A study of dendrochirotid sea cucumbers with a focus on *Cucumaria frondosa* and its potential use in integrated multi-trophic aquaculture

By

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Abstract

Sea cucumbers play fundamental roles in marine ecosystems, and they have long been commercially harvested for seafood. In light of stock declines worldwide, research on cold-water suspension-feeding species is expanding. This thesis explores two species from the North Atlantic. The first chapter validates an aging technique in *Psolus fabricii*, based on the growth rings found in its large calcareous dermal plates. Imaging of plates obtained from juveniles of known age confirmed the annual addition of a layer that extends the plate both vertically and horizontally, generating a pair of dark and light rings that can be used for aging. Wild individuals from shallow inshore areas were aged to a maximum of 28 years and their main morphometrics compared with age. The three other experimental chapters focus on *Cucumaria frondosa*, which has been commercially harvested for decades in North America and was identified as a potential candidate for aquaculture, especially as a biofilter in integrated multi-tropic aquaculture (IMTA). The locomotor and feeding behaviours, and microhabitat selection (spatial distribution) of *C. frondosa* in response to various environmental factors were investigated in the laboratory. Sea cucumbers moved away from static conditions and fled the strongest flows, and their tentacle deployment and insertion rates (i.e. feeding rate) increased with flow. The flow regime favoured by *C. frondosa* was 21-40 cm s^{-1}. Individuals were overall more mobile during the night, whereas no feeding differences were detected between diurnal and nocturnal phases. Gradients in phytoplankton concentration modulated the deployment of feeding tentacles but did not trigger any displacement toward the food source. *C. frondosa* exhibited a clear preference for substrates composed of bare rocks and rocks with coralline algae and displayed a slight preference for darker substrate backgrounds. Finally, *C. frondosa* held in effluent water from salmon culture was determined to assimilate the waste, which modulated its biochemical composition; however, its health condition declined, suggesting that it cannot find appropriate or sufficient nutrients for growth and reproduction in these wastes. The findings presented here aim to improve our understanding of the biology and ecology of cold-water suspension-feeding sea cucumbers to guide and assist fisheries management and aquaculture development.

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Co-authorship Statement

The work described in the present thesis was carried out by Jiamin Sun with supervision and guidance from Annie Mercier, Jean-Francois Hamel, Shawn Robinson, and Sherrylynn Rowe. All chapters were written by Jiamin Sun as journal manuscripts with intellectual and editorial input by co-authors as follows:

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Chapter 1. General Introduction

1.1 Ecological and social values of sea cucumbers

Sea cucumbers, also called holothuroids or holothurians, belong to class Holothuroidea of phylum Echinodermata. They colonize most benthic habitats in temperate, polar and tropical oceans, from the intertidal zone to the deep sea (Conand, 2006). Based on their feeding modes, they can be broadly divided into deposit feeders (order Holothuriida), which gather organic detritus and sediments from the seafloor, and suspension feeders (order Dendrochirotida), which extract phytoplankton, microorganisms and particulate organic matter from the water column.

Overall, sea cucumbers contribute greatly to benthic communities and they play important roles in marine ecosystems (Purcell et al., 2016). Some deposit-feeding species can maintain and improve sediment health through cleaning and bioturbation processes, thereby oxygenating sediments and altering the stratification and stability of soft bottoms (Uthicke, 1999; Purcell et al., 2016). A study conducted on the Great Barrier Reef showed that the species *Holothuria atra* and *Stichopus chloronotus* can bioturbate about 4600 kg year^{-1} 1000 m^{-2} (dry weight), which is approximately the weight of the upper 5 mm of sediment (Uthicke, 1999). Also, some suspension-feeding sea cucumbers can regulate water quality by modulating its carbonate content and pH (Massin, 1982).

Nutrient recycling has been suggested to be one of the most important ecological functions of sea cucumbers (Uthicke, 2001; Massin, 1982; Purcell et al., 2016). The release of inorganic nitrogen as ammonium and phosphate by sea cucumbers can be absorbed by nearby corals, microalgae and bacteria, therefore promoting their growth; in turn, the increased productivity provides food to sea cucumbers (Uthicke, 2001), thus developing a recycling loop in the ecosystem (Purcell et al., 2016). Many symbionts have been found on sea cucumbers, acting as commensals or parasites [see Jangoux and Kinne (1990) and Eeckhaut et al. (2004) for reviews]. Studies have reported that *H. atra* acts as a critical host to the opisthobranch gastropod *Plakobranchus ocellatus*, which uses the sea cucumber as a refuge against predators and as a protected spawning site (Mercier and Hamel, 2005). A study by Caulier et al. (2014) reported that the diet of the harlequin crab *Lissocarcinus orbicularis* is closely associated with its hosts, sea cucumbers belonging to the genera *Thelenota*, *Bohadschia* and *Holothuria*. Finally, sea cucumbers are known to constitute important food sources for other species, both in shallow and in deep waters, thereby transferring energy from microalgae and organic detritus to consumers at higher trophic levels (Purcell et al., 2016). For example, primary productivity enters the food web through predation on the phytoplankton-feeding dendrochirotid *Cucumaria frondosa* by the sea star *Solaster endeca* (So et al., 2010).

In addition to their ecological importance, sea cucumbers represent an economically valuable, conservation-worthy marine resource that supports coastal livelihoods around the world (Anderson et al., 2011). Owing to their nutritional and medicinal values, sea cucumbers have been an important food source for centuries in Asia and have been harvested in China and around the world for at least 400 years (Yang et al., 2015). Currently, over 70 species of holothuroids are commercially exploited and traded (Purcell et al., 2010). The majority of species harvested commercially belong to the order Holothuriida, specifically to the families Holothuriidae and Stichopodidae, which are mostly tropical (Purcell et al., 2012). Processed (cooked and dried) sea cucumber, often called bêche -de-mer or trepang, is exported mostly to Asian markets (Conand, 2004; Purcell et al., 2012).

Over the past few decades, sea cucumber fisheries have developed rapidly because of an increasing demand for this prized seafood (Conand, 2004). Consequent overexploitation and declines in stocks of sea cucumbers have ensued in many parts of the world (Anderson et al., 2011; Friedman et al., 2011; Purcell et al., 2013). The most recent global investigation of the status of sea cucumber fisheries revealed that 20% of the stocks were depleted or collapsed, 38% over-exploited and 14% fully-exploited, with no room for expansion (Purcell et al., 2013). The management of sea cucumber populations is becoming a crucial imperative around the world; however, it is often hampered by a scarcity of biological and stock assessment information (Caddy, 1986; Purcell et al., 2013). Aquaculture has the potential to help replenish depleted sea cucumber stocks and mitigate the impacts of fisheries on wild populations (Bell et al., 2005; Yang et al., 2015). Commercial aquaculture of sea cucumber has begun relatively recently in some countries (Toral-Granda et al., 2008; Purcell et al., 2014) and protocols have only been developed for a small number of temperate and tropical species, such as *Apostichopus japonicus*, *Isostichopus fuscus* and *Holothuria scabra* (Chen, 2005; Eriksson et al., 2012; Hamel et al., 2001; Yang et al., 2015; Mercier and Hamel, 2013). Knowledge of the basic ecology and biology of most commercially important sea cucumbers remains too limited to develop efficient and cost-effective aquaculture programs.

1.2 Overview of focal species *Cucumaria frondosa* and *Psolus fabricii*

1.2.1 Biology and commercial exploitation of *Cucumaria frondosa*

Cucumaria frondosa is the most common sea cucumber in the North Atlantic and Arctic oceans, and one of the most abundant worldwide (Hamel and Mercier, 1996b; Singh et al., 2001). It is distributed off New England (USA), eastern and Arctic Canada, Greenland, and Scandinavia, as well as in the Faroe Islands and Russia (Fig. 1.1) (Hamel and Mercier, 2008; Jordan, 1972). It is therefore a prominent member of cold-water and polar benthic communities. Dense populations are most commonly found at depths of ~30 m (Jordan, 1972; Singh et al., 1998), but the species occurs from shallow tide pools (Klugh, 1924) to 300-400 m (Gosner, 1978), with few specimens found deeper than 800 m (Hamel and Mercier, 2008). *C. frondosa* differs markedly from most harvested and cultured species (Fig. 1.2). It is a cold-water species with annual reproduction and non-feeding (lecithotrophic) larval development (Hamel and Mercier, 1996a). It is also a passive suspension-feeder that captures particulate food, chiefly phytoplankton, by extending its tentacles in the water column (Hamel and Mercier, 1998).

Declines in global sea cucumber resources have fueled an expansion of the industry, from traditional centers in Asia and the Indo-Pacific towards Europe and the Americas. In this context, *C. frondosa* became the target of commercial fisheries in eastern North America at the turn of the century. Like many other commercially important sea cucumber species, *C. frondosa* has been fished extensively in its distribution range to meet the increasing demand from Asia (Hamel and Mercier, 2008). The most commonly traded product is the dried or fresh frozen body wall, generally including the longitudinal muscle bands (Fig. 1.3A). Dried aquapharyngeal bulbs (flowers), liquid or gel extracts and various supplements can also be found on the market (Hamel and Mercier, 2008). The price for *C. frondosa* (i.e. dried body wall or whole sea cucumber) on Asian markets reached approximately US$ 230 kg^{-1} dried weight a few years ago (Sze and Conand, 2015) and its value is steadily increasing (Fig. 1.3B). Recently, the use of sea cucumbers for medicinal and cosmetic purposes has gained popularity. Studies have shown that the tissues of *C. frondosa* are useful sources of antioxidants for human consumption (Mamelona et al., 2007) and Frondanol-A5P extracted from them has the ability to inhibit the growth of pancreatic cancer cells (Roginsky et al., 2010). In addition, it was shown that *C. frondosa* could consume particulate organic material efficiently, both in the laboratory and in the natural environment (absorption efficiency of 68-85%), making it a good candidate biofilter within integrated multi-tropic aquaculture (IMTA) settings (Nelson et al., 2012). The constant decline of wild stocks and the high marketability of *C. frondosa* for food and pharmaceutical/nutraceutical products highlight the importance of efficient management and investigation of its potential for aquaculture. Fisheries and ecological data have been gathered on *C. frondosa* over the past several decades, including on the reproductive cycle, larval development, settlement, and feeding (Hamel and Mercier, 1996b; 1998; Holtz and MacDonald, 2009; Singh et al., 1998; Gianasi et al., 2018; Gianasi et al., 2017). Several aspects of the biology and ecology of *C. frondosa* that would be crucial to the sustainable management of wild stocks and for the development of aquaculture techniques are still incompletely understood, such as age at maturity, recruitment processes, reproductive rate, and natural mortality (DFO, 2018).

1.2.2 Biology of *Psolus fabricii*

Psolus fabricii belongs to family Psolidae in the order Dendrochirotida (Fig. 1.4) (Duben and Koren, 1846). It is an epifaunal suspension feeder occurring commonly in the North Atlantic, from the low tide mark to approximately 150 m depth from the Artic to Cape Cod (WoRMS, 2019). *P. fabricii* is covered with imbricating scales (plates) on the dorsal side. Tube feet are developed exclusively on the ventral sole, except for a few on the soft-skinned introvert (oral complex of dendrochirotids) and in the form of anal papillae around the anal opening (Coady, 1973). Research on *P. fabricii* is very limited because it is not a commercial species. Hopcroft et al. (1985) reported that *P. fabricii* depended less than other sea cucumbers on body surface respiration whereas Hamel et al. (1993) examined the reproductive cycle in relation to various environmental factors. Jennings and Hunt (2010) studied the recruitment patterns in the Bocabec Cove, Bay of Fundy, Canada and found *P. fabricii* settled in the highest densities in early July. This species is also a source of bioactive triterpene glycosides, which have a wide range of biological effects, such as cytotoxic, antifungal and immunomodulatory activities (Kalinin et al., 1989; Kalinin et al., 1983; Gorshkova et al., 1999).

1.3 Major gaps in knowledge

In order to sustainably manage populations of cold-water suspension-feeding sea cucumbers efficiently and generate metrics of strategic value for the development of their aquaculture, much deeper biological and ecological knowledge needs to be acquired (DFO, 2018).

Chief among the current knowledge gaps is the relationship between size and age, which is instrumental for calculating growth, mortality and productivity. Among echinoderms, aging studies have centered on sea urchins (Brady and Scheibling, 2006; Ebert, 1988; Ebert et al., 1999; Ebert and Southon, 2003), brittle stars (Dahm and Brey, 1998; Gage, 2003) and sea stars (Stump and Lucas, 1990) by measuring increments in skeletal plates. However, sea cucumbers have proven very difficult to age, because of their soft-bodied, polymorphic shape, and the small size or lack of permanent hard structures likely to exhibit growth rings (Watanabe et al., 2014). The most common approach so far for this group has relied on methods that are based on the indirect estimation of age, such as modal-progression analysis of size or weight (Poot-Salazar et al., 2014; Herrero-Pérezrul and Reyes-Bonilla, 2008), which is not accurate and often does not apply for juveniles.

The habitat preferences, locomotion, and distribution patterns of sea cucumbers have previously been attributed to a variety of factors including substratum characteristics, food availability, light intensity, temperature and salinity fluctuations, tidal current and predator pressure (Slater and Jeffs, 2010; Navarro et al., 2013; Dissanayake and Stefansson, 2012; Mercier et al., 2000; Navarro et al., 2014; Mercier et al., 1999; Hamel and Mercier, 1996b; Da Silva et al., 1986). Knowledge of the spatial distribution patterns, and habitat preferences of *C. frondosa* are scant apart from field distribution studies conducted in the St. Lawrence Estuary (Hamel and Mercier, 1996b).

Sea cucumbers are becoming popular candidates for IMTA. Some deposit-feeding species have been shown to consume and reduce aquaculture wastes, including *Apostichopus japonicus* (Zhou et al., 2006), and *Parastichopus californicus* (Paltzat et al., 2008). As a suspension-feeder, *C. frondosa* may be held in suspended cages or directly on the substrate. Recent research has revealed that *C. frondosa* is capable of consuming aquaculture waste material and can absorb particulate organic material efficiently, which means it may be a good biofilter within an IMTA setting (Fig. 1.5) (Nelson et al., 2012). However, whether *C. frondosa* in an IMTA system can use energy from the ingested wastes to grow and whether their overall health and biochemical profile will be affected remain unclear.

1.4 Main objectives and thesis structure

Drawing from some of the main knowledge gaps, the present work presents and validates a promising aging technique using the species *P. fabricii*, determines the influence of several external factors on the distribution, feeding and locomotor behaviour of *C. frondosa*, and explores its suitability for integration in IMTA systems.

In an effort to develop a direct aging technique, Chapter 2 uncovered and explored growth rings on the dermal plates (ossicles) of *P. fabricii*. Ossicles are a key characteristic of sea cucumbers and they are of primary importance for taxonomic identification. They are mostly of microscopic size (Stricker, 1985), and they come in a wide variety of simple to complex shapes, such as rods, buttons, and anchors (Massin et al., 2000). The dendrochirotid species *P. fabricii* is covered by imbricating scale-like plates (ossicles), which can reach ~2 cm length in adults, and form a semi-rigid armour (Hopcroft et al., 1985). In Chapter 2, the plates of sea cucumbers that were either wild-caught (unknown age) or laboratory-reared (known age) were examined and compared. The relationship between the number of annual plate rings (age) and other morphological parameters and features was also studied. The findings will be an important addition to the knowledge of sea cucumber biology and may eventually be transferred to other less calcified dendrochirotids, such as *C. frondosa* (from the same geographic location) to provide a valuable tool for stock assessment, fisheries management and aquaculture.

An understanding of the factors that influence the distribution of sea cucumbers is critical to improving the management of their populations and providing some basis for the development of aquaculture protocols (i.e. holding conditions). Two experimental chapters focused on determining locomotor and feeding behaviours, and microhabitat selection (spatial distribution) of *C. frondosa* in response to different flow regimes (Chapter 3), light intensities, levels of phytoplankton concentration (its main source of food), substrate types and background colours (Chapter 4). The putative influence of light on its diel locomotor and feeding activities was also assessed in Chapter 4, and a parallel analysis of behavioural responses to flow in *C. frondosa* was integrated in another publication (Appendix 1).

Chapter 5 compared several metrics between wild individuals of *C. frondosa* and individuals having been held for 4 years in the effluent of an Atlantic salmon culture (i.e. IMTA). The aims were (i) to determine whether *C. frondosa* can assimilate the wastes using stable isotope and specific fish-feed fatty acid biomarkers and (ii) whether they can be sustained on effluent wastes from a salmon culture by comparing health metrics and biochemical composition between the two groups of sea cucumbers.

Finally, Chapter 6 provides a summary of the research findings and highlights the main conclusions, indicating how they may improve our understanding of the biology and ecology of cold-water suspension-feeding sea cucumbers with applications to management and aquaculture. It also presents directions for future research in these areas.

1.5 References

Anderson, S.C., Flemming, J.M., Watson, R., Lotze, H.K., 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries. 12, 317-339.

Bell, J.D., Rothlisberg, P.C., Munro, J.L., Loneragan, N.R., Nash, W.J., Ward, R.D., Andrew, N.L., 2005. Restocking and stock enhancement of marine invertebrate fisheries. Elsevier, San Diego, California, USA.

Brady, S.M., Scheibling, R.E., 2006. Changes in growth and reproduction of green sea urchins, *Strongylocentrotus droebachiensis* (Müller), during repopulation of the shallow subtidal zone after mass mortality. Journal of Experimental Marine Biology and Ecology. 335, 277-291.

Caddy, J., 1986. Stock assessment in data-limited situations–the experience in tropical fisheries and its possible relevance to evaluation of invertebrate resources. Canadian Special Publication of Fisheries and Aquatic Sciences. 92, 379-392.

Caulier, G., Lepoint, G., Van Nedervelde, F., Eeckhaut, I., 2014. The diet of the Harlequin crab *Lissocarcinus orbicularis*, an obligate symbiont of sea cucumbers (holothuroids) belonging to the genera *Thelenota*, *Bohadschia* and *Holothuria*. Symbiosis. 62, 91-99.

Chen, J., 2005. Present status and prospects of sea cucumber industry in China. in: Conand C., Purcell, S., Uthicke, S. Hamel, J.-F., Mercier A. (Eds.), Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries technical paper, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 25-37.

Coady, L.W., 1973. Aspects of the reproductive biology of *Cucumaria frondosa* (Gunnerus, 1770) and *Psolus fabricii* (Düben and Koren, 1846)(Echinodermata: Holothuroidea) in shallow waters of the Avalon Peninsula, Newfoundland. Memorial University of Newfoundland, pp. 117.

Conand, C., 2004. Present status of world sea cucumber resources and utilisation: an international overview. in: Conand C., Purcell, S., Uthicke, S. Hamel, J.-F., Mercier A. (Eds.), Advances in Sea cucumber Aquaculture and Management, FAO Technical Paper Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 13-23.

Conand, C., 2006. Sea cucumber biology, taxonomy, distribution and conservation status. in: Bruckner, A. (Ed.), Proceedings of the CITES Workshop on the Conservation of Sea Cucumbers in the Families Holothuriidae and Stichopodidae. Sliver Spring, MD, USA, pp. 33.

Da Silva, J., Cameron, J.L., Fankboner, P.V., 1986. Movement and orientation patterns in the commercial sea cucumber *Parastichopus californicus* (Stimpson)(Holothuroidea: Aspidochirotida). Marine and Freshwater Behaviour and Physiology. 12, 133-147.

Dahm, C., Brey, T., 1998. Determination of growth and age of slow growing brittle stars (Echinodermata: Ophiuroidea) from natural growth bands. Journal of the Marine Biological Association of the United Kingdom. 78, 941-951.

DFO, 2018. Sea cucumber stock status update in NAFO subdivision 3Ps, DFO Canadian Science Advisory Secretariat Science Response 2018/010.

Dissanayake, D., Stefansson, G., 2012. Habitat preference of sea cucumbers: *Holothuria atra* and *Holothuria edulis* in the coastal waters of Sri Lanka. Journal of the Marine Biological Association of the United Kingdom. 92, 581-590.

Ebert, T.A., 1988. Calibration of natural growth lines in ossicles of two sea urchins, *Strongylocentrotus purpuratus* and *Echinometra mathaei*, using tetracycline. in: Burke R.D., Mladenov P.V., Lambert P., Parsley R.L. (Ed.), Echinoderm Biology: Proceedings 6th International Echinoderm Conference. Balkema, Rotterdam, the Netherlands, pp. 435-443.

Ebert, T.A., Southon, J.R., 2003. Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb 14 carbon. Fishery Bulletin. 101, 915-922.

Ebert, T.A., Dixon, J.D., Schroeter, S.C., Kalvass, P.E., Richmond, N.T., Bradbury, W.A., Woodby, D.A., 1999. Growth and mortality of red sea urchins *Strongylocentrotus franciscanus* across a latitudinal gradient. Marine Ecology Progress Series. 190, 189-209.

Eeckhaut, I., Parmentier, E., Becker, P., Gomez da Silva, S., Jangoux, M., 2004. Parasites and biotic diseases in field and cultivated sea cucumbers. in: Lovatelli, A., Conand, C., Purcell, S., Uthicke, Hamel, J-F., Mercier, A. (Eds.), Advances in Sea Cucumber Aquaculture and Management, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 311-325.

Eriksson, H., Robinson, G., Slater, M.J., Troell, M., 2012. Sea cucumber aquaculture in the Western Indian Ocean: challenges for sustainable livelihood and stock improvement. Ambio. 41, 109-121.

Friedman, K., Eriksson, H., Tardy, E., Pakoa, K., 2011. Management of sea cucumber stocks: patterns of vulnerability and recovery of sea cucumber stocks impacted by fishing. Fish and Fisheries. 12, 75-93.

Gage, J.D., 2003. Growth and production of *Ophiocten gracilis* (Ophiuroidea: Echinodermata) on the Scottish continental slope. Marine Biology. 143, 85-97.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2018. Morphometric and behavioural changes in the early life stages of the sea cucumber *Cucumaria frondosa*. Aquaculture. 490, 5-18.

Gianasi, B.L., Parrish, C.C., Hamel, J.-F., Mercier, A., 2017. Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquaculture Research. 48, 3413-3432.

Gorshkova, I.A., Kalinin, V.I., Gorshkov, B.A., Stonik, V.A., 1999. Two different modes of inhibition of the rat brain Na^+, K^+-ATPase by triterpene glycosides, psolusosides A and B from the holothurian *Psolus fabricii*. Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology. 122, 101-108.

Gosner, K.L., 1978. A field guide to the Atlantic seashore from the Bay of Fundy to Cape Hatteras. Houghton Mifflin, New York, NY, USA.

Hamel, J.-F., Mercier, A., 1996a. Gonad morphology and gametogenesis of the sea cucumber *Cucumaria frondosa*. SPC Beche-de-mer Information Bulletin. 8, 22-33.

Hamel, J.-F., Mercier, A., 1996b. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences. 53, 253-271.

Hamel, J.-F., Mercier, A., 1998. Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. Canadian Journal of Zoology. 76, 1194-1198.

Hamel, J.-F., Mercier, A., 2008. Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 293-306.

Hamel, J.-F., Himmelman, J.H., Dufresne, L., 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). The Biological Bulletin. 184, 125-143.

Hamel, J.-F., Conand, C., Pawson, D.L., Mercier, A., 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as beche-de-mer. Advances in Marine Biology. 41, 129-223.

Herrero-Pérezrul, M.D., Reyes-Bonilla, H., 2008. Weight-Length relationship and relative condition of the holothurian *Isostichopus fuscus* at Espíritu Santo Island, Gulf of California, Mexico. Revista de Biologia Tropical. 56.

Holtz, E., MacDonald, B.A., 2009. Feeding behaviour of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the laboratory and the field: relationships between tentacle insertion rate, flow speed, and ingestion. Marine Biology. 156, 1389-1398.

Hopcroft, R.R., Ward, D.B., Roff, J.C., 1985. The relative significance of body surface and cloacal respiration in *Psolus fabricii* (Holothuroidea: Dendrochirotida). Canadian Journal of Zoology. 63, 2878-2881.

Jangoux, M., Kinne, O., 1990. Diseases of echinodermata. Diseases of Marine Animals. 3, 439-567.

Jennings, L.B., Hunt, H.L., 2010. Settlement, recruitment and potential predators and competitors of juvenile echinoderms in the rocky subtidal zone. Marine Biology. 157, 307-316.

Jordan, A.J., 1972. On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothurioidea) at Lamoine Beach, Maine. University of Maine, Orono.

Kalinin, V., Stepanov, V., Stonik, V., 1983. Psolusoside A—A new triterpene glycoside from the holothurian *Psolus fabricii*. Chemistry of Natural Compounds. 19, 753-754.

Kalinin, V., Kalinovskii, A., Stonik, V., Dmitrenok, P., El'Kin, Y.N., 1989. Structure of psolusoside B—A nonholostane triterpene glycoside of the holothurian genus Psolus. Chemistry of Natural Compounds. 25, 311-317.

Klugh, A.B., 1924. Factors controlling the biota of tide-pools. Ecology. 5, 192-196.

Mamelona, J., Pelletier, E., Girard-Lalancette, K., Legault, J., Karboune, S., Kermasha, S., 2007. Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber, *Cucumaria frondosa*. Food Chemistry. 104, 1040-1047.

Massin, C., 1982. Effects of feeding on the environment: Holothuroidea. in: Jangoux, M., Lawrence, J. (Eds.), Echinoderm Nutrition. AA Balkema, Rotterdam, Netherlands, pp. 494-497.

Massin, C., Mercier, A., Hamel, J.-F., 2000. Ossicle change in *Holothuria scabra* with a discussion of ossicle evolution within the Holothuriidae (Echinodermata). Acta Zoologica. 81, 77-91.

Mercier, A., Hamel, J.-F., 2005. Note on the association between *Plakobranchus ocellatus* (Mollusca: Gastropoda: Opisthobranchia) and *Holothuria atra* (Echinodermata: Holothuroidea). Cahiers de Biologie Marine. 46, 399.

Mercier, A., Hamel, J.-F., 2013. Sea cucumber aquaculture: Hatchery production, juvenile growth and industry challenges. in: Allan, G., Burnell, G. (Eds.), Advances in aquaculture hatchery technology. Woodhead Publishing Ltd, Cambridge, pp. 431-454.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology. 239, 125-156.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 2000. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands, Island, Ocean and Deep-Sea Biology. Springer, pp. 81-100.

Navarro, P., García-Sanz, S., Barrio, J., Tuya, F., 2013. Feeding and movement patterns of the sea cucumber *Holothuria sanctori*. Marine Biology. 160, 2957-2966.

Navarro, P.G., García-Sanz, S., Tuya, F., 2014. Contrasting displacement of the sea cucumber *Holothuria arguinensis* between adjacent nearshore habitats. Journal of Experimental Marine Biology and Ecology. 453, 123-130.

Nelson, E., MacDonald, B., Robinson, S., 2012. The absorption efficiency of the suspension-feeding sea cucumber, *Cucumaria frondosa*, and its potential as an extractive integrated multi-trophic aquaculture (IMTA) species. Aquaculture. 370, 19-25.

Paltzat, D., Pearce, C., Barnes, P., McKinley, R., 2008. Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture. 275, 124-137.

Poot-Salazar, A., Hernández-Flores, Á., Ardisson, P.-L., 2014. Use of the SLW index to calculate growth function in the sea cucumber *Isostichopus badionotus*. Scientific Reports. 4.

Purcell, S.W., Samyn, Y., Conand, C., 2012. Commercially important sea cucumbers of the world, FAO Species Catalogue for Fishery Purpose, Rome, Italy, pp. 180.

Purcell, S.W., Lovatelli, A., Vasconcellos, M., Ye, Y., 2010. Managing sea cucumber fisheries with an ecosystem approach. FAO Fisheries and Aquaculture Technical Paper. 520.

Purcell, S.W., Conand, C., Uthicke, S., Byrne, M., 2016. Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review. 54, 367-386.

Purcell, S.W., Polidoro, B.A., Hamel, J.-F., Gamboa, R.U., Mercier, A., 2014. The cost of being valuable: predictors of extinction risk in marine invertebrates exploited as luxury seafood. Proceedings of the Royal Society of London B: Biological Sciences. 281, 20133296.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M.V., Lovatelli, A., Uthicke, S., 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries. 14, 34-59.

Roginsky, A.B., Ding, X.-Z., Woodward, C., Ujiki, M.B., Singh, B., Bell Jr, R.H., Collin, P., Adrian, T.E., 2010. Anti-pancreatic cancer effects of a polar extract from the edible sea cucumber, *Cucumaria frondosa*. Pancreas. 39, 646-652.

Singh, R., MacDonald, B.A., Lawton, P., Thomas, M.L., 1998. Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. Canadian Journal of Zoology. 76, 1842-1849.

Singh, R., Macdonald, B.A., Lawton, P., Thomas, M.L., 2001. The reproductive biology of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuriodea) using new quantitative methods. Invertebrate Reproduction & Development. 40, 125-141.

Slater, M.J., Jeffs, A.G., 2010. Do benthic sediment characteristics explain the distribution of juveniles of the deposit-feeding sea cucumber *Australostichopus mollis*? Journal of Sea Research. 64, 241-249.

So, J.J., Hamel, J.-F., Mercier, A., 2010. Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology. 17, 473-484.

Stricker, S.A., 1985. The ultrastructure and formation of the calcareous ossicles in the body wall of the sea cucumber *Leptosynapta clarki* (Echinodermata, Holothuroida). Zoomorphology. 105, 209-222.

Stump, R., Lucas, J., 1990. Linear growth in spines from *Acanthaster planci* (L.) involving growth lines and periodic pigment bands. Coral Reefs. 9, 149-154.

Sze, C.P., Conand, C., 2015. Sea cucumber products at the China Fisheries Seafood Expo 2014. SPC Beche-de-mer Information Bulletin. 35, 60-63.

Toral-Granda, V., Lovatelli, A., Vasconcellos, M., 2008. Sea cucumbers: a global review of fisheries and trade. Food and Agriculture Organization of the United Nations, Rome, Italy.

Uthicke, S., 1999. Sediment bioturbation and impact of feeding activity of *Holothuria (Halodeima) atra* and *Stichopus chloronotus*, two sediment feeding holothurians, at Lizard Island, Great Barrier Reef. Bulletin of Marine Science. 64, 129-141.

Uthicke, S., 2001. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology. 265, 153-170.

Watanabe, S., Sumbing, J.G., Lebata-Ramos, M.J.H., 2014. Growth pattern of the tropical sea cucumber, *Holothuria scabra*, under captivity. Japan Agricultural Research Quarterly. 48, 457-464.

WoRMS, 2019. World Register of Marine Species. *Psolus fabricii* (Düben & Koren, 1846). http://www.marinespecies.org/aphia.php?p=taxdetails&id=124703, accessed on 18 Feb, 2019.

Yang, H., Hamel, J.-F., Mercier, A., 2015. The sea cucumber *Apostichopus japonicus*: history, biology and aquaculture. Academic Press, New York, NY, USA.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X., Zhang, F., 2006. Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. Aquaculture. 256, 510-520.

1.6 Figures

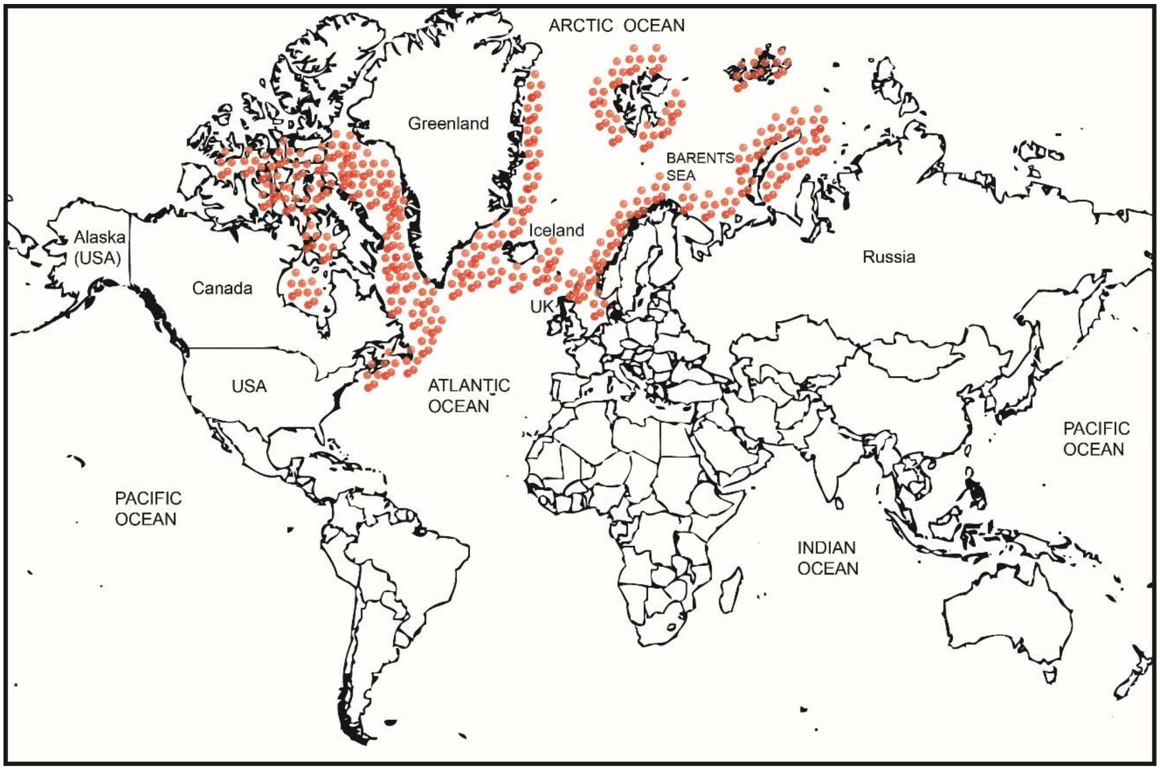

Figure 1.1 Known distribution of *Cucumaria frondosa* around the world (excluding *C. frondosa japonica*).

Figure 1.2 Three adults of *Cucumaria frondosa*, displaying the most common colour morphs. The individual on top is actively feeding.

Figure 1.3 (A) Dried body wall (from Ocean Choice International) and (B) commercial bags of *Cucumaria frondosa* (from a Chinese grocery store).

Figure 1.4 Adult of *Psolus fabricii*.

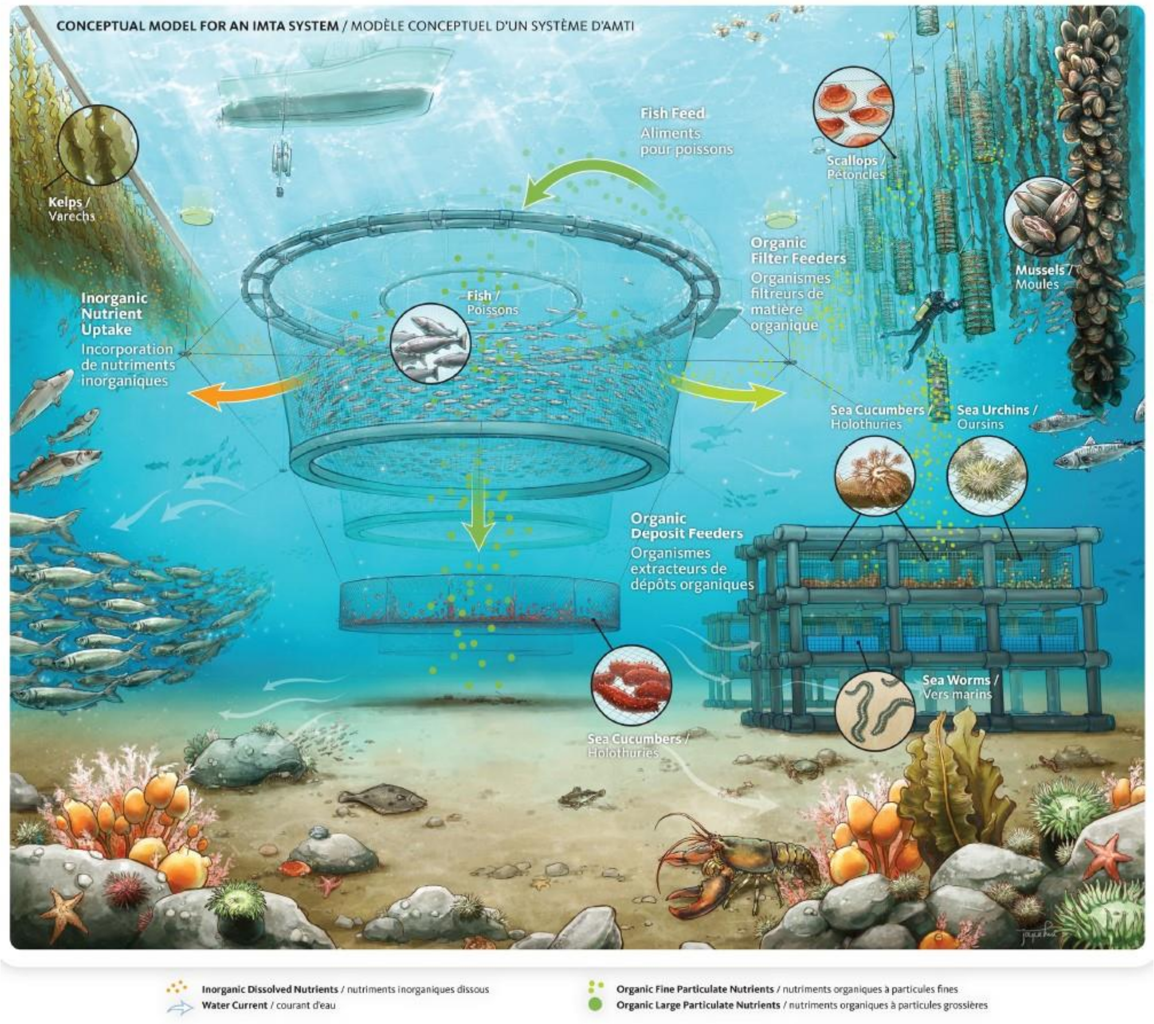

Figure 1.5 Illustration of a conceptual model for an integrated multi-trophic aquaculture (IMTA) system that includes caged suspension-feeding sea cucumbers (from DFO, 2013).

Chapter 2. Age determination in echinoderms: first evidence of annual growth rings in holothuroids [1]

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2.1 Abstract

While age is fundamental in animal biology, forming the basis of critical concepts such as life-history strategies, longevity and population structures, measuring this variable in some taxa remains problematic. Such is the case of holothuroid echinoderms, which play key roles in marine benthic communities from the shore to the abyss, and are extensively fished in many regions across the globe. Here we present and validate a promising aging technique using the cold-water species *Psolus fabricii*. The method involves the extraction of the oldest dermal plates (largest dorsal ossicles) to preserve their original pigments and structure. While plates initially appear to have a uniform texture, polishing and dying them reveals layered ring patterns. A study of laboratory-reared juveniles from settlement to 40 months of age, confirmed that one layer is added annually, making plates both larger and thicker, and generating successive light and dark rings, the latter representing the transition (overlap) between two layers. Therefore, each pair of rings represents an annual growth band. Size-at-age data obtained using this method revealed that growth of *P. fabricii* is slow and that wild individuals collected at diving depths had reached an age of several decades.

Key words: sea cucumber, skeletal plates, age metrics, size at age, ossicles, morphometrics

2.2 Introduction

Understanding the age metrics of marine organisms is fundamental for estimating baseline parameters such as growth rate, population age structure, mortality rate, productivity, and recruitment (Campana, 2001; Moltschaniwskyj and Cappo, 2009). The availability of a reliable aging tool is instrumental to the assessment of longevity as a basis for developing fishery management plans and conservation or recovery strategies, as well as understanding the role of focal species in maintaining and modifying the ecosystems they inhabit (Moltschaniwskyj and Cappo, 2009).

The determination of age in certain marine organisms may be hampered by a lack of valid assessment methods, causing inaccuracy and/or imprecision (Campana, 2001; Bertignac and De Pontual, 2007). Within the phylum Echinodermata, all five extant classes possess ossicles, i.e. dermal calcareous elements that form part of the endoskeleton and provide rigidity and protection (Ruppert et al., 2004), which can display different forms and arrangements (Evamy and Shearman, 1965). Aging studies on echinoderms have centered on taxonomic classes exhibiting well-developed skeletons, such echinoids, i.e. sea urchins (Agatsuma and Nakata, 2004; Brady and Scheibling, 2006; Ebert et al., 1999), ophiuroids, i.e. brittle stars (Dahm and Brey, 1998; Gage, 2003) and asteroids, i.e. sea stars (Stump and Lucas, 1990). Among them, echinoids are the most calcified and the most widely studied. Two techniques are currently used to estimate their age: mark-recapture and sclerochronology (Moltschaniwskyj and Cappo, 2009). In the mark-recapture method, the diameter of the calcareous test is measured, and the skeletal elements (ossicles or plates) are tagged using baths or injections of chemicals such as tetracycline and calcein. Using sclerochronology methods, the bands on plates or plate-like ossicles, similar to the rings on trees, are used to estimate age (Ouréns et al., 2013). An essential assumption for using this technique is that a complete cycle (one translucent or dark ring and one opaque or light ring) is added each year (or following any other quantifiable periodicity), independent of individual size. However, these assumptions are not unanimously supported by experiments and their accuracy remains open to discussion (Russell and Meredith, 2000; Narvaez et al., 2016).

Echinoderms belonging to the class Holothuroidea (sea cucumbers) contribute significantly to species diversity, abundance and biomass, and they play fundamental roles in benthic communities of the world ocean (Purcell et al., 2016). They are also prized seafood (Yang et al., 2015), which has led to the overexploitation and consequent collapse of wild stocks in many regions (Anderson et al., 2011; Purcell et al., 2013). Therefore, the management of sea cucumbers has become a worldwide concern, and conservation plans are often hampered by the scarcity or unreliability of biological and ecological knowledge (Purcell et al., 2013). Management of commercial sea cucumber stocks is, to a significant extent, based on the estimation of population dynamics, which in turn depends on the determination of recruitment, growth rate, mortality and size at age. However, sea cucumbers have proven very difficult to age, because of their soft-bodied, polymorphic shape, and the small size of hard structures that could exhibit growth rings (Watanabe et al., 2014). Marking of ossicles of sea cucumbers with fluorochromes, such as tetracycline and calcein, may provide a valuable tool for mark-recapture studies and was tested in some of those species, such as *Holothuria scabra* (Purcell et al., 2006; Purcell and Blockmans, 2009). However, ossicles in holothuroids may grow discontinuously and can take several months to reach full size (Stricker, 1985), making it difficult to age slow-growing and long-lived species. Consequently, the most common approach so far for aging them has relied on indirect estimation, such as modal-progression analysis of size or weight (Poot-Salazar et al., 2014; Morgan, 2012; Hannah et al., 2012; Watanabe et al., 2014; Ebert, 1978; Herrero-Pérezrul and Reyes-Bonilla, 2008). For example, the growth pattern of *Holothuria* *atra* has been studied using the Brody-Bertalanffy growth function (Ebert, 1978). One problem with such techniques is that there is not always a correlation between size (or weight) and age because environmental and endogenous factors may cause differences in growth rate or even negative growth (i.e. shrinking) in some individuals (Hamel and Mercier, 1996; So et al., 2010; Gianasi et al., 2017).

In this study, we present and validate a technique for aging sea cucumbers based on the growth rings found in those calcareous plates using the species *Psolus fabricii* (Duben and Koren, 1846), a Psolidae sea cucumber belonging to the order Dendrochirotida. The species is an epifaunal suspension feeder occurring commonly from the low tide mark to approximately 150 m depth on hard substrates around the North Atlantic and Arctic Oceans (Brinkhurst et al., 1976). Like other psolids, *P. fabricii* is covered by imbricating scale-like plates, which form a semi-rigid armour (Hopcroft et al., 1985). Calcareous plates were first analyzed from their initial appearance in newly settled captive-reared juveniles over 40 months of growth. The results showed clearly and consistently that one layer is added annually, in the form of a light (new) and a dark (overlapping) section. Plates of wild-caught individuals of various sizes were then examined and their age determined using the number of rings, showing that this species lives in the order of decades. Plate metrics were also plotted against other morphological parameters that might be used to estimate age in a preliminary exploration of growth patterns. To our knowledge, this is the first evidence of the occurrence of growth rings in Holothuroidea, offering a promising tool as well as preliminary data on longevity and mean growth rates in a cold-water sea cucumber.

2.3 Material and methods

2.3.1 Collections and cultures

Fifty *P. fabricii* of various sizes (wet weight of 0.5-187.7 g) were collected between 5 and 11 m depths in Logy Bay (47°39'12.8"N: 52°41'45.3"W) off southeastern Newfoundland, Canada. Over the holding period (2015-2018), these sea cucumbers were cultured in ten 40-L tanks with running seawater (20 L h^{-1}) at ambient temperatures ranging from 1 to 7°C under a naturally fluctuating photoperiod (9L/15D to 16L/8D) where light intensities ranged daily from 5 to 450 lx (Mercier and Hamel, 2010). Continuous input of new seawater in the tanks provided natural plankton and particulate organic matter as food sources (Gianasi et al., 2018b). Also, one individual (5.2 cm in maximum length; 19.2 g in wet weight) was collected opportunistically from 1050 m depth using a bottom-trawl operated by Fisheries and Oceans Canada (DFO), from the CCGS *Teleost* research vessel, in eastern Newfoundland (49°11'23.5"N: 50°12'42.3"W) in 2008.

Hundreds of juveniles were reared from gametes that were naturally spawned in the laboratory on April 12, 2015 and April 28, 2018. Details of the rearing method are reported in the Supplementary Material, Method. During the 40 months of this study, tanks holding juveniles and adults were periodically examined for the presence of plates by siphoning the bottom of the tanks and examining the debris under a microscope (Leica M205FA), and no natural shedding of plates was detected.

2.3.2 Plate extraction and identification

In early juveniles of *P. fabricii* (to about 4 mm in length, laboratory-reared), the plates are clearly visible and do not overlap. To extract them, whole individuals were placed individually into Eppendorf tubes (1.5 mL) and soft tissues dissolved by adding 1-2 drops of commercial bleach (2% NaClO, sodium hypochlorite). After 10-15 seconds, the plates deposited on the bottom of the tubes; they were subsequently rinsed 10 times with distilled water, transferred into depression slides and dried at room temperature (~20 °C) for 1-2 h. The plates were first examined using a microscope and intact plates then placed onto stubs with pre-mounted carbon tabs and imaged using a scanning electron microscope (SEM, Phenom ProX) to characterize their ultrastructure, especially the ring patterns.

In older juveniles and adults (wild-caught), the body wall is covered with several overlapping plates (Fig. S.2.1) firmly attached to tissues of the body wall. Two sampling methods were therefore used and compared. The first involved placing the body wall (without muscle bands) in a concentrated commercial bleach solution (8.25% NaClO) for 12 h. The second method involved boiling the body wall in distilled water inside a slow cooker (Crock-Pot®), at low temperature (79-93 °C) for 12 h. Three individuals were tested for each method. Once soft tissues had been dissolved, the remaining plates were rinsed 10 times in distilled water and dried in the oven at 60 °C for 6 h. The plates were then examined under light microscopy and SEM. The plates obtained with the bleaching method showed thin node points (connection points) with frequent breakage between them, an irregular surface and an accentuated porous appearance, yielding an overall weaker texture. Extraction through low-temperature boiling did not cause any visual alteration of the surface of the plates and ensured preservation of their original shape, dimension and red pigmentation (details in Fig. S.2.2 and Supplementary Material, Results). The boiling method also had the advantage of not requiring careful monitoring of immersion time and pH associated with the bleaching technique. Therefore, it was adopted for the remainder of the sampled individuals.

As plates extracted from older juveniles and adults are thicker and opaque (compared to those of early juveniles up to 40-month old that are mainly translucent), they were polished manually with sandpaper (800-2000 grits) on both sides to decrease their thickness and increase their transparency. They were then bathed in ~1 mL red tissue marking dye (2-aminoethanol 2%, formaldehyde 0.05%, N-dimethylaminoethanol 2%, water 66%; Triangle Biomedical Sciences, Inc), rinsed using distilled water and lightly blotted to improve the ring visualization. The plates were immersed in xylene before examination under a microscope to further accentuate the rings, consisting of alternating light and dark regions.

2.3.3 Validation of annual ring deposition

The plates of laboratory-reared juveniles of various ages (3, 20, 30, and 40 months old; n = 3-5 from each age class) were detached. Based on preliminary experiments and by monitoring the primary plate from time 0 (settlement) in early juveniles (and their persistence during subsequent growth), the oldest plates with the highest number of rings were determined to be the largest plates from the middle body between the mouth and the anus (Fig. S.2.3). A subsample of 3-4 of these oldest plates were examined under light microscopy and SEM, and the number of growth rings of each plate was determined and compared with the actual age of each individual.

2.3.4 Correlation between growth rings and other morphometrics

The actual age (ring counts on oldest plates) of laboratory-reared juveniles was compared to the following metrics: maximum body length (ventrally, from anterior to posterior end), total number of plates and size of the oldest plates.

For wild-caught individuals, a suite of the following selected morphometrics were also collected prior to the soft tissues being rendered by boiling, and compared with their age (ring counts). Immersed weights of each individual (n = 37, including all organs) were obtained using an underhook weighing system (Ohaus® V21PW6) to which a net basket immersed in seawater was attached. Total wet weights were also obtained after wiping excess water. Maximum length and width (Fig. S.2.1B), distance mouth-to-anus (Fig. S.2.1A) and girth (circumference around the maximum width) were measured using rope. The ventral area of this species is flat (sole-like), rimmed with a marginal band of tube feet and a sparser row of tube feet in the middle (Fig. S.2.1B). The sole surface area of each individual was photographed and measured using ImageJ software. Eviscerated weight (body wall without aquapharyngeal bulb and muscle bands) was recorded after all other metrics were collated. The sexual maturity of each individual was determined by the gonad color and the presence of mature gametes in the gonad tubule using a microscope (Hamel et al., 1993). The plates of each individual were detached and their total number and combined dry weight were determined. Four of the oldest plates (the largest plate with the highest number of rings, described in section 2.3.3, Fig. S.2.1) in each of the 37 individuals were processed and photographed. Their Feret diameter and thickness measured using a digital caliper (Traceable®), and the oldest plates were used to determine the age of sea cucumbers. The widths of light and dark section of the rings on the plates were measured under the microscope. In addition, the freshly collected deep-sea individual was processed in the same way, and its age was determined.

To preliminarily model individual growth of wild sea cucumbers, the von Bertalanffy growth function (VBGF), which has been widely used with other species (Herrero-Pérezrul et al., 1999; Morgan, 2012; Poot-Salazar et al., 2014; Watanabe et al., 2014), was fitted to size-at-age data, including maximum length, maximum width, distance mouth-to-anus, and girth (all in cm) using:

$$L_t = L_\infty(1 - e^{-K\,(t - t_0)}),$$

where L_t is the expected size at age t, L_∞ is the asymptotic size (theoretical average maximum length, width, distance mouth-to-anus or girth, in cm), K (year^{-1}) is the growth rate coefficient, and t_0 (year) is a modeling artifact of age at which size would be zero. Growth parameters of the VBGF were calculated for wild-caught individuals (excluding captive juveniles) using the FSA package in R (Ogle et al., 2018).

2.4 Results

2.4.1 Plate morphology and layered ring patterns

The oldest plates are the largest among the plates located in the middle body section between the mouth and the anus and have the highest number of rings (Fig. S.2.1C). Inversely, the newest (smallest) plates occur along the margins close to the sole and have the lowest number of rings (Fig. S.2.1D). After the detachment of plates from the dermis, each of them showed a rhomboidal or irregular shape from a top view and a dome shape from a side view, with a relatively hunched-up top and flat bottom (the latter being in contact with the soft tissue of the body wall).

In juveniles, most plates were porous and nearly round; under scanning electron microscopy (SEM), the central perforations were larger than the marginal ones (Fig. 2.1). Fresh plates collected from adults were reddish in colour and superficially appeared to be uniform and composed of loosely compacted materials (Fig. S.2.2A). After being boiled, polished and dyed, the plates generally became whitish and opaque with a light brown colour in the center. Clear ring-like patterns became visible to the naked eye, which were shown to consist of broad light rings, alternating consistently with narrow dark rings under light microscopy (Fig. 2.2).

2.4.2 Validation of annual ring deposition in juveniles of known age

SEM revealed the details of the accretion pattern, showing that the plates from 3, 20, 30, and 40-month-old (i.e. <1 to nearly 4 years) had one, two, three, and four layers, respectively (Fig. 2.1). By comparing the number of layers with the actual age of the juveniles, an annual growth pattern was obtained (Fig. 2.3). Specifically, from birth to one-year-old, the plates increased in both diameter and thickness, but remained uniform in appearance, with no visible ring pattern. From one year onward, the plates added one layer each year, which made them both larger and thicker (Fig. 2.3A). The new layer is always added on the surface in contact with the soft body wall tissue. The plate increase in size was a combination of both an increase diameter of the newly added layer that was consistently greater than that of the previous layer (Fig. 2.3B), which made the plate expand on the horizontal axis with only the last layer visible from bottom view (Fig. 2.1E) and the rings visible from top view (section naturally exposed to the surrounding environment).

Based on this analysis of plates in known age juveniles, the light rings are those generated each year, and the dark rings represent the transition (vertical overlap) between two layers (i.e. two years). Hence, in the study of plates from larger (wild) individuals, paired light and dark rings were counted as one-year growth.

2.4.3 Analysis of age-related metrics in juvenile, wild and deep-sea individuals

The laboratory-reared juveniles aged 3-40 months ranged in length from 1.0-4.1 mm and displayed 12 to 52 plates, the oldest plate measuring 398-1089 µm in diameter and 65-156 µm in thickness (Fig. 2.1A). The specific morphological metrics of juveniles of various sizes are provided in the Supplementary Material, Results. Overall, the average number and size of plates and body length increased with age (Figs. 2.1, S.2.2). The mean increase in body length for juveniles was 1.1 ± 0.2 mm year^{-1} and the diameter and thickness of plates increased by 295 ± 56 µm and 42 ± 11 µm per year, respectively.

The wild-caught individuals ranged from 0.1 to 18.9 g immersed weight, 0.5 to 187.7 g total wet weight, 0.2 to 65.5 g eviscerated weight and 2.0 to 114.8 cm^2 in sole surface area. They had between 211-1992 plates, totalling a dry weight between 0.1-19.5 g, and measuring 2.9-13.8 mm in diameter, and 0.54-1.96 mm in thickness. The number of annual rings on the plates varied from 4 to 28 (Fig. 2.2), indicating that age of the wild individuals examined was between 4-28 years. Based on the fact that no fully developed gonad (containing mature gametes) was detected in individuals with less than 10 annual rings, it can be concluded that the age at sexual maturity is around 10 years. The mean widths of the light and dark rings were 74 ± 72 µm and 43 ± 24 µm, respectively. This metric was highly variable across a given plate and the plates of different individuals of the same age (Fig. 2.2). For the individual collected from a deep-sea location, the number of annual rings was established at 25 and the widths of light and dark rings were 56 ± 20 µm and 40 ± 17 µm, respectively.

Fit of the von Bertalanffy growth function to size-at-age data available from the wild-caught individuals is presented in Fig. 2.4. The asymptotic (maximum) parameters were estimated to be 13.3 cm length, 9.9 cm width, 7.9 cm mouth-to-anus and 26.6 cm girth. The corresponding growth rate coefficients were 0.056, 0.059, 0.077, and 0.058 year^{-1}, respectively.

2.5 Discussion

The present study combined the use of captive-reared juveniles of known age and wild juveniles and adults to validate the annual deposition of ring patterns on the dorsal plates of *Psolus fabricii*, representing the first successful direct aging method for sea cucumbers. It highlights a new research avenue involving growth rings in Holothuroidea and paves the way for future research in other holothuroid families and species.

In Holothuroidea, ossicles occur in the tentacles, podia and body wall; they can be shaped like rods, tables, or anchors (Massin et al., 2000), and they are generally small, i.e. 20-145 µm in *Leptosynapta clarki* (Stricker, 1985) and 35-250 µm in *Holothuria scabra* (Massin et al., 2000). However, the focal species, *P. fabricii*, exhibits permanent imbricated scale-like ossicles (i.e. plates) on the surface of the body wall, common to all members of the Psolidae family, that measure between 450 µm in diameter in 3-month-old juveniles and 1.4 cm in the largest adults, making them easier to distinguish. While investigators had reported the presence of these plates in psolid species before (Bingham and Braithwaite, 1986; McEuen and Chia, 1991; O'Loughlin and Whitfield, 2010; Giménez and Penchaszadeh, 2010), their porous aspect and uniform colour probably prevented them from being considered potential indicators of age. Moreover, these plates can be challenging to extract without damaging their fine structure. The boiling method developed here preserved the original pigments of plates and did not change their shape, dimension or fine details. Moreover, the rings on the plate obtained by boiling were more obvious than those on plates obtained by bleaching after polishing and dying them.

Even though plate rings in echinoderms, especially in echinoids, have been studied for decades, very little is known about the mechanisms that underlie their addition and growth patterns. Rings were generally interpreted to reflect zones of rapid skeletal growth (coarse-pored stereo, opaque rings that appear light in reflected light, dark in transmitted light) possibly over a short period in spring/summer, followed by zones of slower growth (fine-pored stereo, translucent rings that appear dark in reflected light, light in transmitted light) probably encompassing a longer growth period in autumn/winter driven by temperature and food availability fluctuations (Pearse and Pearse, 1975). In the present study, the dual light and dark zones were also added each year; however, the light ring was added marginally and the dark ring consisted of the overlap between successive layers rather than representing a different density, i.e. the dark section grows over the margin of the plate from the previous year, and the light zone extends outward. While it is still unclear why the plates add one layer each year and how they are connected, it is obvious that the dual ring pattern in *P. fabricii* is not the result of differences between rapid and slow growth periods reported in other echinoderms (Moltschaniwskyj and Cappo, 2009).

The shallow-water population of *P. fabricii* examined here was exposed to seasonal fluctuations in both primary productivity and temperature, identifying these as potential drivers of the rings on the plates. Phytoplankton, which represents the principal food source of *P. fabricii* (Hamel et al. 1993), blooms between March and June, whereas seawater temperatures range from -1 to 8°C. The former factor is a more likely candidate since growth bursts were reported to coincide with peaks in phytoplankton production in another dendrochirotid sea cucumber from the same geographic location (So et al., 2010). Furthermore, the deep-sea specimen of *P. fabricii* examined opportunistically was exposed relatively stable temperature around 2-4°C, DFO station 27 (47°33'N, 52°35'24"W, Fisheries and Oceans Canada), minimizing the role of temperature relative to food availability. Deep-sea echinoids also contain cyclical growth rings (e.g. *Echinus affinis* (Gage and Tyler, 1985) and *Echinosigra phiale* (Gage, 1987)), and are exposed to relatively constant temperature. Annual rings in these species have been reported to mirror annual pulses in the downward flux of detrital food from the euphotic zone (Gage, 1987). Here, the difference in thickness between rings developed in the shallow-water versus deep-sea individuals of *P. fabricii* could reflect the quality and intensity of the food supply they are submitted to (fresh and abundant vs. degraded and sparse, respectively).

The dependence of ring patterns on food supply may explain some of the inconsistencies reported to date in calcified echinoderms. While sclerochronology and the addition of a complete cycle of growth rings was validated annually in chemically tagged individuals of the echinoids *Paracentrotus lividus* (Ouréns et al., 2013) and

Strongylocentrotus droebachiensis (Robinson and MacIntyre, 1997), another study found that less than half of the individuals in the latter added a full cycle of growth rings for a given year (Russell and Meredith, 2000). Similarly, growth rings in the congener *S. purpuratus* were not necessarily laid down annually (Ebert, 1988). In the present study, imaging of plates obtained from juveniles of known age confirmed that the plates of the holothuroid *P. fabricii* consistently added one layer underneath the previously deposited one each year (in all individuals examined). Further studies will be required to confirm that this annual pattern persists throughout the adult life. The diameter of the newly added layer was consistently greater than that of the previous layer, which made the plate expand horizontally as well as vertically, so that only the last layer could be seen from bottom view. This growth pattern underlies the dome shape of the plate, with a relatively flat bottom and hunched-up top. Since the bottom of the plates is firmly imbedded in the body wall dermis, it can be assumed that they grow through basal nourishment. Because plates become more abundant with age, it is important to determine age by counting the number of rings in the oldest (largest) dorsal plates located in the proximal area between mouth and anus (as determined in the juveniles of known age); smaller plates located elsewhere on the body wall display fewer growth rings, as they are synthetized later in the life of the sea cucumbers.

Annual rings in plates not only constitute a proxy of age but can also be used to derive useful information on growth patterns. Data from the present study suggest that *P. fabricii* is a long-lived, slow-growing animal. Since the individuals under study were obtained from shallow inshore locations, where smaller individuals are expected to occur compared to deeper habitats (30-60 m deep), longevity is assumed to be significantly longer than the 28 years measured here. A majority of benthic marine animals display asymptotic growth patterns, with rapid growth rates during the juvenile period followed by a slower growth once individuals reach sexual maturity (Moltschaniwskyj and Cappo, 2009). Fitting the size-at-age data from ring counts in wild individuals of *P. fabricii* to the commonly used von Bertalanffy growth function (VBGF) provided theoretical average maximum sizes in line with what we know of the species, and an overall stronger fit with the distance mouth-to-anus metric. There was no clear growth asymptote within the range of the data, supporting that the individuals sampled had not yet reached maximum age. The unrealistic t_0 values (age at which the size metrics would be zero) are not uncommon and illustrate the complexity and limitations of growth function fitting in echinoderms (Rogers-Bennett et al., 2003; Gage, 1987; Gage, 2003). Keeping in mind that direct aging of sea cucumbers has not been possible until now, it is difficult to explore growth models properly without going beyond the scope of the present study. Future investigations using larger samples sizes across a broader range of age classes are needed to provide greater insights into growth with age in this species.

Among the challenges ahead is the considerable variability in size (length or weight) at age among individuals of wild populations, which may result from heredity and/or sex, or even from the fusion of embryos generating larger juveniles at a corresponding age (Gianasi et al., 2018a). Environmental factors may also be at play, as exemplified by differences between laboratory-reared and wild juveniles. The latter reached 0.4 cm in length after 40 months (3.3 years), consistent with growth of another dendrochirotid species from the same area inside similar mesocosm setups (So et al., 2010; Gianasi et al., 2018b). However, the smallest wild-caught *P. fabricii* in the present study measured 1.4 cm in length and was determined to be 4 years old, i.e. it was just eight months older but 3.5 times larger. Similarly, the individual collected from the deep-water location was older (25 years old) than shallow-water counterparts of similar size (13-17 years old), and it had thinner ring intervals, consistent with slower growth rates in the deep-sea environment (Gage, 1987). Inter-individual variability in size at age has been reported in holothuroids during early ontogeny (Liang et al., 2010), and has long been challenging studies of population structures. In addition, intra-individual variability in growth was evidenced here, which may reflect annual shifts in environmental conditions or a change in food supply following relocation to a different habitat (Hamel et al., 2019).

Although it has yet to be extended to other sea cucumbers, solid evidence for annual periodicity in the size increment of the primary ossicle plates was provided here for *P. fabricii*, generating visible rings that allow the determination of age and growth rates at the individual and population level. Similar growth rings were also recently observed in a congener species, *P. phantapus*, from shallow waters of eastern Canada (J. Sun, 2018). The use of this new method in psolids and its potential application to other species with calcareous structures, such as circum-oral rings and smaller ossicles, provides a valuable tool for exploring longevity, population turnover, as well as patterns of growth under different environmental conditions and in various habitats. These may improve our understanding of population dynamics in sea cucumbers, which are key members of benthic communities worldwide and prized marine resources worldwide.

2.6 Acknowledgements

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2.7 References

Agatsuma, Y., Nakata, A., 2004. Age determination, reproduction and growth of the sea urchin *Hemicentrotus pulcherrimus* in Oshoro Bay, Hokkaido, Japan. Journal of the Marine Biological Association of the United Kingdom. 84, 401-405.

Anderson, S.C., Flemming, J.M., Watson, R., Lotze, H.K., 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries. 12, 317-339.

Bertignac, M., De Pontual, H., 2007. Consequences of bias in age estimation on assessment of the northern stock of European hake (*Merluccius merluccius*) and on management advice. ICES Journal of Marine Science. 64, 981-988.

Bingham, B.L., Braithwaite, L.F., 1986. Defense adaptations of the dendrochirote holothurian *Psolus chitonoides* Clark. Journal of Experimental Marine Biology and Ecology. 98, 311-322.

Brady, S.M., Scheibling, R.E., 2006. Changes in growth and reproduction of green sea urchins, *Strongylocentrotus droebachiensis* (Müller), during repopulation of the shallow subtidal zone after mass mortality. Journal of Experimental Marine Biology and Ecology. 335, 277-291.

Brinkhurst, R.O., Linkletter, L.E., Lord, E.I., Connors, S.A., Dadswell, M.J., 1976. A preliminary guide to the littoral and sublittoral marine invertebrates of Passamaquoddy Bay. Barns Hopkins Ltd., Saint John, New Brunswick.

Campana, S., 2001. Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. Journal of Fish Biology. 59, 197-242.

Dahm, C., Brey, T., 1998. Determination of growth and age of slow growing brittle stars (Echinodermata: Ophiuroidea) from natural growth bands. Journal of the Marine Biological Association of the United Kingdom. 78, 941-951.

Ebert, T.A., 1978. Growth and size of the tropical sea cucumber *Holothuria (Halodeima) atra* Jager at Enewetak Atoll, Marshall Islands. Pacific Science. 32, 183-191.

Ebert, T.A., 1988. Calibration of natural growth lines in ossicles of two sea urchins, *Strongylocentrotus purpuratus* and *Echinometra mathaei*, using tetracycline. in: Burke R.D., Mladenov P.V., Lambert P., Parsley R.L. (Eds.), Echinoderm Biology: Proceedings 6th International Echinoderm Conference. Balkema, Rotterdam, the Netherlands, pp. 435-443.

Ebert, T.A., Dixon, J.D., Schroeter, S.C., Kalvass, P.E., Richmond, N.T., Bradbury, W.A., Woodby, D.A., 1999. Growth and mortality of red sea urchins *Strongylocentrotus franciscanus* across a latitudinal gradient. Marine Ecology Progress Series. 190, 189-209.

Evamy, B., Shearman, D., 1965. The development of overgrowths from echinoderm fragments. Sedimentology. 5, 211-233.

Fisheries and Oceans Canada, 2005. Hydographic Data. http://www.meds-sdmm.dfo-mpo.gc.ca/isdmgdsi/azmp-pmza/hydro/index-eng.html (accessed on 09 February 2019).

Gage, J., 1987. Growth of the deep-sea irregular sea urchins *Echinosigra phiale* and *Hemiaster expergitus* in the Rockall Trough (NE Atlantic Ocean). Marine Biology. 96, 19-30.

Gage, J., Tyler, P., 1985. Growth and recruitment of the deep-sea urchin *Echinus affinis*. Marine Biology. 90, 41-53.

Gage, J.D., 2003. Growth and production of *Ophiocten gracilis* (Ophiuroidea: Echinodermata) on the Scottish continental slope. Marine Biology. 143, 85-97.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2018a. Full allogeneic fusion of embryos in a holothuroid echinoderm. Proceedings of the Royal Society B: Biological Sciences. 285, 20180339.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2018b. Morphometric and behavioural changes in the early life stages of the sea cucumber *Cucumaria frondosa*. Aquaculture. 490, 5-18.

Gianasi, B.L., Parrish, C.C., Hamel, J.-F., Mercier, A., 2017. Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquaculture Research. 48, 3413-3432.

Giménez, J., Penchaszadeh, P.E., 2010. Brooding in *Psolus patagonicus* (Echinodermata: Holothuroidea) from Argentina, SW Atlantic Ocean. Helgoland Marine Research. 64, 21-26.

Hamel, J.-F., Mercier, A., 1996. Evidence of chemical communication during the gametogenesis of holothuroids. Ecology. 77, 1600-1616.

Hamel, J.-F., Himmelman, J.H., Dufresne, L., 1993. Gametogenesis and spawning of the sea cucumber *Psolus fabricii* (Duben and Koren). The Biological Bulletin. 184, 125-143.

Hamel, J.-F., Sun, J., Gianasi, B.L., Montgomery, E.M., Kenchington, E.L., Burel, B., Rowe, S., Winger, P.D., Mercier, A., 2019. Active buoyancy adjustment increases dispersal potential in benthic marine animals. Journal of Animal Ecology. 88, 820-832.

Hannah, L., Duprey, N., Blackburn, J., Hand, C.M., Pearce, C.M., 2012. Growth rate of the California sea cucumber *Parastichopus californicus*: measurement accuracy and relationships between size and weight metrics. North American Journal of Fisheries Management. 32, 167-176.

Herrero-Pérezrul, M., Bonilla, H.R., García-Domínguez, F., Cintra-Buenrostro, C., 1999. Reproduction and growth of *Isostichopus fuscus* (Echinodermata: Holothuroidea) in the southern Gulf of California, Mexico. Marine Biology. 135, 521-532.

Herrero-Pérezrul, M.D., Reyes-Bonilla, H., 2008. Weight-Length relationship and relative condition of the holothurian *Isostichopus fuscus* at Espíritu Santo Island, Gulf of California, Mexico. Revista de Biologia Tropical. 56.

Hopcroft, R.R., Ward, D.B., Roff, J.C., 1985. The relative significance of body surface and cloacal respiration in *Psolus fabricii* (Holothuroidea: Dendrochirotida). Canadian Journal of Zoology. 63, 2878-2881.

Liang, M., Dong, S., Gao, Q., Wang, F., Tian, X., 2010. Individual variation in growth in sea cucumber *Apostichopus japonicus* (Selenck) housed individually. Journal of Ocean University of China. 9, 291-296.

Massin, C., Mercier, A., Hamel, J.-F., 2000. Ossicle change in *Holothuria scabra* with a discussion of ossicle evolution within the Holothuriidae (Echinodermata). Acta Zoologica. 81, 77-91.

McEuen, F., Chia, F.-S., 1991. Development and metamorphosis of two psolid sea cucumbers, *Psolus chitonoides* and *Psolidium bullatum*, with a review of reproductive patterns in the family Psolidae (Holothuroidea: Echinodermata). Marine Biology. 109, 267-279.

Mercier, A., Hamel, J.-F., 2010. Synchronized breeding events in sympatric marine invertebrates: role of behavior and fine temporal windows in maintaining reproductive isolation. Behavioral Ecology and Sociobiology. 64, 1749-1765.

Moltschaniwskyj, N., Cappo, M., 2009. Alternatives to sectioned otoliths: the use of other structures and chemical techniques to estimate age and growth for marine vertebrates and invertebrates. in: Green B.S., Carlos G., Begg G.A. (Eds.), Tropical Fish Otoliths: Information for Assessment, Management and Ecology, Reviews: Methods and Technologies in Fish Biology and Fisheries. Springer, New York, pp. 133-173.

Morgan, A.D., 2012. Use of a growth model to estimate size at age in the temperate sea cucumber *Australostichopus mollis*. SPC Beche-de-mer Information Bulletin. 32, 24-32.

Narvaez, C.A., Johnson, L.E., Sainte-Marie, B., 2016. Growth bands are an unreliable indicator of sea urchin age: evidence from the laboratory and the literature. Limnology and Oceanography: Methods. 14, 527-541.

O'Loughlin, P.M., Whitfield, E., 2010. New species of *Psolus* Oken from Antarctica (Echinodermata: Holothuroidea: Psolidae). Zootaxa. 2528, 61-68.

Ogle, D.H., Wheeler, P., Dinno, A., 2018. FSA: Fisheries Stock Analysis. R package version 0.8.22.9000. See https://github.com/droglenc/FSA.

Ouréns, R., Flores, L., Fernández, L., Freire, J., 2013. Habitat and density-dependent growth of the sea urchin *Paracentrotus lividus* in Galicia (NW Spain). Journal of Sea Research. 76, 50-60.

Pearse, J.S., Pearse, V.B., 1975. Growth zones in the echinoid skeleton. American Zoologist. 15, 731-751.

Poot-Salazar, A., Hernández-Flores, Á., Ardisson, P.-L., 2014. Use of the SLW index to calculate growth function in the sea cucumber *Isostichopus badionotus*. Scientific Reports. 4.

Purcell, S.W., Blockmans, B.F., 2009. Effective fluorochrome marking of juvenile sea cucumbers for sea ranching and restocking. Aquaculture. 296, 263-270.

Purcell, S.W., Blockmans, B.F., Nash, W.J., 2006. Efficacy of chemical markers and physical tags for large-scale release of an exploited holothurian. Journal of Experimental Marine Biology and Ecology. 334, 283-293.

Purcell, S.W., Conand, C., Uthicke, S., Byrne, M., 2016. Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review. 54, 367-386.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M.V., Lovatelli, A., Uthicke, S., 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries. 14, 34-59.

Robinson, S.M.C., MacIntyre, A.D., 1997. Aging and growth of the green sea urchin. Bulletin Aquaculture Association of Canada. 97, 56-60.

Rogers-Bennett, L., Rogers, D.W., Bennett, W.A., Ebert, T.A., 2003. Modeling red sea urchin (*Strongylocentrotus franciscanus*) growth using six growth functions. Fishery Bulletin. 101, 614-626.

Ruppert, E.E., Barnes, R.D., Fox, R.S., 2004. Invertebrate zoology: a functional evolutionary approach. Brooks/Cole-Thamson Learning, Belmont, CA, USA.

Russell, M.P., Meredith, R.W., 2000. Natural growth lines in echinoid ossicles are not reliable indicators of age: a test using *Strongylocentrotus droebachiensis*. Invertebrate Biology. 119, 410-420.

So, J.J., Hamel, J.-F., Mercier, A., 2010. Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology. 17, 473-484.

Stricker, S.A., 1985. The ultrastructure and formation of the calcareous ossicles in the body wall of the sea cucumber *Leptosynapta clarki* (Echinodermata, Holothuroida). Zoomorphology. 105, 209-222.

Stump, R., Lucas, J., 1990. Linear growth in spines from *Acanthaster planci* (L.) involving growth lines and periodic pigment bands. Coral Reefs. 9, 149-154.

Watanabe, S., Sumbing, J.G., Lebata-Ramos, M.J.H., 2014. Growth pattern of the tropical sea cucumber, *Holothuria scabra*, under captivity. Japan Agricultural Research Quarterly. 48, 457-464.

Yang, H., Hamel, J.-F., Mercier, A., 2015. The sea cucumber *Apostichopus japonicus*: history, biology and aquaculture. Academic Press, New York, NY, USA.

2.8 Figures

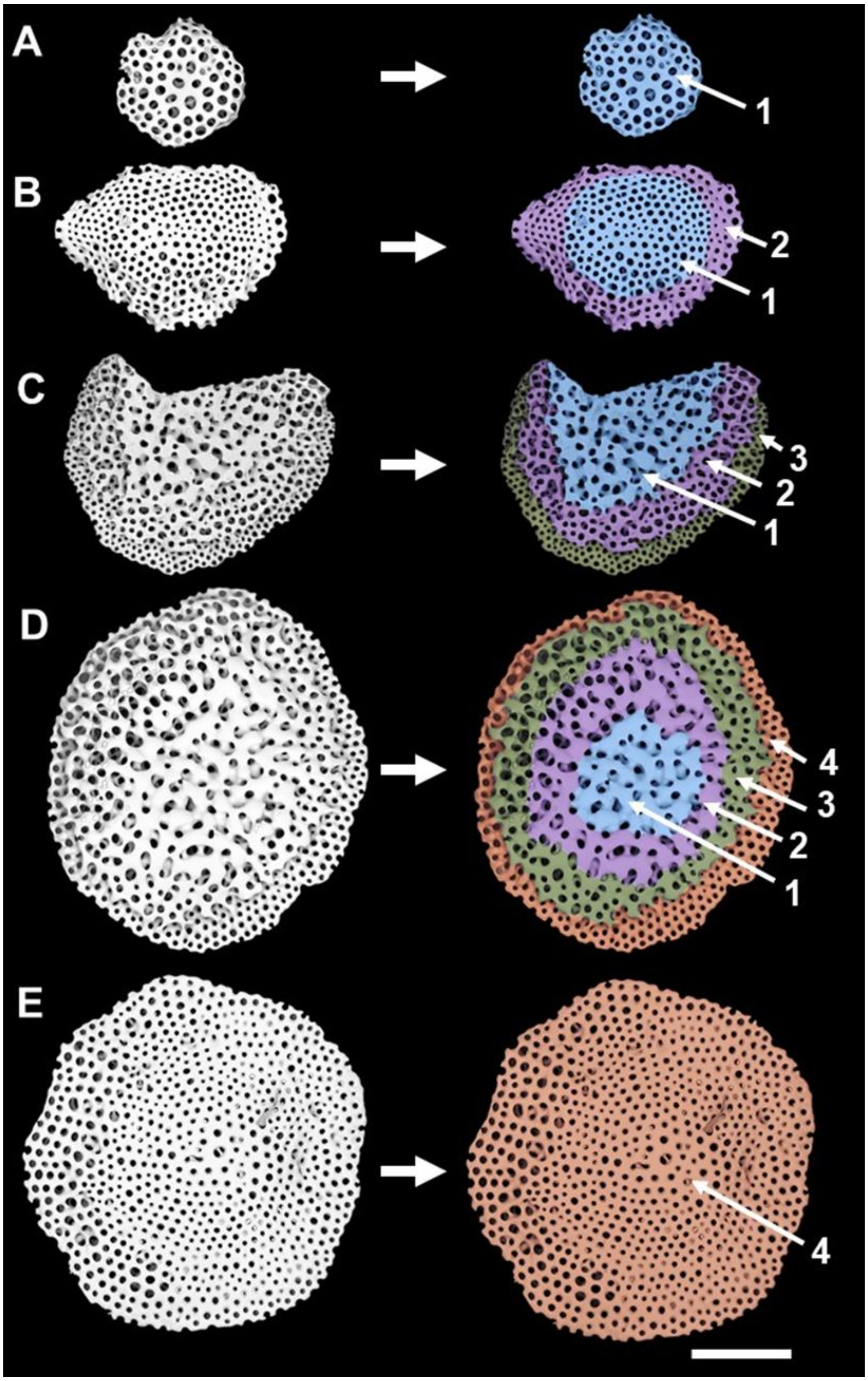

Figure 2.1 Scanning electron microscopy (SEM) of the oldest plates in 3, 20, 30, and 40-month-old juveniles of *Psolus fabricii*. Images on the right show the pattern more clearly using different colours for each layer. (A) Top view of the plate from a three-month-old (0.25 y) juvenile showing round shape with holes, and central holes larger than marginal holes. (B) Top view of the plate from a twenty-month-old (1.7 y) juvenile showing two layers (arrows). (C) Top view of the plate from a thirty-month-old (2.5 y) juvenile showing three layers (arrows). (D) Top view of the plate from a forty-month-old (3.3 y) juvenile showing four layers (arrows). (E) Bottom view of the plate from a forty-month-old (3.3 y) juvenile showing the fourth layer (arrow). The scale bar represents 200 μm in all panels.

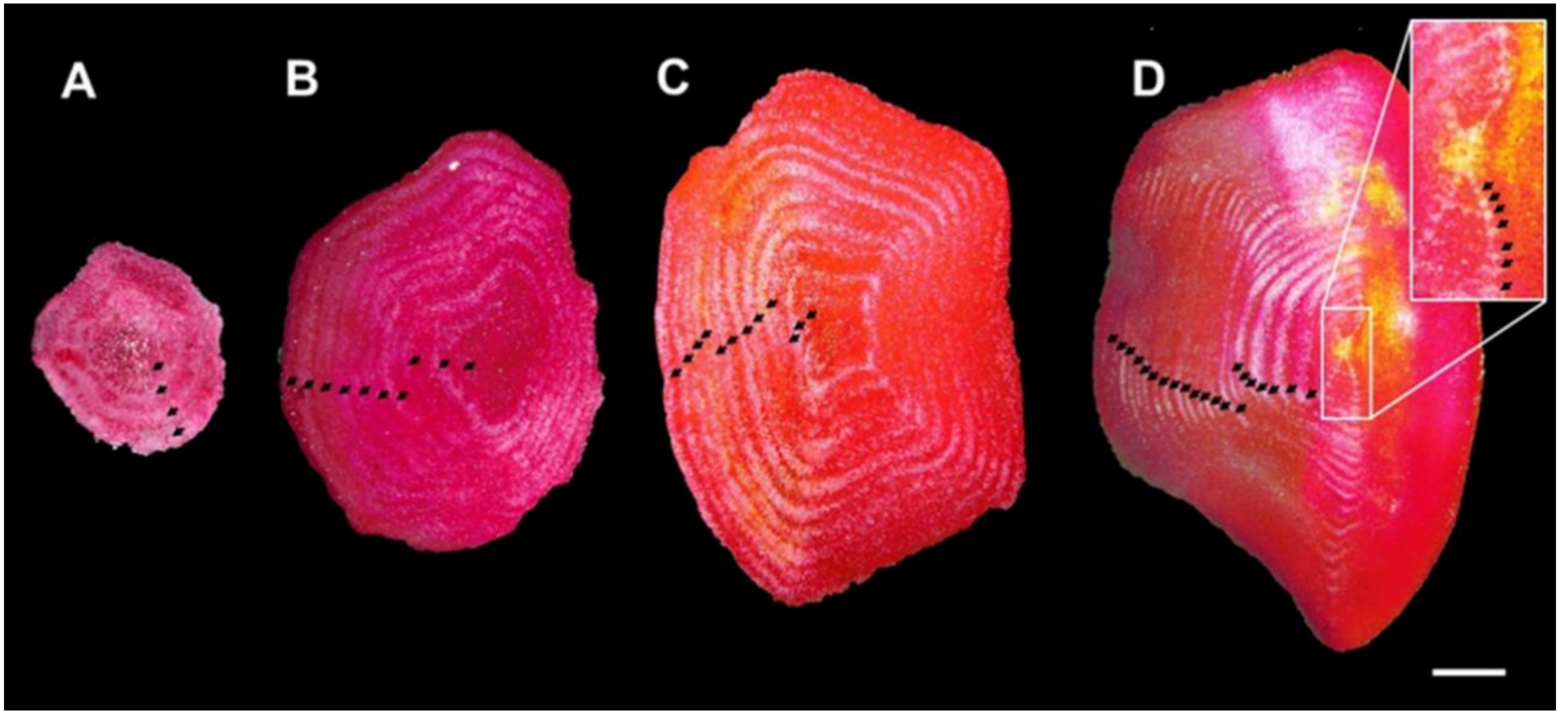

Figure 2.2 Plates from wild-caught individuals of *Psolus fabricii* of different sizes, with different numbers of growth rings (A: 4 rings; B: 10 rings; C: 12 rings; D: 28 rings). One pair, consisting of a dark and a light ring, was considered to represent 1 year of growth. Therefore, the innermost rings of individuals A, B, C, and D were respectively developed in 2013, 2007, 2005, and 1989 and all the outermost rings were developed in 2017. The scale bar at the bottom represents 200 µm.

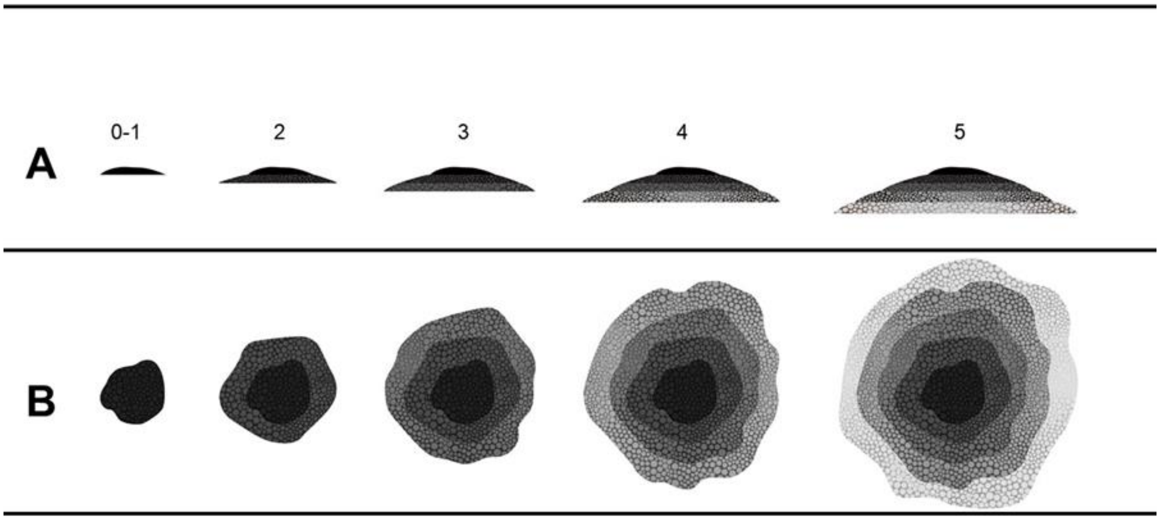

Figure 2.3 Schematic diagram of 2-D plate growth in the sea cucumber *Psolus fabricii*. (A) Vertical addition of one layer every year, shown from the side view. (B) Annual ring expansion on the horizontal plane, shown from the top view. The values above the panels correspond to the relative year.

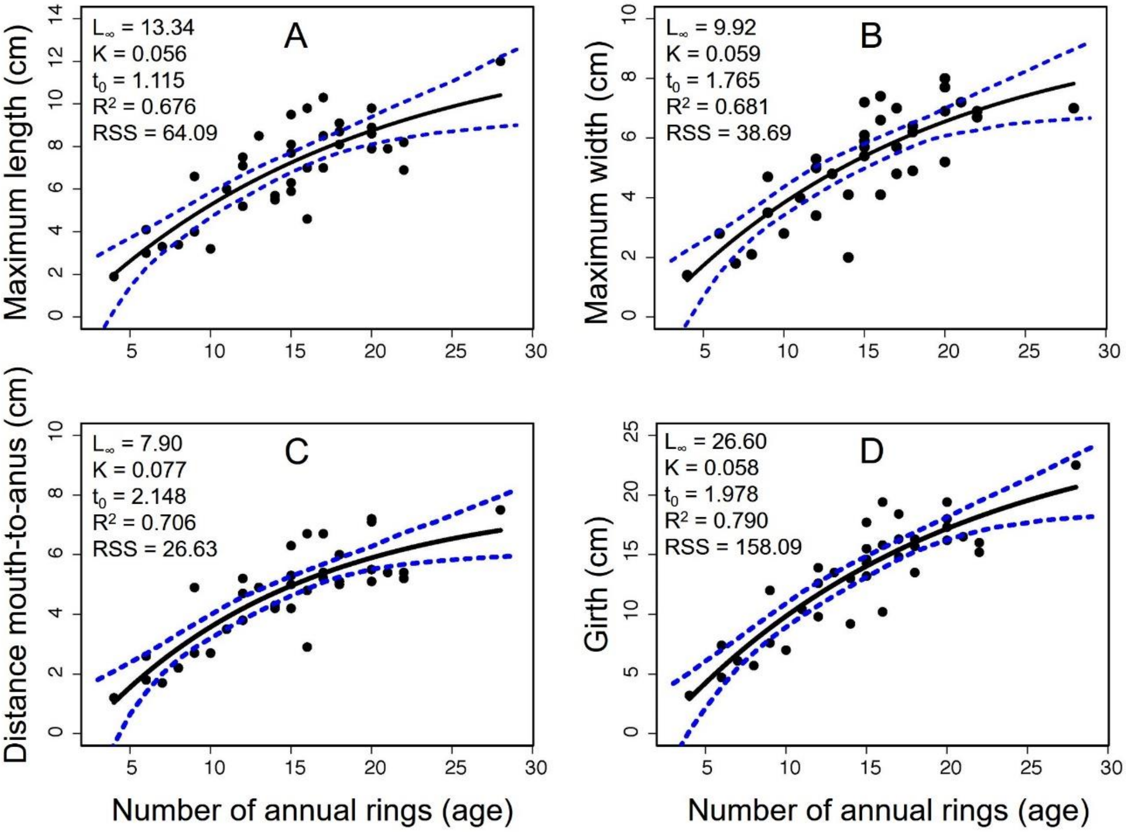

Figure 2.4 von Bertalanffy function fitted to size-at-age data collected from wild individuals of *Psolus fabricii* (n = 37). (A) Maximum length-at-age; (B) Maximum width-at-age; (C) Distance mouth-to-anus-at-age; (D) Girth-at-age. L_∞ (cm) represents the asymptotic size, K (year^{-1}) represents the growth rate coefficient, t_0 (year) is the calculated age at which the selected size metrics would be zero, R^2 is the coefficient of determination, and RSS is the residual sum of squares. Dotted lines represent 95% confidence intervals.

2.9 Supplementary Material

Methods

Rearing methods for juveniles of P. fabricii

Fertilized oocytes (eggs) were collected and were incubated in flow-through rearing vessels. The latter consisted of 4-L round plastic containers with black bottom and walls, placed inside a 40-L tank supplied with ambient running seawater. In order to ensure a constant water flow into the rearing tanks, four equally spaced holes (40 cm^2 each) were made on the walls close to the bottom of the round containers and covered with 1-mm mesh.

Results

Optimal method to extract plates in wild individuals

Images obtained under the light microscope showed that the slow-cooking method preserved the original pigments (Fig. S.2.2A), whereas the use of hypochlorite solution bleached the plate (Fig. S.2.2D). SEM images of the plates also revealed striking differences in the structure and surface of the plates obtained with each method (Fig. S.2.2B, C, E, F). The hypochlorite solution clearly corroded the calcite crystals and modified the original plate shape, as well as its dimension and fine details. The plate obtained by low-temperature boiling showed thick node points with no breakage between them (Fig. S.2.2B); in contrast, the plate obtained by bleaching showed thin node points with frequent breakage between them, and a frailer appearance overall (Fig. S.2.2E). The plate obtained by bleaching also showed an irregular surface and a porous appearance (Fig. S.2.2C) contrasting with that of the plate obtained by boiling, where the entire surface remained smooth (Fig. S.2.2F). The plate obtained by bleaching was frailer than the plate obtained by boiling when they were polished.

Morphological metrics of laboratory-reared and wild-caught individuals of various size

Three-month-old juveniles were 1.2 ± 0.2 mm in length (Fig. S.2.3A) and displayed 12 to 16 plates, the oldest measuring 448 ± 52 µm in diameter and 73 ± 9 µm in thickness (Fig. 2.1A). At 20 months, juveniles were 2.8 ± 0.2 mm long (Fig. S.2.3B), the number of plates ranged from 20 to 24, with the oldest ones measured 645 ± 44 µm in diameter and 95 ± 11 µm in thickness (Fig. 2.1B). Thirty-month-old juveniles reached 3.4 ± 0.4 mm in length, had between 32 and 35 plates the oldest of which measured 746 ± 105 µm in diameter and 126 ± 24 µm in thickness (Fig. 2.1C). At 40 months of age, juveniles were 3.7 ± 0.4 mm long (Fig. S.2.3C), with 41 to 52 plates of 985 ± 81 µm in diameter and 138 ± 19 µm in thickness (Fig. 2.1D, E).

Figures

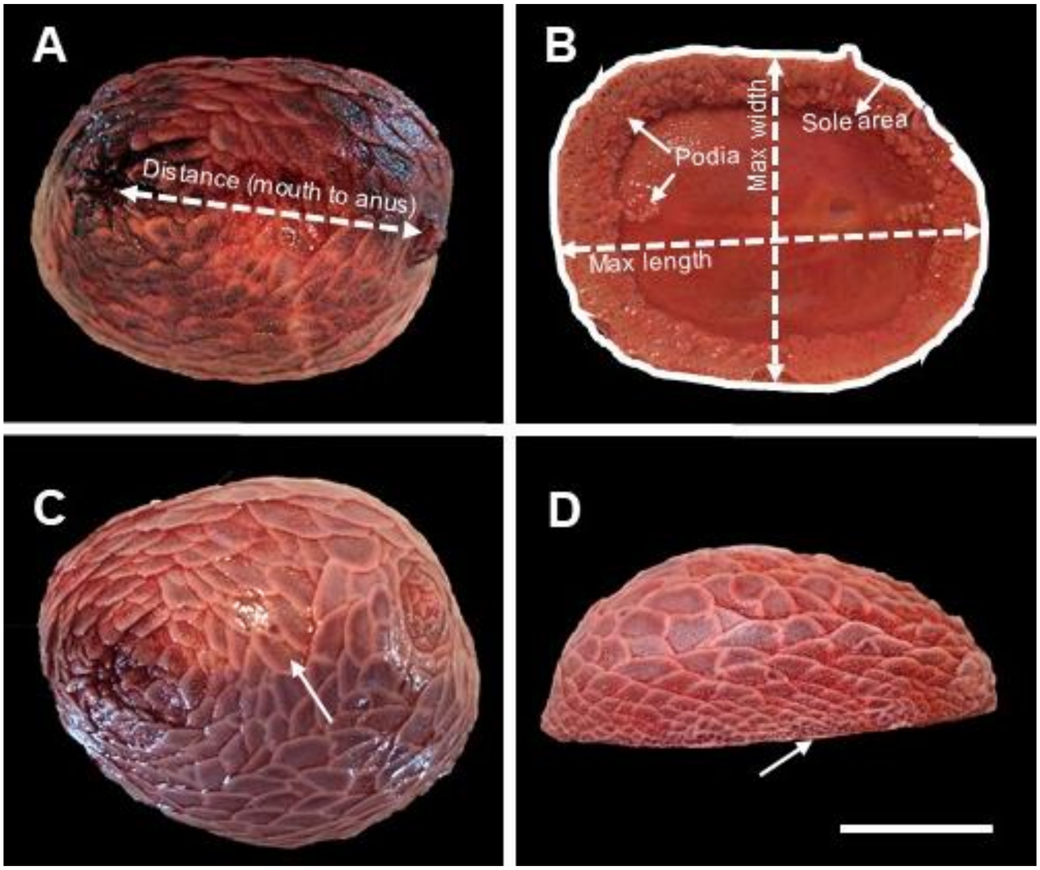

Figure S.2.1 Diagram of main morphometrics measured in adults of *Psolus fabricii*. (A) The dorsal body wall was covered with an "armour" of imbricating plates. The distance from mouth to anus measured in this study is illustrated. (B) The ventral area is flat (sole-like) and rimmed with a marginal band of tube feet and a weaker row down (arrow) the middle. The maximum length, maximum width and sole area measured in this study are shown. (C) The largest (i.e. oldest) plates (arrow) are located in the middle body section between mouth and anus. (D) The smallest (newest) plates (arrow) occur along the margins close to the sole. The scale bar presents 3 cm in all panels.

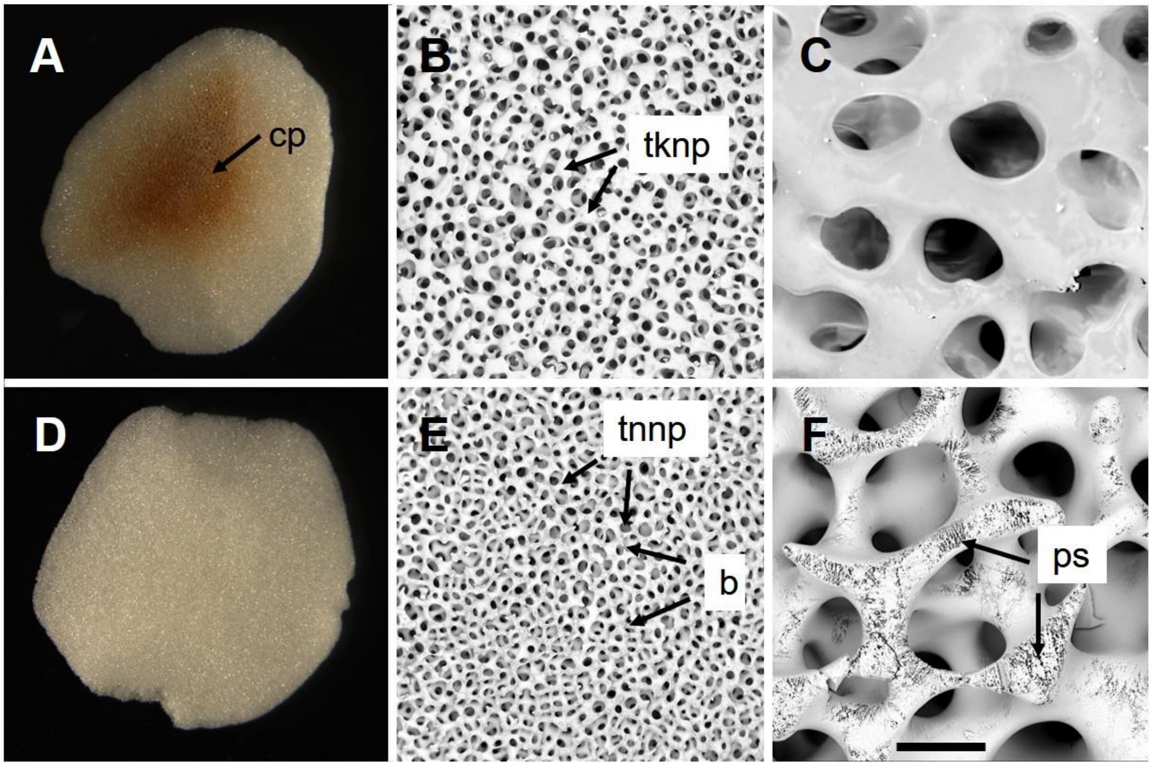

Figure S.2.2 Plates of *Psolus fabricii* obtained from either low-temperature boiling (A, B, C) or bleaching (D, E, F) examined under a light stereomicroscope (A, D) or a scanning electron microscope (B, C, E, F). (A) Caramel colour pigment (cp, arrow) present in the centre of the plate. (B) Plate with thick node points (tknp, arrows) and no breakage between them. (C) Plate displaying a solid structure without cavities. (D) Plate showing loss of pigmentation. (E) Plate showing thin node points (tnnp, arrows) and breakage (b, arrows) between node points. (F) Porous surface (ps, arrows) of plate. The scale bar at the bottom represents 1000 μm in (A, D), 200 μm in (B, E) and 30 μm in (C, F).

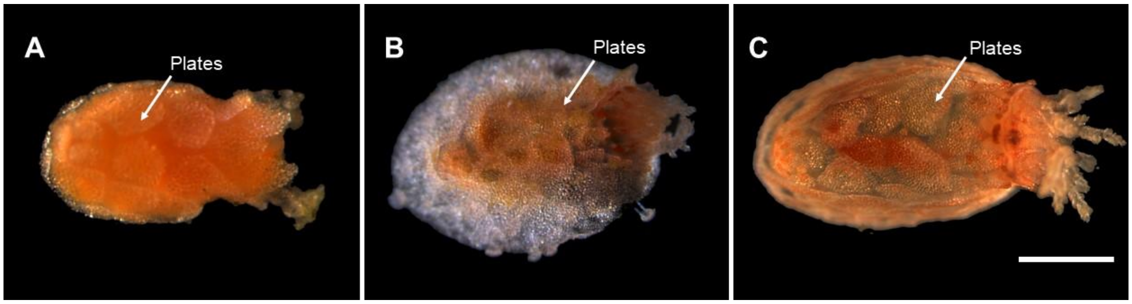

Figure S.2.3 Juveniles of *Psolus fabricii* are covered with imbricating plates, the oldest of which increase in size and number with age (A: 3 months; B: 20 months; and C: 40 months). The scale bar represents 0.47 mm in (A), 0.88 mm in (B) and 1.0 mm in (C).

Chapter 3. Influence of flow on locomotion, feeding behaviour and spatial distribution of a suspension-feeding sea cucumber [2]

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[2] A version of this manuscript was published in Journal of Experimental Biology (2018) 221, jeb189597. doi: 10.1242/jeb.189597

3.1 Abstract

Although movement in response to environmental conditions represents a fundamental link between animal behaviour and population ecology, it is rarely investigated in suspension feeders because they are generally perceived as sessile. Here, the interplay between water flow and fine locomotor and feeding behaviours was experimentally investigated for the first time in a free-moving suspension-feeding sea cucumber (*Cucumaria frondosa*; Echinodermata: Holothuroidea) using time-lapse videography in a mesocosm setting. Individuals moved away from static conditions in the weakest flow treatment and fled the strongest flows (>40 cm s^{-1}) in the more dynamic treatments. The tentacles of individuals located in areas with flows of ≥40 cm s^{-1} was aligned with the direction of the current, whereas in flows <40 cm s^{-1}, they were typically perpendicular to the direction of flow. Tentacle deployment and insertion rates (i.e. feeding rate) increased with flow, from 0.95 min^{-1} at 10 cm s^{-1} to 1.13 min^{-1} at 40 cm s^{-1}. Three modes of locomotion were detected. Forward crawling was most frequent at flows ≤40 cm s^{-1}, passive rolling dominated at flows >40 cm s^{-1}, and active rolling occurred randomly at flows between 0 and 120 cm s^{-1}. Overall, the flow regime favoured by *Cucumaria frondosa* was determined to be between 21 and 40 cm s^{-1}, under which an optimal balance between efficient food capture and energy expenditure for attachment to the bottom was presumably found. These findings provide insight into the distribution and population dynamics of suspension-feeding holothuroids, and may also assist the fisheries management and aquaculture development of commercial species.

Key words: Suspension feeding; Benthic animals, Sea cucumbers, Flow, Feeding, Locomotion.

3.2 Introduction

Almost all organisms need to move at some point during their lives, either through active locomotion or through passive transport by physical means (e.g. water, winds) or other agents (Holyoak et al., 2008). The short-term goals of movement mainly relate to reproduction, feeding and survival, and the longer-term goals are to avoid inbreeding and population extinction (Holyoak et al., 2008). The causes, patterns, mechanisms, and consequences of movement play an important role in determining the structure and dynamics of populations, communities, and ecosystems; as well as the evolution and diversity of life (Nathan et al., 2008; Holyoak et al., 2008).

Sea cucumbers are echinoderms belonging to class Holothuroidea. They are ubiquitous, sometimes dominant, and they play fundamental roles in marine ecosystems (Purcell et al., 2016). Deposit-feeding sea cucumbers influence the stratification and stability of the sediment via ingestion and bioturbation, and suspension-feeding sea cucumbers modulate water quality by altering its carbonate content and pH (Massin, 1982; Uthicke, 2001). Sea cucumbers are also known to constitute important food sources for other species, such as cod, salmon, and walruses (Hamel and Mercier, 2008a; 2008b). Finally, they represent an economically-valuable conservation-worthy marine resource that supports coastal livelihoods around the world (Anderson et al., 2011; Purcell et al., 2013). The habitat requirements, movement, population densities and broad spatial distribution of sea cucumbers have previously been linked to a variety of factors including depth (Woodby et al., 2000; Mercier et al., 2000b), substratum characteristics (Slater and Jeffs, 2010; Hamel and Mercier, 1996; Woodby et al., 2000; Young and Chia, 1982; Dissanayake and

Stefansson, 2012; Mercier et al., 2000b; 2000a), food availability (van Dam-Bates et al., 2016; Navarro et al., 2014; Navarro et al., 2013), light intensity, temperature and salinity fluctuations (Navarro et al., 2014). Under most circumstances, sea cucumbers are considered sedentary or sessile, although they may move in direct response to environmental conditions (Young and Chia, 1982). Substrate type is a well-known driver of distribution; for example, the densities of the deposit-feeding *Parastichopus californicus* in the vicinity of Sitka Sound, Alaska (USA) were highest on shell debris and gravel, and lowest on mud and silt bottoms (Woodby et al., 2000). In a study of another deposit feeder, *Holothuria scabra*, the smallest juveniles (10-40 mm) buried themselves around sunrise and emerged close to sunset, and larger juveniles (40-140 mm) buried when the temperature declined and emerged around mid-day (Mercier et al., 1999). Other deposit-feeding species, such as *Australostichopus mollis* and *Holothuria sanctori*, have been shown to congregate towards areas offering organic-rich food sources (Slater et al., 2011; Slater and Jeffs, 2010; Navarro et al., 2013). Water motion has also been shown to influence distribution in suspension-feeding sea cucumbers, which depend upon currents to bring food particles within reach of their tentacles (Fankboner, 1978; McKenzie, 1991). Clumping in response to wave action was documented along the west coast of South Africa (Barkai, 1991), and dense populations of the dendrochirotid *Aslia lefevrei* were most commonly found in areas of moderately strong water movement and hard substratum (Costelloe and Keegan, 1984a; 1984b).

Cucumaria frondosa (Holothuroidea: Dendrochirotida) is the most abundant sea cucumber in the North Atlantic and Arctic Oceans, and it has been the target of commercial fisheries since the 1980s (Hamel and Mercier, 2008b). The long-lived species is distributed along the coast of New England (USA), the eastern coast of Canada, Iceland and Greenland, down the coast of northern Europe and Scandinavia, as well as in the Faroe Islands (Jordan, 1972). As a suspension feeder, *C. frondosa* ingests suspended particulate food, chiefly phytoplankton, by extending its tentacles in the water column (Hamel and Mercier, 1998). Dense populations are most commonly found on the rocky substrate at depths of ~30 m (Jordan, 1972), but the species occurs from shallow tide pools down to 300-400 m (Klugh, 1924), with few specimens found deeper than 800 m (Hamel and Mercier, 2008b). Fisheries and ecological data have been gathered on *C. frondosa* over the past several decades, including on the reproductive cycle (Hamel and Mercier, 1995), larval development and settlement (Hamel and Mercier, 1996), and feeding (Singh et al., 1998; Hamel and Mercier, 1998; Singh et al., 1999; Holtz and MacDonald, 2009). Knowledge of the spatial distribution patterns, and habitat preferences of *C. frondosa* are scant apart from distribution studies conducted in the St. Lawrence Estuary (Hamel and Mercier, 1996), which showed that smaller individuals mainly inhabited shallow water (<20 m) and larger ones concentrated in deeper water (≥20 m).

The purpose of the present study was to explore locomotion in this seemingly slow moving benthic animal and assess how flow influences key behavioural metrics and ultimately determines the spatial distribution of this species. Experiments were first conducted to assess whether the presence or absence of current affected locomotor and feeding behaviours. Another set of experiments was conducted in a large mesocosm to refine our understanding and determine: (i) whether locomotor behaviour and proportions of immobile *vs* moving individuals vary among different water flows; (ii) whether cloacal respiration and feeding behaviour, indicated by proportions of individuals with tentacles deployed and tentacle insertion rates (TIRs), are affected by water flows; and (iii) how the water flow will modulate the ultimate spatial distribution of suspension-feeding sea cucumbers. This knowledge will refine our ecological understanding of one of the dominant benthic species in the North Atlantic Ocean, provide tools for more efficient management of suspension-feeding sea cucumber populations, and generate metrics of strategic value for the development of optimal flow conditions during captive breeding and sea ranching.

3.3 Materials and methods

3.3.1 Collection, holding and experimental conditions

Approximately 1000 adult sea cucumbers were collected by a fishing vessel (commercially licensed by the federal authority; Fisheries and Oceans Canada) on the southwest Grand Banks of Newfoundland (46°20’43.5” N: 56°23’0.28” W), eastern Canada, at depths between 20 and 30 m. They were kept in a flow-through raceway (11.5 m × 2.5 m × 1.2 m) supplied with 30-60 L min^{-1} of ambient running seawater that was pumped directly from the ocean at 37 m depth. Healthy undamaged sea cucumbers of similar size, with a contracted body length of 15.6 ± 2.5 cm and a wet body mass of 293 ± 36 g were selected for the experiments. Over the holding and study periods, the water temperature varied seasonally from 1 to 7°C, the salinity was 34-35, the pH was 7.8-8.2, and the dissolved oxygen remained >9.0 mg L^{-1} (all parameters were measured with a YSI® 556 MPS probe every other day). Illumination was provided by multiple fluorescent lights suspended above the holding and experimental tanks. The maximum light intensity was 80-270 lx (Traceable® 3252 light meter) and the light/dark (day-night) cycle varied seasonally from 12 h: 12 h to 16 h:8 h light:dark. These ranges of environmental conditions are in line with those occurring in the native habitat of *C. frondosa* (Gianasi et al., 2015). Natural planktonic food (seston biomass in dry weight: 26.7-34.2 mg L^{-1}) in ambient running seawater was available to sea cucumbers during the study.

3.3.2 Small-scale preliminary experiments

A first set of trials was conducted to verify the assumption that current is a factor in the movement and distribution of *C. frondosa*. They were conducted in two tanks (80 cm × 74 cm × 26 cm) supplied with ambient seawater and covered by black tarps to prevent interference from external factors; these served as control and experimental tanks. At the onset of a trial, a group of ten sea cucumbers was placed simultaneously into each of the two tanks, using new individuals for each trial. Over 12 h of acclimation under static conditions, the sea cucumbers moved around freely and distributed themselves randomly in the experimental tanks. Afterward, a small submersible pump (Hydor Koralia Nano 240 Aquarium Circulation Pump) placed at one end of the tank was turned on to generate a nominal bottom current speed of ~27 cm s^{-1} toward the outflow at the other end (the pump in the control tank remained off). This nominal value, which was reduced gradually away from the source, is in the middle of the range determined to be suitable for feeding in *C. frondosa* (Holtz and MacDonald, 2009). Each trial was run for 48 h, after which the individuals were removed and the tanks were drained, cleaned and refilled; the whole process was repeated four consecutive times. To minimize tank effects, the control and experimental tanks were permuted after each replicate. Each trial was recorded via time lapse cameras sensitive to normal and infrared light (Brinno TLC 200 Pro and MAC 200 DN) set to take one picture of the entire arenas every 10 min and stitch them together into a video output. At night, an LED infrared illuminator (DC 12V) was automatically turned on as soon as the lights went off to allow recording of nocturnal activity. A grid dividing the tank into six equal rectangles was overlaid on the output videos and the number of line crossed by the sea cucumbers was used as an index of horizontal distance travelled along the bottom of the tank over 24 h. The time spent travelling and the time spent with tentacles deployed (Hamel and Mercier, 1998; Fankboner, 1978) over 24 h were determined in the presence and absence of flow.

3.3.3 Large-scale mesocosm experiments

Following the results of the small-scale trials, a set of experiments was conducted in a mesocosm mimicking natural conditions, consisting of a large flow-through raceway (8.25 m× 2.5 m× 0.85 m, length × width × depth). The bottom of the raceway was covered with 20-30 cm of gravel (1-3 cm diameter), with several scattered pebbles and small boulders (8-13 cm diameter). Gravelly and rocky substrates are reported to yield the highest densities of *C. frondosa* (Hamel and Mercier, 1996; So et al., 2010). A plate (5.5 m long) partially divided the tank longitudinally, thus creating a circular flume with unobstructed flow (Fig. 3.1). The two sections of the raceway on each side of the dividing plate, labeled A and B, were used alternately during the replicate trials to minimize any tank effect. Grid markings were made on the dividing plate and along the raceway, at 50-cm intervals, creating 11 equal areas on each side of the raceway and providing a reference for spatial analysis. The diameters of the inlet and outlet were 4.5 cm and 10 cm, respectively. Three nominal flow regimes at the water inlet (i.e. maximum flow measured using a hand-held Global Water FP211 probe) were used to create three treatments: weakly dynamic (≤ 20 cm s^{-1}), mildly dynamic (≤ 100 cm s^{-1}) and highly dynamic (≤ 200 cm s^{-1}). The three nominal flows generated an increasingly broad continuum of flow regimes across the experimental arena, all consistent with field values (Holtz and MacDonald, 2009). The near-bottom flows (speed and directionality) in the mesocosm were determined by pipetting fluorescein sodium salt (FSA, Sigma-Aldrich) 5 cm from the bottom and recording the movement of FSA with a camera. As the experimental arena is a raceway, a nearly laminar flow with minor turbulence was generated. The experimental design and the nominal flow regimes for each treatment are illustrated in Fig. 3.1.

At the beginning of each experiment in the morning (09:00 h), 100 sea cucumbers were evenly spread between areas 2 and 11 at a density of 16 individuals m^{-2} (Fig. 3.1). After 5 h of acclimation in static conditions, sufficient to allow sea cucumbers to attach to the substrate (Holtz and MacDonald, 2009), the water flow was turned on (14:00 h) (as described above). A time-lapse video camera (described previously) was mounted 3 m above the experimental raceway to render a full view of the arena and allow measurement of the locomotor and feeding behaviours, and spatial distribution of sea cucumbers. The three flow treatments were conducted randomly. To minimize the possibility of environmental effects, the 4 replicate treatments were conducted alternatingly in side A or B (2 replicates for each; Fig. 3.1). Every replicate run lasted four days. No individual was ever used for two successive trials. The water flow in the tank was determined not only in tanks without sea cucumbers (i.e. nominal flows), but also around individual sea cucumbers using the handheld flow probe at the various time points (1, 3, 6, 18, 48 and 96 h) during each replicate run.

3.3.3.1 Distribution and locomotor behaviour

Based on videos recorded, the distribution of sea cucumbers was determined at various time points (1, 3, 6, 18, 48 and 96 h). The proportion of sea cucumbers positioned under specific flow regimes in each treatment was analyzed over time.

Based on the videos, the proportion of moving individuals in the different flow treatments at various times was calculated as follows: proportion of moving individuals (%) = $N_{Mt}/N_T \times 100$, where N_{Mt} is the number of sea cucumbers that moved more than one body length within 1 h prior to time interval t, and N_T is the total number of sea cucumbers. From the above results, only 0-1% of sea cucumbers moved after 6 h in the mildly- and highly-dynamic treatments. Therefore, the proportions of moving individuals in various sectors of the experimental arena experiencing different flow regimes and the modes of locomotion were compared across treatments at times 1, 3, 6, and 96 h. In addition, the proportion of sea cucumbers in the feeding posture (with tentacles deployed) while using various locomotion modes was determined in the first experimental hour (a universally active phase across treatments).

The proportion of moving individuals in various sectors of the experimental arena experiencing different flow regimes over time was calculated for each treatment as: Proportion of moving individuals (%) = $N_{Ms}/N_{Ts} \times 100$, where N_{Ms} is the number of sea cucumbers that moved more than one body length within 1 h prior to the specific time intervals in sector s, and N_{Ts} is the total number of sea cucumbers in sector s.

3.3.3.2 Tentacle deployment and feeding rate

Based on videos, the proportion of sea cucumbers with tentacles deployed (i.e. deployed individuals) at the various time points (1, 3, 6, 18, 48 and 96 h) was calculated for each treatment as: Proportion of deployed individuals (%) = $N_{Ft}/N_T \times 100$, where N_{Ft} corresponds to the number of sea cucumbers with tentacles fully deployed in the water column at time t, and N_T is the total number of sea cucumbers. In addition, the proportion of deployed individuals in various sectors of the experimental arena experiencing different flow regimes over time was calculated for each treatment as: proportion of deployed individuals (%) = $N_{Fs}/N_{Ts} \times 100$, where N_{Fs} corresponds to the number of sea cucumbers with tentacles fully deployed in the water column (i.e. presumably feeding) in sector s at the various times, and N_{Ts} is the total number of the sea cucumbers in sector s.

Because the highly-dynamic treatment offered the broadest overall range of flow regimes (when considering the distribution of sea cucumbers in the whole mesocosm; see Fig. 3.1C), the relationship between tentacle insertion rates (TIRs; i.e. feeding rate) and flow speed was studied at the end of this treatment, when the spatial distribution of sea cucumbers had stabilized. TIR was defined as the number of tentacles inserted into the mouth per minute (insertions per minute) and is regarded as a useful indicator of food intake (Holtz and MacDonald, 2009; Singh et al., 1999). Four feeding sea cucumbers exposed to each of four main flow rates (10, 20, 30 and 40 cm s^{-1}) in each of the treatment replicates were recorded using an underwater camera (GoPro, Hero 4 Silver). The camera was placed close to the focal individual, using an extension pole, to record tentacle movements. Videos were analyzed to determine TIR and the orientation of the crown of oral tentacles relative to current at the various flow speeds. TIRs were determined using frame-by-frame analysis over 10-min intervals. Mean TIRs from a total of 16 individuals for each flow rate were thus obtained.

3.3.3.3 Cloacal respiration rate

Cloacal movement is regarded as an indicator of respiratory rate, i.e. water circulation in the respiratory tree where oxygenation occurs (Doyle and McNiell, 1964; Jaeckle and Strathmann, 2013; Gianasi et al., 2015). The cloacal movements of sea cucumbers occupying different locations in the tank (submitted to different water flow regimes) were recorded using the GoPro camera at the end of the highly-dynamic treatment to determine the variation of respiratory rates in response to different flow rates. This treatment and time point were selected for reasons stated previously. Cloacal respiratory rates were determined by counting the number of cloacal openings over 10-min intervals. Values of openings per minute were averaged for 16 individuals at each flow rate.

3.3.4 Statistical analysis

All data were tested for normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively. For the small-scale experiment, the average distance travelled, the time spent travelling and the time spent deployed were compared between the two treatments (presence and absence of flow) using *t*-tests. For the large-scale experiment, a two-way repeated-measures analysis of variance (RM-ANOVA) was used to test the proportion of moving individuals and the proportion of deployed individuals among different time points and flow treatments. When interaction between the factors was significant, each factor was analysed separately using one-way RM ANOVA, followed by a Bonferroni test. Significant differences between flow rates and the proportion of moving individuals, the proportion of deployed individuals, the locomotion modes, TIRs and cloacal movement were determined using one-way ANOVA followed by post hoc multiple comparisons with Tukey's method or *t*-test. Significance level in all tests was set at $P < 0.05$.

3.4 Results

3.4.1 Small-scale preliminary experiments

There were significant differences between treatments in both the time sea cucumbers spent travelling ($t = -3.57$, $df = 14$, $P = 0.003$) and the distance they travelled ($t = -4.56$, $df = 14$, $P < 0.001$) over 24 h, which both were less in the presence of flow (mean of 65 min and 91 cm, respectively) than in its absence (mean of 97 min and 140 cm; Fig. 3.2A, B). Inversely, the time spent with tentacles deployed over 24 h was significantly greater in the presence of flow (10 h) than in its absence (3 h) ($t = 11.42$, $df = 14$, $P < 0.001$; Fig. 3.2C).

3.4.2 Large-scale experiments

3.4.2.1 Distribution and locomotor behaviour

Overall, marked flow preference was determined and no aggregation behaviour was detected. The sea cucumbers typically spread in the experimental arena under all flows tested. They moved away from near-zero flow conditions over time under weak flow, whereas under mildly- and highly-dynamic flows, individuals consistently moved away from the strongest flow regimes >40 cm s^{-1}.

Specifically, in the weakly-dynamic treatment, the proportion of sea cucumbers located in areas with flows of <10 cm s^{-1} decreased from 91% at the beginning of the experiment (0 h) to 80% at the end of the experiment (96 h), whereas the proportion of sea cucumbers located in areas with flows of 10-20 cm s^{-1} increased from 9 to 20% (Fig. 3.3A). In the mildly-dynamic treatment, the proportion of sea cucumbers located in areas with flows of >80 cm s^{-1} decreased from 7 to 0% after only 3 h and no sea cucumbers were found in areas with flows >60 cm s^{-1} after 6 h (Fig. 3.3B). In the highly-dynamic treatment, the proportion of sea cucumbers located in areas with the strongest flows (>120 cm s^{-1}) decreased from 10 to 0% after only 1 h, whereas the proportion of sea cucumbers located in areas with the lowest flows (≤20 cm s^{-1}) increased from 41 to 63%. No sea cucumbers were found in areas with flows >80 cm s^{-1} after 6 h in the highly-dynamic treatment (Fig. 3.3C). At the end of the mildly- and highly-dynamic treatments (96 h), no sea cucumbers were found in areas with flows > 40 cm s^{-1}.

A two-way RM ANOVA showed that both treatment ($F_{2,6} = 43.44$, $P < 0.001$) and time ($F_{5,15} = 116.78$, $P < 0.001$) had a significant influence on the proportion of moving sea cucumbers, but also revealed a significant interaction between the two factors ($F_{10,30} = 19.746$, $P < 0.001$; Fig. 3.4). Independent analyses at each level (one-way RM ANOVA) showed that the proportion of moving sea cucumbers was significantly affected by treatment at all time points (Table S.3.1). During the first experimental hour, the proportion of moving sea cucumbers in the highly-dynamic treatment was significantly higher than in the weakly- and mildly-dynamic treatments (Bonferroni test, $P < 0.05$). From the third hour (3 h) to the end of the experiment (96 h), the proportion of moving sea cucumbers in the weakly-dynamic treatment was significantly higher than in the two other treatments ($P < 0.05$), and no significant differences were found between the mildly- and highly-dynamic treatments ($P > 0.05$). No significant differences in the proportion of moving individuals were found across time points in the weakly-dynamic treatment ($F_{5,15}$ = 1.07, P = 0.415), but significant differences over time were found in the mildly- ($F_{5,15}$ = 35.95, P < 0.001) and highly-dynamic treatments ($F_{5,15}$ = 78.93, P < 0.001). Specifically, the proportion of moving individuals was significantly greater at 1 h than at any other time in both treatments (Bonferroni test, P < 0.05), but no significant differences were found among 6, 18, 48 and 96 h (P > 0.05).

In the weakly-dynamic treatment, the proportion of moving sea cucumbers located in more dynamic areas (flows of 10-20 cm s^{-1}) was significantly greater than in areas with flow of <10 cm s^{-1} at 1 h and 96 h (Table S.3.2; Fig. 3.5A). In the mildly-dynamic treatment, the proportion of moving sea cucumbers was significantly affected by flow regimes at all time points except 96 h (Table S.3.2; Fig. 3.5B). During the first hour of the mildly-dynamic treatment, the proportions of moving sea cucumbers located in areas with flows of 61-80 and >80 cm s^{-1} were significantly greater than in other areas (Tukey's test, P < 0.05; Fig. 3.5B). In the highly-dynamic treatment, the proportion of moving individuals was also significantly affected by flow regimes at 1 h and 3 h (Table S.3.2; Fig. 3.5C). Movements at other times points were nearly null. During the first hour of the highly-dynamic treatment, the proportions of moving sea cucumbers located in areas with flow of 41-80, 81-120 and >120 cm s^{-1} were significantly greater than in other areas (Tukey's test, P < 0.05; Fig. 3.5C).

3.4.2.2 Tentacle deployment and feeding rate

A two-way RM ANOVA showed that both treatment ($F_{2,6}$ = 42.22, P < 0.001) and time ($F_{5,15}$ = 75.07, P < 0.001) had a significant influence on the proportion of deployed sea cucumbers, but also revealed a significant interaction between the two factors ($F_{10,30}$ =

10.52, $P < 0.001$; Fig. 3.6). The results of independent one-way RM ANOVA showed that the proportion of deployed sea cucumbers was significantly affected by treatment at all time points (Table S.3.3). From the beginning to the end of the experiment, the proportion of deployed sea cucumbers in the weakly-dynamic treatment remained significantly lower than in the mildly- and highly-dynamic treatments (Bonferroni test, $P < 0.05$). In addition, the proportion of deployed sea cucumbers varied significantly over time under all treatments (weakly-dynamic, $F_{5,15} = 10.80$, $P < 0.001$; mildly-dynamic, $F_{5,15} = 31.26$, $P < 0.001$; highly-dynamic, $F_{5,15} = 44.84$, $P < 0.001$). The proportion of sea cucumbers with tentacles deployed at 1 h was significantly lower than at all other times in all treatments (Bonferroni test, $P < 0.05$), and no significant differences were found among 6, 18, 48, and 96 h in all treatments ($P > 0.05$).

In the weakly-dynamic treatment, the proportion of deployed sea cucumbers located in areas with flows of 0-10 cm s^{-1} was significantly lower than in areas with flows of 10-20 cm s^{-1} at all time points (Table S.3.4; Fig. 3.7A). Only 11-18% of sea cucumbers located in areas with flows of <10 cm s^{-1} had tentacles deployed at all time points, compared with 49-60% of sea cucumbers located in areas with flows of 10-20 cm s^{-1}. In the mildly-dynamic treatment, the proportion of deployed sea cucumbers was significantly affected by flow regimes at all time points except at 1 h (Table S.3.4; Fig. 3.7B). The proportion of deployed sea cucumbers located in areas with flows of 21-40 cm s^{-1} was highest (31-85%) at all time points (Fig. 3.7B). In the highly-dynamic treatment, the proportion of deployed sea cucumbers was significantly affected by flow regimes at all time points (Table S.3.4; Fig. 3.7C). The proportion of deployed sea cucumbers located in areas with flows of 81-120 cm s^{-1} was lowest at 1 h (13 ± 10%), and the proportion of deployed sea cucumbers located in areas with flows of 21-40 cm s^{-1} was highest (49-90%) at all time points (Fig. 3.7C).

TIRs varied significantly among sea cucumbers positioned in different flow regimes across the experimental arena ($F_{3,60} = 15.50$, $P < 0.001$, Fig. 3.8A). TIRs increased from a low of 0.95 ± 0.09 insertions min^{-1} at flows of 10 cm s^{-1} to a high of 1.13 ± 0.06 insertions min^{-1} at flows of 40 cm s^{-1} (Fig. 3.8A).

3.4.2.3 Cloacal respiration rate

Cloacal movements measured during the highly-dynamic treatment did not differ significantly among sea cucumbers positioned in different flow regimes across the experimental arena ($F_{3,60} = 0.79$, $P = 0.503$; Fig. 3.8B). Nevertheless, individuals located in stronger flow regimes generally had higher respiration rates, which ranged from 1.95 ± 0.25 opening min^{-1} at a flow regime of 10 cm s^{-1} to 2.09 ± 0.27 opening min^{-1} at 40 cm s^{-1}.

3.4.3 Behavioural observations

3.4.3.1 Tentacle orientation

Videos and still pictures revealed that the crown of tentacles of the sea cucumbers had different orientations under different water flow regimes. The crown of tentacles of individuals located in areas with flows ≥40 cm s^{-1} tended to follow the direction of the current and was not fully deployed (Fig. 3.9A). The crown of tentacles of individuals located in areas with flows between 10 and 40 cm s^{-1} typically faced the current or were perpendicular to the direction of water flow (Fig. 3.9B, C). The crown of tentacles of individuals located in areas with flows＜10 cm s^{-1} was typically perpendicular to the direction of water flow (Fig. 3.9D). Overall, tentacles were only fully deployed at flows between 0 and 40 cm s^{-1} (Fig. 3.9B, C, and D).

3.4.3.2 Locomotion modes

Three different modes of locomotion were detected; namely, forward crawling (moving forward through contraction-extension and using podia on ventral surface; movie S1), active rolling (on the longest body axis without significant body shape change; movie S2), and passive rolling (carried by water movement; movie S3). Overall, 42 ± 10% of sea cucumbers that used crawling, 25 ± 19% that used active rolling, and 10 ± 6% that used passive rolling were found to extend their tentacles when moving. The movement speed of forward crawling and active rolling was similar, ranging from 0.2 to 0.9 m h^{-1}. However, the movement speed of passive rolling was 180-3000 m h^{-1}, which mainly depended on the water flow rate.

In the weakly-dynamic treatment of the large-scale experiments, 68-85% of moving sea cucumbers located in areas with flows of <10 cm s^{-1} were crawling, and 15-32% used active rolling or alternated crawling and active rolling at all time points. Between 38 and 75% of moving sea cucumbers located in areas with flows of 10-20 cm s^{-1} were crawling, and 25-62% used active rolling or alternated between crawling and active rolling at all time points. During the first experimental hour of the mildly-dynamic treatment, no sea cucumbers located in areas with flows of $\leq$40 cm s^{-1} used passive rolling, and 75-81% displayed forward crawling. However, 84 ± 16% of moving sea cucumbers located in areas with flows of >80 cm s^{-1} used passive rolling, and only 5 ± 4% displayed forward crawling. During the third experimental hour of the mildly-dynamic treatment, 58-75% of moving sea cucumbers located in areas with flows of 41-80 cm s^{-1} displayed passive rolling. During the first experimental hour of the highly-dynamic treatment, no sea cucumbers located in areas with flows of <40 cm s^{-1} displayed passive rolling, and 60-71% of them exhibited forward crawling. Inversely, 64-93% of moving sea cucumbers located in areas with flows of >80 cm s^{-1} displayed passive rolling, and only 2-6% displayed forward crawling.

3.5 Discussion

Forces imposed by moving water can dramatically influence the locomotor behaviour of marine organisms (Wildish and Kristmanson, 2005). Behavioural responses to flow include rheotaxis, which is a directed response to flow direction involving locomotion or muscular turning of body parts, and rheokinesis, which is a non-directed response causing random movement proportional to flow velocity (Wildish and Kristmanson, 2005). Relatively few suspension feeders have significant locomotor capabilities because most of them are sessile or tube-dwelling. Therefore, previous studies on suspension-feeders in response to flow mainly focused on their feeding behaviour and body/appendage orientation. For sea cucumbers, most behavioural studies have been conducted on deposit-feeding species and food availability was identified as the main factor driving movement (van Dam-Bates et al., 2016; Navarro et al., 2014; Slater et al., 2011). In the present study, water flow was shown to play an important role in eliciting displacement in *C. frondosa*, a free-moving suspension feeder. Individuals tended to move away from near-zero flow conditions over time and avoid the strongest flow areas >40 cm s^{-1}. It can be suggested that in weakly dynamic environments, *C. frondosa* sought stronger flows to find more suitable grounds for passive suspension feeding (i.e. conditions likely to supply phytoplankton and other suspended particles). However, in more dynamic environments, *C. frondosa* fled flow regimes above a certain strength, presumably to minimize drag that would impede suspension feeding and might induce dislodgement. Pan et al. (2015) observed that the sea cucumber *Apostichopus japonicus*, which feeds on deposited organic matter, moved downstream under high flows of 30 cm s^{-1}, and moved either downstream or upstream under flows of 15 cm s^{-1}. For deposit feeders, displacement from dynamic to weaker flows is chiefly about reducing drag effects. For suspension feeders such as *C. frondosa*, it is also and perhaps more importantly, a question of capture/feeding efficiency.

Water flow was determined to drive the movement but also the activity level of *C. frondosa*. In the small-scale experiments conducted under weak flow, the time spent travelling and the distance travelled were less in the presence of flow than in its absence. Similarly, in the large-scale mesocosm experiments, 12-15% of the sea cucumbers kept moving throughout the 96 h of the trial in the weakly-dynamic treatment, suggesting that the available flow regimes were not suitable or optimal. Conversely, few sea cucumbers were moving after 6 h in the mildly- and highly-dynamic treatments, suggesting they had found suitable flow conditions within that interval. This finding contrasts with the locomotion of deposit-feeders such as *A. japonicus*, in which water current consistently acts as a stimulating factor, causing an increase in movement (Pan et al., 2015). It emerges that *C. frondosa* can actively move away from suboptimal flow regimes (that are either too weak or too strong) and towards more suitable intermediate conditions (10-40 cm s^{-1}). Another study conducted on a suspension feeder, the polychaete *Manayunkia speciosa*, showed that it moved from high flow to low flow to increase survivorship (Malakauskas et al., 2013).

An unexpected finding of the present study was that *C. frondosa* exhibits different modes of locomotion, i.e. not only the typical forward crawling, but also active rolling (on the side), and passive rolling, in response to different flow regimes. Forward crawling remained the most frequently observed locomotor behaviour under weak flow regimes. It also commonly occurred in areas with flows <40 cm s^{-1} in the more dynamic treatments, suggesting that it is widely used by *C. frondosa* when flow conditions are below that threshold (40 cm s^{-1}). Low incidence of active rolling (moving sideways while remaining partly attached to the bottom) consistently occurred under all the flows tested up to 120 cm s^{-1}, whereas passive rolling occurred strictly in areas with flows >40 cm s^{-1}. There are two main differences between these two modes of locomotion. First, passive rolling relies partly on the power of flow, whereas active rolling is independent of flow. Second, passive rolling involves a change of shape (ballooning) to increase buoyancy (Hamel et al., 2019), whereas active rolling does not. In the highly-dynamic treatment, the proportion of sea cucumbers located in areas with the lowest flows (≤20 cm s^{-1}) increased from 41 to 63%. Based on the video records, it appears that sea cucumbers initially located in flows >40 cm s^{-1} were passively carried (rolling) to the opposite area. However, sea cucumbers in the weakest flows actively crawled towards areas with flows between 21 and 40 cm s^{-1}, suggesting relocation to a preferred habitat. In other words, passive rolling seems to be used by sea cucumbers to move from strong to weak flow areas, after which they may move by crawling or active rolling towards definitive optimal locations. This diversity in locomotor behaviour is currently being investigated, as it would suggest that sea cucumbers can move much faster and over greater distances than typically assumed (Hamel et al., 2019), and not only during escape responses to a predator (Margolin, 1976). These modes of locomotion may enhance their responsiveness to environmental stimulation and favour broader distribution ranges and massive relocation when required, even at the adult stage.

The present study highlighted a close relationship between water flow and feeding in *C. frondosa*, based on tentacle deployment, orientation and insertion rates. Under flows <20 cm s^{-1}, the crown of tentacles typically faced the current, which may help *C. frondosa* capture more food particles per unit of time (increase efficiency). More typically sessile suspension-feeding benthic organisms, such as scallops (Sakurai and Seto, 2000), sea fans (Leversee, 1976), sea anemones (Anthony, 1997), and crinoids (Leonard et al., 1988), also orient themselves or their feeding appendages relative to currents in order to enhance their capture capacities and exploit their food supply optimally. Here, in flows ≥40 cm s^{-1}, *C. frondosa* oriented its tentacles to follow the direction of the current and did not deploy them fully, which was reported in a previous study (Holtz and MacDonald, 2009), likely to alleviate the deformation of the tentacles caused by the increase in flow. Changes in orientation in response to variable water currents has also been observed in a few elasipodid species, including *Peniagone japonica* distributed in deep bays in Japan (Okada and Ohta, 1993). Moreover, most individuals of the species *Irpa abyssicola* filmed on the bottom of Hayes Deep (2700 m depth, Norwegian and Greenland Seas) faced into the current, whereas on the slope they mainly oriented with the current (Gebruk et al., 2003). To a large extent, tentacle deployment in *C. frondosa* can be associated with feeding behaviour, although the tentacles may be extended without any movements towards the mouth for several hours (Hamel and Mercier, 1998), indicating that its serves other purposes as well. It is possible that *C. frondosa* uses its tentacles to detect the direction and strength of water flow and reacts accordingly by adjusting its body orientation or by moving. Optimal location and orientation relative to flow presumably allow this planktivorous species to maximize food capture and minimize energetic costs.

Cucumaria frondosa, like other passive suspension feeders, depends entirely on the ambient flow to supply food particles to its feeding appendages (Singh et al., 1998; Leichter and Witman, 1997). This may explain why the time spent with tentacles deployed in *C. frondosa* was greater in the presence of flow than in the absence of flow. However, in the mildly- and highly-dynamic treatments, the proportion of sea cucumbers with tentacles deployed was relatively low (8-12%) especially in areas with flows >80 cm s^{-1}. This may be related to an increasing difficulty in deploying tentacles. The cessation of feeding (tentacle retraction) by dendrochirotids in strong currents may occur to avoid damage or may be due to a failure to maintain efficient particle capture (McKenzie, 1987). In the two more dynamic treatments, the proportion of deployed sea cucumbers located in areas with flows of 21-40 cm s^{-1} was greater than in all other areas (with different flows) at given time points, suggesting that this optimal range allows *C. frondosa* to fully deploy its tentacles while providing a sufficient supply of particulate food.

Beyond the proportion of individuals with deployed tentacles, the TIR is a more specific indicator of feeding activity (Holtz and MacDonald, 2009; Singh et al., 1999). Holtz and MacDonald (2009) used a small laboratory flume holding five individuals to determine that TIRs were not affected by water flows <40 cm s^{-1} and were reduced when flow was above a threshold velocity of ~55 cm s^{-1}. In contrast, the present study found that TIRs increased with increasing water flow between 0 and 40 cm s^{-1} in the larger life-size tank setup, which held 100 individuals and may have provided *C. frondosa* with an environment that is closer to natural conditions. As the water flow increases, suspension-feeders will more easily capture food particles because more particles come into contact with their feeding structures (Shimeta and Jumars, 1991). This could mean that *C. frondosa* needs less time to capture food particles before the tentacles are inserted into the mouth with increasing water flow, up to a certain threshold, as discussed previously. From results obtained here combining the proportion of deployed sea cucumbers and TIR, the optimum water flow for *C. frondosa* to feed lies between 21 and 40 cm s^{-1}.

Sea cucumbers respire mainly by two mechanisms; one is by taking up oxygen across the general body surface (Hopcroft et al., 1985), and the other is by drawing and expelling water through the cloaca, in and out of the respiratory tree (Woodby et al., 2000). In the present study, cloacal movement (respiration rate) was not related to the water flow. In contrast, the need for more frequent renewal of water in the respiratory tree is a good indicator of stress in sea cucumbers (Shiell, 2006; Gianasi et al., 2015). Therefore, experimental results of the present study have shown that *C. frondosa* was stress-free at flows ≤40 cm s^{-1} and was otherwise well adapted to a wide range of water flows.

Ultimately, the final spatial distribution displayed by *C. frondosa* in the mesocosm reflected the fact that, as a passive suspension feeder, it must seek optimal water flow to subsist. In areas where the water flow was zero or close to zero, a proportion of sea cucumbers was still moving around even after four days, presumably in search of better conditions. In contrast, strong flow may impede tentacle deployment in *C. frondosa*, as outlined previously, and it also requires more energy to adhere to the bottom as suggested in *Apostichopus japonicus* (Pan et al., 2015). Here, between 50 and 93% of sea cucumbers were moving during the first hour spent in the mildly-dynamic and highly-dynamic treatments, which generated locally high flow regimes of >80 cm s^{-1} and >120 cm s^{-1}, respectively. After 96 h, no sea cucumbers were found in areas with flows >40 cm s^{-1}. However, a previous study revealed that *C. frondosa* can colonize areas of water flow >40 cm s^{-1} in the field (Holtz and MacDonald, 2009). There are a number of possible explanations for this. The high water velocity in the field may have been transient; for example, the sea cucumbers at Jamieson Island were exposed to high velocities of 99-130 cm s^{-1} only 17% of the time (Holtz and MacDonald, 2009). Flow velocities in the field are more variable than in the laboratory, changing with tides and oceanographic conditions (e.g. storms), and the seafloor is more complex (rock, crevices, macrophytes) offering zones with moderate current even under high flows. Furthermore, gregarious or clumping behaviour in the wild can enhance the resistance of organisms to dynamic environments, as exemplified by a study in the sea cucumbers *Thyone aurea* and *Pentacta doliolum* on the west coast of South Africa (Barkai, 1991).

In conclusion, the present study highlighted significant shifts in the locomotor and feeding behaviours of a suspension-feeding sea cucumber in response to water flow. The findings not only provide novel information on the ecology of suspension feeders, but will help our understanding of the broad yet patchy distribution of sea cucumbers in various environments, and will be of strategic value to the management of commercial fisheries. In addition, knowledge of the preferred conditions of water flow in suspension-feeding sea cucumbers will inform the design of holding conditions in the context of captive breeding, integrated multi-trophic aquaculture and sea ranching.

3.6 Acknowledgements

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3.7 References

Anderson, S.C., Flemming, J.M., Watson, R., Lotze, H.K., 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries. 12, 317-339.

Anthony, K.R., 1997. Prey capture by the sea anemone *Metridium senile* (L.): effects of body size, flow regime, and upstream neighbors. The Biological Bulletin. 192, 73-86.

Barkai, A., 1991. The effect of water movement on the distribution and interaction of three holothurian species on the South African west coast. Journal of Experimental Marine Biology and Ecology. 153, 241-254.

Costelloe, J., Keegan, B.F., 1984a. Littoral and Benthic Investigations on the West Coast of Ireland: XIX. Synonomy, Diagnostic Morphology, Distribution and Life-Style of *Aslia lefevrei* (Barrois 1882)(Holothuroidea: Echinodermata), Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science. 84, 29-34.

Costelloe, J., Keegan, B.F., 1984b. Feeding and related morphological structures in the dendrochirote *Aslia lefevrei* (Holothuroidea: Echinodermata). Marine Biology. 84, 135-142.

Dissanayake, D., Stefansson, G., 2012. Habitat preference of sea cucumbers: *Holothuria atra* and *Holothuria edulis* in the coastal waters of Sri Lanka. Journal of the Marine Biological Association of the United Kingdom. 92, 581-590.

Doyle, W.L., McNiell, G.F., 1964. The fine structure of the respiratory tree in *Cucumaria*. Journal of Cell Science. 3, 7-11.

Fankboner, P.V., 1978. Suspension-feeding mechanisms of the armoured sea cucumber *Psolus chitinoides* Clark. Journal of Experimental Marine Biology and Ecology. 31, 11-25.

Gebruk, A.V., Bluhm, H., Soltwedel, T., Thiel, H., 2003. A re-description of the enigmatic deep-sea holothurian *Irpa abyssicola* (Elpidiidae, Elasipodida) with remotely operated vehicle observations on benthic organisms in the Norwegian-Greenland Basin. Sarsia: North Atlantic Marine Science. 88, 49-54.

Gianasi, B.L., Verkaik, K., Hamel, J.-F., Mercier, A., 2015. Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. PLoS ONE. 10, e0127884.

Hamel, J.-F., Mercier, A., 1995. Spawning of the sea cucumber *Cucumaria frondosa* in the St Lawrence Estuary, eastern Canada. SPC Beche-de-mer Information Bulletin. 7, 12-18.

Hamel, J.-F., Mercier, A., 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences. 53, 253-271.

Hamel, J.-F., Mercier, A., 1998. Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. Canadian Journal of Zoology. 76, 1194-1198.

Hamel, J.-F., Mercier, A., 2008a. Population status, fisheries and trade of sea cucumbers in temperate areas of the Northern Hemisphere, in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 257-292.

Hamel, J.-F., Mercier, A., 2008b. Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 293-306.

Hamel, J.-F., Sun, J., Gianasi, B.L., Montgomery, E.M., Kenchington, E.L., Burel, B., Rowe, S., Winger, P.D., Mercier, A., 2019. Active buoyancy adjustment increases dispersal potential in benthic marine animals. Journal of Animal Ecology. 88, 820-832.

Holtz, E., MacDonald, B.A., 2009. Feeding behaviour of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the laboratory and the field: relationships between tentacle insertion rate, flow speed, and ingestion. Marine Biology. 156, 1389-1398.

Holyoak, M., Casagrandi, R., Nathan, R., Revilla, E., Spiegel, O., 2008. Trends and missing parts in the study of movement ecology. Proceedings of the National Academy of Sciences. 105, 19060-19065.

Hopcroft, R.R., Ward, D.B., Roff, J.C., 1985. The relative significance of body surface and cloacal respiration in *Psolus fabricii* (Holothuroidea: Dendrochirotida). Canadian Journal of Zoology. 63, 2878-2881.

Jaeckle, W., Strathmann, R., 2013. The anus as a second mouth: anal suspension feeding by an oral deposit-feeding sea cucumber. Invertebrate Biology. 132, 62-68.

Jordan, A.J., 1972. On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothurioidea) at Lamoine Beach, Maine. PhD thesis. University of Maine, Orono.

Klugh, A.B., 1924. Factors controlling the biota of tide-pools. Ecology. 5, 192-196.

Leichter, J.J., Witman, J.D., 1997. Water flow over subtidal rock walls: relation to distributions and growth rates of sessile suspension feeders in the Gulf of Maine Water flow and growth rates. Journal of Experimental Marine Biology and Ecology. 209, 293-307.

Leonard, A., Strickler, J., Holland, N., 1988. Effects of current speed on filtration during suspension feeding in *Oligometra serripinna* (Echinodermata: Crinoidea). Marine Biology. 97, 111-125.

Leversee, G.J., 1976. Flow and feeding in fan-shaped colonies of the gorgonian coral, *Leptogorgia*. The Biological Bulletin. 151, 344-356.

Malakauskas, D.M., Willson, S.J., Wilzbach, M.A., Som, N.A., 2013. Flow variation and substrate type affect dislodgement of the freshwater polychaete, *Manayunkia speciosa*. Freshwater Science. 32, 862-873.

Margolin, A.S., 1976. Swimming of the sea cucumber *Parastichopus californicus* (Stimpson) in response to sea stars. Ophelia. 15, 105-114.

Massin, C., 1982. Effects of feeding on the environment: Holothuroidea. in: Jangoux, M., Lawrence, J. (Eds.), Echinoderm Nutrition. AA Balkema, Rotterdam, Netherlands, pp. 494-497.

McKenzie, J.D., 1987. The ultrastructure of the tentacles of eleven species of dendrochirote holothurians studied with special reference to the surface coats and papillae. Cell and Tissue Research. 248, 187-199.

McKenzie, J.D., 1991. The taxonomy and natural history of north European dendrochirote holothurians (Echinodermata). Journal of Natural History. 25, 123-171.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology. 239, 125-156.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 2000a. Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*. Journal of Experimental Marine Biology and Ecology. 249, 89-110.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 2000b. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia. 440, 81-100.

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., Smouse, P.E., 2008. A movement ecology paradigm for unifying organismal movement research. Proceedings of the National Academy of Sciences. 105, 19052-19059.

Navarro, P., García-Sanz, S., Barrio, J., Tuya, F., 2013. Feeding and movement patterns of the sea cucumber *Holothuria sanctori*. Marine Biology. 160, 2957-2966.

Navarro, P.G., García-Sanz, S., Tuya, F., 2014. Contrasting displacement of the sea cucumber *Holothuria arguinensis* between adjacent nearshore habitats. Journal of Experimental Marine Biology and Ecology. 453, 123-130.

Okada, H., Ohta, S., 1993. Photographic evidence of variable bottom-current activity in the Suruga and Sagami Bays, central Japan. Sedimentary Geology. 82, 221-237.

Pan, Y., Zhang, L., Lin, C., Sun, J., Kan, R., Yang, H., 2015. Influence of flow velocity on motor behavior of sea cucumber *Apostichopus japonicus*. Physiology & Behavior. 144, 52-59.

Purcell, S.W., Conand, C., Uthicke, S., Byrne, M., 2016. Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review. 54, 367-386.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M.V., Lovatelli, A., Uthicke, S., 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries. 14, 34-59.

Sakurai, I., Seto, M., 2000. Movement and orientation of the Japanese scallop *Patinopecten yessoensis* (Jay) in response to water flow. Aquaculture. 181, 269-279.

Shiell, G.R., 2006. Effect of invasive tagging on the activity of *Holothuria whitmaei* [Echinodermata: Holothuroidea]: a suitable mark-recapture method for short-term field studies of holothurian behaviour. Marine and Freshwater Behaviour and Physiology. 39, 153-162.

Shimeta, J., Jumars, P.A., 1991. Physical mechanisms and rates of particle capture by suspension-feeders. Oceanography and Marine Biology: An Annual Review. 29, l-257.

Singh, R., MacDonald, B.A., Lawton, P., Thomas, M.L., 1998. Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. Canadian Journal of Zoology. 76, 1842-1849.

Singh, R., MacDonald, B.A., Thomas, M.L., Lawton, P., 1999. Patterns of seasonal and tidal feeding activity in the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the Bay of Fundy, Canada. Marine Ecology Progress Series. 187, 133-145.

Slater, M.J., Jeffs, A.G., Sewell, M.A., 2011. Organically selective movement and deposit-feeding in juvenile sea cucumber, *Australostichopus mollis* determined in situ and in the laboratory. Journal of Experimental Marine Biology and Ecology. 409, 315-323.

So, J.J., Hamel, J.-F., Mercier, A., 2010. Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology. 17, 473-484.

Uthicke, S., 2001. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology. 265, 153-170.

van Dam-Bates, P., Curtis, D.L., Cowen, L.L., Cross, S.F., Pearce, C.M., 2016. Assessing movement of the California sea cucumber *Parastichopus californicus* in response to organically enriched areas typical of aquaculture sites. Aquaculture Environment Interactions. 8, 67-76.

Wildish, D., Kristmanson, D., 2005. Benthic suspension feeders and flow. Cambridge University Press, Cambridge, United Kingdom.

Woodby, D., Smiley, S., Larson, R., 2000. Depth and habitat distribution of *Parastichopus californicus* near Sitka, Alaska. Alaska Fishery Research Bulletin. 7, 22-32.

Young, C., Chia, F.-S., 1982. Factors controlling spatial distribution of the sea cucumber *Psolus chitonoides*: Settling and post-settling behaviour. Marine Biology. 69, 195-205.

3.8 Figures

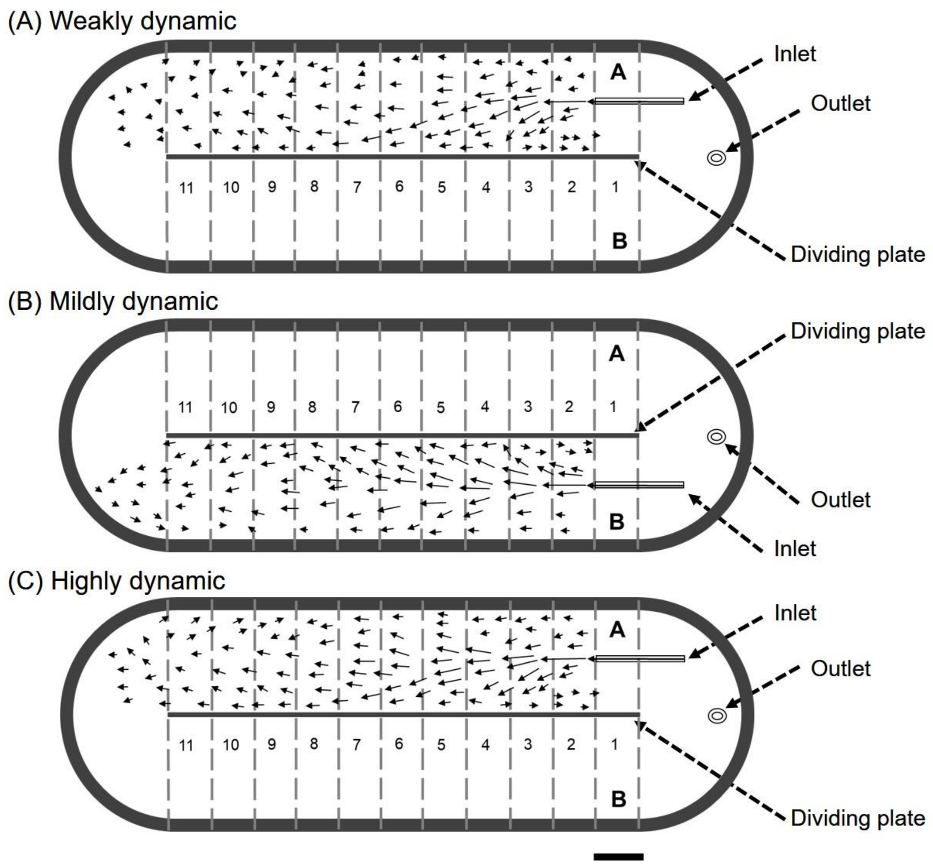

Figure 3.1 Top view of the experimental raceway (8.25 m × 2.5 m× 0.85 m, length × width × depth) used in the large-scale experiments. (A) Weakly dynamic (20 cm s^{-1}), (B) mildly dynamic (100 cm s^{-1}), and (C) highly dynamic (200 cm s^{-1}) treatments. The vectors show the direction and strength of the nominal flow in each treatment. The scale bar at the bottom represents 10 cm s^{-1} in (A), 50 cm s^{-1} in (B) and 100 cm s^{-1} in (C). The black numbers identify 11 tank sections that provided a reference for spatial analysis. Side A or B was used alternately in the replicates of each treatment (shown here alternating across the panels only to illustrate the position of the inlet on both sides).

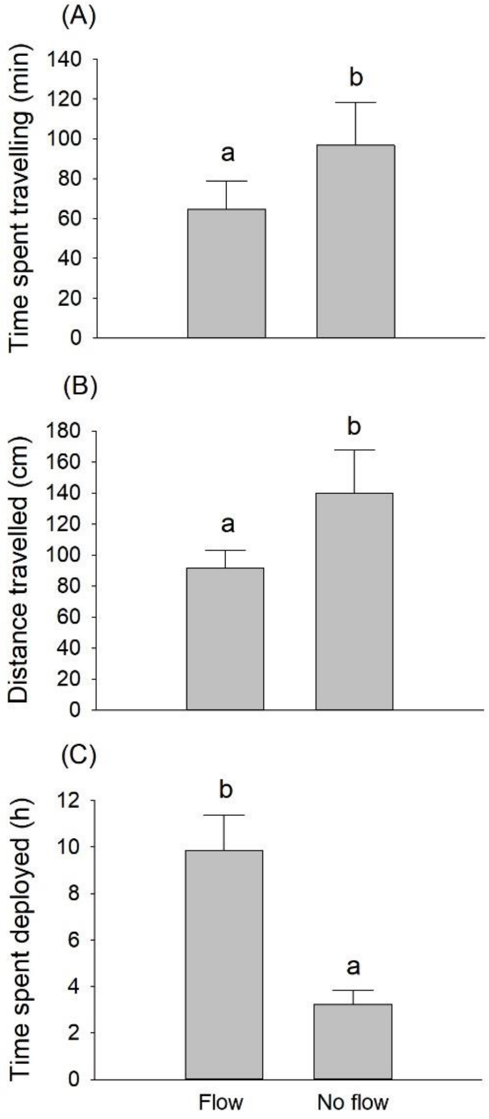

Figure 3.2 Feeding and locomotion behaviour of *C. frondosa* with and without flow. Effect of the presence/absence of flow on (A) time spent travelling, (B) horizontal distance travelled, and (C) time spent with tentacles deployed over 24 h by *C. frondosa*. Data are shown as mean ± s.d. for 10 individuals in each treatment (n = 4 replicate runs per treatment). Means with different letters are significantly different (*t*-test, $P < 0.05$).

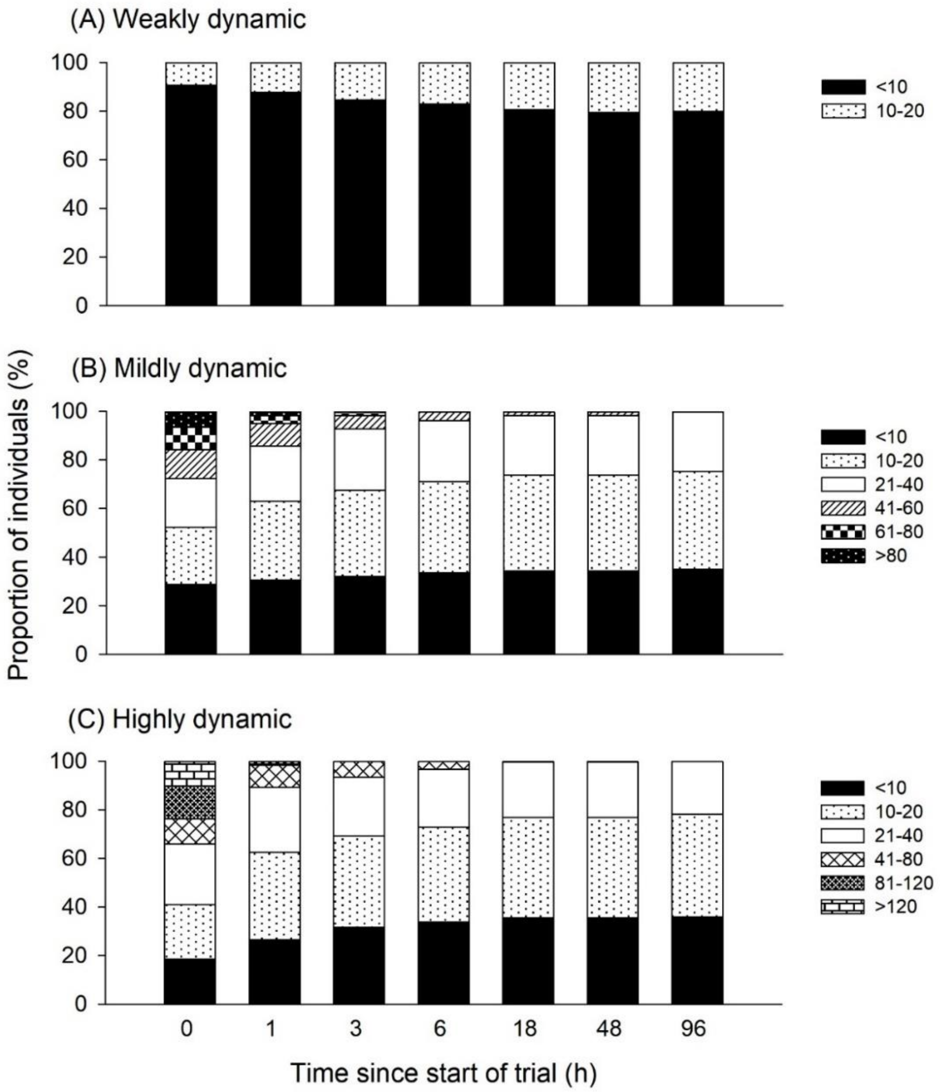

Figure 3.3 Distribution of 100 individuals of *C. frondosa* in various sectors of the experimental arena experiencing different flow regimes (from 0-10 to 120-200 cm s^{-1}) over time (0, 1, 3, 6, 18, 48, and 96 h). (A) Weakly dynamic (20 cm s^{-1}), (B) mildly dynamic (100 cm s^{-1}), and (C) highly dynamic (200 cm s^{-1}) treatments. Data are shown as means (n = 4).

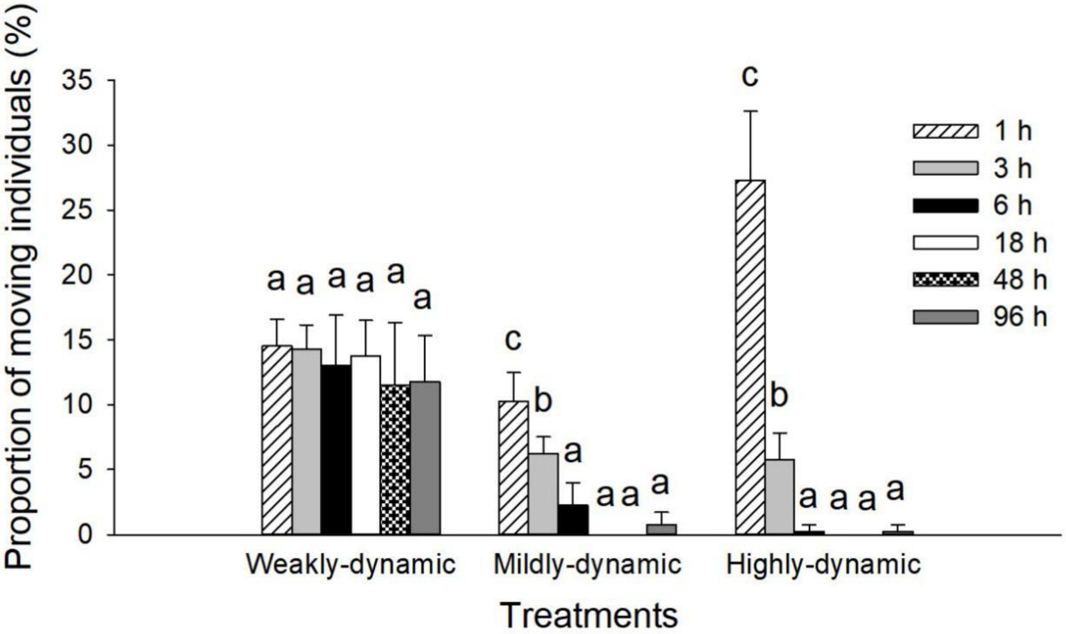

Figure 3.4 Percentage of individuals of *C. frondosa* that were scored as moving at the different time points (1, 3, 6, 18, 48, and 96 h) under the three treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}). Data are shown as mean ± s.d. for 100 individuals in each treatment (n = 4 replicate runs per treatment). Different letters highlight significant differences between different time points within treatments (two-way RM ANOVA, $P < 0.05$).

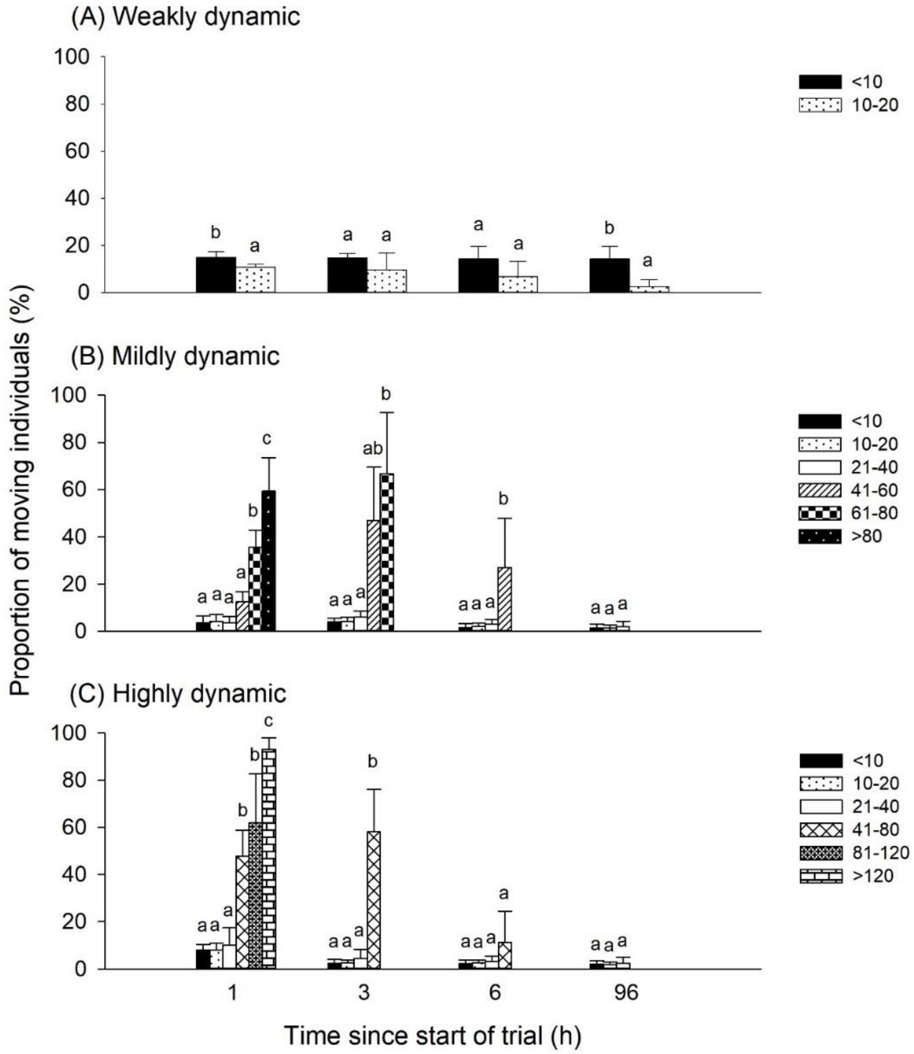

Figure 3.5 Percentage of individuals of *C. frondosa* that were scored as moving in various sectors of the experimental arena experiencing different flow regimes (from 0-10 to 120-200 cm s^{-1}) over time (1, 3, 6, and 96 h). (A) Weakly dynamic (20 cm s^{-1}), (B) mildly dynamic (100 cm s^{-1}), and (C) highly dynamic (200 cm s^{-1}) treatments. Data are shown as means ± s.d. (n = 4). Means with different letters are significantly different (ANOVA or *t*-test, $P < 0.05$).

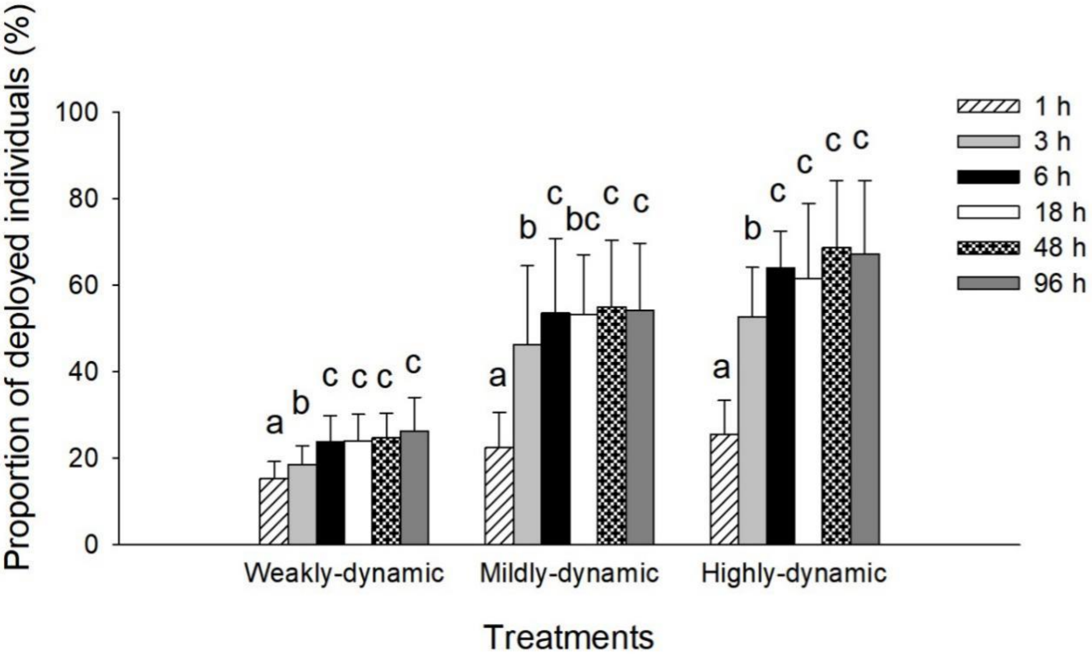

Figure 3.6 Percentage of individuals of *C. frondosa* with tentacles deployed at various time points (1, 3, 6, 18, 48, and 96 h) under different treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}). Data are shown as means ± s.d. for 100 individuals in each treatment (n = 4 replicate runs per treatment) with standard deviation. Means with different letters show significant differences between time points within treatments (two-way RM-ANOVA, $P < 0.05$).

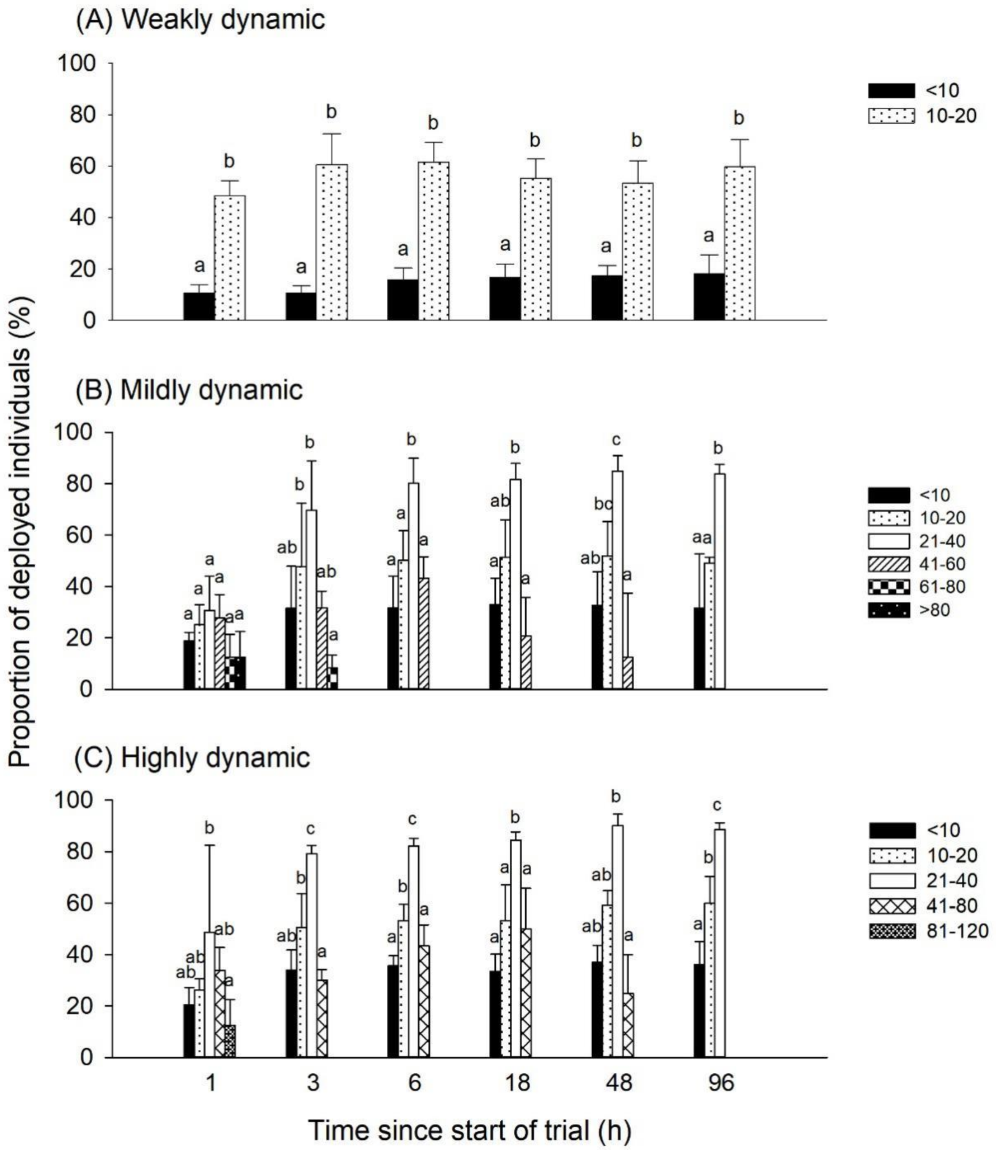

Figure 3.7 Percentage of individuals of *C. frondosa* with tentacles deployed in various sectors of the experimental arena experiencing different flow regimes (from 0-10 to 120-200 cm s^{-1}) over time. (A) Weakly dynamic (20 cm s^{-1}), (B) mildly dynamic (100 cm s^{-1}), and (C) highly dynamic (200 cm s^{-1}) treatments. Data are shown as means ± s.d. (n = 4). Means with different letters are significantly different (one-way ANOVA or *t*-test, $P < 0.05$).

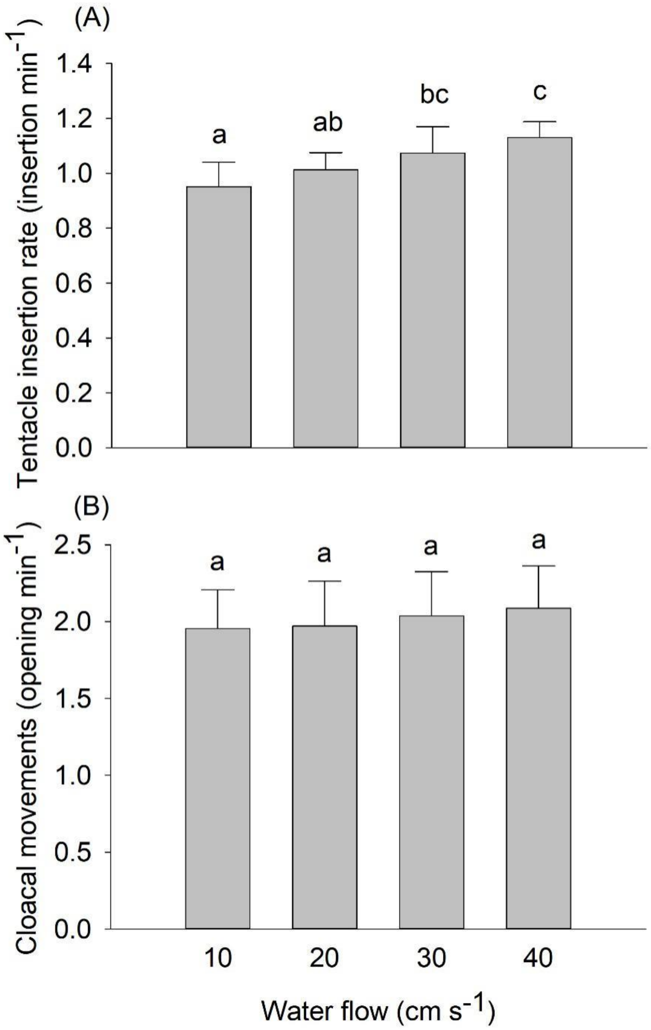

Figure 3.8 Effect of flow on the feeding and cloacal (respirator) behaviours of *C. frondosa*. Tentacle insertion rates (A) and cloacal movements (B) under different flow regimes. Data are shown as mean with standard deviation (n = 16 individuals per flow regime). Means with different letters are significantly different (ANOVA, $P < 0.05$).

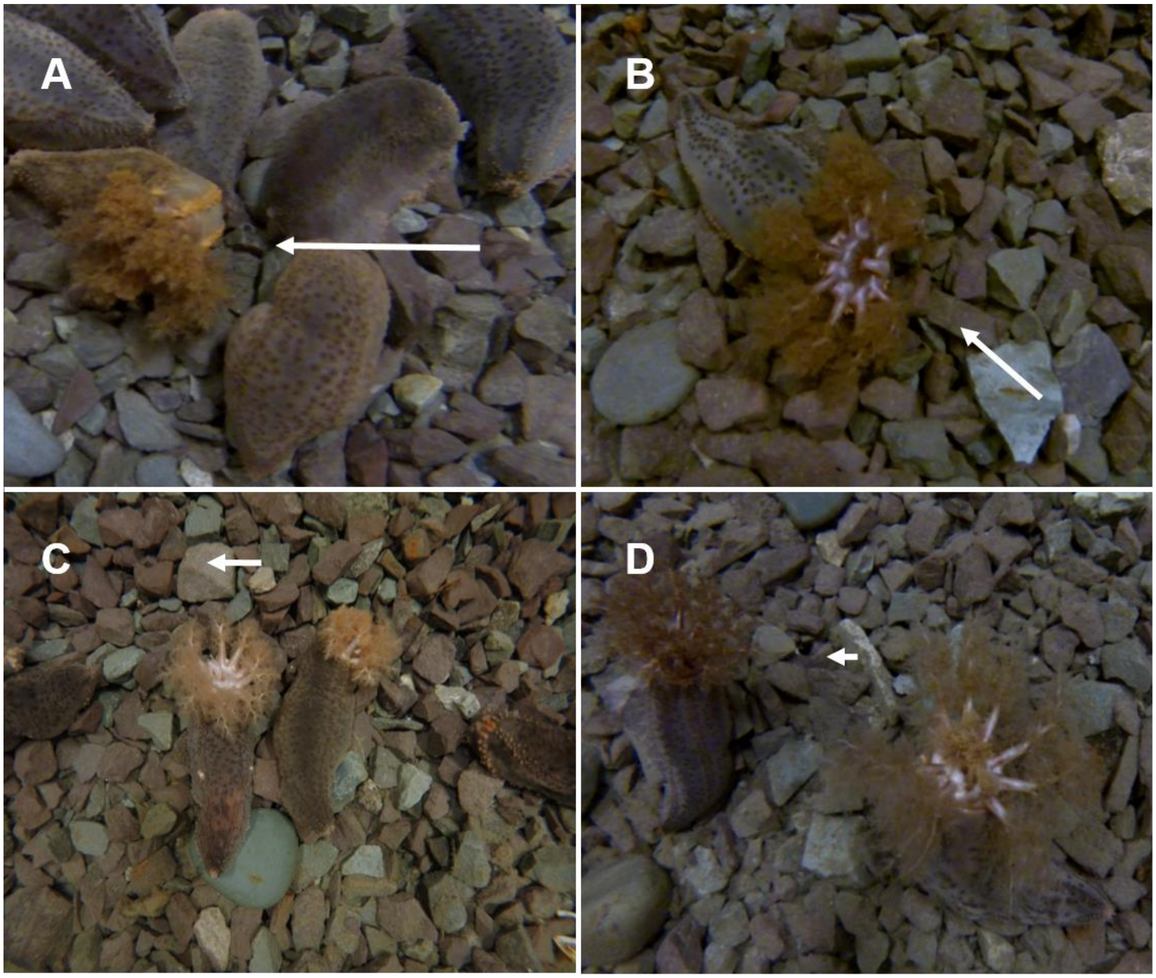

Figure 3.9 Orientation of the crown of tentacles of *C. frondosa* under different flow regimes. The arrow shows the direction of the flow and its length is proportional to the flow speed. (A) The crown of tentacles follows the direction of the flow at ≥40 cm s^{-1}. (B) The crown of tentacles directly faces the direction of the flow at 20 cm s^{-1}. (C) The crown of tentacles is perpendicular to the direction of the flow at 10 cm s^{-1}. (D) The crown of tentacles is perpendicular to the direction of the flow at <10 cm s^{-1}.

3.9 Supplementary Material

Table S.3.1 Statistical comparison of the proportion of moving sea cucumbers among the three flow treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}) at determined time points, using one-way repeated-measures ANOVA

Time since start of trial (h)	***F***	***df***	***P***
1	19.20	2	0.002
3	19.98	2	0.002
6	33.36	2	0.001
18	99.73	2	<0.001
48	23.00	2	0.002
96	28.70	2	0.001

Table S.3.2 Statistical comparison of the proportion of moving sea cucumbers among the 2-6 sectors of the experimental arena (experiencing different flow regimes) at selected time points in each of the flow treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}), using *t*-test or one-way ANOVA

Flow treatments	**Time since start of trial (h)**	***F(t)***	***df***	***P***
Weakly dynamic	1	3.03	6	0.023
	3	1.42	6	0.206
	6	1.79	6	0.124
	96	3.89	6	0.008
Mildly dynamic	1	44.00	5	<0.001
	3	6.29	4	0.004
	6	5.62	3	0.012
	96	0.17	2	0.850
Highly dynamic	1	47.35	5	<0.001
	3	35.40	3	<0.001
	6	1.65	3	0.231
	96	0.07	2	0.935

Table S.3.3 Statistical comparison of the proportion of deployed sea cucumbers among the three flow treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}) at selected time points, using one-way repeated-measures ANOVA

Time since start of trial (h)	***F***	***df***	***P***
1	17.62	2	0.003
3	24.20	2	0.001
6	37.16	2	<0.001
18	33.03	2	0.001
48	41.86	2	<0.001
96	31.51	2	0.001

Table S.3.4 Statistical comparison of the proportion of deployed sea cucumbers among 2-6 sectors of the experimental arena (experiencing different flow regimes) at selected time points for each of the flow treatments (weakly, 20 cm s^{-1}; mildly, 100 cm s^{-1}; and highly dynamic, 200 cm s^{-1}), using *t*-test or one-way ANOVA

Flow treatments	**Time since start of trial (h)**	***F(t)***	***df***	***P***
Weakly dynamic	1	-11.58	6	<0.001
	3	-8.15	6	<0.001
	6	-10.31	6	<0.001
	18	-8.43	6	<0.001
	48	-7.46	6	<0.001
	96	-6.45	6	0.001
Mildly dynamic	1	0.93	5	0.485
	3	6.57	4	0.003
	6	15.32	3	<0.001
	18	11.44	3	0.001
	48	15.09	3	<0.001
	96	18.18	2	0.001
Highly dynamic	1	3.26	4	0.041
	3	29.97	3	<0.001
	6	50.88	3	<0.001
	18	2.03	3	0.015
	48	5.04	3	0.017
	96	42.15	2	<0.001

Movie S.3.1 Illustration of forward crawling (arrow)

Movie S.3.2 Illustration of active rolling (arrow)

Movie S.3.3 Illustration of passive rolling (arrows)

All the movies are available:

http://jeb.biologists.org/lookup/doi/10.1242/jeb.189597.supplemental

Chapter 4. Effect of light, phytoplankton, substrate types and colour on locomotion, feeding behaviour and microhabitat selection in the sea cucumber *Cucumaria frondosa* [3]

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[3] A version of this manuscript is currently under review in Aquaculture.

4.1 Abstract

While the suspension-feeding sea cucumber *Cucumaria frondosa* is commercially exploited in the North Atlantic and is considered to have potential for integrated aquaculture, the impact of environmental conditions on its behaviour and population structure remains incompletely understood. The present study showed that adults are not photosensitive; they do not exhibit any preference for either illuminated or shaded areas. Within each photoperiod treatment, analyses of the daily activity cycle revealed that the proportion of individuals with feeding tentacles deployed did not change over time; however, the proportion of moving individuals increased in the dark phase under the 12 h light/12 dark regime, but remained relatively constant under continuous light or darkness. Hence, from an aquaculture perspective, long days (i.e. summer photoperiod) might decrease the time spent moving in favour of feeding, potentially shifting the energy budget towards faster growth. Variations in phytoplankton concentration modulated the deployment of tentacles but did not trigger any displacement toward the food source, indicating that feeding responds directly to the presence of food. Moreover, *C. frondosa* exhibited a clear preference for substrates composed of bare rocks and rocks with coralline algae and displayed a weak preference for darker substrate backgrounds. Together, these findings highlight how some key environmental factors can govern the feeding, locomotor activities, and eventual distribution of cold-water suspension-feeding sea cucumbers.

Key words: Environmental factors; Diel rhythm, Behaviour; Distribution; Holothuroid, Echinoderm

4.2 Introduction

Sea cucumbers, or holothuroid echinoderms, colonize most benthic habitats in temperate, polar and tropical oceans, where they may occur from the intertidal zone to the deep sea (Conand, 2006). They play important roles in marine ecosystems, such as regulating water chemistry/quality and modulating sediment stratification and nutrient cycling (Massin, 1982; Uthicke, 2001; Purcell et al., 2016). Owing to their nutritional and medicinal values, sea cucumbers have been an important food source for centuries in Asia and have been harvested in China and around the world for at least 400 years (Yang et al., 2015). However, an increasing demand from Asian markets combined with the lack of effective management measures have led to overexploitation and severe decline in stocks of high-value species in many parts of the world (Anderson et al., 2011; Purcell et al., 2013). Aquaculture has the potential to help replenish depleted sea cucumber stocks and mitigate fisheries impacts on wild populations (Bell et al., 2005; Yang et al., 2015). However, commercial aquaculture protocols have only been developed for a small number of temperate and tropical species, such as *Apostichopus japonicus* and *Holothuria scabra*, to supply markets and/or assist the natural recovery of wild stocks (Chen, 2005; Eriksson et al., 2012; Hamel et al., 2001; Yang et al., 2015; Mercier and Hamel, 2013). Beyond these species, knowledge of the basic ecology and biology of most commercially important sea cucumbers remains too limited to develop efficient and cost-effective aquaculture programs.

Many marine benthic species are capable of extensive movement during their different life stages (Grantham et al., 2003). Movement at various scales enables benthic animals to find better feeding conditions, to promote increased rates of growth and reproduction

(Scheibling, 1981; Pittman and McAlpine, 2003; Brady and Scheibling, 2006), or to escape sub-optimal environmental conditions (Hamel et al., 2019). Factors that influence sea cucumber distribution, growth, and behaviours have been studied in many respects to better understand population persistence, community dynamics and species-specific aquaculture prerequisites. Light cycles are known to drive daily activity rhythms, such as in juveniles of *Apostichopus japonicus*, which displayed nocturnal activity and hid during daytime (Dong et al., 2010). Similarly, juveniles of *Holothuria scabra* and *Stichopus* cf. *horrens* were shown to display a diel cycle, being the most active at night, exhibiting intermediate activity during twilight, and minimal to no activity during daytime (Mercier et al., 1999; Palomar-Abesamis et al., 2017). Sea cucumbers typically concentrate in areas that provide the best habitat, environmental conditions or food availability while reducing the risk of predation (Yingst, 1982; Sibuet, 1985). For example, the deposit-feeding sea cucumbers *Stichopus variegatus*, *Australostichopus mollis* and *H. scabra* exhibited a very distinct patch selectivity based on total organic matter in the sediments (Uthicke and Karez, 1999; Mercier et al., 2000; Slater et al., 2011). Juveniles of *H. scabra* studied in the Solomon Islands preferred seagrass habitat characterized by muddy sand with 7-15% organic content, and cover against some predators (Mercier et al., 2000). Moreover, a study of *Parastichopus californicus* showed that densities were highest on shell debris and gravel, and lowest on mud and silt bottoms in the vicinity of Sitka Sound, Alaska (USA). Hard substrates could provide more solid footing to keep individuals from drifting away under high dynamic flows (Woodby et al., 2000).

The sea cucumber *Cucumaria frondosa* (Holothuroidea: Dendrochirotida) has been the focus of commercial fisheries in the North Atlantic since the 1980s (Hamel and Mercier, 2008). The species is widely distributed in cold-water habitats, from the Arctic Ocean to Cape Cod (USA) in the Northwest Atlantic, as well as along the coast of northern Europe and Russia (Hamel and Mercier, 2008). It has recently been identified as a potential candidate for integrated multi-trophic aquaculture (Nelson et al., 2012). Unlike the most commonly studied species of sea cucumbers, which are deposit-feeders, *C. frondosa* feeds on suspended particulate materials by deploying ramified tentacles into the water column (Hamel and Mercier, 1998). Previous studies on *C. frondosa* have explored local field distributions (Hamel and Mercier, 1996), population genetics (So et al., 2011), diet and feeding patterns (Singh et al., 1998; Hamel and Mercier, 1998; Singh et al., 1999), movement in response to flow (Sun et al., 2018) and disturbances (Hamel et al., 2019), sexual dimorphism, reproductive cycle and spawning (Singh et al., 2001; Montgomery et al., 2018b; Hamel and Mercier, 1995), larval swimming capacity and photosensitivity (Montgomery et al., 2017; 2018a), and juvenile growth and behaviour in the laboratory (Hamel and Mercier, 1996; Gianasi et al., 2018; So et al., 2010). So far, only one study has documented photosensitivity in *C. frondosa* and it was restricted to early juveniles showing an increasing tolerance to light intensity with age (Gianasi et al., 2018), which explains why they congregate under rocks or in crevices during the first few years of life (Hamel and Mercier, 1996). The quality and quantity of food was also found to affect the feeding behaviour and growth of *C. frondosa* (Singh et al., 1998; Hamel and Mercier, 1998; Singh et al., 1999; Gianasi et al., 2017); however, whether food availability is a driver of locomotion and spatial distribution has never been tested. Similarly, dense populations of *C. frondosa* are commonly found on rocky bottoms (Hamel and Mercier, 1996), but the substrate preferences of adults have not been assessed experimentally.

The present study was conducted to determine the locomotor and feeding behaviours, and the microhabitat selection (spatial distribution) of *C. frondosa* in response to different light intensities, levels of phytoplankton concentration (its main source of food), substrate types and background colours. The putative influence of light on its diel locomotor and feeding activities was also assessed. Understanding the distribution as well as the feeding and locomotor behaviours of *C. frondosa* in response to various stimuli will provide baseline information that could be useful for stock management in identifying the most probable high-density areas, and also assist in the eventual development of aquaculture programs.

4.3 Materials and methods

4.3.1 Sea cucumber collection and maintenance

Adults of *C. frondosa* were collected through Fogo Island Co-operative Society Ltd (commercially licensed by the federal authority; Fisheries and Oceans Canada) on the Southwest Grand Banks of Newfoundland, eastern Canada, at depths between 20 and 30 m (Northwest Atlantic Fisheries Organization, NAFO Subdivision 3Ps). Individuals were kept in a flow-through tank (11.5 m × 2.5 m × 1.2 m, length × width × depth) with running water (30-60 L min^{-1}) at ambient temperature (1-7 °C). Light was provided by multiple fluorescent lights ranging from 5-450 lux (over the daily cycle) and naturally fluctuating photoperiod (from 15L/9D in the summer to 8L/16D during winter). These ranges of environmental conditions are in line with those occurring in the native habitat of *C. frondosa* (Gianasi et al., 2015). Planktonic food present in the ambient seawater was available to sea cucumbers as food (as per Hamel and Mercier, 1998). Healthy and undamaged individuals displaying normal feeding activity, firm attachment to the substrate and no skin lesions were selected for the experiments. They had an average total wet weight of 280.6 ± 76.3 g and contracted body length of 15.2 ± 3.9 cm.

4.3.2 Effect of light

4.3.2.1 Response to illuminated/shaded area*s*

Experiments were conducted in a tank composed of two circular sections (81 cm diameter × 40 cm depth) linked by a narrow passage (width: 7.5 cm) that could be opened or closed by a guillotine door. The whole tank was covered with either a transparent or black fiberglass cover and each circular section had independent seawater inflow (70 L h^{-1}) and outflow systems (Fig. S.4.1). For each trial, ten sea cucumbers (size described above) were placed in the centre of each of the circular section with the guillotine door closed and left to acclimate for 12-16 h until they had attached firmly and/or resumed normal activity (tentacle deployment and retraction). Two ambient light intensities (low: 90-126 lux; high: 420-560 lux, gradient ranging from the border to the centre of the tank) were tested successively by changing the height of the fluorescent light fixture (spectrum of 350-750 nm) above the experimental tank. Both light intensity ranges tested are commonly found where *C. frondosa* occurs in nature (Hamel and Mercier, 1996). The light intensity at the surface of the tanks was measured using a light meter (Traceable® 3252).

At the onset of a trial, the guillotine door was slid upward to provide an opening height of 5.0 cm, which was enough for sea cucumbers to get through. Some faint light could penetrate from the middle opening and the light intensity gradient in the shaded section varied from 4 to 2 lux in the low light intensity and from 6 to 2 lux in the high light intensity (weakening towards the shaded area). To control for potential tank effects, the shaded and illuminated sections were permuted across four replicate trials, each of which lasted four days. No individual was ever used for two successive trials. A time-lapse camera (Brinno TLC 200 Pro) was set to take one photograph of the entire experimental field (illuminated section) every 10 minutes. Pictures were automatically stitched together into a video output by the camera. Based on the videos, the distribution of the sea cucumbers in the illuminated area was analyzed at various time points (0/initial distribution, 8, 24, 48, 96 h) and compared using one-way repeated measures (RM) ANOVA after confirming normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively.

4.3.2.2 Diel rhythms under different photoperiod regimes

This experiment was conducted to determine the daily rhythm of feeding (based on tentacle deployment in the water column, as per Sun et al. 2018) and locomotor activities of *C. frondosa* under 12 h light/12 h dark, 24 h light and 24 h dark photoperiod regimes. The 12 h light/12 h dark photoperiod was set at 12 hours of light starting at 08:00 (maximum intensity of 230-270 lux; Hamel and Mercier, 1996) and 12 hours of darkness starting at 20:00 (corresponding to springtime photoperiod when sea cucumbers feed the most; Hamel and Mercier, 1998). More precisely, the photoperiod occurring in nature during the experimental period, 08:00-20:00 was defined as "day" and 20:00-08:00 was defined as "night" in the present study. Photoperiod was controlled using Hydro Farm light timers (TM 01015). All experiments were conducted in one rectangular tank (80 cm length × 74 cm width × 26 cm depth) supplied with ambient seawater. Ten sea cucumbers were placed in the center of the tank and acclimated for 24 h. Each trial lasted 24 h and was replicated four times. No individual was ever used for two successive trials. A time-lapse infrared-sensitive camera (described previously) was set to take one photograph of the entire experimental field every 60 seconds. In the dark phase, a LED infrared illuminator (DC 12V, ICAMI) was automatically turned on as soon as the lights went off to allow recording of nocturnal activity.

The number of individuals with tentacles deployed for more than 30 minutes (feeding proxy) and the number of individuals that underwent displacement of more than three body lengths (by forward crawling or rolling; Sun et al., 2018; Hamel et al., 2019) were measured every 2 h to estimate feeding and locomotion rhythms, respectively (individuals that writhed around, i.e. back-and-forth swaying, were not regarded as moving). The difference among different 2-h intervals (6 intervals between 08:00-20:00, and another 6 intervals between 20:00-08:00) was compared using one-way RM ANOVA followed by Bonferroni test. The proportions of individuals with deployed tentacles or moving under different photoperiods were analysed with one-way ANOVA followed by post hoc multiple comparisons with Tukey's test. A mean value of the proportions of deployed/feeding and moving individuals was also calculated separately for period of daytime (from 08:00 to 20:00) and night time (from 20:00 to 08:00) and compared using paired sample *t*-test.

In addition, the distance travelled by each individual was analysed using the video-tracking algorithm in ImageJ/Fiji with the MTrackJ plug-in (Schneider et al., 2012) at intervals of 1 frame per minute. Each video was first calibrated to convert pixels into real distances (cm) using reference marks. The average distance travelled by the ten sea cucumbers over 24 h from each trial among different photoperiod treatments was then determined and compared using one-way ANOVA followed by post-hoc multiple comparisons with Tukey's method. The distance travelled during the day (08:00-20:00, 12 h) and night (20:00-08:00, 12 h) periods were compared using paired sample *t*-test. All data were tested for normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively.

4.3.3 Effect of phytoplankton

4.3.3.1 Small-scale experiments

A first set of trials were conducted to verify the assumption that phytoplankton concentration (a preferred food in *C. frondosa*: Hamel and Mercier, 1998) is a factor in both feeding response and movement of *C. frondosa*. They were conducted in two tanks as described in section 4.3.2.2 above. At the onset of a trial, ten sea cucumbers were placed into each of the two tanks for a period of 12-h acclimation under static conditions. Food was distributed by gravity in each tank from 20-L reservoirs. One reservoir was filled with natural seawater to which 2 mL of commercial phytoplankton was added to generate a phytoplankton concentration in the reservoir of ~15 mg L^{-1}, comparable to that found during the peak of autumn plankton blooms in the northern Atlantic Ocean (i.e. ~17.5 mg L^{-1}, Henson et al., 2009). The other reservoir was filled solely with natural seawater representing the baseline phytoplankton concentration (~5 mg L^{-1}). The phytoplankton used (commercial Shellfish Diet 1800, Reed Mariculture) consisted of a mix of *Isochrysis* sp., *Pavlova* sp., *Chateoceros calcitrans*, *Thalassiosira weissflogii*, and *T. pseudonana*, which is well accepted as food by *C. frondosa* (Gianasi et al., 2019). The contents of both reservoirs (one per experimental tank) were mixed continuously through bubbling and trickled at a flow rate of 400-500 ml h^{-1} through a small hose submerged into the tanks 5 cm below the surface. The phytoplankton concentration was determined in the reservoir by collecting 600 ml of water, filtering it on pre-weighed 25-mm Whatman GF/C filters, drying at 60 °C for 24 h, and reweighing after having cooled to ambient temperature in a desiccator (Thermo Scientific^{TM}). Phytoplankton concentration by weight was calculated from dilution factors using an average of triplicate measures for each seawater sample. Each trial was run for 24 h, after which individuals were removed, and the tanks drained, cleaned and refilled; the whole process was repeated four consecutive times. To minimize tank effects, the two tanks were permuted after each replicate. The time sea cucumbers spent with tentacles deployed and the distance each individual travelled over 24 h were determined under the two conditions and compared using a *t*-test after testing for normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively.

4.3.3.2 Large-scale mesocosm experiments

Based on the results of the small-scale experiments, another experiment was conducted in a large mesocosm, to determine whether variable phytoplankton concentrations could drive a change in the spatial distribution of *C. frondosa* in a less constrained, more life-size environment, using live phytoplankton. The experiment was conducted in a tank (11.5 m length × 2.5 m width × 0.9 m depth) supplied with flow-through seawater (200 L min^{-1}). To maintain the water quality while minimizing the influence of flow on the locomotion and distribution (Sun et al., 2018), the seawater inflow and outflow were located in the upper half of the tank, separated from the experimental arena (subsection of the entire mesocosm: 5.75 m × 2.5 m × 0.9 m). Two 20-L reservoirs described previously were set on opposite sides of the experimental arena of the tank. One was filled with live diatom *Chaetoceros muelleri* (~4–9 μm diameter, 4.7×10^6 cell ml^{-1}), a species of phytoplankton that is well accepted as food by *C. frondosa* (Gianasi et al., 2017) and the other was filled with natural seawater (control for water agitation). The water in each of the reservoirs was mixed continuously through bubbling. Two food concentrations (low: 250–300 ml h^{-1}; high: 550–700 ml h^{-1}, see phytoplankton concentration details below) were tested by adjusting the inflow of phytoplankton supply (mirrored by the flow of the corresponding agitation control). A small hose connected to the reservoirs was submerged into the tank, 50 cm below the surface, to trickle the contents into the experimental arena. The phytoplankton concentration in the experimental arena was measured at mid-day on days 1 and 2 by collecting triplicate 1-L samples of seawater 20 cm from the phytoplankton and control inlets, 70 cm below the surface (close to the sea cucumber). Both concentrations tested generated a gradient from the food source to the opposite side. Specifically, the phytoplankton concentration varied from 18.7 to 10.7 mg L^{-1} in the low-food concentration treatment (corresponding to the natural food in winter) and from 32.2 to 12.5 mg L^{-1} in the high-food concentration (corresponding to the natural food in spring).

At the beginning of the experiment, fifty sea cucumbers were placed in the center of the experimental arena. A time-lapse video camera (described previously) was mounted 3 m above the experimental arena with a view of the entire experimental arena to allow measurement of the spatial distribution of sea cucumbers over time. Each trial lasted two days and was replicated four times. To minimize the possibility of tank effects, replicate treatments were conducted by alternating the placement of the food and control reservoirs on either side of the raceway. Based on videos recorded, the spatial distribution of individuals in both food gradients was determined at various time points (0, 6, 12, 24, and 48 h) and compared between the two sides of the tank (food side vs opposite side) using *t*-test after testing for normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively.

4.3.4 Effect of substrate type and colour

This experiment was designed to assess whether adults of *C. frondosa* exhibit any substrate preference. The trials were conducted in one rectangular tank (80 cm length × 74 cm width × 26 cm depth) supplied with ambient seawater under natural photoperiod. Three types of substrate were tested: (1) bare rocks, (2) rocks with coralline algae (*Lithothamnion glaciale*), (3) rocks with kelp (L*aminaria longicruris*). All substrates had a similar surface area (18-24 cm long and 12-17 cm wide, ~350 cm^2). Three replicates of each substrate were haphazardly distributed into the experimental tank, leaving an open area (bottom of tank) in the center to place ten sea cucumbers at time 0. Each trial lasted four days (96 h) and was replicated four times. No individual was ever used for two successive trials. To minimize the possibility of tank effects, the distribution of the three substrates (n=9) were haphazardly rearranged after each replicate. Based on the time lapse videos, the number of individuals on each substrate was recorded at various time points (8, 24, 48, 96 h) using the camera system described previously and was tested using a chi-square test.

Another experiment was designed to verify whether substrate colour can affect the distribution of *C. frondosa*. This experiment was conducted in one 50-L rectangular tank (90 cm length × 35 cm width × 16 cm depth) with a white background on the first third of the tank and black background in the last third of the tank, with uniform grey portion between the two (~ 1000 cm^2 for each colour). The grey portion was considered a transitional neutral zone between the white and black sections. The tank was supplied with ambient seawater under a natural photoperiod. One sea cucumber was placed in each of the three colour quadrants, as assigned by a random number computer generator (https://www.random.org/widgets). Each trial lasted 24 h and was replicated four times.

No individual was ever used for two successive trials. Between each replicate the tank was drained, scrubbed and rotated 180° (to reduce tank effect). Based on the time lapse videos (as described previously), the number of individuals on each substrate colour was measured at various time points (0, 6, 12, 18, 24 h) and compared using one-way ANOVA or one-way ANOVA on ranks.

4.4 Results

4.4.1 Response to illuminated/shaded areas

Under the low light intensity condition, the proportion of sea cucumbers distributed in the illuminated area of the experimental arena did not show any significant change over time (one-way RM ANOVA; $F_{3,4} = 0.36$, $P = 0.829$, Fig. 4.1A). Under the high light intensity condition, the proportion of individuals distributed in the illuminated *vs* shaded area increased from 50.0 ± 0 to 57.5 ± 6.5% over time; but not significantly ($F_{3,4} = 2.12$, $P = 0.141$, Fig. 4.1B).

4.4.2 Diel rhythms under different photoperiods

Overall, the proportion of individuals with tentacles deployed was significantly affected by the photoperiod treatments ($F_{2,9} = 17.20$, $P < 0.001$, Fig. 4.2A). Specifically, the proportion of individuals with tentacles deployed in the 24 h light treatment (20 ± 2%) was significantly lower than other photoperiod treatments (Tukey's test, $P < 0.05$), but no significant differences were detected between 12 h light/12 h dark (27 ± 2%) and 24 h dark conditions (27 ± 2%, $P > 0.05$, Fig. 4.2A). When each photoperiod treatment was considered separately, the proportion of individuals with deployed tentacles did not significantly change across 2-h intervals over the 24-h cycle in any of the photoperiod treatments (one-way RM ANOVA; 12 h light/12 h dark, $F_{3,11} = 1.67$, $P = 0.124$, Fig. 4.2B; 24 h light, $F_{3,11} = 0.777$, $P = 0.66$, Fig. 4.2C; 24 h dark, $F_{3,11} = 0.317$, $P = 0.977$, Fig. 4.2D). In addition, no significant differences in the proportion of individuals with tentacles deployed were found between the 'day' (08:00-20:00) and 'night' periods (20:00-08:00) in any photoperiod treatment (12 h light/12 h dark, $t = 0.40$, $df = 3$, $P = 0.718$; 24 light, $t = -1.19$, $df = 3$, $P = 0.319$; 24 h dark, $t = -0.68$, $df = 3$, $P = 0.544$; Fig. 4.2E).

Overall, the proportion of moving individuals was significantly affected by the photoperiod treatment ($F_{2,9} = 28.83$, $P < 0.001$, Fig. 4.3A). Specifically, the proportion of moving individuals under 12 h light/12 h dark (11 ± 1%) and 24 h dark (15 ± 2%) was significantly greater than under 24 h light (6 ± 2%, Tukey's test, $P < 0.05$). When each photoperiod treatment was considered separately, the proportion of moving sea cucumbers changed significantly across 2-h intervals of the 24-h cycle under 12 h light/12 h dark (one-way RM ANOVA, $F_{3,11} = 6.34$, $P < 0.001$; Fig. 4.3B), but not under 24-h light ($F_{3,11} = 0.67$, $P = 0.753$; Fig. 4.3C) or 24-h dark ($F_{3,11} = 1.49$, $P = 0.181$; Fig. 4.3D). During the transition period between the light and dark phases (18:00 to 22:00) and between the dark and light phases (06:00-10:00) of 12 h light/12 h dark, the proportion of moving individuals increased rapidly from 6 ± 5% to 14 ± 6% and decreased rapidly from 17 ± 9% to 8 ± 6% (Fig. 4.3B), respectively. In addition, the proportion of moving individuals was significantly lower in the 'day' than in the 'night' periods under 12 h light/12 h dark ($t = -6.71$, $df = 3$, $P = 0.007$) and 24 h dark treatments ($t = -5.75$, $df = 3$, $P = 0.011$); however, no significant differences were found between 'day' and 'night' under 24 h light ($t = -1.99$, $df = 3$, $P = 0.141$; Fig. 4.3E).

The average distance travelled by sea cucumbers in a day varied significantly depending on the photoperiod (one-way ANOVA, $F_{2,9} = 20.17$, $P < 0.001$, Fig. 4.4A). Specifically, individuals traveled significantly greater daily distances (Tukey's test, $P < 0.05$) when held under 12 h light/12 h dark (95.4 ± 21.9 cm d^{-1}) and continuous darkness (96.5 ± 8.6 cm d^{-1}), with no difference between the two ($P > 0.05$), than they did under continuous light (42.3 ± 4.2 cm d^{-1}). In addition, sea cucumbers travelled significantly shorter distances during the 'day' (from 08:00 to 20:00) than during the 'night' (20:00 to 8:00) both under 12 h light/12 h dark (mean of 20.9 vs 74.5 cm; $t = -9.82$, $df = 3$, $P = 0.002$) and in continuous darkness (mean of 40.4 vs 56.1 cm; $t = -3.88$, $df = 3$, $P = 0.030$). However, under 24-h light, individuals moved equal mean distances in both phases (20.5 *vs* 21.8 cm; $t = -1.04$, $df = 3$, $P = 0.374$; Fig. 4.4B).

4.4.3 Effect of phytoplankton

Overall, the small-scale experiment showed that the presence of phytoplankton increased the feeding behaviour (tentacles deployed), but did not elicit any detectable change in locomotor behaviour in *C. frondosa*. Specifically, there was a significant difference between treatments in the time spent with tentacles deployed over 24 h, which was greater in the presence of phytoplankton (185.6 ± 14.1 mins) than in the control (103.7 ± 8.9 mins; $t = 9.80$, $df = 6$, $P < 0.001$, Fig. 4.5A). However, no significant differences in the distance travelled by sea cucumbers over 24 h were found between control (99.9 ± 16.0 cm) and phytoplankton conditions (85.8 ± 11.6 cm; $t = -1.43$, $df = 6$, $P = 0.204$; Fig. 4.5B).

In the larger mesocosm, phytoplankton concentration did not stimulate locomotion or influence spatial distribution in sea cucumbers. Specifically, at either low or high phytoplankton concentrations, most sea cucumbers (low: 72 ± 4%; high: 66 ± 9%)

remained in the center (initial position) of the arena after 48 h; only a few individuals moved toward (low: 16 ± 2%; high: 17 ± 2%) or away from the food source (low: 13 ± 2%; high: 18 ± 5%; Fig. 4.5CD). Under the two nominal phytoplankton concentrations tested, the proportions of individuals close to the food source were similar to the opposite (no-food) side, with no significant differences between the two at any time point (Table S.4.1, Fig. 4.5CD).

4.4.4 Effect of substrate type and background colour

In these trials, sea cucumbers changed their distribution over time and showed strong substrate preferences (Fig. 4.6A). The proportion of individuals located on the bare tank bottom/walls decreased from the predetermined value of 100% at the beginning of the experiment (0 h) to 20 ± 12% at the end of the experiment (96 h), whereas the proportion of individuals on bare rocks and on rocks with coralline algae increased from 0 to 35 ± 5% and from 0 to 40 ± 7%, respectively. The proportion of individuals on rocks with kelp was 10 ± 2% after 8 h and had decreased to 5 ± 2% at the end of the experiment (96 h). Analysis showed that the proportions of individuals on bare rocks and rocks with coralline algae were greater than would be expected on the basis of random distribution across surface areas alone; inversely, the proportions of individuals on rocks with kelp and bare tank sections were significantly lower than expected (Table 4.1).

Despite the proportions of individuals distributed on the darker substrates generally being greater (black, 33-42%; grey, 33-50%) than on the white substrate section (17-33%, Fig. 4.6B), no significant differences were found at any time point (6 h, $F_{2,9} = 1.5$, $P = 0.274$; 12 h, $H = 0.9$, $P = 0.632$; 18 h, $H = 0$, $P = 1$; 24 h, $F_{2,9} = 1.5$, $P = 0.274$). Overall, sea cucumbers showed a weak preference for darker substrate backgrounds.

4.5 Discussion

In the natural environment, species have evolved multiple behavioural strategies to maximise their chances of surviving and optimizing fitness when faced with various ecological pressures, such as the shifting availability of food in space and time, and the presence of predators (Davies et al., 2012). Among environmental drivers, light was identified as one of the most important factors affecting ecological processes in aquatic species, including rhythms in daily activity, reproductive cycle as well as migratory and aggregative behaviours (Lythgoe, 1988; Mercier and Hamel, 2009). Spatial distribution is also commonly modulated by substrate type, which can facilitate food acquisition and/or provide shelter to marine benthic species (Jones et al., 1994).

Findings suggests that adults of *Cucumaria frondosa* do not exhibit any direct reaction to light (photosensitivity) since they did not show a preference for either illuminated or shaded areas under any light intensity, in contrast to their early juveniles, which were determined to display clear photo-negativity (Hamel and Mercier, 1996). More precisely, Gianasi et al. (2018) reported that tolerance to light increased with age, from 25 lux in 1-month-old to >50 lux in 6, 12, and 21-month-old individuals. The present findings complete this trend, suggesting that while *C. frondosa* never becomes photopositive, adults become less sensitive to light. The photo-negativity documented in just-settled juveniles of *C. frondosa* was demonstrated to play a role in their capacity to choose appropriate locations on the settlement substrate, generally undersurfaces, to minimize predatory pressure from sea stars (Legault and Himmelman, 1993; Hamel and Mercier, 1996; So et al., 2010) and grazing sea urchins (Hamel and Mercier, 1996). While a greater abundance of larger individuals of *C. frondosa* occur in dimmer waters down to 60 m (Hamel and Mercier, 1996), the present findings suggest that they are not doing so because they are actively seeking darker environments, as initially assumed.

Diel activity cycles vary among different holothuroid species, with peaks in activity being diurnal (Shiell, 2006), nocturnal (Mercier et al., 1999; 2000; Dong et al., 2011; Sun et al., 2015), or crepuscular (Graham and Battaglene, 2004), in response to a variety of environmental factors, such as light and water temperature. In *C. frondosa*, more individuals exhibited locomotor activity during the nocturnal phase of the 24-h cycle, and the distance travelled was consequently greater at night than during the day, when they remained mostly immobile (with or without tentacles extended for feeding). Nocturnal displacement may point to a protective strategy against predators (Nelson and Vance, 1979; Mercier et al., 1999), especially for *C. frondosa*, which is known to be preyed upon by several large diurnal hunters such as fishes, seals and walruses (Hamel and Mercier, 2008). The daily mean distance travelled was 0.95 m under the 12 h light/12 h dark regime, which is lower than in deposit-feeding species, i.e. 8 m in *H. arguinensis* (Navarro et al., 2014), 3 m in *Actinopyga mauritiana* (Graham and Battaglene, 2004), and 4 m in *Parastichopus californicus* (Da Silva et al., 1986). Greater movements were associated with enhanced food acquisition in deposited-feeding sea cucumbers since they generally ingest and process organic matter as they move (Mercier et al., 2000; van Dam-Bates et al., 2016). In contrast, *C. frondosa* is a suspension feeder that does not need to forage for food; instead, water flow was demonstrated as one of the key factors involved in food capture for this species (Sun et al., 2018) since it carries the planktonic particles that represent its main food source (Hamel and Mercier, 1998).

The present study determined that greater nocturnal movement in *C. frondosa* was maintained under constant darkness for 24 h (similar to 12 h light-12 h dark regime), in line with endogenous control reported in *A. japonicus* (Dong et al., 2010; Sun et al., 2015) and *Stichopus* cf. *horrens* (Palomar-Abesamis et al., 2017). Also, the proportion of moving individuals and the distance travelled did not differ between 12-h segments corresponding to 'day' and 'night' phases under 24-h light, suggesting the absence of any internal rhythm; instead, the cue that stimulates movement is the decrease in light intensity, as shown in other sea cucumbers (Wheeling et al., 2007; Dong et al., 2010). The proportion of moving individuals and the distance travelled in 24-h darkness were overall greater compared to individuals under 12 h light/12 dark and 24-h light, which further supports that *C. frondosa* is more active, especially more mobile, in darkness. Interestingly, increased activity in darkness may explain why more individuals of *C. frondosa* ended up in the illuminated section of the tank under high light intensities. Because they were more mobile in the dark, sea cucumbers were more likely to move from the shaded to the illuminated section than inversely. Whether the enhanced locomotor patterns of *C. frondosa* would persist after longer-term exposure to constant darkness remains to be clarified.

In deposit-feeding sea cucumbers, nocturnal behaviour has fueled research into improved culture conditions. For instance, Dong et al. (2011) showed that *A. japonicus* maintained in darkness can exhibit longer periods of feeding, which potentially increases its growth rate. In the present study of a suspension-feeding species, no significant differences in the proportion of sea cucumbers with tentacles deployed were found over time or between diurnal and nocturnal phases under 12 h light/12 h dark. This was consistent with the work of Singh et al. (1999), where no marked difference was found in the number of adult sea cucumbers with tentacles extended and feeding in the day or at night from the field. Also, no major differences in the proportion of sea cucumbers with tentacles deployed were found over time or between diurnal and nocturnal periods under constant light and constant darkness, further supporting that suspension-feeding in *C. frondosa* is not photosensitive, unlike deposit-feeding in other species (Mercier et al., 1999; Dong et al., 2010). The interspecific and ontogenetic differences in the photosensitivity of sea cucumbers may therefore be linked to feeding guilds, i.e. planktivore for suspension feeders and detritivore for deposit feeders. Even though no obvious eye-like structure was evidenced in sea cucumbers, pigmented photoreceptors at the base of the tentacles have been identified in some species (Yamamoto and Yoshida, 1978; Pawson et al., 2010). The suspension feeders' tentacles are oriented upwards (toward the light) and the deposit feeders' tentacles are generally oriented downwards (away from light) when feeding. This may result in greater tolerance and lower sensitivity to light in suspension feeders. Unlike members of Holothuriidae that feed when moving at night (Mercier et al., 1999; Dong et al., 2011; Sun et al., 2015), nocturnal displacement in *C. frondosa*, which may last as long as ~100 min per night (Sun et al., 2018), prevents feeding (as tentacles are retracted) and requires energy expenditure. Therefore, shifting the photoperiod in favour of longer days (e.g. 16 h light and 8 h dark) during captive rearing could potentially translate into improved growth rates (more feeding, less movement) in this notoriously slow growing species (Hamel and Mercier, 1996). On the other hand, individuals maintained under continuous 24-h light exhibited reduced feeding activity compared to other treatments, suggesting photo-inhibition under constant exposure to light, which should thus be avoided from an aquaculture perspective.

Although *C. frondosa* was found to be able to feed around the clock, the time spent with tentacles deployed was greater in response to phytoplankton spikes than under baseline food conditions, in line with the field studies of Hamel and Mercier (1998) and Singh et al. (1998) who showed that changing tides (and concurrent influx of phytoplankton) increased the proportion of individuals with tentacles deployed. The fact that the presence of food alone can stimulate tentacle deployment separates the roles played by tidal current and food supply in the feeding response of *C. frondosa*. On the other hand, there were no significant differences in the locomotor behaviours of *C. frondosa* based on food gradients (i.e. no movement towards the food source). This finding differs from the locomotor pattern of most deposit feeders, whereby individuals may travel long distances to find a suitably rich sediment patches to feed on (Navarro et al., 2014). For example, the movement of juveniles of *H. scabra* was greater on bare surfaces, or substrates deprived of organic matter (OM), relative to substrates with high OM (Mercier et al., 1999). Also, *A. mollis* and *H. arguinensis* rapidly moved through sediment with low organic content, whereas they slowed down, or even stopped, when an area with available food was found (Slater et al., 2011; Navarro et al., 2014). For suspension feeders, these imperatives are not as strong, as the food is coming to them passively through currents. In the large-mesocosm experiments, the distribution of individuals did not vary across the phytoplankton gradient at any time point, suggesting that food may not be a major driver of locomotor behaviour in *C. frondosa*. While unlikely, it cannot be totally excluded that the difference between the spiked and baseline food side was not steep enough to stimulate movement. Previous studies have shown that the feeding response of *C. frondosa* may be initiated by both chemical and tactile stimuli (Jordan, 1972; Hamel and Mercier, 1998; Singh et al., 1998);

however, the low turbulence level conditions in this experiment may not have been aligned perfectly with the food detection abilities of this species.

Both newly-settled juveniles and adults of *C. frondosa* have been suggested to favour rocky substrates in the wild, e.g. in the St. Lawrence Estuary and over the St. Pierre Bank (Hamel and Mercier, 1996; So et al., 2010), and sea cucumbers found on rocky substrates are reportedly heavier than those found on other substrates (So et al., 2010). Hard bottoms are believed to possess optimal water flow and provide solid attachment for this species compared to soft bottoms, enabling full deployment of their feeding tentacles. In line with these assumptions, the present study confirmed that, when given the choice, *C. frondosa* favoured substrates composed of bare rocks and rocks covered coralline algae. It further highlighted that rocks with coralline algae were slightly more attractive compared to bare rocks, consistent with preferences recorded in juveniles during field and laboratory investigations (Medeiros-Bergen and Miles, 1997; Gianasi et al., 2018). Preference for rocks covered with coralline algae could be attributed to the fact that they signal optimal conditions of light and/or current on which this species depends to identify ideal feeding grounds. The relatively coarse surface offered by coralline algae may also enable firmer attachment than the smooth bare rocky surface. Moreover, individuals were rarely found on the rock covered with kelp, possibly because the swaying movement of seaweed hampered tentacle deployment and feeding and/or disrupted food-carrying water flow. Why darker shades of background were also preferred is still unclear, although it may offer camouflage against pelagic predators viewing sea cucumbers from above.

The present work suggests that while a tendency towards increased nocturnal movement/activity was detected, gradients in light (at intensities typical of their depth range) and food concentrations did not emerge as direct drivers of spatial distribution in fully-grown individuals. However, *C. frondosa* was influenced by substrate types and background colours, clearly preferring darker coloured bare rocks and rocks covered in coralline algae. The findings not only provide baseline information of value for stock management, but will inform the eventual development of aquaculture programs. Importantly, in contrast to common aquaculture practices for deposit-feeding species where constant darkness prolongs feeding and stimulates growth, the absence of light is unlikely to enhance growth in the suspension-feeding *C. frondosa* (except perhaps during the earliest life stages). Constant light would not enhance feeding either. Overall, long days (i.e. summer photoperiod) and a continuous supply of food might offer the optimal aquaculture conditions, since it would decrease the time spent moving in favour of feeding, creating an energy budget that might translate into faster growth.

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4.7 References

Anderson, S.C., Flemming, J.M., Watson, R., Lotze, H.K., 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries. 12, 317-339.

Bell, J.D., Rothlisberg, P.C., Munro, J.L., Loneragan, N.R., Nash, W.J., Ward, R.D., Andrew, N.L., 2005. Restocking and stock enhancement of marine invertebrate fisheries. Elsevier, San Diego, California, USA.

Brady, S.M., Scheibling, R.E., 2006. Changes in growth and reproduction of green sea urchins, *Strongylocentrotus droebachiensis* (Müller), during repopulation of the shallow subtidal zone after mass mortality. Journal of Experimental Marine Biology and Ecology. 335, 277-291.

Chen, J., 2005. Present status and prospects of sea cucumber industry in China. in: Conand C., Purcell, S., Uthicke, S. Hamel, J.-F., Mercier A. (Eds.), Advances in Sea Cucumber Aquaculture and Management. FAO Fisheries technical paper, Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 25-37.

Conand, C., 2006. Sea cucumber biology, taxonomy, distribution and conservation status. in: Bruckner, A. (Ed.), Proceedings of the CITES Workshop on the Conservation of Sea Cucumbers in the Families Holothuriidae and Stichopodidae. Sliver Spring, MD, USA, pp. 33.

Da Silva, J., Cameron, J.L., Fankboner, P.V., 1986. Movement and orientation patterns in the commercial sea cucumber *Parastichopus californicus* (Stimpson)(Holothuroidea: Aspidochirotida). Marine and Freshwater Behaviour and Physiology. 12, 133-147.

Davies, N.B., Krebs, J.R., West, S.A., 2012. An introduction to behavioural ecology. Wiley-Blackwell, Oxford, UK.

Dong, G., Dong, S., Wang, F., Tian, X., 2010. Effects of light intensity on daily activity rhythm of juvenile sea cucumber, *Apostichopus japonicus* (Selenka). Aquaculture Research. 41, 1640-1647.

Dong, G., Dong, S., Tian, X., Wang, F., 2011. Effects of photoperiod on daily activity rhythm of juvenile sea cucumber, *Apostichopus japonicus* (Selenka). Chinese Journal of Oceanology and Limnology. 29, 1015-1022.

Eriksson, H., Robinson, G., Slater, M.J., Troell, M., 2012. Sea cucumber aquaculture in the Western Indian Ocean: challenges for sustainable livelihood and stock improvement. Ambio. 41, 109-121.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2018. Morphometric and behavioural changes in the early life stages of the sea cucumber *Cucumaria frondosa*. Aquaculture. 490, 5-18.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2019. Triggers of spawning and oocyte maturation in the commercial sea cucumber *Cucumaria frondosa*. Aquaculture. 498, 50-60.

Gianasi, B.L., Verkaik, K., Hamel, J.-F., Mercier, A., 2015. Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. PLoS ONE. 10, e0127884.

Gianasi, B.L., Parrish, C.C., Hamel, J.-F., Mercier, A., 2017. Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquaculture Research. 48, 3413-3432.

Graham, J.C., Battaglene, S.C., 2004. Periodic movement and sheltering behaviour of *Actinopyga mauritiana* (Holothuroidea: Aspidochirotidae) in Solomon Islands. SPC Beche-de-mer Information Bulletin. 19, 23-31.

Grantham, B.A., Eckert, G.L., Shanks, A.L., 2003. Dispersal potential of marine invertebrates in diverse habitats. Ecological Applications. 13, 108-116.

Hamel, J.-F., Mercier, A., 1995. Spawning of the sea cucumber *Cucumaria frondosa* in the St Lawrence Estuary, eastern Canada. SPC Beche-de-mer Information Bulletin. 7, 12-18.

Hamel, J.-F., Mercier, A., 1996. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences. 53, 253-271.

Hamel, J.-F., Mercier, A., 1998. Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. Canadian Journal of Zoology. 76, 1194-1198.

Hamel, J.-F., Mercier, A., 2008. Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 293-306.

Hamel, J.-F., Conand, C., Pawson, D.L., Mercier, A., 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as beche-de-mer. Advances in Marine Biology. 41, 129-223.

Hamel, J.-F., Sun, J., Gianasi, B.L., Montgomery, E.M., Kenchington, E.L., Burel, B., Rowe, S., Winger, P.D., Mercier, A., 2019. Active buoyancy adjustment increases dispersal potential in benthic marine animals. Journal of Animal Ecology. 88, 820-832.

Henson, S.A., Dunne, J.P., Sarmiento, J.L., 2009. Decadal variability in North Atlantic phytoplankton blooms. Journal of Geophysical Research: Oceans. 114.

Jones, C.G., Lawton, J.H., Shachak, M., 1994. Organisms as ecosystem engineers. in: Samson, F.B., Knopf, F.L. (Eds.), Ecosystem Management. Springer, New York, NY, USA, pp. 130-147.

Jordan, A.J., 1972. On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothurioidea) at Lamoine Beach, Maine. University of Maine, Orono.

Legault, C., Himmelman, J.H., 1993. Relation between escape behaviour of benthic marine invertebrates and the risk of predation. Journal of Experimental Marine Biology and Ecology. 170, 55-74.

Lythgoe, J.N., 1988. Light and vision in the aquatic environment. in: Atema, J., Fay, R.R., Popper, A.N., Tavolga, W.N. (Eds.), Sensory Biology of Aquatic Animals. Springer, New York, NY, USA, pp. 57-82.

Massin, C., 1982. Effects of feeding on the environment: Holothuroidea. in: Jangoux, M., Lawrence, J. (Eds.), Echinoderm Nutrition. AA Balkema, Rotterdam, Netherlands, pp. 494-497.

Medeiros-Bergen, D.E., Miles, E., 1997. Recruitment in the holothurian *Cucumaria frondosa* in the Gulf of Maine. Invertebrate Reproduction & Development. 31, 123-133.

Mercier, A., Hamel, J.-F., 2009. Gametogenesis. Advances in Marine Biology. 55, 7-72.

Mercier, A., Hamel, J.-F., 2013. Sea cucumber aquaculture: Hatchery production, juvenile growth and industry challenges. in: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead Publishing Ltd, Cambridge, pp. 431-454.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 1999. Daily burrowing cycle and feeding activity of juvenile sea cucumbers *Holothuria scabra* in response to environmental factors. Journal of Experimental Marine Biology and Ecology. 239, 125-156.

Mercier, A., Battaglene, S.C., Hamel, J.-F., 2000. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. Hydrobiologia. 440, 81-100.

Montgomery, E., Hamel, J.-F., Mercier, A., 2017. Ontogenetic shifts in swimming capacity of echinoderm propagules: a comparison of species with planktotrophic and lecithotrophic larvae. Marine Biology. 164, 43.

Montgomery, E., Hamel, J.-F., Mercier, A., 2018a. Ontogenetic variation in photosensitivity of developing echinoderm propagules. Journal of Experimental Marine Biology and Ecology. 500, 63-72.

Montgomery, E., Ferguson-Roberts, J., Gianasi, B., Hamel, J.-F., Kremenetskaia, A., Mercier, A., 2018b. Functional significance and characterization of sexual dimorphism in holothuroids. Invertebrate Reproduction & Development, 1-11.

Navarro, P.G., García-Sanz, S., Tuya, F., 2014. Contrasting displacement of the sea cucumber *Holothuria arguinensis* between adjacent nearshore habitats. Journal of Experimental Marine Biology and Ecology. 453, 123-130.

Nelson, B., Vance, R., 1979. Diel foraging patterns of the sea urchin *Centrostephanus coronatus* as a predator avoidance strategy. Marine Biology. 51, 251-258.

Nelson, E.J., MacDonald, B.A., Robinson, S.M., 2012. A review of the Northern sea cucumber *Cucumaria frondosa* (Gunnerus, 1767) as a potential aquaculture species. Reviews in Fisheries Science. 20, 212-219.

Palomar-Abesamis, N., Juinio-Meñez, M.A., Slater, M.J., 2017. Effects of light and microhabitat on activity pattern and behaviour of wild and hatchery-reared juveniles of *Stichopus* cf. *horrens*. Journal of the Marine Biological Association of the United Kingdom, 1-11.

Pawson, D.L., Pawson, D.J., King, R.A., 2010. A taxonomic guide to the Echinodermata of the South Atlantic Bight, USA: 1. Sea cucumbers (Echinodermata: Holothuroidea). Zootaxa. 2449, 1-48.

Pittman, S., McAlpine, C., 2003. Movements of marine fish and decapod crustaceans: process, theory and application. Advances in Marine Biology. 44, 205-294.

Purcell, S.W., Conand, C., Uthicke, S., Byrne, M., 2016. Ecological roles of exploited sea cucumbers. Oceanography and Marine Biology: An Annual Review. 54, 367-386.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M.V., Lovatelli, A., Uthicke, S., 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries. 14, 34-59.

Scheibling, R., 1981. Optimal foraging movements of *Oreaster reticulatus* (L.)(Echinodermata: Asteroidea). Journal of Experimental Marine Biology and Ecology. 51, 173-185.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nature Methods. 9, 671.

Shiell, G.R., 2006. Effect of invasive tagging on the activity of *Holothuria whitmaei* [Echinodermata: Holothuroidea]: a suitable mark-recapture method for short-term field studies of holothurian behaviour. Marine and Freshwater Behaviour and Physiology. 39, 153-162.

Sibuet, M.A., 1985. Quantitative distribution of echinoderms (Holothuroidea. Asteroidea. Ophiuroidea. Echinoidea) in relation to organic matter in the sediment, in deep-sea basins of the Atlantic Ocean, Keegan B.F., O'Connor B.F. (eds) Echinodermata Balkema, Rotterdam, pp. 99-103.

Singh, R., MacDonald, B.A., Lawton, P., Thomas, M.L., 1998. Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. Canadian Journal of Zoology. 76, 1842-1849.

Singh, R., MacDonald, B.A., Thomas, M.L., Lawton, P., 1999. Patterns of seasonal and tidal feeding activity in the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) in the Bay of Fundy, Canada. Marine Ecology Progress Series. 187, 133-145.

Singh, R., Macdonald, B.A., Lawton, P., Thomas, M.L., 2001. The reproductive biology of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuriodea) using new quantitative methods. Invertebrate Reproduction & Development. 40, 125-141.

Slater, M.J., Jeffs, A.G., Sewell, M.A., 2011. Organically selective movement and deposit-feeding in juvenile sea cucumber, *Australostichopus mollis* determined in situ and in the laboratory. Journal of Experimental Marine Biology and Ecology. 409, 315-323.

So, J.J., Hamel, J.-F., Mercier, A., 2010. Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology. 17, 473-484.

So, J.J., Uthicke, S., Hamel, J.-F., Mercier, A., 2011. Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (Echinodermata: Holothuroidea). Marine Biology. 158, 859-870.

Sun, J., Hamel, J.-F., Mercier, A., 2018. Influence of flow on locomotion, feeding behaviour and spatial distribution of a suspension-feeding sea cucumber. Journal of Experimental Biology, jeb. 189597.

Sun, J., Zhang, L., Pan, Y., Lin, C., Wang, F., Kan, R., Yang, H., 2015. Feeding behavior and digestive physiology in sea cucumber *Apostichopus japonicus*. Physiology & Behavior. 139, 336-343.

Uthicke, S., 2001. Nutrient regeneration by abundant coral reef holothurians. Journal of Experimental Marine Biology and Ecology. 265, 153-170.

Uthicke, S., Karez, R., 1999. Sediment patch selectivity in tropical sea cucumbers (Holothurioidea: Aspidochirotida) analysed with multiple choice experiments. Journal of Experimental Marine Biology and Ecology. 236, 69-87.

van Dam-Bates, P., Curtis, D.L., Cowen, L.L., Cross, S.F., Pearce, C.M., 2016. Assessing movement of the California sea cucumber *Parastichopus californicus* in response to organically enriched areas typical of aquaculture sites. Aquaculture Environment Interactions. 8, 67-76.

Wheeling, R.J., Verde, E.A., Nestler, J.R., 2007. Diel cycles of activity, metabolism, and ammonium concentration in tropical holothurians. Marine Biology. 152, 297-305.

Woodby, D., Smiley, S., Larson, R., 2000. Depth and habitat distribution of *Parastichopus californicus* near Sitka, Alaska. Alaska Fishery Research Bulletin. 7, 22-32.

Yamamoto, M., Yoshida, M., 1978. Fine structure of the ocelli of a synaptid holothurian, *Opheodesoma spectabilis*, and the effects of light and darkness. Zoomorphologie. 90, 1-17.

Yang, H., Hamel, J.-F., Mercier, A., 2015. The sea cucumber *Apostichopus japonicus*: history, biology and aquaculture. Academic Press, New York, NY, USA.

Yingst, J.Y., 1982. Factors influencing rates of sediment ingestion by *Parastichopus parvimensis* (Clark), an epibenthic deposit-feeding holothurian. Estuarine, Coastal and Shelf Science. 14, 119-134.

4.8 Tables

Table 4.1 Results of chi-square test of the distribution of *C. frondosa* on different substrates over time (8, 24, 48, 96 h)*

Substrates	**Surface area (m^2)**	**No. settled**				**Expected**	**χ^2**			
		8 h	24 h	48 h	96 h		8 h	24 h	48 h	96 h
Bare rocks	0.42 (17.6%)	8	12	14	14	7.04	0.13	3.49	6.88	6.88
Rocks with kelp	0.42 (17.6%)	4	3	3	2	7.04	1.31	2.32	2.32	3.61
Rocks with coralline algae	0.42 (17.6%)	9	14	15	16	7.04	0.55	6.88	9.00	11.40
Bare tank	1.12 (47.2%)	19	11	8	8	18.88	0	3.29	6.27	6.27
Totals	2.38			40		-	1.99 ($P = 0.574$)	15.98 ($P = 0.001$)	24.47 ($P < 0.001$)	28.16 ($P < 0.001$)

*Individuals were initially placed in the middle of the experimental tank (on bare tank substrate) at time 0 h.

4.9 Figures

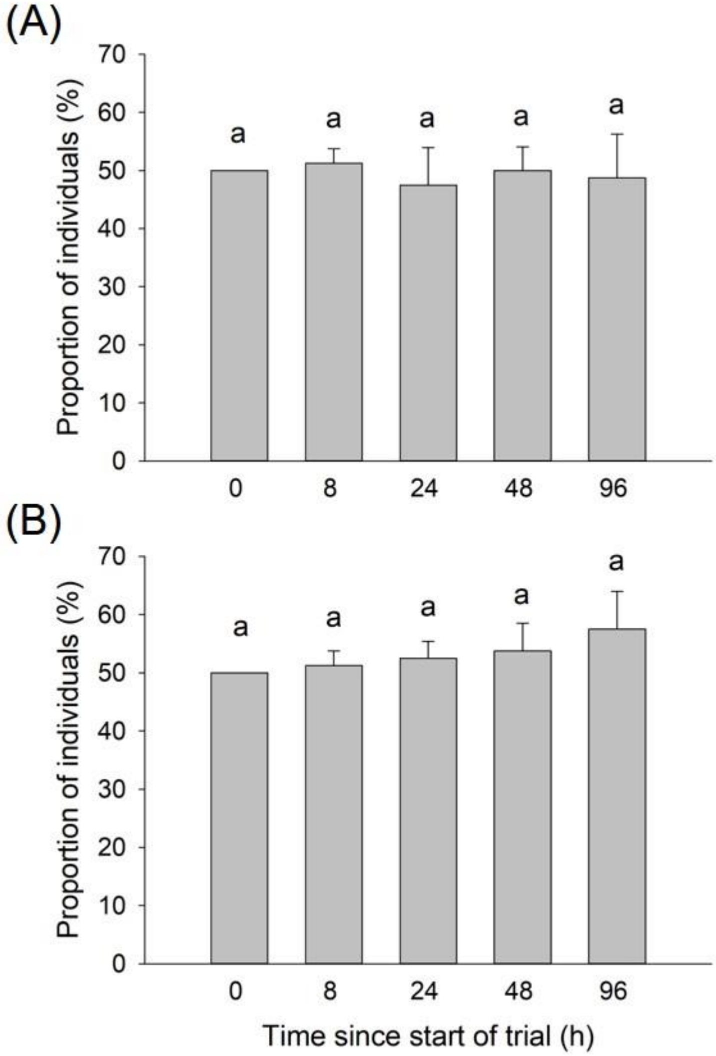

Figure 4.1 Distribution of *C. frondosa* in the illuminated section of the tank under (A) low and (B) high light intensity over time. At time 0 h, the guillotine door between illuminated and shaded sections was closed and there was an equal number of individuals in each. Data are shown as mean with standard deviation (n = 4) and no significant differences were detected among different time points (one-way RM ANOVA, $P > 0.05$).

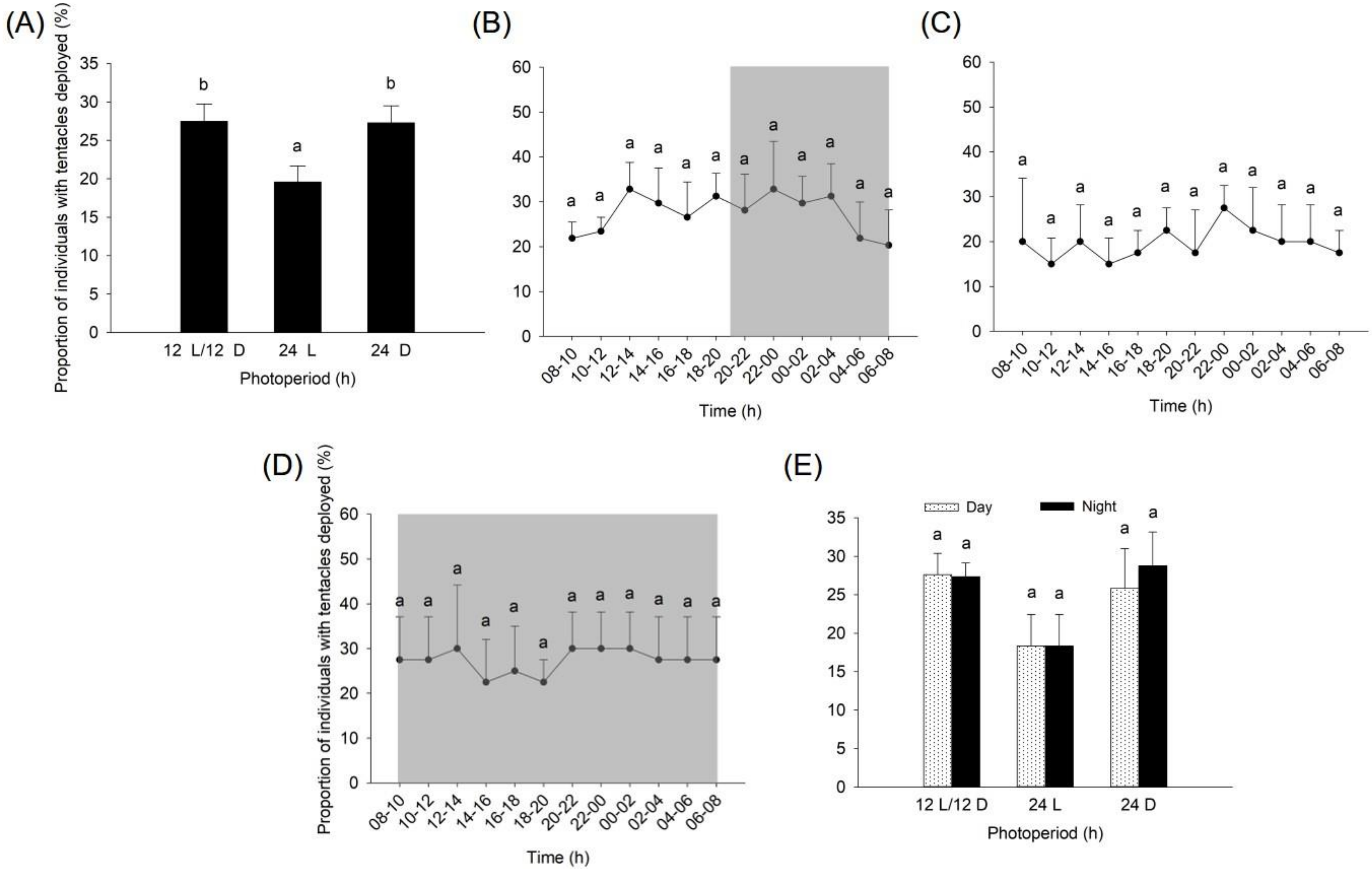
(A)
Proportion of individuals with tentacles deployed (%)
0
5
10
15
20
25
30
35
b
a
b
12 L/12 D
24 L
24 D
Photoperiod (h)
(B)
0
10
20
30
40
50
60
a
a
a
a
a
a
a
a
a
a
a
a
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(C)
0
10
20
30
40
50
60
a
a
a
a
a
a
a
a
a
a
a
a
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(D)
Proportion of individuals with tentacles deployed (%)
0
10
20
30
40
50
60
a
a
a
a
a
a
a
a
a
a
a
a
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(E)
Day
Night
0
5
10
15
20
25
30
35
a
a
a
a
a
a
12 L/12 D
24 L
24 D
Photoperiod (h)

Figure 4.2 Effect of photoperiod on feeding behaviour in *C. frondosa*. Mean proportion of deployed individuals (A) under different photoperiods. Proportion of individuals with deployed tentacles at 2-h intervals over the daily cycle under (B) 12 h light/12 h dark (normal photoperiod), (C) 24 h light and (D) 24 h darkness. Mean proportion of deployed individuals (E) during the diurnal (08:00-20:00) versus nocturnal (20:00-08:00) phases of the cycle. Grey areas in panels B and D correspond to periods of darkness. Data are shown as mean with standard deviation (n = 4). Statistical differences were detected among different photoperiod regimes in A (one-way ANOVA followed by Tukey's test, $P < 0.05$), but no statistical differences were detected among different time points in B, C and D (RM-ANOVA, $P > 0.05$) or between day/night in E (paired sample *t*-test, $P > 0.05$).

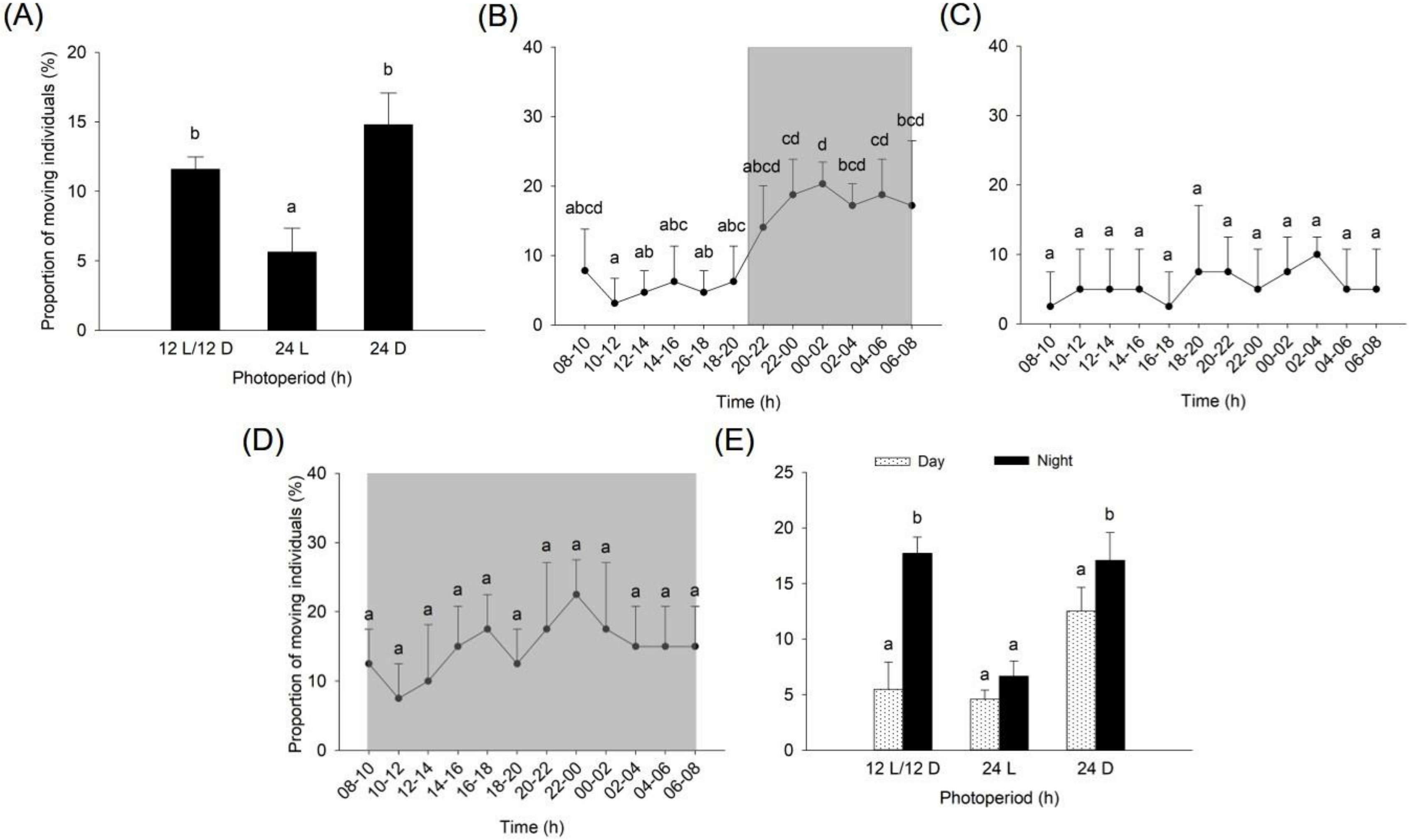
(A)
Proportion of moving individuals (%)
0
5
10
15
20
b
a
b
12 L/12 D
24 L
24 D
Photoperiod (h)
(B)
0
10
20
30
40
abcd
a
ab
abc
ab
abc
abcd
cd
d
bcd
cd
bcd
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(C)
0
10
20
30
40
a
a
a
a
a
a
a
a
a
a
a
a
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(D)
Proportion of moving individuals (%)
0
10
20
30
40
a
a
a
a
a
a
a
a
a
a
a
a
08-10
10-12
12-14
14-16
16-18
18-20
20-22
22-00
00-02
02-04
04-06
06-08
Time (h)
(E)
Day
Night
0
5
10
15
20
25
a
b
a
a
a
b
12 L/12 D
24 L
24 D
Photoperiod (h)

Figure 4.3 Effect of photoperiod on locomotor behaviour in *C. frondosa*. Mean proportion of moving individuals (A) under different photoperiod regimes. Proportion of moving individuals at 2-h intervals over the daily cycle under (B) 12 h light/12 h dark, (C) 24 h light and (D) 24 h darkness. Mean proportion of moving individuals (E) during the diurnal (08:00-20:00) versus nocturnal (20:00-08:00) phases of the cycle. Grey areas in panels B and D correspond to periods of darkness. Data are shown as mean with standard deviation (n = 4). Superscript letters illustrate statistical differences among different photoperiod regimes in A (one-way ANOVA followed by Tukey's test, $P < 0.05$), among different time points in B (RM-ANOVA followed by Bonferroni test, $P < 0.05$), and between day/night in E (paired sample *t*-test, $P < 0.05$), but no statistical differences were detected among different time points in C and D (RM-ANOVA, $P > 0.05$).

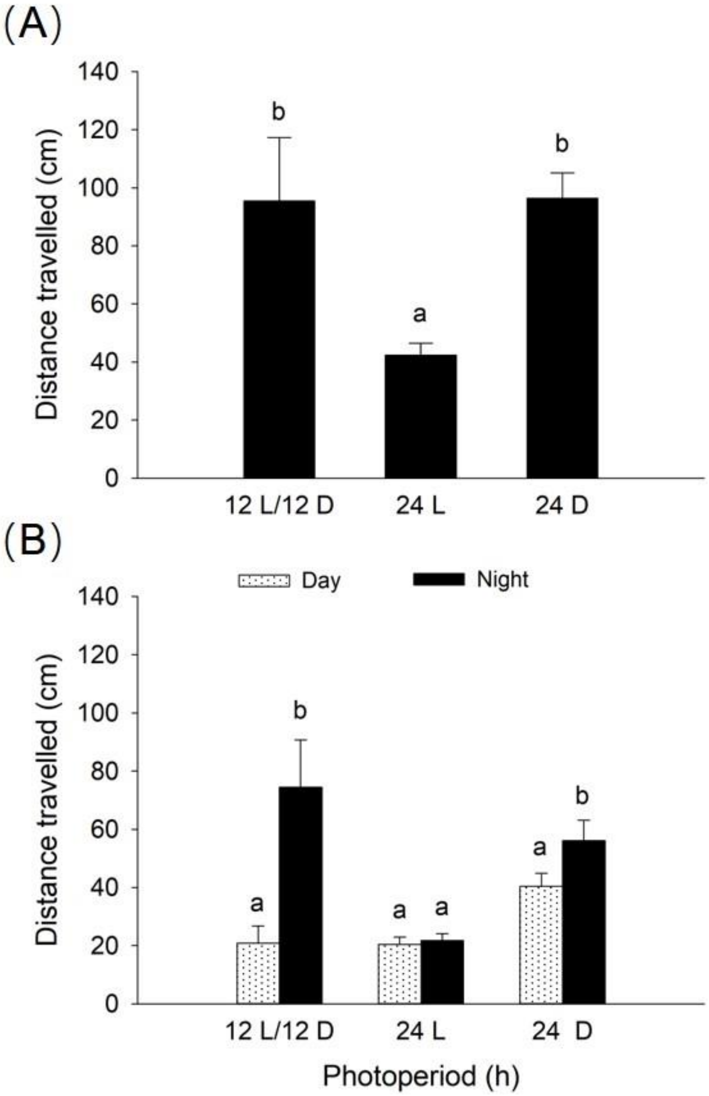

Figure 4.4 Effect of photoperiod on distance travelled by *C. frondosa*. (A) Distance travelled under different photoperiod regimes over the 24-h cycle. (B) Distance travelled during the diurnal (08:00-20:00, 12 h) versus nocturnal (20:00-08:00, 12 h) phases of the cycle. Data are shown as mean with standard deviation (n = 4) and means with different letters are significantly different (one-way ANOVA followed by Tukey's test or paired sample *t*-test, $P < 0.05$).

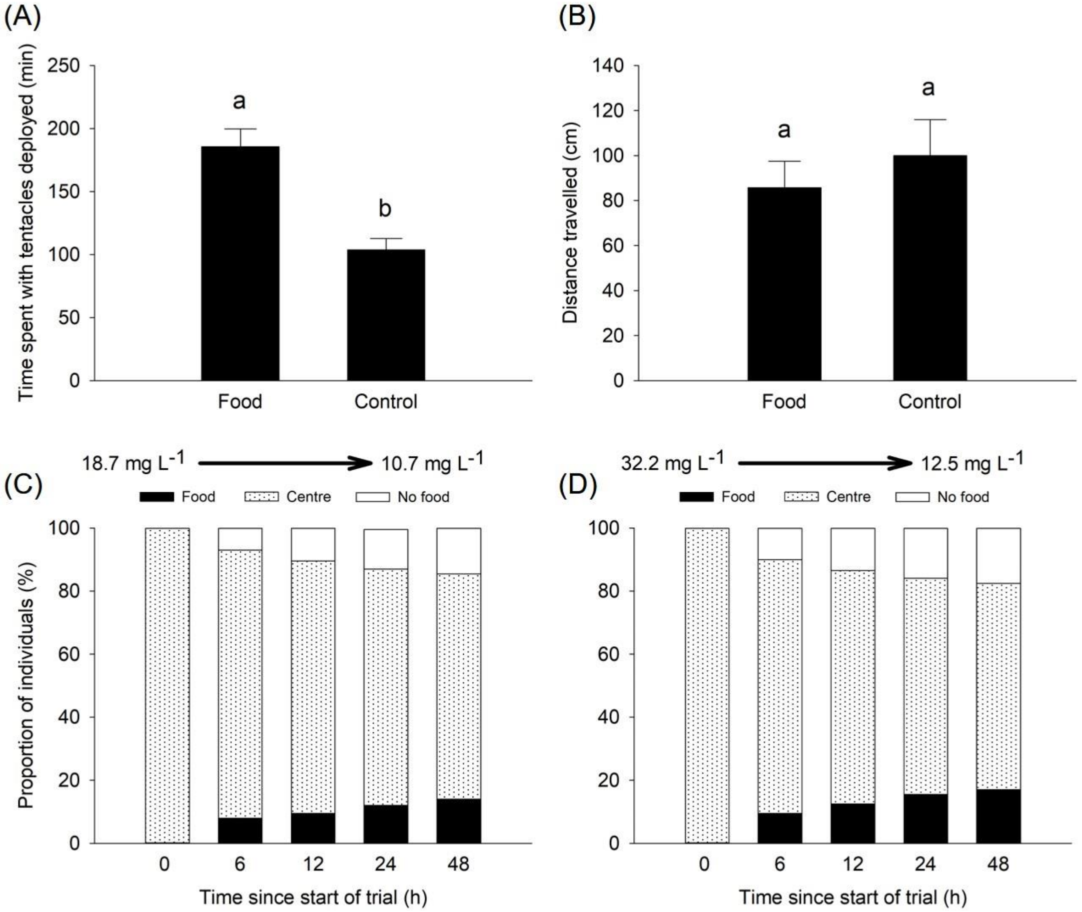

Figure 4.5 Effect of phytoplankton on feeding and locomotor behaviours in *C. frondosa*. (A) Time spent with tentacles deployed and (B) distance travelled over 24 h under control and spiked food conditions (small-scale experiments). Spatial distribution, shown by the proportion of individuals in three locations (food side, centre, no-food side) of the experimental arena (mesocosm) under gradients of (C) low phytoplankton concentration and (D) high phytoplankton concentration treatments over time (0, 6, 12, 24, and 48 h). The values above each panel in C and D illustrate the decreasing phytoplankton concentration gradient. Data are shown as mean (n = 4) with standard deviation in A and B, and different letters highlight significant differences between treatment and control (*t*-test, $P < 0.05$).

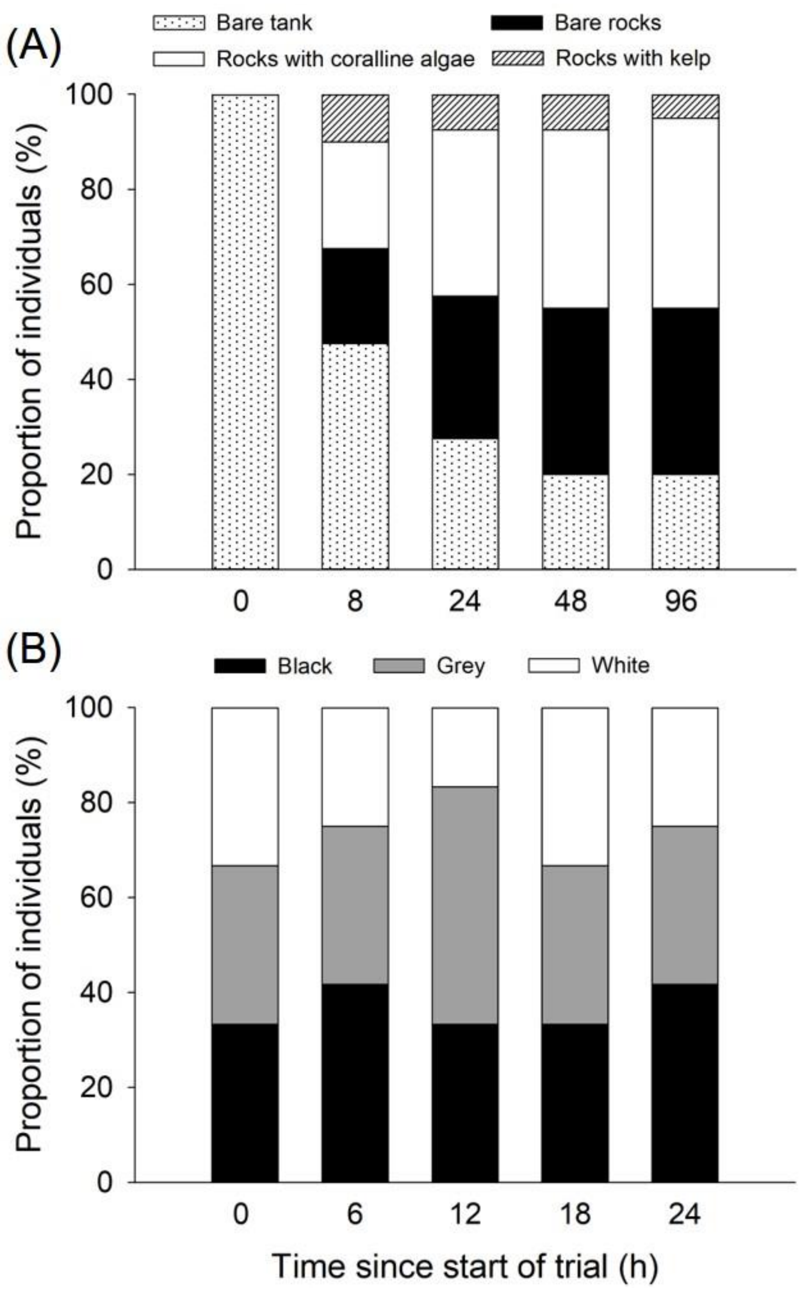

Figure 4.6 Effect of substrate type and background colour on distribution in *C. frondosa*. Mean proportion (n = 4) of individuals on various (A) substrate types and (B) background colours over time.

4.10 Supplementary material

Table S.4.1 Results of *t*-test of the distribution of *C. frondosa* between food and no food sections of the tank under low and high food concentrations over time (6, 12, 24 and 48 h)

Treatments	**Time since start of trial (h)***	***t***	***P***
Low food concentration	6	0.66	0.537
	12	-0.63	0.550
	24	-0.29	0.780
	48	1.81	0.121
High food concentration	6	-0.29	0.780
	12	-0.34	0.743
	24	-0.17	0.868
	48	-0.17	0.870

* Individuals were placed in the centre of the experimental arena (initial position) at time 0 h

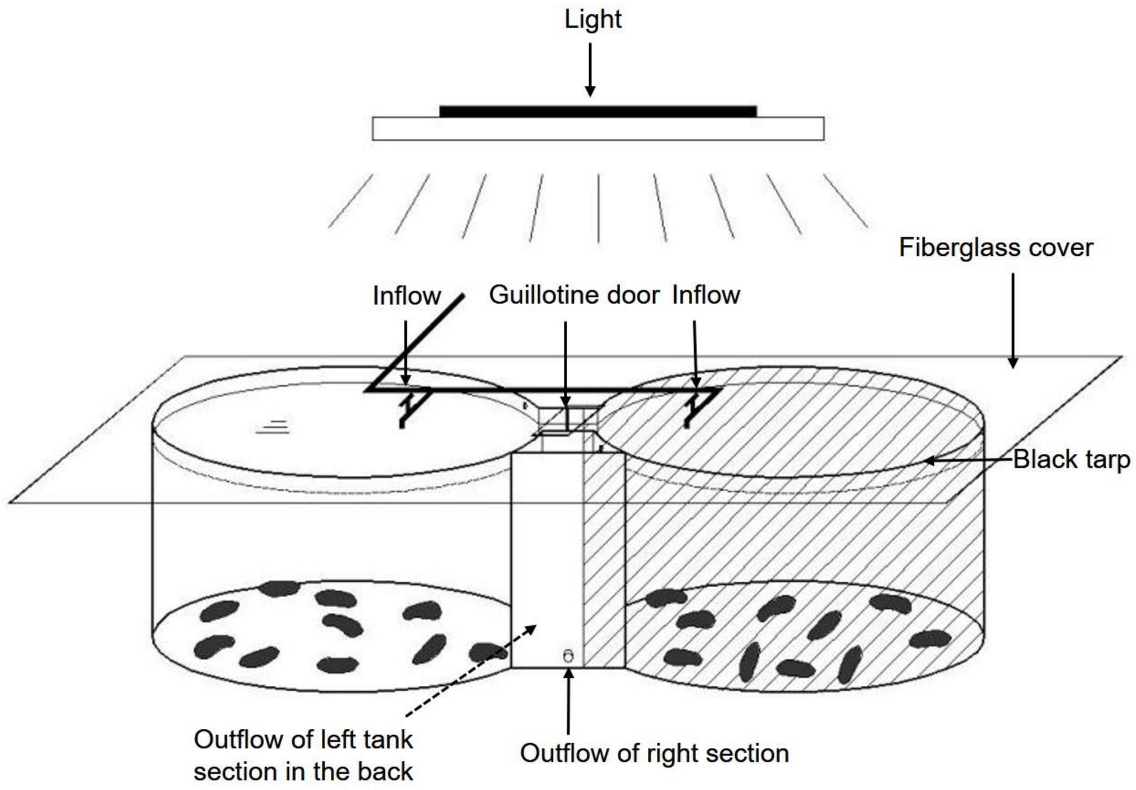

Figure S.4.1 Diagram of the experimental setup of a dual round tank system. Each section (81 cm diameter × 40 cm depth) had an independent inflow and outflow system. The two sections were linked by a narrow corridor equipped with a guillotine door (width: 7.5 cm). Half of the fiberglass cover was wrapped with black tarp to keep either one of the two tank sections in shade. The light intensities in the illuminated section varied between 90-126 lux under the weak light treatment and 420-560 lux under the strong light treatment; whereas light intensities in the shade section were 2-6 lux in both treatments.

Chapter 5. Health condition of the sea cucumber *Cucumaria frondosa* after multi-year holding in effluent waters of land-based salmon cultures[4]

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[4] A version of this manuscript is currently being prepared for submission to Aquaculture.

5.1 Abstract

Aquaculture activities are known to release excess dissolved and particulate matter into the surrounding environment and several methods have been proposed to mitigate this footprint, including co-culture of species occupying different trophic levels. In the present study, sea cucumbers either held in effluent water from salmon culture over 4 years or collected from the field were compared using stable isotope, lipid and fatty acid (FA) signatures as indicators of waste assimilation, health condition and biochemical composition. Enrichment of $\delta^{13}C$ in muscle bands and intestine and of $\delta^{15}N$ in all tissues was detected in captive individuals relative to wild individuals, indicating the uptake and assimilation of waste from salmon culture. The higher levels of FA biomarkers typical of salmon feed (18:1ω9, 18:2ω6 and 20:1ω9) and lower ω3/ω6 ratio in the captive sea cucumbers also implied assimilation of the waste. However, male and female sea cucumbers from the co-culture became smaller with time, their organ indices were lower than those of wild individuals (e.g. poorly developed gonad), and their biochemical composition differed in various aspects: triacylglycerols (TAG) content was greater in wild individuals and phospholipids (PL) content was greater in captive individuals. Also, FA profiles of all the tissues differed between the two groups of sea cucumbers, whereas total lipid contents of muscle bands and gonad remained similar. Overall, results are consistent with assimilation by *C. frondosa* of some of the particulate matter generated by salmon culture, suggesting it may help mitigate the footprint of this industry to some extent. In turn, the biochemical composition of the sea cucumbers changed and their reduced size and body indices suggested that this food source did not provide suitable nutrients to sustain growth and reproduction.

Key words: Co-culture, suspension-feeder, salmon, IMTA, stable isotope, fatty acid

5.2 Introduction

Atlantic salmon (*Salmo salar*) is one of the most intensively-farmed marine fish, owing to its amenability to high stocking densities and rapid growth, and to well established international markets (Jobling et al., 2010). Canada is the world's fourth largest producer of Atlantic salmon after Norway, United Kingdom and Chile (Liu and Sumaila, 2008). Although salmon farming brings unquestionable economic benefits, concern about its environmental costs is growing. Whether the expansion of intensive marine-finfish aquaculture can be sustained is being questioned in light of the potential effect of inorganic and organic wastes produced by fish farms (Navarrete-Mier et al., 2010; Wang et al., 2012). Atlantic salmon culture releases extra nitrogen (N) and phosphorus (P) and produce organic wastes in the form of unconsumed feed and feces (Pillay, 2008). An estimated 3% of salmon feed remains uneaten, representing approximately 12-17% of the total solid waste (Cromey et al., 2002). Consequently, salmon farming using sea cages may lead to eutrophication of the water column, while solid waste accumulates on the ocean floor, impacting the oxygen demand at the substrate/benthos, and leading to a decline of oxygen-sensitive species in favour of more resistant opportunistic species (Mayor and Solan, 2011; Pillay, 2008).

To make fish farming more sustainable in the long term, emphasis has been placed on exploring land-based models (Shpigel et al., 1993) and/or using integrated multi-trophic aquaculture (IMTA), which has the potential to reduce waste loading and environmental impacts and to increase the efficiency and productivity of intensive monoculture systems (Neori et al., 2004). In brief, IMTA makes use of by-products, including wastes, from one aquatic species to provide nutrients to another (Troell et al., 2009; Chopin et al., 2012). IMTA involves the culture of aquatic animals and the use of their waste products (excess feed and feces) as a food source for other commercially viable extractive species to favor environmental remediation, economic stability and social acceptability (Ridler et al., 2007; Troell et al., 2009). Many species have successfully been integrated into IMTA systems to extract inorganic and organic waste, such as seaweed (Abreu et al., 2011; Kang et al., 2008), mussels (MacDonald et al., 2011; Irisarri et al., 2015; Reid et al., 2010), and sea urchins (Orr et al., 2014; Sterling et al., 2016).

Sea cucumbers are high-value products from marine aquaculture and fisheries; they have also been identified as prospective extractive species for IMTA due to their ability to feed on the particulate waste generated by other animals (Zamora et al., 2018). Many studies have shown them to be good candidates for co-culture with finfish (Ahlgren, 1998; Yokoyama, 2013; Hannah et al., 2013), bivalves (Zhou et al., 2006; Slater and Carton, 2007; Paltzat et al., 2008; Yokoyama, 2015), gastropods (Kang et al., 2003; Maxwell et al., 2009), shrimps (Purcell et al., 2006; Bell et al., 2007) and jellyfish (Ren et al., 2014). In one case, Kang et al. (2008) co-cultured the deposit-feeding sea cucumber *Apostichopus japonicus* and the abalone *Haliotis discus* in circulating culture tanks to clean up the hatchery effluent during the overwintering period. They showed that ammonia and nitrite contents in seawater decreased, and that the survival and growth rates increased in co-cultured abalone compared to abalone cultured alone in the same experimental setup.

Moreover, Hannah et al. (2013) suggested that another deposit-feeding species (*Parastichopus californicus*) suspended directly below net pens of sablefish (*Anoplopoma fimbria*) grew faster and reduced the total organic carbon and total nitrogen contents of the fish feces by an average of 60% and 62%, respectively. The potential use of suspension-feeding sea cucumbers remains comparatively understudied and is only starting to be explored.

Cucumaria frondosa is the most common sea cucumber in the North Atlantic and one of the most abundant worldwide, occurring off New England (USA), in eastern and Arctic Canada, Greenland, and Scandinavia, as well as in the Faroe Islands and Russia (Hamel and Mercier, 2008; Jordan, 1972). Like many other commercially important sea cucumber species, *C. frondosa* has been fished extensively in its distribution range to meet the increasing demand from Asia (Hamel and Mercier, 2008). However, *C. frondosa* differs from most previously cultured holothuroid species. It is a cold-water species and a passive suspension-feeder that captures a wide range of particulate food (4-1500 µm), by extending its tentacles in the water column (Hamel and Mercier, 1998; Gianasi et al., 2017). A recent interest has arisen to utilize *C. frondosa* as a bio-filter within IMTA systems to help reduce particulate organic loading (Nelson et al., 2012b). It was shown that *C. frondosa* could ingest excess salmon feed and feces efficiently, both in the laboratory and in the natural environment, making it a candidate biofilter within an IMTA setting (Nelson et al., 2012a). What remains untested is whether individuals in this IMTA system can use energy from the ingested wastes to grow and whether their overall health and biochemical profile differ from those of wild individuals.

Organic matter can be incorporated into the tissue of an animal via its diet (Peterson and Fry, 1987) and its assimilation can be measured by a shift in the carbon and nitrogen stable isotope signatures (Post, 2002). Moreover, fatty acid biomarkers can be used to trace specific lipid sources and make inferences about the diet of aquatic animals. In recent years, greater levels of plant oils have been incorporated into salmon feed to reduce costs and minimize dependence on fish oil sources; such terrestrial lipid sources naturally contain high proportions of 18:1ω9, 18:2ω6 and 18:3ω3 (Skog et al., 2003; Narváez et al., 2008), which can be used as tracers of fish feed.

The present study was designed to compare several key metrics between wild individuals of *C. frondosa* and individuals held for 4 years in the effluent of an Atlantic salmon culture (i.e. IMTA system). The stable carbon and nitrogen isotope signatures and specific fatty acids in tissues were studied to confirm whether the co-cultured sea cucumbers assimilated the waste products coming from the salmons. Also, their general health condition and biochemical composition were measured to determine if sea cucumbers could be sustained on the effluent waste from salmon culture. In particular, whether co-cultured sea cucumbers could not only survive but grow and eventually reproduce in captivity was assessed.

5.3 Material and methods

5.3.1 Captive holding conditions (IMTA)

The experiment was performed in a land-based aquaculture facility (Wave Energy Research Centre of College of the North Atlantic) in Lord's Cove, Newfoundland (Canada), which was designed to demonstrate the viability of cascaded IMTA in an open flow-through tank system. Seawater was pumped into the facility from 120 m offshore at 10 m depth. The water was filtered to 200 µm by the filtration system (sand filters) before passing through a degassing column on the way to a 7000-L header tank. The filtered seawater was delivered directly to three 8000-L cylindrical tanks (2.9 × 1.3 m; diameter × depth). Around 200 juveniles (70-80 g) of Atlantic salmon (*Salmo salar*) were cultured in the three tanks (~ 70 individuals per tank) with flow-through water (~ 3000 L h^{-1}). They were fed the equivalent of 5-90 g of feed (EWOS®) per day per fish, depending on the size of fish. Effluent water from the salmon tanks was thoroughly mixed in a common head tank before being directed through a valve system at a rate of 25-30 L min^{-1}, to two 2000-L rectangular tanks (3.2 × 1.6 × 0.4 m; length × width × depth), each containing ~350 adults of *C. frondosa*. Light was provided through several windows and multiple fluorescent lights (32-Watt, 5000 Kelvin white T8) following natural photoperiod. All sea cucumbers had been collected by licensed harvesters at depths between 20 and 30 m in October 2013.

5.3.2 Sample collection for body indices and chemical analysis

To assess the state of sea cucumbers in the IMTA system, 40 individuals were sampled haphazardly after 20 months (June 2015) and after 32 months (June 2016) and their weights were measured. At the end of the study in August 2017, after 45 months under IMTA conditions, 12 sea cucumbers were collected from each of two holding tanks (n = 24 total). For comparison purposed, 24 adults were collected from the wild at approximately the same date (~10 day earlier). These wild sea cucumbers were of similar size, came from the same area (Grand Bank) and were caught using the same fishing gear as those placed in the IMTA 4 years prior. Individuals from the IMTA system and from the wild were transported to the laboratory in coolers with iced seawater (Gianasi et al., 2016), and separately held in two 500-L tanks with filtered seawater (200 µm) for 4 days to allow all feces to be evacuated.

The total wet weight of each individual (n = 48) was obtained and their contracted length (mouth-to-anus) measured using rope. The sex of each individual was determined with a gonad smear when determination from the genital papilla described by Montgomery et al. (2018) could not be made. All individuals were dissected and their different organs/tissues (body wall, longitudinal and circular muscle bands, intestine, respiratory tree) were separately weighted and stored at -20°C for further analysis. The wet weight of the body wall (without the aquapharyngeal bulb and muscle bands) was chosen as a denominator to establish the different organ indices (Hamel and Mercier, 1996).

In addition, the fish feed and the particulate wastes, representing the main food sources for the sea cucumbers held in the IMTA system, were collected from four locations on of the bottom of the effluent mixed tank (described above), by siphoning and passing through a 100-µm mesh. The filtrates were stored at -20°C until further analysis.

5.3.3 Sample analysis

The organs (muscle bands, intestine, and gonad) of 4 males and 4 females from either the IMTA system or the wild, together with the waste samples from effluent water, were submitted to the following analyses to confirm whether captive sea cucumbers assimilated the waste from salmon tanks and determine their respective biochemical composition.

5.3.3.1 Stable isotope and elemental analyses

Samples were oven-dried at 70°C for 24 hours, and then ground into a fine powder using mortar and pestle. To get rid of carbonates that might affect the stable C isotope ratio data, hydrochloric acid (HCl, 1 M) was added dropwise to waste samples until bubbles stopped forming. Samples were then rinsed 3 times with distilled water, and oven dried again. Subsamples of 1 mg were packed into tin cups and simultaneously analyzed for stable carbon and nitrogen isotope ratios, and for elemental C and N, at the Earth Resources Research and Analysis (TERRA) of the Core Research Equipment and Instrument Training Network (CREAIT) of Memorial University, using a Delta V Plus (Carlo Erba) continuous-flow isotope-ratio mass spectrometer. Isotope ratios are expressed in the conventional notation (i.e. $\delta^{13}C$ and $\delta^{15}N$) in parts per thousand (‰), following the equation:

$$\delta^{15}N \text{ or } \delta^{13}C = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R_{sample} is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The average standard deviation of selected replicates was ± 0.1‰ for $\delta^{15}N$ and ± 0.1‰ for $\delta^{13}C$. Total elemental C and N were measured as % of dry mass, and the average standard deviation was ± 3.2 for %C and ± 0.1 for %N. L-valine, USGS-24 graphite, IAEA-CH-6 sucrose, LSVEC, MUN-CO-1, and MUN-CO-2 were used as standards for stable carbon isotopes. IAEA-N-1, USGS-25, USGS-26, and L-valine were used to assess accuracy and precision of stable nitrogen isotope data. B2155 protein was used as a standard for $\delta^{13}C$ and $\delta^{15}N$.

5.3.3.2 Lipid content and lipid class analyses

Samples of muscle bands, gonad and intestine (0.5-1.0 g) were collected from each still-frozen individual to limit lipid oxidation and hydrolysis. Also, four samples of 3.5-4.5 g of waste were collected from the effluent mixing tank. Each sample was immersed in chloroform (8 ml), topped up with nitrogen gas (N_2), sealed with Teflon tape and stored in a freezer at -20 ºC until extraction.

Lipids were extracted and analyzed following Parrish (1999). Briefly, samples were homogenized in a chloroform:methanol:water (2:1:1) mixture, sonicated for 5 min, and centrifuged for 2 min. The bottom, organic layer was removed using a double pipetting technique involving placing two pipettes inside one another. Chloroform was then added to the sample, and the procedure was repeated three times. The top layer (lipid extracts) was pooled into a lipid-clean vial, and the total amount was blown down to volume under a gentle stream of nitrogen. Vials were sealed and stored at -20ºC until lipid and fatty acid analysis.

Lipid classes were determined by thin layer chromatography with flame ionization detection using an Iatroscan MK-6 and a three-stage development system to separate lipid classes. The first separation consisted of 25 min and 20 min developments in a mixture of hexane:diethyl ether:formic acid (98.95:1:0.05). The second development used a mixture of hexane, diethyl ether, and formic acid (79.9:20:0.1) for 40 min. The last separation consisted of a 15 min development of 100% acetone followed by two 10 min developments in chloroform:methanol:chloroform-extracted-water (5:4:1). Lipid classes were identified and quantified through comparison with a prepared standard. Data were processed using the PeakSimple Chromatography software (V4.51, SRI Instruments, US).

5.3.3.3 Fatty acid analysis

Fatty acids were determined from the same samples previously used for lipid class analysis. The fatty acid derivatization procedure is based on Parrish (1999). Briefly, an aliquot of the lipid extracts (muscle bands, 100 µl; intestine and gonad, 30 µl; waste, 10 µl), calculated in relation to the total amount of lipids within each sample, was transferred into a lipid clean vial and dried under N_2. After the addition of 1.5 ml of dichloromethane and 3 ml of Hilditch reagent (i.e. H_2SO_4 dissolved in methanol), the vials were sonicated, sealed with Teflon tape, and heated for 1 h at 100ºC. After cooling, 0.5 ml of saturated sodium bicarbonate and 1.5 ml of hexane were added to each vial, thus creating two layers. The upper, organic layer was removed and transferred into a new lipid clean vial. Finally, the solution was blown dry under N_2, and 0.5 ml of hexane was added to each vial. Vials were then sealed and kept at -20ºC until analysis. The samples were analyzed as fatty acids methyl esters (FAME) on a HP 6890 Gas Chromatograph FID equipped with a 7683 autosampler. Chromatograms were compared to a prepared standard and analysed using the software Varian Galaxie Chromatography Data System. Shorthand FA notations of the form A:BωX were used, where A represents the number of carbons atoms, B the number of double bonds and X is the position of the first double bond closest the terminal methyl group (CH_3).

5.3.4 Statistical analysis

All data were tested for normality and equal variance using Kolmogorov-Smirnov and Levene's tests ($\alpha = 0.05$), respectively. Differences in health condition between the sea cucumbers from the field (wild individuals) and from the IMTA system (captive individuals), were assessed using *t*-test for each organ index (muscle bands, intestine, gonad and respiratory tree). Stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and elemental N (%N) and C (%C), and total lipid content were also compared between the two groups using *t*-test. Permutational multivariate ANOVA (PERMANOVA) was performed to explore the FA composition variability among captive and wild individuals, and waste. Data in the text are expressed as mean ± standard deviation. Statistical analyses were conducted with Sigmaplot® (Systat Software, San Jose, CA, USA) and Primer® (Primer-E, Lutton, UK).

5.4 Results

5.4.1 Morphological metrics and organ indices

Overall, the survival rate of sea cucumbers under IMTA over 4 years was around 90% and the wet weight of captive individuals varied significantly over time ($F_{3,124} = 18.56$, $P < 0.001$). The wet weight of captive individuals decreased from 253 ± 52 g in June 2015 to 122 ± 60 g in August 2017 (Fig. 1). The freshly-collected wild sea cucumbers (229 ± 48 g) were significantly larger than the captive individuals at the end of this study (122 ± 60 g, Tukey's test, $P < 0.001$) and showed higher organ indices (Fig. 5.1, 5.2). All the organ indices of wild individuals were significantly greater than those of captive individuals, in both males (muscle bands, $t = 3.78$, $df = 16$, $P = 0.002$; intestine, $t = 3.30$, $df = 16$, $P = 0.005$; gonad, $t = 3.48$, $df = 16$, $P = 0.003$; respiratory tree, $t = 2.38$, $df = 16$, $P = 0.030$) and females (muscle bands, $t = 5.76$, $df = 20$, $P < 0.001$; intestine, $t = 2.16$, $df = 20$, $P = 0.043$; gonad, $t = 3.53$, $df = 20$, $P = 0.002$; respiratory tree, $t = 2.28$, $df = 20$, $P = 0.017$; Fig. 5.2). All the wild sea cucumbers had a well-developed gonad with the presence of reddish vitellogenic oocytes and small yellowish oocytes in the gonad tubules (Fig. S.5.1A), whereas only 67% of captive sea cucumbers had a gonad, and when present it was consistently smaller than in wild counterparts (Fig. S.5.1B). The gonad index displayed the greatest difference; with 22.0 ± 11.7% in wild males and 27.0 ± 15.1% in wild females, which was roughly three times the gonad index measured in captive males (7.2 ± 3.0%) and females (7.5 ± 4.4%) (Fig. 5.2).

5.4.2 Stable isotope and elemental analyses

Stable N isotope ratios ($\delta^{15}N$) of all organs of captive individuals were significantly greater than those of wild individuals, for both sexes (Table S.5.1). Specifically, a marked difference in $\delta^{15}N$ of intestine occurred between captive (male, 12.0 ± 0.8‰; female, 12.1 ± 0.5‰) and wild individuals (male, 7.1 ± 0.2‰, t = -12.51, df = 6, P < 0.001; female, 7.4 ± 0.7‰, t = -11.17, df = 6, P < 0.001). Stable C isotope ratios ($\delta^{13}C$) of muscle bands of captive sea cucumbers were -17.4 ± 0.2‰ in males and -17.3 ± 0.2‰ in females, significantly greater than in wild individuals (male, -18.3 ± 0.4‰, t = -3.83, df = 6, P = 0.009; female, -18.1 ± 0.5‰, t = -3.10, df = 6, P = 0.020). Also, $\delta^{13}C$ of intestine was significantly greater in captive than wild individuals, in both males (captive, -19.8 ± 0.5‰; wild, -21.8 ± 0.3‰, t = -6.19, df = 6, P < 0.001) and females (captive, -19.6 ± 0.1‰; wild, -21.1 ± 0.8‰, t = -3.52, df = 6, P = 0.013). However, no significant differences in $\delta^{13}C$ of the gonad was found between the two groups for both males (captive, -21.1 ± 0.4‰; wild, -21.1 ± 0.4‰, t = 0.06, df = 6, P = 0.953) and females (captive, -20.9 ± 0.3‰; wild, -20.9 ± 0.7‰, t = -0.19, df = 6, P = 0.857). Overall, sea cucumbers held in the IMTA system displayed enrichment in $\delta^{13}C$ and $\delta^{15}N$ relative to those collected from the wild (Fig. 5.3). In addition, $\delta^{13}C$ and $\delta^{15}N$ values obtained for the samples of salmon waste were -22.4 ± 0.3‰ and 11.8 ± 0.2‰, respectively.

The concentration of elemental N was greater in captive than wild individuals for muscle bands and intestine, but not for the gonad. Specifically, the concentration of elemental N in percent dry mass was significantly greater in the muscle bands (*t* = -3.35, *df* = 6, *P* = 0.015) and intestine (*t* = -3.49, *df* = 6, *P* = 0.013) of captive males (11.8 ± 0.5%, 9.5 ± 0.4%) than wild males (10.4 ± 0.7%, 8.6 ± 0.3%). The same was true in females for both muscle bands (captive, 12.0 ± 0.1%; wild, 10.8 ± 0.6%, *t* = -3.86, *df* = 6, *P* = 0.008) and intestine (captive, 9.9 ± 0.5%; wild, 8.4 ± 0.6%, *t* = -3.85, *df* = 6, *P* = 0.008). However, the percent dry mass of N in the gonad was significantly greater in wild than in captive individuals for males (captive, 6.5 ± 0.7%; wild, 8.2 ± 1.5%, *t* = 2.13, *df* = 6, *P* = 0.048) and females (captive, 7.1 ± 0.9%; wild, 8.6 ± 0.8%, *t* = 2.66, *df* = 6, *P* = 0.038). The concentration of elemental C in percent dry mass was significantly greater in the muscle bands (*t* = -3.12, *df* = 6, *P* = 0.021) and gonad (*t* = -6.86, *df* = 6, *P* < 0.001) of captive males (46.0 ± 1.9%, 55.6 ± 1.1%) than wild males (41.8 ± 1.9%, 48.0 ± 2.0%). The same was true in females for both muscle bands (captive, 46.1 ± 0.4%; wild, 42.7 ± 1.5%, *t* = -4.18, *df* = 6, *P* = 0.006) and gonad (captive, 54.1 ± 0.7%; wild, 50.2 ± 1.4%, *t* = -4.92, *df* = 6, *P* = 0.003). The percent dry mass of elemental C in the intestine was significantly greater in captive than in wild females (captive, 49.2 ± 2.0%; wild, 45.7 ± 1.8%, *t* = -2.57, *df* = 6, *P* = 0.042) but no significant difference occurred in males (captive, 48.9 ± 2.4%; wild, 47.4 ± 3.1%, *t* = -0.81, *df* = 6, *P* = 0.450).

5.4.3 Lipid classes and total lipids

The following lipid classes were found in all tissues of wild and captive sea cucumbers: acylated glyceryl ethers (AGE), triacylglycerols (TAG), sterols (ST) and phospholipids (PL). However, hydrocarbons (HC) were only found in gonad tissue, and acetone mobile polar lipids (AMPL) occurred in the gonad and intestine but not in muscle bands. For both groups of sea cucumbers, total lipid content was generally higher in the gonad (males: captive, 26.0 mg g^{-1}; wild, 27.2 mg g^{-1}, females: captive, 25.9 mg g^{-1}; wild, 26.6 mg g^{-1}) than in the intestine (males: captive, 12.0 mg g^{-1}; wild, 14.1 mg g^{-1}, females: captive, 9.2 mg g^{-1}; wild, 11.4 mg g^{-1}) and muscle bands (males: captive, 3.2 mg g^{-1}; wild, 3.3 mg g^{-1}, females: captive, 3.1 mg g^{-1}; wild, 3.6 mg g^{-1}, Fig. 5.4).

For all tissues examined in both sexes, total lipid contents were consistent between wild and captive sea cucumbers, except for the intestine of males, where the total lipid levels were significantly greater in wild (14.1 ± 1.0 mg g^{-1}) than in captive individuals (12.0 ± 1.0 mg g^{-1}, $t = 2.98$, $df = 6$, $P = 0.025$; Fig. 5.4). When comparing each lipid class between wild and captive sea cucumbers, the gonad of the latter displayed low content of AMPL (males, 0.20 ± 0.05 mg g^{-1}; females, 0.23 ± 0.06 mg g^{-1}), whereas AMPL was not detected in the gonad of wild individuals. The PL content was greater in captive than wild individuals and, inversely, the TAG content was higher in wild individuals for both sexes (Table 5.1).

5.4.4 Fatty acids

For both groups of sea cucumbers, the most abundant FA in muscle bands were: eicosapentaenoic acid, 20:5ω3 (EPA; 27.8-36.0%); docosatetraenoic acid, 22:4ω6 (9.0-20.0%); and hexadecatetraenoic acid, 16:4ω3 (6.9-13%) and the majority of FA found in the gonad comprised palmitoleic acid, 16:1ω7 (20.4-22.5%) and EPA (12.7-22.5%). Also, large proportions of EPA (31.3-36.4%) were found in the intestine of both wild and captive sea cucumbers.

The FA composition of all the tissues (muscle bands, gonad and intestine) differed between wild and captive sea cucumbers, in both sexes, and between sea cucumber tissues and waste material (Fig. 5.5, 5.6, 5.7); these differences were significant according to the PERMANOVA analysis (Table S.5.2). When comparing each fatty acid between wild and captive individuals, 16:1ω7 content was greater in all tissues of the former for both sexes (Table 5.2); however, the contents of oleic acid (18:1ω9), linoleic acid (18:2ω6) and gadoleic acid (20:1ω9) were greater in the tissues of captive than wild individuals. Regardless of sex, the arachidonic acid (ARA, 20:4ω6) content of muscle bands and intestine were greater in captive than in wild individuals. The EPA content in muscle bands and intestine of wild individuals were similar to those of captive individuals, in both males and females, but the contents in the gonad were nearly twice greater in wild (21.0-22.5%) than captive individuals (12.7-13.5%). The docosahexaenoic acid (DHA, 22:6ω3) was detected in the gonads of wild individuals but not in the gonads of captive individuals; moreover, DHA content in the intestine of both males and females of captive individuals (3.9-4.7%) was roughly three times that found in wild counterparts (1.1-1.3%).

5.5. Discussion

While *C. frondosa* has been identified as a potential candidate for IMTA, limited information currently exists on the suitability of suspension-feeding sea cucumbers in such systems. From a study of sea cucumbers either held close to a fish farming site or held in the laboratory supplemented with fish feed and feces, Nelson et al. (2012) found that *C. frondosa* was capable of capturing and ingesting excess salmon feed and feces, suggesting its potential use as an extractive species. The isotope and fatty acid profiles determined here support that *C. frondosa* not only ingests but can assimilate the organic waste of effluent water from salmon farming; however, the net outcome for its health appears to be negative.

Assimilation of food resources is known to be reflected in the stable isotope signature of animal tissues (Peterson and Fry, 1987), which gets shifted towards that of the food source (Post, 2002). The present results are in line with this description. Individuals held in a land-based IMTA system along with salmon for nearly 4 years displayed enrichment in $\delta^{13}C$ and $\delta^{15}N$ relative to those collected from the wild. The food available in the IMTA was chiefly composed of salmon waste (residual feed and feces) as the water supplying the system was filtered, thereby minimizing the availability of natural plankton. In contrast, previous studies have shown that seston, including plankton and particulate organic and inorganic matter, are the main food sources of wild *C. frondosa* (Singh et al., 1998; Hamel and Mercier, 1998). The isotopic signatures of these food sources differed accordingly, with waste material displaying -22.4‰ for $\delta^{13}C$ and 11.8‰ for $\delta^{15}N$, whereas Ostrom and Macko (1992) and Ostrom et al. (1997) reported lower δ13C and δ15N values for seston (plankton and particulate materials) in Newfoundland (-23.5‰ and 3‰, respectively). The enrichment in the $\delta^{13}C$ and $\delta^{15}N$ signatures of captive individuals is therefore consistent with processing and assimilation of the salmon waste materials. Another study conducted on a deposit feeder, *Australostichopus mollis*, showed that isotope signatures of sea cucumbers at the farm site were more enriched relative to those of individuals from the natural site (Slater and Carton, 2010). Alternately, or additionally, some of the changes in $\delta^{13}C$ and $\delta^{15}N$ signatures might be the result of long-term starvation (discussed later on).

Certain specific fatty acids (FA) have been successfully used as tracers of fish feed to determine its assimilation by aquatic animals (Gao et al., 2006; Redmond et al., 2010; Irisarri et al., 2015). Here, all the tissues of the individuals held in the IMTA system had greater levels of FA biomarkers of fish feed (18:1ω9, 18:2ω6 and 20:1ω9) than wild individuals of both sexes, in line with assimilation of residual feed by sea cucumbers. In addition, fish feed now use plant oils (Skog et al., 2003; Narváez et al., 2008), which are depleted in ω3 PUFA and rich in ω6 PUFA (Menoyo et al., 2007). Therefore, a lower ω3/ω6 ratio in all the tissues of captive sea cucumbers compared to wild individuals further suggests that salmon feed was assimilated by sea cucumbers. Similarly, previous studies showed the assimilation of fish feed by mussels cultured close to fish pens or supplemented with crushed fish pellets or fish effluents, based on the lower ω3/ω6 ratio in their digestive gland and mantle tissue relative to those of mussels from the natural site (Gao et al., 2006; Redmond et al., 2010; Irisarri et al., 2015; Handå et al., 2012).

While the shift in biochemical composition supports an assimilation of particulate waste present in effluent water from salmon tanks, *C. frondosa* may not have been able to ingest enough of it (due to rapid sinking) or did not get nutrients of sufficient quality through this system based on its decreasing body size over the years. Insufficient or poor-quality food was described to induce auto-digestion of stored nutrient reserves in the different organs (including body wall and muscle bands) resulting in progressive weight loss (see below). Previous studies suggested that stable isotope analysis might serve as an index of nutritional stress (Gannes et al., 1997). Specifically, nitrogen isotope ratios appear to be particularly sensitive in that $\delta^{15}N$ of the whole body should increase with the duration of starvation (Hatch, 2012). Therefore, the enrichment in the $\delta^{15}N$ signatures of captive individuals in the present study may be related to their long-term starvation. However, a number of studies have challenged this assumption (Gorokhova and Hansson, 1999; Hatch, 2012) so whether starvation affected the stable isotope signatures of sea cucumbers in the present study remains to be clarified. What is clear is that the physical condition of sea cucumbers held for 4 years under IMTA differed from that of wild individuals; they were overall smaller, suggesting that the salmon waste did not provide adequate nourishment for their sustenance and growth. Muscle bands are one of the most important energy storage organs in *C. frondosa*, and their weight-based proportion is closely related to food supply and reproductive state (Hamel and Mercier, 1996; Gianasi et al., 2017). The lower index of muscle bands in captive sea cucumbers further suggests long-term malnutrition, whereby the energy stored in the muscle bands was used for other basal activities. In addition, spawning in captive sea cucumbers was only recorded in the first year of the 4-year study, likely enabled by stored nutrients still present in the gonad. A third of captive individuals did not have a gonad at the end of the study, and in those that did, the gonad was not well developed.

Previous studies have suggested that the biochemical composition of sea cucumbers was reflective of their diet (Yu et al., 2015; Wen et al., 2016; Gianasi et al., 2017) as also described in other taxa (Kanazawa et al., 1979). In the present study, differences in specific lipid classes between wild and captive sea cucumbers were observed. TAG in male and female tissues was greater in wild than captive individuals; this lipid class was described to play a role as energy storage in echinoderms and can be accumulated during periods of high food availability (Prowse et al., 2017). The variability of TAG likely reflects the different energy allocation strategies, i.e. how energy is distributed towards growth, survival, and reproduction (Parzanini et al., 2018). For example, the proportion of TAG displayed increasing trends in the pre-spawning period, and declined in ovaries of *C. frondosa* post spawning (Verkaik et al., 2016; Gianasi et al., 2017). The reduction of TAG in captive individuals may suggest that they maximized their survival at the expense of preserving energetic lipid storage. Similarly, TAG decreased more slowly in fed than unfed larvae of the sea urchin *Heliocidaris tuberculata* (Prowse et al., 2017). However, the greater content of PL in captive *C. frondosa* may be related to the high content of PL in waste (main food source). The present study also revealed that the FA composition of all the tissues of wild individuals differed from captive individuals of both sexes. The FA biomarker of diatom, 16:4ω1, was detected in all tissues of wild sea cucumbers but not in any tissue of captive individuals, and the content of 16:1ω7 was greater in all the tissues of wild individuals than captive individuals. This supports the absence of diatoms as a food source in the IMTA system. However, the presence of bacterial fatty acids (i.e. i15:0, ai15:0, 15:0, i16:0, ai16:0) (Kaneda, 1991) in the tissues of both wild and captive individuals provides an indicator of bacterial contribution to their diet. In aquatic organisms, ARA, EPA and DHA are considered essential nutrients because they are required for optimal health (growth, reproduction, and immunity) and most of them are unable to synthesize them *de novo* (Parrish, 2009). Here, the proportion of EPA in the gonad was lower in captive than wild individuals of both sexes and DHA was not detected at all in the captive individuals. This may explain the poor development of gonad and the lack of reproduction in captive sea cucumbers.

The results of this study showed that suspension-feeding sea cucumbers held downstream of salmon cultures exhibited shifts in both stable carbon and nitrogen isotopes, and in some specific fatty acids indicative of salmon feed. This suggests that *C. frondosa* can perform the role of an extractive species, assimilating and incorporating salmon-farming waste, and mitigating the accumulation of heavier solids, while providing an additional remunerative product. However, the poor physical condition, associated with the change in biochemical composition of captive sea cucumbers indicates that waste from fish farming may not completely meet their nutritional requirements for normal growth and reproduction, questioning the possibility of growing juveniles of *C. frondosa* to commercial size under such conditions. In other words, a natural diet including phytoplankton or lipid-rich suspended particulate matter may be crucial to maintain overall health in *C. frondosa*, which was not provided under the present salmon IMTA system. Further experimental trials will be required to obtain more precise information on the effects of salmon waste, what nutrients may be missing, whether supplements can offset any deficiencies, and whether the flavor and texture of sea cucumbers from IMTA systems will be changed. Importantly, the present study was conducted in a land-based aquaculture facility using filtered seawater, whereas the most popular salmon farming method uses cages (pens) at sea. It is possible that such an open-ocean design could provide enough natural seston for *C. frondosa* to remain healthy, although whether it would still ingest and assimilate salmon waste is uncertain. Additional work is needed to measure the assimilative capacity of suspension-feeding sea cucumbers inside various IMTA designs, including salmon floating pens and co-culture with other extractive species, such as bivalves.

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5.7 References

Abreu, M.H., Pereira, R., Yarish, C., Buschmann, A.H., Sousa-Pinto, I., 2011. IMTA with *Gracilaria vermiculophylla*: productivity and nutrient removal performance of the seaweed in a land-based pilot scale system. Aquaculture. 312, 77-87.

Ackefors, H., Enell, M., 1994. The release of nutrients and organic matter from aquaculture systems in Nordic countries. Journal of Applied Ichthyology. 10, 225-241.

Ahlgren, M.O., 1998. Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: implications for polyculture. Journal of the World Aquaculture Society. 29, 133-139.

Bell, J.D., Agudo, N.N., Purcell, S.W., Blazer, P., Simutoga, M., Pham, D., Della Patrona, L., 2007. Grow-out of sandfish *Holothuria scabra* in ponds shows that co-culture with shrimp *Litopenaeus stylirostris* is not viable. Aquaculture. 273, 509-519.

Chopin, T., Cooper, J.A., Reid, G., Cross, S., Moore, C., 2012. Open-water integrated multi-trophic aquaculture: environmental biomitigation and economic diversification of fed aquaculture by extractive aquaculture. Reviews in Aquaculture. 4, 209-220.

Cromey, C.J., Nickell, T.D., Black, K.D., 2002. DEPOMOD—modelling the deposition and biological effects of waste solids from marine cage farms. Aquaculture. 214, 211-239.

Folke, C., Kautsky, N., 1992. Aquaculture with its environment: prospects for sustainability. Ocean & Coastal Management. 17, 5-24.

Gannes, L.Z., O'Brien, D.M., Del Rio, C.M., 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology. 78, 1271-1276.

Gao, Q.-F., Shin, P.K., Lin, G.-H., Chen, S.-P., Cheung, S.G., 2006. Stable isotope and fatty acid evidence for uptake of organic waste by green-lipped mussels *Perna viridis* in a polyculture fish farm system. Marine Ecology Progress Series. 317, 273-283.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2016. Experimental test of optimal holding conditions for live transport of temperate sea cucumbers. Fisheries Research. 174, 298-308.

Gianasi, B.L., Parrish, C.C., Hamel, J.-F., Mercier, A., 2017. Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquaculture Research. 48, 3413-3432.

Gorokhova, E., Hansson, S., 1999. An experimental study on variations in stable carbon and nitrogen isotope fractionation during growth of *Mysis mixta* and *Neomysis integer*. Canadian Journal of Fisheries and Aquatic Sciences. 56, 2203-2210.

Hamel, J.-F., Mercier, A., 1996. Studies on the reproductive biology of the Atlantic sea cucumber *Cucumaria frondosa*. SPC Beche-de-mer Information Bulletin. 8, 22-33.

Hamel, J.-F., Mercier, A., 1998. Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. Canadian Journal of Zoology. 76, 1194-1198.

Hamel, J.-F., Mercier, A., 2008. Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 293-306.

Handå, A., Ranheim, A., Olsen, A.J., Altin, D., Reitan, K.I., Olsen, Y., Reinertsen, H., 2012. Incorporation of salmon fish feed and feces components in mussels (*Mytilus edulis*): Implications for integrated multi-trophic aquaculture in cool-temperate North Atlantic waters. Aquaculture. 370, 40-53.

Hannah, L., Pearce, C., Cross, S., 2013. Growth and survival of California sea cucumbers (*Parastichopus californicus*) cultivated with sablefish (*Anoplopoma fimbria*) at an integrated multi-trophic aquaculture site. Aquaculture. 406, 34-42.

Hatch, K.A., 2012. The use and application of stable isotope analysis to the study of starvation, fasting, and nutritional stress in animals. in: McCue M.D. (Eds), The Comparative Physiology of Fasting, Starvation. Springer, Berlin/Heidelberg, Germany, pp. 337-364.

Irisarri, J., Fernández-Reiriz, M.J., Labarta, U., Cranford, P.J., Robinson, S.M., 2015. Availability and utilization of waste fish feed by mussels *Mytilus edulis* in a commercial integrated multi-trophic aquaculture (IMTA) system: a multi-indicator assessment approach. Ecological Indicators. 48, 673-686.

Jobling, M., Arnesen, A.-M., Befey, T., Carter, C., Hardy, R., LeFrancois, N., Keefe, R., Koskela, J., Lamarre, S., 2010. The salmonids (family: salmonidae). Finfish Aquaculture Diversification, 234-288.

Jordan, A.J., 1972. On the ecology and behavior of *Cucumaria frondosa* (Echinodermata: Holothurioidea) at Lamoine Beach, Maine. University of Maine, Orono.

Kanazawa, A., Teshima, S., Ono, K., 1979. Relationship between essential fatty acid requirements of aquatic animals and the capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. Comparative Biochemistry and Physiology. B, Comparative Biochemistry. 63, 295-298.

Kaneda, T., 1991. Iso-and anteiso-fatty acids in bacteria: biosynthesis, function, and taxonomic significance. Microbiology and Molecular Biology Reviews, 55, 288-302.

Kang, K.H., Kwon, J.Y., Kim, Y.M., 2003. A beneficial coculture: charm abalone *Haliotis discus hannai* and sea cucumber *Stichopus japonicus*. Aquaculture. 216, 87-93.

Kang, Y.H., Shin, J.A., Kim, M.S., Chung, I.K., 2008. A preliminary study of the bioremediation potential of *Codium fragile* applied to seaweed integrated multi-trophic aquaculture (IMTA) during the summer. Journal of Applied Phycology. 20, 183-190.

Liu, Y., Sumaila, U.R., 2008. Can farmed salmon production keep growing? Marine Policy. 32, 497-501.

MacDonald, B.A., Robinson, S.M., Barrington, K.A., 2011. Feeding activity of mussels (*Mytilus edulis*) held in the field at an integrated multi-trophic aquaculture (IMTA) site (*Salmo salar*) and exposed to fish food in the laboratory. Aquaculture. 314, 244-251.

Maxwell, K.H., Gardner, J.P., Heath, P.L., 2009. The effect of diet on the energy budget of the brown sea cucumber, *Stichopus mollis* (Hutton). Journal of the World Aquaculture Society. 40, 157-170.

Mayor, D.J., Solan, M., 2011. Complex interactions mediate the effects of fish farming on benthic chemistry within a region of Scotland. Environmental Research. 111, 635-642.

Menoyo, D., Lopez-Bote, C.J., Diez, A., Obach, A., Bautista, J.M., 2007. Impact of n − 3 fatty acid chain length and n − 3/n − 6 ratio in Atlantic salmon (*Salmo salar*) diets. Aquaculture. 267, 248-259.

Montgomery, E., Ferguson-Roberts, J., Gianasi, B., Hamel, J.-F., Kremenetskaia, A., Mercier, A., 2018. Functional significance and characterization of sexual dimorphism in holothuroids. Invertebrate Reproduction & Development. 62, 191-201.

Narváez, M., Freites, L., Guevara, M., Mendoza, J., Guderley, H., Lodeiros, C., Salazar, G., 2008. Food availability and reproduction affects lipid and fatty acid composition of the brown mussel, *Perna perna*, raised in suspension culture. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology. 149, 293-302.

Navarrete-Mier, F., Sanz-Lázaro, C., Marín, A., 2010. Does bivalve mollusc polyculture reduce marine fin fish farming environmental impact?. Aquaculture, 306, 101-107.

Nelson, E.J., MacDonald, B.A., Robinson, S.M., 2012a. The absorption efficiency of the suspension-feeding sea cucumber, *Cucumaria frondosa*, and its potential as an extractive integrated multi-trophic aquaculture (IMTA) species. Aquaculture. 370, 19-25.

Nelson, E.J., MacDonald, B.A., Robinson, S.M., 2012b. A review of the Northern sea cucumber *Cucumaria frondosa* (Gunnerus, 1767) as a potential aquaculture species. Reviews in Fisheries Science. 20, 212-219.

Neori, A., Chopin, T., Troell, M., Buschmann, A.H., Kraemer, G.P., Halling, C., Shpigel, M., Yarish, C., 2004. Integrated aquaculture: rationale, evolution and state of the art emphasizing seaweed biofiltration in modern mariculture. Aquaculture. 231, 361-391.

Orr, L.C., Curtis, D.L., Cross, S.F., Gurney-Smith, H., Shanks, A., Pearce, C.M., 2014. Ingestion rate, absorption efficiency, oxygen consumption, and fecal production in green sea urchins (*Strongylocentrotus droebachiensis*) fed waste from sablefish (*Anoplopoma fimbria*) culture. Aquaculture. 422, 184-192.

Ostrom, N.E., Macko, S.A., 1992. Sources, cycling, and distribution of water column particulate and sedimentary organic matter in northern Newfoundland fjords and bays: a stable isotope study. in: Whelan, J.K., Farrington, J.W. (Eds.), Organic Matter: Productivity, Accumulation, and Preservation in Recent and Ancient Sediments. Columbia University Press, New York, pp. 55-81.

Ostrom, N.E., Macko, S.A., Deibel, D., Thompson, R.J., 1997. Seasonal variation in the stable carbon and nitrogen isotope biogeochemistry of a coastal cold ocean environment. Geochimica et Cosmochimica Acta. 61, 2929-2942.

Paltzat, D., Pearce, C., Barnes, P., McKinley, R., 2008. Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). Aquaculture. 275, 124-137.

Parrish, C.C., 1999. Determination of total lipid, lipid classes, and fatty acids in aquatic samples. in: Arts, M.T., Wainman, B.C. (Eds.), Lipids in Freshwater Ecosystems. Springer, New York, NY, pp. 4-20.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. in: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, NY, pp. 309-326.

Parzanini, C., Parrish, C.C., Hamel, J.F., Mercier, A., 2018. Functional diversity and nutritional content in a deep-sea faunal assemblage through total lipid, lipid class, and fatty acid analyses. PLoS ONE. 13.

Peterson, B.J., Fry, B., 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics. 18, 293-320.

Pillay, T.V.R., 2008. Aquaculture and the Environment. Blackwell Publishing Ltd., Oxford, UK.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology. 83, 703-718.

Prowse, T.A.A., Sewell, M.A., Byrne, M., 2017. Three-stage lipid dynamics during development of planktotrophic echinoderm larvae. Marine Ecology Progress Series. 583, 149-161.

Purcell, S.W., Patrois, J., Fraisse, N., 2006. Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson). Aquaculture Research. 37, 515-522.

Redmond, K.J., Magnesen, T., Hansen, P.K., Strand, Ø., Meier, S., 2010. Stable isotopes and fatty acids as tracers of the assimilation of salmon fish feed in blue mussels (*Mytilus edulis*). Aquaculture. 298, 202-210.

Reid, G., Liutkus, M., Bennett, A., Robinson, S., MacDonald, B., Page, F., 2010. Absorption efficiency of blue mussels (*Mytilus edulis* and *M. trossulus*) feeding on Atlantic salmon (*Salmo salar*) feed and fecal particulates: implications for integrated multi-trophic aquaculture. Aquaculture. 299, 165-169.

Ren, Y., Dong, S., Wang, X., Gao, Q., Jiang, S., 2014. Beneficial co-culture of jellyfish *Rhopilema esculenta* (Kishinouye) and sea cucumber *Apostichopus japonicus* (Selenka): implications for pelagic-benthic coupling. Aquaculture Research. 45, 177-187.

Ridler, N., Wowchuk, M., Robinson, B., Barrington, K., Chopin, T., Robinson, S., Page, F., Reid, G., Szemerda, M., Sewuster, J., 2007. Integrated multi− trophic aquaculture (IMTA): A potential strategic choice for farmers. Aquaculture Economics & Management. 11, 99-110.

Shpigel, M., Neori, A., Popper, D.M., Gordin, H., 1993. A proposed model for “environmentally clean” land-based culture of fish, bivalves and seaweeds. Aquaculture. 117, 115-128.

Singh, R., MacDonald, B.A., Lawton, P., Thomas, M.L., 1998. Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. Canadian Journal of Zoology. 76, 1842-1849.

Skog, T.E., Hylland, K., Torstensen, B.E., Berntssen, M.H., 2003. Salmon farming affects the fatty acid composition and taste of wild saithe *Pollachius virens* L. Aquaculture Research. 34, 999-1007.

Slater, M.J., Carton, A.G., 2007. Survivorship and growth of the sea cucumber *Australostichopus (Stichopus) mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. Aquaculture. 272, 389-398.

Sterling, A.M., Cross, S.F., Pearce, C.M., 2016. Co-culturing green sea urchins (*Strongylocentrotus droebachiensis*) with mussels (*Mytilus spp.*) to control biofouling at an integrated multi-trophic aquaculture site. Aquaculture. 464, 253-261.

Troell, M., Joyce, A., Chopin, T., Neori, A., Buschmann, A.H., Fang, J.-G., 2009. Ecological engineering in aquaculture—potential for integrated multi-trophic aquaculture (IMTA) in marine offshore systems. Aquaculture. 297, 1-9.

Verkaik, K., Hamel, J-F., Mercier, A., 2016. Carry-over effects of ocean acidification in a cold-water lecithotrophic holothuroid. Marine Ecology Progress Series. 557, 189-206.

Wang, X., Olsen, L. M., Reitan, K. I., Olsen, Y., 2012. Discharge of nutrient wastes from salmon farms: environmental effects, and potential for integrated multi-trophic aquaculture. Aquaculture Environment Interactions, 2, 267-283.

Wen, B., Gao, Q.-F., Dong, S.-L., Hou, Y.-R., Yu, H.-B., Li, W.-D., 2016. Effects of different feed ingredients on growth, fatty acid profiles, lipid peroxidation and aminotransferases activities of sea cucumber *Apostichopus japonicus* (Selenka). Aquaculture. 454, 176-183.

Wu, R., 1995. The environmental impact of marine fish culture: towards a sustainable future. Marine Pollution Bulletin. 31, 159-166.

Yokoyama, H., 2013. Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages—potential for integrated multi-trophic aquaculture. Aquaculture. 372, 28-38.

Yokoyama, H., 2015. Suspended culture of the sea cucumber *Apostichopus japonicus* below a Pacific oyster raft–potential for integrated multi-trophic aquaculture. Aquaculture Research. 46, 825-832.

Yu, H.-B., Gao, Q.-F., Dong, S.-L., Wen, B., 2015. Changes in fatty acid profiles of sea cucumber *Apostichopus japonicus* (Selenka) induced by terrestrial plants in diets. Aquaculture. 442, 119-124.

Zamora, L.N., Yuan, X., Carton, A.G., Slater, M.J., 2018. Role of deposit-feeding sea cucumbers in integrated multitrophic aquaculture: progress, problems, potential and future challenges. Reviews in Aquaculture. 10, 57-74.

Zhou, Y., Yang, H., Liu, S., Yuan, X., Mao, Y., Liu, Y., Xu, X., Zhang, F., 2006. Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets. Aquaculture. 256, 510-520.

5.8 Tables

Table 5.1 Lipid classes in salmon culture waste and in tissues (muscle bands, gonad and intestine) of male and female individuals of wild and captive sea cucumbers. The hyphen indicates that the concentration was below detection. Data are shown as means ± s.d. (n = 4) in mg g^{-1} wet weight

		Male						**Female**					
		Muscle bands		**Gonad**		**Intestine**		**Muscle bands**		**Gonad**		**Intestine**	
Lipid classes	**Waste**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**
HC	0.09 ± 0.01	-	-	0.32 ± 0.11	0.29 ± 0.06	-	-	-	-	0.30 ± 0.03	0.28 ± 0.03	-	-
AGE	-	0.11 ± 0.03	0.10 ± 0.02	7.89 ± 3.17	8.63 ± 2.67	0.56 ± 0.17	0.55 ± 0.15	0.10 ± 0.03	0.08 ± 0.02	9.31 ± 2.18	8.44 ± 3.73	0.53 ± 0.26	0.56 ± 0.21
TAG	0.83 ± 0.10	1.39 ± 0.45	0.19 ± 0.08	10.76 ± 4.22	8.67 ± 3.27	3.60 ± 1.62	0.95 ± 0.24	1.24 ± 0.41	0.21 ± 0.05	10.78 ± 5.36	8.12 ± 3.73	3.67 ± 0.56	0.95 ± 0.20
FFA	3.20 ± 0.46	-	-	2.51 ± 0.35	3.20 ± 0.95	2.48 ± 1.32	0.78 ± 0.27	-	-	2.43 ± 1.21	3.32 ± 0.44	2.05 ± 0.73	0.73 ± 0.25
ALC	-	-	0.09 ± 0.02	-	0.31 ± 0.11	-	0.05 ± 0.01	-	0.09 ± 0.05	-	0.29 ± 0.02	-	0.05 ± 0.01
ST	0.37 ± 0.05	0.09 ± 0.01	0.19 ± 0.03	1.12 ± 0.35	1.07 ± 0.10	0.23 ± 0.08	0.95 ± 0.24	0.09 ± 0.01	0.20 ± 0.01	0.97 ± 0.29	1.08 ± 0.18	0.29 ± 0.09	0.74 ± 0.14

AMPL	0.18 ± 0.06	-	-	-	0.20 ± 0.05	0.60 ± 0.07	0.63 ± 0.30	-	-	-	0.23 ± 0.06	0.62 ± 0.11	0.61 ± 0.15
PL	4.07 ± 0.50	1.68 ± 0.33	2.68 ± 0.38	3.65 ± 1.14	4.63 ± 1.16	6.60 ± 1.64	8.06 ± 0.94	1.58 ± 0.30	2.56 ± 0.32	2.84 ± 0.21	4.15 ± 1.42	4.26 ± 0.44	5.52 ± 0.98

*Lipid classes: HC, hydrocarbons; AGE, acylated glyceryl ethers; TAG, triacylglycerols; FFA, free fatty acids; ALC, alcohols; ST, sterols; AMPL, acetone mobile polar lipids; PL, phospholipids

Table 5.2 Fatty acid composition of waste from salmon farming, and of tissues (muscle bands, gonad and intestine) in male and female indivuduals of wild and captive sea cucumbers. The hyphen indicates that the concentration was below detection. Data are shown as means ± s.d. (n = 4) as proportions (%)

		Male						**Female**					
		Muscle bands		**Gonad**		**Intestine**		**Muscle bands**		**Gonad**		**Intestine**	
Fatty acid	**Waste**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**
14:0	8.5 ± 1.9	4.6 ± 0.7	0.4 ± 0.1	3.9 ± 0.6	0.5 ± 0.2	6.4 ± 1.6	0.3 ± 0.1	3.5 ± 1.6	0.3 ± 0.1	3.6 ± 0.7	0.8 ± 0.4	6.2 ± 2.1	0.4 ± 0.1
i15:0	0.4 ± 0.0	-	-	-	-	-	-	-	-	-	-	-	-
ai15:0	-	1.4 ± 0.2	0.6 ± 0.2	13.4 ± 3.4	12.0 ± 2.1	2.0 ± 0.5	1.5 ± 0.3	1.1 ± 0.2	0.5 ± 0.2	13.3 ± 3.3	13.4 ± 2.1	2.0 ± 0.3	1.3 ± 0.4
15:0	0.8 ± 0.04	-	-	0.7 ± 0.2	1.0 ± 0.2	0.2 ± 0.1	-	-	-	0.6 ± 0.2	0.9 ± 0.1	0.2 ± 0.1	-
i16:0	-	0.2 ± 0.1	0.2 ± 0.1	1.8 ± 0.3	4.0 ± 0.1	0.7 ± 0.2	-	0.2 ± 0.1	0.2 ± 0.1	2.0 ± 0.2	3.7 ± 0.3	0.6 ± 0.2	-
ai16:0	-	0.2 ± 0.1	-	0.3 ± 0.0	0.3 ± 0.1	-	-	0.2 ± 0.1	-	0.2 ± 0.1	0.3 ± 0.1	-	-
16:0	32.0 ± 0.8	4.3 ± 0.6	2.1 ± 0.3	4.6 ± 1.1	2.2 ± 0.4	2.3 ± 0.2	1.8 ± 0.2	3.4 ± 0.5	2.0 ± 0.2	3.5 ± 0.5	2.7 ± 0.5	2.3 ± 0.3	1.7 ± 0.3
16:1ω7	7.0 ± 0.3	4.0 ± 0.8	1.2 ± 0.2	22.5 ± 1.4	20.4 ± 1.8	13.4 ± 2.2	1.4 ± 0.8	6.4 ± 0.8	0.8 ± 0.5	22.1 ± 1.4	20.1 ± 2.0	15.5 ± 2.9	3.5 ± 2.0
16:1ω5	0.3 ± 0.1	0.2 ± 0.1	-	0.7 ± 0.2	1.3 ± 0.2	-	-	0.2 ± 0.1	-	0.7 ± 0.1	0.8 ± 0.1	-	-
ai17:0	0.2 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	1.2 ± 0.1	5.4 ± 1.3	0.2 ± 0.1	0.9 ± 0.3	0.3 ± 0.1	0.4 ± 0.2	1.4 ± 0.4	5.6 ± 1.4	0.2 ± 0.0	1.1 ± 0.2
16:2ω4	0.4 ± 0.0	0.4 ± 0.2	-	0.9 ± 0.2	0.3 ± 0.1	0.9 ± 0.1	-	0.5 ± 0.2	-	0.9 ± 0.1	0.3 ± 0.1	1.1 ± 0.2	-
16:3ω4	0.1 ± 0.0	0.1 ± 0.0	-	0.5 ± 0.2	1.5 ± 0.1	0.6 ± 0.2	-	0.2 ± 0.1	-	0.6 ± 0.3	1.2 ± 0.7	0.7 ± 0.2	-

17:1	-	-	-	0.3 ± 0.2	0.6 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	-	-	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.2
16:4ω3	-	6.9 ± 3.5	13.0 ± 0.5	2.5 ± 0.3	2.1 ± 0.5	4.2 ± 1.7	8.2 ± 3.5	9.4 ± 2.0	9.9 ± 3.7	2.0 ± 1.0	2.4 ± 0.6	3.9 ± 1.0	7.8 ± 2.0
16:4ω1	0.1 ± 0.1	0.7 ± 0.7	-	1.8 ± 0.4	-	1.9 ± 0.5	-	0.9 ± 0.5	-	1.3 ± 0.4	-	2.5 ± 0.7	-
18:0	7.6 ± 0.3	4.0 ± 0.6	2.0 ± 0.4	3.0 ± 0.9	1.0 ± 0.3	4.2 ± 0.4	2.6 ± 1.1	3.6 ± 0.4	1.9 ± 0.2	2.9 ± 1.1	1.4 ± 0.4	4.1 ± 0.1	3.2 ± 1.4
18:1ω9	11.5 ± 0.4	1.1 ± 0.2	2.0 ± 0.4	3.8 ± 0.9	4.7 ± 1.0	2.5 ± 0.5	3.4 ± 0.5	1.5 ± 1.2	1.8 ± 0.4	3.9 ± 0.5	5.4 ± 1.1	3.2 ± 0.4	4.2 ± 0.3
18:1ω7	6.0 ± 0.1	1.7 ± 1.6	2.2 ± 0.6	3.6 ± 0.4	9.1 ± 1.3	2.9 ± 0.3	3.1 ± 0.4	3.2 ± 0.2	2.6 ± 0.8	4.1 ± 0.6	8.2 ± 1.4	3.0 ± 0.4	3.5 ± 1.0
18:2ω6	4.1 ± 0.1	0.8 ± 0.3	1.7 ± 0.3	0.4 ± 0.0	1.5 ± 0.4	0.8 ± 0.0	3.1 ± 0.8	1.0 ± 0.3	1.4 ± 0.9	0.4 ± 0.1	1.9 ± 0.1	0.8 ± 0.1	3.0 ± 0.9
20:1ω9	2.0 ± 1.1	2.8 ± 1.9	6.5 ± 0.8	1.0 ± 0.2	1.7 ± 0.7	0.9 ± 0.2	4.9 ± 0.5	2.9 ± 0.5	5.4 ± 3.1	1.2 ± 0.4	1.6 ± 0.4	1.5 ± 0.8	4.0 ± 1.5
20:4ω6	1.1 ± 0.1	2.2 ± 0.6	5.9 ± 0.3	-	-	0.5 ± 0.3	3.0 ± 0.8	2.2 ± 0.4	6.3 ± 1.1	-	-	0.5 ± 0.1	2.2 ± 0.7
20:5ω3	2.8 ± 0.2	36.0 ± 2.8	27.8 ± 0.8	22.5 ± 3.3	12.7 ± 0.8	35.3 ± 3.5	36.4 ± 6.6	35.1 ± 1.3	32.0 ± 3.9	21.0 ± 3.2	13.5 ± 1.4	34.0 ± 2.5	31.3 ± 7.9
22:1ω7	-	-	-	0.8 ± 0.1	1.0 ± 0.1	1.3 ± 0.4	-	-	-	0.7 ± 0.2	0.7 ± 0.4	0.9 ± 0.2	-
22:4ω6	1.7 ± 0.1	9.0 ± 1.0	19.3 ± 1.4	2.0 ± 0.4	5.4 ± 0.5	3.6 ± 1.4	12.5 ± 2.6	9.9 ± 2.8	20.0 ± 2.1	2.3 ± 0.7	5.6 ± 1.3	3.5 ± 1.5	9.9 ± 5.4
22:6ω3	3.0 ± 0.2	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.2	-	1.3 ± 0.1	3.9 ± 0.5	0.7 ± 0.4	0.5 ± 0.4	0.9 ± 0.3	-	1.1 ± 0.3	4.7 ± 0.7
24:1	0.6 ± 0.1	2.1 ± 0.4	1.5 ± 0.4	2.7 ± 0.3	1.8 ± 0.2	2.2 ± 0.4	0.6 ± 0.2	2.0 ± 0.3	1.7 ± 0.3	2.3 ± 0.3	1.3 ± 0.3	1.9 ± 0.2	0.8 ± 0.3
∑ SFA	51.1 ±1.9	14.5 ± 2.5	7.4 ± 0.7	12.7 ± 2.6	6.0 ± 1.5	16.4 ± 1.2	7.2 ± 3.0	11.6 ± 2.4	6.3 ± 1.8	11.1 ± 3.7	6.4 ± 1.2	15.0 ± 2.4	8.9 ± 5.8
∑ MUFA	32.3 ± 1.9	22.6 ± 2.2	13.4 ± 0.8	36.1 ± 2.5	43.6 ± 3.9	25.4 ± 3.9	14.9 ± 3.4	19.3 ± 2.5	15.4 ± 2.6	39.2 ± 3.9	41.2 ± 2.3	28.0 ± 2.7	17.9 ± 7.4

∑ PUFA	15.5 ± 0.8	60.3 ± 4.5	78.1 ± 1.1	34.5 ± 4.8	28.8 ± 1.4	55.0 ± 5.4	76.9 ± 6.3	67.1 ± 4.8	77.2 ± 1.9	32.8 ± 4.4	29.6 ± 1.6	54.1 ± 5.5	70.5 ± 13.4
ω3/ω6	0.8 ± 0.1	3.6 ± 0.3	1.5 ± 0.2	10.8 ± 1.5	2.1 ± 0.3	8.3 ± 2.1	2.6 ± 0.4	3.5 ± 0.7	1.5 ± 0.3	8.9 ± 2.1	2.1 ± 0.3	8.1 ± 1.3	2.9 ± 0.4

SFA = saturated fatty acids, MUFA = monounsaturated fatty acids and PUFA = polyunsaturated fatty acids

5.9 Figures

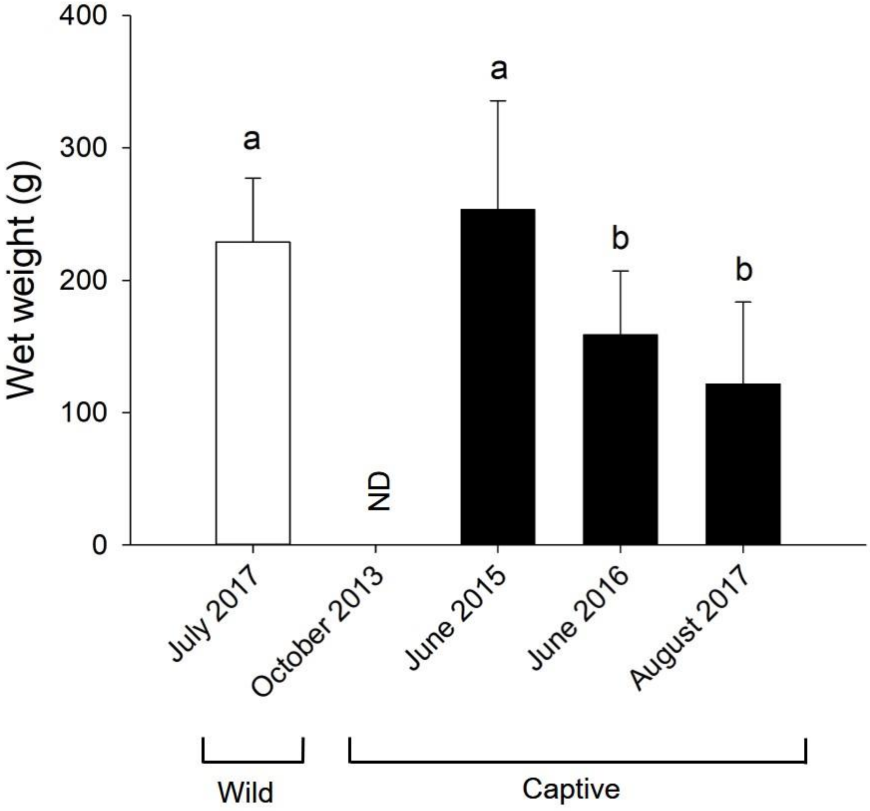

Figure 5.1 Wet weight of wild individuals (collected in 2017) and change in the wet weight of captive individuals over time (2013-2017). Data are shown as means ± s.d. (n = 24 for wild and captive individuals in August 2017; n = 40 for 2015 and 2016). Means with different letters are significantly different (ANOVA, $P < 0.05$). ND: not determined.

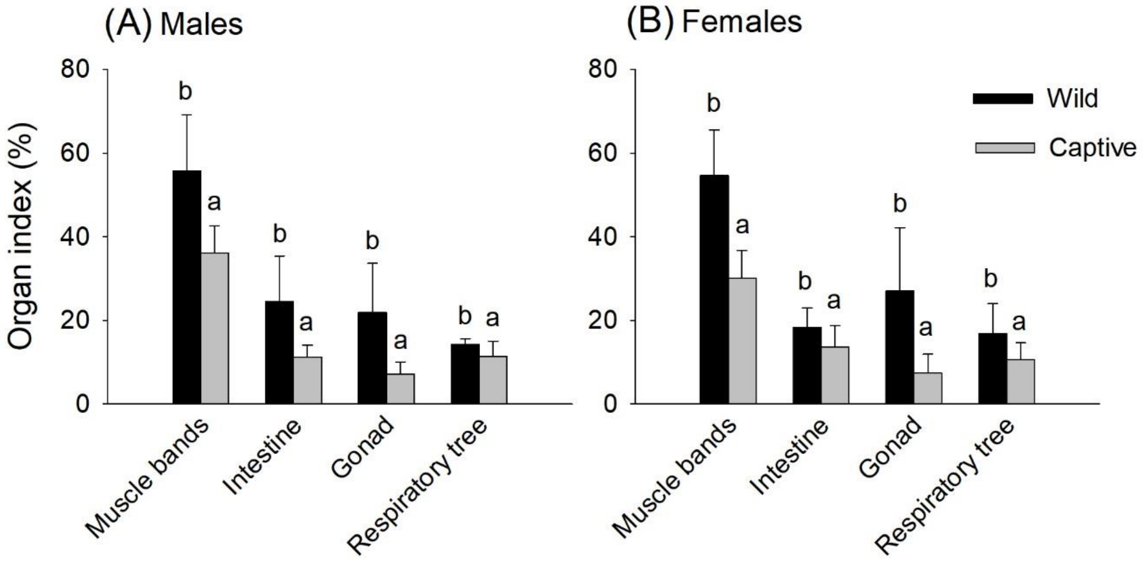

Figure 5.2 Organ indices of (A) male and (B) female individuals of wild and captive sea cucumbers. Data are shown as means ± s.d. (n = 10 for wild and n = 8 for captive in A; n = 14 for wild and n = 8 for captive in B). Different letters show statistically significant differences (*t*-test, $P < 0.05$).

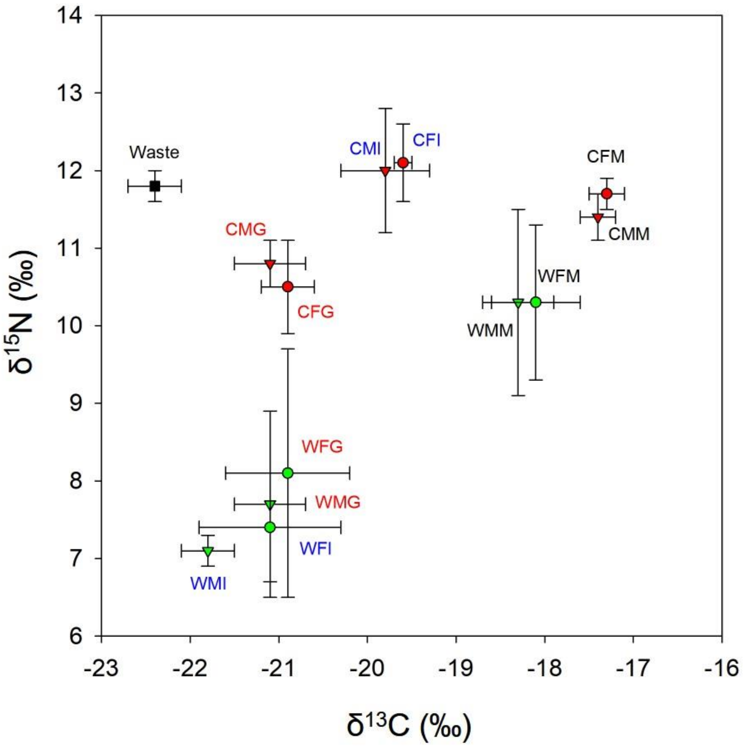

Figure 5.3 Biplot of δ^{13}C and δ^{15}N in the muscle bands, intestine and gonad of wild and captive sea cucumbers (males and females), and in the waste from salmon tanks (food for captive individuals). All the data are shown as means ± s.d. (n =4). WMM = wild male muscle bands, WFM = wild female muscle bands, WMI = wild male intestine, WFI = wild female intestine, WMG = wild male gonad, WFG = wild female gonad; CMM = captive male muscle bands, CFM = captive female muscle bands, CMI = captive male intestine, CFI = captive female intestine, CMG = captive male gonad, CFG = captive female gonad. The triangles and circles represent male and female individuals, respectively, and the green and red colours represent wild and captive individuals, respectively. The black square corresponds to waste material.

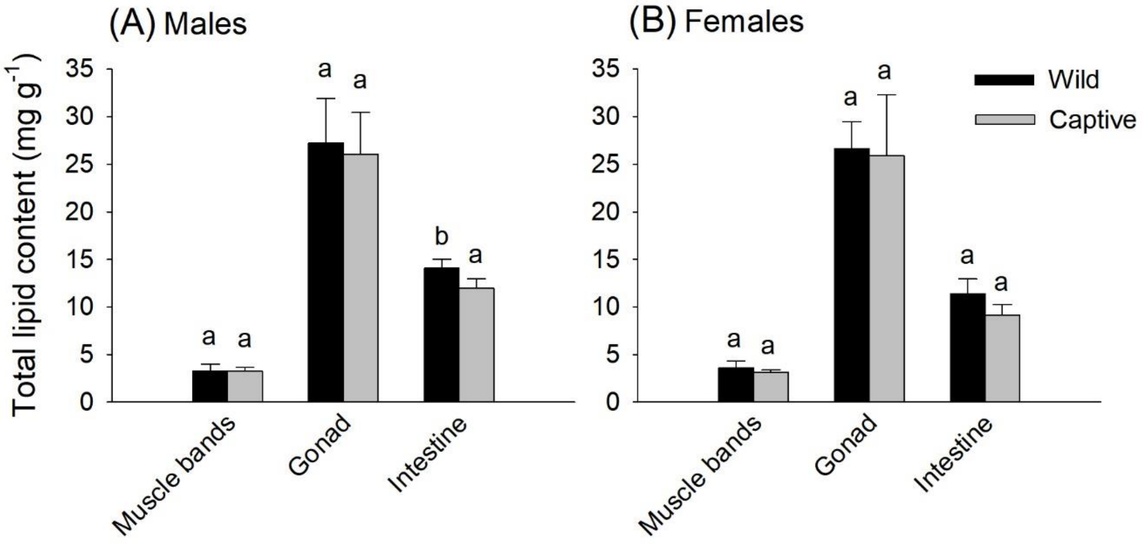

Figure 5.4 Total lipids in muscle bands, gonad and intestine of (A) male and (B) female individuals of wild and captive sea cucumbers. Data are shown as means ± s.d. (n = 4). Comparsions were made between wild and captive individuals (*t*-test), and means with different letters are significantly different ($P < 0.05$).

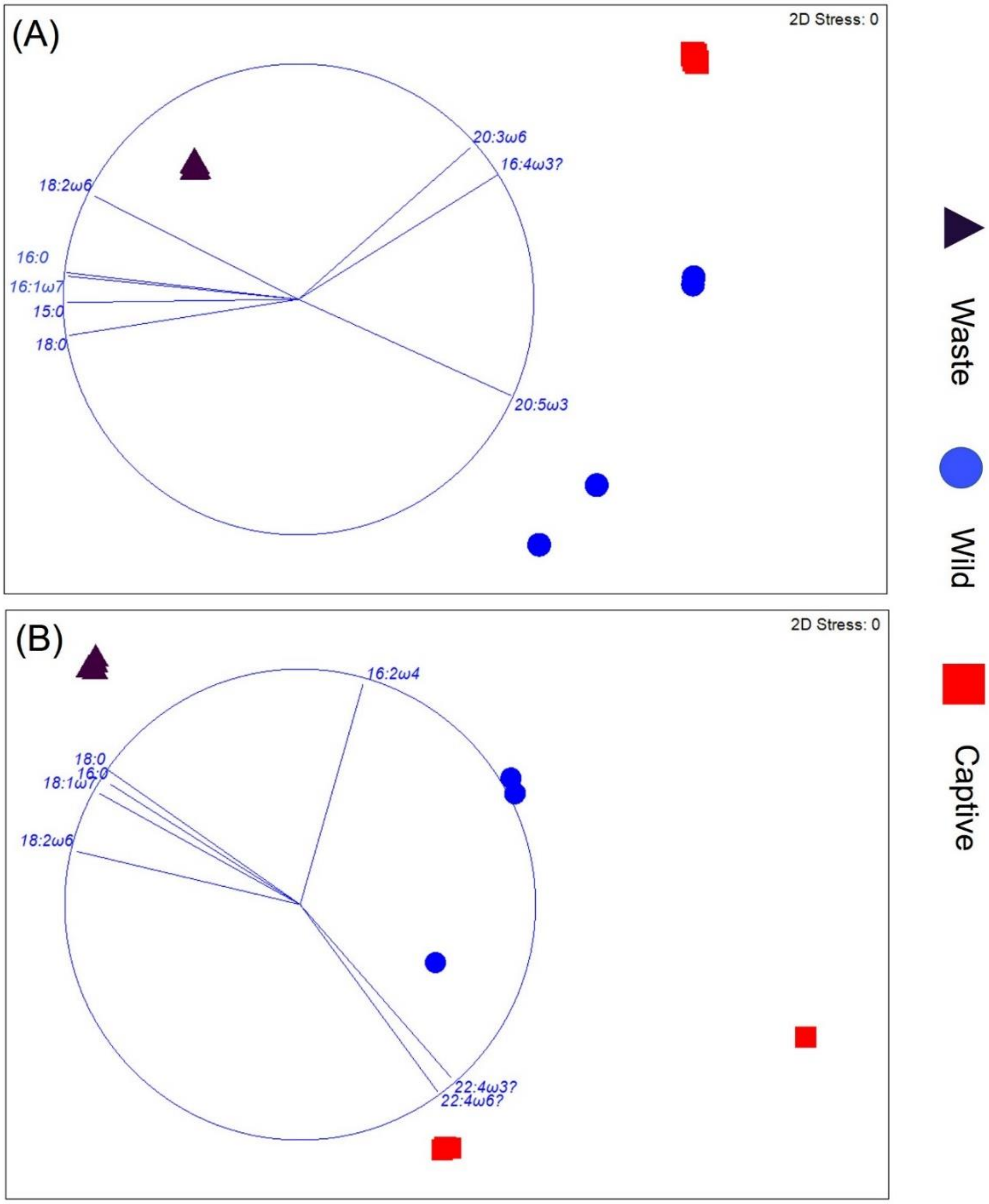

Figure 5.5 MDS plot of fatty acid composition of muscle bands in (A) male and (B) female individuals of wild and captive sea cucumbers, and in waste from salmon tanks (food for captive individuals).

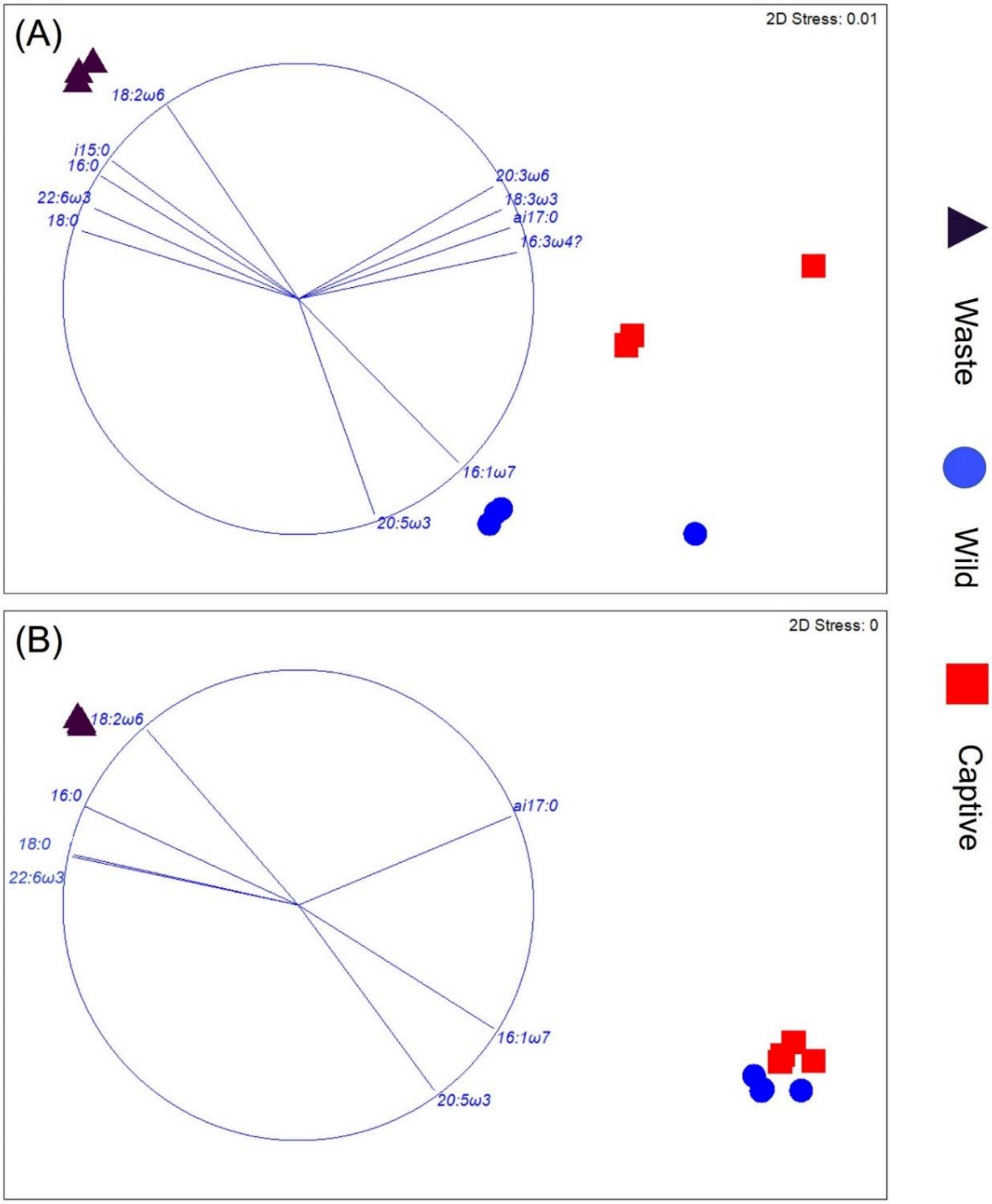

Figure 5.6 MDS plot of fatty acid composition of gonad in (A) male and (B) female individuals of wild and captive sea cucumbers, and in waste from salmon tanks (food for captive individuals).

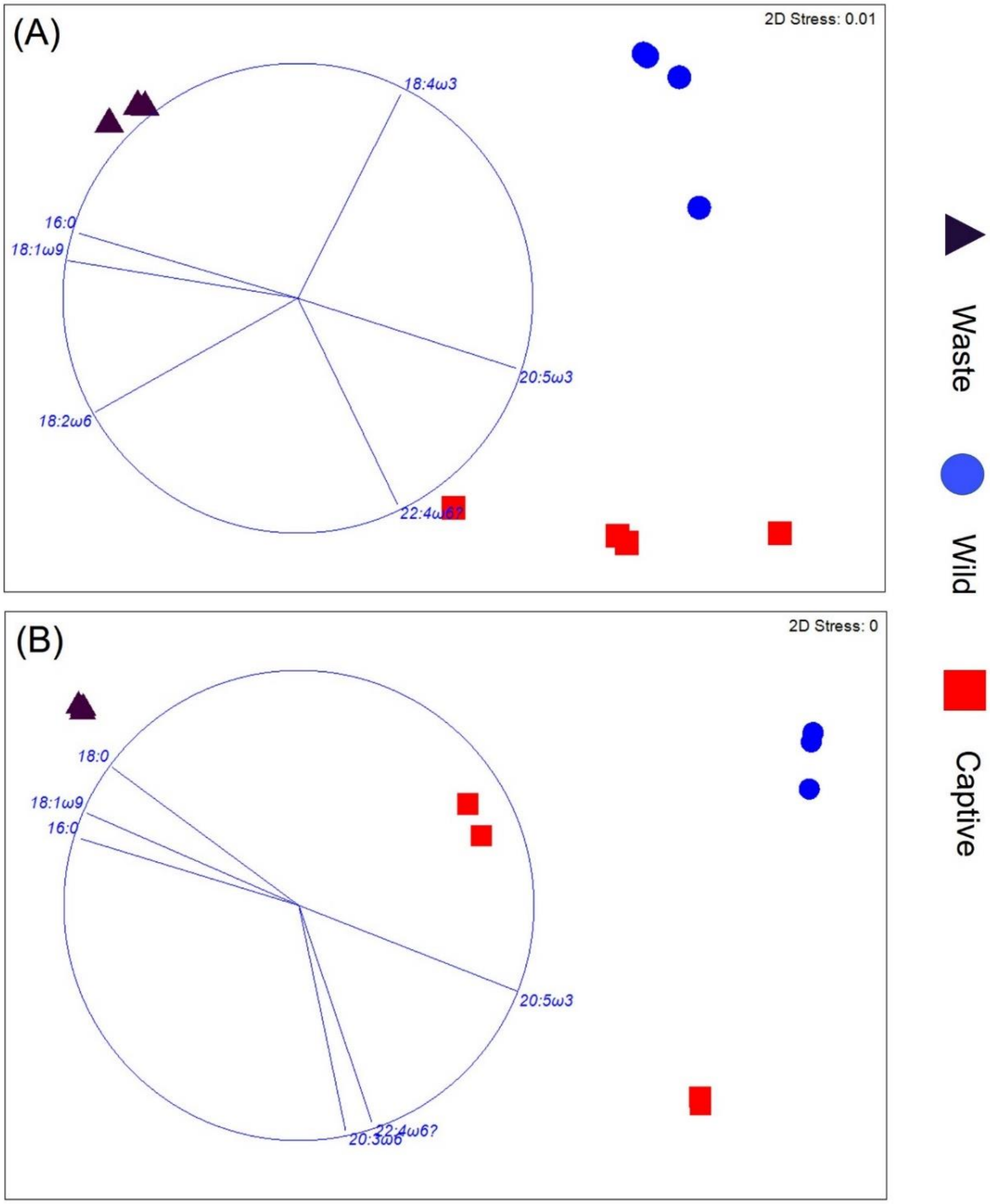

Figure 5.7 MDS plot of fatty acid composition of intestine in (A) male and (B) female individuals of wild and captive sea cucumbers, and in waste from salmon tanks (food for captive individuals).

5.10 Supplementary Material

Table S.5.1 Stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) and elemental N (%N) and C (%C) composition of waste from salmon tanks, and of tissues (muscle bands, gonad, and intestine) in captive and wild sea cucumbers. Data are shown as means ± s.d. (n = 4). Values for captive and wild individuals were compared using *t*-test; different letters indicate statistical differences.

		Male						**Female**					
		Muscle bands		**Gonad**		**Intestine**		**Muscle bands**		**Gonad**		**Intestine**	
	Waste	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**	**Wild**	**Captive**
%N	3.2 ± 0.1	10.4 ± 0.7^{a}	11.8 ± 0.5^{b}	8.2 ± 1.5^{b}	6.5 ± 0.7^{a}	8.6 ± 0.3^{a}	9.5 ± 0.4^{b}	10.8 ± 0.6^{a}	12.0 ± 0.1^{b}	8.6 ± 0.8b	7.1 ± 0.9^{a}	8.4 ± 0.6^{a}	9.9 ± 0.5^{b}
%C	27.0 ± 0.9	41.8 ± 1.9^{a}	46.0 ± 1.9^{b}	48.0 ± 2.0^{a}	55.6 ± 1.1^{b}	47.4 ± 3.1^{a}	48.9 ± 2.4^{a}	42.7 ± 1.5^{a}	46.1 ± 0.4^{b}	50.2 ± 1.4^{a}	54.1 ± 0.7^{b}	45.7 ± 1.8^{a}	49.2 ± 2.0^{b}
$\delta^{15}N$	11.8 ± 0.2	10.3 ± 1.2^{a}	11.4 ± 0.3^{b}	7.7 ± 1.2^{a}	10.8 ± 0.3^{b}	7.1 ± 0.2^{a}	12.0 ± 0.8^{b}	10.3 ± 1.0^{a}	11.7 ± 0.2^{b}	8.1 ± 1.6^{a}	10.5 ± 0.6^{b}	7.4 ± 0.7^{a}	12.1 ± 0.5^{b}
$\delta^{13}C$	-22.4 ± 0.3	-18.3 ± 0.4^{a}	-17.4 ± 0.2^{b}	-21.1 ± 0.4^{a}	-21.1 ± 0.4^{a}	-21.8 ± 0.3^{a}	-19.8 ± 0.5^{b}	-18.1 ± 0.5^{a}	-17.3 ± 0.2^{b}	-20.9 ± 0.7^{a}	-20.9 ± 0.3^{a}	-21.1 ± 0.8^{a}	-19.6 ± 0.1^{b}

Table S.5.2 Results of PERMANOVA analysis conducted on fatty acid profiles of salmon waste and of various tissuses in males and females of *Cucumaria frondosa*.

Sex	Tissues	Contrast	*P* (perm)
Male	Muscle bands	Captive vs Wild	0.046
		Captive vs Waste	0.031
		Wild vs Waste	0.038
	Gonad	Captive vs Wild	0.027
		Captive vs Waste	0.024
		Wild vs Waste	0.023
	Intestine	Captive vs Wild	0.025
		Captive vs Waste	0.027
		Wild vs Waste	0.025
Female	Muscle bands	Captive vs Wild	0.031
		Captive vs Waste	0.029
		Wild vs Waste	0.033
	Gonad	Captive vs Wild	0.029
		Captive vs Waste	0.029
		Wild vs Waste	0.031
	Intestine	Captive vs Wild	0.034
		Captive vs Waste	0.034
		Wild vs Waste	0.027

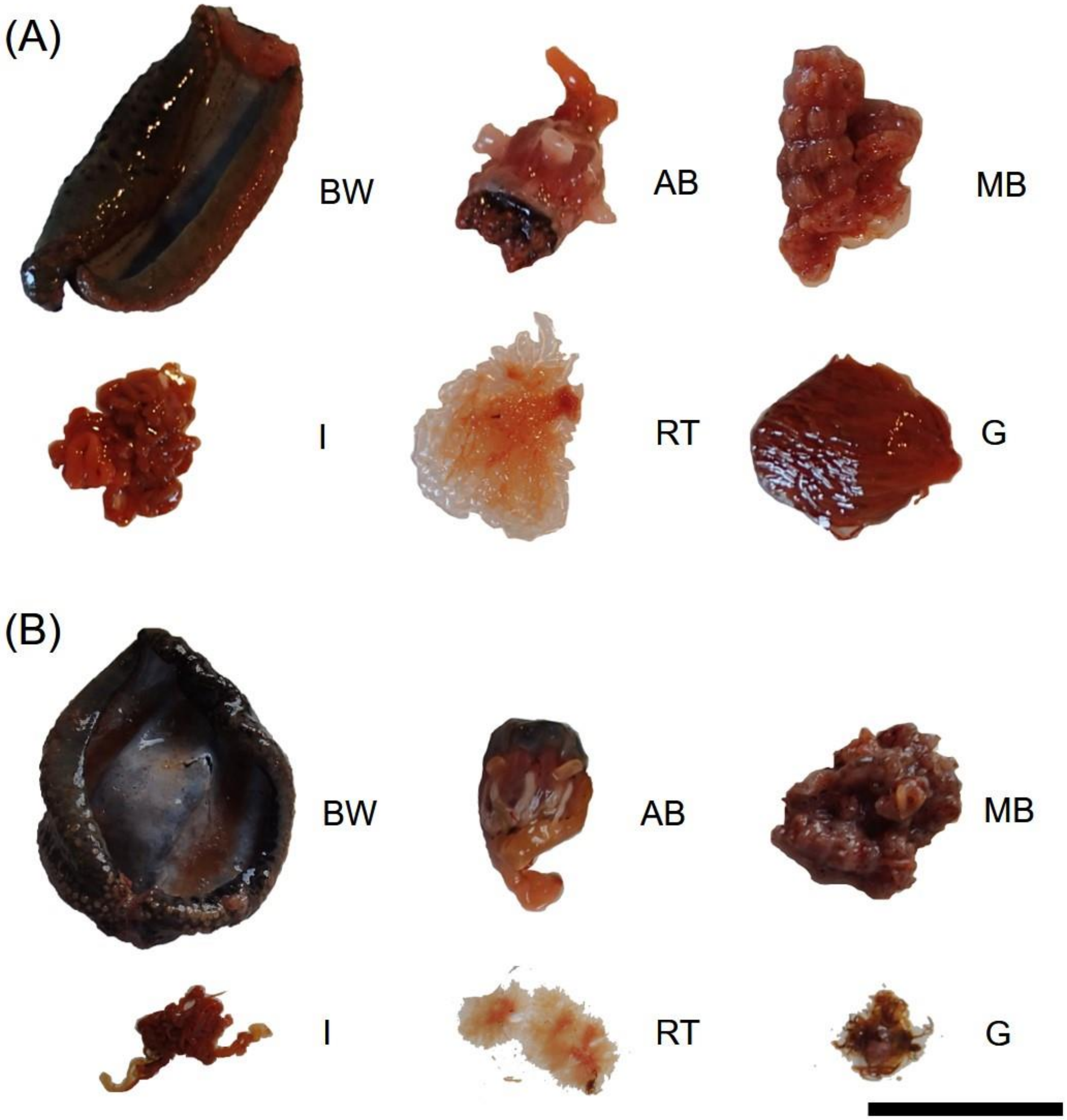

Figure S.5.1 Morphometrics of (A) wild and (B) captive sea cucumbers. BW: body wall; AB: aquapharyngeal bulb; MB: muscle bands; I: intestine; RT: respiratory tree; G: gonad. Scale bar represents 3 cm.

Chapter 6. General Conclusions

6.1 Summary

Sea cucumber is a high value seafood product that is exploited through wild fisheries and commercial aquaculture (Conand, 2004). It is perceived as healthy food, used in traditional medicine and as dietary supplement, and it has been consumed for centuries, especially in East Asia (Kiew and Don, 2012; Yang et al., 2015). The high demand and market prices for sea cucumber have led to the expansion of fisheries and to stock depletion for the most highly prized species (Anderson et al., 2011; Purcell et al., 2013). In this context, the sea cucumber *Cucumaria frondosa* is currently the target of an emerging fishery in Atlantic Canada and becoming one of the predominant commercial species on the global market (Hamel and Mercier, 2008). Developing a sustainable management strategy is a key factor in the emergence of any fishery. However, according to the Department of Fisheries and Oceans Canada (DFO), the information available on *C. fronsosa* does not currently provide enough of a scientific basis for determining the sustainable exploitation rate and assessing the risk of any increase in harvest level (DFO, 2018). Therefore, more scientific data must be collected to manage this emerging fishery species and conserve natural populations. In addition, *C. frondosa* is considered to have potential as an aquaculture candidate and as an extractive species in integrated multi-tropic aquaculture (IMTA) systems, i.e. to reduce the environmental footprint of salmon and mussel farming (Nelson et al., 2012). In this thesis, key biological and ecological aspects of *C. frondosa* were investigated to provide managers with additional information necessary to develop sustainable management strategies and aquaculture protocols. The work focused on developing a direct method to determine age (using the sibling dendrochirotid *Psolus fabricii*), examined the potential factors that may underlie the spatial distribution of adults of *C. frondosa*, and explored its suitability for integration in IMTA systems.

Chapter 2 presented and validated an aging technique using the cold-water dendrochirotid species *P. fabricii*. A novel method of detaching plates (ossicles) from tissues was developed. Compared to the traditional bleaching method, boiling the body wall at low-temperature preserved the original pigments and structure of plates. Freshly extracted plates superficially appeared to be uniform and composed of loosely compacted materials; however, polishing and dying them revealed ring patterns (light and dark rings) visible to the naked eye and under light microscopy. SEM images revealed that the plates from 3, 20, 30, and 40-month-old laboratory-reared juveniles had one, two, three, and four layers, respectively. By mirroring the results of the growth rings of the very small juveniles on the plates of larger individuals (wild individuals), it was implied that one layer is added annually, making plates both larger and thicker, and generating successive light and dark rings, the latter representing the transition (overlap) between two layers. Therefore, paired light and dark rings were counted as annular rings, representing one-year growth. Age determination using this technique suggested that growth of *P. fabricii* is slow and that wild individuals collected at diving depths had reached 28 years old. The study also highlighted that individuals of similar sizes may be of different ages and that some environmental factors contributed greatly to the difference. The use of this new method provides a valuable tool for exploring longevity, population turnover, as well as patterns of growth under different environmental conditions and in various habitats. Further research will hopefully show that this aging method may be applied in other commercial species, including *C. frondosa*, to provide new data of value to management and conservation.

Chapter 3 explored the influence of water flow on the fine locomotor and feeding behaviours, and spatial distribution in adults of *C. frondosa* using time-lapse videography. In the small-scale experiments, both the time sea cucumbers spent travelling and the distance they travelled were less in the presence of flow than in its absence; inversely, the time spent with tentacles deployed was significantly greater in the presence of flow than in its absence. In the large-scale mesocosm experiments, the sea cucumbers moved away from near-zero flow conditions over time, whereas under mildly- and highly-dynamic flow treatments, individuals consistently moved away from the strongest flow regimes >40 cm s^{-1}. The crown of tentacles of individuals located in areas with flows ≥40 cm s^{-1} tended to follow the direction of the current and were not fully deployed, whereas in flows <40 cm s^{-1} they typically faced the current or were perpendicular to the direction of water flow. Tentacle deployment and insertion rates increased with flow and individuals located in stronger flow regimes generally had higher cloacal respiration rates. Three modes of locomotion were detected. Forward crawling was most frequent at flows ≤40 cm s^{-1}, passive rolling dominated at flows >40 cm s^{-1}, and active rolling occurred randomly at flows between 0 and 120 cm s^{-1}. Using the same experiment, locomotion aided by an active increase in buoyancy under various flows was further explored in a separate publication (Appendix 1). The final spatial distribution displayed by *C. frondosa* in the mesocosm reflected the fact that, as a passive suspension feeder, it sought optimal water flow (21-40 cm s^{-1}) that can balance between efficient food capture and energy expenditure for attachment to the bottom.

Chapter 4 explored the influence of other stimuli such as light, food, substrate types and colour on the locomotion, feeding behaviour and microhabitat selection in adults of *C. frondosa*. Individuals did not exhibit any strong preference for either illuminated or shaded areas. Under three photoperiod treatments (12 h light/12 h dark, full light and full darkness), the proportion of individuals with feeding tentacles deployed did not change significantly over 24 h. The proportion of moving sea cucumbers changed significantly over 24 h under 12 h light/12 h dark, but not under 24-h light and 24-h dark. Individuals maintained under continuous light did exhibit overall reduced feeding and locomotor activities compared to those kept under the natural photoperiod (12 h light/12 h dark) and continuous darkness. *C. frondosa* was overall more mobile during the night, whereas no feeding differences were detected between diurnal and nocturnal phases. In the small-scale experiment, the presence of phytoplankton increased the feeding behaviour, but did not elicit any detectable change in locomotor behaviour. In the large mesocosm experiment, gradients in phytoplankton concentration did not trigger any displacement toward the food source. Moreover, *C. frondosa* exhibited a clear preference for substrates composed of bare rocks and rocks with coralline algae and displayed a slight preference for darker substrate backgrounds. Taken together, the findings of Chapters 3 and 4 highlighted how some key environmental factors can govern the feeding, locomotor activities, and eventual distribution of *C. frondosa*, which will not only provide baseline information of value for stock management, but will inform the eventual development of aquaculture programs.

Chapter 5 compared several key metrics between wild individuals of *C. frondosa* and individuals held for 4 years in the effluent of an Atlantic salmon culture (i.e. IMTA). The greater content of $\delta^{13}C$ in muscle bands and intestine and $\delta^{15}N$ in all tissues of cultured sea cucumbers, and the higher levels of salmon feed fatty acid (FA) biomarkers 18:1ω9, 18:2ω6 and 20:1ω9 and lower ω3/ω6 ration in the cultured individuals as compared to those in wild individuals indicated the uptake and assimilation of waste from salmon farming by cultured individuals. However, individuals from the IMTA system were smaller and their organ indices lower than those of wild individuals. All the wild sea cucumbers had a well-developed gonad; whereas only two thirds of the cultured individuals had a gonad and it was consistently smaller that that of wild counterparts. Moreover, the biochemical composition of cultured individuals differed from that of wild individuals. The triacylglycerols (TAG) content was greater in wild individuals, whereas phospholipids (PL) content was greater in cultured individuals. The arachidonic acid (ARA, 20:4ω6) content of muscle bands and intestine were greater in cultured than in the wild individuals, whereas eicosapentaenoic acid (EPA, 20:5ω3) content of the gonad was nearly twice higher in wild than cultured individuals. The docosahexaenoic acid (DHA, 20:6ω3) was detected in the gonad of wild individuals but not in cultured individuals. The findings suggested that *C. frondosa* could be used as an extractive species in IMTA systems, and provided novel information that will help to develop protocols or tools for aquaculture programs. However, whether the species itself could yield high-quality products following maintenance in such an IMTA system was questioned.

Appended to the thesis is a published paper that partly relied on data collected during the experiments described in Chapter 3. It demonstrated the occurrence of active buoyancy adjustment (ABA) in *C. frondosa* and another species (*Holothruria scabra*), whereby individuals can achieve neutral or positive buoyancy, leading them to tumble or float at speeds orders of magnitudes faster than during benthic crawling. ABA involves an increase in seawater to flesh ratio (by up to 740% in *C. frondosa*, in the most severe response), and simultaneous detachment from the substrate while changing shape and spatial orientation. This reaction was confirmed to start occurring as early as 6 months post settlement in juveniles and was also documented in wild adult populations. Based on video footage, tumbling assisted by currents yielded displacement speeds of up to 90 km d^{-1}. In both species, ABA was triggered by high conspecific density, decreasing salinity and increasing water turbidity. Taken together, these findings imply that displacement during planktonic larval stages may not supersede the locomotor capacity of benthic adult stages, challenging the notion of their sedentary lifestyle. ABA emerges as a potentially generalized means of dispersal among holothuroids, with critical implications for worldwide conservation, management and sea ranching of commercial species.

Overall, the studies presented in this thesis provided solid knowledge on the biology of two dendrochirotid species, which is not only significant from an ecological viewpoint but also provide managers with tools to develop appropriate management strategies. Beyond these, the findings also contribute significantly to current efforts being deployed toward the aquaculture of *C. frondosa*.

6.2 Future directions

Research on *C. frondosa* has so far provided basic information on the species. For instance, previous work has explored settlement, early growth and spatial distribution (Hamel and Mercier, 1996a; Gianasi et al., 2018), reproductive biology (Hamel and Mercier, 1996b; Gianasi et al., 2019; Singh et al., 2001), juvenile growth and behaviour in the laboratory (Hamel and Mercier, 1996a; Gianasi et al., 2018; So et al., 2010), population genetics (So et al., 2011), diet and feeding behaviour (Singh et al., 1998; Hamel and Mercier, 1998; Gianasi et al., 2017), and ABA behavior (Hamel et al., 2019). The present thesis contributed a potential aging technique as well as better knowledge of the environmental drivers of locomotor and feeding behaviours. However, several aspects of the biology and ecology of *C. frondosa* still need to be investigated from the fishery management perspective. Although great progress has been made, there is still a long way to go before the development of an aquaculture industry can become possible. Some areas that deserve further attention are outlined below.

6.2.1 Age determination in sea cucumbers

Chapter 2 presented and validated an aging technique using *P. fabricii*. The method may be applied confidently in other psolid species with large plates. However, it may be more difficult to apply directly to other groups of commercial sea cucumbers possessing ossicles that are smaller and/or grow discontinuously (Stricker, 1985). Nevertheless, the findings provide a valuable starting point for further studies on age determination in commercial species, which is imperative for their management and conservation. Statolith in the statocysts have been successfully used to determine the age in many aquatic species, such as sea lampreys (Volk, 1986), whelks (Richardson et al., 2005; Hollyman et al., 2018), and gonatid squids (Arkhipkin and Bjørke, 2000; Natsukari and Komine, 1992; Jackson, 1994). Previous studies have also shown that apodous sea cucumbers possess statocysts (Clark, 1907; Ehlers, 1997). Further studies need to be conducted to determine whether *C. frondosa* and other commercial species have statoliths, which may give more possibilities to determine their ages. In addition, whether the aging technique developed here can be applied to other calcareous structures present in sea cucumbers, such as circum-oral rings and smaller ossicles, needs to be tested.

Interestingly, the low-temperature boiling method developed in Chapter 2 did not cause any visual alteration of the dermal plates' surface and ensured preservation of their original shape, dimension and red pigmentation. Given its great advantage, further studies can be conducted to determine whether this method can be applied to other echinoderms to extract well-preserved dermal ossicles (or other calcareous structures). Based on the SEM images of the plates in juveniles of various age, it was concluded that the light ring was added marginally and the dark ring consisted of the overlap between successive layers rather than representing a different density, i.e. the dark section grows over the margin of the plate from the previous year, and the light zone extends outward. However, why the plates add one layer each year and how they are connected is still unclear. Further studies of the plates using tetracycline and calcein incorporation might shed some light on this, and improve our understanding of growth of dermal plates. The results of Chapter 2 also showed the significance of environmental factors on the growth of *P. fabricii*; however, only one deep-sea individual was compared with individuals from shallow waters. In future studies, more deep-sea individuals should be collected to solidify our knowledge of growth patterns in deep-sea environments.

6.2.2 Effect of various environmental factors on *C. frondosa*

The results of Chapter 3 highlighted that locomotor and feeding behaviours of the suspension-feeding *C. frondosa* varied in response to water flow, and that water flow could affect the final spatial distribution. However, the seawater used in Chapter 3 was pumped directly from the ocean, which means natural planktonic food was available in the water. Chapter 4 suggested that the presence of phytoplankton increased the feeding behaviour; however, gradients in phytoplankton concentration did not trigger any displacement toward the food source in *C. frondosa*. In other words, the action of flow carrying food can modulate locomotion in sea cucumbers, while food alone cannot. Further studies might use filtered seawater to confirm whether flow in isolation can elicit locomotion and alter spatial distribution. The present work also suggested that light is not one of the main factors affecting the feeding behaviour and spatial distribution of fully-grown individuals of *C. frondosa*, which differed from results obtained with other deposited-feeding species. Even though no obvious eye-like structure was evidenced in sea cucumbers, pigmented photoreceptors at the base of the tentacles were identified in some species (Yamamoto and Yoshida, 1978; Pawson et al., 2010). Further studies can explore whether *C. frondosa* displays any photoreceptive structures. Furthermore, environmental conditions occurring in the field are more complex than those offered in the laboratory (even in large-scale mesocosms). More field studies should ideally be conducted to confirm the results obtained from the laboratory work to better manage wild stocks of *C. frondosa*. Finally, a study (Appendix 1) showed that high flow regimes, high conspecific density, low salinity and turbidity can stimulate alternate (faster) means of locomotion through active buoyancy adjustment (ABA). Determining the mechanisms underlying this reaction and whether it can be triggered by other environmental factors requires further research.

The growth rate of *C. frondosa* is thought to be rather slow under naturally fluctuating conditions. From current knowledge, *C. frondosa* is expected to require between 12 to 25 years to grow from larva to commercial adult size, based on previous field and laboratory studies (Hamel and Mercier, 1996a; So et al., 2010). Slow growth is one of the most important factors that hampers the development of aquaculture in *C. frondosa*. A quantitative study of the effects of water temperature, food quantity and quality and stock density on the growth rates of *C. frondosa* may help find ways to increase their growth rate. Moreover, all the relevant factors will need to be integrated to determine the optimal culture conditions for *C. frondosa*.

6.2.3 Use of *C. frondosa* in IMTA systems

The study in Chapter 5 suggested that *C. frondosa* could assimilate and incorporate the wastes from a land-based salmon culture, but that resulting health metrics were not optimal. Further experimental trials will be required to obtain more precise information on the effects of salmon wastes, what nutrients may be missing, and whether supplements can offset the detrimental effects. Some aspects of acceptance by consumers, such as smell, flavor and texture, should be compared between sea cucumbers from IMTA systems and wild individuals. A semi-open system that would give access to live phytoplankton may be considered. Further studies are also needed to ascertain the feasibility of scaling up such IMTA systems and to further explore the assimilative capacity of *C. frondosa*. As a suspension-feeder, *C. frondosa* may be held in suspended cages or directly on the substrate. However, which method is more suitable and whether the biology of *C. frondosa* is compatible with the notions of captive holding are still unknown. Further work is also needed to adapt these designs for a variety of species and conditions because *C. frondosa* may be cultured with other extractive species, such as mussels. Any potential positive or negative relationship between *C. frondosa* and other co-occurring suspension feeders will need to be determined in future work.

6.3 References

Anderson, S.C., Flemming, J.M., Watson, R., Lotze, H.K., 2011. Serial exploitation of global sea cucumber fisheries. Fish and Fisheries. 12, 317-339.

Arkhipkin, A.I., Bjørke, H., 2000. Statolith shape and microstructure as indicators of ontogenetic shifts in the squid *Gonatus fabricii* (Oegopsida, Gonatidae) from the Norwegian Sea. Polar Biology. 23, 1-10.

Clark, H.L., 1907. The apodous holothurians: A monograph of the Synaptidae and Molpadiidae, including a report on the representatives of these families in the collections of the United States National Museum. Smithsonian Contributions to Knowledge, 35, 1-231.

Conand, C., 2004. Present status of world sea cucumber resources and utilisation: an international overview. in: Conand C., Purcell, S., Uthicke, S. Hamel, J.-F., Mercier A. (Eds.), Advances in Sea cucumber Aquaculture and Management, FAO Technical Paper Food and Agriculture Organization of the United Nations, Rome, Italy, pp. 13-23.

DFO, 2018. Sea cucumber stock status update in NAFO subdivision 3Ps, DFO Canadian Science Advisory Secretariat Science Response 2018/010.

Ehlers, U., 1997. Ultrastructure of the statocysts in the apodous sea cucumber *Leptosynapta inhaerens* (Holothuroidea, Echinodermata). Acta Zoologica. 78, 61-68.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2018. Morphometric and behavioural changes in the early life stages of the sea cucumber *Cucumaria frondosa*. Aquaculture. 490, 5-18.

Gianasi, B.L., Hamel, J.-F., Mercier, A., 2019. Triggers of spawning and oocyte maturation in the commercial sea cucumber *Cucumaria frondosa*. Aquaculture. 498, 50-60.

Gianasi, B.L., Parrish, C.C., Hamel, J.-F., Mercier, A., 2017. Influence of diet on growth, reproduction and lipid and fatty acid composition in the sea cucumber *Cucumaria frondosa*. Aquaculture Research. 48, 3413-3432.

Hamel, J.-F., Mercier, A., 1996a. Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). Canadian Journal of Fisheries and Aquatic Sciences. 53, 253-271.

Hamel, J.-F., Mercier, A., 1996b. Studies on the reproductive biology of the Atlantic sea cucumber *Cucumaria frondosa*. SPC Beche-de-mer Information Bulletin. 8, 22-33.

Hamel, J.-F., Mercier, A., 1998. Diet and feeding behaviour of the sea cucumber *Cucumaria frondosa* in the St. Lawrence estuary, eastern Canada. Canadian Journal of Zoology. 76, 1194-1198.

Hamel, J.-F., Mercier, A., 2008. Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. in: Toral-Granda, M.V., Lovatelli, A., Vasconcellos, M. (Eds.), Sea cucumbers: A Global Review of Fisheries and Trade. FAO Fisheries and Aquaculture Technical Paper, Rome, pp. 293-306.

Hamel, J.-F., Sun, J., Gianasi, B.L., Montgomery, E.M., Kenchington, E.L., Burel, B., Rowe, S., Winger, P.D., Mercier, A., 2019. Active buoyancy adjustment increases dispersal potential in benthic marine animals. Journal of Animal Ecology. 88, 820-832.

Hollyman, P., Leng, M., Chenery, S., Laptikhovsky, V., Richardson, C., 2018. Statoliths of the whelk *Buccinum undatum*: a novel age determination tool. Marine Ecology Progress Series. 598, 261-272.

Jackson, G.D., 1994. Application and future potential of statolith increment analysis in squids and sepioids. Canadian Journal of Fisheries and Aquatic Sciences. 51, 2612-2625.

Kiew, P.L., Don, M.M., 2012. Jewel of the seabed: sea cucumbers as nutritional and drug candidates. International Journal of Food Sciences and Nutrition. 63, 616-636.

Natsukari, Y., Komine, N., 1992. Age and growth estimation of the European squid, *Loligo vulgaris*, based on statolith microstructure. Journal of the Marine Biological Association of the United Kingdom. 72, 271-280.

Nelson, E.J., MacDonald, B.A., Robinson, S.M., 2012. A review of the Northern sea cucumber *Cucumaria frondosa* (Gunnerus, 1767) as a potential aquaculture species. Reviews in Fisheries Science. 20, 212-219.

Pawson, D.L., Pawson, D.J., King, R.A., 2010. A taxonomic guide to the Echinodermata of the South Atlantic Bight, USA: 1. Sea cucumbers (Echinodermata: Holothuroidea). Zootaxa. 2449, 1-48.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M.V., Lovatelli, A., Uthicke, S., 2013. Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. Fish and Fisheries. 14, 34-59.

Richardson, C., Saurel, C., Barroso, C., Thain, J., 2005. Evaluation of the age of the red whelk *Neptunea antiqua* using statoliths, opercula and element ratios in the shell. Journal of Experimental Marine Biology and Ecology. 325, 55-64.

Singh, R., MacDonald, B.A., Lawton, P., Thomas, M.L., 1998. Feeding response of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea) to changing food concentrations in the laboratory. Canadian Journal of Zoology. 76, 1842-1849.

Singh, R., Macdonald, B.A., Lawton, P., Thomas, M.L., 2001. The reproductive biology of the dendrochirote sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuriodea) using new quantitative methods. Invertebrate Reproduction & Development. 40, 125-141.

So, J.J., Hamel, J.-F., Mercier, A., 2010. Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. Fisheries Management and Ecology. 17, 473-484.

So, J.J., Uthicke, S., Hamel, J.-F., Mercier, A., 2011. Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (Echinodermata: Holothuroidea). Marine Biology. 158, 859-870.

Stricker, S.A., 1985. The ultrastructure and formation of the calcareous ossicles in the body wall of the sea cucumber *Leptosynapta clarki* (Echinodermata, Holothuroida). Zoomorphology. 105, 209-222.

Volk, E.C., 1986. Use of calcareous otic elements (statoliths) to determine age of sea lamprey ammocoetes (*Petromyzon marinus*). Canadian Journal of Fisheries and Aquatic Sciences. 43, 718-722.

Yamamoto, M., Yoshida, M., 1978. Fine structure of the ocelli of a synaptid holothurian, *Opheodesoma spectabilis*, and the effects of light and darkness. Zoomorphologie. 90, 1-17.

Yang, H., Hamel, J.-F., Mercier, A., 2015. The sea cucumber *Apostichopus japonicus*: history, biology and aquaculture. Academic Press, New York, NY, USA.

Appendix

Appendix 1 Active buoyancy adjustment increases dispersal potential in benthic marine animals[5]

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Abstract

1. While the study of dispersal and connectivity in the ocean typically centers on pelagic species and planktonic larval stages of benthic species, the present work explores an overlooked locomotor means in post-settlement benthic stages that redefines their dispersal potential.
2. Members of the echinoderm class Holothuroidea colonize a diversity of marine environments worldwide, where they play key ecological and economical roles, making their conservation a priority. Holothuroids are commonly called sea cucumbers or sea slugs to reflect their slow movements and are assumed to disperse chiefly through pelagic larvae.
3. The present study documents and explores their unexpected ability to actively modify their buoyancy, leading them to tumble or float at speeds orders of magnitudes faster than through benthic crawling. Two focal species representing different taxonomic orders, geographic distributions and reproductive strategies were studied over several years.
4. Active buoyancy adjustment (ABA) was achieved through a rapid increase in seawater to flesh ratio by up to 740%, leading to bloating, and simultaneously detachment from the substrate. It occurred as early as 6 months post settlement in juveniles and was recorded in wild adult populations. In experimental trials, ABA was triggered by high conspecific density, decreasing salinity and increasing water turbidity. Based on field video footage, ABA-assisted movements generated speeds of up to 90 km d^{-1}.

5. These findings imply that displacement during planktonic larval stages may not supersede the locomotor capacity of benthic stages, challenging the notion of sedentarity. Combining the present results and anecdotal reports, ABA emerges as a generalized means of dispersal among benthic animals, with critical implications for worldwide management and conservation of commercially and ecologically significant species.

Key words: benthic organisms, dispersal, echinoderm, locomotor behaviour, marine organisms, movement ecology, sea cucumber

Introduction

Movement is among the fundamental components of life, and a key determinant of community structure (Bie et al., 2012), population and ecosystem connectivity (Baguette, Blanchet, Legrand, Stevens & Turlure, 2013), and ecological and evolutionary processes (Nathan et al., 2008). Understanding why and how organisms disperse is also central to wildlife management and conservation (Allen & Singh, 2016). Overall, the interplay between adaptation and dispersal determines the persistence of species in a dynamic and ever changing world (Berg et al., 2010). Various dispersal attributes and strategies have evolved among terrestrial and aquatic organisms to offset the associated costs of movement (Bonte et al., 2012). Some organisms are motile throughout their lives, whereas others are adapted to undergo movement at precise, limited phases of their life cycles, commonly called the dispersive phase(s) (Allen, Metaxas & Snelgrove, 2018). The life-history strategies of organisms are often driven by the nature and circumstances of their dispersive phases (e.g. restricted or prolonged, active or passive). In the marine realm, movement ecology focuses on large pelagic megafauna (Hays et al., 2016; Sequeira et al., 2018), with fewer data on non-vertebrate benthic taxa (Holyoak, Casagrandi, Nathan, Revilla & Spiegel, 2008) despite the fact that they form the bulk of marine macrofaunal biodiversity.

Most benthic organisms exhibit a complex life-history, whereby the early life stages are pelagic and the adults are either sessile (permanently anchored), such as barnacles, sponges and corals, or sedentary (exhibiting limited movement) such as many molluscs and echinoderms. While benthic stages can display transient movement (Winston, 2012), dispersal is commonly presumed to occur predominantly during planktonic embryonic and larval phases lasting days to weeks (Grantham, Eckert & Shanks, 2003). Consequently, research on marine population structures and connectivity is chiefly centered on pelagic propagules (Cowen & Sponaugle, 2009), and secondarily on rafting of benthic juveniles or adults (Thiel & Gutow, 2005; Macfarlane et al., 2013), although a more holistic view is increasingly being advocated (Pilditch, Valanko, Norkko & Norkko, 2015; Allen et al., 2018).

Holothuroids (Echinodermata: Holothuroidea), also commonly known as sea cucumbers or sea slugs, are ubiquitous members of benthic communities extending from the poles to the equator and from the shores to the abyssal trenches, where they may represent up to 95% of the whole biomass (Heezen & Hollister, 1971). Many species have broad geographic distributions; for instance *Holothuria scabra* occurs throughout the Indo-Pacific and along most of the tropical Asian and Eastern African coasts (Hamel, Conand, Pawson & Mercier, 2001), and *Cucumaria frondosa* is common in the Arctic and on both sides of the North Atlantic Ocean (Hamel & Mercier, 2008a). Several holothuroid species, including *H. scabra* and *C. frondosa*, are also commercially exploited in several regions of the globe (Purcell, Samyn & Conand, 2012; 2013). The notorious boom-and-bust pattern of wild fisheries has led the most prized species to the brink of extinction, earning them a spot on the IUCN Red List of endangered species (Anderson, Flemming, Watson & Lotze, 2011; Purcell, Polidoro, Hamel, Gamboa & Mercier, 2014). Apart from being one of the most sought after luxury seafoods, holothuroids play critical roles in several marine environments, from bioturbation to nutrient recycling (Purcell, Conand, Uthicke & Byrne, 2016).

Our current understanding of holothuroid biology, including life-history strategy, population structure/connectivity and biogeography, revolves around the notion of a sedentary adult with a dispersive larval stage. Many species are classified as sessile (Grantham et al., 2003), and estimates of benthic displacement through forward crawling in juveniles and adults range from a few centimeters to a few meters a day. For instance, *H. scabra* was found to cover 40-80 cm d^{-1} through crawling as a juvenile (Mercier, Battaglene & Hamel, 2000) and 1.3 m d^{-1} as an adult (Purcell & Kirby, 2006). Short-term average movement rates were 2–8 m d^{-1} in *Bohadschia argus* and 5–9 m d^{-1} in *Thelenota ananas*, yielding a long-term range of 15–47 m over 2 years (Purcell, Piddocke, Dalton & Wang, 2016). Average displacements of 3.9 m d^{-1} have been reported in *Parastichopus californicus* (Da Silva, Cameron & Fankboner, 1986). In *C. frondosa*, 12-month old juveniles were shown to move up to 5 cm h^{-1} (Gianasi, Hamel & Mercier, 2018), equivalent to 1.2 m d^{-1}. In addition, some coastal species (including *C. frondosa*) can display enhanced contractions when encountering a predator (e.g. Margolin, 1976; Legault & Himmelman, 1993; So, Hamel & Mercier, 2010), although these escape responses are of short duration and not considered true means of locomotion.

The present study revisits the dispersal capacity of juvenile and adult holothuroids in subtidal and intertidal environments, based on evidence that they commonly and predictably rely on semi-pelagic means of locomotion that can be orders of magnitude faster than their podia-assisted benthic crawling movements. A combination of experimental trials and observational data from the field, gathered in two species belonging to different taxonomic orders and native environments, suggest that juveniles and adults are capable of moving as efficiently as pelagic embryos/larvae. In challenging the notion that these long-lived macrobenthic organisms strictly disperse during a brief period in their early life history, the findings have crucial implications for management and conservation initiatives.

Materials and Methods

The two holothuroid species under study, *Cucumaria frondosa* and *Holothuria scabra*, are among the well studied echinoderms, both from biological/ecological and commercial perspectives, including studies in wild and captive individuals. Among others, papers have been published on their reproductive cycle, embryonic and larval development, settlement, growth, population genetics, feeding, movement, prey-predator interactions, chemical composition, as well as on the effect of various environmental stressors on their health (e.g., Hamel et al., 2001; Hamel & Mercier, 2008a, b; Mercier & Hamel, 2013).

Field studies

Adults of *Cucumaria frondosa* in Newfoundland and Nova Scotia (eastern Canada)

At-sea monitoring in Newfoundland was conducted off the south coast on St. Pierre Bank in the Northwest Atlantic Fisheries Organization (NAFO) Subdivision 3Ps (around

46°13’N 56°30’W). A first survey was conducted in August 2004 aboard the CCGS *Shamook* at depths of 45-52 m (3.5-4.5°C) and a second survey was conducted in August 2005 aboard the CCGS *Templeman* at depths of 41-57 m (2.9 to 5.0 °C). Video footage of the seafloor was collected using a benthic sled (Lauth, Wakefield & Smith, 2004) deployed over the stern of the vessel. An underwater camera (Simrad OE 1367) was mounted on the front of the sled and was angled slightly downward. Digital video was stored on an autonomous recording unit (Underwood, Winger & Legge, 2012). Descent of the sled was monitored using a SCANMAR depth sensor and bottom temperature was recorded using a VEMCO Minilog-TR thermograph. Successful video transects were conducted at a total of 3 stations in 2004 and 6 in 2005, at a towing speed of 2.0 knots with on-bottom durations ranging from 0.09 to 0.62 h. All transects were made during daylight hours.

The video survey in Nova Scotia was conducted in the Shortland Canyon on the Scotian Shelf in NAFO Subdivision 4Vs (around 44°14’N: 58°28’W) using a Campod unit deployed from the CCGS *Hudson* between 220 and 300 m depth in July 2008. Campod, which is a static observation platform equipped with a high-resolution video camera for viewing the seabed directly below and an oblique video camera for viewing the seabed ahead (Gordon et al., 2000), was deployed while the ship was slowly drifting (<1 knot; ~2 m from the seafloor) over the investigation site for 20-30 min on each set. The ship’s position was used as a proxy for on-bottom location.

For both locations, video footage was used to conduct an analysis of displacement and behaviour in *C. frondosa*. The number of individuals tumbling or floating as well as their proportion relative to other individuals present on the seafloor were assessed. Tumbling individuals are defined as those that drift with the current while remaining in constant or partial contact with the substrate (i.e. rolling and bouncing movements). Floating individuals are those that drift with the current without touching the substrate. Displacement speeds of individuals were calculated as the number of body lengths travelled per interval of 15 s relative to a reference point on the bottom (successive measures were taken to obtain a mean for each focal individual). The speed value over 15 s was extrapolated to m min^{-1} and daily displacement (km d^{-1}). Because only individuals traveling parallel to the viewpoint were considered, speed sample sizes (number of individuals analyzed) varied as provided in the results. Based on their estimated length of 21-26 cm mouth-anus (CSAR, 2006), individuals in the videos from Newfoundland and Nova Scotia were all adults.

Holothuria scabra in Madagascar

This study was conducted in offshore enclosures (Indian Ocean Trepang, IOT) located off Belaza, 25 km south of Toliara, Madagascar (23°29'S; 43°45'E). They were spread over 100 Ha, in the upper intertidal zone, 0.5 to 1.5 km from the high water mark, consistent with the typical habitat of *H. scabra*. Each of the seven focal enclosures covered an area of ~15,000 m^2 and was seeded with a mean of 24,500 (± 5,500 SD) individuals obtained from spawning local wild broodstock. They were submerged with 1.5 to 2.5 m of water at high tide and 0.10 m at low tide. Freshwater channels a few meters wide and a few centimeters deep fed the enclosures at low tide (decreasing salinity to 18-30 psu). The other enclosures not under the influence of freshwater runoff were at 35-36 psu. Juveniles and adults of *H. scabra* were maintained in these enclosures at a density not exceeding 2 ind m^{-2}, which is at the low end of densities reported for individuals in the wild (Mercier et al., 2000).

Every 15 days between April 2014 and November 2016, the number of tumbling/floating individuals was counted in 9-10 subsamples per enclosure (using standardized effort), and mean proportions derived from counts based on the total number of individuals held in each enclosure (established at night on a weekly basis). In addition, video footage was taken at night when most individuals were surfacing and tumbling/floating events were detected, towards the end of ebb tide when the water level was 0.3 m. The speed of displacement of tumbling or floating individuals was calculated using video clips, whereby the time to travel across 0.7-m markers was measured.

Laboratory studies

Early juveniles of *Cucumaria frondosa*

Adults of *C. frondosa* (n = 200) measuring 11.0 ± 1.7 cm (± SD; n = 30) contracted body length were kept in two 500-L tanks supplied with running ambient seawater (20 L h^{-1}). Males and females spawned freely during the natural breeding season. Embryos and larvae (n=150 per vessel) were incubated in three rearing vessels (~0.4 embryo ml^{-1}) consisting of 4-L round containers with meshed openings (1-mm in diameter), placed inside a 40-L tank supplied with running ambient seawater (20 L h^{-1}). Natural light was provided through large windows following ambient photoperiod (from 15 h light/9 h dark in the summer to 8 h light/16 h dark during winter). Natural instances of active buoyancy adjustment or ABA (e.g. bloating, floating) were visually recorded on a daily basis in several cohorts of juveniles between post-settlement until 2 years of age (~5-6 mm long). Photos and measurements were taken under an automated stereomicroscope (Leica M205FA) using the associated software (Leica LAS-X).

Adults of *Cucumaria frondosa*

To identify the drivers of ABA, trials were conducted in 20-L tanks. The response of adult individuals (as described above) to ecologically relevant factors (from general knowledge of the species and preliminary experiments) was measured, using 4 population densities (1, 5, 10 and 15 individual m^{-2}), 3 salinities [32, 26 and 22 psu; based on threshold tolerance for the species in So et al. (2010)] and 2 turbidity levels (pristine vs. 1-L solution of detritic organic matter; mimicking turbidity flows generated by storms, tidal currents or trawling activities close to the seafloor). Except when otherwise mentioned, trials were conducted in triplicate with 4 individuals per tank following 2 h of acclimation. The salinity was adjusted by slowly adding freshwater (~50 ml min^{-1}) to the tank; salinity was measured with a multiparameter probe (YSI 556 MPS). The load of turbidity water (suspended sediment concentration) was adjusted by adding the whole volume once; the number of particles and bacteria was established to be 1.4-2.2 X 10^{9} ml^{-1} (measured with a hemocytometer). Response metrics were recorded for 2 h after the targeted parameter of each treatment was established, including orientation of the tentacles and their level of extension (relative to the substrate and the water surface), the shape of the body (degree of bloating) as well as the orientation of the anus (relative to the substrate and the water surface).

In distinct experiments, individuals of a similar size (as described above) that were either in the normal state or undergoing ABA (showing signs of morphological and behavioural changes) were compared for the strength of their attachment to the substrate, the amount of water they contained, and the rhythm of their cloacal respiration. The strength of attachment to the substrate was assessed in 24 normal individuals and in individuals undergoing ABA (22 stimulated by high conspecific density, 17 by turbidity and 20 by low salinity). This was done by gently placing a zip-tie around their middle section to hook them to a digital precision spring balance (WeiHeng®) as per a method used with sea urchins (Santos & Flammang, 2007). The force (in Newton) necessary to detach the individual from the substrate was determined, and the number of podia used for anchorage was counted through the transparent glass wall of the tank. Another set of individuals exhibiting either weak ABA (induced by conspecific density) or severe ABA (from turbidity) were used to assess behaviour and importance of water intake during ABA. The shapes of the two groups of individuals (n=19 of each) were monitored, including the precise orientation of the tentacles and anus relative to the rest of the body, the level of tentacle extension, and the ratio between total length (distance mouth-anus) and mid-body diameter. After they had reached the maximum size established during preliminary observations, their wet weight (bloated) was measured before making a longitudinal slit across the body wall to expose the organs (Polian vesicle, intestine, respiratory tree), which were punctured and drained. The water content was collected and weighed. The same procedure was repeated on a group of normal individuals (n=18). A final experiment was undertaken to measure cloacal respiration rate (opening min^{-1}) in weakly and severely bloated individuals (n=9 of each, induced by density and turbidity, respectively). The dilatation of the anus was also measured with a ruler at different times during ABA. Triplicates of all measurements were obtained from each individual. The same procedures were repeated on 7 individuals under normal holding conditions, which were firmly attached to the substrate and not showing signs of ABA.

To fine-tune our understanding of the relationship between flow regime and the occurrence of ABA, trials were conducted in a large mesocosm. A population of ~1000 adults of *C. frondosa* was held in a 34,500-L flow-through tank (11.5 m long × 2.5 m wide × 1.2 m deep) in ambient running seawater (30-60 L min^{-1}). An experimental flow-through raceway (8.25 m long × 2.5 m wide × 0.85 m deep) was covered with gravel (1-3 cm diameter) to mimic the natural habitat where *C. frondosa* can be found. Grid markings were made on the dividing plate and the raceway, at 50-cm intervals to allow measurement of movements and speed of displacement.

For the trials, 100 individuals of similar size (described earlier) were evenly spread, at a density considered equivalent to the high-density level in experiments described above, and left to acclimate for 5 h in static conditions before effecting a nominal flow of 200 cm sec^{-1} (at the inflow). Precise flows were measured around focal individuals in the experimental arena using a hand-held flow probe (Global Water, FP211). A time-lapse video camera (Brinno, TLC 200 Pro) placed above the arena took one picture every minute for 3 h. Pictures were automatically stitched together into a video output. To minimize the possibility of tank effects, 4 successive replicates were conducted, alternatingly placing the inflow in different locations. No individual was ever used for two successive trials. Based on analysis of the video footage, the proportions of crawling, tumbling and floating individuals were determined for the various flow regimes, and their respective speeds of displacement were measured.

Data analysis

Data on strength of attachment in adults of *C. frondosa* did not meet the assumptions of normal distribution and equal variance; the effect of ABA type on this variable was therefore tested using one-way analysis of variance (ANOVA) on ranks followed by pairwise comparisons using Dunn's method. Data on water to flesh ratio (weight based) among independent groups of *C. frondosa* adults exhibiting different ABA levels were not normally distributed but exhibited equal variance, and were thus tested using one-way ANOVA followed by Holm-Sidak pairwise comparisons (non-parametric counterparts yielded the same results). The same approach was applied to proportions of tumbling individuals among different water flows. In *H. scabra*, data on proportions of individuals displaying tumbling/floating across locations did not meet the normality and equal variance assumptions; analysis was conducted using one-way ANOVA on ranks followed by pairwise comparisons (Tukey's method). Data on the intensity of ABA reaction across seasons and lunar cycles in *H. scabra* were normally distributed and displayed equal variance, and were thus compared using two-way ANOVA and Holm-Sidak post-hoc tests. All data in the text are reported as mean and standard deviation (SD).

Results

Adults of *Cucumaria frondosa* in the field

Occurrences of tumbling individuals (Supporting Information S1) were recorded in two geographic locations (located >400 km apart) in eastern Canada. In Newfoundland, the proportion of tumbling individuals ranged from 1.22 to 45% with individuals moving at speeds of 30 ± 6 m min^{-1} (n=13). In Nova Scotia, the proportion of tumbling individuals reached 100% and they were determined to move faster, around 55 ± 9 m min^{-1} (n=21). The holothuroids in Nova Scotia were tumbling across an area of muddy sand where no attached individuals could be detected. Conversely, a mix of attached and tumbling individuals was observed in Newfoundland, where the substrate was mostly composed of pebbles, small boulders or compacted sand. Based on the calculated speeds, it was estimated that individuals could travel 41.1 ± 7.7 km d^{-1} (Newfoundland) and 79.3 ± 7.6 km d^{-1} (Nova Scotia). In both locations, tumbling was recorded in the day time (no data obtained at night). No special weather events (e.g. storms) or unusual environmental conditions were noted in the study areas at the time. Tumbling individuals were characteristically bloated with ambulacral podia and tentacles retracted (Supporting Information S1).

Juveniles of *Cucumaria frondosa* in the laboratory

A capacity to expand the entire body into a balloon shape was first detected when juveniles were 6 months old, in ~35% of the population (all individuals exhibiting this behaviour were doing it synchronously in 3 independent culture vessels). Bloated individuals were up to 3.8 times larger in volume than the normal juveniles (Fig. A.1A, B). The length of normal juveniles (mouth-anus) was 1.8 ± 0.2 mm and their height (maximum distance from the dorsal to the ventral side) was 0.4 ± 0.1 mm compared to 2.1 ± 0.2 mm in length and 1.3 ± 0.1 mm in height for bloated individuals, representing an average increase of ~17% in length and ~225% in height. The ossicles covering the body wall of normal individuals were tightly packed and often overlapped. However, ossicles of bloated juveniles were spaced out over the body wall (Fig. A.1A, B). In the absence of current, the majority of bloated juveniles remained attached to the wall of the rearing tanks; however, 9% of individuals (n=14) were seen floating at the surface of the water. The ABA reaction persisted for ~2 days before individuals resumed their normal body shape and position on the bottom of the tanks. No mortality was observed as a result of this reaction. Sporadic

ABA was also noticed in older juveniles (>6 months of age), however, the frequency of occurrence was lower (~1% of the population). This behaviour was recorded both during the day and at night. No perceptible change in environmental factors (temperature, salinity) were noted while juveniles were exhibiting ABA.

Adults of *Cucumaria frondosa* in the laboratory

Undisturbed (normal) individuals of *C. frondosa* remained firmly attached to the substrate with their tentacles either retracted or extended; the oral end occupied the highest position, i.e. farthest from the substrate, or closest to the water surface (Fig. A.1C).

After the treatments (density, salinity, turbidity) were applied, the following changes in general behaviour were recorded: decrease in the strength of attachment to the substrate, retraction of the tentacles (when initially extended), bloating of the whole body, and in some cases change in orientation whereby the anus was in an upright position (above the oral end), followed by tumbling or floating when water current was present. These behaviours were categorized in two distinct intensity levels that were determined to be related to the intensity of the stimulus. The responses recorded during these experiments highlighted the fact that, depending on the severity of the stressor (from mild to life-threatening), the sequence and/or intensity of responses varied.

Normal state (baseline metrics)

When kept in pristine natural seawater at low population densities (≤5 ind m^{-2}) and normal salinity (~32-34 psu), individuals remained firmly attached, requiring a force of 803.4 ± 57.9 g (7.87 ± 0.57 Newtons) to detach them from the substrate (Fig. A.2). The number of podia used for anchorage fluctuated around 390 ± 55 ; several of these podia were torn during forcible detachment. The average wet weight of normal individuals was

352 ± 32 g, corresponding to an underwater weight of 6.7 g, ~12.5 cm length and 6.5 cm diameter (contracted condition). Water on average represented 31 ± 2% of the wet weight of normal individuals corresponding to a water:flesh ratio of 0.45 (Fig. A.3), and their tentacle crown was consistently upward (Fig. A.1C). The opening diameter of the anus was 1.7 ± 0.2 mm in the normal state, with a cloacal respiration rate of 0.99 ± 0.13 opening min^{-1}.

Weak reactions

When exposed to high densities of 10 or 15 ind m^{-2}, individuals generally displayed a posture and behaviour similar to those of the normal holothuroids except that the number of podia involved was reduced and the corresponding strength of attachment was significantly weakened (Fig. A.2). Inside a 20 min period, the number of anchored podia varied from 53.7 ± 14.1 overall, and the force required to detach them varied between 38.0 ± 24.6 g (0.37 ± 0.24 Newtons; Fig. A.2) with a cloacal respiration rate of 0.9 ± 0.1 opening min^{-1} and an opening diameter of the anus of 1.7 ± 0.3 mm. Water content increased by ~75% compared to the normal state, representing on average 39 ± 5% of the body weight of individuals (Fig. A.3), which displayed a more rounded shape. The water:flesh ratio increased to 0.65 (Fig. A.3). A light water current was enough to detach such individuals.

Severe reactions

Compared to weak reactions induced by high conspecific density, ABA reactions to turbidity and low salinities (22-26 psu) developed more quickly and were more severe (Fig. A.2). Firstly, the number of attached podia decreased to 0.8 ± 2.2 within 5 min. The body shape started to become rounded, and the cloacal respiration increased to 3.9 ± 0.2 openings min^{-1} until maximum bloating of the whole body, after which it decreased to 0.7 ± 0.4

opening min^{-1}. The opening diameter of the anus increased to a maximum of 3.7 ± 0.4 mm at the peak of the bloated state. The reaction culminated with retraction of all podia and complete detachment of the bloated individuals from the substrate (Fig. A.1D, E) inside 10-15 min, corresponding to an almost null or null force of attachment (1.4 ± 2.7 g, 0.01 ± 0.03 Newtons; Fig. A.2). Finally, there was a consistent change in the body orientation, switching from mouth-up (Fig. A.1C) to anus-up (Fig. A.1F, G). The total wet weight increased to an average of 1044 ± 134 g in fully bloated individuals; their underwater weight was close to 0 g; the average body length was 20.0 cm and the diameter 12.5 cm. Severely bloated individuals displayed a ~742 % increase on average in their flesh:water ratio relative to normal individuals; water filled the respiratory tree, intestine, Polian vesicle and coelomic cavity, representing 78 ± 5% of the whole weight on average or a water:flesh ratio of 3.79 (Fig. A.3). These individuals were extremely buoyant, and the slightest water movement was enough to carry them away.

Flow experiments

There was a clear increment in the proportion of tumbling individuals from ABA responses induced by increased current (Fig. A.4). At flow regimes ≤40 cm s^{-1}, most individuals exhibited forward crawling and a few were rolling on their side while remaining in contact with the substrate (but not using their ambulacral podia). When exposed to flows between 41 and 120 cm s^{-1}, a majority of individuals started tumbling (Fig. A.4), and many were bouncing (periodically losing contact with the substrate). The ranges of displacement speeds measured in the tumbling individuals were 9.3 ± 3.0, 22.0 ± 5.2, 39.3 ± 13.2, and 54.7 ± 26.3 cm s^{-1} when the flow speeds were 0-20, 21-40, 41-80, and 81-120 cm s^{-1}, respectively. In normal individuals, the maximum forward crawling speeds averaged 0.013 ± 0.006 cm s^{-1}.

Juveniles and adults of *Holothuria scabra*

In contrast to adults of *C. frondosa* where tumbling close to the bottom was the main ABA behaviour detected in the field, adults of *H. scabra* (>270 g wet weight) displayed both tumbling and floating (Supporting Information S2). Thousands of individuals were seen displaying ABA since July 2014; whereby they either float near the sea surface at low tide (in 0.2-0.7 m depth), or they tumble on the sediment, and get carried away by the current. The speed of floating *H. scabra* was estimated to be 0.1 m sec^{-1}, equivalent to 6 m min^{-1} whereas tumbling speed was around 0.02 m sec^{-1}. Normal individuals of this species exhibit a length:width ratio of 2:3, depending on contraction state, whereas bloated individuals had a 1:3 ratio. The wet weight of tumbling/floating individuals was 329.3 ± 91.0 g, compared to normal values of 298.7 ± 53.4 g. When tumbling or floating, both males and females became flabby, crescent-shaped, and slightly transparent under direct light (Supporting Information S2). ABA was related to the uptake of seawater in the respiratory trees and coelomic cavity. Tumbling and floating behaviours were only recorded during ebb tides (never during flood tides).

Between 33 and 73 tumbling/floating individuals of *H. scabra* were recorded by each observer during rounds conducted at the full or new moons every month for over two years, which was extrapolated to constitute between 0.5 and 4% of the entire surveyed population on any occasion. Instances of tumbling/floating occurred at unequal frequencies in the different enclosures monitored (mean conspecific density of 1.5 ind m^{-2}). The individuals housed in the offshore pens (35-36 psu) did not exhibit the behaviour (data not illustrated), whereas those in the 6 focal enclosures closest to land (18-27 psu) displayed the behaviour at frequencies that varied between 0.8±0.1% and 2.9±0.6% (Fig. A.5) with an average of 2.0±0.8% (n=294) over the course of the study. Enclosure B11 was the least affected by drops of salinities (27-30 psu), being located 600 m from the shore; it also showed the lowest proportion of individuals with ABA, i.e. 0.8±0.1% (Fig. A.5). Overall, occurrences of ABA were higher during full moons than during new moons and more frequent during the cool than during the warm season (Fig. A.6). Although most frequent at night, ABA also occurred during the day. The location of tumbling/floating individuals at the full moon and at the new moons carried them to the west side of the enclosures where they accumulated, especially in the northwest corner, corresponding to the open sea. Several floating individuals found their way over the fences (Supporting Information S3) and were later seen hundreds of meters out at sea.

Discussion

Movement ecology represents a strategic link between animal behaviour and population dynamics that can be defined as the interplay between an individual's internal state, its motion capacity, its navigation capacity, and external factors (Nathan et al., 2008). These topics are understudied in the marine realm compared to the terrestrial environment, in part because movement patterns in the ocean are uniquely shaped by the pelago-benthic life history of most animal species, whereby adults are benthic and early life stages are pelagic (Walther, Munguia & Fuiman, 2015). While focus has been placed on pelagic larvae that drift with the currents, it has been postulated that benthic adults may be the missing link in population connectivity relevant to fisheries biology and marine conservation studies (Frisk, Jordaan & Miller, 2014). A recent review of movement ecology in marine animals with complex life cycles (i.e. the bulk of marine faunal biodiversity) further emphasized the importance of holistically considering all life stages (Allen et al., 2018). Interestingly, the movement of sessile or sedentary benthic adults through physical transport by currents was presented only as being either passive, i.e. following dislodgment, or indirect through dispersal of colonized substrates, i.e. rafting (Allen et al., 2018).

The present study highlights a different strategy, i.e. active alternation between self-directed motion and physical transport, which evokes potentially more frequent and more predictable patterns of benthic locomotion under environmental control. Two species from drastically different climes and environments (*C. frondosa* and *H. scabra*) were shown to consistently react in a matter of minutes to undesirable environmental conditions and/or abnormally high conspecific densities. Instead of using their podia to crawl away, they actively underwent a change in body shape, water content (buoyancy) and strength of attachment to the substrate that allowed them to be carried away passively at speeds >1000 times greater than crawling. Thereby, population metrics of sedentary organisms may be responding more quickly than expected to environmental, social and reproductive imperatives. Overall, a change in our perception of sedentarity in marine benthic animals appears to be warranted, towards a deeper integration of how behavioural adaptations modulate mobility and dispersal across all life stages.

Until now, accounts of "swimming" in adult holothuroids were thought to be unique to certain deep-sea species (Rogacheva, Gebruk & Alt, 2012), except for burst responses to predator encounters (Margolin, 1976; Legault & Himmelman, 1993). The unprecedented evidence provided here for subtidal/intertidal species, from combined experimental and observational work, underscores that dispersal in holothuroids (and other macrobenthic animals) may not be limited to the pelagic larval phase, but also occur during the benthic juvenile/adult stages through ABA. Such findings shed new light on a growing body of anecdotal reports (Supporting Information S3), including “ballooning” in cultured holothuroid juveniles (C. Hair pers. comm., University of the Sunshine Coast, Australia), “balling” in asteroid echinoderms (Sheehan & Cousens, 2017) and “inflating” in pennatulacean corals (Chimienti, Angeletti & Mastrototaro, 2018), supporting that ABA-assisted locomotion could be widespread, if not generalized. While most larval forms disperse through mid-column or near-surface currents, the potential contribution of bottom currents to ABA-assisted migration suggests that dispersal of benthic species may not only occur over longer periods, but also take advantage of broader oceanographic processes than typically accounted for in plankton-centric models (e.g. Cowen & Sponaugle, 2009). This could even lead to a shift in dispersal paradigms; i.e. over a lifetime, displacement might be equal or greater during the adult benthic stage than through larval dispersal. The present study also hints at predictable patterns of ABA-assisted migrations (e.g. monthly, seasonally) likely to have significant impacts, both from ecological and economical perspectives.

Already, the occurrence of ABA and associated behaviours in Holothuroidea can help understand population structures, and variations thereof, which are not easily reconciled with a slow-moving sedentary benthic lifestyle. For one, it may contribute to the broad or cosmopolitan distributions, as those seen in the focal species. In addition, large aggregations of *C. frondosa* have been reported around Newfoundland (CSAR, 2006). These patchy distribution patterns could be the result of mass migrations, such as those depicted here in the videos, perhaps in response to transient currents or turbidity flows (e.g. tides, storms). ABA and tumbling in *C. frondosa* may also explain bathymetric trends in size frequency distributions, whereby large individuals are typically found in greater number at deeper depths (Hamel & Mercier, 1996). Interestingly, sudden mass beaching of holothuroids and other echinoderms has been reported, which could have involved ABA reactions (Supporting Information S3).

While the general ABA behaviours were similar in the two focal species, nuances were detected, which may or may not be related to the fact that the study on *C. frondosa* involved wild individuals and field surveys, whereas that on *H. scabra* involved captive individuals (albeit of first generation studied in close-to-natural settings). Only tumbling was commonly recorded in *C. frondosa*, whereas tumbling and floating were both consistently documented in *H. scabra*. In general, *C. frondosa* is found at subtidal depths on rocky or gravely bottoms (Hamel & Mercier, 1996), while *H. scabra* typically occurs on soft substrate at shallower depths, sometimes <1 m (Hamel et al., 2001). The latter therefore have a greater likelihood of coming in contact with air, which is suspected to assist in the floating behaviour by combining ABA with accumulation of air in the body cavity. Another difference between the focal species was the more predictable ABA behaviour evidenced in *H. scabra* juveniles and adults during certain lunar/tidal phases, which may be a dispersal strategy to counter drops in the quality or quantity of benthic food supply (organic matter) in sea-ranching settings. In contrast, *C. frondosa* is a suspension feeder that captures plankton carried by the currents, virtually eliminating any need for periodic movement related to localized depletion of food.

The experimental segment on *C. frondosa* highlighted different strengths of ABA, which might afford a certain level of control over its use and outcome. When triggered by an increase in conspecific density (weak response) only minimum bloating (~40% increase in seawater to flesh ratio) and detachment were noted in *C. frondosa*. Under laboratory conditions this behaviour persisted for days or weeks, as long as conspecific density was not decreased, and it disrupted normal feeding (which requires firm attachment to the substrate and deployment of the oral tentacles). This weak ABA response may be akin to a standby state, allowing the individual to be carried away opportunistically. This mechanism may prevent accumulations of holothuroids at bottlenecks or boundaries by helping to redistribute individuals more evenly after mass physical-migration events. The latter appear to occur in response to greater stress, such as a sudden decrease in salinity or surge in turbidity, which triggered a more severe ABA response in the trials (>700% increase in water:flesh ratio). Like other echinoderms, holothuroids have a limited capacity for osmoregulation that makes them susceptible to low salinities (Meng, Dong, Dong, Yu & Zhou, 2011). Under such adverse conditions, ABA was also linked to body reorientation in *C. frondosa*, from a normal upward to an unusual downward (anus-up) posture, persisting as long as the stressor was present. Moreover, cloacal respiration, which is a known indicator of stress level in *C. frondosa* (Gianasi, Verkaik, Hamel & Mercier, 2015), increased immediately after exposure to low salinity or high turbidity, possibly to help fill the body cavity and aid ABA. Once the response was established, slower cloacal respiration rates were recorded, presumably to limit exchanges with the adverse external environment.

Most ABA reactions, with the exception of positive buoyancy observed at the air-sea interface (discussed earlier), were not immediately conducive to movement, but instead dependent on the presence and strength of water flow. Therefore, ABA-generated speeds were highly variable, but generally yielded greater displacements than the 1-2 m d^{-1} typically effected by forward crawling in the focal species (Purcell & Kirby, 2006; Gianasi et al., 2018) and the maximum crawling speeds measured here in adults of *C. frondosa* (12 m d^{-1}). Tumbling of *C. frondosa* (from camera tows) was estimated to generate displacement rates of up to ~90 km d^{-1}. In comparison, the pelagic embryos/larvae that develop over 40-45 days (Hamel & Mercier, 1996) are estimated to cover 17-20 km d^{-1} over that period (So, Uthicke, Hamel & Mercier, 2011). Weak ABA generated by high conspecific densities was maintained as long as the stressor persisted (in the order of weeks under laboratory setting); similarly, severe ABA reactions started to subside only when conditions of turbidity or salinity returned to normal, supporting that ABA may underlie both short- and long-term travel. Over the lifetime of the species, which is likely in the order of several decades (Ebert & Southon, 2003), dispersal of adults through ABA could be several orders of magnitude greater than larval dispersal. Movements through ABA are probably not rare either, since they were captured on video during random benthic surveys conducted hundreds of km and years apart. Conditions that trigger them may occur relatively frequently. Based on results obtained in the mesocosm, water flows between 20-120 cm s^{-1} induced tumbling behaviour. Overall, tumbling under laboratory conditions effected displacement speeds between 5 and 86 cm s^{-1} that represented ~70% of the concurrent flow regimes to which they were exposed. Tumbling events documented in the field may correspond to periods of changing tide and/or may have followed residual storm-induced currents, which can fetch up to 110 cm s^{-1} over the Newfoundland Grand Banks (Wu, Tang, Li & Prescott, 2011). Similarly, tumbling and floating in *H. scabra* was favoured by tidal currents along the coast of Madagascar, as evidenced by greater occurrence during the full/new moons and ebbing tides, although the greater intensity of ABA events at the full than at the new moon remains unresolved. Moreover, *H. scabra* exhibited ABA behaviour when the current was in an offshore direction, presumably to avoid being washed ashore. It was demonstrated during the mesocosm trials that individuals of *C. frondosa* were able to re-anchor to the substrate after ABA events under water flows of similar strengths, emphasizing the "active" component of ABA. However, more experiments should be performed to better understand the limitation of this dispersive strategy.

While no direct comparison can be made with terrestrial taxa, due to the distinctiveness of the aquatic medium, a few interesting parallels may be drawn. Tumbling behaviour on land is best exemplified by tumbleweeds that break free of attachment to be carried by the wind (Baker, Beck, Bienkiewicz & Bjostad, 2008). However, this adaptation involves the death of the parental unit to enable the dispersal of seeds. Indeed, much like dispersal in the ocean (Cowen & Sponaugle, 2009), dispersal in air largely centers on propagules (Howe & Smallwood, 1982; Cain, Milligan & Strand, 2000), rather than on the adult and juvenile stages discussed here. One exception may be the transient aerial dispersal of adult arachnids using silk (dubbed "ballooning"), which has been described as a mixed Evolutionary Stable Strategy (Bell, Bohan, Shaw & Weyman, 2005). Overall, the drivers and implications of mixing directed small-scale movements and undirected long-distance dispersal (e.g. crawling and tumbling; walking and ballooning) deserve better integration in the conceptual frameworks of movement ecology (Nathan et al., 2008).

The occurrence of ABA in Holothuroidea is also of great significance for their management and conservation, since they are the target of important fisheries and aquaculture programs (Hamel & Mercier, 2008a; Purcell et al., 2013; 2014). Rapid ABA responses could explain why well-known fishing grounds for *C. frondosa* can start to yield dramatically decreased catches over short periods, and recover again (Quin Sea Ltd. meeting, 13 Dec 2017). Bottom trawling activity is known to generate increased turbidity (Palanques, Guillén & Puig, 2001), which likely triggers ABA and momentarily reduces overall catchability (as neutrally-buoyant individuals likely bounce off the gear, and could drift away). Such sudden variations in the catchability of *C. frondosa* due to ABA not only impact the fisheries, but also need to be factored in during stock assessments. On the other hand, certain areas may function as bottlenecks where holothuroids accumulate transiently after mass ABA events, as was suspected to occur in the highly dynamic tidal environment of the Passamaquoddy Bay (S. Rowe, pers. comm.). Should these areas become targeted by fisheries, increased catches could deplete the resource at much faster rates than estimated by monitoring programs. The occurrence of ABA also has major implications for aquaculture and sea ranching. Until now, the loss of juveniles (seedlings) during the grow-out phase was principally attributed to either mortality or predation. However, the main culprit may well be ABA, as shown here with *H. scabra* in Madagascar and suspected by other stakeholders in several regions, including Vietnam, Papua New Guinea and Malaysia (C. Hair pers. comm., World Aquaculture Conference symposium; E. Nesher pers. comm., Malaysia; Supporting Information S3). Moreover, climatic changes will likely drive increases in the occurrence and/or severity of storm events and rainy seasons, thereby stimulating ABA and tumbling/floating behaviours over the coming years, which could change the population dynamics and distribution range of focal holothuroids. For all these reasons, more attention should henceforth be given to ABA in painting a more holistic picture of the ecology and biogeography of so-called sedentary benthic organisms.

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Author Contributions

JFH and AM conceived and coordinated the study, analyzed the data and led the writing of the manuscript; JS, BLG and EMM collected and analyzed laboratory data on *C. frondosa*; BB compiled the data on *H. scabra*; EK led the collection of field video data on *C. frondosa* in Nova Scotia; SR helped collect data on *C. frondosa* and provided intellectual input; PW led the collection of field video data on *C. frondosa* in Newfoundland; all authors contributed critically to the drafts and gave final approval for publication.

Data Accessibility

Data associated with this study are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.90103c0 (Hamel et al., 2018).

References

Allen, A.M. & Singh, N.J. (2016) Linking movement ecology with wildlife management and conservation. *Frontiers in Ecology and Evolution*, *3*, 155.

Allen, R.M., Metaxas, A., & Snelgrove, P.V.R. (2018) Applying movement ecology to marine animals with complex life cycles. *Annual Review of Marine Science*, *10*, 3.1-3.24.

Anderson, S.C., Flemming, J.M., Watson, R., & Lotze, H.K. (2011) Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries*, *12*, 317–339.

Baguette, M., Blanchet, S., Legrand, D., Stevens, V.M., & Turlure, C. (2013) Individual dispersal, landscape connectivity and ecological networks. *Biological Reviews*, *88*, 310-326.

Baker, D.V., Beck, K.G., Bienkiewicz, B.J., & Bjostad, L.B. (2008) Forces necessary to initiate dispersal for three tumbleweeds. *Invasive Plant Science and Management*, *1*, 59-65.

Bell, J.R., Bohan, D.A., Shaw, E.M., & Weyman, G.S. (2005) Ballooning dispersal using silk: world fauna, phylogenies, genetics and models. *Bulletin of Entomological Research*, *95*, 69-114.

Berg, M.P., Kiers, E., Driessen, G., Van Der Heijden, M., Kooi, B.W., Kuenen, F., Liefting, M., Verhoef, H.A., & Ellers, J. (2010) Adapt or disperse: understanding species persistence in a changing world. *Global Change Biology*, *16*, 587-598.

Bie, T., Meester, L., Brendonck, L., Martens, K., Goddeeris, B., Ercken, D., Hampel, H., Denys, L., Vanhecke, L., & Gucht, K. (2012) Body size and dispersal mode as key traits determining metacommunity structure of aquatic organisms. *Ecology Letters*, *15*, 740-747.

Bonte, D., Van Dyck, H., Bullock, J.M., Coulon, A., Delgado, M., Gibbs, M., Lehouck, V., Matthysen, E., Mustin, K., & Saastamoinen, M. (2012) Costs of dispersal. *Biological Reviews*, *87*, 290-312.

Cain, M.L., Milligan, B.G., & Strand, A.E. (2000) Long-distance seed dispersal in plant populations. *American Journal of Botany*, *87*, 1217-1227.

Chimienti, G., Angeletti, L., & Mastrototaro, F. (2018) Withdrawal behaviour of the red sea pen *Pennatula rubra* (Cnidaria: Pennatulacea). *The European Zoological Journal*, *85*, 64-70.

Cowen, R.K. & Sponaugle, S. (2009) Larval dispersal and marine population connectivity. *Annual Review of Marine Science*, *1*, 443-466.

CSAR (2006). Habitat utilization and density of sea cucumber (*Cucumaria frondosa*) on St. Pierre Bank, Newfoundland: observations using a towed camera sled in 2004 and 2005, Fisheries and Marine Institute of Memorial University, St. John's, NL, Canada.

Da Silva, J., Cameron, J.L., & Fankboner, P.V. (1986) Movement and orientation patterns in the commercial sea cucumber *Parastichopus californicus* (Stimpson)

(Holothuroidea: Aspidochirotida). *Marine and Freshwater Behaviour and Physiology*, *12*, 133-147.

Ebert, T.A. & Southon, J.R. (2003) Red sea urchins (*Strongylocentrotus franciscanus*) can live over 100 years: confirmation with A-bomb ^{14}carbon. *Fishery Bulletin*, *101*, 915-922.

Frisk, M.G., Jordaan, A., & Miller, T.J. (2014) Moving beyond the current paradigm in marine population connectivity: are adults the missing link? *Fish and Fisheries*, *15*, 242-254.

Gianasi, B.L., Hamel, J.-F., & Mercier, A. (2018) Morphometric and behavioural changes in the early life stages of the sea cucumber *Cucumaria frondosa*. *Aquaculture*, *490*, 5-18.

Gianasi, B.L., Verkaik, K., Hamel, J.-F., & Mercier, A. (2015) Novel use of PIT tags in sea cucumbers: promising results with the commercial species *Cucumaria frondosa*. *PLoS One*, *10*, e0127884.

Gordon, D., Kenchington, E., Gilkinson, K., McKeown, D., Steeves, G., Chin-Yee, M., Vass, W., Bentham, K., & Boudreau, P. (2000) Canadian imaging and sampling technology for studying marine benthic habitat and biological communities. In ICES 2000 annual science conference. ICES, Copenhagen, Bruges, Belgium.

Grantham, B.A., Eckert, G.L., & Shanks, A.L. (2003) Dispersal potential of marine invertebrates in diverse habitats. *Ecological Applications*, *13*, 108–116.

Hamel, J.-F. & Mercier, A. (1996) Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 253–271.

Hamel, J.-F. & Mercier, A. (2008a). Population status, fisheries and trade of sea cucumbers in temperate areas of the northern hemisphere. In *Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical paper 516* (eds V. Toral-Granda, A. Lovatelli & M. Vasconcellos), Vol. 516, pp. 257-292. FAO, Rome.

Hamel, J.-F. & Mercier, A. (2008b). Precautionary management of *Cucumaria frondosa* in Newfoundland and Labrador, Canada. In *Sea cucumbers. A global review of fisheries and trade. FAO Fisheries and Aquaculture Technical paper 516* (eds V. Toral-Granda, A. Lovatelli & M. Vasconcellos), pp. 293-306. FAO, Rome.

Hamel, J.-F., Conand, C., Pawson, D.L., & Mercier, A. (2001) The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): its biology and exploitation as beche-de-mer. *Advances in Marine Biology*, *41*, 129–223.

Hamel, J.-F., Sun, J., Gianasi, B.L., Montgomery, E.M., Kenchington, E.L., Burel, B., . . . Mercier, A. (2018) Data from: Active buoyancy adjustment increases dispersal potential in benthic marine animals. *Dryad Digital Repository*, https://doi.org/10.5061/dryad.90103c0.

Hays, G.C., Ferreira, L.C., Sequeira, A.M.M., Meekan, M.G., Duarte, C.M., Bailey, H., Bailleul, F., Bowen, W.D., Caley, M.J., Costa, D.P., Eguíluz, V.M., Fossette, S., Friedlaender, A.S., Gales, N., Gleiss, A.C., Gunn, J., Harcourt, R., Hazen, E.L., Heithaus, M.R., Heupel, M., Holland, K., Horning, M., Jonsen, I., Kooyman, G.L., Lowe, C.G., Madsen, P.T., Marsh, H., Phillips, R.A., Righton, D., Ropert-Coudert, Y., Sato, K., Shaffer, S.A., Simpfendorfer, C.A., Sims, D.W., Skomal, G., Takahashi, A., Trathan, P.N., Wikelski, M., Womble, J.N., & Thums, M. (2016) Key questions in marine megafauna movement ecology. *Trends in Ecology & Evolution*, *31*, 463-475.

Heezen, B.C. & Hollister, C.D. (1971) *The face of the deep* Oxford University Press, London.

Holyoak, M., Casagrandi, R., Nathan, R., Revilla, E., & Spiegel, O. (2008) Trends and missing parts in the study of movement ecology. *Proceedings of the National Academy of Sciences*, *105*, 19060.

Howe, H.F. & Smallwood, J. (1982) Ecology of seed dispersal. *Annual Review of Ecology and Systematics*, *13*, 201-228.

Lauth, R.R., Wakefield, W.W., & Smith, K. (2004) Estimating the density of thornyheads, *Sebastolobus* spp., using a towed video camera sled. *Fisheries Research*, *70*, 39-48.

Legault, C. & Himmelman, J.H. (1993) Relation between escape behaviour of benthic marine invertebrates and the risk of predation. *Journal of Experimental Marine Biology and Ecology*, *170*, 55-74.

Macfarlane Colin, B.A., Drolet, D., Barbeau Myriam, A., Hamilton Diana, J., & Ollerhead, J. (2013) Dispersal of marine benthic invertebrates through ice rafting. *Ecology*, *94*, 250-256.

Margolin, A.S. (1976) Swimming of the sea cucumber *Parastichopus californicus* (Stimpson) in response to sea stars. *Ophelia*, *15*, 105-114.

Meng, X.-l., Dong, Y.-w., Dong, S.-l., Yu, S.-s., & Zhou, X. (2011) Mortality of the sea cucumber, *Apostichopus japonicus* Selenka, exposed to acute salinity decrease and related physiological responses: osmoregulation and heat shock protein expression. *Aquaculture*, *316*, 88-92.

Mercier, A. & Hamel, J.-F. (2013). Sea cucumber aquaculture: hatchery production, juvenile growth and industry challenges. In *Advances in aquaculture hatchery* *technology* (eds G. Allan & G. Burnell), pp. 431-454. Woodhead Publishing Ltd, Cambridge.

Mercier, A., Battaglene, S.C., & Hamel, J.-F. (2000) Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia*, *440*, 81-100.

Nathan, R., Getz, W.M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D., & Smouse, P.E. (2008) A movement ecology paradigm for unifying organismal movement research. *Proceedings of the National Academy of Sciences*, *105*, 19052-19059.

Palanques, A., Guillén, J., & Puig, P. (2001) Impact of bottom trawling on water turbidity and muddy sediment of an unfished continental shelf. *Limnology and Oceanography*, *46*, 1100-1110.

Pilditch, C.A., Valanko, S., Norkko, J., & Norkko, A. (2015) Post-settlement dispersal: the neglected link in maintenance of soft-sediment biodiversity. *Biology Letters*, *11*.

Purcell, S.W. & Kirby, D.S. (2006) Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling. *Fisheries Research*, *80*, 53-61.

Purcell, S.W., Samyn, Y., & Conand, C. (2012) *Commercially important sea cucumbers of the world* FAO Species Catalogue for Fishery Purposes No 6, Rome.

Purcell, S.W., Conand, C., Uthicke, S., & Byrne, M. (2016) Ecological roles of exploited sea cucumbers. *Oceanography and Marine Biology: An Annual Review*, *54*, 367-386.

Purcell, S.W., Piddocke, T.P., Dalton, S.J., & Wang, Y.-G. (2016) Movement and growth of the coral reef holothuroids *Bohadschia argus* and *Thelenota ananas*. *Marine Ecology Progress Series*, *551*, 201-214.

Purcell, S.W., Polidoro, B.A., Hamel, J.-F., Gamboa, R.U., & Mercier, A. (2014) The cost of being valuable: predictors of extinction risk in marine invertebrates exploited as luxury seafood. *Proceedings of the Royal Society B: Biological Sciences*, *281*, 20133296.

Purcell, S.W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, V., Lovatelli, A., & Uthicke, S. (2013) Sea cucumber fisheries: global review of stock status, management measures and drivers of overfishing. *Fish and Fisheries*, *14*, 34–59.

Rogacheva, A., Gebruk, A., & Alt, C.H.S. (2012) Swimming deep-sea holothurians (Echinodermata: Holothuroidea) on the northern Mid-Atlantic Ridge. *Zoosymposia*, *7*, 213-224.

Santos, R. & Flammang, P. (2007) Intra-and interspecific variation of attachment strength in sea urchins. *Marine Ecology Progress Series*, *332*, 129-142.

Sequeira, A.M.M., Rodríguez, J.P., Eguíluz, V.M., Harcourt, R., Hindell, M., Sims, D.W., Duarte, C.M., Costa, D.P., Fernández-Gracia, J., Ferreira, L.C., Hays, G.C., Heupel, M.R., Meekan, M.G., Aven, A., Bailleul, F., Baylis, A.M.M., Berumen, M.L., Braun, C.D., Burns, J., Caley, M.J., Campbell, R., Carmichael, R.H., Clua, E., Einoder, L.D., Friedlaender, A., Goebel, M.E., Goldsworthy, S.D., Guinet, C., Gunn, J., Hamer, D., Hammerschlag, N., Hammill, M., Hückstädt, L.A., Humphries, N.E., Lea, M.A., Lowther, A., Mackay, A., McHuron, E., McKenzie, J., McLeay, L., McMahon, C.R., Mengersen, K., Muelbert, M.M.C., Pagano, A.M., Page, B., Queiroz, N., Robinson, P.W., Shaffer, S.A., Shivji, M., Skomal, G.B., Thorrold, S.R., Villegas-Amtmann, S., Weise, M., Wells, R., Wetherbee, B., Wiebkin, A., Wienecke, B., & Thums, M. (2018)

Convergence of marine megafauna movement patterns in coastal and open oceans. *Proceedings of the National Academy of Sciences*, *115*, 3072.

Sheehan, E.V. & Cousens, S.L. (2017) “Starballing”: a potential explanation for mass stranding. *Marine Biodiversity*, *47*, 617-618.

So, J.J., Hamel, J.-F., & Mercier, A. (2010) Habitat utilisation, growth and predation of *Cucumaria frondosa*: implications for an emerging sea cucumber fishery. *Fisheries Management and Ecology*, *17*, 473–484.

So, J.J., Uthicke, S., Hamel, J.-F., & Mercier, A. (2011) Genetic population structure in a commercial marine invertebrate with long-lived lecithotrophic larvae: *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Marine Biology*, *158*, 859–870.

Thiel, M. & Gutow, L. (2005) The ecology of rafting in the marine environment. II. The rafting organisms and community. *Oceanography and Marine Biology: an annual review*, *43*, 279-418.

Underwood, M.J., Winger, P.D., & Legge, G. (2012) Development and evaluation of a new high definition self-contained underwater camera system to observe fish and fishing gears in situ. *Journal of Ocean Technology*, *71*, 59-70.

Walther, B.D., Munguia, P., & Fuiman, L.A. (2015) Frontiers in marine movement ecology: mechanisms and consequences of migration and dispersal in marine habitats. *Biology Letters*, *11*.

Winston, J.E. (2012) Dispersal in marine organisms without a pelagic larval phase. *Integrative and Comparative Biology*, *52*, 447-457.

Wu, Y., Tang, C.C.L., Li, M.Z., & Prescott, R.H. (2011) Modelling extreme storm-induced currents over the grand banks. *Atmosphere-Ocean*, *49*, 259-268.

Figures

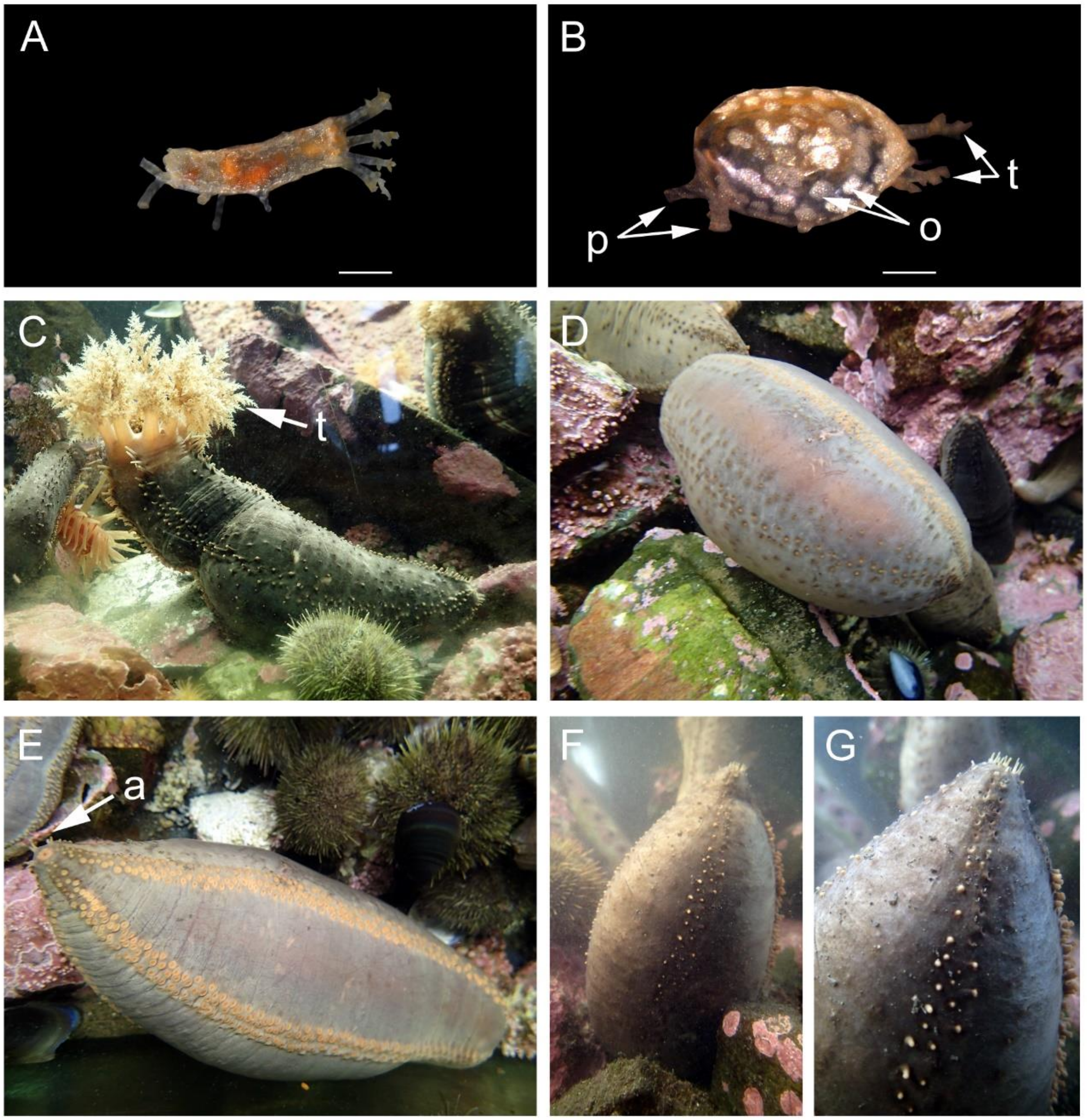

Figure A.1 Illustration of posturing and behaviour involved during active buoyancy adjustment (ABA) in *Cucumaria frondosa*. A) Juvenile in normal state exhibiting typical elongated shape, with tentacles deployed (right). B) Juvenile exhibiting bloating (balloon shape) typical of ABA, with podia (p) and tentacles (t) deployed, and space visible between ossicles (o) on the surface of the body wall. C) Adult in normal state with ventral podia attached, oral end elevated and tentacles deployed (t). D) Adult detaching from the substrate and assuming a bloated shape during initiation of ABA. E) Adult in middle stage of ABA with anus (a) becoming elevated. F-G) Posture at culmination of ABA in adult, i.e. fully bloated, completely detached, neutrally buoyant, with anal end pointing upwards. Scale bars in A and B represent 0.5 mm; individuals in C to G are ~24 cm long (relaxed).

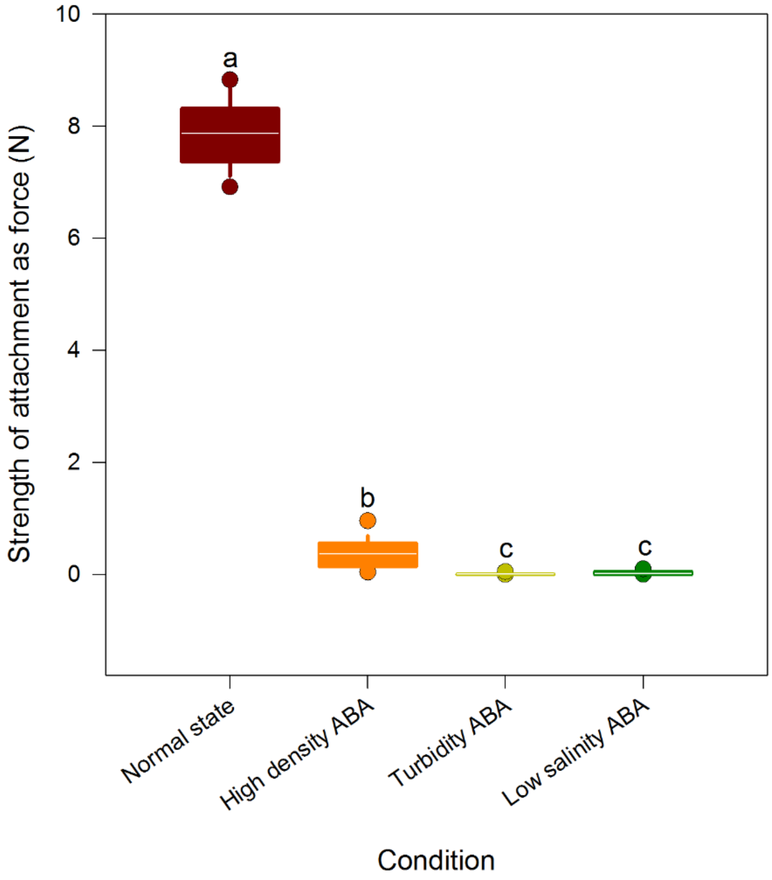

Figure A.2 Box plot of strength of attachment to the substrate in *Cucumaria frondosa* measured as force (N) necessary to forcibly pull away individuals in different states (at the peak of the reaction), comparing normal adults to adults exhibiting ABA in response to high conspecific density (weak ABA), water turbidity and low salinity (severe ABA). The box shows the mean in white (n=17-24) with upper and lower quartiles, the whiskers show minimum and maximum values, and the circles show the outliers (5th and 95th percentile). Different letters denote statistically significant differences (one-way ANOVA on ranks, H = 73.51, df = 3, $p < 0.001$; post-hoc Dunn's method, $p < 0.05$).

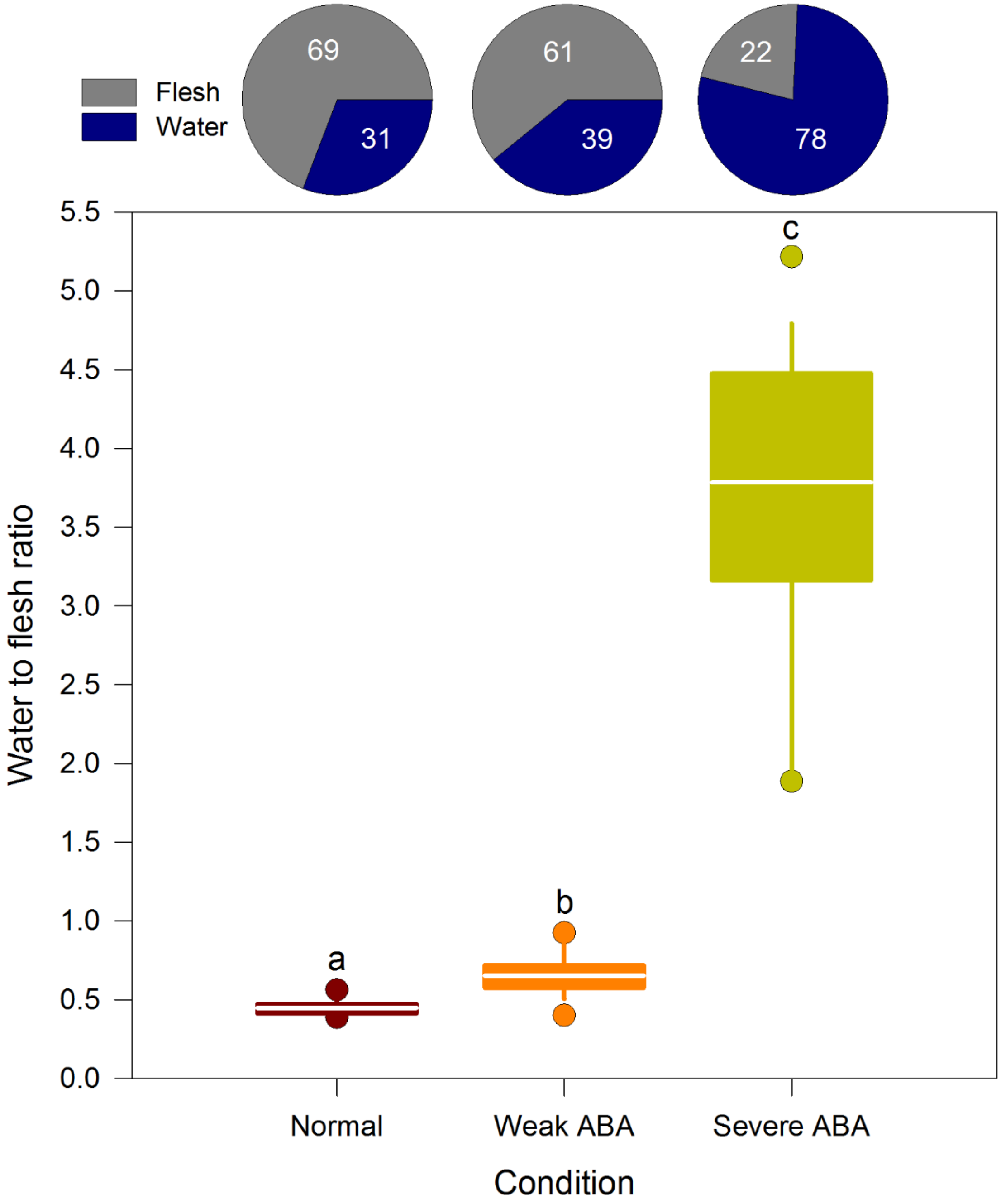

Figure A.3 Pie charts showing percent water content and box plot depicting water to flesh ratio (weight based) in normal adults and in bloated adults exhibiting weak or severe ABA. The pies show mean proportions. Each box shows the mean in white (n=18-19) with upper and lower quartiles, whiskers show minimum and maximum values, and the circles show the outliers (5th and 95th percentile). Different letters denote statistically significant differences between conditions (one-way ANOVA, $F_{2,53} = 671.03$, $p < 0.001$; post-hoc Holm-Sidak method, $p < 0.05$).

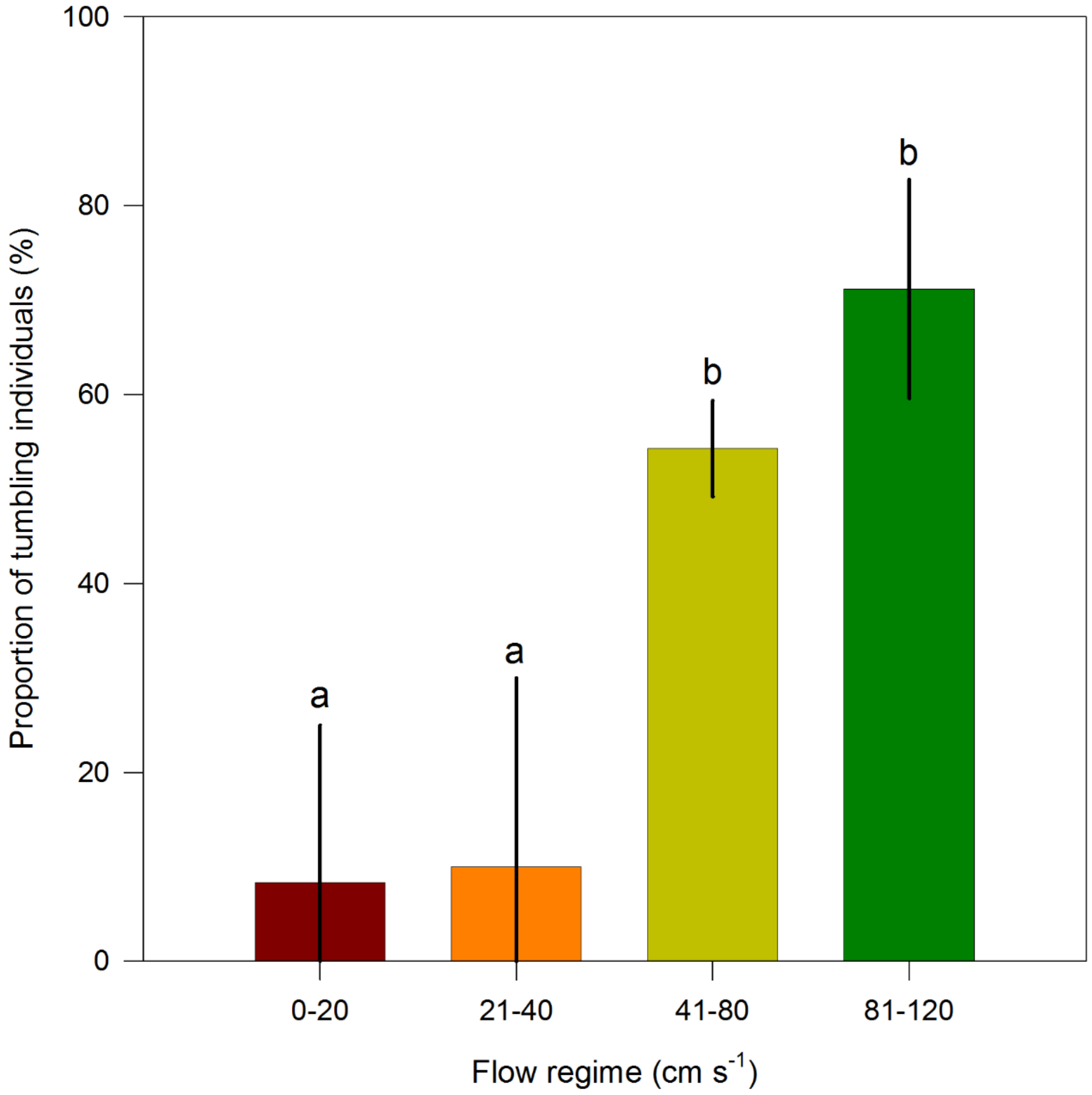

Figure A.4 Proportion of tumbling individuals of *Cucumaria frondosa* under various flow regimes in a large mesocosm. Data shown as mean (±SD) across 4 replicate trials. Different letters denote statistically significant differences between conditions (one-way ANOVA, $F_{3,12} = 19.19$, $p < 0.001$; post-hoc Holm-Sidak method, $p < 0.05$).

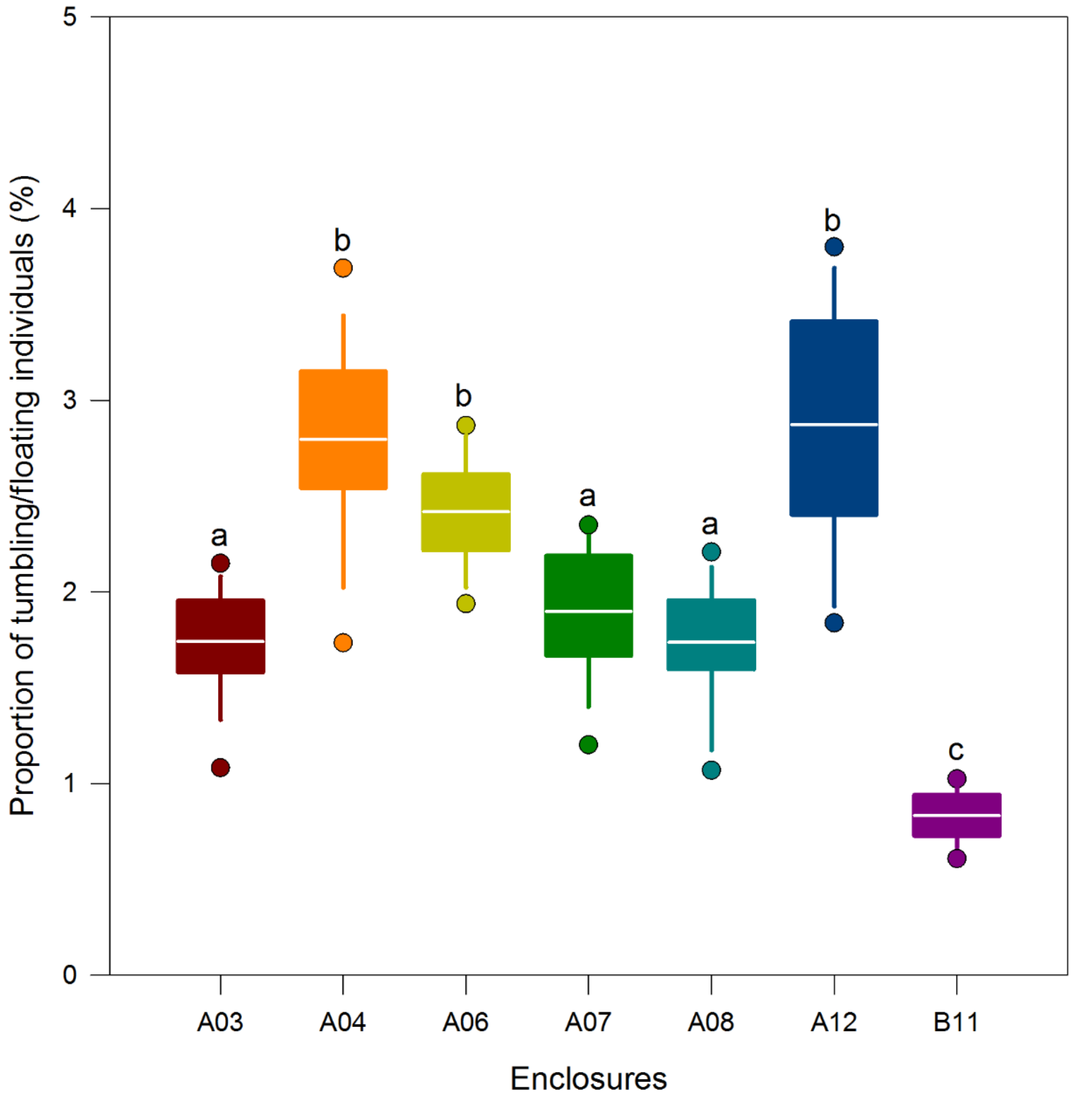

Figure A.5 Proportion of individuals of *Holothuria scabra* displaying tumbling/floating from ABA in 7 focal enclosures around the full and new moons over the study. The box shows the mean in white (n=42) with upper and lower quartiles, the whiskers show minimum and maximum values, and the circles show the outliers (5th and 95th percentile). Different letters denote statistically significant differences between pens (one-way ANOVA on ranks, H = 219.16, df = 6, p <0.001; post-hoc Tukey's method, p <0.05).

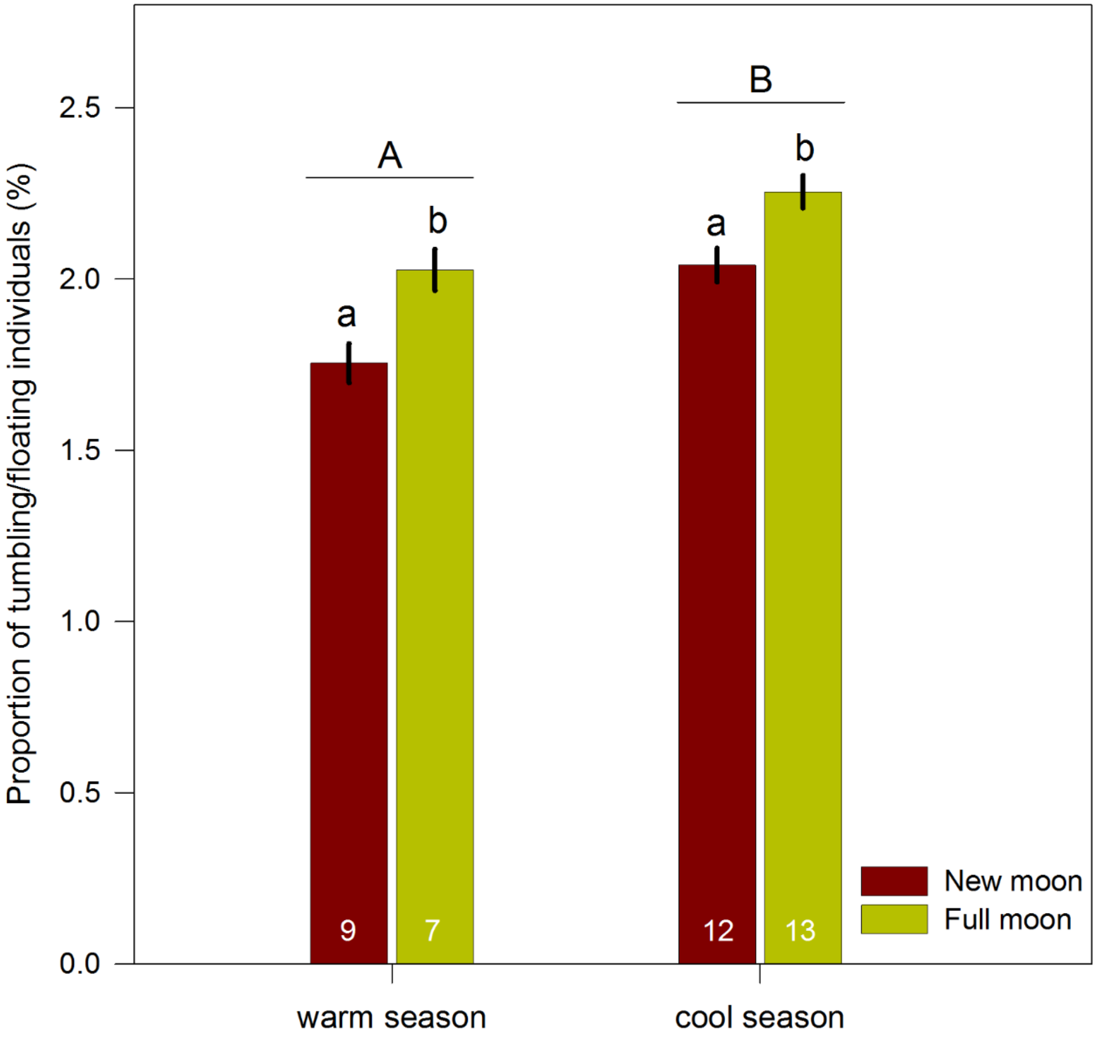

Figure A.6 Intensity of ABA reaction across seasons and lunar cycles in *Holothuria scabra*, shown as mean proportion (±SD) of individuals scored as "floating" or "tumbling" while monitoring the seven sea pens every 15 days in 2015-2016. Sample size is shown on each bar. Different lowercase letters denote statistically significant differences between moon phases in each season (two-way ANOVA, $F_{1,38} = 20.43$, $p < 0.001$; post-hoc Holm-Sidak method, $p < 0.005$), and different capital letters denote statistically significant differences between seasons (two-way ANOVA, $F_{1,38} = 22.73$, $p < 0.001$).

Supplementary Material

Video (S.A.1) showing example of tumbling in *Cucumaria frondosa* (Nova Scotia, Canada).

Video (S.A.2) showing example of tumbling and floating in *Holothuria scabra* (Madagascar).

Anecdotal reports (S.A.3) of ABA-related behaviours in Holothuroidea and other Echinodermata.

All the supplementary materials are available: https://besjournals.onlinelibrary.wiley.com/doi/full/10.1111/1365-2656.12943