention of adverse effects on ecosystems caused by invasive species.
New Zealand	Act Biosecurity Act	To provide a legal basis for the management of pest and unwanted organisms.	46. Duty to report notifiable organisms: (1) Every person who—(a) at any time suspects the presence of an organism in any place in New Zealand; and (b) suspects that it is for the time being declared to be a notifiable organism under subsection (2) of section 45; and (c) believes that it is not at the time established in that place; and (d) has no reasonable grounds for believing that the chief technical officer is aware of its presence or possible presence in that place at that time,— shall without unreasonable delay report to the chief technical officer its presence or possible presence in that place at that time.52 Communication of pest or unwanted organism: no person shall knowingly communicate, cause to be communicated, release, or cause to be released, or otherwise spread any pest or unwanted organism except— (a) in the course of and in accordance with a pest management plan; or (b) as provided in an emergency regulation made under section 150; or (c) for a scientific purpose carried out with the authority of the Minister; or (d) as permitted either generally or specifically by a chief technical officer. Part 5: Pest Management. 54. Purpose of this PartThe purpose of this part is to provide for the eradication or effective management of harmful organisms that are present in New Zealand by providing for— (a)

Country	Document Type and Name	Objective	Pertinent Details of Document
			the development of effective and efficient instruments and measures that prevent, reduce, or eliminate the adverse effects of harmful organisms on economic wellbeing, the environment, human health, enjoyment of the natural environment, and the relationship between Māori, their culture, and their traditions and their ancestral lands, waters, sites, wāhi tapu, and taonga; and (b) the appropriate distribution of costs associated with the instruments and measures.
New Zealand	Management Strategy Regional Pest Management Strategies 2010-2015	To provide a strategic and governing framework for the efficient and effective management of invasive species (or pests) in Northland.	Section 6 of the plan specifically deals with marine pest management. This section outlines the reasoning for declaring species as pests, provides management objectives, and lists the associated regulations for each pest designation. The European Green Crab is considered an 'exclusion marine pest' or a potential pest species not known to be established in New Zealand. Exclusion marine pests are unwanted and notifiable organisms under the Biosecurity Act; therefore rules 46, 52, and 53 of the Biosecurity Act apply.
United States	Act National Invasive Species Act of 1996	To prevent unintentional introductions and spread of aquatic nuisance species through ballast water.	Section 1204 of this act calls for state management plans addressing introductions of aquatic invasive species.
	Strategic Plan Aquatic Nuisance Species Task Force Strategic Plan (2013-2017)	To prevent, monitor, and control aquatic invasive species, and increase public awareness regarding these species.	This plan contains a set of strategic goals, and associated objectives and action items that are intended to be completed in the next 5 years. Prevention, early detection, and rapid response are recognized as the best management tools to control aquatic nuisance species. It is also apparent that collaboration, cooperation, and coordination among jurisdictions, industry, and other groups are essential to effectively manage aquatic nuisance species.
	Management Plan Washington State Aquatic Nuisance Species Management Plan	To minimize the unauthorized or unintentional introduction of non-native aquatic species, especially the introduction and spread of aquatic invasive species.	The goal of this plan is to coordinate all current aquatic invasive species management actions in Washington, and to identify further management actions, especially those relating to aquatic invasive species. This plan acknowledges that the aquaculture and live seafood industries as major pathways for aquatic invasive species introductions. Washington has identified European Green Crabs as an aquatic nuisance species, and has established regulations, as well as a Green Crab monitoring and control program. This plan identifies gaps in the management of Green Crabs and provides further management strategies. Some of the management strategies are listed below: • Collaborate efforts with the live seafood industry, aquaculture industry, and Department of Fish and Wildlife shellfish biologists to prevent further introductions through these pathways. • Deem water bodies with invasive species to be designated as 'infested waters'. • Prepare and publish a list of all water bodies infested with European Green Crabs in Washington and other states. • Continue Green Crab monitoring and control efforts. • Develop and distribute information as part of an outreach program to the aquaculture industry to alert them of potential aquatic invasive species impacts from their operations. • Trace, assess, and manage pathways by which non-indigenous species enter Washington state, including live seafood and aquaculture pathways.
	Management Plan Oregon Aquatic Nuisance Species Management Plan	To minimize the adverse ecological, economic, and social impacts of aquatic nuisance species by preventing and managing introductions, population growth, and dispersal of these species into, within, and from Oregon.	This plan recognizes the pathway for unintentional introductions of fish, mollusks, and crustaceans through aquaculture practices. It also acknowledges the need for an implementation program that reviews and regulates intentional introductions, and also monitors pathways by which non-indigenous species can be unintentionally transported into the state of Oregon.

Country	Document Type and Name	Objective	Pertinent Details of Document
	Management Plan Alaska Aquatic Nuisance Species Management Plan	To prevent introductions, and identify and respond to invasive threats. This management plan addresses non-native aquatic nuisance species that have been and could be introduced into Alaskan waters.	This plan recognizes the European Green Crab as a potential invasive species of concern. The plan also identifies aquaculture as a major pathway for non-native aquatic species introductions. The main goal of the plan is "to coordinate with the public and federal, state, local, and tribal governments for the prevention and monitoring of invasive species and the development of an effective public information system."
United States	Code Washington Administrative Code (WAC) 232-12-016: Non-native Aquatic Species	To prevent introductions and spread of European Green Crabs and other non-native species into water bodies of Washington by restricting movement of aquaculture product from pest infested waters.	WAC 232-12-016 Non-native Aquatic Species. (4) Aquaculture provisions. It is unlawful to fail to comply with the following provisions regarding aquaculture and waters containing prohibited aquatic animal species or invasive aquatic plant species. (a) When a natural body of water is designated by rule as infested, ongoing aquaculture operations in that body of water are restricted from transferring product, equipment or associated materials until such time as the operator of the aquaculture operation submits to the department a plan to prevent the spread of invasive aquatic plants and prohibited aquatic animal species, and has received approval from the department of such plan
	Code Washington Administrative Code (WAC) 232-12-01701: Aquatic Nuisance Specie	To prevent introductions and spread of European Green Crabs and other non-native species into water bodies of Washington by designating them as deleterious species.	WAC 232-12-01701 Aquatic Nuisance Species (1) The following species are designated as deleterious exotic wildlife and aquatic nuisance species: (a) Zebra Mussels, including *Dreissena polymorpha* and other species commonly known as Quagga; (b) European Green Crabs, *Carcinus maenas*; and (c) Chinese Mitten Crabs, including all members of the genus *Eriocheir*.
	Code Alaska Administrative Code (AAC) 05 AAC 41.005: Permit Required	To restrict the import and export of live aquatic animals in the state of Alaska.	Transportation, Possession, and Release of Live Fish; Aquatic Farming 05 AAC 41.005. Permit required (a) Except as otherwise provided, a person may not transport, possess, export from the state, or release into the waters of the state, any live fish unless the person holds a fish transport permit issued by the commissioner, and the person is in compliance with all conditions of the permit and the provisions of this chapter. A fish transport permit will be issued for a fixed term subject to the provisions of (c) of this section. Notwithstanding the provisions of this subsection, and except as restricted under AS 16.05.240, a licensed processor may export live shellfish out of the waters of the state for human consumption without a fish transport permit only after complying with all applicable reporting requirements. (b) A fish transport permit authorizes only that operation specified in the permit. Any change of species, brood stock, or location requires a new permit. Any other change requires an amendment to the permit. (c) The commissioner shall suspend the permit, or particular provisions of the permit including amendments, if the commissioner finds (1) on the basis of new information or changed circumstances, that the permitted activity will adversely affect the continued health and perpetuation of native, wild, or hatchery stocks of fish; or (2) the permittee has failed to comply with permit terms or the provisions of this chapter. (d) Notwithstanding the expiration, termination or suspension of a fish transport permit, each permittee is responsible for the obligations arising under the terms and conditions of the permit, and under the provisions of this chapter. (e) Unless otherwise provided in regulation or by emergency order, a permit is not required for transportation of fish caught in a sport, personal use, subsistence, or commercial fishery from the place of harvest to a place within the state for processing, or commercially caught fish to a place within the state for sale.

Country	Document Type and Name	Objective	Pertinent Details of Document
	Regulation Oregon Administrative Rule (OAR) 635-005-0900: Oyster Import Applications and Permit	Prevent import of oysters that are diseased or harbour pests into the state of Oregon.	OAR 635-005-0900: Oyster import applications and permit (1) It is unlawful for any person to import oysters into this state for the purpose of planting or to plant the same in the waters of this state without first having obtained a permit to do so from the Director. (2) Such application shall be in the form of a letter and shall include the following information: maximum quantity to be imported, name of exporter, the approximate time the shipment will be made, and the name of the person or agency that will inspect the seed including a notarized certification from such person or agency at the time the oysters are inspected, declaring them to the best of his knowledge free from disease, infestation pests, and other substances which might endanger shellfish in the waters of this state.(3) The Director shall issue a permit to import oysters for planting in the waters of this state when it has been established to his satisfaction that a qualified person or agency will inspect the oysters and certify them as being free of disease, infestation pests, and other substances which might endanger shellfish in the waters of this state.
	Statute Alaska Statutes (AS) 16.40.100: Aquatic Farm and Hatchery Permits	To restrict the movement of shellfish from one mariculture to another within the state of Alaska to protect natural fish and wildlife resources.	AS 16.40.100. Aquatic farm and hatchery permits. (a) A person may not, without a permit from the commissioner, construct or operate (1) an aquatic farm; or (2) a hatchery for the purpose of supplying aquatic plants or shellfish to an aquatic farm. (b) A permit issued under this section authorizes the permittee, subject to the conditions of AS 16.40.100 - 16.40.199 and AS 17.20, to (1) acquire, purchase, offer to purchase, transfer, possess, sell, and offer to sell stock and aquatic farm products that are used or reared at the hatchery or aquatic farm; and (2) except as provided in (f) of this section, harvest and, without further cultivation, sell an insignificant population that may be present at the aquatic farm site of a wild stock of a shellfish species intended to be cultured at the site. (c) The commissioner may attach conditions to a permit issued under this section that are necessary to protect natural fish and wildlife resources.

8.2. INTERNATONAL AQUATIC NON-INDIGENOUS SPECIES MITIGATION POLICIES WITH THEIR JURISDICTIONAL AREA, RELEASE DATE AND REFERENCE

Country	Policy Level and Region	Document Type and Name	Year	Department or Authority	Target Group or Species	Reference
International	International	Report Report of the Working Group on Marine Shellfish Culture (WGMASC)	2010	International Council for the Exploration of the Sea	Marine shellfish culture	ICES. 2010. Report of the Working Group on Marine Shellfish Culture (WGMASC), 29 March–2 April 2010, Galway, Ireland . ICES CM 2010/SSGHIE:07 . 94 pp.
	International	Report Report of the ICES Working Group on Introduction and Transfers of Marine Organisms (WGITMO)	2012	International Council for the Exploration of the Sea	Marine invasive species	ICES. 2012. Report of the ICES Working Group on Introduction and Transfers of Marine Organisms (WGITMO), 14 - 16 March 2012, Lisbon, Portugal. ICES CM 2012/ACOM: 31. 301 pp.

Country	Policy Level and Region	Document Type and Name	Year	Department or Authority	Target Group or Species	Reference
	European Union	Regulation Council Regulation (EC) No 708/2007 of 11 June 2007 Concerning Use of Alien and Locally Absent Species in Aquaculture	2007	The Council of the European Union	Aquatic Invasive Species (AIS)	Europa summaries of EU legislation accessed January 2015; last updated 27/07/2001;
Australia	National	Control Plan Controlling the Northern Pacific Sea Star in Australia	2004	Department of Sustainability and Environment	Northern Pacific Sea star	Michaela Dommisse and Don Hough. March 2004. Final report for the Australian government department of the environment and heritage controlling the Northern Pacific Sea Star (*Asterias amurensis*) in Australia. Marine Strategy Department of Sustainability and Environment (DSE).
	National	Control PlanNational Control Plan for the Northern Pacific Sea Star	2008	Government of Australia	Northern Pacific Sea Star	National Control Plan for the Northern Pacific Sea Star *Asterias amurensis*. Prepared for the Australian Government by Aquenal Pty Ltd 2008
Australia	National	Control Plan National Control Plan for the European Green Shore Crab	2008	Government of Australia	European Green Crab	National Control Plan for the European Green Crab *Carcinus maenas*. Prepared for the Australian Government by Aquenal Pty Ltd 2008
Canada	National	Act Fisheries Act	1985	Government of Canada	Fisheries	Canada Fisheries Act 1985
Canada	National	Regulation Fishery (General) Regulations	1993	Government of Canada	Fisheries	Canada Fishery (General) Regulations
	National	Code National Code on Introductions and Transfers of Aquatic Organisms	2003	Department of Fisheries and Oceans Canada	Aquatic species	Canada National Code on Introductions and Transfers of Aquatic Organisms 2003
Canada	National	Action Plan A Canadian Action Plan to Address the Threat of Aquatic Invasive Species	2004	Canadian Council of Fisheries and Aquaculture Ministers Aquatic Invasive Species Task	Aquatic invasive species	A Canadian Action Plan to Address the Threat of AIS-2004

Country	Policy Level and Region	Document Type and Name	Year	Department or Authority	Target Group or Species	Reference
				Group		
	National	Restriction Canadian Shellfish Sanitation program - Manual of Operations	2011	Canadian Food Inspection Agency; Department of Fisheries and Oceans Canada and Environment Canada	Shellfish aquaculture	Canadian Shellfish Sanitation Program 2011
	Regional and Provincial; Prince Edward Island (PEI)	Restriction Restricting a Body of Water Protocol		Department of Fisheries and Oceans Canada	Invasive tunicates	Containment and Mitigation of Nuisance Tunicates on Prince Edward Island to Improve Mussel Farm Productivity
	Regional and Provincial; British Columbia (BC)	License Conditions Shellfish Aquaculture License Under the Pacific Aquaculture Regulations 2012	2012	Department of Fisheries and Oceans Canada	European Green Crab	DFO Shellfish Aquaculture Licence Under the Pacific Aquaculture Regulations 2012
Finland	National	Act Animal Disease Act (55/1980)	1980 (2008)	Government of Finland; Ministry of Agriculture and Forestry; Finnish Food Safety Authority Evira	Animals and animal products carrying diseases	Finland National Strategy on Invasive Alien Species 2012; Appendix 1
	National	Strategic Plan Finland's National Strategy on Invasive Alien Species	2012	Ministry of Agriculture and Forestry in Finland	Invasive species	Finland National Strategy on Invasive Alien Species 2012

Country	Policy Level and Region	Document Type and Name	Year	Department or Authority	Target Group or Species	Reference
Ireland	National	Code of Practice Marine Aquaculture Code of Practice (Draft)	2009	Invasive Species Ireland	Aquatic invasive species	Ireland Marine Aquaculture Code of Practice 2009:
Japan	National	Act Invasive Alien Species Act (Law No. 78)	2004	Government of Japan	Invasive species	Invasive alien species act (Law No. 78 (June 2, 2004)
	National	Policy Basic Policy for Preventing Adverse Effects on Ecosystems Caused by Invasive Alien Species	2004	Government of Japan	Invasive species	Basic policy for preventing adverse effects on ecosystems caused by invasive alien species (Cabinet Decision as of October 15, 2004)
New Zealand	National	Act Biosecurity Act	1993	Government of New Zealand	Pests and unwanted organisms	New Zealand_Biosecurity Act 1993:
	Regional; Northland	Management Strategy Regional Pest Management Strategies 2010-2015	2010	Northland Regional Council	Invasive species	New Zealand_Marine Pest Management Strategy - Section 6
United States	National	Act National Invasive Species Act of 1996	1996	United States Government	Aquatic invasive species	National Invasive Species Act 1996
	National	Strategic Plan Aquatic Nuisance Species Task Force Strategic Plan (2013-2017)	2012	Aquatic Nuisance Species Task Force	Aquatic invasive species	United States AIS Task Force Strategic Plan 2013-2017
United States	Washington State	Management Plan Washington State Aquatic Nuisance Species Management Pln	2001	Washington Department of Fish and Wildlife (WDFW)	Aquatic invasive species	Washington_Aquatic Nuisance Species Management Action Plan:

Country	Policy Level and Region	Document Type and Name	Year	Department or Authority	Target Group or Species	Reference
United States	Oregon State	Management Plan Oregon Aquatic Nuisance Species Management Plan	2002	Center for Lakes and Reservoirs (Portland State University)	Aquatic invasive species	Oregon_Aquatic Nuisance Species Management Plan:
	Alaska State	Management Plan Alaska Aquatic Nuisance Species Management Plan	2002	Alaska Department of Fish and Game	Aquatic Invasive Species	Alaska_Aquatic Nuisance Species Management Plan
	Regional and State; Washington	Code Washington Administrative Code (WAC) 232-12-016: Non-native Aquatic Species		Washington Department of Fish and Wildlife (WDFW)	European Green Crabs and other non-native aquatic species	Washington WAC Non-native Aquatic Species
	Washington State	Code Washington Administrative Code (WAC) 232-12-01701: Aquatic Nuisance Specie		Washington Department of Fish and Wildlife (WDFW)	European Green Crabs and other non-native aquatic species	Washington WAC Aquatic Nuisance Species
	Alaska State	Code Alaska Administrative Code (AAC) 05 AAC 41.005: Permit Required		Alaska Department of Fish and Game	Live aquatic animals	Alaska_05 AAC 41.005 Permit Required
	Oregon State	Regulation Oregon Administrative Rule (OAR) 635-005-0900: Oyster Import Applications and Permit		Oregon Department of Fish and Wildlife	Oysters	Oregon_OAR Oyster Import Applications and Permit
	Alaska State	Statute Alaska Statutes (AS) 16.40.100: Aquatic Farm and Hatchery Permits		Alaska Department of Fish and Game	Aquatic farms and hatcheries	Alaska_Statute 16.40.100