Integrated Multi-Trophic Aquaculture with the California Sea Cucumber (*Parastichopus californicus*): Investigating Grow-out Cage Design for Juvenile Sea Cucumbers Co-cultured with Pacific Oysters (*Crassostrea gigas*)

by

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B.Sc., Simon Fraser University, 2013

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Abstract

Excess nutrients in the form of uneaten food or waste from intensive, monospecies aquaculture farms can have negative effects on the surrounding natural ecosystem, causing eutrophication and benthic habitat degradation. Biomitigative techniques such as Integrated Multi-Trophic Aquaculture (IMTA) are being investigated for their ability to reduce these negative environmental impacts. IMTA is the co-culture of multiple species from complementary trophic levels, physically orientated in such a way that excess waste nutrients from the fed component are intercepted by the extractive species. For IMTA systems to become a sustainable aquaculture design alternative, it is important to ensure that infrastructure orientation and stocking densities of the extractive species maximize the amount of excess nutrients intercepted and overall system efficiency. Previous research has shown that the majority of wastes from fed finfish are made up of large organic particulates which sink rapidly to the benthos underneath or near the fish cages and which would be available to benthic deposit-feeding species. The California sea cucumber **(***Parastichopus californicus*) is a promising extractive species for IMTA on the west coast of Canada due to its deposit-feeding behaviour and its relatively high market price. Owing to the sea cucumber's morphology and ability to move through restricted spaces, containment can be difficult without reducing nutrient transfer and overall IMTA system efficiency (i.e. mesh sizes needed to contain small sea cucumbers may restrict flow of farm particulates to them). The overall goal of the present work is to effectively contain juvenile sea cucumbers in such a way that maximizes benthic extraction of large-particulate nutrients within an IMTA system.

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Chapter 1: Integrated Multi-Trophic Aquaculture with the California Sea Cucumber (*Parastichopus californicus*): Background and Introduction

1.0 Abstract

Excess nutrients in the form of uneaten food or waste from intensive, monospecies aquaculture farms can have negative effects on the surrounding natural ecosystem, causing eutrophication and benthic habitat degradation. Biomitigative techniques such as Integrated Multi-Trophic Aquaculture (IMTA) are being investigated for their ability to reduce these negative environmental impacts. IMTA is the co-culture of multiple species from complementary trophic levels, physically orientated in such a way that excess waste nutrients from the fed component are intercepted by the extractive species. For IMTA systems to become a sustainable aquaculture design alternative, it is important to ensure that infrastructure orientation and stocking densities of the extractive species maximize the amount of excess nutrients intercepted and overall system efficiency. Previous research has shown that the majority of wastes from fed finfish are made up of large organic particulates which sink rapidly to the benthos underneath or near the fish cages and which would be available to benthic deposit-feeding species. The California sea cucumber **(***Parastichopus californicus*) is a promising extractive species for IMTA on the west coast of Canada due to its deposit-feeding behaviour and its relatively high market price. Owing to the sea cucumber's morphology and ability to move through restricted spaces, containment can be difficult without reducing nutrient transfer and overall IMTA system efficiency (i.e. mesh sizes needed to contain small sea cucumbers may restrict flow of farm particulates to them). The overall goal of the present work is to effectively contain juvenile sea cucumbers in such a way that maximizes benthic extraction of large-particulate nutrients within an IMTA system.

2.0 Aquaculture

The aquaculture industry has been rapidly increasing over the past 40 years and is currently the fastest growing food production industry in the world (Béné et al., 2015). It is estimated that in 2014 aquaculture produced 44.1% (73.8 million tonnes, excluding aquatic plants and macroalgae) of the world's seafood, with an annual average growth rate of 5.8% (FAO, 2016). Aquaculture production by tonnage in 2014 consisted of 49.3% finfish, 27% aquatic plants, 15.9% molluscs, 6.8% crustaceans, and 0.9% other aquatic animals (Table 1.1). Similar to the rapid expansion of the agriculture industry in the 1940s–60s, coined the 'green revolution', this rapid growth of the aquaculture industry has been termed the 'blue revolution'. These increases in food production have occurred both due to market demand and technological advances, which have allowed for large crop production, usually in the form of large intensive mono-cultures, at minimized costs. Although these intensive mono-cultures have been demonstrated to be highly efficient in terms of production and labour costs, there has been a movement within the agriculture industry towards a more sustainable ecosystem approach, such as polyculture, to help promote healthy functioning ecosystems (DeFries et al., 2004; Kass, 1978; Tscharntke et al., 2005). Large intensive mono-cultures on land have been shown to have negative effects such as soil erosion, increased dependence on synthetic fertilizers, decreased biodiversity, and a reduction in ecosystem functions such as pollination (DeFries et al., 2004; Petreanu et al., 1997; Tscharntke et al., 2005). Large intensive mono-cultures in the marine environment have a different set of challenges and negative environmental impacts, but these could also be mitigated by an ecosystem management approach such as polyculture (Chopin et al., 2001; Troell et al., 2003).

Excess nutrients from intensively-fed monospecies aquaculture farms, in the form of uneaten food and waste, released into the surrounding environment can have negative impacts such as eutrophication and benthic habitat degradation (Frankic and Hershner, 2003; Gowen and Bradbury, 1987). Folke and Kautsky (1992) found that the characteristics of intensive mono-culture systems are similar to those of stressed ecosystems, where large inputs of energy are required for the survival of these open-throughput-based operations. These types of systems lead to reduced resource-use efficiency, decreased positive ecosystem interactions, and a farm more susceptible to parasitism, disease, and waste accumulation (Folke and Kaustky, 1992). To avoid this unbalanced and unsustainable situation, researchers have suggested polyculture techniques that would utilise ecosystem functions without degrading the resource base it depends on (Folke and Kautsky, 1992; Neori et al., 2007). Biomitigation technologies are currently being researched and implemented within various aquaculture systems to lessen the environmental impacts of excess nutrients on the surrounding ecosystems. The most common example of this is using seaweed species within or near an aquaculture farm as a way of capturing excess inorganic nutrients like nitrogen, a major cause of eutrophication (Abreu et al., 2011; Chopin et al., 2001; 2012; He et al., 2008). This potential solution of polyculture as a biomitigative measure to reduce negative environmental impacts and encourage ecosystem-based management is a more sustainable way to manage our aquaculture systems (Folke and Kautsky, 1992).

3.0 Integrated Multi-Trophic Aquaculture (IMTA)

Integrated multi-trophic aquaculture (IMTA) is the co-culture of multiple species from complementary trophic levels, farmed in proximity to one another in such a way that nutrients are recycled within this engineered ecosystem (Chopin et al., 2012). IMTA is an example of the extensive integrated polyculture suggested by Folke and Kautsky (1992) and by Frankic and Hershner (2003). The goal of IMTA is to increase the environmental sustainability of an aquaculture farm by recycling excess nutrients within the site. This is done through species diversification by incorporating profitable nutrient-extractive species, which not only increase ecosystem function via their uptake of wastes produced by the farm, but also are of commercial value (Chopin, 2012). This crop diversification may increase the economic sustainability of the farm and subsequently increase the farmer’s willingness to adopt this ecosystem-based approach over the highly-efficient and potentially environmentally-degrading mono-culture method. In a case study of aquaculture farms in Sanggou Bay, China, IMTA farms had a better cost-benefit analysis and higher net present value when assessed against comparable mono-culture sites (Shi et al., 2013).

IMTA farms can vary greatly depending on location and species cultured, although the general concept of IMTA comprises four major components: fed species, inorganic nutrient-extractive species, organic filter-feeding species, and organic deposit-feeding species. First, there is the fed-species component, examples of which include finfish and prawns (Chopin et al., 2012; Martínez-Porchas et al., 2010). Second, the inorganic nutrient-extractive species are often seaweeds, which readily absorb excess nitrogen and phosphorous from the aquatic environment (Chopin et al., 2001). Nitrogen is often limiting in the ocean while phosphorous is often limiting in freshwater environments and the addition of these nutrients in excess is often the cause of unwanted toxic algal blooms and eutrophication (Pitois et al., 2001). Many studies have demonstrated the ability of various seaweed species to absorb excess nitrogen from the fed-species component within an IMTA farm (Abreu et al., 2011; Ahn et al., 1998; Chopin et al., 2001; He et al., 2008; Neori et al., 2004; Petrell et al., 1993; Reid et al., 2013). Third, there is the organic filter-feeding species, which is often a suspended bivalve species such as oysters, scallops, and mussels. These bivalves extract the small organic particulates suspended within the water column and have been shown to be effective water filters (Nelson et al., 2012; Ren et al., 2012). Finally, there is the organic deposit-feeding species, which are generally benthic detritivores, capable of extracting larger organic particles from areas directly below or adjacent to the above fed-species component of the IMTA system (Cubillo et al., 2016; Hannah et al., 2013; MacDonald et al., 2013; Orr et al., 2014; Paltzat et al., 2008).

Currently, IMTA systems are in experimental development or near commercial scale in a number of countries including Canada, Chile, China, Ireland, South Africa, United Kingdom, USA (FAO, 2009), Korea (Kim et al., 2014), and Norway (Handå et al., 2012). Within Canada, on the east coast experimental IMTA farms have been in operation in the Bay of Fundy since 2001, culturing Atlantic salmon, blue mussels, and kelps (FAO, 2009). On the west coast of Canada, the first commercially licenced IMTA farm has been operating at a pre-commercial level since 2006, culturing sablefish, oysters, scallops, and kelps off the northwest coast of Vancouver Island in Kyuquot Sound (Cross, 2012). This SEAfood System, Sustainable Ecological Aquaculture, expands upon the ecological design of IMTA to address other issues such as use of chemicals in mono-culture (organic certifications) and carbon footprint (alternate energy) (Cross, 2012).

4.0 Sea Cucumber Aquaculture

Sea cucumbers have been identified as an ideal species to adopt in an IMTA system due to their deposit-feeding behaviour which enables them to utilize the large organic waste particulates which settle below an aquaculture farm (Cubillo et al., 2016; Lopes and Lemos, 2015; Zamora et al., 2016). This is especially important for IMTA nutrient recycling as benthic degradation via bio-deposition is a major concern for open-water and coastal aquaculture (FAO, 2009; Frankic and Hershner, 2003; Gowen and Bradbury, 1987; Kalantzi and Karakassis, 2006), as the majority of large organic particulates settle directly below or adjacent to an aquaculture farm (Mente et al., 2006). Sea cucumbers have the ability to directly ingest and assimilate these waste particulates as well as cause horizontal nutrient redistribution through bioturbation of the sediment as they move and feed across the benthos, decreasing benthic impacts and increasing benthic primary production (Crozier, 1918; Hannah et al., 2013; Hauksson, 1979; Moriarty, 1982; Orr, 2012; Slater and Carton, 2009; Uthicke, 1999, 2001; Yuan et al., 2015). In contrast, the suspended filter-feeding IMTA component is generally limited to small organic particulates suspended within the water column; thus restricted by waste particulate size and by spatial and temporal variations in particle concentrations as they move through the water column (Filgueira et al., 2017; Lander et al., 2013; MacDonald et al., 2011; Reid et al., 2009, 2010). Sea cucumbers have also been shown to feed selectively on sediments of high organic content and are capable of feeding directly on wastes produced by a number of fed aquaculture species (Hannah et al., 2013; Orr, 2012; Slater et al., 2009, 2011; van Dam-Bates et al., 2016). Sea cucumbers are also an ideal IMTA nutrient-extractive species due to their relatively high market value (Purcell et al., 2014), which is an important factor when selecting a species for IMTA systems as farmers are unlikely to adopt IMTA to cultivate low-value organisms (Chopin, 2012).

Sea cucumbers have been harvested in China for more than 400 years (Máñez and Ferse, 2010) to be consumed as a delicacy known as Bêche-de-mer (whole dried sea cucumbers) or used in traditional medicines (Purcell et al., 2014). Throughout ancient human history and carried through to current times, Chinese culture has highly valued the traditional medicinal properties of food. Within Chinese culture, sea cucumbers share the same ancient Chinese calligraphy character as ginseng, another highly-valued traditional Chinese medicine and are sometimes referred to as '*haishen'* which roughly translates to '*ginseng from the sea*' (Yang and Bai, 2015). Sea cucumbers have been prized for their traditional medicinal use as early as the Ming Dynasty (1368–1644 AD) and are still highly valued today, as Bêche-de-mer and traditional Chinese medicines make up the majority of the commercial interest and consumption of sea cucumbers globally (Slater, 2015; Yang and Bai, 2015). Other uses for sea cucumbers include: the longitudinal muscle bands are consumed in North America and Europe (Matthews et al., 1990; Slater, 2015) while the gonads are harvested and consumed in some traditional cultures (Conand, 1990). An additional growing interest in sea cucumbers is their use in biochemical research for the abundant bioactive compounds found in and isolated from the body wall and mussel bands, including: anti-cancer polymers (anti-tumorigenic and anti-angiogenic) and joint lubrication, anti-inflammatory, hemolytic, anti-fungal (Collin and Adrian, 2010; Yibmanstasiti et al., 2012), anti-bacterial, and anti-retroviral compounds (Kouzuma et al., 2003; McClure et al., 1992).

As the Chinese economy grows, there has been a large demographic shift with a rapidly-increasing middle class in this highly populated country (Ravallion, 2010). With this growing middle class, equipped with a disposable income, has come an increased market demand in recent years for delicacy sea cucumber products, with prices reaching up to an astonishing \$1,668 USD kg^{-1} and average price ranging from \$15 to \$385 USD kg^{-1} (Purcell et al., 2014). Due to this high value and market demand, many tropical species of sea cucumbers are being overfished, with a number in decline or listed as threatened (Bell et al., 2008; Fabinyi and Liu, 2014; Purcell et al., 2012, 2013). As adult sea cucumbers do not have an abundance of natural predators, they are slow moving and easy targets for wild harvesting by humans.. Their limited range and slow movement as well as high market value makes them prone to overfishing in jurisdictions where regulations are lacking or unenforced (Anderson et al., 2011).

As sea cucumber populations decline in many countries, fishermen who collect sea cucumbers by SCUBA, hooka, or free diving are forced to push their limits by continually diving deeper to access sea cucumbers. This has become a health concern for the fishermen as many have encountered or are at risk of physical health issues such as decompression sickness. Decompression sickness is caused by unsafe diving practices (including diving too long, too deep, or ascending too rapidly) which can lead to joint pain, inner ear issues, convulsions, paralysis, and death if not treated by hyperbaric oxygen therapy in a recompression chamber (Pauley, 1965). Hyperbaric oxygen therapy is costly and may be inaccessible to many fishermen, especially those of low socioeconomic standing or those located in remote areas (Alessia Kockel, marine biologist and dive instructor in rural Philippines, personal communication, August 5, 2016). Risk of decompression sickness increases with diving depth, yet desperate sea cucumber fishermen will often risk diving deep to access available populations or more valuable sea cucumbers, like the Whiteteat fish (*Holothuria fuscogilva*). This is a high-value species, with a retail value of \$128–274 USD kg^{-1}, which is often found in reef habitats at 10–50 m depth, recruiting in shallow areas and migrating to deeper areas (Purcell et al., 2012b). Akamine (2001) recorded experiences of sea cucumber fishermen of Mangsee Island in the southern part of the Palawan province in the Philippines and documented how the fishermen have adapted to sea cucumber resource depletion. At the time of the study, Mangsee Island had a population of approximately 6,000 people and the main fishing activities included dynamite fishing and diving for sea cucumbers. Akamine (2001) described how hooka diving fishermen, using air compressors, would often dive to 30–60 m depth during multi-day fishing trips lasting up to 43 consecutive days. He reported first-hand accounts of tragedy from unsafe diving practices including the following:

> *"In the mid-1990s, deeper fishing grounds were sought and the use of echo-sounders increased to explore underwater topography…fishing depth increased to 50 or 60 meters in search of H. fuscogilva. Naturally, they encountered several decompression accidents. For a year from July 1997, there were at least three divers who died from decompression accidents. In December 1998, there were three fishing vessels that sank with only two divers surviving out of more than thirty at the Jackson Atolls when they*

> *were hit by the typhoon. After this tragedy, the fishing shifted back to shallow waters in Malaysian territory of the Spratly Islands."* (Akamine, 2001, p. 605)

Aquaculture of these declining species has been shown to help relieve pressure from fisheries and can aid in re-stocking depleted populations (Battaglene, 1999; Purcell et al., 2012). Re-stocking wild populations of marine species with hatchery-raised juveniles is not a new concept and has been implemented at a large scale for Pacific salmon for over 100 years (ODFW, 2015). Re-stocking with hatchery-raised juveniles helps not only to restore declining populations but also to reconnect genetically-isolated populations (Bell et al., 2008) (due to the relatively limited spatial distribution and movement of sea cucumbers (e.g. 3.9 m d^{-1} for *Parastichopus californicus* (Da Silva et al., 1986) and 2 m d^{-1} for *H. fuscogilva* (Reichenbach, 1999)) as reproduction occurs via broadcast spawning. Bell et al. (2008) suggested that some management actions needed to restore small-scale tropical sea cucumber fisheries would include re-stocking no-take zones with hatchery-reared juveniles and rearing small wild-caught sea cucumbers in an aquaculture setup, such as sea pens, until they reach minimum harvestable size.

There are over 60 species of sea cucumbers of commercial interest throughout the world, with many populations overfished, at risk of depletion, or lacking information on population status (Table 1.2). Of these species, most are harvested within southeast Asia and the Indian ocean, where small-scale artisanal and commercial fishermen make up a large majority of the wild harvest, using SCUBA, hooka, or free-diving methods to collect the sea cucumbers by hand. Over the last three decades, sea cucumber wild harvest has been steadily increasing as fishermen exploit new areas and deeper depths (Fig. 1.1). Since 2002, culture of sea cucumbers has exponentially increased, due in large part to refined hatchery production techniques of the Japanese sea cucumber, *Apostichopus japonicus* (Fig. 1.1; Han et al., 2016). This has allowed production of sea cucumbers to more than double, with aquaculture now producing over 60% of total sea cucumber production (Table 1.2; Fig. 1.1). Globally, sea cucumber wild harvest and farming is approximately a $5 billion USD industry in China (Zhang et al., 2015), with over 90% of total global production coming from two species which are harvested and farmed in Asia; *A. japonicus* and *Holothuria scabra* (63.7 and 27.8%, respectively (Table 1.2)), with China alone producing 200,969 tonnes of cultured sea cucumber in 2014 (Han et al., 2016).

4.1 Farming Methods

Aquaculture production of sea cucumbers primarily consists of three farming methods: pond culture, ranching, and suspended/benthic contained culture (i.e. cages or pens), with the majority of sea cucumbers currently being produced via ranching or pond culture. Culture type can depend on location, physical attributes of an area (i.e. habitat type, water temperature, and salinity), species, and which method is most economical. The Japanese sea cucumber contributes to 99% of sea cucumber aquaculture production and over 60% of overall global sea cucumber production (Table 1.2).

Pond culture has become popular in Malaysia, Philippines, Indonesia, Vietnam, and northern China, with *A. japonicus* farmed in the latter and *H. scabra* farmed elsewhere. This culture method increased in popularity following a detrimental virus outbreak within the shrimp farming industry, where vacant shrimp ponds were converted to culture the more profitable sea cucumber (Chen and Chang, 2015). In some cases, the sea cucumbers and shrimp are grown together or in a crop rotation style, since co-culture has been found to improve shrimp growth rates and benthic conditions in the ponds (Chen et al., 2015a, 2015b; Ren et al., 2010; Thu, 2003). These marine ponds are generally 150 cm in depth and vary in area from 0.2 to 1 hectare. *Holothuria scabra* is solely cultured in southeast Asia due to its relatively high tolerance to salinity fluctuations as this can be an issue in pond culture (Tuwo and Tresnati, 2015). In order to convert the ponds from shrimp to sea cucumber culture, farmers add substrate including stone, tile, nets, tubes, and cement pipe. Adding substrate to the ponds is vital for culture success as it provides surface area for larval settlement as well as shelter for juveniles and adults (Chen and Chang, 2015).

Ranching, sometimes referred to as marine enhancement, is widely used for *A. japonicus* throughout China, Japan, and Russia, although the majority of production occurs in northern China within the Yellow Sea. Ranching is a culture method where hatchery-raised juveniles (3 cm or more in length) are released onto artificial reefs by divers and later collected by divers when they have reached harvestable size (Zhang et al., 2015). This culture method allows sea cucumbers to feed on natural sediment and has lower production costs, although harvesting is more difficult, juvenile survival is lower, and initial material costs are high (Chen and Chang, 2015). Site selection, size of juveniles at release, and improvement of benthic habitat (i.e. artificial reef) are all important factors in successful sea cucumber ranching. Site selection should consider water depth, current, salinity, temperature, benthic habitat, and substrate type as well as proximity to sources of pollution (Chen and Chang, 2015). Initial survival is low for small sea cucumbers, as low as 0.5% survival for very small juveniles (Chen and Chang, 2015), and increases with size. Once individuals reach 20 g, survival is almost 100% (*H. scabra*, Tuwo and Tresnati, 2015).

Sea cucumber ranching mostly consists of *A. japonicus*, although *H. scabra* is considered a good candidate due to their limited movement of 2 m a day (Tuwo and Tresnati, 2015), declining wild population, and high value. There has also been significant research interest in benthic ranching of *Australostichopus mollis* in New Zealand (Davey et al., 2010; Heath et al., 2015; Slater et al., 2009, 2011; Slater and Carton, 2007, 2009; Stenton-Dozey and Heath, 2009; Zamora and Jeffs, 2011, 2012a, 2013; Zamora et al., 2014) and some interest in *P. californicus* in Canada (van Dam-Bates et al., 2016) beneath shellfish farms, as the sea cucumbers are often found in naturally high abundances at these sites. Research has shown that *A. mollis* is retained within the impact footprint of a mussel farm, most likely due to the high amount of organics below the bivalves (Slater and Carton, 2010). Relocation experiments with *A. mollis* at a mussel farm and a control site found that a higher proportion of adult sea cucumbers remained within the farm area, compared to the control sites (Davey et al., 2010; Heath et al., 2015). Similarly, *P. californicus* has been shown to reduce random movement in the presence of higher organic sediments, comparable to high organic sediments below an aquaculture farm (van Dam-Bates et al., 2016), a trait which may keep them within farm boundaries. Adult *P. californicus* have been shown to move 27 cm h^{-1} in the summer and 4 cm h^{-1} in the winter (McCloskey, 2006).

Suspended/benthic contained culture (i.e. cages or pens) is an additional and slightly less popular culture method for sea cucumbers, the form of which varies depending on location, species, and life stage of the sea cucumber. Suspended cage culture is a more common grow-out method in the more southern provinces (Zhejiang and Fujian provinces) in China, where farmers take *A. japonicus* of approximately 50 g from northern locations and grow them in suspended cages in the south over the winter months (Chen and Chang, 2015). This is done to avoid the sea cucumber's dormant winter stage, increasing growth and shortening the culture period by six months or more (Chen and Chang, 2015). The sea cucumbers are kept within stacked suspended cages made of rigid plastic or net cages and fed an artificial diet (Chen and Chang, 2015). Pen and cage trials have been conducted with *H. scabra* using large pens (20–40 m^2 made of 5-mm mesh) in shallow coastal areas (Conand and Tuwo, 1996; Tuwo, 2004), as well as fully enclosed rigid cages of 1–3-mm mesh (with 50% survival for juveniles 1.5–6.3 g) (Pitt and Duy, 2004).

Experimental cage trials have also been conducted with *P. californicus* within salmon pens (18 m in diameter and 7.5-m deep with 5-mm mesh) (Ahlgren, 1998) and within modified oyster grow out trays (L×W×H: 56.25×56.25×21.25 cm, reinforced with mesh (5–20 mm) on the walls and lids, as well as solid or very fine mesh (0.5 mm) bottoms) (Hannah et al., 2013; Paltzat et al., 2008).

Alternative sea cucumber culture methods such as “full-cycle” land-based culture (Katow et al., 2015) and various IMTA culture methods are currently being investigated and developed. This includes seeding sea cucumbers within kelp aquaculture farms (Namukose et al., 2016), contained in cages below finfish pens (Yu et al., 2012), suspended in offshore containment with abalone (Kim et al., 2014). Co-culture with green-lipped mussels in New Zealand (Slater and Carton, 2009; Slater et al., 2007, 2009), with shrimp in China (Chang et al., 2004; Martínez-Porchas et al., 2010; Purcell, 2004; Purcell et al., 2006; Zheng et al., 2009), as well as others (Table 1.2). The largest challenges facing land-based culture are the prevalence of lesion disease, which can cause mass mortality in adult sea cucumbers kept long term in tanks, as well as artificial feed development (Katow et al., 2015).

Since survival of sea cucumbers in all culture types (pond, ranching, and cage) depends on the size of juveniles, hatchery growth is an important culture period which often includes an intermediate nursery stage to increase juvenile size before transferring to ponds, artificial reefs, or cages (Chen and Chang, 2015; Katow et al., 2015; Tuwo and Tresnati, 2015; Yu et al., 2015). For *A. japonicus*, these intermediate nursery stages can include tanks with corrugated PVC plates which are kept in the hatchery, or a juvenile marine nursery grow-out period where 50–100 juveniles are placed into “onion bags filled with balled-up polyethylene mesh” for 6–10 months until juveniles are an appropriate size for stocking pond or reef culture (ideally >3 cm, although one batch of hatchery-reared individuals can vary in length from 2 to 100 mm) (Chen and Chang, 2015; Katow et al., 2015). For *H. scabra*, there are three juvenile nursery stages, the first being outdoor tanks with a fiberglass, flexible PVC-cloth liner, or concrete surfaces with the tanks initially heavily shaded (Pitt and Duy, 2004). Once juveniles reach 1–3 mm they are moved to mesh bags (L×W×H: 2×2×1 m, with 450-µm mesh) which are placed within ponds (Pitt and Duy, 2004). When juveniles reach 1 g in size, after an average of 41 days, they are transferred to larger mesh bags (L×W×H: 4×4×1.2 m or 6×6×1.2 m, with 1-mm mesh) for approximately 2 months. This intermediate juvenile nursery stage provides protection from predators for the very small juveniles as well as keeps them within an area of higher dissolved oxygen levels than at the bottom of the ponds, increasing survival (Pitt and Duy, 2004).

In addition to sea cucumber aquaculture having the potential for relieving fishing pressure as well as re-stocking declining wild populations (Battaglene, 1999; Bell et al., 2008; Purcell et al., 2012), it could also reduce the negative impacts of aquaculture farms on the surrounding environment by reducing the amount of excess nutrients discharged from the system (Cubillo et al., 2016; Orr, 2012). Some examples of sea cucumbers already being co-cultured in an IMTA setting include: *A. japonicus* and *H. scabra* commercially cultured with shrimp in ponds in China (Chang et al., 2004; Martínez-Porchas et al., 2010; Purcell, 2004; Purcell et al., 2006; Zheng et al., 2009) and *A. japonicus* commercially cultured in suspended offshore systems with abalone within the Yellow Sea (Barrington et al., 2009) or seeded below kelp longline areas also in China (Yang et al., 1999). Many experimental or pilot-scale trials have been completed or are currently underway for IMTA culture with sea cucumbers including: *Cucumaria frondosa* with Atlantic salmon (*Salmo salar*), blue mussels (*Mytilus edulis*), and kelp (*Saccharina latissima*) in Canada (McPhee et al., 2015; Nelson et al., 2012); *Holothuria tubulosa* cultured in bottom cages beneath sea bream (*Sparus aurata*) pens in Spain (Macías et al., 2008); *Holothuria leucospilota* placed under fish (*Lutjanus erythopterus*, *Epinephelus fario*, and *Rachycentron canadum*) farms in bottom cages in southern China (Yu et al., 2012); and the Australian sea cucumber (*Australostichopus mollis*) co-cultured with green-lipped mussels (*Perna canaliculus*) in New Zealand (Slater and Carton, 2009; Slater et al., 2007; 2009), as well as others (Table 1.2).

4.2 Sea Cucumber IMTA Challenges

The potential for sea cucumbers to have a positive effect on nutrient recycling and to increase profit by incorporating them into an IMTA system is being increasingly recognized around the globe by scientists and commercial producers (Zamora et al., 2016). The challenges currently halting the expansion of sea cucumber IMTA farming include: genetic effects on wild populations by the introduction of hatchery-raised juveniles, estimating disease transfer risk to wild and other cultured populations, accurately evaluating their economic potential, and finding practical farming systems (Zamora et al., 2016). Disease transfer risk to both wild populations of sea cucumbers and to other species cultured within the IMTA system is difficult to estimate, but additional research should be conducted on diseases that affect sea cucumber populations and any potential sea cucumbers may have to harbour pathogens of other cultured species (Eriksson et al. 2012; Granada et al., 2015). With any new innovation for commercial production, it can be difficult to accurately evaluate the true economic potential as few examples exist which are directly comparable. Choosing a high-value species of sea cucumber, finding more efficient and streamlined production and processing methods, and evaluating start up or aquaculture gear conversion costs as well as local labour costs are some of the many variables to consider before integrating sea cucumbers into an IMTA system (Zamora et al., 2016).

Possible negative genetic effects due to releasing hatchery-raised juveniles within an area for IMTA farming can be mitigated in two ways. The first technique is restricting interactions between wild and cultured animals through containment structures or utilizing site selection and habitat type as biological barriers between the populations. The second mitigation technique would be to consider local genetics and reduce genetic bottlenecking during broodstock collection and spawning for hatchery production. By using these techniques, in combination when possible, any potential negative effect on the genetics of surrounding wild populations will be greatly reduced (Blankenship and Leber, 1995; Eriksson et al., 2012). For example, in Japan, for broodstock spawning, a hatchery producer utilizes at least 100 individuals collected annually from an area where hatchery-raised juveniles have not previously been released and the resulting hatchery-raised juveniles are only released in the area where the broodstock were collected from (Katow et al., 2015). Additionally, developing a method for differentiating wild and cultured sea cucumbers would be useful. Research for this is on-going, although no cost-effective and long-term method currently exists (Gianasi et al., 2015; Purcell et al., 2006).

Finding practical farming methods for sea cucumbers to be incorporated into an IMTA system is a challenge that will require innovative research and development for each culture method and will vary by location, species, and farm type. Any potential methods will need to aim for minimizing costs of materials, building/implementation, maintenance, and labour. In addition, sea cucumber culture methods should not be disruptive to the culture of other IMTA species in terms of farm cycles, operations, and the physical structures of the farm. If sea ranching is found to be the more appropriate method for integrating sea cucumbers into a farm site, then understanding movement of cultured sea cucumbers and their interactions with wild populations of sea cucumbers are important. Developing methods to restrict movement of the cultured sea cucumbers to the area affected by the farm and effective identification or tagging methods to distinguish cultured from wild individuals will be needed (Hair et al., 2006, Purcell et al., 2006a; Purcell and Blockmans, 2009; Stenton-Dozey, 2007b). For any culture type it is also important to consider the stocking density in terms of the primary goal of the sea cucumbers within the farm. For example, if the primary objective of sea cucumbers within the farm is bioremediation of the sediments, stocking densities may be higher than what would be ideal for sea cucumber production, as growth can be dependent on stocking density and optimal densities for sea cucumber growth may be different than those for nutrient recycling (Zamora et al., 2016; Zhang and Kitazawa, 2016).

For suspended or benthic-cage culture methods, it is important to consider any effect the cage will have on the bioremediation potential of the sea cucumber within the IMTA system and any incurred maintenance costs of the cages. Mesh sizes required to contain the sea cucumbers, especially small adults and juveniles, would likely greatly reduce the amount of farm particulates entering the cages, as well as cause a potential issue with biofouling which would increase labour and maintenance costs. For *A. japonicus* cultured in mono-culture in suspended cages in the southern provinces in China, artificial feed is required as the cages restrict the sea cucumbers from accessing sufficient natural food sources (Chen and Chang, 2015). Future sea cucumber cage designs for IMTA will need to optimise the bioremediation potential of sea cucumbers by allowing the maximum amount of farm particulates to be captured within the cages, while efficiently containing the sea cucumbers and minimizing biofouling, maintenance and water quality issues. There are now several prototypes of closed containment fish culture structures that can be moored at sea. Waste can be controlled and easily pumped into extractive containment structures. This may fit very well into sea cucumber culture.

5.0 The California Sea Cucumber (*Parastichopus californicus*)

5.1 Wild Fishery

The species of commercial interest on the west coast of North America is the California sea cucumber (*P. californicus*), which has been commercially harvested in Canadian waters since 1971 (DFO, 2015). The main market for this species is Hong Kong and mainland China for Bêche-de-mer and traditional medicines, where *P. californicus* is considered a mid-range value sea cucumber. Fishermen receive \$3.70–11.00 USD kg^{-1} split weight and retail value ranges from \$264.55 to \$639.30 USD kg^{-1} dried (Table 1.2).

Commercial harvesting consists of a dive fishery where sea cucumbers are collected by hand using SCUBA. In British Columbia, Canada, where approximately 38.5% of the wild sea cucumbers are captured, the fishery is managed with a fishing season (October–September), limited license holders with total allowable catch and individual quotas, as well as limited-entry licensing and area quotas and licensing, where areas are harvested on a three-year rotation (DFO, 2015; O'Regan, 2015). The wild fishery for *P. californicus* within Canada is thought to be managed sustainably, although experienced harvesters are concerned about overfishing and feel that management should lower quotas and further restrict licences (O'Regan, 2015). The remaining 61.5% of sea cucumber harvest occurs within the USA in Alaska, Washington, and California, with 33.9%, 18.9%, and 8.7% of total landings, respectively.

In the United States, *P. californicus* is harvested by divers in Alaska and Washington or by trawl and or divers in California. Each State has different management strategies, with estimate population harvest rates of 6.4% on 3-year rotational fisheries areas in Alaska, Management areas, quotas and limited licences in Washington, and limited number of licences with minimum landings per licence in California. In 2015, the State of Hawaii implemented an emergency ban on sea cucumber harvesting that was highly publicised gaining much media and public attention, this incident although not pertaining to *P. californicus*, has helped bring attention to the management of sea cucumber harvesting in the USA (Loomis, 2016).

Overall the fishery for *P. californicus* was valued at approximately 14 million USD in 2014, before processing, 5.4 million USD in Canada and 8.6 million USD in the USA (Table 1.2). Within Canada it has been shown that value-added processing, including packaging of muscle bands and drying, salting, or pickling sea cucumber skin, increases total value by 4 million USD, adding further economic incentive (DFO, 2015). Although this species only makes up approximately 0.5% of the global sea cucumber production, there is interest from aquaculture farmers to culture this species, due not only to its relatively high market value but also its potential for ecological benefit, resulting in increased social acceptability (Barrington et al., 2010).

5.2 Biology

5.2.1 Morphology and Distribution

The California sea cucumber, also referred to as the giant red sea cucumber, is commonly found in low-intertidal and subtidal rocky areas in quiet bays, ranging from Alaska to Baja California (Brumbaugh, 1980; Kozloff, 1983; Lambert, 1997). *Parastichopus californicus* can be found at a wide range of depths from intertidal to areas as deep as 183 m (Zhou and Shirley, 1996). They are thought to exhibit seasonal vertical migration behaviour, with higher densities in shallower water during spring and summer months (Cieciel, 2004).

Similar to other sea cucumber species, *P. californicus* has a soft body with an endoskeleton consisting of small calcareous ossicles. The ventral side of the sea cucumber is lined with rows of tube feet which use internal hydrostatic pressure from the water vascular system for locomotion, a defining characteristic of the Echinodermata family (Lambert, 1997; Fig. 1.2: A,C). Within the body there are five longitudinal muscle bands which allow the sea cucumber to expand and contract its body (Cameron, 1985). The sea cucumber has a distinct anterior oral and posterior end where the anus or cloaca is located (Fig. 1.2: A). The anterior end of the sea cucumber contains twenty buccal or peltate oral tentacles used for feeding, which are modified tube feet and also utilise the water vascular system for movement (Fig. 1.2: B; Pechenik, 2010). The pentactula larvae stage of the sea cucumber have five oral tentacles, and once they grow more than five they are considered juvenile sea cucumbers yearlings, age 0, with a very large variation in size and development in juvenile sea cucumbers 0-1 year of age post settlement (Cameron, 1985).

Within the body is the coelomic cavity, mostly consisting of water, where the visceral organs are located. Visceral organs consist of the sea cucumbers digestive system, respiratory trees and gonads. Holothurians are the only echinoderms that have truly specialized internal respiratory system; respiratory trees (Fig. 1.2: D), the respiratory tree are evaginations of the posterior digestive system and connected to the cloaca which pumps seawater in the respiratory trees, and water is pumped back out by the contraction of the respiratory tree tubules (Jaeckel and Strathmann, 2012; Pechenik, 2010). The California sea cucumber has separate sexes, with a 1:1 ratio of male to female in the wild, with no external sexual dimorphism. The gonads, either testis or ovaries, are located within the coelom consisting of two tufts of elongate bifurcating tubules, ripe gonad tubules in adults are cream-white in males and bright orange in females (Cameron, 1985).

The soft body wall, longitudinal muscle bands, as well as the nature of their respiratory process (coelomic fluid easily passed in and out of the body and respiratory trees), make mass and linear measurements of body size difficult and unreliable (Cameron and Fankboner, 1989). As such, alternative techniques of measurement have been developed, such as size index, S.I. (S.I. = length × width × 0.01) (Yingst, 1982) and immersed wet weight (Hannah et al., 2012). Although lethal, the most accurate form of measurement, used commercially, is split weight, where the body wall is split longitudinally and the coelomic fluid removed before measuring (Hannah et al., 2012). New methods using image analysis software are currently being developed to measure and estimate sea cucumber body size and weight *in situ* for *A. japonicus* (Liu et al., 2015) that could possibly be used for *P. californicus* studies in the future.

5.2.2 Feeding Behaviour and Movement

The California sea cucumber is a deposit-feeding detritivore which ingests benthic detritus, absorbing the organic content. The detritus is typically made up of phytoplankton, bacteria, and decaying plant and animal matter that settle to the benthos. Mucous-covered peltate oral tentacles, which have been described as “cauliflower-like structures” (Bouland et al., 1982; Fig. 1.2: B), are mechanically powered by the water-vascular system and collect detritus by adhesion, bringing it to the pharynx (Cameron, 1985). The sea cucumbers are also capable of suspension feeding using their oral tentacles and because the sea cucumber’s respiratory tree is an evagination of the posterior digestive system, *P. californicus* is also capable of suspension feeding via its anus (Jaeckle and Strathmann, 2013). Pervious research has shown that *P. californicus* demonstrates selective feeding on sediments with higher amounts of organics (Paltzat et al., 2008). van Dam-Bates et al. (2016) found that *P. californicus* demonstrated selective feeding behaviour on sediments of higher organics, as well as increased random movement in the presence of higher organic sediments, although their overall movement is non-directional.

Sea cucumbers move along the benthos (or any structure at any angle) using suction via their tube feet which is an extension of their water vascular system and well as their longitudinal muscle bands to contract and expand their body. *Parastichopus californicus* is thought to be continuously feeding as it moves non-directionally across the sea floor (Da Silva et al., 1986, Cieciel, 2004). During the fall and winter months the California sea cucumber shows reduced feeding and reduced movement, moving 19.2 ± 2.08 cm h^{-1} in the summer and 7.9 ± 1.25 cm h^{-1} in the fall for adult sea cucumbers (Cieciel et al., 2005; McCloskey, 2006).

5.2.3 Visceral Atrophy, Evisceration, and Aestivation

Sea cucumbers, especially tropical species, are known for their ability to eviscerate their respiratory and digestive organs in response to stress and as a defensive mechanism (Bakus, 1973). For most species of sea cucumbers, evisceration of their intestine and respiratory tree will occur through their cloaca in times of stress (Mashanov and García-Arrarás, 2011), two exceptions being *Eupentacta fraudatrix* and *Holothuria difficilis* which will eviscerate through their mouth (Vladimir and Yu, 2011) or through a tear in the body wall (Kille, 1937). Loss of the digestive and respiratory organs is not lethal for the sea cucumbers as they will regenerate them. Regeneration time of visceral organs varies by species, from seven days for complete regeneration in *H. scabra* (Bai, 1971) to approximately 30 days for *Thyone briarues* (Kille, 1935), *A. japonicus* (Zheng et al., 2006b), *Holothuria glaberrima* (García-Arrarás et al., 1998), and *P. californicus* (Fankboner and Cameron, 1985), and up to 145 days for *A. mollis* (Dawbin, 1948).

Although evisceration is seen less often as a 'defensive' response in temperate sea cucumber species, it has been suggested that adult *P. californicus* undergo seasonal evisceration in the fall and early winter (Swan, 1961). Fankboner and Cameron (1985), however, have shown that *P. californicus* undergoes seasonal atrophy, where visceral organs are absorbed, rather than eviscerated, during winter months due to reduced food availability. During this winter season, many of the adult *P. californicus* enter this dormant stage, resulting in reduced body weight as feeding ceases and stored energy in the body wall is utilized (Fankboner and Cameron, 1985; Hannah et al., 2013; Paltzat et al., 2008).

Similarly, the temperate and commercially-important sea cucumber *A. japonicus*, also has a seasonal dormant state known as aestivation, which occurs in most mature adult sea cucumbers during the summer months (Wang et al., 2015). Aestivation is a strategy used by various animals to survive during extreme conditions and lack of food (Abe, 1995; Bartholomew and Cade, 1957; Gatten, 1985; Storey, 2002; Storey and Storey, 1990). *Apostichopus japonicus* will respond to high summer temperatures by migrating to deeper environments, reducing movement, stopping feeding, and reducing its size (Wang et al., 2015). During this dormant state, *A. japonicus* undergoes biochemical and physiological changes, including digestive organ degradation and a change in digestive enzyme activity. Yang et al. (2006) demonstrated how sea cucumber metabolic rate is dependent on temperature as well as body size, with small (21.2 ± 4.7 g) sea cucumbers' metabolic rate peaking at 25°C, while large (148.5 ± 15.4 g) and medium

(69.3 ± 6.9 g) sizes peaked at 20°C. Yuan et al. (2007) discuss how the aestivation state alters the sea cucumbers' energy budget, as defecation and respiration accounts for approximately 50% and 19.8–39.4% of the budget, respectively. Aestivation allows for a reduced oxygen consumption of 54.4–79.7% and eliminates the energy used for feeding and defecation (Yuan et al., 2007).

Reducing metabolic activity to survive extreme conditions and lack of food is a widely used survival strategy for many forms of life (Gatten, 1985; Geiser, 2004; Guppy et al., 1994; Guppy and Withers, 1999; Hand and Hardewig, 1996; Storey, 2002; Storey and Storey, 1990). Guppy et al. (1994) stated that "metabolic depression, in the face of environmental stress, has been reported in all major invertebrate phyla with the exception of Echinodermata, and in all vertebrate classes" (Guppy et al., 1994, pp. 175). As seen with aestivation in *A. japonicus*, visceral atrophy in *P. californicus*, and evisceration in most other sea cucumber species, Holothuroids are not an exception of metabolic depression survival strategies, but rather an extreme version of it, where organs are either largely reduced or completely expelled. Digestive organs can be energetically costly to an animal and when food availability is scarce or caloric value is low the animals can reduce metabolic rate by either reducing the physical size of the digestive organs or by relocating to an area of lower temperature, since metabolic rate depends on both size of the animal and temperature (Hand and Hardewig, 1996; Storey and Storey, 1990). This behavioural response to lower metabolic rate by relocating to an area of lower temperature may also provide insight to the adult sea cucumber's vertical migration to deeper waters, in the winter for *P. californicus* and the summer for *A. japonicus*.

5.2.4 Reproduction and Life History

Although *P. californicus* does not exhibit sexual dimorphism, the sexes are separate and the animal reproduces via broadcast spawning, releasing either sperm or eggs into the water column. The eggs, or oocytes, are translucent spheres with a diameter of 150 µm, of which one female can produce hundreds of thousands per spawning event (Cameron and Fankboner, 1989), although as like other sea cucumber species there is a positive relationship between body size and reproductive output (Lawrence, 1987). In the wild, spawning occurs throughout the spring and summer months, but researchers have been successful at artificially inducing spawning in the laboratory year-round (Cameron and Fankboner, 1989). Once fertilization in the water column occurs, three pelagic stages develop including: gastrula, auricularia, and doliolaria (Cameron and Fankboner, 1989). The gastrula stage occurs around 40–64 hours after fertilization and development of the auricularia stage begins on the 6th day with feeding beginning around the 13th day after fertilization (Cameron and Fankboner, 1989). This feeding larval auricularia stage remains in the water column for approximately 65–125 days until it undergoes a short metamorphosis into the doliolaria larval stage and within 24–48 hours it will develop into the pentactula stage (Cameron and Fankboner, 1989). The ‘juvenile’ that arises from the metamorphosis of a doliolaria larva is morphologically similar to an adult and has settled out of the water column. It is referred to as pentactula larva until it possesses more than the five original buccal tentacles. When a pentactula larva grows additional buccal tentacles, it is then considered a juvenile (0+ year class). Although aging techniques have yet to be developed for this species, and little quantitative information is available for growth rates of *P. californicus,* animals often reach a harvestable size of approximately 500 g in 4 to 5 years (DFO, 2014), with mature animals thought to be ≥56 months (Cameron, 1985). Size at first maturity is unknown for *P. californicus,* although it would be suspected to be similar to the temperate Japanese sea cucumber *A. japonicus*, which first reaches sexual maturity at about two years, when individuals weigh approximately 250 g (wet weight) (Wang et al., 2015). Broodstock collection for *A. japonicus* is suggested for individuals aged two years or more with relaxed body length greater than 20 cm and total wet weight greater than 200 g (Liu et al., 2015).

5.2.5 Predation

Although reports of predation on *P. californicus* are few, Cameron and Fankboner (1989) found that predatory sea stars, *Solaster dawsoni* and *Solaster stimpsoni,* would occasionally attack adult *P. californicus*. The adults were never consumed by the sea stars during observation, but *S. dawsoni* predated and consumed juvenile sea cucumbers in aquaria experiments (Cameron and Fankboner, 1989). The researchers also reported juvenile *P. californicus* being consumed by hermit crabs (*Pagurus hirsutusculus*) (Cameron and Fankboner, 1989). In predation studies, *P. californicus* exhibit a ‘swimming’ behaviour response to predators, involving vigorous sinusoidal or undulating motion. This swimming response will slightly displace the sea cucumber away from its predator, although with juveniles negligible displacement was observed and the efficiency of the swimming escape behaviour increases with animal size (Cameron, 1985). A more recent study by Larson et al. (2013) found that sea otters (*Enhydra lutris*) are predators of *P. californicus*, sea cucumbers making up 2 to 12% of the sea otter’s diet in southeastern Alaska and their presence having a negative effect on sea cucumber populations. This is an especially important predator to consider for any future aquaculture developments as sea otter populations have been increasing and expanding in recent years (Larson et al., 2013). There are no recorded predation attempts on juvenile *P. californicus* by fish species, although juvenile sandfish sea cucumbers, *H. scabra*, are frequently predated upon by triggerfish, emperor fish, and breams (Hamel et al., 2001; Pitt, 2001; Pitt and Duy, 2004) as well as by crabs (Lavitra, 2008; Pitt and Duy, 2004).

5.2.6 Habitat Preference and Juvenile Behaviour

Parastichopus californicus is most often found on, and thought to prefer, hard substrates such as bedrock, boulders, gravel, or crushed shell (Campagna and Hand, 2004; Zhou and Shirley, 1996). Other sea cucumbers, including *A. japonicus*, are also found to prefer hard substrates and it is suggested as a farming technique to create artificial habitat by adding hard substrata (Xing et al., 2012). Juvenile *P. californicus* appear to be more susceptible to predation and exhibit strong cryptic behaviour. They have been found to closely associate with various red algae which provides refuge from predators (Cameron and Fankboner, 1989). Due to this cryptic behaviour, small juveniles are seldom encountered *in situ*, with the exception that they are often found in high abundances on existing oyster farm gear (Cheng and Hillier, 2011). This cryptic behaviour appears only to be demonstrated during the small juvenile stage where they can only be found *in situ* within “fissures or crevices that afforded overhanging protection on a nearly vertical rock wall extending from 2 to 20 m” (Cameron, 1985). *Cucumaria frondosa*, a suspension-feeding sea cucumber with a similar life history to *P. californicus,* also exhibits a strong cryptic behaviour at the juvenile stage and until they reach a length of ~35 mm, when they will move to more exposed areas (Hamel and Mercier, 1996). It is thought that juvenile *P. californicus* in the 2-year class or older are at a size where they are immune to predation by predatory sea stars (Cameron, 1985). The cryptic behaviour of juvenile sea cucumbers is also seen in tropical species like *Actinopyga echinites* (Wiedemeyer, 1994). Wiedemeyer (1994) conducted field experiments on the habitat preference of small juvenile *A. echinites* and found that they had a strong preference for solid substrates and showed cryptic behaviour with a tendency to hide in narrow crevices of hard substrates, suggesting a distinct recruitment strategy.

6.0 *Parastichopus californicus* and IMTA

The earliest research published on the potential of California sea cucumber co-culture (with finfish) was in 1998 in Alaska (Ahlgren, 1998). This study was not focused on IMTA, but on the potential biofouling mitigation ability of the sea cucumber reared in salmon cages. One hundred large adult sea cucumbers (contracted length: 30–40 cm) were placed in circular floating fish pens (diameter: 18 m, depth: 7.5 m, mesh size: 5 mm) holding approximately one million pink (*Oncorhynchus gorbuscha*) or chum (*Oncorhynchus keta*) salmon fry. The study found that the sea cucumbers consumed a significant amount of the fouling debris on the net pens and that they were also capable of consuming decomposing salmon fry, turning obstructing biofouling debris and unwanted waste into a marketable product (Ahlgren, 1998).

Ten years later, Paltzat et al. (2008) investigated the feasibility of co-culture of the California sea cucumber with suspended Pacific oysters at a farm off Quadra Island, British Columbia. For this study, six sub-adult sea cucumbers (contracted length: 8–13 cm) were contained within High Flow™ (Fukui North America, Eganville, ON, Canada) oyster grow-out trays (L × W × H: 56.25 × 56.25 × 21.25 cm), reinforced with wire mesh (0.625 cm) to prevent escapees and a solid PVC insert in the bottom of the tray to retain biodeposits. The trays were suspended 2.5 m below oyster strings (total depth: 8.5 m) for approximately 12 months. This study was successful, with no sea cucumber mortalities, overall positive sea cucumber growth (mean weight increase: 42.9 g), and an average assimilation efficiency of 40.4% (Paltzat et al., 2008), demonstrating the feasibility of California sea cucumber co-culture with shellfish and the ability of the sea cucumber to utilize shellfish waste and pseudofaeces.

Paltzat et al. (2008) also found that *P. californicus* showed a seasonal change in its feeding behaviour, associated with visceral atrophy, the annual resorption of the digestive organs during the dormant winter stage (Yingst, 1982; Fankboner and Cameron, 1985). Although little is known about the factors that trigger this seasonal dormancy in *P. californicus*, Paltzat et al. (2008) suggested that the reduced food quality (significantly lower organic content) may have been a factor in inducing visceral atrophy.

Orr (2012) investigated the potential organic extractive capabilities of a number of invertebrates for IMTA co-culture with sablefish (*Anoplopoma fimbria*). This was accomplished by conducting laboratory feeding trials to assess the organic extractive potential of various invertebrates fed sablefish waste. The invertebrates included were green sea urchins, basket cockles, blue mussels, spot prawns, and California sea cucumbers. Adult *P. californicus* were fed sablefish waste and natural sediment for comparison *in vivo*. Orr (2012) found that the sea cucumbers had a significantly higher absorption efficiency when feeding on the sablefish waste than when consuming the natural sediment diet (45% vs 23%, respectively), the former having a higher organic content.

Hannah et al. (2013) investigated the growth and survival of the California sea cucumber contained within cages suspended beneath a sablefish pen at an IMTA farm on the west coast of Vancouver Island. This 12-month field trial tested the effects of sea cucumber size and stocking density on growth and survival, with ‘small’ and ‘large’ sea cucumbers weighing 7 to 99 g and 100 to 565 g whole wet weight, respectively. Three stocking densities were tested: 12, 17, and 21 individuals m^{-2}. The sea cucumbers were contained within modified oyster-culture trays (L × W × H: 57 × 57 × 21 cm), similar to Paltzat et al. (2008), with added mesh along the sides and top for containment, 20-mm mesh for the large sea cucumbers and 5-mm mesh for the small sea cucumbers, in addition to very small mesh (mesh size: 0.5 mm) on the cage bottom to retain organic particulates. That study found that small sea cucumbers suspended below the sablefish pen grew significantly faster than control individuals ~250 m away from the fish farm and that stocking density had a significant direct effect on growth of both small and large size classes of sea cucumber (Hannah et al., 2013). The small sea cucumbers suspended below the sablefish pen were also efficient at reducing total organic carbon and total nitrogen of the sablefish waste by 60.3% and 62.3%, respectively, and maintained a high survival rate throughout the study (mean survival: 99.5%). Hannah et al. (2013) also observed seasonal feeding behaviour and seasonal change in growth, with decreased growth rates and body mass during the fall and winter months, the effect increasing with sea cucumber size and stocking density.

As part of a research project investigating interactions between wild and ranched sea cucumbers, van Dam-Bates (2014) used laboratory trials to investigate movement and containment of *P. californicus*. He found that fences made with rigid plastic mesh had significantly fewer escapes than that made with flexible nylon netting and suggested that fencing would need to extend beyond the surface of the water to fully prevent any escapes. van Dam-Bates (2014) also concluded that small mesh sizes would be necessary for containment, as he observed sea cucumbers “squeezing their bodies through openings that were up to a third their contracted width”. In further laboratory trials assessing movement of adult *P. californicus* in response to organically-enriched sediments, van Dam-Bates et al. (2016) found that the total organic material (TOM) of sediments altered the sea cucumbers’ foraging behaviour, with more rapid and random movement in relatively high-TOM (~8.0%) sediment than in areas of relatively low TOM (~1.4%). This difference in foraging behaviour in response to available organic material may help to explain how aquaculture tenures could retain benthic-ranching populations at a farm site, but also may be a mechanism for how wild individuals are retained and found in higher abundances at some aquaculture sites (van Dam-Bates et al., 2016).

6.1 Potential IMTA Farming Options for the California Sea Cucumber

Potential culture options for *P. californicus* include: benthic ocean ranching, benthic cage culture, and suspended cage culture. Factors that would determine the appropriate culture method would include: interactions with wild populations, retaining sea cucumbers within the tenure area, predation risk, maximizing the biomitigation potential of the sea cucumbers, risk of harmful anoxic conditions, practicality for the farm (i.e. not disruptive to other farm operations), and which method farmers find most economical in terms of building, maintenance, and overall labour costs. Understanding movement of the sea cucumbers and knowing whether or not full containment is necessary (or if the sea cucumbers are naturally retained within the farm area without containment) are important in determining whether or not sea ranching is an appropriate method. It is also important to consider any effect that a containment structure will have on the bioremediation potential of the sea cucumber within the IMTA system, and any effect on the hydrodynamics of the area (e.g. reducing benthic flows can increase risk of anoxic conditions).

6.1.1 Benthic Ocean Ranching

Benthic ocean ranching is likely to be the best economical option for sea cucumber culture as building, labour, and maintenance costs would most likely be lower than containment culture (Zamora et al., 2016). The lack of restricting cages or mesh eliminates any potential biofouling issues that are associated with other culture types. Since the sea cucumbers' movement along the benthos is unrestricted, the entire benthic area of the farm is available as a food source for them, maximizing the nutrient recycling capability of the IMTA system, including benthic sediment horizontal bioturbation. This culture method would most likely provide a greater culture surface area as well as food availability for the sea cucumbers, increasing production potential within the farm.

Benthic ocean ranching is limited to farm locations with an appropriate depth for harvesting by divers and with a substratum type suitable for the sea cucumbers. It is suggested by *A. japonicus* farmers that an area with fine sand with an artificial reef or added oyster shell is best as these add an additional incentive for the sea cucumbers to remain within the farm area, as they are thought to prefer hard substrates (Gavrilova and Kucheryavenko, 2011; Zhang et al., 2015). Although the sea cucumbers are more likely to be retained in the high organic areas under a farm (Davey et al., 2010; Heath et al., 2015; Slater and Carton, 2010; van Dam-Bates et al., 2016) they are not contained by a physical barrier, and thus are not protected from predators or kept separate from wild populations of sea cucumbers. Without effective tagging techniques, the farmer cannot differentiate between the hatchery-reared out-planted individuals and wild sea cucumbers within the lease area, creating a potential conflict between wild harvesters and farmers (Zamora et al., 2016). An additional possible issue is the difficulty in harvesting and monitoring the sea cucumbers as this requires SCUBA divers. Also, if a farm site has high levels of organics, particularly before the release of hatchery-raised sea cucumbers, there is a greater risk of harmful anoxic conditions detrimentally affecting the juveniles; in this case a juvenile-nursery intermediate stage may be required (Yu et al., 2013).

One option may be benthic ranching with a fence structure around the perimeter of the tenure with a mesh size and wall height that would limit the cultured sea cucumbers to the area affected by the farm and restrict wild sea cucumbers from entering the tenure, although it will most likely not provide protection from most predators and would not exclude wild sea cucumbers that settle on the aquaculture gear or in the tenure as larvae. Additionally, the fence structure may have to extend past the water line to be fully effective (van Dam-Bates, 2014) and would increase maintenance and labour costs, as biofouling as well as damage due to wave action and tides would be additional issues with a fence structure. This option could also affect hydrodynamics of the farm, reducing flow rates and increasing risk of anoxic conditions, especially under circumstances of heavy biofouling.

6.1.2 Cage Culture

Benthic cage culture is an option that would ensure that sea cucumbers are retained within the farm tenure and minimize interactions with wild populations, provided appropriate mesh sizes were used. This method may be the most impractical culture option, however, as it could restrict nutrients available to the sea cucumbers and farm particulates and biofouling organisms could clog the mesh of the cage creating maintenance issues and increased risk of anoxic conditions (Yu et al., 2013, 2014). This method would also be restricted to sites with appropriate depth and may not completely protect against predators. Predatory sea stars have been observed consuming sea cucumbers in benthic cages through the mesh wall and double walls around the cages were necessary to reduce sea star predation (Hannah et al., 2013).

By raising cages from the benthos, suspended cage culture would further protect sea cucumbers from predators, such as sea stars, and reduce risk of death by anoxia (Yu et al., 2013), in addition to not being restricted by depth of the farm site. Although, to make this a viable culture method, suspended cage design needs to maximize the amount of farm particulates captured within the cages, while efficiently containing the sea cucumbers and minimizing biofouling and maintenance issues. Due to the sea cucumber's morphology, containment can be difficult. *Parastichopus californicus*' soft and plastic body wall, longitudinal muscles, and ability to alter the amount of coelomic fluid allow the sea cucumber to drastically change length and width dimensions. Additionally, the rows of tube feet on the ventral side used for locomotion allow the animal to attach to many surface types at all angles. This combination of flexible body wall, limited hard body structures (calcareous oral ring), and 'sticky' tube feet makes for an indubitable escape artist, an issue for any chosen method of containment including benthic cages or fencing. Innovative design that considers the sea cucumber's behaviour and ecology is needed to tackle this issue of containment and nutrient capture in order for the sea cucumbers to act efficiently as a key nutrient recycling species within an IMTA system.

7.0 Research Objective

As previously outlined, *P. californicus* is a promising deposit-feeding species for IMTA (Hannah et al., 2013; Orr, 2012; Paltzat et al., 2008). However, issues of containment and nutrient transfer between the IMTA species remain obstacles for farmers that wish to incorporate this valuable extractive species. It has been suggested that additional research and improvements are needed in sea cucumber cage design that will allow for optimal containment of sea cucumbers and retention of organic particulates (Hannah et al., 2013; Yu et al., 2013; van Dam-Bates, 2014). This is an important area of research for the future of incorporating sea cucumbers, an important nutrient-recycling species, into an IMTA system. The addition of the California sea cucumber to existing aquaculture or incorporated into new IMTA sites could help move the industry toward sustainable, integrated, and ecosystem-based aquaculture practices. The objective of this research is to investigate containment design for juvenile California sea cucumbers (*P. californicus*) with the goal of increasing uptake of farm nutrients, thereby increasing overall IMTA nutrient-recycling efficiency, and maximizing sea cucumber containment. In order to do this, design elements of suspended sea cucumber cages, which accounted for the juvenile sea cucumber's behaviour and habitat preference, were tested in the laboratory and *in situ* to address the research question: *How can juvenile California sea* *cucumbers be practically contained within an IMTA farm while maximizing the amount of nutrients recycled and the overall IMTA system efficiency?*

Chapter 2: Integrated Multi-Trophic Aquaculture with the California sea cucumber (*Parastichopus californicus*): Investigating cage design elements for juvenile sea cucumbers

1.0 Abstract

The California sea cucumber (*Parastichopus californicus*) has been identified as an ideal species for integrated multi-trophic aquaculture (IMTA), due to its nutrient recycling capability and relatively high market value. The overall goal of the present study was to design a cage that effectively contains juvenile sea cucumbers in such a way that maximizes benthic extraction of large-particulate nutrients within an IMTA system. Because of the sea cucumber's morphology and behaviour, small mesh sizes are required for effective containment, which would restrict the flow of farm particulates to the sea cucumbers, hence reducing overall IMTA system efficiency. Animal behaviour and habitat preference were carefully considered when developing new suspended cage designs. Cage modifications included: the addition of nylon mesh (<1 mm) on the walls/lid of commercial oyster trays, oyster shell as substrate, and a novel mesh fringe (instead of a cage lid) encircling the upper rim of the cage. Six cage designs were chosen based on a preliminary laboratory trial. These designs were tested at a commercial Pacific oyster (*Crassostrea gigas*) farm where they were suspended beneath oyster rafts and at a reference site 320 m away for a 4 or 6-month period (May 24 and August 1 to November 14, 2015). Containment, growth, and occurrence of visceral atrophy of the juvenile sea cucumbers were monitored over time. Oyster faeces/pseudo-faeces deposition and particle retention efficiency of each cage design were also examined. Fine mesh (<1 mm) increased containment of juvenile sea cucumbers in the field study (77.1 ± 7.9 % compared to 21.4 ± 5.4 % for the unmodified cages without fine mesh), but significantly reduced total organic matter (TOM) deposition rates (0.03 ± 0.01 g m^{-2} day^{-1} compared to 0.15 ± 0.06 g m^{-2} day^{-1} for the unmodified cages). The novel fringe cage design had initially high containment success in the short-term laboratory trial (95.1 ± 2.8 %), with much lower containment success in the field trial (28.6 ± 4.8 %) but had significantly higher rates of TOM deposited within the cages (0.37 ± 0.10 g m^{-2} day^{-1}) than all other cage designs. Oyster shell substrate increased containment of sea cucumbers in the laboratory study from 24.9 ± 3.1 % in the unmodified cage without oyster shell to 75.3 ± 5.8 % in the unmodified cage with oyster shell, but oyster shell presence/absence was non-significant in the field trial. Significantly more sea cucumbers showed evidence of visceral atrophy in the fine mesh cages with and without oyster shell (7.3 ± 2.4, 8.3 ± 2.6 individuals, respectively) and

in the fringe cage with oyster shell (3.3 ± 1.6) at the end of the field study than all other cage designs. A positive farm effect was found, with increased containment, increased organics deposited within the cages, and more newly-recruited sea cucumbers in the experimental cages than in the control cages. Fall growth rates for sea cucumbers were not significantly different among cage types and showed a trend of reduced growth from October 1 to November 14. Sea cucumber growth for August to October was postive. As predicted, with lower food availability due to restricting mesh size and/or lids came significantly lower average growth rates, with 0.01 ± 0.02 g d^{-1} for fine mesh closed lid cages, 0.19 ± 0.05 g d^{-1} and 0.22 ± 0.05 g d^{-1} for large mesh and open lid cages.

2.0 Introduction

With a rapidly growing human population, food security and sustainable resource management have emerged as important areas of concern and research. Globally, wild fisheries catches have plateaued over the past few decades (FAO, 2016) while the human population and global demand for seafood is increasing. As a result, the aquaculture industry has been rapidly expanding over the past 40 years and is currently the fastest-growing, food production industry in the world (Béné et al., 2015), producing 52% of the world's seafood (Table 1.1). With the rapid expansion of the aquaculture sector, it is important to consider the possible environmental impacts of this food-production method.

One negative environmental impact of intensive, fed, mono-species aquaculture is the flow of excess nutrients, in the form of uneaten food and waste, into the surrounding ecosystem, causing eutrophication and benthic habitat degradation (Frankic and Hershner, 2003; Gowen and Bradbury, 1987). Biomitigative solutions are currently being researched and implemented in various experimental and commercial aquaculture systems to lessen the environmental impacts caused by excess nutrients. One example of this is integrated multi-trophic aquaculture (IMTA), which is the co-culture of multiple species from complementary trophic levels, positioned in such a way that excess nutrients are intercepted by extractive species components. The goal of IMTA is to improve the ecological sustainability of aquaculture by reducing environmental impacts through bio-mitigation and removal or recycling of excess nutrients, as well as increasing economic sustainability through crop diversification (Chopin et al., 2012, 2013; Troell et al. 2003). There are many possible variations of the IMTA concept, including marine or freshwater, open-water or land-based, and temperate or tropical and there is an endless variety and combination of potential species. Although systems will vary, depending on location and chosen species, the general IMTA concept includes four major components: a fed species, an inorganic-nutrient extractive species, an organic-particulate filter-feeding species, and an organic-particulate deposit-feeding species. Examples of these species groups, respectively, include finfish or prawns (Chopin et al., 2012; Martínez-Porchas et al., 2010); macroalgae (Abreu et al., 2011; Ahn et al., 1998; Chopin et al., 2001; He et al., 2008; Neori et al., 2004; Petrell et al., 1993; Reid et al., 2013); suspended bivalves such as oysters, scallops, or mussels (Nelson et al., 2012; Ren et al., 2012); and benthic detritivores such as sea cucumbers and sea worms (Cubillo et al., 2016; Hannah et al., 2013; MacDonald et al., 2013; Orr et al., 2014; Paltzat et al., 2008; Slater et al., 2009). For IMTA systems to become a sustainable aquaculture-design alternative, it is important to ensure that the overall efficiency of nutrient transfer between trophic levels is optimized and that the majority of the excess nutrients produced by fed species are successfully intercepted by the various extractive ones. Previous research has shown that the majority of the organic particles from a fed aquaculture species accumulate directly below or adjacent to the aquaculture containment structures and that they do not have a large horizontal spatial dispersal (Filgueira et al., 2017; Lander et al., 2013). A major implication of this finding is that the deposit-feeding species of the IMTA system are extremely important for the overall system efficiency of recycling organic nutrients, as long as they have access to the nutrients being released from the farm.

When selecting potential species for IMTA systems, their nutrient extractive capability is an important factor to consider, as is their market value—farmers are unlikely to adopt IMTA to cultivate low-value species (Chopin et al., 2012). Sea cucumbers have a high potential as a deposit-feeding extractive component of IMTA systems and are a valuable product due to the high demand in Asian countries. The market value of sea cucumbers may be as high as 1,668 USD/kg dried weight, depending on species and quality of product, with average prices ranging from 15 to 385 USD/kg (Purcell, 2014). Due to this high value and market demand, many tropical sea cucumbers are unsustainably harvested, causing many of these species to be in decline or listed as threatened (Bell et al., 2008; Fabinyi and Liu, 2014; Purcell, 2014; Purcell et al., 2013; 2014). Aquaculture of these particular species has helped relieve pressure from fisheries as well as allowing restocking of depleted populations (Battaglene, 1999; Purcell et al., 2012). Some examples of sea cucumbers cultured in a commercial IMTA setting include: the Australian sea cucumber (*Australostichopus mollis*) co-cultured with green-lipped mussels

(*Perna canaliculus*) in New Zealand (Slater and Carton, 2009; Slater et al., 2007, 2009), the sandfish (*Holothuria scabra*) cultured with shrimp in China (Chang et al., 2004; Martínez-Porchas et al., 2010; Purcell, 2004; Purcell et al., 2006; Zheng et al., 2009), and the Japanese sea cucumber (*Apostichopus japonicus*) co-cultured with abalone (Qi et al., 2013), jellyfish (Ren et al., 2014), and fish (Yokoyama, 2013).

The California sea cucumber (*Parastichopus californicus*) has been shown to be a promising extractive species for IMTA on the west coast of Canada, due to its deposit-feeding behaviour, nutrient extractive capabilities, and valuable market price (Hannah et al., 2013; Orr, 2012; Paltzat et al., 2008). A lucrative wild fishery currently exists in British Columbia (BC) for the species, with over 600 tonnes harvested a year with a landed value of approximately 7 million CAD (DFO, 2014). Wild fisheries for *P. californicus* also exist throughout the west coast of the United States, totaling over 980 tonnes per year valued at over 8.6 million USD, with Alaska, Washington, and California producing 545.7, 303.5, and 140 tonnes, respectively (FAO, 2014). Although wild-harvested *P. californicus* only makes up 0.5% of global sea cucumber markets (Table 1.3, FAO, 2014), aquaculture farmers have recently become interested in this species due to its high market value, potential ecological benefits, and resulting increased social acceptability of aquaculture (Barrington et al., 2010; DFO, 2014). However, due to the sea cucumber's morphology and ability to move through restricted spaces, containment can be difficult without reducing nutrient transfer and overall IMTA system efficiency (*i.e.* mesh sizes needed to contain small sea cucumbers may restrict flow of farm particulates to them). For the California sea cucumber to become a viable component of IMTA systems, a containment solution must be found that allows for the maximum amount of excess nutrients to accumulate within the sea cucumber cage while minimizing the emigration of animals out of the cage (Hannah et al., 2013).

The California sea cucumber was first identified as a potential species for co-culture in Alaska at a salmon aquaculture site (Ahlgren, 1998), although the study was not focused on IMTA, but rather on the potential biofouling mitigation ability of the species. Almost a decade later, the feasibility of sea cucumber and oyster co-culture was tested using juvenile sea cucumbers (contracted length: 8–13 cm, stocking density: 6 inds per 0.31 m^2), kept in modified oyster trays with mesh (mesh size: 0.625 cm) and a solid PVC insert in the bottom of the cage, suspended 2.5 m below oyster strings for approximately 12 months. The sea cucumbers in the study had overall positive growth and had an average assimilation efficiency of 40.4% on the shellfish waste and pseudofaeces produced by the oysters (Paltzat et al., 2008). Orr (2012) investigated the potential organic extractive capabilities of a number of marine invertebrate species [including green sea urchin (*Strongylocentrotus droebachiensis*), basket cockle (*Clinocardium nuttallii*), blue mussel (*Mytilus edulis*), spot prawn (*Pandalus platyceros*), and California sea cucumber] for IMTA culture with sablefish (*Anoplopoma fimbria*). This was accomplished by conducting laboratory feeding trials to assess the organic extractive potential of the invertebrates fed sablefish waste versus control natural diets. A conclusion of that study was that the California sea cucumber is capable of extracting organic material from sablefish waste and would be an appropriate species for IMTA culture with this species (Orr, 2012). Co-culture of sablefish and *P. californicus* was then found to be successful in field trials at a commercial IMTA site on the west coast of Vancouver Island (Hannah et al., 2013). The project investigated adult sea cucumber growth and survival at various stocking densities in an IMTA context. The study found that sea cucumbers grew significantly faster below the sablefish pen than control individuals, held approximately 250 m away, and that sea cucumbers were efficient at reducing total organic carbon and total nitrogen by 60.3% and 62.3%, respectively (Hannah et al., 2013). From laboratory experiments on movement and containment of *P. californicus*, van Dam-Bates (2014) found that small mesh sizes would be necessary for containment, as he observed sea cucumbers “squeezing their bodies through openings that were up to a third their contracted width” (van Dam-Bates, 2014). In addition, experiments on the movement of adult *P. californicus* in response to organically-enriched sediments showed that the total organic material (TOM) of sediments altered the sea cucumbers’ foraging behaviour, with more rapid and random movement in high-TOM (~8.0%) sediment than in low-TOM (~1.4%) sediment (van Dam-Bates et al., 2016). No published research, however, has examined the design of holding systems for *P. californicus.* This will be crucial, especially in IMTA systems where the goal is to maximize interception of fish wastes while ensuring that the sea cucumbers remain contained.

The overall objective of the present study was to examine how to effectively contain juvenile sea cucumbers in such a way that maximizes benthic extraction of large-particulate nutrients, overall IMTA system efficiency, and containment of the sea cucumbers. Since standard aquaculture gear does not allow for the practical containment of *P. californicus* without a reduction in the amount of nutrient transfer from the above, fed-aquaculture species (*i.e.* mesh sizes needed to contain small sea cucumbers may restrict flow of farm particulates to them), animal behaviour and habitat preference were carefully considered when developing new suspended-cage designs while trying to address this containment and system-efficiency issue. Various cage designs were tested, both in the laboratory and field, that take into account juvenile sea cucumbers' cryptic behaviour (Cameron and Fankboner, 1989; Wiedemeyer, 1994) and apparent preference for oyster shell substrate (Cheng and Hillier, 2011; C. Pearce, Fisheries and Oceans Canada, personal communication), as well as the amount of organic material able to accumulate within the cage. Cage design elements tested included: various mesh sizes, cages with and without lids, novel cage fringes, and oyster shells as substrate within the cages.

2.1 Hypotheses Tested

The research was designed to test the following hypotheses:

H_{O1}: Presence/absence of a cage lid will not significantly affect containment of *P. californicus* or nutrient delivery rates of particulates to the sea cucumbers.

H_{A1}: Presence/absence of a cage lid will significantly affect containment of *P. californicus* and/or nutrient delivery rates of particulates to the sea cucumbers.

H_{O2}: Mesh size will not significantly affect containment of *P. californicus* or nutrient delivery rates of particulates to the sea cucumbers.

H_{A2}: Mesh size will significantly affect containment of *P. californicus* and/or nutrient delivery rates of particulates to the sea cucumbers.

H_{O3}: Presence/absence of a cage fringe will not significantly affect containment of *P. californicus* or nutrient delivery rates of particulates to the sea cucumbers.

H_{A3}: Presence/absence of a cage fringe will significantly affect containment of *P. californicus* and/or nutrient delivery rates of particulates to the sea cucumbers.

H_{O4}: Oyster shell substrate will not significantly affect containment of *P. californicus.*

H_{A4}: Oyster shell substrate will significantly affect containment of *P. californicus.*

H_{O5}: Cage type will not significantly affect food availability or occurrence of visceral atrophy in juvenile *P. californicus*.

H_{A5}: Cage type will significantly affect food availability and/or occurrence of visceral atrophy in juvenile *P. californicus*.

H_{O6}: Presence/absence of the oyster farm will not significantly affect containment of *P. californicus* or nutrient delivery rates of particulates to the sea cucumbers.

H_{A6}: Presence/absence of the oyster farm will significantly affect containment of *P. californicus* and/or nutrient delivery rates of particulates to the sea cucumbers.

3.0 Materials and Methods

3.1 Sea Cucumber Cage Designs

High Flow™ oyster grow-out trays (Fukui North America, Eganville, Ontario, Canada: L × W × H: 56.25 × 56.25 × 21.25 cm, mesh size: 1.2–3.0 cm) (Fig. 2.1) were chosen as the basic cage as they are commercially available, relatively cheap, and presently there are no commercial cages designed specifically for sea cucumber culture. These oyster trays were modified using various cage-design elements detailed below. The cages were initially tested in the laboratory to assess sea cucumber containment with a subset of the most effective cage designs (described below) then being tested in a 6-month field trial at a Pacific oyster (*Crassostrea gigas*) farm, examining containment, growth, nutrient capture, and occurrence of sea cucumber visceral atrophy.

3.1.1 Solid Cage Bottom

All cage treatments had a solid PVC sheet (thickness: 0.5 cm; colour: gray) added to the bottom of the cage that served as a hard surface to collect the shellfish waste and allow the sea cucumbers to graze on it. Without this, the waste material would fall through the mesh bottom of the High Flow™ trays. In the laboratory experiment, a control treatment of a PVC sheet without a cage was used to see if the animals would move off the flat surface if not contained by any walls or lids (Fig. 2.1).

3.1.2 Cage Mesh Insert and Lid

Some cage treatments had a mesh lining (mesh size: 1 mm) added to the inside of the walls and lids of the cage (Fig. 2.1). This mesh size was chosen based on findings by van Dam-Bates (2014) showing that the mesh size required for containing small juvenile California sea cucumbers must be less than half of the animal's maximum contracted width. Sea cucumber contracted widths in the laboratory and field studies were 5.9 ± 1.6 mm (mean ± SD (wet weight: 0.35 ± 0.26 g)) and 19 ± 8.7 mm (wet weight: 9.3 ± 10.7 g), therefore the 1-mm mesh size was sufficient for containing the animals. This mesh size is similar to that of mosquito net material (mesh size: 2–5 mm) used to contain juvenile *A. japonicus* of 0.001–4.2 g (Gavrilova and Kucheryavenko, 2011) and the 1-mm mesh size used by Yokoyama (2013) to contain juvenile *A. japonicus* of 0.03–9.5 g. Cages lacking this small mesh, with only the standard oyster tray mesh size of 1.2–3.0 cm, were also tested as well as cages without lids (Fig. 2.1).

3.1.3 Cage Fringes

The modification seen in cage designs 6, 7, and 8 (Fig. 2.1), the floating mesh frame encircling the inner rim of the cage, is based on Wiedemeyer's (1994) experimental box design. He used a floating mesh frame in a lid-free box to contain juvenile sea cucumbers (*Actinopyga echinites*) in a field experiment to acquire basic biological information on the life stages and habitat preference of small individuals. From laboratory and field studies, Wiedemeyer (1994) found that this mesh frame prevented emigration of the juvenile cucumbers out of the boxes. The 'small' (mesh size: 250 μm) and 'large' (mesh size: 500 μm) nylon mesh fringes used to create the encircling mesh frame in the present study were chosen based on their flexibility and soft texture more so than on their specific mesh sizes. It was envisioned that, by having a flexible (*i.e.* not solid or rigid) substrate surrounding the top of the cage, this may deter the sea cucumbers from climbing around the fringe and leaving the cage.

3.1.4 Oyster Shell Substratum

Additional treatments with cages that contained 2 kg of Pacific oyster shells placed in the bottom were chosen based on previous IMTA sea cucumber research by Yokoyama (2013). In a field experiment, *A. japonicus* were cultured below red seabream fish cages with 2 kg of oyster shells added to sea cucumber cages (diameter: 30 cm, height: 20 cm, mesh size: 1-mm mesh on the bottom and 5-mm mesh on the sides and top of the cage). The oyster shells were placed within the cages to increase surface area (Yokoyama, 2013). *Parastichopus californicus* may benefit from having an oyster shell substratum as well, as juveniles are often found naturally recruited within oyster grow-out containers (Cheng and Hillier, 2011). In a field trial by Slater and Carton (2007), culturing *A. mollis* beneath a mussel farm, natural rock was added to each cage as substratum and refuge for the sea cucumbers. For other farmed sea cucumber species that prefer hard substrates, like *A. japonicus*, creating artificial habitat by adding hard substrata has been suggested as a possible farming technique (Xing et al., 2012). Since *P. californicus* are cryptic during the small juvenile stage (Cameron and Fankboner, 1989), providing them with extra surface area and crevices to hide in may help retain the sea cucumbers within the cage.

A combination of the cage design elements listed above resulted in eight cage designs (Fig. 2.1) that were tested in the laboratory. Based on the results from the initial laboratory experiment, designs 2, 4, and 7 (unmodified cage, cage with small mesh, and cage with small fringe, respectively) were chosen for further investigation in the field trial. Each of these three designs was tested with and without oyster shells, giving six cage types tested in the 6-month field trial (Fig. 2.2).

3.2 Sea Cucumber Measurements

3.2.1 Whole Wet Weight in Air

Although immersed whole weight is a more accurate non-lethal weight measurement for adult sea cucumbers (Hannah et al., 2012), and was initially attempted in the laboratory study, it was deemed impractical for the small juvenile sizes of sea cucumbers used in this study. Whole wet weight in air (referred to subsequently as simply "weight") was used as the measurement of sea cucumber weight. This was accomplished by gently rolling the sea cucumbers on paper towel for 10 seconds to remove excess external water before weighing the individuals.

3.2.2 Split Weight in Air

Split weight in air (referred to subsequently as simply "split weight") was calculated by weighing the animal after removing the visceral organs and coelomic fluid and blotting the body wall for 10 seconds on a paper towel to remove excess external water. Split weight is the most accurate form of sea cucumber weight measurement, although this method is lethal to the sea cucumber and inappropriate for repeated measures of growth of individuals over time (Hannah et al., 2012).

3.2.3 Whole Length and Whole Width

Sea cucumbers were handled for at least 10 seconds to induce contraction before measuring length and width. The former is the contracted length of the sea cucumber from anterior to posterior excluding protruding buccal tentacles. The latter is the contracted width of the sea cucumber measured mid-dorsally or at the widest point, measured from body wall to body wall, excluding papillae spikes.

3.2.4 Size Index

Size index is a scaled index factor utilizing both length and width measurements of the sea cucumbers (Yingst, 1982), with the equation given below. Due to the sea cucumber's plastic body shape, length or width measurements alone can often be inaccurate and highly variable. Size index provides a way of representing comparable sizes of sea cucumbers which accounts for both length and width of the individual.

$$\text{Size Index} = (\text{Length, cm}) \times (\text{Width, cm}) \times 0.01 \text{ (Yingst, 1982)}$$

3.3 Laboratory Study

3.3.1 Collection and Maintenance of Experimental Animals

Juvenile *P. californicus* were collected by hand off shellfish aquaculture gear in Fanny Bay, BC and transported to the Fisheries and Oceans Canada Pacific Biological Station (PBS), located in Nanaimo, BC. Mean ± SD ($n = 177$) intial sizes were: wet weight: 0.35 ± 0.26 g, contracted length: 2.35 ± 0.84 cm, and size index: 0.015 ± 0.009. Animals were transported in coolers containing ambient seawater and the trip took approximately 3 hours. Oxygen and temperature levels were monitored during transportation and were within acceptable limits for maintaining sea cucumbers. Animals were then held in outdoor flow-through tanks (volume of seawater: 372–412 L, flow rate: 6 L min^{-1}) for a minimum acclimation period of 2 weeks. Tanks were supplied with ambient, sand-filtered, and UV-treated seawater pumped from depth. Sea cucumbers were fed every 72 h a mixture of ground commercial salmon feed and dried and powdered seaweed *Ulva* sp., in a 70:30 ratio of fish feed to seaweed (Xia et al., 2012). This powdered feed was mixed with 240–1000 ml of seawater and a sparse layer of feed was evenly distributed on the bottom of the tanks. During this feeding process, water flows to tanks were temporarily reduced to allow food particles to settle on the bottom. During daily checks, any uneaten food and faeces were removed via siphon, with tanks fully cleaned (scrubbed and disinfected) a minimum of once a week.

3.3.2 Experimental Set-up

The eight cage designs tested were: (1) PVC bottom insert lacking a cage, (2) unmodified cage with a lid, (3) cage without a lid with fine mesh (< 1 mm) on the walls, (4) cage with a lid and fine mesh on the walls, (5) unmodified cage with 2 kg of oyster shells, (6) cage without a lid with fine mesh on the walls and a fringe made of 500-µm mesh ("large" mesh), (7) cage without a lid with fine mesh on the walls and fringe made of 250-µm mesh ("small" mesh), and (8) cage without a lid with fine mesh on the walls, a fringe made of 500-µm mesh, and 2 kg of oyster shells (Fig. 2.1). All cage designs had the solid PVC bottom insert.

The eight cage types were suspended within eight indoor tanks (diameter: 1.27 m, height: 0.75 m, volume: 0.86 m^3), with one cage per tank. Flow rate to the tanks was 6 L min^{-1}, giving a tank turnover rate of 2.4 h^{-1}. Eight replicates per treatment (cage type) were run over time, with one replicate of each treatment being conducted at each time and in each tank (*i.e.* Latin-square design) (Fig. 2.3). The order of the treatments within the tanks at any given time point was randomized, given the constriction of the Latin-square design. The duration of each individual trial was 48 h, with a minimum of 48 h between trials. The sea cucumbers were starved for 24 h prior to each trial to standardize hunger levels and to provide incentive for them to leave their cages. The experiment was run from September to November 2014.

Animals were randomized between trials and arbitrarily chosen from the outdoor holding tanks. At the beginning of each 48-h trial period, cages were stocked with 24 ind. $cage^{-1}$. This stocking density was chosen to replicate the "high" stocking density used by Hannah et al. (2013). In the final experimental replicate, stocking density was reduced to 12 ind. $cage^{-1}$ due to mortality and insufficient numbers of sea cucumbers being available. Weight and size index (Yingst, 1982) of experimental animals were recorded before commencing each trial.

GoPro™ cameras (GoPro Inc., San Mateo, California, USA) were mounted above four of the eight tanks to conduct time-lapse photography (one photograph per minute) for the duration of each trial. A natural photoperiod regime, 12 h light : 12 h dark using overhead fluorescent lights, was used throughout the laboratory experiment, with red light used during dark hours to illuminate the experimental set-up for tanks with cameras. All tanks were shaded from direct fluorescent light exposure using corrugated opaque plastic covers. Each cage was checked every 6–8 h during the trials, during which the approximate locations of sea cucumbers were recorded, with minimal disturbance of the animals. At the end of each 48-h trial, final locations of the sea cucumbers were recorded to quantify the total number of sea cucumbers in each area of the cages or outside of the cages, in a similar method to that used by Slater et al. (2011).

3.3.3 Statistical Analyses

Statistical analyses were performed using the open-source statistical software R, version 3.2.3 (R, 2015). Percent containment of sea cucumbers by cage type was analysed using three-way analysis of variance (ANOVA), with a Tukey-HSD multiple comparison post-hoc test, with cage type as the main factor and tank and time as blocking factors. Statistical significance was reported when the p-value was < 0.05. Percent containment was used instead of number of individual sea cucumbers to account for the change in stocking density in the last trial. Containment was defined as sea cucumbers located on the inside of the cage, either on the PVC bottom insert, walls, or on the inside of the lid at the end of the 48-h trial. For the solid bottom lacking a cage (Fig. 2.1, 1), containment was defined as sea cucumbers still attached to the suspended structure. The Levene's test was used to assess homogeneity of variance and the Shapiro-Wilk test for normality. Data was arcsin-transformed due its nature (percentage data) and due to violations of variance and normality. Transformed data was homogeneous (Levene's test, p-value = 0.187), although non-normal (Shapiro Wilk test, p-value = 0.0000217). Due to the robust nature of ANOVA with regard to small departures from normality, we chose to continue with the parametric analysis despite the violation of normality assumption.

3.4 Field Study

3.4.1 Study Site

The field study was performed at Effingham Oysters Ltd., a commercial Pacific oyster farm located at 49° 1' 22.18"N, 125° 8' 55.34"W in Effingham Inlet, within Barkley Sound, on the west coast of Vancouver Island, BC (Fig. 2.4). This farm cultures oysters in High Flow™ oyster grow-out trays (L × W × H: 56.25 × 56.25 × 21.25 cm, mesh size: 1.2–3.0 cm) stacked approximately 1.8 m high and suspended 2–6 m below the water surface, for a maximum depth of oysters of 8 m (Fig. 2.2). The field study was conducted from May 21 to November 14, 2015. During this period, the oyster farm continued in full operation with oysters of various ages growing above the experimental sea cucumber cages as well as the harvesting of oysters from rafts above the cages.

3.4.2 Water Quality Measurements

Three temperature data loggers (HOBO® Pro v2 TidbiT, Onset Computer Corporation, Bourne, Massachusetts, USA) were deployed at 9 m to record temperature every 30 min throughout the field trial. Oxygen and salinity levels were measured at each sampling period, approximately every 2 months, using a Van Dorn sampler to collect water at 0 and 3 m and a handheld YSI salinity/temperature probe (YSI 30, YSI, Poway, California, USA). Water turbidity was also measured at each sampling period using a secchi disc.

3.4.3 Collection of Experimental Animals

Sea cucumbers were collected on site by hand picking them off the commercial oyster trays during routine farm operations. Abundance of sea cucumbers found within the oyster trays varied greatly, with one tray containing the maximum of 30 individuals, but many trays containing none. Sizes of sea cucumbers also varied greatly among trays, ranging from < 0.1 g whole wet weight to > 400 g. Sea cucumbers were collected on two occasions, May 21–23 and July 28–31, 2015. The aim was to find as many small juvenile sea cucumbers as close in size range as possible with a contracted length between 1 and 10 cm. The initial mean ± SD (n = 574) sizes were: whole wet weight: 9.3 ± 10.74 g, contracted length: 5.5 ± 2.0 cm, and size index: 0.120 ± 0.093 (Table 2.1). They were temporarily held within oyster trays lined with 500–1000 µm mesh, either suspended at 6-m depth or placed within a floating upwelling system (FLUPSY), for a maximum of 72 h prior to the experiment.

3.4.4 Experimental Set-up

Three cage designs were used: (1) unmodifed standard oyster cage and lid, (2) cage with fine mesh (mesh size: < 1 mm) on the sides and lid, and (3) cage with fine mesh (mesh size: <1 mm) on the sides, with no lid, and an encircling mesh fringe (mesh size: 250 µm) along the inner top rim. In addition, all three cage designs were also fitted with a solid PVC sheet (thickness: 3 mm) on the bottom (Fig. 2.1). Each of these three cage designs had either no oyster shell or oyster shell (weight: 2 kg) as a substratum, for a total of six cage types, as well as three cages (one of each of unmodified, fine mesh, and fringe cage without oyster shell) without sea cucumbers as controls (Fig. 2.2). Five replicates of each of these six cage types were deployed at the farm in May and August, until November 2015, one of each type deployed 9 m below five randomly chosen oyster rafts (rafts 1–5, Fig. 2.4). In order for the experimental cages to not distrupt farm operations, the locations on the rafts from which the cages were suspended were hapazardly chosen and varied by raft. Due to the varied abundance and sizes of sea cucumbers found during collection, experimental sea cucumber cage replicates were deployed in two phases. The first two replicates (rafts 1 and 2) were deloyed on May 24, 2015 with a stocking density of 21 ind. cage^{-1} while the remaining three replicates (rafts 3–5) were deployed on August 1, 2015 with a stocking density of 14 ind. cage^{-1}.

To account for the influence of the oysters on the sea cucumbers held at the commercial oyster farm, a control site was located 320 m away from the oyster rafts, on an abandoned floating fish-farm structure (Fig. 2.4). Here five replicates of the fringe with oyster shell cage type were suspended at 9-m depth from August 1 to November 14, 2015. This cage type was chosen as the farm control as it appeared to be the most promising design from the initial laboratory containment study.

3.4.5 Sampling Experimental Sea Cucumbers

Sea cucumbers were sampled August 1–2 (rafts 1 and 2 only), October 1–5, and November 10–14, 2015. At each sample time, each cage was carefully and slowly pulled to the surface by hand. The number of sea cucumbers and their location in each cage were recorded.

The contracted length, contracted width, and whole wet weight of each sea cucumber were measured, as described previously for the laboratory trial. Size index was later calculated for each individual. Percent changes in mean whole wet weight, absolute growth rate (AGR, in g day^{-1}), and specific growth rate (SGR, in % day^{-1}) were calculated for the sea cucumbers using the mean wet weight of each cage of sea cucumbers between adjacent sampling periods. Calculations were as follows:

$$\text{Percent change} = ((W_2 - W_1) / W_1) \times 100$$

$$\text{AGR} = (W_2 - W_1) / T$$

$$\text{SGR} = ((\ln W_2 - \ln W_1) / T) \times 100$$

where W_2 is the mean wet weight at time of analysis (g), W_1 is the wet weight at the time of the previous sampling period (g), and T is the length of the growth period (days).

To assess final sea cucumber density, animals in the cages at the final sampling period were categorized into experimental sea cucumbers with and without organs and newly settled sea cucumbers. Experimental sea cucumbers were dissected to determine the state of their visceral organs and to obtain a split weight at the end of the study (Fig. 2.5). Newly settled sea cucumbers were identified as any individual found within the cage that was smaller than the experimental sea cucumbers at the start of the trial. These were generally significantly smaller, with typical, young-juvenile, white opaque skin colouring, through which visceral organs were easily observed. The mean wet weight of newly-recruited sea cucumbers (0.4 ± 0.7 g, mean ± SD) was much smaller compared to the initial mean wet weight of experimental sea cucumbers (9.3 ± 10.7 g).

3.4.6 Sediment Collection and Processing

An additional three replicates of each cage type without oyster shell (unmodified, small mesh, and fringe) (Fig. 2.2) were suspended below three experimental oyster rafts without sea cucumbers to assess the immigration of sea cucumbers into the experimental cages suspended below the oyster rafts. The zero-stocking density cages were also used to assess the amount of organics collected and retained in each cage type without experimental animals present. Sediment traps (height: 110 cm, capture area: 0.16 m^2) (Fig. 2.6) were also suspended from the same three rafts as the zero-stocking density cages for comparison (Fig. 2.2). The sediment traps were made of a PVC cylinder (60 cm in length and 5 cm inner diameter, with the top edge beveled to prevent material buildup and a capture cone made up of aluminum sheeting. The capture cone was designed to increase the area (diameter: 45 cm, area: 0.16 m^2), from which organics were collected. The same sediment trap design and length of deployment (~30 days) were previous used by Cross (2005) for a study on IMTA.

The zero-stocking density cages were deployed at rafts 3, 4, and 5 at 9-m depth on August 1, 2015 (Fig. 2.2). During the October and November sampling periods, all sediments and organics retained within most cages (including the zero-stocking density cages and cages with sea cucumbers, but excluding cages containing oyster shell substrate) were collected at rafts 3–5. Sediments within the oyster shell substrate cages were not collected at each sampling period due to time restraints and the difficulty of obtaining all the organics settled throughout the oyster shell. Organics within the oyster shell treatment cages were removed and oyster shells rinsed with seawater to ensure consistent handling of all cages throughout the experiment. During the final sample in November 2015, sediments were also collected from the five fringe with oyster shell control replicates deployed 320 m away from the oyster rafts. For this sediment collection, additional time was planned so that each oyster shell was carefully rinsed with filtered seawater to collect all the organics settled within these control cages. Three sediment traps (Fig. 2.6) were additionally deployed beneath rafts 3, 4, and 5 at 9-m depth from October 1, 2015 to November 14, 2015.

Sediments collected from the experimental cages, as well as the sediment traps, were analysed for total dry weight (g m^{-2} day^{-1}), total organic matter (TOM) (ash-free dry weight, g m^{-2} day^{-1}), as well as total nitrogen (TN) and total organic carbon (TOC) (percent and g m^{-2} day^{-1}). All sediment collected was initially frozen at -4°C in the field, for a maximum of 6 days, and then at -18°C in the laboratory until processing, approximately 3 months later. Sediments were thawed, washed with distilled water, and dried to a constant weight at 60°C. Dried samples were homogenized using a mortor and pestle. A subsample of 0.5 g was taken from the dried homogenized samples and sent to an external laboratory for TOC and TN content analysis, which was determined using a high-temperature combustion technique similar to Paltzat et al. (2008) and Orr et al. (2014). Total organic matter or ash-free dry weight was determined for each sediment sample by measuring the difference between the pre-ash dried weight of the sample and the post-ash weight of the sample after ashing at 500°C for at least 6 h or until constant weight (Paltzat et al., 2008; Orr et al., 2014). All sediment data was analysed in g m^{-2}

day^{-1} or percent of g m^{-2} day^{-1}, to account for any difference in deployment time as well as area of collection (0.16 m^2 for the sediment traps and 0.27 m^2 for the cages).

3.4.7 Statistical Analyses

Sea cucumber containment success was calculated by dividing the number of experimental sea cucumbers found within the cages at the end of the experiment by the initial stocking density and expressing as a percentage. As individuals within cages were not independent, cage means were calculated for growth data, with 2 large unmodified mesh ($C_LL_YF_NO_{Y/N}$) replicates with less than five sea cucumbers being omitted from the analyses due to high variation is sea cucumber size resulting in unrepresentative means in cage replicates with low sea cucumber numbers. Statistical analyses were performed using the open-source statistical software R, version 3.2.3 (R, 2015) and significance reported when the *p*-value was < 0.05. Sediment retention rates of the various cage types was initially assessed by comparing the dry weight of sediments collected from cages in g m^{-2} day^{-1} for both summer (August–September) and fall (October–November) to examine possible seasonal effects. An initial 2-way ANOVA determined that season had no significant main effect or interaction with cage type (*p*-values = 0.41, 0.45, respectively), therefore all summer and fall data for sediment collection were combined for subsequent sediment analyses.

A 3-way ANOVA was used to assess containment of the sea cucumbers by cage type (fixed factor, three levels), oyster shell presence/absence (fixed factor, two levels), and raft location (random blocking factor, five levels), with an interaction term between cage type and oyster shell presence/absence. Similar 3-way ANOVAs were also used to analyse total sediment collected, TOM, TN, and TOC with cage type (fixed factor, three levels), sea cucumber presence/absence (fixed factor, two levels), and raft location (random factor, three levels), with an interaction term between cage type and sea cucumber presence/absence. All ANOVAs were followed by a Tukey-HSD multiple comparison post-hoc test to determine treatment differences. As only the fringe plus oyster shell cages were suspended at the control site, these were not included in the initial 3-way ANOVAs. A one-way ANOVA was used to analyse sediment data (total sediment collected, TOM, TN, and TOC) by cage type including the fringe oyster shell control cages. A *t*-test was used to compare containment success of the sea cucumbers between the fringe with oyster shell cages suspended below the oyster farm and at the control site.

A 3-way ANOVA was used to assess final stocking density of sea cucumbers, including newly-recruited sea cucumbers, by cage type (fixed factor, three levels), oyster shell presence/absence (fixed factor, two levels), and raft location (random blocking factor, five levels), with an interaction term between cage type and oyster shell presence/absence. This was done for each category of sea cucumber found in each cage type at the terminus of the field study (experimental sea cucumbers with or without visceral organs, newly settled sea cucumbers, and total number of sea cucumbers). A *t*-test was used to compare final stocking density of the sea cucumbers between the fringe with oyster shell cages suspended below the oyster farm and at the control site. And finally, a 3-way ANOVA was used to assess sea cucumber growth rates (percent change in wet weight, AGR, and SGR) by cage type (fixed factor, three levels), oyster shell presence/absence (fixed factor, two levels), and raft location (random blocking factor, five levels) for both summer and fall growth periods.

All datasets were assessed prior to ANOVAs using Levene's test for homogeneity of variance and Shapiro-Wilk tests and Q-Q plots for normality. Datasets that failed either assumption were transformed by either the natural logarithm (ln) or logit transformation for proportional datasets (log (p/[1 p]) (Warton and Hui, 2011). These transformations were successful in normalising the data and creating variance homoscedasticity.

3.5 Treatment Designations

The following abbreviations will be used throughout the chapter to designate the various treatments:

(1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm), and C_N = no mesh (flat PVC plate alone)

(2) lid: L_Y = yes lid and L_N = no lid

(3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe

(4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell

(5) sea cucumbers: $S_{Y=}$yes sea cucumbers, S_N = no sea cucumbers

So a treatment abbreviation of $C_SL_NF_LO_Y$ would be a cage with small mesh, no lid, large-mesh fringe, and oyster shell.

4.0 Results

4.1 Laboratory Study

A 3-way ANOVA showed significant cage type and time effects, but no significant tank effect (Table 2.2). The results can be grouped into high, medium, and low sea cucumber containment success with each category statistically significant from one another and each cage type within categories not significantly different from one another (Fig. 2.7). The three cage designs with small mesh, no lid and fringes ($C_SL_NF_SO_N$: 95.1 ± 2.8%, $C_SL_NF_SO_Y$: 98.4 ± 1.1%, $C_SL_NF_LO_N$: 95.8 ± 1.8%) contained significantly more sea cucumbers than all other designs tested, while the large mesh cage with oyster shell ($C_LL_YF_NO_Y = 75.3 \pm 5.8\%$) and the fine mesh cage ($C_SL_YF_NO_N = 72.5 \pm 6.7\%$) treatments contained significantly more individuals than the remaining tested cage designs: $C_NL_NF_NO_N$ (9.7 ± 3.9%), $C_LL_YF_NO_N$ (24.9 ± 3.1%), and $C_SL_NF_NO_N$ (27. 6 ± 8.1%) (Fig. 2.7).

4.2 Field Study

4.2.1 Evironmental Parameters

At 9-m depth, seawater temperature showed both seasonal and daily variation (Fig. 2.8). Average temperature from May 21 to July 1 was 12.8°C (min: 10.8°C, max: 16.7°C). Higher temperatures and daily fluctuations were recorded throughout July and August until mid September, with an average temperature of 14.8°C (min: 12.1°C, max: 18.4°C). From September 15 to November 15 the average seawater temperature dropped to 13.1°C (min: 11.8°C, max 15.0°C). Secchi disc depths were recorded only during the four sampling periods. There was a notable increase in water clarity on July 30 which was also the day of the maximum water temperature of 18.4°C (Fig. 2.8).

Dissolved oxygen concentration showed a slight decrease in the summer at both 3- and 9-m depths and a large increase in the fall at all three depths (Fig. 2.9A). Salinity at 3- and 9-m depths remained relatively constant throughout the experiment, but decreased substantially at 0 m in the fall, due to heavy rain and surrounding topography (Fig. 2.9B).

4.2.2 Sea Cucumber Containment

4.2.2.1 Experimental Sea Cucumbers with/without Organs

A 3-way ANOVA on all sea cucumbers (with/without organs) showed a significant cage type effect on sea cucumber containment success at the oyster farm, but no significant effects of oyster shell presence/absence, raft, or interactions (Table. 2.3). The fine mesh cages ($C_SL_YF_NO_{N/Y}$ = 77.1 ± 7.9%) contained significantly more sea cucumbers than the unmodified ($C_LL_YF_NO_{N/Y}$ = 21.4 ± 5.4%) and fringe ($C_SL_NF_SO_{N/Y}$ = 28.6 ± 4.8%) cages (Fig. 2.10). A *t*-test revealed that the open-lid fringe cages at the oyster site contained significantly more sea cucumbers ($C_SL_NF_SO_Y$ = 38.7 ± 6.3%) than the open-lid fringe cages suspended at the control site ($C_SL_NF_SO_Y$ = 20.0 ± 2.7%) (p-value = 0.028, t = -3.015, df = 5.255) (Fig. 2.10).

4.2.2.2 Experimental Sea Cucumbers without Organs

A 3-way ANOVA examining only experimental sea cucumbers which showed signs of visceral atrophy or evisceration revealed that cage type, oyster shell presence/absence, and the interaction were all significant (Table 2.4). There was no significant difference in numbers of sea cucumbers in fine mesh cages ($C_SL_YF_NO_N$ = 8.3 ± 2.6, $C_SL_YF_NO_Y$ = 7.3 ± 2.4), and the open-lid fringe with oyster shell ($C_SL_NF_SO_Y$ = 3.3 ± 1.6) cages, but these were all significantly higher than both unmodified large mesh cage ($C_LL_YF_NO_Y$ = 0.8 ± 0.8) and open-lid fringe cages without oyster shell ($C_SL_NF_SO_N$ = 0.2 ± 0.2) (Fig. 2.11). There were no sea cucumbers found in either the large mesh without oyster shell ($C_LL_YF_NO_N$) cages or control open-lid fringe ($C_SL_YF_SO_Y$) cages.

4.2.2.3 Experimental Sea Cucumbers with Organs

A 3-way ANOVA examining only experimental sea cucumbers with intact visceral organs showed insignificant effects of cage type, oyster shell presence/absence, and interaction between the two factors, but indicated a significant raft effect (Table 2.4, Fig. 2.11).

4.2.2.4 Newly-Recruited Sea Cucumbers

For newly recruited sea cucumbers found in cages at the end of the study, a 3-way ANOVA showed no significant effect of cage type, oyster shell presence/absence, raft, or interaction (Table 2.4). The numbers of newly-recruited sea cucumbers in the three cage types were: open-lid fringe cages ($C_SL_NF_SO_{Y/N}$ = 7.9 ± 2.8), fine mesh cages ($C_SL_YF_NO_{Y/N}$ = 4.3 ± 1.3), and large mesh unmodified cages ($C_LL_YF_NO_{Y/N}$ = 4.2 ± 1.4) (Fig. 2.11). No newly settled sea cucumbers were found in the open-lid fringe ($C_SL_NF_SO_Y$) control trays away from the oyster farm.

4.2.2.5 Experimental Sea Cucumbers with/without Organs and Newly-recruited Sea Cucumbers

A 3-way ANOVA on all sea cucumbers revealed a significant cage effect, but no other significant main or interactive effects (Table 2.4). The fine mesh cages ($C_SL_YF_NO_{Y/N}$ = 17.8 ± 1.0) had significantly more sea cucumbers than the large mesh unmodified ($C_LL_YF_NO_{Y/N}$) ones (8.7 ± 1.9), whereas the open-lid fringe cages ($C_SL_NF_SO_{Y/N}$ = 13.6 ± 3.4) were not significantly different from either of the other treatments (Fig. 2.11). The experimental open-lid fringe cages ($C_SL_NF_SO_Y$ = 13.5 ± 7.2) had significantly more individuals than the control open-lid fringe cages ($C_SL_NF_SO_Y$ = 2.8 ± 0.4).

4.3 Sea Cucumber Size and Visceral Atrophy/Evisceration

For all cage types, observations of visceral atrophy occurred only in larger experimental sea cucumbers (Figs. 2.12). For the open-lid fringe and large unmodified mesh ($C_SL_NF_SO_{Y/N}$ and $C_LL_YF_NO_{Y/N}$) cages, only sea cucumbers greater than 5.0 g whole wet weight showed signs of visceral atrophy, whereas only sea cucumbers 1.0 g and larger in the fine mesh ($C_SL_YF_NO_{Y/N}$) cages underwent visceral atrophy (Figs. 2.12). All sea cucumbers in the control cages located away from the oyster farm had their visceral organs.

4.4 Sediment Retention Efficiency

4.4.1 Dry Weight of Sediments

A 3-way ANOVA on the dry weight of sediments collected from the experimental sea cucumber cages showed both significant cage type and raft effects, but non-significant effects of sea cucumber presence/absence and the interaction (Table 2.5). All three cage types had significantly different sediment retention rates: open-lid fringe cages ($C_SL_NF_SS_{Y/N}$ = 3.93 ± 0.96 g m^{-2} day^{-1}), large unmodified mesh cages ($C_LL_YF_NS_{Y/N}$ = 1.60 ± 0.60 g m^{-2} day^{-1}), and fine mesh cages ($C_SL_YF_NS_{Y/N}$ = 0.12 ± 0.03 g m^{-2} day^{-1}) (Fig. 2.13).

A 1-way randomized block ANOVA performed on all cage types, including the control cages and the sediment traps, revealed significant cage type and raft effects (Table 2.5). The sediment trap had a significantly higher sediment retention rate (8.27 ± 1.46 g m^{-2} day^{-1}) than all other cage types. The open-lid fringe control cages had a significantly lower sediment retention rate ($C_SL_NF_SS_{Y/N}$ = 0.74 ± 0.22 g m^{-2} day^{-1}) than the experimental open-lid fringe cages ($C_SL_NF_SS_{Y/N}$ = 3.93 ± 0.96 g m^{-2} day^{-1}), but not significantly different than the large unmodified mesh ($C_LL_YF_NS_{Y/N}$) cages (Fig. 2.13).

4.4.2 Total Organic Matter

A 3-way ANOVA on TOM collected from the experimental sea cucumber cages also showed both significant cage type and raft effects, but non-significant effects of sea cucumber presence/absence and the interaction (Table 2.6). All three cage types had significantly different TOM retention rates— open-lid fringe ($C_SL_NF_SS_{Y/N}$ = 0.37 ± 0.10 g m^{-2} day^{-1}), large unmodified mesh ($C_LL_YF_NS_{Y/N}$ = 0.15 ± 0.06 g m^{-2} day^{-1}), and fine mesh cage ($C_SL_YF_NS_{Y/N}$ = 0.03 ± 0.01 g m^{-2} day^{-1}) (Fig. 2.14).

A 1-way randomized block ANOVA performed on all cage types, including the control cages and the sediment traps, revealed significant cage type and raft effects (Table 2.6). The sediment trap had a significantly higher TOM retention rate (1.30 ± 0.21 g m^{-2} day^{-1}) than all other cage types. The open-lid fringe control cages had a significantly lower TOM retention rate ($C_SL_NF_SS_{Y/N}$ = 0.04 ± 0.01 g m^{-2} day^{-1}) than the experimental open-lid fringe cages ($C_SL_NF_SS_{Y/N}$ = 0.37 ± 0.10 g m^{-2} day^{-1}), but not significantly different than the large unmodified mesh ($C_LL_YF_NS_{Y/N}$) cages (Fig. 2.14).

4.4.3 Total Nitrogen

A significant cage effect on TN was revealed by a 3-way ANOVA, while the effect of sea cucumber presence/absence, raft, and interaction were non-significant (Table 2.7). All cage types were significantly different from one another—open-lid fringe ($C_SL_NF_SS_{Y/N}$ = 0.23 ± 0.05

g), large unmodified mesh ($C_LL_YF_NS_{Y/N}$ = 0.07 ± 0.02 g), and fine mesh cage ($C_SL_YF_NS_{Y/N}$ = 0.02 ± 0.004 g) (Fig. 2.15).

A 1-way randomized block ANOVA performed on all cage types, including the control cages and the sediment traps, revealed a significant cage type effect (Table 2.7). The sediment traps (0.41 ± 0.08 g) and experimental open-lid fringe cages ($C_SL_NF_SS_{Y/N}$ = 0.23 ± 0.05 g) were not significantly different from one another, but contained significantly more nitrogen than the rest of the cage types, including the large unmodified mesh ($C_LL_YF_NS_{Y/N}$ = 0.07 ± 0.02 g), control open-lid fringe ($C_SL_NF_SS_{Y/N}$ = 0.03 ± 0.01 g), and fine mesh ($C_SL_YF_NS_{Y/N}$ = 0.02 ± 0.004 g) cages (Fig. 2.15).

4.4.4 Total Organic Carbon

A 3-way ANOVA revealed that only the effect of cage type was significant for TOC (Table 2.8). All cage types were significantly different from one another—open-lid fringe ($C_SL_NF_SS_{Y/N}$ = 1.54 ± 0.34 g), large unmodified mesh ($C_LL_YF_NS_{Y/N}$ = 0.47 ± 0.17 g), and fine mesh cage ($C_SL_YF_NS_{Y/N}$ = 0.13 ± 0.03 g) (Fig. 2.16).

A 1-way randomized block ANOVA performed on all cage types, including the control cages and the sediment traps, revealed a significant cage type effect (Table 2.8). The sediment traps contained the highest amount of TOC (3.05 ± 0.47 g) with the experimental open-lid fringe cages having the second highest ($C_SL_NF_SS_{Y/N}$ = 1.54 ± 0.34 g)—both being significantly different from one another and containing significantly higher TOC than the remaining cage types, including large unmodified mesh ($C_LL_YF_NS_{Y/N}$ = 0.47 ± 0.17 g), control open-lid fringe ($C_SL_NF_SS_{Y/N}$ = 0.17 ± 0.05 g), and fine mesh cages ($C_SL_YF_NS_{Y/N}$ = 0.13 ± 0.03 g) (Fig. 2.16).

4.5 Sea Cucumber Growth

4.5.1 Percent Change in Whole Wet Weight

For percent change in whole wet weight of sea cucumbers in the summer, a 1-way completely randomized block ANOVA showed that cage type was significant (Table 2.9), with large unmodified mesh cages ($C_LL_YF_NO_{Y/N}$ = 110.7 ± 27.7 %) being significantly larger than fine mesh cages ($C_SL_YF_NO_{Y/N}$ = 11.2 ± 12.8%) and neither being significantly different than open-lid fringe cages ($C_SL_NF_SO_{Y/N}$ = 83.5 ± 21.4%) (Fig. 2.17). A 1-way completely randomized block

ANOVA for percent change in whole wet weight for the fall resulted in no significant terms (Table 2.9), with large unmodified mesh ($C_LL_YF_NO_{Y/N}$ = -13.7 ± 25.2%), fine mesh ($C_SL_YF_NO_{Y/N}$ = -18.9 ± 15.5%), and open-lid fringe ($C_SL_NF_SO_{Y/N}$ = -32.2 ± 17.7%) cages all being negative and not significantly different from one another (Fig. 2.17).

4.5.2 Absolute Growth Rate

For AGR (g d^{-1}) of sea cucumbers in the summer, a 1-way completely randomized block ANOVA showed that cage type was significant (Table 2.9), with $C_LL_YF_NO_{Y/N}$ (0.22 ± 0.05 g d^{-1}) and $C_SL_NF_SO_{Y/N}$ (0.19 ± 0.05 g d^{-1}) being significantly similar and both being significantly higher than $C_SL_YF_NO_{Y/N}$ (0.01 ± 0.02 g d^{-1}) (Fig. 2.17). A 1-way completely randomized block ANOVA for AGR in the fall resulted in no significant terms (Table 2.9), with $C_LL_YF_NO_{Y/N}$ (-0.19 ± 0.17 g d^{-1}), $C_SL_YF_NO_{Y/N}$ (-0.06 ± 0.02 g d^{-1}), and $C_SL_NF_SO_{Y/N}$ (-0.24 ± 0.15 g d^{-1}) all being negative and not significantly different from one another (Fig. 2.17).

4.5.3 Specific Growth Rate

For SGR (% d^{-1}) of sea cucumbers in the summer, a 1-way completely randomized block ANOVA showed that cage type was significant (Table 2.9), with large unmodified mesh ($C_LL_YF_NO_{Y/N}$ = 1.10 ± 0.23 % d^{-1}) and open-lid fringe ($C_SL_NF_SO_{Y/N}$ = 0.92 ± 0.20 % d^{-1}) being significantly higher than fine mesh cages ($C_SL_YF_NO_{Y/N}$ = 0.11 ± 0.19 % d^{-1}) (Fig. 2.17). A 1-way completely randomized block ANOVA for SGR in the fall resulted in no significant terms (Table 2.9), with large unmodified mesh ($C_LL_YF_NO_{Y/N}$ = -1.21 ± 1.27 % d^{-1}), fine mesh ($C_SL_YF_NO_{Y/N}$ = -0.57 ± 0.18 % d^{-1}), and open-lid fringe ($C_SL_NF_SO_{Y/N}$ = -1.51 ± 0.77 % d^{-1}) cages all being negative and not significantly different from one another (Fig. 2.17).

5.0 Discussion

5.1 Effects of Sea Cucumber Cage Lid and Mesh Size

H_{A1}: *Presence/absence of a cage lid will significantly affect containment of juvenile P. californicus and nutrient delivery rates of particulates to the sea cucumbers.*

H_{A2}: *Mesh size will significantly affect containment of juvenile P. californicus and nutrient delivery rates of particulates to the sea cucumbers.*

The presence of a cage lid significantly increased containment of juvenile *P. californicus* when using fine mesh cages in the laboratory experiment [72.5 ± 6.7 % (mean ± SE) for $C_SL_YF_NO_N$ versus 27.6 ± 8.1 % for $C_SL_NF_NO_N$]. The use of fine mesh also significantly increased containment in the laboratory study, which is shown by the large unmodified cages ($C_LL_YF_NO_N$ = 24.9 ± 3.1 %) having a significantly lower percentage of sea cumbers than the fine mesh ones ($C_SL_YF_NO_N$ = 72.5 ± 6.7 %). Similarly, in the longer-term field experiment, fine mesh significantly increased sea cucumber containment from 21.4 ± 5.4 % in the large unmodified mesh ($C_LL_YF_NO_{Y/N}$) cages to 77.1 ± 7.9 % in fine mesh ($C_SL_YF_NO_{Y/N}$) cages.

Comparing TOM deposition rates between cage types in the field experiment, the presence of a lid and smaller mesh size significantly reduced organic matter retained within the cage types. The fine mesh cage had a significantly reduced TOM deposition rate ($C_SL_YF_NO_{Y/N}$ = 0.03 ± 0.01 g m^{-2} day^{-1}) compared to all the other treatments—five times lower than large unmodified mesh cage ($C_LL_YF_NO_{Y/N}$ = 0.15 ± 0.06 g m^{-2} day^{-1}) and 12 times lower than the open-lid fringe cage ($C_SL_NF_SO_{Y/N}$ = 0.37 ± 0.10 g m^{-2} day^{-1}). All cage types, however, had significantly lower rates of TOM deposition than the sediment traps (1.30 ± 0.21 g m^{-2} day^{-1}).

These results were expected as a cage with fine mesh and/or lid should contain a higher number of sea cucumbers than those with a larger mesh and without lid, but fine mesh and lids will restrict the amount of organic detritus captured within the cage. Although these results are intuitive, they are important to demonstrate the motivation for this study. If the main concern from a policy or management perspective is absolute containment of animals based on mesh size (DFO, 2014) it may not be practical from an IMTA farmer's perspective since a mesh size small enough to contain juvenile sea cucumbers will greatly restrict food availability to the cultured animals in an IMTA setting where animals are not manually fed. From an IMTA perspective this reduces the benthic-extraction, nutrient-recycling function of the cultured sea cucumbers by greatly reducing the organics available to them to ingest and absorb. Previous research on sea cucumbers (*A. japonicus*) in IMTA culture has also identified the need of improving sea cucumber culture equipment in such a way that creates an effective IMTA system and increases food availability to the sea cucumbers cultured in the water column (Yu et al., 2013).

One important limitation worth noting for the field experiment results is that it is unknown if containment success reported is actual containment success *per se*, survival, or a combination of the two, as the fate of the unaccounted-for sea cucumbers is unknown. It is likely that some amount of mortality would occur due to handling and/or predation. Therefore, from the field experiment data we cannot say that “uncontained” sea cucumbers are necessarily escapees from the cages.

5.2 Effects of Sea Cucumber Cage Fringe

H_{A3}: Presence/absence of a cage fringe will significantly affect containment of juvenile P. californicus and nutrient delivery rates of particulates to the sea cucumbers.

The presence of a mesh fringe encircling the inner rim of an open-lid cage significantly increased containment of juvenile sea cucumbers in the initial laboratory experiment (27.6 ± 8.1 % for open-lid no fringe $C_SL_NF_NO_N$ versus 95.1 ± 2.8 % for open-lid fringe $C_SL_NF_SO_N$). In the longer-term field experiment, containment of sea cucumbers in open-lid fringe cage ($C_SL_NF_SO_{Y/N}$ = 28.6 ± 4.8 %) was much lower than in the shorter-term laboratory study, but not significantly different than that in the large unmodified mesh cages ($C_LL_YF_NO_{Y/N}$ = 21.4 ± 5.4%).

In the field experiment, the open-lid fringe cages had a significantly higher rate of TOM deposition ($C_SL_NF_SO_{Y/N}$ = 0.37 ± 0.10 g m^{-2} day^{-1}) than both the large unmodified mesh cages ($C_LL_YF_NO_{Y/N}$ = 0.15 ± 0.06 g m^{-2} day^{-1}) and the fine mesh cages ($C_SL_YF_NO_{Y/N}$ = 0.03 ± 0.01 g m^{-2} day^{-1}). All cage types had a significanly lower rate of TOM deposition compared to the sediment traps suspended below the oyster rafts (1.30 ± 0.21 g m^{-2} day^{-1}).

The goal of this novel open-lid fringe was to test a sea cucumber cage design that would ideally contain the juvenile sea cucumbers within the cage while permitting the most organic material to enter. This fringe design was based on a similar open-lid juvenile sea cucumber containment system which was found to be successful in an 9-month field experiment for juvenile *Actinopyga echinities* (Wiedemeyer, 1994). Wiedemeyer (1994) reported natural mortality of juvenile *A. echinities* at an average of 0.6% month^{-1} for a closed-lid containment system and 3.3% month^{-1} in an open-lid version. Mortality in that study for the open-lid boxes included predation, dislodgement effects, and possible escapees, with an average of 29.7% reduction of sea cucumbers over the 9-month field trial attributed to predation as the boxes were on the benthos and predators were observed in the boxes. The researchers tested the containment effectiveness of the open-lid box design in the laboratory prior to the field trials with no reported results (Wiedemeyer, 1994).

This open-lid fringe cage style was successful in the field experiment for collection of TOM but had much lower containment success than in the shorter-term laboratory experiment. The three most reasonable factors contributing to the difference of these results are time, environment, and sea cucumber size. The laboratory experiment was a short-term (48 h) experiment whereas the field experiment was substantially longer (4–6 months), with the sea cucumbers having a much longer time for escapement in the latter than the former. Another possible explanation for reduced containment success in the field is predation, as the sea cucumbers were not exposed to predators in the laboratory trial. During sampling of experimental cages in the field, no evidence of predation or mortality was observed in any of the cages, although decomposition of these small holothurians occurs quickly (Bakus, 1973; Takahashi, 1974; Fortune, personal observation) and specimens may have disintegrated completely, leaving no evidence between sample periods. In addition, predators may have consumed whole individuals, although no predation was observed during sample periods, small crabs were observed in the sea cucumber cages. Another possible explanation for the differences in containment success between the laboratory and field trials is the larger size of juveniles used for the field versus laboratory experiment (9.32 ± 10.7 g and 0.35 ± 0.26 g, respectively). It is possible this fringe-cage design is more effective at containing very small juveniles of < 1.0 g and < 3.0 cm contracted length. Further testing is recommended as this open-lid cage design should still be considered for a potential nursery grow-out method for small juvenile sea cucumbers in one of their most vulnerable life stages (< 3.0 cm contracted length) (Chen and Chang, 2015; Katow et al., 2015; Pitt and Duy, 2004; Tuwo and Tresnati, 2015; Yu et al., 2015). Different fringe materials, widths and mesh sizes should be further researched and tested for containment success and effectiveness for various animal sizes and life stages, as some designs may be more effective at containing larger individuals.

5.3 Effects of Oyster Shell Substrate in Sea Cucumber Cages

H_{A4}*: Oyster shell substrate will significantly affect containment of juvenile P. californicus.*

The presence of oyster shells in the sea cucumber cages significantly increased containment of sea cucumbers in the short-term laboratory experiment by approximately 50%, from 24.9 ± 3.1 % in the large unmodified mesh with oyster shell ($C_LL_YF_NO_N$) cage to 75.3 ± 5.8 % in the large unmodified mesh without oyster shell ($C_LL_YF_NO_Y$) cage. In the field experiment, the effect of oyster shell substrate was non-significant for sea cucumber containment success. This statistically non-significant result from the field trial was unexpected, based on the significant effect found during the short-term laboratory study. A trend of increased containment in the cages with oyster shell exists (Fig. 2.10), but perhaps would need a greater sample size to be statistically significant.

One possible explanation for the unpredicted non-significant effect of oyster shell substrate on containment success during the field experiment would be possible increased mortality caused by handling and natural physical disturbance (*i.e.* storms and wave action) in the oyster shell treatment cages. Although the presence of oyster shells increased containment in the short-term laboratory experiment and all cages throughout the experiment had equal handling, the potential for physical disturbance by the sometimes sharply-edged oyster shells to the soft-bodied sea cucumbers in the field may have increased mortality in these cages and thus decreased “containment success” as mortality was not observed directly and was not able to be accounted for in this experiment. No evidence of this, however, was observed during the field trial.

5.4 Effects of Sea Cucumber Cage on Food Availability and Occurrence of Visceral Atrophy

H_{A5}: Cage type will significantly affect food availability and occurrence of visceral atrophy in juvenile P. californicus.

Sea cucumbers are unique animals to culture due to their ability to expel or absorb their internal organs, known as evisceration and visceral atrophy, respectively. These behaviours are important to understand from an IMTA perspective because if the sea cucumber does not have a functioning digestive system this affects the animals’ ability to act as a nutrient extractive species. This is also an important process to understand from a farmer’s perspective as seasonal visceral atrophy will cause the sea cucumbers to reduce in size over the winter and farmers obviously want to optimize growth of their cultured animals. It is unknown if seasonal visceral atrophy is triggered by temperature, daylight, or food availability in *P. californicus*, but it has been suggested by various authors that food availability or food quality is a trigger (Fankboner and Cameron, 1985; Hannah et al., 2013; Paltzat et al., 2008). This is an important point for investigating sea cucumber cage design as the type of cage or containment used affects the amount of food available to the sea cucumbers.

Cage type, as previously stated, had a significant effect on TOM deposition rate, with fine mesh cages containing the least amount of TOM ($C_S L_Y F_N O_{Y/N} = 0.03 \pm 0.01$ g m^{-2} day^{-1}), large unmodified mesh cages retaining five times more ($C_L L_Y F_N O_{Y/N} = 0.15 \pm 0.06$ g m^{-2} day^{-1}), and open-lid fringe cages retaining over 12 times more ($C_S L_N F_Y O_{Y/N} = 0.37 \pm 0.10$ g m^{-2} day^{-1}).

In addition, cage type, oyster shell, and cage type/oyster shell interaction were all significant factors in the number of experimental sea cucumbers showing signs of visceral atrophy or evisceration (*i.e.* greatly reduced or lacking visceral organs). The greatest number of sea cucumbers lacking or with reduced digestive systems were found in the fine mesh with and without oyster shell and the open-lid fringe with oyster shell cages ($C_SL_YF_NO_Y$ = 7.3 ± 2.4, $C_SL_YF_NO_N$ = 8.3 ± 2.6, and $C_SL_NF_YO_Y$ = 3.3 ± 1.6 individuals, respectively). Significantly fewer sea cucumbers were found with signs of visceral atrophy in the large unmodified mesh cages with oyster shell ($C_LL_YF_NO_Y$ = 0.8 ± 0.8 individuals) and the open-lid fringe without oyster shell cages ($C_SL_NF_YO_N$ = 0.2 ± 0.2 individuals), with no signs of visceral atrophy found in sea cucumbers in the large unmodified mesh cages without oyster shell ($C_LL_YF_NO_N$) cages and the open-lid fringe ($C_SL_NF_YO_Y$) cages at the control site.

As predicted, the greatest amount of visceral atrophy was found in the fine-mesh cages which had significantly less organic matter available to the sea cucumbers, suggesting, as other studies have, that food availability is a trigger for visceral atrophy in *P. californicus*. Unexpected was the higher number of sea cucumbers with signs of visceral atrophy in the experimental open-lid fringe with oyster shell cages ($C_SL_NF_YO_Y$ = 3.3 ± 1.6), which was significantly higher than the open-lid fringe without oyster shell ($C_SL_NF_YO_N$ = 0.2 ± 0.2) cages. Although the former was not significantly different than the fine mesh cages ($C_SL_YF_NO_Y$ = 7.3 ± 2.4 and $C_SL_YF_NO_Y$ = 8.3 ± 2.6), the high level of variation likely reduced the ability to detect a significant difference.

5.5 Oyster Farm Effects on Sea Cucumber Co-Culture

H_{A6}: Presence/absence of the oyster farm will significantly affect containment of juvenile P. californicus and nutrient delivery rates of particulates to the sea cucumbers.

Open-lid fringe cages suspended below the oyster farm had significantly higher containment success than similar cages at the control site ($C_SL_NF_YO_Y$, 38.7 ± 6.3 and $C_SL_NF_YO_Y$ control, 20.0 ± 2.7 %, respectively) while the former had significantly higher TOM deposition rates than the latter (0.37 ± 0.10 and 0.04 ± 0.01 g m^{-2} day^{-1}, respectively). The increased organic material and increased containment of sea cucumbers within the cages suspended below the oyster farm compared to the control site indicates a positive effect of the oyster farm on both organic matter accumulation and sea cucumber containment. This is an important finding as, not only have sea cucumbers been shown to be an ideal IMTA species for their nutrient extractive ability, but this positive farm effect indicates that a poly-culture or IMTA system is a more ideal culture environment for the sea cucumbers than mono-culture. This finding aligns with other research on sea cucumber aquaculture where poly-culture or IMTA has been shown to be more productive than mono-culture (Chen et al., 2015a, 2015b; Ren et al., 2010; Thu, 2003). This may be due to the sea cucumber's selective feeding behaviour. Previous research has shown that *P. californicus* demonstrates selective feeding on sediments with higher amounts of organics (Paltzat et al., 2008). van Dam-Bates et al. (2016) found that *P. californicus* increased random movement in the presence of higher organic sediments, which the researchers suggested would keep an animal in an area of higher TOM. The sea cucumber cages suspended below the oyster farm contained more TOM than the control cages away from the farm, which may be a contributing factor to the increased containment of sea cucumbers within the cages at the farm compared to the control site.

5.6 Newly-Recruited Sea Cucumbers

During the field experiment, most notably during the final sampling period in November, high numbers of newly-recruited sea cucumbers were found within the experimental sea cucumber cages. Experimental and recruited individuals were easily distinguished by size and colour. The newly-recruited sea cucumbers were <1.0 g and many still had white colouring, which is a phenotype/colouration seen in the early juvenile stage in the first year of growth post-larval settlement (Cameron and Fankboner, 1989), whereas the experimental sea cucumbers were an average of 9.3 ± 10.7 g, with red body colour. The number of newly-recruited sea cucumbers was highly variable among cage treatments and not significantly affected by cage type. It is unknown if the sea cucumbers settled in the cages from their pelagic larval stage, but from the high numbers found and sizes, it would be more likely that they recruited to the commercial oyster cages above and fell into the experimental cages. Juvenile sea cucumbers are known to be found in high abundances on oyster farm gear (Cheng and Hillier, 2011; C.M. Pearce, DFO, personal communication).

When newly-recruited sea cucumbers are accounted for in the final stocking density of the cages at the end of the experiment, no significant difference was found for the number of individuals between the fine mesh ($C_SL_YF_NO_{Y/N}$) and open-lid fringe ($C_SL_NF_YO_{Y/N}$) cages. In fact, there were more sea cucumbers with functioning digestive systems in the latter than in the former. From an IMTA perspective, if the goal of culturing these detritivore animals is to integrate them into a farming system such that they have access to and are feeding on the excess organics, the number of animals that have a functioning digestive system is important. Using this criterion, the open-lid fringe ($C_SL_NF_YO_{Y/N}$) cages out-performed the fine mesh ($C_SL_YF_NO_{Y/N}$) cages.

No newly-recruited sea cucumbers were found in the control cages located away from the commercial oyster farm. This furthers the finding of a positive farm interaction for sea cucumbers and highlights how the oyster farm acts as an artificial reef, providing an ideal settling habitat for sea cucumber larvae. It has been locally known that high abundances of juvenile sea cucumbers are often found on oyster farm gear, when they are nearly impossible to find in the wild otherwise, although no research has quantified to what degree these artificial reef structures contribute to the local sea cucumber population. Cheng and Hillier (2011) attempted to monitor juvenile *P. californicus* recruitment to oyster shell bags in the Puget Sound by randomly placing bags of oyster shells throughout the sound to provide settling habitat for sea cucumbers. Although that study was well motivated, due to the high number of sea cucumbers seen recruited to commercial oyster farms, the researchers only recovered a small number (N = 13, from 24 collection bags) of newly-recruited sea cucumbers (Cheng and Hillier, 2011). Comparatively, our experimental cages suspended under the oyster farm collected 266 juvenile sea cucumbers (in addition to the 574 individuals we were able to easily collect by hand from the oyster farm prior to the experiment), whereas the cages at the control site, only 320 m away, had no newly-recruited sea cucumbers. Further research should be done to understand how aquaculture farms provide ideal protective settling habitat for important native species, like the California sea cucumber, and how they contribute as an artificial reef to local biodiversity and wild fisheries.

5.7 Sea Cucumber Growth

Fall growth rates for sea cucumbers were not significantly different among cage types and showed a trend of reduced growth from October 1 to November 14. This is consistant with previous research by Hannah et al. (2013) and Paltzat et al. (2008) who found that *P. californicus* feeding behaviour and growth changed seasonally, with reduced growth rates and body mass during the fall and winter months. To have overall postive growth throughout the year, growth in the spring and summer must significantly exceed and compensate for the reduced growth over the fall and winter. This makes the spring and summer growth period very important for sea cucumber culture.

Sea cucumber growth for August to October was postive. As predicted, with lower food availability due to restricting mesh size and/or lids came significantly lower AGRs [fine mesh cages ($C_SL_YF_NO_{Y/N} = 0.01 \pm 0.02$ g d^{-1}) versus open-lid fringe ($C_SL_NF_SO_{Y/N} = 0.19 \pm 0.05$ g d^{-1}) and large unmodified mesh ($C_LL_YF_NO_{Y/N} = 0.22 \pm 0.05$ g d^{-1})]. This is an important finding as it furthers the point that if containment is based soley on mesh size it makes it impractical for farmers, as the mesh size needed to physically contain these juvenile sea cucumbers reduced food availability and significantly reduced growth during the important summer growth period. Future studies should more closely exaime growth rates of juvenile *P. californicus* in a farm setting year round. One limitiation of the present study is that majority of cage replicate deloyements were delayed from the initial scheduled ‘spring’ time of May until August 1, as a result we were unable to accurately measure growth earlier in the season.

5.8. Minimum Body Size for Visceral Atrophy

An interesting observation from the final sampling period and dissections of the sea cucumbers from the field experiment is that it appears there is a minimum size of >1 g wet weight before the sea cucumber undergoes visceral atrophy. This observed minimum size may have to do with the age of the sea cucumber, as well as the amount of energy stored in the body wall, which enables them to get rid of their digestive system for this wintering hibernation mode.

From a farmer’s perspective, this reduced metabolic activity that sea cucumbers exhibit seasonally is important to consider as sea cucumbers will reduce in size over the winter months due to this seasonal visceral atrophy. Interestingly, a study in China was able to successful avoid the seasonal summer hibernation stage, or seasonal aestivation, observed in *A. japonicus*, by relocating the sea cucumbers to an area of lower water temperatures, since the environmental trigger for this species’ reduced metabolic activity is high temperature (Xing et al., 2012). Since the environmental trigger for the seasonal visceral atrophy of *P. californicus* appears to be food quality and/or availability, future studies should investigate possible measures sea cucumber farmers could take to avoid seasonal atrophy and increase growth rates of the cultivated animals as well as increasing IMTA system efficiency during the winter months.

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Tables

Table 1.1. Global aquaculture production summary in 2014.

Species Group	Aquaculture Value (USD 1,000)	Aquaculture Quantity (mt)	Wild Harvest (mt)	Total Production (mt)
Freshwater fishes	**67,574,398** *40.8 %*	**42,617,081** 21.9 %	**10,590,021** 5.4 %	**53,207,102** 27.4 %
Diadromous fishes	**24,020,616** *14.5 %*	**4,866,482** 2.5 %	**1,724,835** 0.9 %	**6,591,317** 3.4 %
Marine fishes	**9,706,979** *5.9 %*	**2,378,328** 1.2 %	**65,950,529** 33.9 %	**68,328,857** 35.1 %
Crustaceans	**36,173,409** *21.8 %*	**6,915,072** 3.6 %	**6,870,068** 3.5 %	**13,785,140** 7.1 %
Molluscs	**19,012,406** *11.5 %*	**16,113,194** 8.3 %	**7,674,326** 3.9 %	**23,787,520** 12.2 %
Miscellaneous aquatic animals	**3,664,475** *2.2 %*	**893,568** 0.5 %	**635,455** 0.3 %	**1,529,023** 0.8 %
Aquatic plants	**5,637,414** *3.4 %*	**27,306,965** 14 %	N/A	**27,306,965** 14 %
Global Total	**165,789,697** *100 % of value*	**101,090,690** 52 %	**93,445,234** 48 %	**194,535,924** 100 %

Data retrieved from:

FAO. 2016. Fishery and aquaculture statistics. FAO, Rome, 2016.

Table 1.2. Global commercial sea cucumber industry overview

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Atlantic Ocean and Adjacent Seas:								
Cucumaria frondosa (Gunnerus, 1767)	Orange-footed sea cucumber, Northern sea cucumber	North Atlantic	Fishers Receive: 0.25 per piece, 0.154 kg^{-1}	Harvested by trawling (Canada) and dredging (Barents Sea and Maine). Harvest is managed by seasonal fishing closures and by animal size.	Experimentally: With Atlantic salmon, blue mussels and kelp[3,4]	**5 538.1** Canada: 5 379 USA: 159.1[5]	0	0
Holothuria mammata (Grube, 1840)	–	Mediterranean and adjacent eastern Atlantic[6]	No recent information available	Commercially exploited in Turkey.[7]	None	**~183** [7] Mixed fishery: 550 t / 3 species	0	0
Holothuria polii (Chiaje, 1823)	–	Mediterranean and adjacent eastern Atlantic[6]	No recent information available	Commercially exploited in Turkey.[7]	None	**~183** [7] Mixed fishery: 550 t / 3 species	0	0
Holothuria tubulosa (Gmelin, 1790)	Cotton-spinner, Tubular sea cucumber	Mediterranean and surrounding Atlantic waters[7]	No recent information available	Commercially exploited in Turkey.[7]	Experimentally: In bottom cages beneath sea bream [8]	**~183** [7] Mixed fishery: 550 t / 3 species	0	0
Atlantic Ocean and Adjacent Seas Mixed Fisheries:						**3 929**	**0**	**0**
Actinopyga agassizii (Skelenka, 1867)	Pepino de mar	Atlantic Central America and Gulf of Mexico	No recent information available	Wild harvest banned in Panama.	None	...	0	0
Astichopus multifidus (Sluiter, 1910)	Furry sea cucumber	Caribbean Region, including Gulf of Mexico, Florida, the Bahamas, Panama	Market value not determined	Overexploited in Panama with a fishing ban as of 2003.	None	...	0	0
Holothuria arguinensis (Koehler and Vaney, 1906)	–	North-east Atlantic, from the Berlengas Island (Portugal) to Morocco and Mauritania, including Canary Islands[12]	Market value not determined	Experimental hatchery production in Portugal.[13]	None	...	0	0
Holothuria forskali (Chiaje, 1823)	Black sea cucumber, Cotton-spinner	North-east Atlantic[14]	No recent information available	No recent information available.	Experimentally: In Recirculating Aquaculture System (RAS) with sea bass[15] and in RAS with Flounder[16]	...	0	0
Holothuria mexicana (Ludwig, 1875)	Donkey dung sea cucumber	Atlantic Central America and Gulf of Mexico	Retail Value: 64-106 kg^{-1}	Harvest in the Gulf of Mexico and Caribbean, often illegally.	None	...	0	0
Holothuria sanctori (Chiaje, 1823)	–	Mediterranean and adjacent eastern Atlantic[6]	No recent information available	Commercially exploited in Turkey.[17]	Experimentally: In suspended cages with floating sea bass [10,11]	...	0	0

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Atlantic Ocean and Adjacent Seas Mixed Fisheries Continued:								
Isostichopus badionotus (Selenka, 1867)	Four-sided sea cucumber, Chocolate chip sea cucumber, Sea pudding	Caribbean Sea, subtropical Atlantic, Brazil, Venezuela, Colombia, Panama, the Bahamas, South Carolina (USA). Mid Atlantic at Ascensions Island and the Gulf of Guinea off Western Africa	Fishers Receive: 22 kg^{-1} salted Retail Value: 203-402 kg^{-1} dried	One of the most important commercial species in the Caribbean. Exploited in Cuba, Nicaragua and Venezuela. Illegal and unregulated fishing occurs in Colombia. Of potential interest in Florida (USA), Puerto Rico and the United Sates Virgin Islands.	Experimentally: With finfish, filter feeder bivalves and macroalgae [18,19]	…	0	0
Stichopus regalis (Cuvier, 1817)	Royal cucumber	Northwestern Mediterranean, eastern and western Atlantic[20]	Retail Value: Up to 145 kg^{-1} [20]	Commercially exploited in Spain.[20]	Experimentally: In bottom cages beneath sea bream fish [9]	…	0	0
					Atlantic Ocean and Adjacent Seas Total:	**10 017.1**	**0**	**0**
Pacific, Indian and Southern Ocean:								
Actinopyga spinea (Cherbonnier, 1980)	Bury blackfish, New Caledonia blackfish	New Caledonia and Northeastern Australia and Great Barrier Reef (GBR)	Fishers Receive: 2.4 kg^{-1} wet Sold at: 2-4 piece, 20-30 kg^{-1} Wholesale: 63-95 kg^{-1}	Managed in New Caledonia with no-take marine reserves and fishing permits. Size limits, restricted areas and fishing licences used in Australia.	None	**150** (GBR[1])	0	0
Apostichopus japonicus (Selenka, 1867)	Japanese sea cucumber	Western Pacific Ocean, the Yellow Sea, the Sea of Japan, the Sea of Okhotsk. North to the Russian Federation and Alaska (USA). Southern limit is Tanega-shima in Japan	Sold at: 2-3 per piece, 120-130 kg^{-1} in brine and up to 400-500 kg^{-1} dried Retail Value: 970-2 950 kg^{-1} dried	Most important commercial species in Northeast Asia. Fished on an industrial scale. Exploitation regulated by fishing permits. In Japan there are size limits, restricted breeding reserves and restricted fishing during spawning season. Farming Method: Ponds and various sea ranching systems in China, South Korea and the Russian Federation[21-24].	Commercially: In ponds with shrimp [21,22,25], in suspended offshore systems with abalone [26], seeded below kelp long-line areas in China [22,27]. Piloted with scallop, jellyfish, crab, and shrimp in varying combinations [25,28-32] Experimentally: With abalone and sea urchins [33]. Within land-based systems with abalone [34-36]. With oysters and scallops [37-41], placed in bottom cages under fish farms [42], open water red sea bream farms [43] and under Yellowtail pens in Japan [44]. Also with tunicates *Styela clava*[74]	**10 596**	**201 069**	**698 362**
Australostichopus mollis (Hutton, 1872)	Brown mottled sea cucumber	New Zealand and south-eastern parts of Australia	Sold at: 275 kg^{-1} dried	Semi-industrial exploitation in New Zealand.	Experimentally: In a land-based system with abalone [45], with Pacific oysters in intertidal mud flats [46] and co-culture with mussels [47-57]	**6**[1]	0	0
Holothuria fuscogliva (Cherbonnier, 1980)	White teatfish	Indo-Pacific, Madagascar and Red Sea to Easter Islands	Fishers Receive: 7 kg^{-1} wet, 30-55 per piece Sold at: 17-33 kg^{-1} dried Traded at: 42-88 kg^{-1} dried Wholesale: 25-165 kg^{-1} Retail Value: 128-274 kg^{-1}	Overexploited in many areas. In Seychelles it is considered fully exploited. Fishing is banned in a number of countries with size regulated and zone closures in others.	None	**126**	0	0
Holothuria nobilis (Selenka, 1867)	Black teatfish	Western Indian Ocean, Red and Arabian Seas	Sold at: 20-80 kg^{-1} Retail Value: 106-139 kg^{-1}	Valuable and overexploited in much of its range. Currently banned in Egypt.	None	**10**	0	0

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
<u>**Pacific, Indian and Southern Ocean Continued:**</u>								
Holothuria scabra (Jaeger, 1833)	Sandfish	Indo-Pacific east to Fiji	Fishers Receive: 4-8 kg^{-1} split, 3 per piece Sold at: 33-90 kg^{-1} dried, 0.8 per piece Exported at: 60-110 kg^{-1} Wholesale: 108-200 kg^{-1} Retail Value: 115-1 668 kg^{-1}	Exploited throughout its range. Heavily exploited in Madagascar, New Caledonia, Oman, Vietnam, Australia and Mauritius. Overfished in India, Papua New Guinea, Solomon Islands and Vanuatu leading to fishing moratoria. <u>Farming Method:</u> Pond culture, Sea ranching, and pen or cage culture is currently being used to farm *H. scabra*. [58]	<u>Commercially:</u> Small scale in ponds with shrimp (crop rotation) [59,60] and with lobsters and mussels [61,62] <u>Experimentally:</u> With carnivorous Babylon snails[63,64]. With fish (milkfish, pompano and Asian sea bass) in earthen ponds[65]). With red seaweed *Kappaphycus straitum* in lagoon systems [66]. With sea urchins in RAS [16]	**92 000** [58]	**173**	**936**
Parastichopus californicus (Stimpson, 1857)	California sea cucumber, Giant red sea cucumber	Along the Pacific Coast of North America, from the Aleutian Islands (Alaska, USA) to the Gulf of California	Fishers Receive: 3.7-11 kg^{-1} split weight [1,67] Wholesale: 44 kg^{-1} [68] Retail Value: 264.55-639.30 kg^{-1} [75]	Commercially exploited throughout its range along the west coast of North America. Commercial fishery began in 1970s-1980s. Animals are collected by hand via SCUBA or by trawling. Harvest is regulated by permits for gear type and limited entry (USA) and by fishing seasons, limited licences and no-take zones (Canada).	<u>Experimentally:</u> With Pacific oyster [69]. Pilot-scale test with sable-fish, *Anoplopoma fimbria*, Pacific hybrid scallops and kelp, *Saccharina latissima*, in an open-water system [70]	**1 609.4,** USD 13.99 million Canada: 619.9 t, USD 5.39 million USA: 989.5 t, USD 8.6 million [Alaska: 545.7 t, Washington: 303.5 t, California: 140 t]	0	0
Thelenota ananas (Jaeger, 1833)	Prickly redfish, *Holothurie ananas*	Indo-Pacific, the Red Sea, eastern Africa, southeast Asia as far east to French Polynesia	Fishers Receive: 11-18 per piece	Exploited throughout its Indo-Pacific distribution. Managed by size limits and no-take zones in some areas. The Great Barrier Reef also utilizes rotational harvest strategies and limited entry and permits.	None	**125**	0	0
<u>**Pacific, Indian and Southern Ocean Mixed Fisheries:**</u>						**15 243**	**956**	**6361**
Actinopyga echinites (Jaeger, 1833)	Deep-water redfish, Brownfish	Western central Pacific, Asia, Africa and Indian Ocean	Fishers Receive: 2 kg^{-1} dried Traded at: 28-54 kg^{-1} Exported at: 20-30 kg^{-1} Wholesale: up to 63 kg^{-1}	Overexploited in many areas. Fishing is banned in a number of countries with size regulated and zone closures in others.	None	...	...	...
Actinopyga lecanora (Jaeger, 1835)	Stonefish	North Australia, southeast Asia and south Pacific Islands, East Indies, east Africa and Madagascar	Fishers Receive: 5-8 piece, 7 kg^{-1}dried Traded at: 6-20 kg^{-1} Wholesale: 79-108 kg^{-1}	Wild harvest throughout entire range distribution. Restricted and size regulations in some areas.	None	...	...	...
Actinopyga mauritiana (Quoy and Gaimard, 1833)	Surf redfish	North Australia, southeast Asia and south Pacific Islands, Indian Ocean, east Africa and Madagascar	Fishers Receive: 2 kg^{-1} wet, 2-4 per piece Sold at: 10-15 kg^{-1}dried Traded at: 13-47 kg^{-1} dried Exported at: 30 kg^{-1} Wholesale: 57-79 kg^{-1} Retail Value: up to 145 kg^{-1}	Overexploited in many areas. Fishing is banned in a number of countries with size regulated and zone closures in others. Research in Egypt for aquaculture-based restocking.	None	...	...	...
Actinopyga milaris (Quoy and Gaimard, 1833)	Blackfish, Hairy blackfish	North Australia, southeast Asia and south Pacific Islands, Indian Ocean, east Africa and Madagascar	Fishers Receive: 2.4 kg^{-1}, 1-5 per piece Sold at: 15 kg^{-1} dried Wholesale: 63-92 kg^{-1} dried	Wild harvest in more than a dozen countries. Heavily fished in China, Indonesia and the Philippines. Fishing is banned in a number for countries and size regulations in others.	None	...	...	...

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Pacific, Indian and Southern Ocean Mixed Fisheries Continued:								
Actinopyga palauensis (Panning, 1944)	Deepwater blackfish, Panning's blackfish	Eastern Australia and western Pacific Islands	Fishers Receive: 2.4 kg^{-1} wet, 2-16 per piece Exported at: 35 kg^{-1} Wholesale: 95-116 kg^{-1} dried	Harvested in 8 countries, with fishing permits required in some as well as rotational harvest strategies in some (Australia).	None	…	…	…
Apostichopus parvimensis (Clark, 1913)	Warty sea cucumber	Eastern Pacific, Gulf of California north to Point Conception, California (USA)	Fishers Receive: 1 kg^{-1} wet Sold at: 9 kg^{-1} dried	Commercially exploited on the west coast of Mexico and southern California. Collected opportunistically within the sea urchin fishery permits. Managed by No-take marine reserves, fishing permits and trawling is prohibited in some conservation areas.	None	…	…	…
Athyondium chilensis (Semper, 1868)	Pepino de mar, Ancoco blanco	West coast of South America	Sold at: 10 kg^{-1} dried	Commercially exploited in Peru and Chile. Not regulated.	Experimentally: with clams [71]	…	…	…
Bohadschia arguys (Jaeger, 1833)	Leopard fish, Tiger fish	Australia, south Pacific Islands, southeast Asia, East Indies	Fishers Receive: 2-4 kg^{-1} Sold at: 6-20 kg^{-1} Traded at: 15-27 kg^{-1} Wholesale: 49-63 kg^{-1}	Heavily fished in Indonesia, over fished in Vietnam and Kiribati. Considered to be medium-low commercial importance in China.	None	…	…	…
Bohadschia atra (Massin et al., 1999)	Papiro (Madagascar)	Southwest region of the Indian Ocean	No recent information available	No recent information available.	None	…	…	…
Bohadschia marmorata (Jaeger, 1833)	Brown-spotted sandfish, Chalkfish	East Africa, Madagascar, East Indies, southeast Asia, Pacific Islands, north Australia	Fishers Receive: 1.4-2.0 kg^{-1} split weight Traded at: 9-22 kg^{-1} dried	Harvested in a number of countries, although not of commercial interest in some due to low value. Fishing moratorium exists in some areas, although many regions lack regulation for this species.	None	…	…	…
Bohadschia subrubra (Quoy and Gaimard, 1833)	Falalyjaka (Madagascar)	Indian Ocean	No recent information available	Harvest by snorkel diving as part of multi-species fisheries. No regulations determined.	None	…	…	…
Bohadschia vitiensis (Semper, 1868)	Brown sandfish	Indo-Pacific	Fishers Receive: 0.4-1.4 piece, 6 kg^{-1} dried Wholesale: 49 kg^{-1} dried Retail Value: 103-167 kg^{-1}	Commercially exploited in most countries of the Indo-Pacific, overfished in some areas. Fishing banned in some areas (Samo).	None	…	…	…
Cucumaria japonica (Semper, 1868)	Japanese cucumaria, Kinko, Black sea cucumber	Northwest Pacific Ocean (Russian Federation, Japan, Bearing Sea, Yellow Sea)	No recent information available	Commercially harvested in Japan and the Russian Federation. Harvest is managed by seasonal fishing closures, no-take zones and gear limitations.	None	…	…	…
Holothuria arenicola (Semper, 1868)		Indo-Pacific, Red Sea, some locations in the western Pacific	Retail Value: up to 2 kg^{-1}	Artisanal fishing in China, Madagascar and Egypt without regulations.	None	…	…	…
Holothuria atra (Jaegar, 1833)	Lollyfish, Black lollyfish, Black beauty and Mani	Indo-Pacific, central and eastern tropical Pacific	Fishers Receive: 0.6-1.4 kg^{-1} split weight Sold at: 2.5 kg^{-1} dried Traded at: 4-20 kg^{-1} dried Wholesale: up to 63kg^{-1} dried Retail Value: up to 210 kg^{-1}	Harvested in at least 20 countries. Commercially important in China, Japan, Malaysia, Thailand, Indonesia, Philippines, Viet Nam, Tanzania, Mauritius and Eritrea. Some size regulations exist in limited areas. Illegally fished in the Galapagos Islands.	None	…	…	…

Species	Common Name^1	Distribution^1	Market Value (USD)^1	Wild Stock Status^1 and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014^2	Aquaculture Quantity (t) 2014^2	Aquaculture Value (USD 000) 2014^2
Pacific, Indian and Southern Ocean Mixed Fisheries Continued:								
Holothuria cinerascens (Brandt, 1835)	–	Indo-Pacific, Pacific Islands as far east as Easter Islands	Unknown, considered low value	Fished in Guam and Southern Cook Islands for its gonads in subsistence fisheries. Commercially important in China.	None	…	…	…
Holothuria coluber (Semper, 1868)	Snakefish	Southeast Asia and northern Australia	Fishers Receive: 1.4- 2.5 kg^{-1} split Sold at: 4-20 kg^{-1} dried Wholesale: up to 38 kg^{-1}	Fished within many countries of its range, heavily exploited in Indonesia and the Philippines.	None	…	…	…
Holothuria edulis (Lesson, 1830)	Pinkfish	Indo-Pacific and Pacific Islands	Traded at: 4-20 kg^{-1} dried	Low value species. Artisanal fishing in most of its range in the Indo-Pacific, heavily exploited in Indonesia and the Philippines.	None	…	…	…
Holothuria flavomaculata (Semper, 1868)	Red snakefish	Indo-Pacific	Fishers Receive: 2 kg^{-1} split	Low value species. Few regulations. Commercially harvested in Palau, the Federated States of Micronesia, Solomon Islands and Vanuatu, and artisanal fishing elsewhere.	None	…	…	…
Holothuria fuscocinerea (Cherbonnier, 1980)	–	Indo-Pacific, Galapagos Islands and Gulf of California	Traded at: 3 kg^{-1} dried	Commercially important in China (low commercial importance), Malaysia and the Philippines.	None	…	…	…
Holothuria fuscopunctata (Jaeger, 1833)	Elephant trunkfish	Indo-Pacific	Fishers Receive: 0.8-1.7 piece Sold at: 2.7 kg^{-1} dried Traded at: 8 kg^{-1} dried Wholesale: 11-19 kg^{-1}	Harvested mostly by hand, commercially exploited in western Pacific region east to Tonga. Considered underexploited in some areas (Seyechelles). Size limit regulations in Australia.	None	…	…	…
Holothuria hilla (Lesson, 1830)	Tiger tail	Indo-Pacific, Pacific Islands and Central America	Traded at: 3 kg^{-1} dried	Low value species. Fished commercially in Philippines, Indonesia and Madagascar.	None	…	…	…
Holothuria impatiens (Forsskål, 1775)	Brown spotted sea cucumber, Impatient sea cucumber, Bottleneck sea cucumber	Indo-Pacific, Pacific Islands and Central America	Traded at: 2 kg^{-1} dried	Low value species. Unexploited in western central Pacific. Commercial importance in China, Indonesia and Mexico. Limited harvesting in Madagascar.	None	…	…	…
Holothuria kefersteini (Selenka, 1867)	–	Eastern Pacific, Gulf of Baja California to Peru and the Galapagos Islands	Retail Value: 126 kg^{-1} dried	Illegally and severely over-exploited in the Galapagos Islands, El Salvador and Mexico.	None	…	…	…
Holothuria lessoni (Massin et al., 2009)	Golden sandfish	Indo-Pacific, east Africa to Tonga	Fishers Receive: 8 per piece, 4-6 kg^{-1} split Sold at: 8 kg^{-1} split Exported at: > 100 kg^{-1} Traded at: 70-80 kg^{-1} dried Retail Value: 242-787 kg^{-1} dried	Exploited throughout its range. Currently banned in Papua New Guinea, Solomon Islands and Vanuatu. Banned from export in Tonga. Size limits, no-take zones and licensing regulations exist in Australia.	None	…	…	…
Holothuria leucospilota (Brandt, 1835)	White threadfish	Broad distribution throughout Indo-Pacific, western central Pacific, Pacific Islands	Sold at: 1.3 kg^{-1} Traded at: 5 kg^{-1}	Harvest where low-value species are exploited. Exploited for gonads and subsistence fishing in the Cook Islands, Samoa and Tonga.	Experimentally: Placed under fish farms in bottom cages in southern China [72]	…	…	…

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Pacific, Indian and Southern Ocean Mixed Fisheries Continued:								
Holothuria notabilis (Ludwig, 1875)	Dorilisy	Indo-Pacific and Great Barrier Reef	No recent information available	Heavily exploited in Madagascar.	None	...	...	...
Holothuria pervicaz (Selenka, 1867)	–	Indo-Pacific, Pacific Islands and northern Australia	Traded at: 3 kg^{-1}	Low-value species. Fished in China, Madagascar and Indonesia.	None	...	...	...
***Holothuria* sp.** (type 'Pentard')	–	Comoros, Madagascar, Tanzania, Maldives and Sri Lanka	Sold at: 17-26 kg^{-1}, Retail Value: 188 kg^{-1} dried	Considered the highest value and main species in trade in Seychelles. Fisheries managed by limited permits and no-take zones.	None	...	...	...
Holothuria spinifera (Théel, 1886)	Brownfish	Red Sea, Persian Gulf, Sri Lanka, northern Australia, the Philippines	Retail Value: 160-188 kg^{-1} dried	Commercially important in India until banned in 2001. Considered one of the most valuable species in Tanzania. Exploited in Sri Lanka and other islands of the Indian Ocean.	None	...	...	...
Holothuria whitemaei (Bell, 1887)	Black teatfish	Western Australia east to Hawaii. South China Sea to 31 Degrees south eastern Australia.	Fishers Receive:4-6 kg^{-1}, 18 piece Traded at: 23-104 kg^{-1} dried Exported at: 40-50 kg^{-1} dried Wholesale: 25-116 kg^{-1} Retail Value: 37-231 kg^{-1}	Commercially exploited throughout its range. Overfished in Papua New Guinea, Solomon Islands and Vanuatu leading to fishing moratoria. Overexploited on the Great Barrier Reef, reducing stocks by 80 percent leading to a moratorium on this species. Heavily fished in Indonesia, China and the Philippines.	None	...	...	...
Isostichopus fuscus (Ludwig, 1875)	Brown sea cucumber, Giant sea cucumber	Baja California to Ecuador, including eastern Pacific Islands	Sold at: 1.4 piece	Commercially exploited in Ecuador, Mexico, Panama and Peru. Managed by no-take zones, restricted fishing seasons, size limits and permits in the Galapagos Islands and Mexico. Fishing is banned in Ecuador and the species is Listed in the Convention on International Trade in Engendered Species of Wild Fauna and Flora (CITES).	Experimentally: In shrimp ponds (crop rotation) [73]	...	...	...
Pearsonothuria graeffei (Semper, 1868)	Blackspotted sea cucumber, Flowerfish, Orange fish	Indo-Pacific	Fishers Receive: 0.3-0.6 kg^{-1} split weight Sold at: 2-5 kg^{-1} dried	Commercially exploited in a number of countries.	None	...	...	...
Stichopus chloronotus (Brandt, 1835)	Greenfish	Indo-Pacific, east Africa, Madagascar, Sri Lanka, East Indies, north Australia, most islands of the central western Pacific	Fishers Receive: 0.4-0.7 piece Sold at: 12-20 kg^{-1} dried Traded at: 60 kg^{-1} dried Exported at: 25 kg^{-1} dried Wholesale: 63-95 kg^{-1} dried	Commercially important in China, Japan, Malaysia, Thailand, the Philippines, Vietnam and heavily exploited in Indonesia. Harvest in Kenya and Madagascar but of low commercial value. Artisanal fisheries throughout its distribution (e.g. Fiji, Tonga, Vietnam) and some semi-industrial fisheries (Mauritius).	None	...	...	...
Stichopus herrmanni (Semper, 1868)	Curreyfish	Indo-Pacific, east Africa east to Tonga, Japan south to Lord Howe Island (Australia), also Red Sea, southeast Arabia, Persian Gulf	Fishers Receive: 1-5 piece Sold at: 12-20 kg^{-1} dried Traded at: 35-58 kg^{-1} dried Exported at: 20-30 kg^{-1} dried Wholesale: 79-159 kg^{-1} dried Retail Value: 182-214 kg^{-1}	Commercially exploited in China, Malaysia, Thailand, the Philippines, Vietnam and heavily exploited in Indonesia. Artisanal fisheries exist throughout its distribution.	None	...	...	...

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Pacific, Indian and Southern Ocean Mixed Fisheries Continued:								
Stichopus horrens (Selenka, 1868)	Selenka's sea cucumber, Warty sea cucumber, Dragonfish	Red Sea, east Africa, Maldives, East Indies, north Australia, the Philippines, China and southern Japan, the Pacific Islands	Sold at: 39 kg^{-1} dried Wholesale: 56-83 kg^{-1} dried	Heavily exploited in Indonesia, Madagascar and highly sought after in the Philippines. It is collected illegally in the Galapagos Islands.	None	...	...	...
Stichopus monotuberculatus (Quoy and Gaimard, 1833)	Often confused with *S. horrens*	Patchy distribution throughout Indo-Pacific	Sold at: 39 kg^{-1} dried Exported at: 11-16 kg^{-1} dried Wholesale: 111-133 kg^{-1} dried	Sold as *S. horrens*, because of similarity in appearance. Extent of exploitation is unknown.	None	...	...	...
Stichopus naso (Semper, 1868)	Often confused with *S. horrens*	Indo-Pacific, Madagascar east to the Philippines, Japan to northwestern Australia	Sold at: 39 kg^{-1} dried	Sold as *S. horrens*, because of similarity in appearance. Extent of exploitation is unknown.	None	...	...	...
Stichopus ocellatus (Massin et al., 2002)	Curryfish, Ocellated sea cucumber	Papua New Guinea, northern Great Barrier Reef and Torres Strait (Australia), Malaysia, the Philippines, Vietnam, possibly Indonesia	Traded at: 35-58 kg^{-1} dried. Wholesale: up to 111 kg^{-1} dried	Artisanal fisheries (Philippines, Malaysia, Vietnam), and collected in small quantities at a semi-industrial scale (Australia). Managed in Australia, with permits and no-take zones. No regulation in southeast Asian countries.	None	...	...	...
Stichopus vastus (Sluiter, 1887)	Curryfish, Zebrafish	Indonesia, the Philippines, Papua New Guinea, Palau Islands, Yap and northeastern Australia	Traded at: 35-58 kg^{-1} dried	Exploited in artisanal and semi-industrial fisheries. Heavily exploited in Indonesia. Managed in Australia with permits, no-take zones, and rotational harvest strategies.	None	...	...	...
Thelenota anax (Clark, 1921)	Amber fish	Tropical Indian Ocean, East Africa, southeast Asia, Tropical Pacific, northern Australia	Sold at: 3-4 kg^{-1} dried Traded at: 13 kg^{-1} Wholesale: 14-32 kg^{-1} dried	No recent information available.	None	...	...	...
Thelenota rubralineata (Massin and Lane, 1991)	Lemonfish, Candycanefish, Red-lined sea cucumber	Southeast Asia, south Pacific Islands	Traded at: 13 kg^{-1}	Prior to moratoria, it was commercially harvested in Papua New Guinea and Solomon Islands. Few regulations for exploitation elsewhere.	None	...	...	...
					Pacific, Indian and Southern Ocean Total:	**119 865.4**	**202 198**	**705 659**
					Global Total:	**129 882.5**	**202 198**	**705 659**

Species	Common Name[1]	Distribution[1]	Market Value (USD)[1]	Wild Stock Status[1] and Farming Methods	Integrated Multi-Trophic Aquaculture	Wild Harvest (t) 2014[2]	Aquaculture Quantity (t) 2014[2]	Aquaculture Value (USD 000) 2014[2]
Pacific, Indian and Southern Ocean Mixed Fisheries Continued:								
Stichopus horrens (Selenka, 1868)	Selenka's sea cucumber, Warty sea cucumber, Dragonfish	Red Sea, east Africa, Maldives, East Indies, north Australia, the Philippines, China and southern Japan, the Pacific Islands	Sold at: 39 kg^{-1} dried Wholesale: 56-83 kg^{-1} dried	Heavily exploited in Indonesia, Madagascar and highly sought after in the Philippines. It is collected illegally in the Galapagos Islands.	None	...	...	...
Stichopus monotuberculatus (Quoy and Gaimard, 1833)	Often confused with *S. horrens*	Patchy distribution throughout Indo-Pacific	Sold at: 39 kg^{-1} dried Exported at: 11-16 kg^{-1} dried Wholesale: 111-133 kg^{-1} dried	Sold as *S. horrens*, because of similarity in appearance. Extent of exploitation is unknown.	None	...	...	...
Stichopus naso (Semper, 1868)	Often confused with *S. horrens*	Indo-Pacific, Madagascar east to the Philippines, Japan to northwestern Australia	Sold at: 39 kg^{-1} dried	Sold as *S. horrens*, because of similarity in appearance. Extent of exploitation is unknown.	None	...	...	...
Stichopus ocellatus (Massin et al., 2002)	Curryfish, Ocellated sea cucumber	Papua New Guinea, northern Great Barrier Reef and Torres Strait (Australia), Malaysia, the Philippines, Vietnam, possibly Indonesia	Traded at: 35-58 kg^{-1} dried. Wholesale: up to 111 kg^{-1} dried	Artisanal fisheries (Philippines, Malaysia, Vietnam), and collected in small quantities at a semi-industrial scale (Australia). Managed in Australia, with permits and no-take zones. No regulation in southeast Asian countries.	None	...	...	...
Stichopus vastus (Sluiter, 1887)	Curryfish, Zebrafish	Indonesia, the Philippines, Papua New Guinea, Palau Islands, Yap and northeastern Australia	Traded at: 35-58 kg^{-1} dried	Exploited in artisanal and semi-industrial fisheries. Heavily exploited in Indonesia. Managed in Australia with permits, no-take zones, and rotational harvest strategies.	None	...	...	...
Thelenota anax (Clark, 1921)	Amber fish	Tropical Indian Ocean, East Africa, southeast Asia, Tropical Pacific, northern Australia	Sold at: 3-4 kg^{-1} dried Traded at: 13 kg^{-1} Wholesale: 14-32 kg^{-1} dried	No recent information available.	None	...	...	...
Thelenota rubralineata (Massin and Lane, 1991)	Lemonfish, Candycanefish, Red-lined sea cucumber	Southeast Asia, south Pacific Islands	Traded at: 13 kg^{-1}	Prior to moratoria, it was commercially harvested in Papua New Guinea and Solomon Islands. Few regulations for exploitation elsewhere.	None	...	...	...
					Pacific, Indian and Southern Ocean Total:	**119 865.4**	**202 198**	**705 659**
					Global Total:	**129 882.5**	**202 198**	**705 659**

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73 Mercier, A. et al. (2012) Asia-Pacific Tropical Sea Cucumber Aquaculture. ACIAR Proceedings No. 136, Noume, New Caledonia,

74 Ju, B. et al. (2016) Aquaculture International, DOI: 10.1007/s10499-016-027-8

75 Ebay, 2016. Retrieved July 1st 2016 from www.ebay.ca, search "California sea cucumber"

Table 1.3. Global sea cucumber production summary 2014.

Species	Wild Harvest (mt)	Aquaculture Quantity (mt)	Total Production (mt)
Apostichopus japonicus (Asia)	**10,596** 3.2 %	**201,069** 60.5 %	**211,665** 63.7 %
Holothuria scabra (Asia)	**92,000*** 27.7 %	**173** 0.1 %	**92,173** 27.8 %
Pacific Mixed Fisheries (47 Species)	**15,660** 4.7 %	**956** 0.3 %	**16,616** 5.0 %
Cucumaria frondosa (Canada & USA)	**5,538.1** 1.7 %	**0** 0 %	**5,538.1** 1.7 %
Atlantic Mixed Fisheries (11 Species)	**4,479** 1.3 %	**0** 0 %	**4,479** 1.3 %
Parastichopus californicus (Canada & USA)	**1,609.4** 0.5 %	**0** 0 %	**1,609.4** 0.5 %
Global Total	**129,882.5** 39.1 %	**202,198** 60.9 %	**332,080.5** 100 %

Data retrieved from:

FAO. 2014. Fisheries and Aquaculture Statistics. www.fao.org/fishery/statistics

* Tuwo, A. and Tresnati, J. 2015. Sea cucumber farming in southeast Asia (Malaysia, Philippines, Indonesia, Vietnam). In Echinoderm Aquaculture, First Edition. Edited by Brown, N.P. and Eddy, S.D. published by John Wiley and Sons, Inc., Hoboken, New Jersey, 331–352

Table 2.1. *Parastichopus californicus* size measurements.

	Mean	SD	Min	Max	*N*
Laboratory Study:					
Wet Weight (g)	0.35	0.26	0.02	1.69	177
Whole Length (cm)	2.35	0.84	0.71	5.41	-
Whole Width (cm)	0.59	0.16	0.26	1.17	-
Size Index	0.015	0.009	0.002	0.057	-
Field Study:					
<u>Initial Size:</u>					
Wet Weight (g)	9.3	10.74	0.1	85.8	574
Whole Length (cm)	5.5	2.0	1.8	12.1	-
Whole Width (cm)	1.9	0.87	0.4	5.1	-
Size Index	0.120	0.093	0.007	0.550	-
<u>Final Size Excluding Newly Recruited Sea Cucumbers:</u>					
Wet Weight (g)	11.3	13.7	0.2	95	227
Whole Length (cm)	6.5	3.1	1.3	16.4	-
Whole Width (cm)	1.4	0.6	0.4	3.8	-
Size Index	0.109	0.095	0.005	0.623	-
Split Weight (g)	6.3	5.8	0.1	28.5	173
<u>Final Size of Sea Cucumbers Lacking Visceral Organs Excluding Newly Recruited Sea Cucumbers:</u>					
Wet Weight (g)	11.8	11.7	1.5	68.0	63
Whole Length (cm)	7.0	2.4	3.1	16.4	-
Whole Width (cm)	1.5	0.55	0.8	3.8	-
Size Index	0.118	0.092	0.026	0.623	-
Split Weight (g)	6.3	5.2	1.2	28.5	-
<u>Final Size of Sea Cucumbers with Visceral Organs Excluding Newly Recruited Sea Cucumbers:</u>					
Wet Weight (g)	10.5	14.8	0.2	95	144
Whole Length (cm)	6.1	3.4	1.3	15.7	-
Whole Width (cm)	1.3	0.7	0.4	3.2	-
Size Index	0.101	0.099	0.005	0.442	-
Split Weight (g)	5.9	6.3	0.1	25.6	90
<u>Newly Recruited Sea Cucumbers:</u>					
Wet Weight (g)	0.4	0.7	0.01	5.6	266
Whole Length (cm)	2.0	1.5	0.5	15.1	-
Whole Width (cm)	0.6	0.3	0.2	2.7	-
Size Index	0.016	0.034	0.001	0.399	-
<u>Final Size All Sea Cucumbers Including Newly Recruited:</u>					

Wet Weight (g)	5.6	11.2	0.01	95	495
Whole Length (cm)	4.1	3.3	0.5	16.4	-
Whole Width (cm)	0.95	0.65	0.2	3.8	-
Size Index	0.058	0.083	0.001	0.6	-
Split Weight (g)	6.0	5.9	0.03	28.5	194

Table 2.2. ANOVA results for containment success of *Parastichopus californicus* in the laboratory trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Cage	7	17.34	2.48	43.93	**<0.00001**
Time	7	1.52	0.22	6.08	**<0.0001**
Tank	7	0.18	0.03	0.70	0.68
Error	42	1.52	0.04		

Table 2.3. ANOVA results for percent containment of *Parastichopus californicus* in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Cage	2	75.15	37.58	11.99	**<0.001**
Oyster	1	9.27	9.27	2.96	0.11
Raft	4	13.91	3.48	1.11	0.39
Cage x Oyster	2	4.54	2.27	0.72	0.50
Error	16	50.16	3.14		

Table 2.4. ANOVA results for final count of *Parastichopus californicus* in the field trial (sub-divided by state of visceral organs).

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value

Experimental sea cucumbers without organs:					
Cage	2	832.7	416.4	32.71	**<0.00001**
Oyster	1	186.7	186.7	14.67	**<0.0025**
Raft	4	131.4	32.8	2.58	0.08
Cage x Oyster	2	150.0	75.0	5.89	**<0.025**
Error	16	203.7	12.7		
Experimental sea cucumbers with organs:					
Cage	2	14.88	7.44	1.18	0.33
Oyster	1	5.80	5.80	0.92	0.35
Raft	4	165.65	41.41	6.57	**<0.005**
Cage x Oyster	2	3.00	1.50	0.24	0.79
Error	16	100.83	6.30	6.30	
Newly recruited sea cucumbers:					
Cage	2	78.6	39.28	1.36	0.29
Oyster	1	8.6	8.60	0.30	0.59
Raft	4	242.8	60.71	2.10	0.13
Cage x Oyster	2	92.0	45.99	1.59	0.23
Error	16	462.5	28.91		
All sea cucumbers:					
Cage	2	351.7	175.83	3.79	**<0.05**
Oyster	1	4.1	4.05	0.09	0.77
Raft	4	327.0	81.74	1.76	0.19
Cage x Oyster	2	83.6	41.80	0.90	0.43
Error	16	743.1	46.45		

Table 2.5. ANOVA results for dry sediment deposition rate (g m^{-2} day^{-1}) in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Experimental cages suspended below oyster farm:					
Cage	2	63.55	31.78	47.24	**<0.00001**
Sea cucumbers (SC)	1	0.11	0.11	0.16	0.70

Raft	2	8.35	4.17	6.20	**<0.01**
Cage x SC	2	0.45	0.23	0.34	0.72
Error	25	16.82	0.67		
Experimental cages including control and sediment traps:					
Cage	4	82.49	20.62	39.62	**<0.00001**
Raft	2	8.11	4.06	7.70	**<0.0025**
Error	34	17.90	0.53		

Table 2.6. ANOVA results for total organic matter deposition rate (g m^{-2} day^{-1}) in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Experimental cages suspended below oyster farm:					
Cage	2	35.52	17.76	27.55	**<0.00001**
Sea cucumbers (SC)	1	0.02	0.02	0.04	0.85
Raft	2	4.40	2.20	3.42	**<0.05**
Cage x SC	2	0.23	0.12	0.18	0.84
Error	26	16.12	0.65		
Experimental cages including control and sediment traps:					
Cage	4	57.13	14.28	29.08	**<0.00001**
Raft	2	4.26	2.13	4.34	**<0.025**
Error	34	16.70	0.49		

Table 2.7. ANOVA results for total nitrogen (g) deposited in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Experimental cages suspended below oyster farm:					
Cage	2	34.98	17.49	30.13	**<0.00001**
Sea cucumbers (SC)	1	0.15	0.15	0.27	0.61
Raft	2	3.50	1.75	3.02	0.07
Cage x SC	2	0.54	0.27	0.47	0.63

Error	25	14.51	0.58		
Experimental cages including control and sediment traps:					
Cage	4	0.57	0.14	14.42	**<0.00001**
Raft	2	0.06	0.03	2.98	0.06
Error	34	0.34	0.01		

Table 2.8. ANOVA results for total organic carbon (g) deposited in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Experimental cages suspended below oyster farm:					
Cage	2	33.45	16.72	27.09	**<0.00001**
Sea cucumbers (SC)	1	0.18	0.18	0.30	0.59
Raft	2	2.96	1.48	2.40	0.11
Cage x SC	2	0.51	0.26	0.41	0.67
Error	25	15.43	0.62		
Experimental cages including control and sediment traps:					
Cage	4	28.66	7.16	16.25	**<0.00001**
Raft	2	2.27	1.13	2.57	0.09
Error	34	14.99	0.44		

Table 2.9. ANOVA results for growth of *Parastichopus californicus* in the field trial.

Term	*df*	Sum of squares	Mean square	*F*-ratio	*p*-value
Sea cucumber percent change in whole wet weight during summer:					
Cage	2	29851	14925	5.78	**<0.025**
Raft	2	3034	1517	0.59	0.57
Error	16	30995	2583		
Sea cucumber percent change in whole wet weight during fall:					

Cage	2	1027	513.3	0.27	0.77
Raft	2	77	38.5	0.02	0.98
Error	12	23128	1927.3	6.30	
Sea cucumber absolute growth rate (g d^{-1}) during summer:					
Cage	2	0.15	0.07	6.43	**<0.025**
Raft	2	0.01	0.003	0.232	0.80
Error	12	0.14	0.01		
Sea cucumber absolute growth rate (g d^{-1}) during fall:					
Cage	2	0.10	0.05	0.48	0.63
Raft	2	0.001	0.001	0.005	0.99
Error	12	1.26	0.11		
Sea cucumber specific growth rate (% d^{-1}) during summer:					
Cage	2	3.16	1.58	6.04	**<0.025**
Raft	2	0.25	0.13	0.49	0.63
Error	12	3.14	0.26		
Sea cucumber specific growth rate (% d^{-1}) during fall:					
Cage	2	2.76	1.38	0.33	0.73
Raft	2	0.23	0.12	0.03	0.97
Error	12	50.61	4.22		

Figures

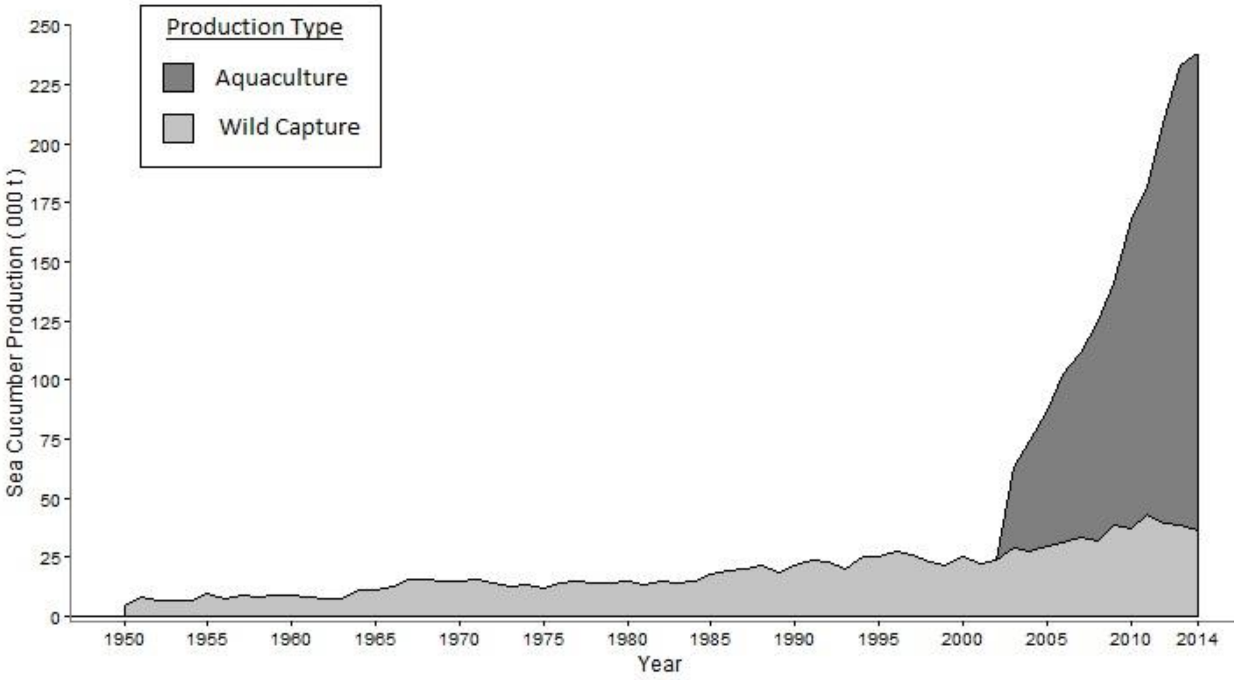


Figure 1.1. FAO statistics on global sea cucumber production.
Data retrieved from: FAO. 2014. Fisheries and Aquaculture Statistics, www.fao.org/fishery/statistics.

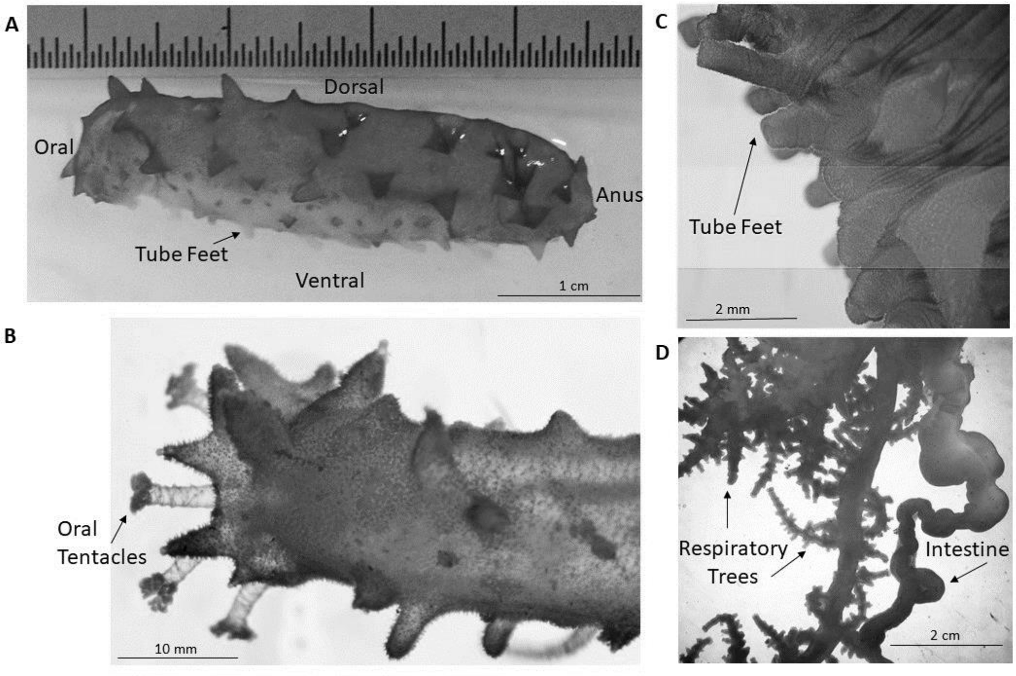


Figure 1.2. *Parastichopus californicus* morphology. (A) Juvenile *P. californicus*, profile view showing distinct oral/aboral sides or anterior/ posterior ends, dorsal side with epithelial red spikes, and ventral side with tube feet. (B) Juvenile *P. californicus*, retaining its mostly white, post-settlement phenotype, displaying oral tentacles. (C) Juvenile *P. californicus* tube feet. (D) Digestive tract and respiratory tree of an adult *P. californicus* post-evisceration.

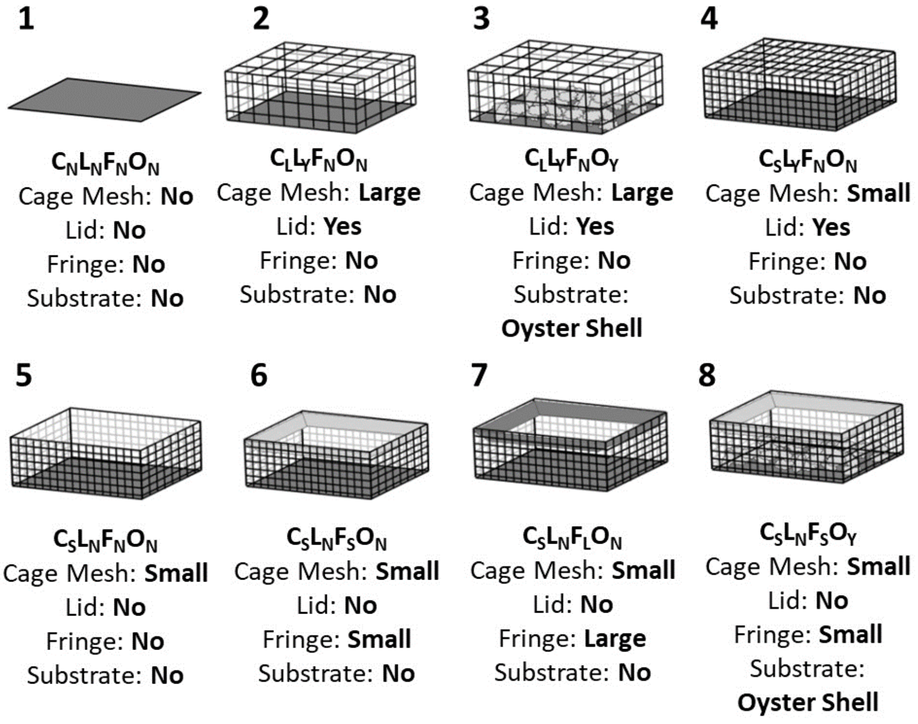
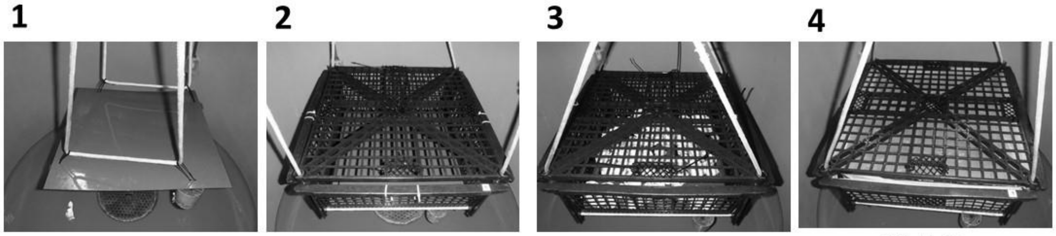


Figure 1.3. Sea cucumber cage designs. Commercial high-flow oyster trays were modified for testing juvenile *P. californicus* containment. All cages were modified with a solid bottom of PVC sheeting. Cages 4, 5, 6, 7, and 8 were modified with nylon mesh (‘Small’ < 1 mm) on the walls and lid for cage 4. Cages 6, 7, and 8 were modified with mesh fringe encircling the inner rim (‘Large’ = 500-µm mesh, ‘Small’ = 250-µm mesh). Cages 3 and 8 had 2 kg of Pacific oyster (*Crassostrea gigas*) shell added.

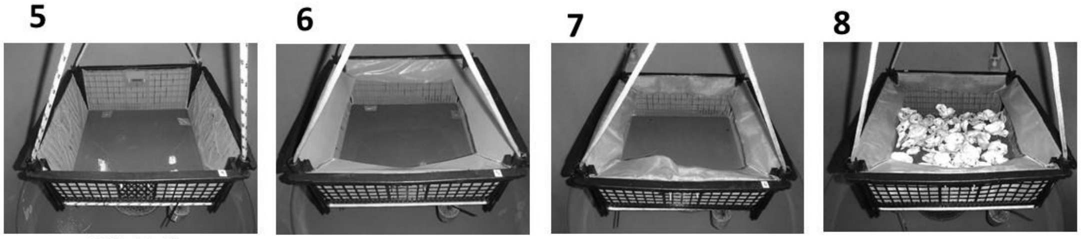


$C_NL_NF_NO_N$
Cage Mesh: **No**
Lid: **No**
Fringe: **No**
Substrate: **No**

$C_LL_YF_NO_N$
Cage Mesh: **Large**
Lid: **Yes**
Fringe: **No**
Substrate: **No**

$C_LL_YF_NO_Y$
Cage Mesh: **Large**
Lid: **Yes**
Fringe: **No**
Substrate:
Oyster Shell

$C_SL_YF_NO_N$
Cage Mesh: **Small**
Lid: **Yes**
Fringe: **No**
Substrate: **No**



$C_SL_NF_NO_N$
Cage Mesh: **Small**
Lid: **No**
Fringe: **No**
Substrate: **No**

$C_SL_NF_SO_N$
Cage Mesh: **Small**
Lid: **No**
Fringe: **Small**
Substrate: **No**

$C_SL_NF_LO_N$
Cage Mesh: **Small**
Lid: **No**
Fringe: **Large**
Substrate: **No**

$C_SL_NF_SO_Y$
Cage Mesh: **Small**
Lid: **No**
Fringe: **Small**
Substrate:
Oyster Shell

Figure 2.1. Sea cucumber cage designs. Commercial high-flow oyster trays were modified for testing juvenile *P. californicus* containment. All cages were modified with a solid bottom of PVC sheeting. Cages 4, 5, 6, 7, and 8 were modified with nylon mesh (‘Small’ < 1 mm) on the walls and lid for cage 4. Cages 6, 7, and 8 were modified with mesh fringe encircling the inner rim (‘Large’ = 500-µm mesh, ‘Small’ = 250-µm mesh). Cages 3 and 8 had 2 kg of Pacific oyster (*Crassostrea gigas*) shell added.

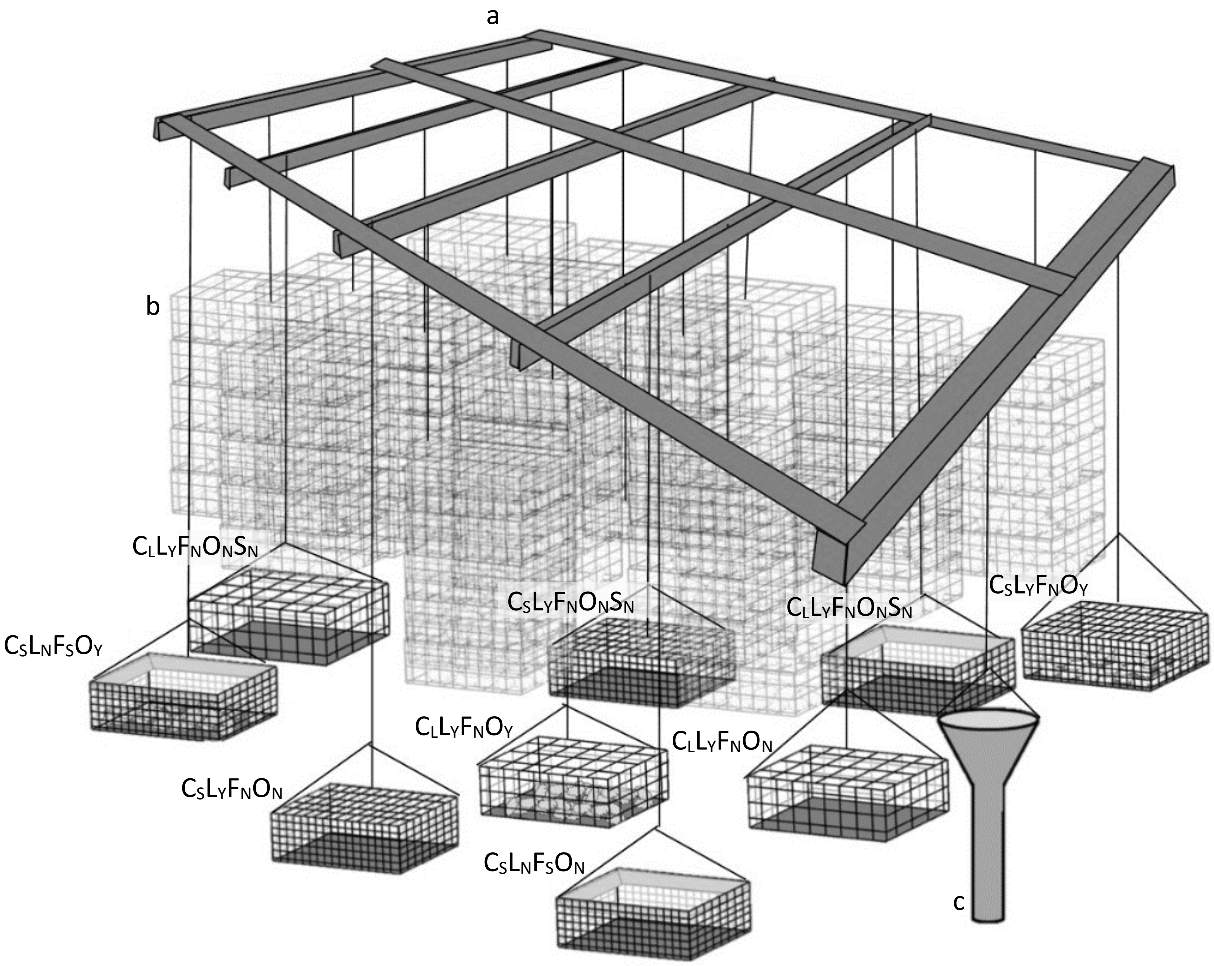
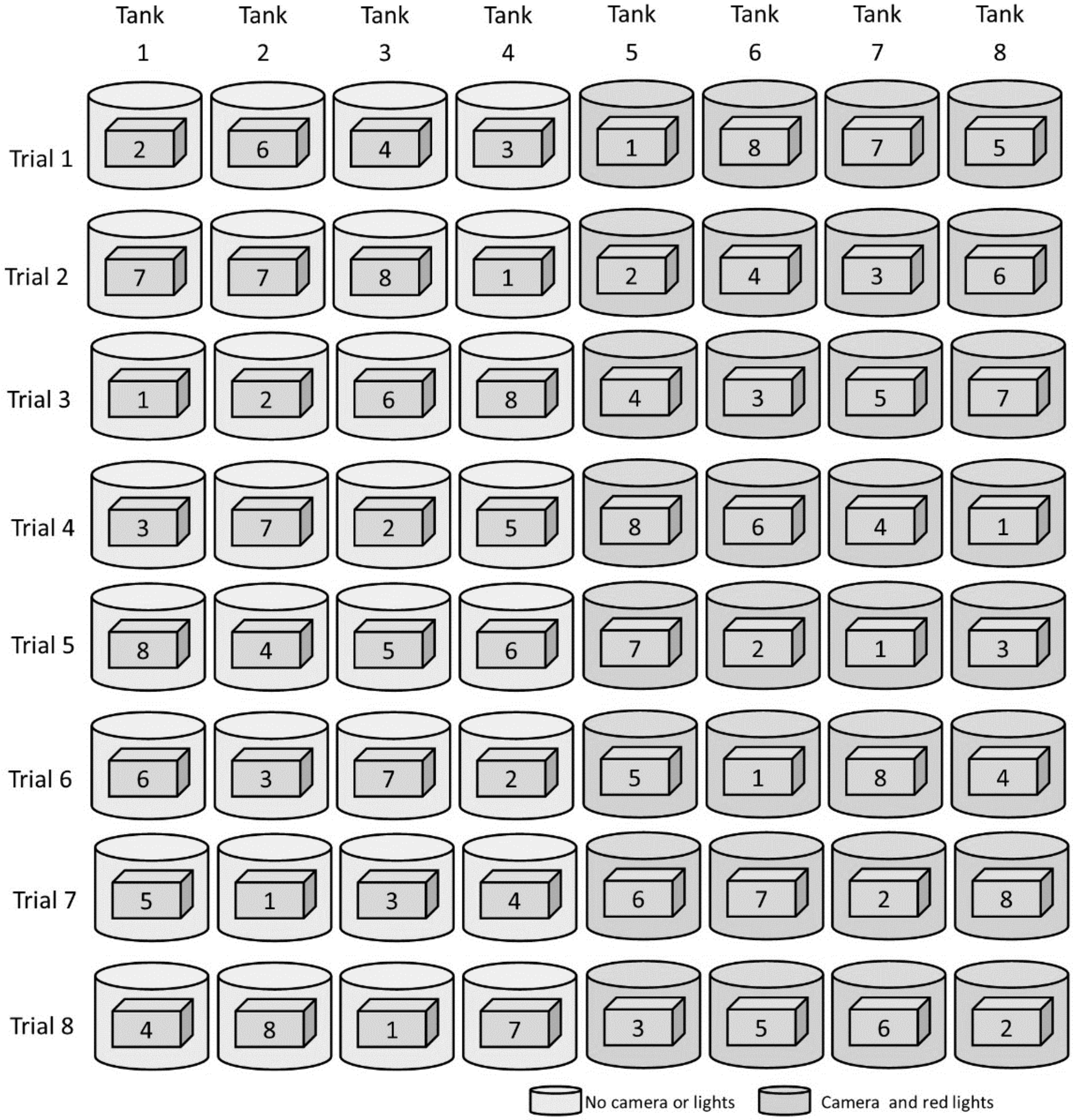


Figure 2.2. Schematic of one oyster raft with experimental sea cucumber cages. (a) Oyster raft floating structure, approximately 8 x 7.5 m, made of wood, Styrofoam, and plastic materials. (b) Commercial oyster stacks, approximately 1.8 m in height, suspended from the oyster raft to a maximum depth of 8 m. Cage abbreviations are: (1) cage mesh size: C_L=large mesh (1.2–3.0 cm) and C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_S = small mesh (250 µm) and F_N = no fringe; (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell; (5) Sea cucumbers: S_N = zero stocking density sea cucumber cage. (c) Sediment trap (height: 110 cm, capture area: 0.16 m^2 at top of sediment trap suspended) at 9 m depth. Figure not to scale.



	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5	Tank 6	Tank 7	Tank 8
Trial 1	2	6	4	3	1	8	7	5
Trial 2	7	7	8	1	2	4	3	6
Trial 3	1	2	6	8	4	3	5	7
Trial 4	3	7	2	5	8	6	4	1
Trial 5	8	4	5	6	7	2	1	3
Trial 6	6	3	7	2	5	1	8	4
Trial 7	5	1	3	4	6	7	2	8
Trial 8	4	8	1	7	3	5	6	2

Figure 2.3. Diagram of experimental laboratory Latin-square design, blocked over time and by tank. Each tray was suspended in an 860 L tank with the order of trays randomized. Trials were 48 h with a minimum 48 h rest period between trials. *Parastichopus californicus* were starved for 24 h and randomized among tray types prior to each trial. An ambient photoperiod was kept throughout the experiment, with tanks equipped with cameras illuminated with red lights.

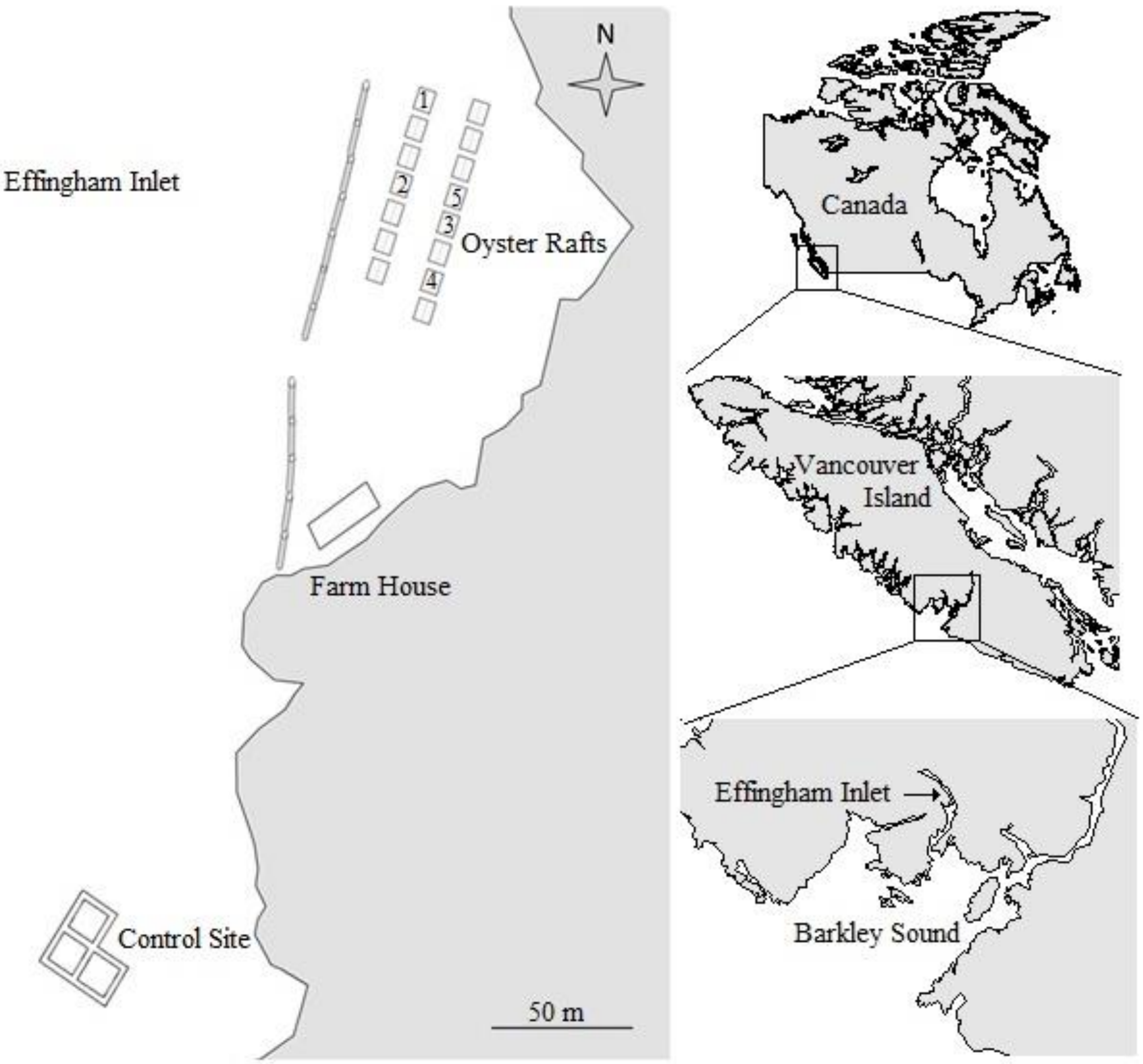


Figure 2.4. Field experiment location. Effingham Oysters Ltd., a Pacific oyster (*Crassostrea gigas*) farm, is located on the west coast of Vancouver Island, British Columbia, Canada, within Barkley Sound and Effingham Inlet (49° 1'22.18''N, 125° 8'55.34''W). Experimental *Parastichopus californicus* cages were suspended from randomly-chosen rafts labeled 1–5 as well as at a control site 320 m away located on an abandoned fish farm structure.

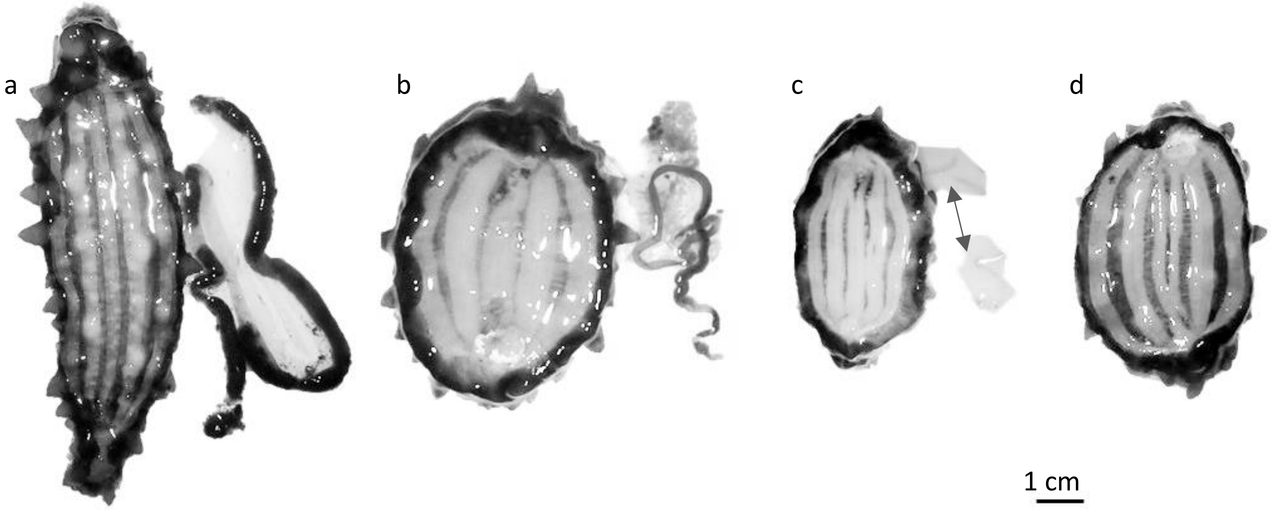


Figure 2.5. Split *Parastichopus californicus* with various states of visceral organs. (a) Individual with a fully-functioning digestive system. (b) Individual with a reduced digestive tract. (c) Individual with greatly reduced visceral organs, remnants of which being highlighted by arrows. (d) Individual with no visceral organs present.

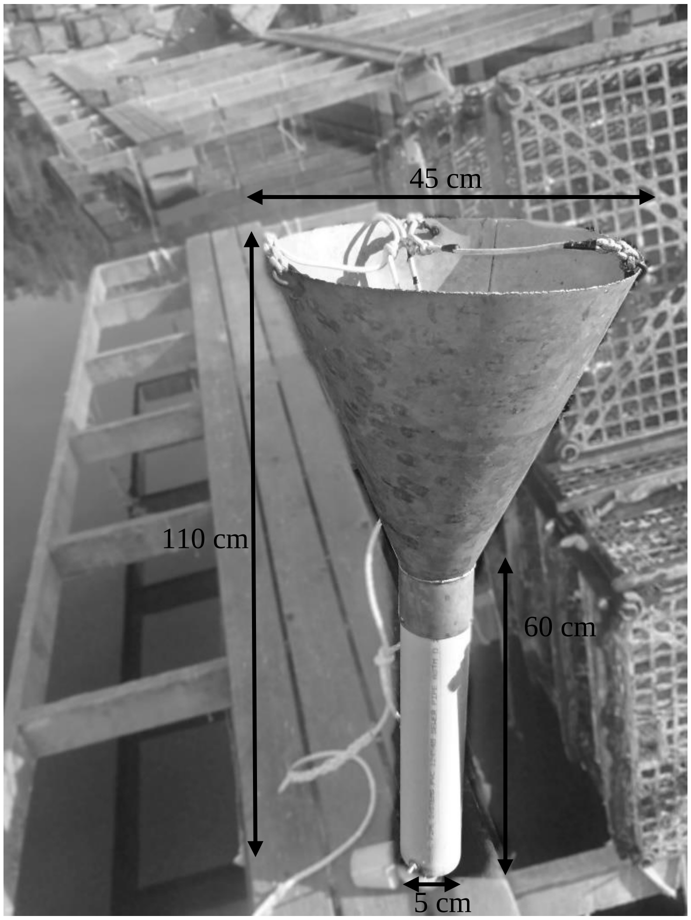


Figure 2.6. Sediment trap deployed at 9 m depth below oyster farm rafts from October 1 to November 14, 2015 in Effingham Inlet, Vancouver Island, British Columbia, Canada. The collection canister consists of a PVC cylinder 60 cm in length and with a 5 cm inner diameter, with the top edge beveled to prevent material buildup. The 45 cm diameter capture cone was manufactured out of aluminum sheeting. Concrete weights were attached to the bottom to keep the trap vertical.

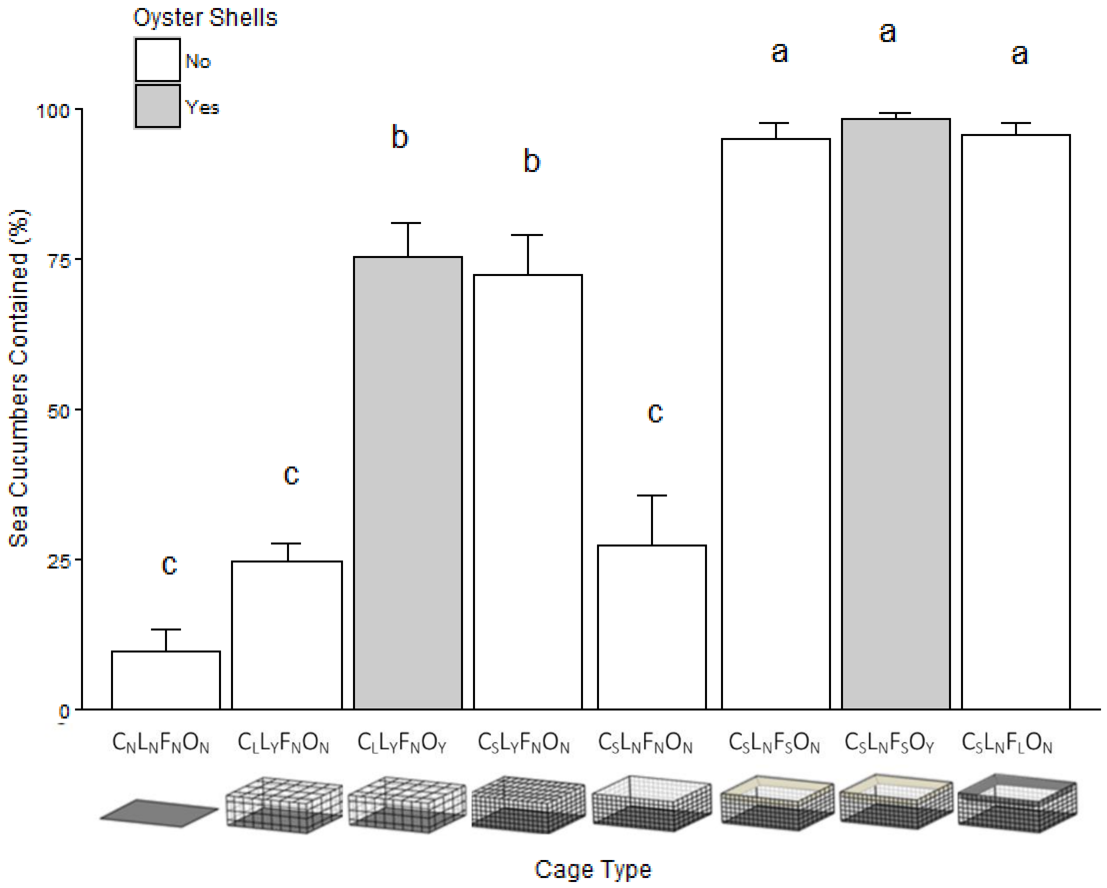


Figure 2.7. Mean (+SE) percent containment of *Parastichopus californicus* in the various cage types in the laboratory trial after 48 h. Initial stocking density was 21 juveniles per cage and *n* = 8. Different letters above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm), and C_N = no mesh (flat PVC plate alone); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

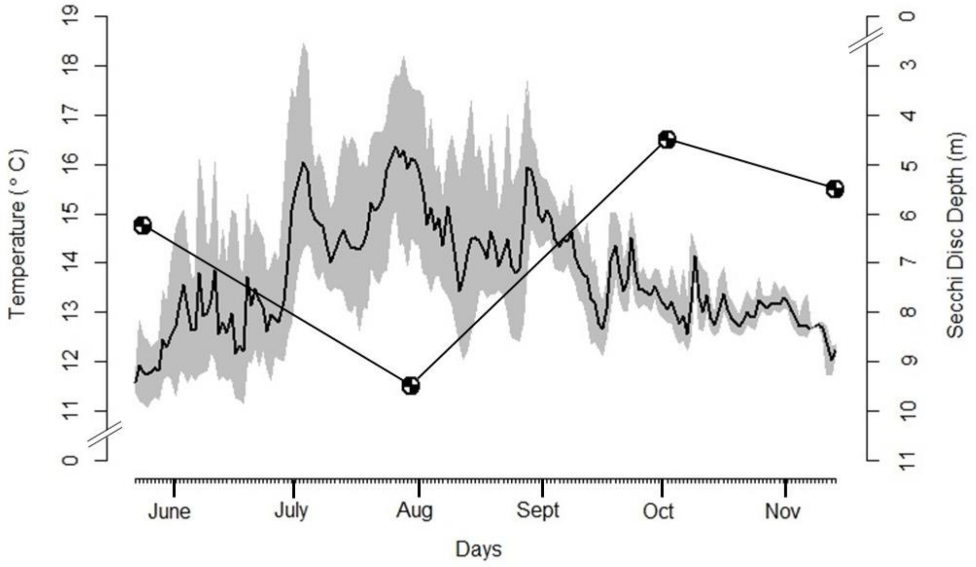


Figure 2.8. Water temperature and secchi disc depth during the study period, May 23 to November 15, 2015 in Effingham Inlet, Vancouver Island, British Columbia, Canada. Water temperature was recorded at 9 m depth. The dark temperature line indicates daily average temperature, with daily minimum and daily maximum displayed in the grey-shaded area.

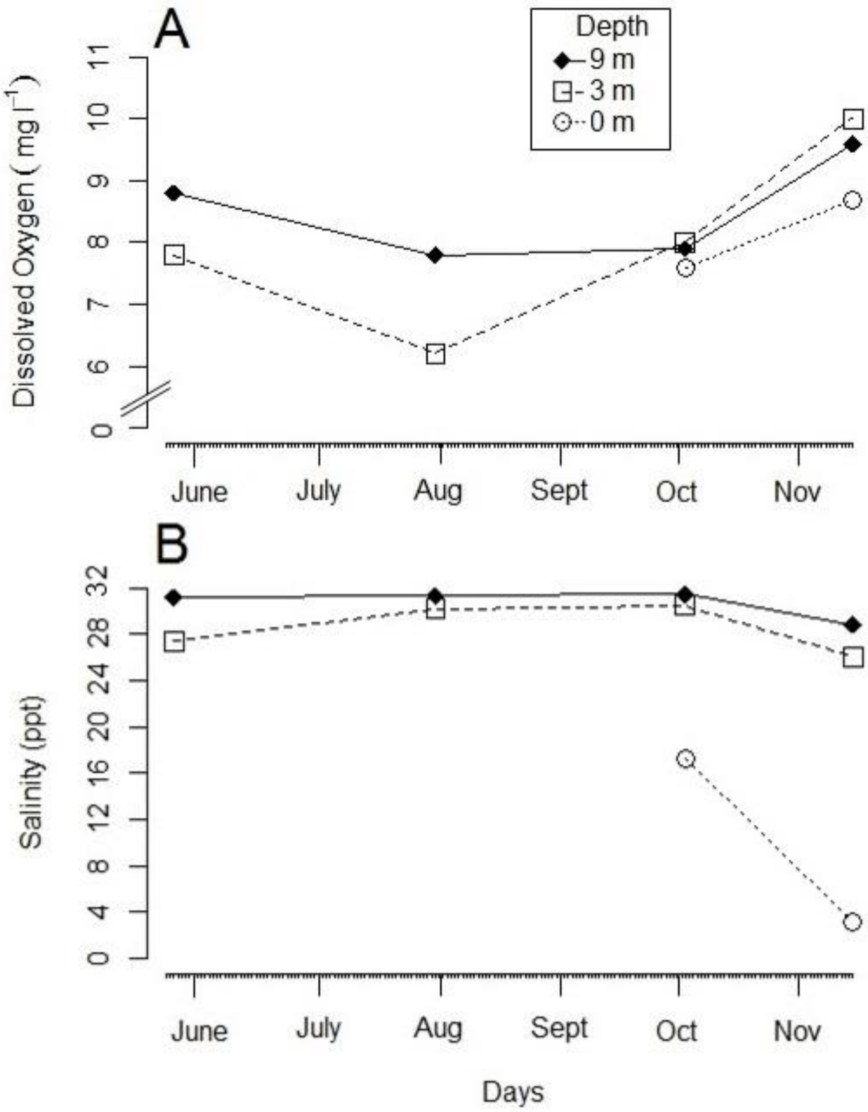


Figure 2.9. Water salinity and dissolved oxygen during the study period, May 23 to November 15, 2015 in Effingham Inlet, Vancouver Island, British Columbia, Canada.

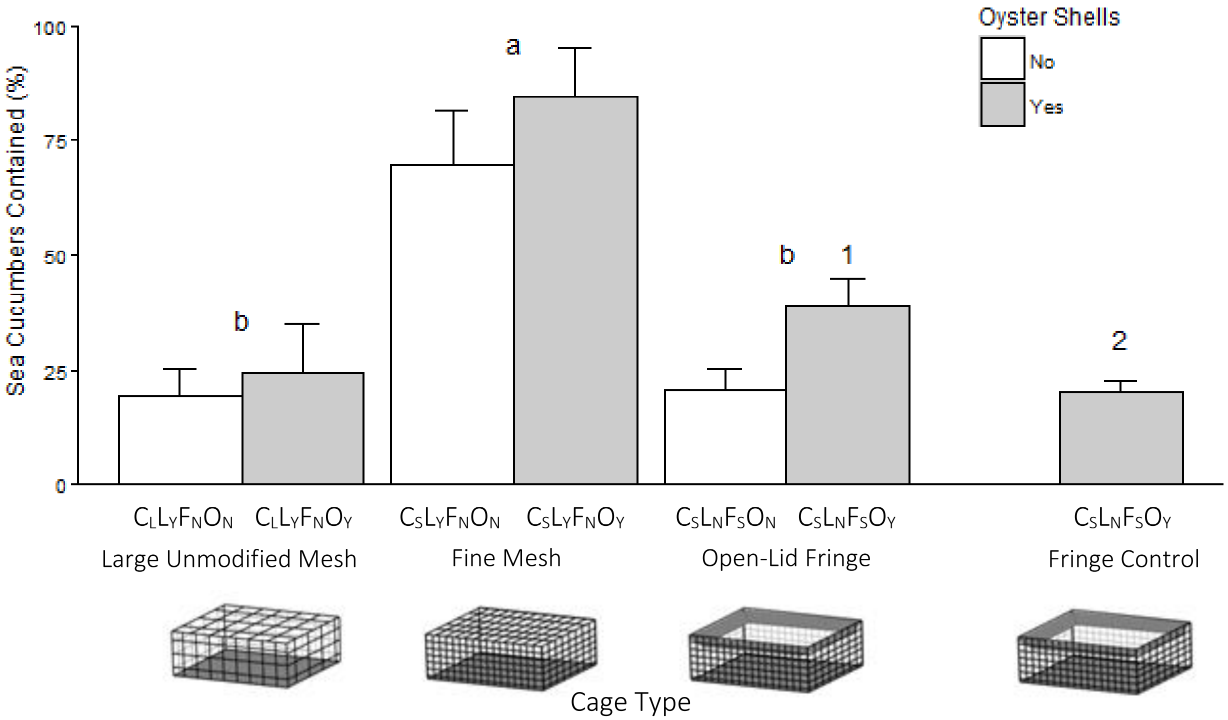


Figure 2.10. Mean (+SE) percent containment of *Parastichopus californicus* in the various cage types in field trials. Initial stocking density of 21 juvenile sea cucumbers for rafts 1 and 2, deployed May 23rd and 14 juvenile sea cucumbers per cage for rafts 3-5 deployed August 1st, *n* = 5, trial ended November 15th, 2015. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test or two sample t-test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

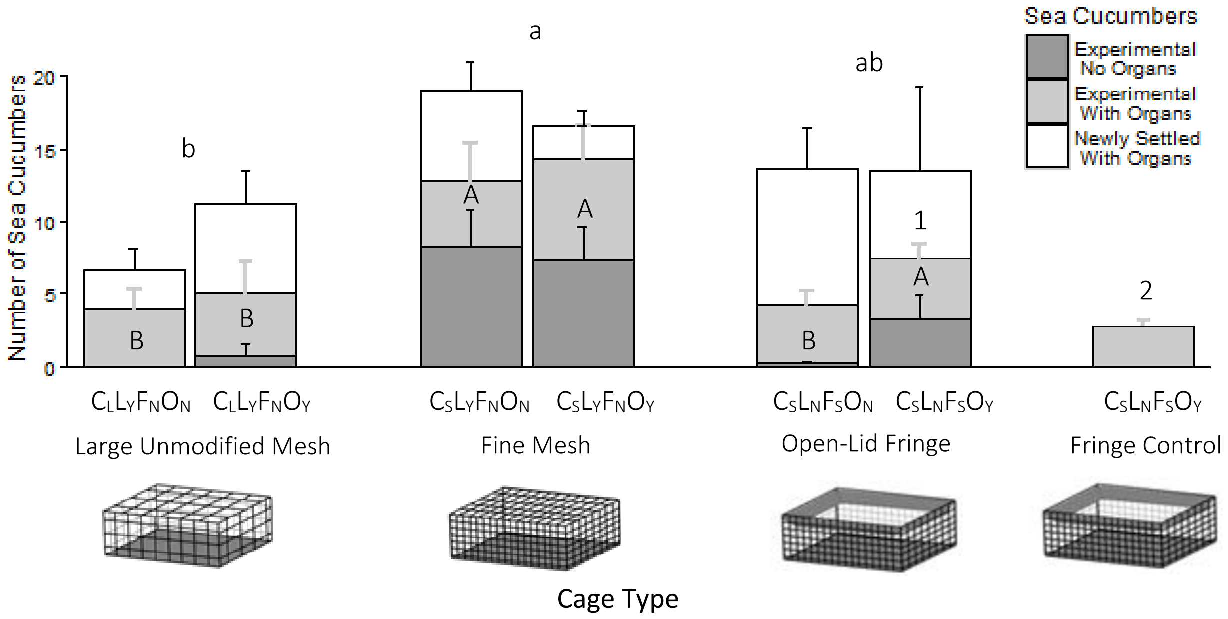


Figure 2.11. Final sea cucumber count by cage type and state of visceral organs. Experimental cages, *n* = 5, were suspended 9 m below a pacific oyster farm and fringe control cages were suspended at 9 m depth 320 m away from farm. Initial stocking density of 21 juvenile sea cucumbers for rafts 1 and 2, deployed May 23rd and 14 juvenile sea cucumbers per cage for rafts 3-5 deployed August 1st, *n* = 5, trial ended November 15th, 2015. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test or two sample t-test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

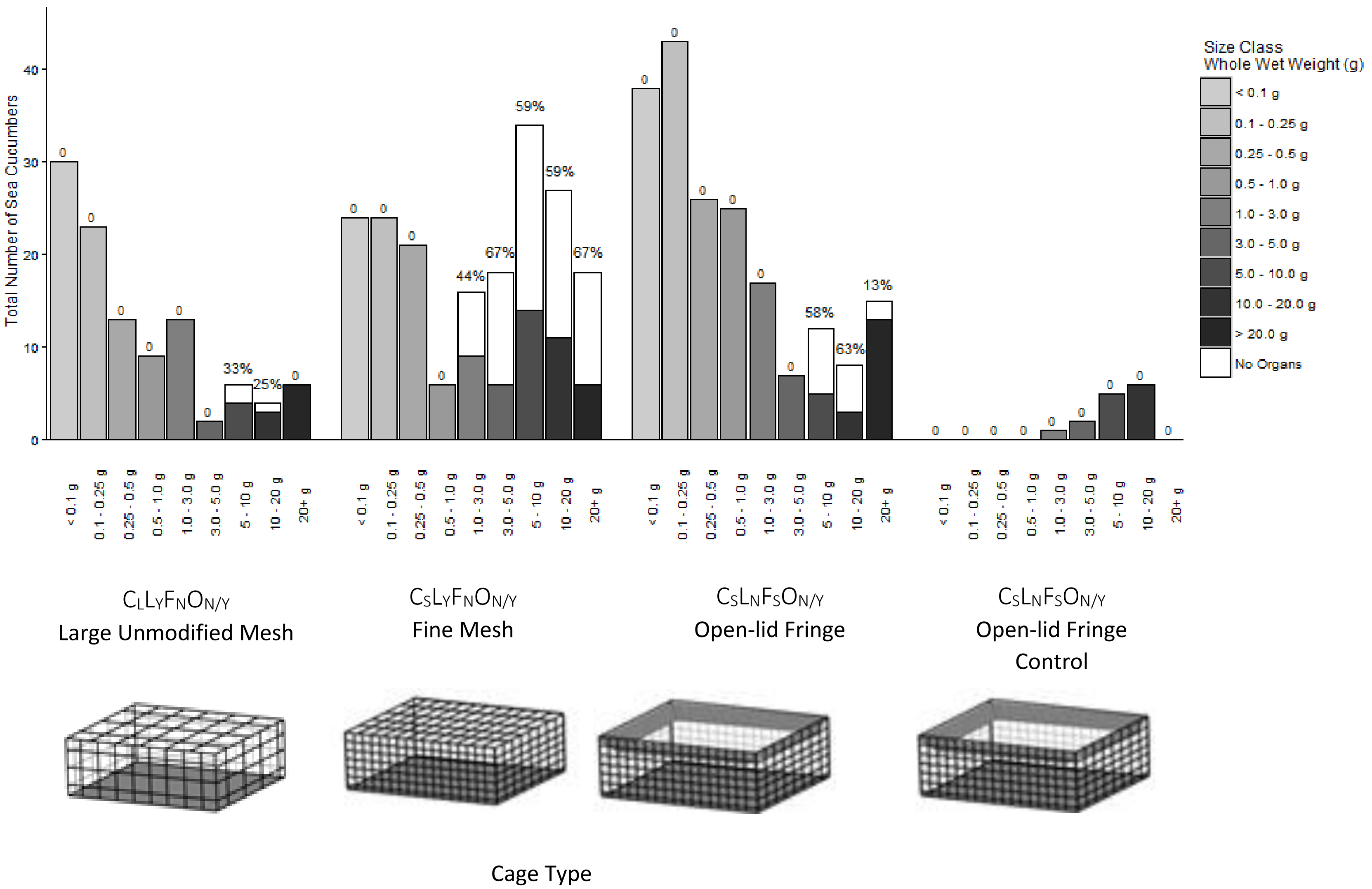


Figure 2.12. Count data of sea cucumbers by cage type, size class and state of visceral organs. The numbers above bars is percent of sea cucumbers without organs. Experimental cages *n* = 5, were suspended below a pacific oyster farm at 9 m depth and fringe control cages, *n* = 5, were suspended at 9 m depth 320 m away. Field trial occurred from May to November 2015. Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

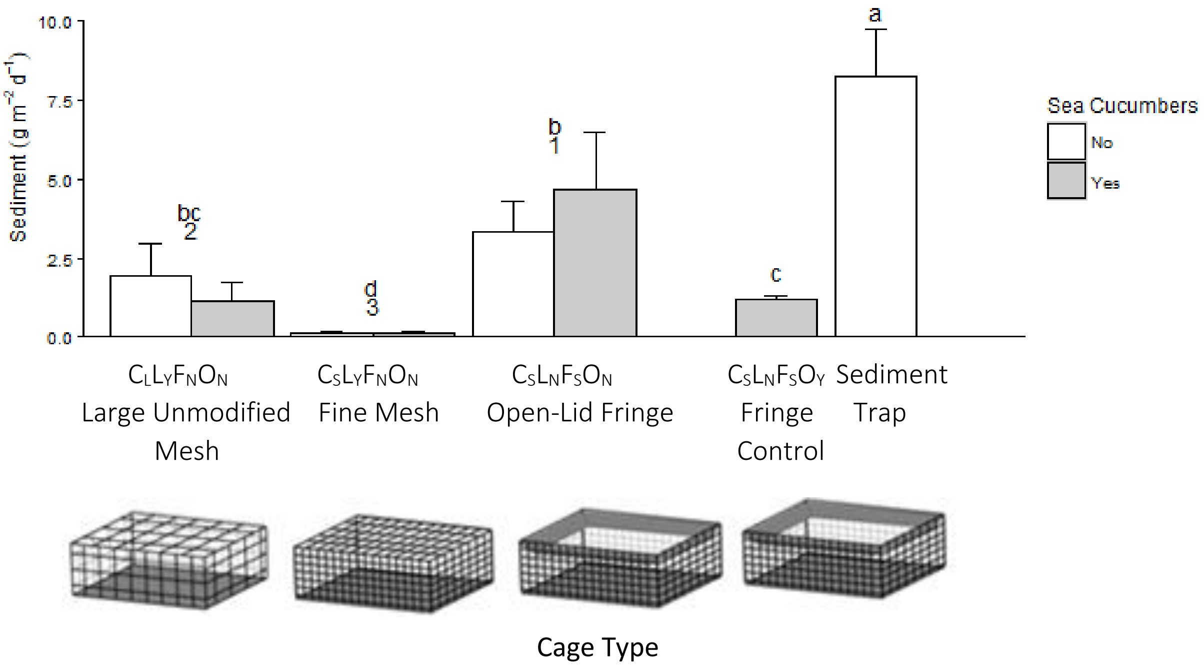


Figure 2.13. Field trial sediment retention rates by cage type, dry weight of sediments collected from cages in g m^{-2} day^{-1} (mean +SE), for both summer (August-September) and fall (October-November) 2015 season. Experimental cages, *n* = 6, and sediment traps, *n* = 3, were suspended 9 m depth below pacific oyster, *Crassostrea gigas*, farm. Fringe control cages, *n* = 5, were suspended 320 m. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

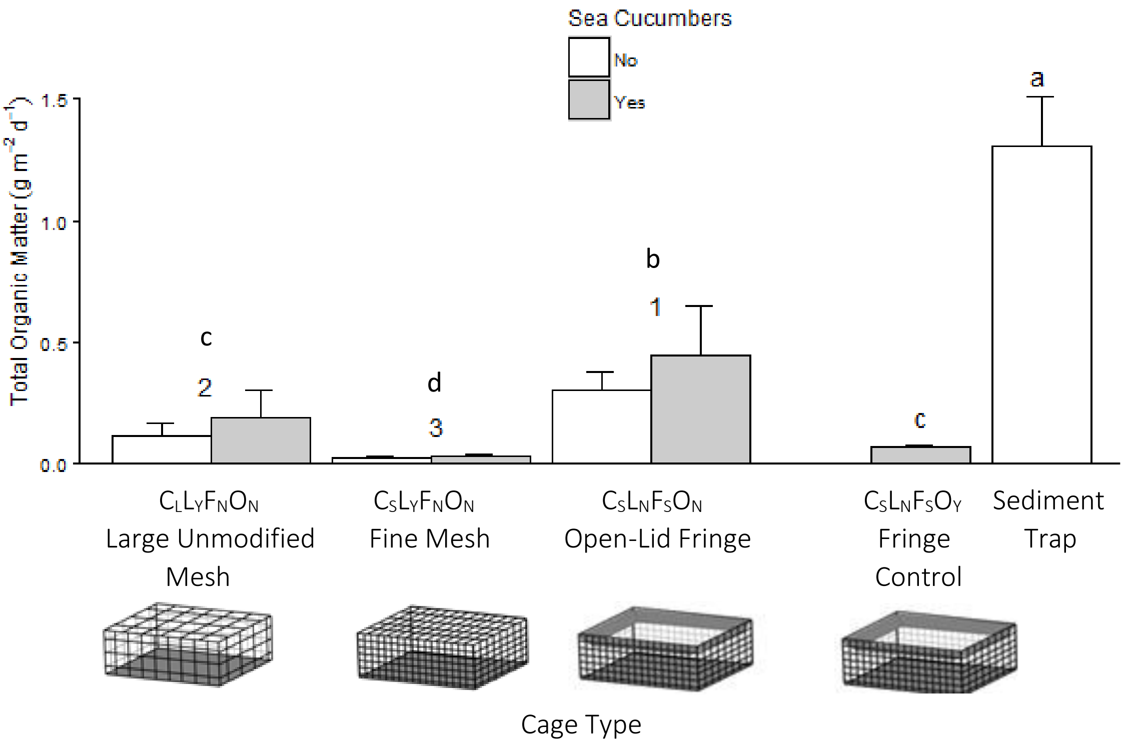


Figure 2.14. Total organic matter retention rates by cage type, dry weight of total organic matter collected from cages in g m^{-2} day^{-1} (mean +SE), for both summer (August-September) and fall (October-November) 2015 season. Experimental cages, *n* = 6, and sediment traps, *n* = 3, were suspended 9 m depth below pacific oyster, *Crassostrea gigas*, farm. Fringe control cages, *n* = 5, were suspended 320 m away. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

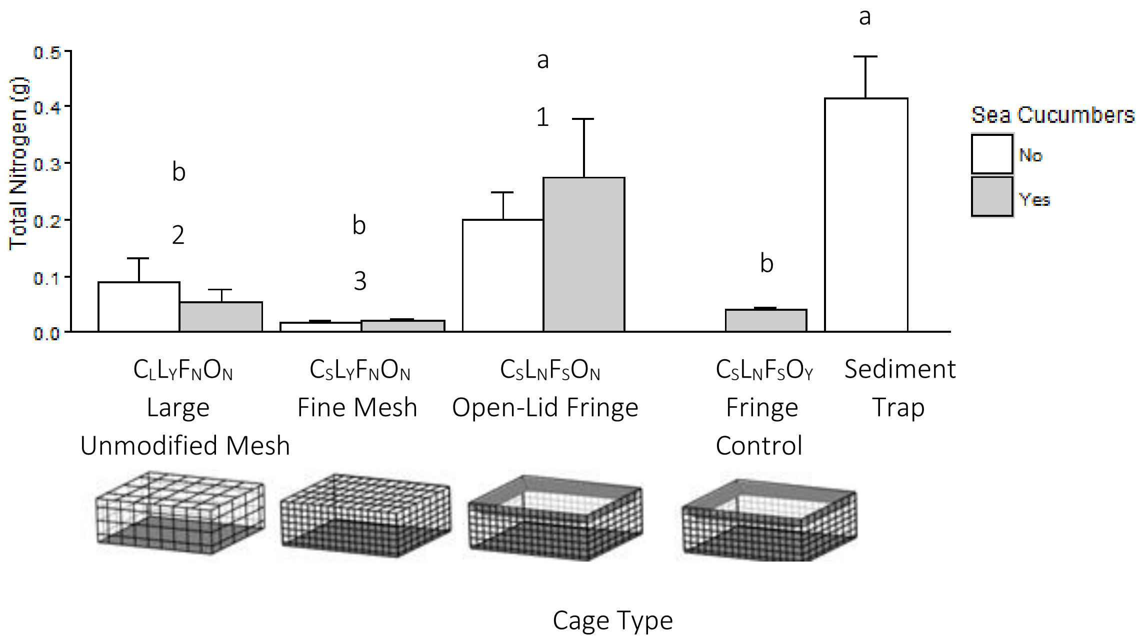


Figure 2.15. Total nitrogen retention by cage type (mean g dry weight nitrogen, +SE), for both summer (August-September) and fall (October-November) 2015 season. Experimental cages, *n* = 6, and sediment traps, *n* = 3, were suspended 9 m depth below pacific oyster, *Crassostrea gigas*, farm. Fringe control cages, *n* = 5, were suspended 320 m away. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

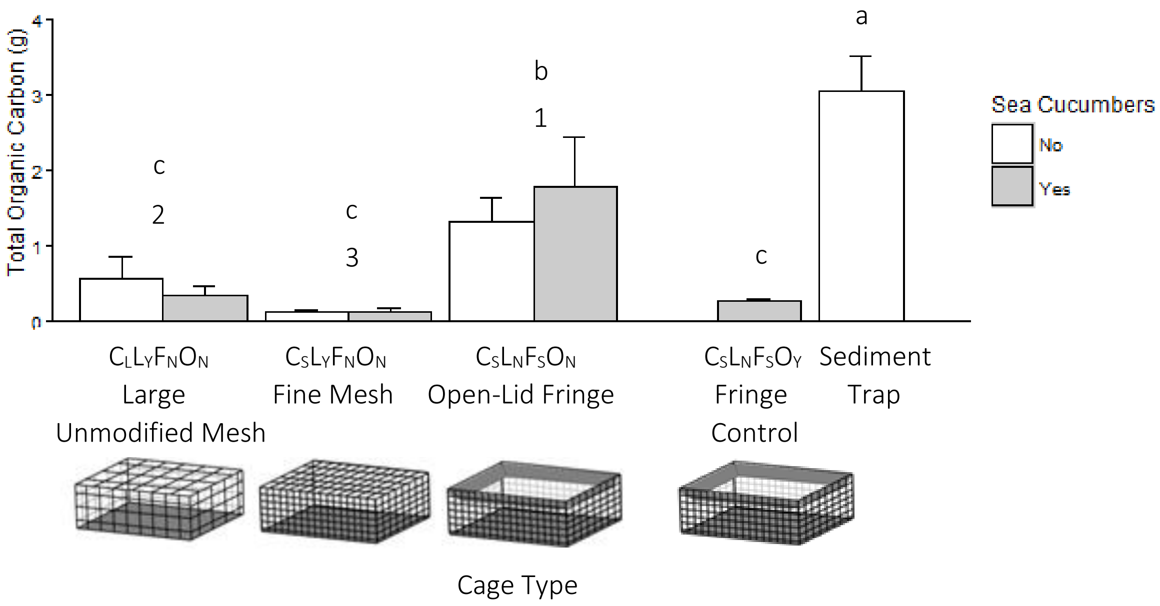


Figure 2.16. Total organic carbon retention by cage type (mean g dry weight organic carbon, +SE), for both summer (August-September) and fall (October-November) 2015 season. Experimental cages, *n* = 6, and sediment traps, *n* = 3, were suspended 9 m depth below pacific oyster, *Crassostrea gigas*, farm. Fringe control cages, *n* = 5, were suspended 320 m away. Different letters or numbers above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.

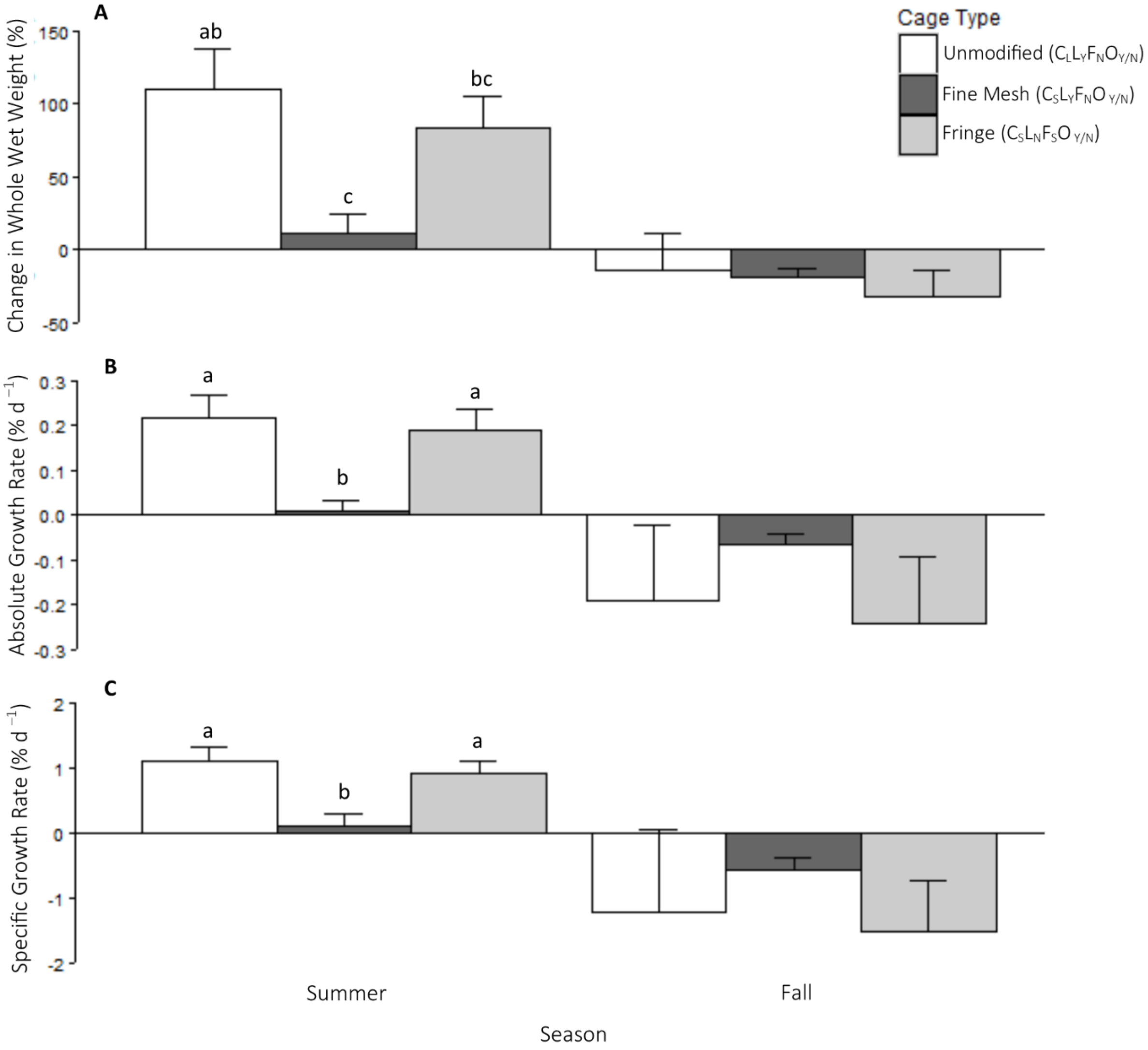


Figure 2.17. Sea cucumber whole wet weight growth (mean +SE) for both summer (August-September) and fall (October-November) 2015 season, $n = 6$. A: Percent change whole wet weight, B: Absolute growth rate (g d^{-1}), C: Specific growth rate (% d^{-1}). Different letters above bars indicate means which are significantly ($p < 0.05$) different (Tukey HSD test). Cage abbreviations are: (1) cage mesh size: C_L = large mesh (1.2–3.0 cm), C_S = small mesh (1 mm); (2) lid: L_Y = yes lid and L_N = no lid; (3) fringe mesh size: F_L = large mesh (500 µm), F_S = small mesh (250 µm), and F_N = no fringe; and (4) oyster shell: O_Y = yes oyster shell and O_N = no oyster shell.