# Characterization and role of major deep-sea pennatulacean corals in the bathyal zone of Eastern Canada

by

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# Abstract

Pennatulaceans (Octocorallia: Pennatulacea), commonly called sea pens, are colonial corals that typically anchor themselves into soft sediment (mud, sand), allowing them to colonize large areas of the sea floor from the intertidal zone down to the abyssal plain. Sea pens can occur sparsely or form large aggregations, suggesting that they may provide an important structural habitat to other organisms by increasing the complexity of the muddy seabed. The investigation of the three most common sea pens (Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata) of the continental slope of Newfoundland and Labrador (Northwest Atlantic) showed different morphologies and adaptations to environmental parameters. Variations in their morphology were visible along bathymetric and latitudinal gradients, following food availability. This study identified different feeding strategies according to stable isotope signatures and macromorphologies (polyp diameter, colony length, shape). Different defense strategies were also identified based on the presence and localisation of sclerites in the tissues. Reproductive strategies were determined for A. grandiflorum and H. finmarchica, with both species presenting a seasonal spawning between April (Southern Newfoundland) and July/August (Labrador). The latitudinal shift in spawning followed the development of the surface phytoplankton bloom (i.e. sinking of phytodetritus). Low fecundity at the polyp level (13 and 6 oocytes polyp$^{-1}$, respectively) yielded similar whole-colony fecundity in the two species (500-6000 oocytes colony$^{-1}$). The measure of fecundity is discussed to highlight the importance of standardizing metrics (mature oocyte just before spawning) to avoid an overestimation of the fecundity and to allow comparison among

species. Only ~20% of the oocyte matured in both species. The remaining oocytes in A. grandiflorum disappeared, indicating that oogenesis develops and culminates over 12 months. In H. finmarchica the persistence of the small oocytes indicates protracted oogenesis (>12 months). Finally, an analysis of the associated biodiversity showed that sea pens have relatively few associates but that they play an important role in their life history. Some associates are obligate (e.g. parasitic copepods) and others facultative symbionts (the sea anemone Stephanauge nexilis). The seasonal (April-May) presence of fish larvae (Sebastes spp.) and shrimp larvae (Pandalus borealis, Pasiphae multidentata and Acanthephyra pelagica) emphasize the role of sea pens as nursery habitat, and provides an argument to recognize them as an essential fish habitat.

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*"Ne crois pas que tu t'*es trompé de route quand *tu n'es pas allé assez loin"*

Claude Aveline

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## Co-authorship statement

The research described in this thesis was carried out by Sandrine Baillon, with guidance from Annie Mercier and Jean-François Hamel. Sandrine Baillon was responsible for data collection and analysis. Manuscripts resulting from this thesis were prepared by Sandrine Baillon, with editing assistance and intellectual input from co-authors as follows:

Authorship for publications arising from Chapter 2 will be S. Baillon, J.-F. Hamel, M. English and A. Mercier.

Authorship for publications arising from Chapter 3 and 6 will be S. Baillon, J.-F. Hamel, V.E. Wareham and A. Mercier.

Authorship for publications arising from Chapter 4 and 5 will be S. Baillon, J.-F. Hamel and A. Mercier.

The manuscripts in chapters 2 to 6 have been prepared for publication in different scientific journals, and the format of chapters varies according to the specific journal requirements.

# Chapter 1 : General introduction

# 1. Corals

Corals are a paraphyletic group of organisms and are members of the phylum Cnidaria, class Anthozoa and class Hydrozoa, that live from the Arctic circle to Antarctica, between the surface and >6000 m depth (Williams 1995; Longo et al. 2005; Etnoyer et al. 2006; Roberts et al. 2006; Cairns 2007). To date 5080 species of corals have been described, 66% of which (3336) live below 50 m and are azooxanthellate (i.e., lacking symbiotic algae) (Cairns 2007). Zooxanthellate corals from warm and shallow-water environments (e.g., tropical coral reefs) have received considerable attention; however, cold-water and deep-sea corals remain comparatively understudied despite increasing interest in recent years.

Shallow and deep-water corals form an important habitat for other species (Moberg and Folke 1999; Buhl-Mortensen and Mortensen 2004). However, they are highly sensitive to human-related disturbance (Roberts et al. 2002; Roberts et al. 2006) including climate change, dynamite fishing, eutrophication due to erosion, pollution (e.g. oil leaking), and trawling. Therefore, it is important to know and understand their biology and ecology to determine their potential for resilience and ultimately manage and protect these habitat-forming species.

## 1.1 Coral morphology

Knowledge of the morphology of coral species allows a better understanding of their ecology and biology (e.g. small polyps harbour a lower fecundity than larger polyps). Coral morphology varies with surrounding environmental conditions including light (for shallow-water species), water movement, sedimentation, depth and competition

for space (Chappell 1980; Prada et al. 2008; Todd 2008). To date, morphological variations have been examined in coral species from shallow waters across limited depth ranges (spanning <50 m) due to the easier access to these coral colonies as well as to a better knowledge of environmental conditions along the coasts. In contrast, deep-sea corals that colonize a wide range of depths (spanning >1000 m) and occasionally present a cosmopolitan distribution (Roberts et al. 2006) are more rarely considered (Pasternak 1989; Rice et al. 1992). Their wide geographic distributions make them ideal for studies of intra-specific morphological variation across depths and geographic regions.

Species or individual morphology can be described/discriminated using biometrics. Coral biometric parameters include a large number of macrometric characteristics: colony length, colony height, weight, polyp density, polyp diameter, polyp length. All of these parameters have previously been shown to vary along environmental gradients. For example, the shallow-water coral Briareum asbestinum sampled in the Bahamas showed different colony sizes at 5 and 35 m depth (West et al. 1993). The shallow colonies were shorter and stouter, with more polyps than the deeper colonies. The authors suggest that the decrease in polyp density with depth is an adaptation to a decrease in light intensity; lower polyp density minimizes shading of adjacent polyps of the same colony, decreasing any impact on the photosynthesis of the mutualistic algae.

Biometrics can also be measured at a smaller scale: micrometric parameters include the shape, length and number of tentacles and pinnules[1] (Grossowicz and Benayahu 2012), or length, stoutness, concentration and abundance of sclerites (West

[1] The pinnules are small lateral extensions on the tentacles; they are involved in the capture of prey.

1998). Sclerites are small calcareous elements found in the tissues of corals (Bayer et al. 1983). They are present in most taxa and are often used to identify coral species (Lewis and Von Wallis 1991; Carlo et al. 2011). Sclerites have two main functions: (1) support to the colony and (2) defense against predation (West 1998). Their prevailing role can be determined by their shape, density, concentration, size and arrangement. For example, it has been found that long sclerites are less palatable than short ones (West 1998; Puglisi et al. 2000; Clavico et al. 2007).

### 1.2 Reproduction

The vulnerability of corals to disturbance highlights the necessity to understand their resilience capacity, which depends on different factors including reproductive strategy (fecundity, gametogenic cycle and spawning periodicity). Corals exhibit a variety of reproductive strategies. Some species are gonochoric[2], others hermaphroditic[3]; some are broadcast spawners while others brood their offspring internally (Roberts et al. 2009). Fecundity corresponds to the number of offspring that an organism produces in a period of time (Ramirez Llodra 2002). When it is not possible to count the number of offspring produced, due to a limited access to the organisms (e.g. deep-sea species) or in the case of broadcast spawners, fecundity is determined by the number of oocytes in the reproductive organs of an individual. In corals, fecundity is usually determined by the number of oocytes or spermatocysts per polyp (Ramirez Llodra 2002) as corals do not have any defined gonads; gametes are produced along the mesenteries. Different methods have been used to estimate coral fecundity: number of oocytes per $cm^3$, per leaf (for the

[2] One individual or colony is either male or female.
[3] One individual or colony is male and female (simultaneous or sequential).

Pennatulacea, see below) or per polyp, which preclude inter-species comparison. Another impediment to comparisons is the fact that fecundity is measured at different moments throughout oogenesis even though all primary oocytes will not necessarily mature and be released (Eckelbarger and Watling 1995). These different measures may lead in some cases to an overestimation of the potential number of offspring produced.

The periodicity of the reproductive cycle can be examined at two different levels: (1) the pace at which gametes undergo full development (gametogenesis) and (2) the frequency of gamete release (spawning). The two cycles typically (but not always) follow each other. Reproductive cycles have often been defined as continuous, semi-continuous, seasonal or aseasonal. Usually, a diagnosis of continuous reproduction is based on the presence of different oogenic stages[4] in the same polyp (Pires et al. 2009). However, the presence of oocytes in different stages of development may also be evidence of multiple cohorts resulting from protracted oogenesis. Maturation of oocytes can occur over a few months (Waller and Tyler 2005; Mercier and Hamel 2011) or take > 12 months (Orejas et al. 2002; Orejas et al. 2007; Mercier and Hamel 2011; Brooke and Järnegren 2013). A prolonged oogenesis with overlapping cohorts does not always indicate that gamete release is also continuous or aseasonal, i.e. spawning may follow a shorter and/or more discreet period. The simplest example of this is when gametes take two years to mature but, because a new cycle is initiated every 12 months, one cohort is ready to be released annually (Edwards and Moore, 2008).

[4] Stages (or level of maturity) are determined by the size and cellular content of the oocytes and spermatocysts.

# 2 Cold-water corals

## 2.1 Overview

While the existence of cold-water corals has been known since the 18th century, the extent and importance of deep-sea coral ecosystems has only recently been shown, due in part to the emergence of new technologies (e.g. acoustic surveys and submersibles) and the deepening forays of the fishing and oil industries (Roberts et al. 2006). Unfortunately, many of these emerging technologies also have negative impacts on deep-sea corals, which are now finding themselves threatened by human activities including bottom trawling and oil exploration as well as by ocean acidification (Roberts et al. 2006). Their slow growth (Andrews et al. 2002; Sherwood and Edinger 2009) compounds the necessity to protect them before irreparable damage has been done.

Deep-sea corals can occur as solitary forms, as isolated colonies, or form large thickets or reefs that may reach 300 m in height and several kilometres in diameter (Buhl-Mortensen and Mortensen 2005; Longo et al. 2005; Roberts et al. 2006). They form one of the most complex biological habitats of the deep sea, offering a variety of microhabitats that serve as feeding and spawning sites, or shelter, to other species, including cnidarians, polychaetes, crustaceans, molluscs, echinoderms and fishes (Buhl-Mortensen and Mortensen 2005; Longo 2005; Murillo et al. 2011). The biodiversity associated with deep-sea corals is comparable to that encountered in shallow tropical coral reefs (Roberts et al. 2006) and is generally higher than in deep-sea areas without corals (Jensen and Frederiksen 1992; Mortensen et al. 2008).

Most of the deep-sea corals studied so far are gonochoric and present a continuous reproductive cycle (Waller 2005; Watling et al. 2011) which has been considered as the typical pattern in the deep sea due to the presumed homogeneity of this environment (Gage 1992). However, accounts of seasonality in the deep sea are multiplying (Brooke and Young 2003; Orejas et al. 2007; Sun et al. 2010b; Mercier and Hamel 2011). This seasonality is often explained by variations in food availability, linked with surface blooms of phytoplankton (Sun et al. 2010a; Mercier and Hamel 2011).

## 2.2 Pennatulacean corals

Most of the limited studies on the distribution and biology of deep-sea corals have focused on the subclass Hexacorallia, order Scleractinia (stony corals), and to a lesser degree on some species of the subclass Octocorallia. Within the latter, members of the order Alcyonacea (soft corals) have received more attention than the Pennatulacea (commonly called sea pens). Unlike other octocorals, sea pens are formed by a primary large polyp called the oozooid. The oozooid is divided in two parts: the peduncle, which anchors the colony in the sediment, and the rachis, where the secondary polyps are formed. Four different secondary polyps exist: (1) the autozooids, (2) the siphonozooids, (3) the mesozooids, and (4) the acrozooids (Williams et al. 2012). The autozooids and siphonozooids are present in all sea pen species, while the mesozooids have been observed only in Pennatula and Pteroeides and the acrozooids only on Pteroeides (Williams et al. 2012). The autozooids are large, bearing 8 tentacles and mesenteries; they are used for feeding and reproduction. The siphonozooids are small ciliated polyps employed for the regulation of the inhalant movements of water in the colony (Williams 1995; Williams 2011) while the mesozooids, when present, are used for exhalant

movements (Williams et al. 2012). The acrozooids have only recently been discovered, and have been suggested to allow asexual reproduction (Williams et al. 2012). In most studies, the general term polyp is used to designate the autozooids, and this will be the case in the present thesis.

Pennatulaceans are reported from the shallows to >6100 m (Williams 1995). Some species exhibit worldwide distributions, possibly due to their adaptation to live anchored in the soft sediment (Williams 2011). In addition to having large geographic distributions, sea pens can either be solitary (Figure 1-1A) or cover extensive areas, forming fields (Figure 1-1B and D; Baker et al. 2012). The presence of sea pens on mud or sand flats, whether as solitary colonies or in large aggregations, may provide an important structural habitat to other organisms (Tissot et al. 2006). However, not all sea pens anchor in soft sediments. At least three species are known that have the ability to attach to hard substratum (Williams and Aderslade, 2011).

Studies have already shown clear evidence of the negative impact of human activities on sea pens, which have been identified as vulnerable species in both shallow and deep environments (NAFO 2008; Donaldson et al. 2010; Murillo et al. 2011). Gates and Jones (2012) recently demonstrated that sea pens, in spite of being the most common species on soft sediment in Norwegian Sea (380 m), were less abundant in areas disturbed by drilling. Another study noted the absence of the sea pen Virgularia mirabilis in areas disturbed by dredging and boat moorings in the North Atlantic (Hoare and Wilson 1977). Moreover, due to their slow growth (Wilson et al. 2002), a long period might be necessary for the recovery of many sea pens.

## 3. Study areas and focal species

Five high-density areas of deep-sea corals have been identified along the continental slope of Newfoundland and Labrador in eastern Canada: Southwest Grand Banks Slope, Flemish Pass, Northeast Edge of the Northeast Newfoundland Shelf, Labrador Shelf Edge and Upper Slope, and Hudson Strait (Figure 1-2). This continental slope shelters 60 known species of deep-sea corals, including at least 16 sea pens (Gilkinson and Edinger 2009; Murillo et al. 2011; V.E. Wareham, personal communication) occupying nearly all five high-density areas (Laurentian Channel, Southwest Grand Banks, Flemish Pass, Orphan Basin, Figure 1-2).

This thesis focuses on three of the most common species of sea pens found along the continental slope of Newfoundland and Labrador: Anthoptilum grandiflorum (Verrill, 1879), Halipteris finmarchica (Sars, 1851) and Pennatula aculeata (Danielssen, 1860). A. grandiflorum (Figure 1-1A) is a cosmopolitan species with confirmed occurrences in the North and South Atlantic, North and South Pacific, and Indian and Antarctic Oceans (Williams 2011) from 100 to >2000 m (Baker et al. 2012). A. grandiflorum is easily distinguishable by its question-mark shape (Figure 1-1A). The polyps are arranged around the central axis and it either lacks or has only minute sclerites (Hecker et al. 1980; Williams 1990, 1995; López-González et al. 2001). H. finmarchica and P. aculeata are found in the North Atlantic (Williams 2013a, b) between 100 and >2000 m (Baker et al. 2012). Both species are highly calcified, with sclerites found from the peduncle to the tip of the rachis. H. finmarchica (Figure 1-1C) can be longer than 150 cm while P. aculeata (Figure 1-1E) reaches a maximum of 25 cm. Polyps of H. finmarchica are arranged into a

ridge (Figure 1-1C) while those of P. aculeata are fused to create leaves (Figure 1-1E). A. grandiflorum usually occurs as a solitary colony (Figure 1-1A), but can also form large fields as is commonly observed for H. finmarchica and P. aculeata (Figure 1-1B and D).

## 4. Objectives and chapter structure

The main goal of my thesis is to expand knowledge on the biology and role of deep-sea pennatulaceans and thereby provide arguments for their protection and management. The thesis is further divided into three main objectives: (1) to gain a better understanding of their morphology and trophic position (Chapter 2), (2) to determine their reproductive cycles and strategies (Chapters 3 and 4) and (3) to establish their importance as biogenic substrates for other species (Chapters 5 and 6). Chapter 2 considered the three species presented earlier while Chapters 3 to 6 considered only the two most common sea pens in the Northwest Atlantic: A. grandiflorum and H. finmarchica.

In Chapter 2, I studied different macrobiometric parameters (colony length, wet weight, peduncle length, polyp diameter, polyp density) and microbiometric parameters (sclerite length, sclerite stoutness, sclerite abundance, sclerite concentration, sclerite elemental composition). I compared them among the three species to identify potential biological differences. The study was completed by an analysis of the stable isotope signatures of the species to highlight potential differences in their diet, and in their feeding strategies. Finally, I determined the influence of region and depth on all these parameters. Samples for this study came from a large area, between the Laurentian Channel and the lower Arctic, spanning 2,200 km, and were collected between 98 and

1415 m depth. It is likely that different regions and depths are submitted to different environmental conditions, which can lead to morphological adaptations in the species.

In Chapter 3, I used colonies sampled in 2006 and 2007 to determine the reproductive cycle of A. grandiflorum and relationships with environmental parameters (temperature and food availability). I characterized the gametogenesis and spawning cycles of the species, determined fecundity at the polyp level in different sections of the colony and estimated whole colony fecundity based on the presence of mature oocytes just before spawning. This chapter emphasizes the importance of well-defined measures of fecundity by comparing fecundity values based on the number of mature oocytes just before spawning and the fecundity measured at other moments during the cycle (total number of oocytes per polyp).

In Chapter 4, I elucidated the reproductive cycle and fecundity of H. finmarchica using colonies sampled in 2006-2007. The presence of two cohorts of oocytes in the polyps of H. finmarchica (i.e., maintenance of a pool of small oocytes and maturation of a portion of the oocytes every year) was evidenced and suggested a protracted oogenesis. The chapter discusses the factors which may underlie this type of reproductive strategy and critically reviews the use of the term "continuous" to describe gametogenesis and spawning.

In Chapters 5 and 6 I analysed the associated species[5] of focal sea pens to determine their importance as a biogenic habitat. In chapter 5, I looked at the biodiversity and distribution of various associates of A. grandiflorum and H. finmarchica. The

[5] The associated species refer to the organisms that live inside and at the surface of the corals (Buhl-Mortensen and Mortensen 2005).

different species were classified as endobiont, ectobiont or free-living according to their position on the sea pen. Their distribution was discussed according to sea pen morphology, season, and depth. The three most common associates were further examined and their relationship with their hosts characterized. I determined whether they were parasites, commensals or mutualists, and assessed their level of dependence to the sea pen host. Using isotopic analysis and examination of gastro-vascular contents, I also characterized the respective diets of the sea pens and their common associates.

Finally, in Chapter 6, I focused on some of the most important and common associated species: fish larvae of Sebastes spp. (redfish), which were found to use sea pens as a nursery habitat. These associates were considered separately due to their economic importance (fisheries). I determined the abiotic and biotic factors influencing the presence of fish larvae on the sea pens. Moreover, to gain a larger view of the importance of deep-sea corals as nursery habitat, I determine the presence of fish larvae on other species of sea pens and octocorals found at sites where the presence of larvae on A. grandiflorum or H. finmarchica had been observed.

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## Figures

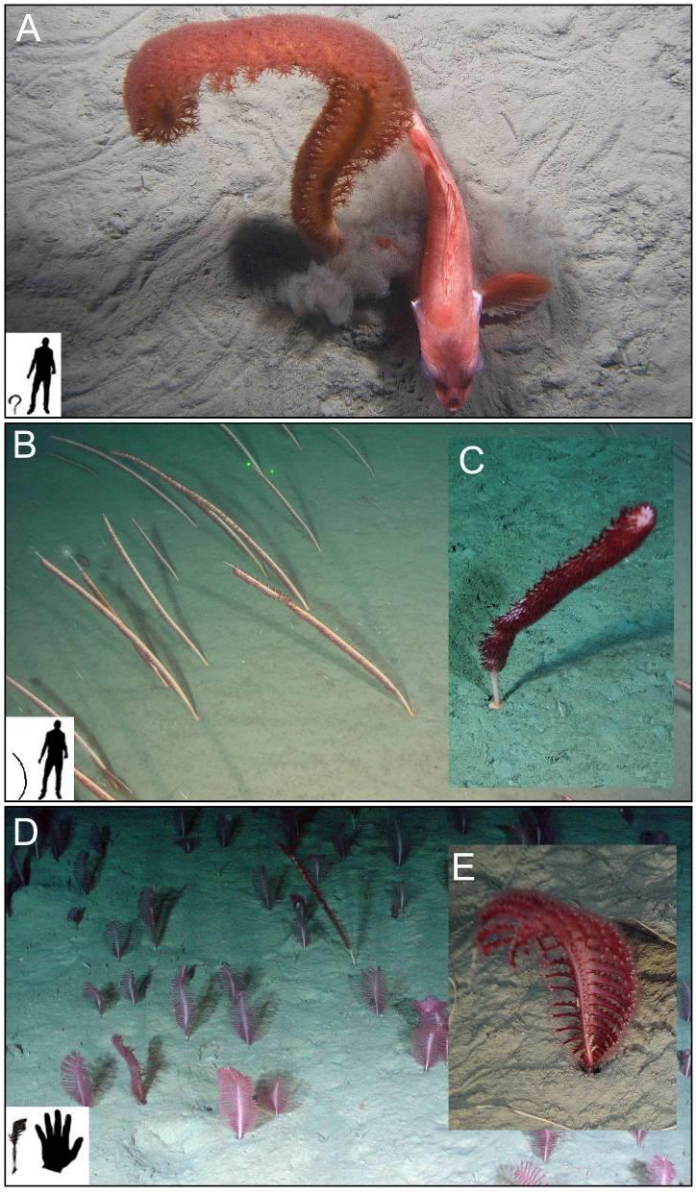


Figure 1-1 Photos in situ of (A) Anthoptilum grandiflorum in presence of an adult redfish (Sebastes sp.), (B) a field and (C) a single colony of Halipteris finmarchica, (D) a field and (E) a single colony of Pennatula aculeata. Photos courtesy of DFO.

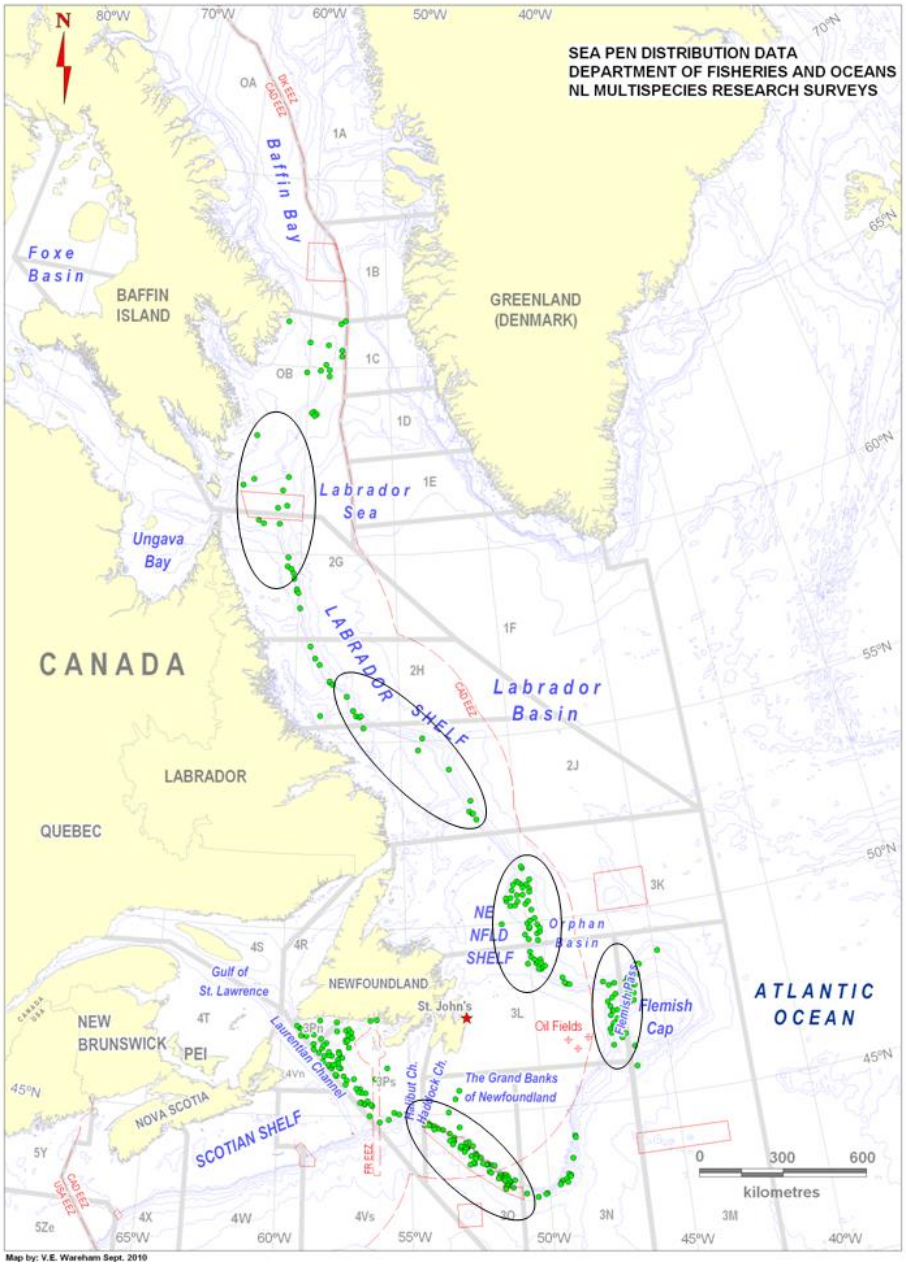


Figure 1-2 Distribution of observation/collection stations of sea pen (dots) and location of the five high-density areas of deep-sea corals (combining all known species; ovals) along the continental slope of Newfoundland and Labrador. Map courtesy to Vonda E. Wareham (DFO)

# Chapter 2 : Comparative biometry and isotopy of three dominant pennatulacean corals in the Northwest Atlantic

## Abstract

Animal biometrics quantify phenotypic character to help identify species and detect individuals, behaviours and morphological variations relevant to biogeography, population ecology and behavioural studies. Biometric analyses were conducted on three common deep-water pennatulacean corals (sea pens) in the Northwest Atlantic: Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata. These three species show different morphological characters and adaptations to their environment. Analyses of colony length, ratio of peduncle to colony length, weight/length ratio, polyp size and density as well as sclerite shape, location and abundance indicate that their plasticity is modulated by external factors, including latitude and depth of collection, food availability and anti-predator strategies. Moreover, the three species had different carbon and nitrogen stable isotope signatures, primarily linked with polyp diameters as well as different colony shapes, suggesting that they rely on slightly different food sources (varying proportions of phytodetritus and zooplankton). Finally, the Mg/Ca ratio of sclerites differed among types of sclerites in P. aculeata, potentially indicating different pathways in their formation related to their different roles (support or defense).

# 1. Introduction

Animal biometrics provide quantitative measures of phenotypic characteristics, typically based on appearance and behaviour (Kühl and Burghardt 2013). They can be used to identify and classify species and to provide information about their biology. Like a number of colonial organisms, corals present high intra-specific phenotypic variations, especially in external morphological characters. This has largely been documented in tropical reef corals, where variable morphologies have sometimes misled taxonomic identifications (Todd 2008). Variations in morphology can be explained by genetic differentiation, phenotypic plasticity, or both (Todd et al. 2001). Phenotypic plasticity allows an organism to adjust its attributes to a specific environment during its life-time, whereas genotypic differentiation leads to the maintenance of these characteristics across generations (Bradshaw 1965).

Different factors influence the morphology of shallow-water scleractinians and octocorals, such as light, water movement, sedimentation, depth and competition for space (Prada et al. 2008; Todd 2008). However, it is generally difficult to determine which specific parameter(s) might drive the different components of the phenotype (Todd et al. 2001). Morphological characters prone to vary include colony size, shape and weight, polyp diameter and density, and sclerite size and shape (Chappell 1980; West 1997; Costa et al. 2011). Biometric analyses using these variables can provide insights into biological adaptations. For example, studies showed that shallow colonies (5 m) of the gorgonian Briareum asbestinum in the Bahamas were plumper and shorter with a higher density of polyps and shorter sclerites than deeper colonies (35 m) (West et al.

1993). In turn, greater polyp density in corals can lead to increased gamete production (fecundity) and greater fitness (Sakai 1998). Furthermore, the shape, density, concentration, size and arrangement of sclerites (minute skeletal elements present in the tissues) can provide information on their main function, which is generally divided between defense against predators and colony support (West 1998). Long sclerites are less palatable than short ones, suggesting a trade-off between resistance to flow and resistance to predation with increasing sclerite length (West 1998; Puglisi et al. 2000; Clavico et al. 2007).

The trophic level occupied by a species also ties into its morphological and biological adaptations. Different methods can be used to study trophic niches, including analysis of polyp diameter and of stable isotope signatures. Polyp diameter might provide general information on potential prey size. According to Sebens (1987), there is a correlation between size of polyp and size of prey; however other studies have shown the absence of relationship (Palardy et al. 2005, 2006). Stable isotope analysis determines the trophic level (nitrogen signature) and dominant carbon source (i.e. in benthic communities, detritivores have a higher $\delta^{13}C$ than suspensivores (Sherwood et al., 2008). Isotopic signatures of deep-sea corals from Newfoundland showed possible ranges of feeding modes from fresh phytodetritus consumers (e.g. Paragoria) to carnivorous species (e.g. Flabellum, Sherwood et al., 2008). Potential links between nitrogen or carbon signatures and polyp diameter have not been verified so far.

Morphological variations/adaptations and their implications have mostly been examined in coral species from shallow waters across restricted depth ranges (spanning <50 m). A very limited number of studies relate to cold-water and deep-water corals

(Pasternak 1989; Rice et al. 1992) despite the fact that they constitute hotspots of marine biodiversity comparable to tropical coral reefs (Buhl-Mortensen et al. 2010). These corals colonize a wide range of depths (spanning >1000 m) and occasionally present a cosmopolitan distribution (Roberts et al. 2006), making them ideal for studies of intra-specific morphological variations across depths (Pasternak 1989) and geographic regions. To date, some 60 species of deep-sea corals have been identified along the continental slope of Newfoundland and Labrador (Northwest Atlantic, Canada), nearly one third of which are sea pens (Octocorallia: Pennatulacea) (Wareham and Edinger 2007; Baker et al. 2012). Sea pens are considered to be the most advanced octocorals, based on colonial complexity, functional specialization of polyps, and colonial integration (Bayer 1973). They are formed by one primary axial polyp: the oozooid, a unique feature that distinguishes pennatulaceans from other octocorals, further divided in two sections, the peduncle and the rachis. The peduncle allow the anchorage of the colony in the sediment while the rachis exhibits lateral budding of the body wall to give rise to secondary polyps (i.e. autozooids and siphonozooids) (Williams 2011). The oozooid can be supported by a central axis of calcite, which provides support to the colony. The functions of autozooids include feeding and gamete synthesis, while the ciliated siphonozooids control water movement for the expansion and contraction of the colony (Parker 1920; Soong 2005; Williams 2011).

Despite the abundance and diversity of sea pens worldwide, studies on their biometrics and morphology are scarce (Pasternak 1989; Rice et al. 1992). Sclerites are usually mentioned in the taxonomic description of a species (Williams 1995; López-González et al. 2001; López-González and Williams 2011); however the only

investigation of their role in sea pens was conducted on Renilla muelleri from shallow waters off Brazil (Alonso 1979; Clavico et al. 2007). Here, we examine biometric variations in three of the most common deep-water sea pens in the Northwest Atlantic, Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata, which were recently found to act as nurseries for larvae of commercial fish (Baillon et al. 2012, Chapter 6). The three sea pen species are distinguishable by their general external morphology. P. aculeata is the smallest and presents a feather or quill shape with polyps arranged along leaves. A. grandiflorum is of medium length in the form of a question mark and with polyps arranged in rows. H. finmarchica is the longest with a whip-like shape and polyps arranged in fused rows, forming ridges. Variations in macrometric (colony length, wet weight, polyp diameter and density) and micrometric traits (sclerite metrics) were investigated along depth gradients (98-1415 m) and among various regions of the continental slope spanning ~2500 km. Variations in the stable isotope signature among species were analysed in relation to their polyp diameters. Sclerite metrics were examined to gain biological knowledge under the assumption that primarily structural sclerites would exhibit more environmentally-mediated variations than those functioning as a defense mechanism; i.e. structural sclerites would respond more directly to hydrological and geological conditions present at different depths and/or in different regions.

# 2. Material and methods

## 2.1 Sampling

This study took advantage of routine multi-species research surveys conducted by Fisheries and Oceans Canada (DFO) between 2005 and 2010 along the continental slope of Newfoundland and Labrador (eastern Canada; Figure 2-1). Surveys followed a stratified random sampling design using a Campellen 1800 trawl towed for 15 min on ~1.4 km of seafloor. By-catch corals were collected and frozen at -20°C. A total of 252 colonies of Anthoptilum grandiflorum (98-1347 m), 102 colonies of Halipteris finmarchica (214-1333 m), and 172 colonies of Pennatula aculeata were analysed (98-1415 m; Appendix 2-A).

## 2.2 Biometrics

### 2.2.1 Colonies and polyps

Each sea pen colony was divided into four sections: peduncle (anchoring the sea pen in the sediment) and lower, middle and upper sections of the rachis (bearing the secondary polyps, autozooids and siphonozooids) similar to previous studies on sea pens (Soong 2005; Pires et al. 2009). A. grandiflorum (Figure 2-2) and H. finmarchica (Figure 2-3) present the same general organisation with the autozooids positioned directly on the oozooid, whereas in P. aculeata the autozooids are arranged along leaves (Figure 2-4). In this study we considered only the autozooids and used the term polyp to designate them. The following macro-morphological traits were measured for each colony: total wet weight, total colony length (from the base of the peduncle to the tip of the colony), colony height (from the base of the rachis, corresponding to the sea floor, to the highest point of

the colony), central axis length, and length and maximum diameter of the peduncle. The percent peduncle length (PPL) was defined as the peduncle length/colony length×100 and the colony weight-length ratio (W/L ratio) as the colony weight/colony length. Measures of polyp density around a 1-cm segment of the colony and of the basal diameter of 10 of those polyps were taken in each section of A. grandiflorum and H. finmarchica. Within each section of P. aculeata, the number of leaves was noted, then the length of three leaves per section were measured. Finally, the number of polyps on the three leaves was recorded as well as the basal diameter of 10 polyps on those three leaves.

### 2.2.2 Sclerites

The flesh around a 1-cm segment of each of the four colony sections was removed on 17 A. grandiflorum, 24 H. finmarchica and 27 P. aculeata. The samples were dried in an oven at 60 °C for 48 h and their dry weight measured with an analytical balance (precision of 0.1 mg). Each dry sample was placed in a vial with 0.5 M NaOH and boiled until all the organic matter was dissolved as per Clavico et al. (2007). The solution was filtered according to the minimum size of sclerite reported by Williams (1995) in the same or similar species (i.e., 20 µm for A. grandiflorum; 40 µm for H. california; 120 µm for P. phosphorea). The isolated sclerites were then rinsed with distilled water five times, dried in an oven at 60 °C for 48 h and weighed.

For quantitative analyses, photos of sclerite monolayers were taken with a stereomicroscope (Nikon SMZ1500) coupled to a digital camera (Nikon DXM1200F) using ACT-1 imaging software. The unbroken sclerites were counted, and the lengths and middle widths of each measured (minimum n=20) using Simple PCI 6 software. Photos of the sclerites were also taken with a scanning electron microscope (SEM; Phenom ProX). The stoutness of the sclerites was defined as their length to width ratio. Sclerite

metrics were also defined by: (1) sclerite concentration ([scl]) as percent weight of sclerite in the different colony sections ([scl]=sclerite weight divided by 1-cm tissue dry mass×100) and (2) sclerite abundance (Ab) corresponding to the number of sclerites per 1-g of dry soft tissue in each colony section (Ab=number of sclerites divided by dry weight of 1-cm tissue minus dry weight of sclerites in 1-cm tissue).

To obtain a finer picture of the repartition of sclerites, samples taken from specific areas were analyzed separately in 5 colonies of each species using the techniques described previously. These samples included the tissue surrounding the central axis, the column and polyp tentacles for H. finmarchica (Figure 2-3), and the dorsal track (sensu Bayer et al. 1983), leaves and polyp tentacles for P. aculeata (Figure 2-4).

Elemental composition of the tentacle sclerites, peduncle sclerites and long sclerites (including leaf sclerites and dorsal track sclerites for P. aculeata) were measured using the SEM operated at 15 kV coupled with energy dispersive spectroscopy (EDS). Measures were taken over a period of 30 seconds and elemental concentrations recorded as weight percentage. This method allows determination of surface composition of sclerites. The magnesium to calcium (Mg/Ca) ratio was calculated. To limit the potential influence of region and/or depth of collection, three colonies of H. finmarchica and P. aculeata sampled in GB at 623-773 m were analysed, in which three measures were recorded on three sclerites of each type. As well, to limit the influence of colony section on the calcium and magnesium concentrations (Weinbauer and Vellmirov 1995), only sclerites from the middle section of the rachis were used for the tentacle sclerites and long sclerites.

2.3 Isotopic analysis

The three species showed significant differences in polyp diameters (see results) which might lead to different diets. To determine their trophic level and potential food sources, analyses of stable isotopes were conducted according to Sherwood et al. (2008) on 9 colonies (2 A. grandiflorum, 3 H. finmarchica and 4 P. aculeata) sampled in April 2007 in the Laurentian Channel. Briefly, dried samples were ground to powder and treated with 5% (v/v) HCl to remove carbonates, then rinsed three times with de-ionised water and dried again for 24 h at 60 °C. Between 0.6 and 2.3 mg of sample was placed into 10×10 mm ultralight Sn capsules. The analyses were carried out using a Carlo Erba 1500 elemental analyser connected via a ConFlo-II interface to a FinniganTM MAT 252 isotope ratio mass spectrometer in the Department of Earth Sciences at Memorial University. The carbon and nitrogen isotopic values are provided using the standard δ-notation: $\delta X=[(R_{sample}/R_{standard})-1]\times10^3$, where X corresponds to $^{13}C$ or $^{15}N$ and R is $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively.

Trophic level (TL) was estimated from the $\delta^{15}N$ values using the following equation (Nilsen et al. 2008): $TL_{consumer}=[(\delta^{15}N_{consumer}-\delta^{15}N_{base})/\Delta\delta^{15}N]+TL_{base}$ where $\delta^{15}N_{consumer}$ corresponds to the $\delta^{15}N$ of the taxa considered, while $\delta^{15}N_{base}$ and $TL_{base}$ correspond to the value of the baseline of the trophic web considered, and $\Delta\delta^{15}N$ is the trophic fractionation for $\delta^{15}N$ (average 3.8‰ for polar and deep-sea studies (Iken et al. 2005). Here the base values were considered similar to the ones use in Gale et al. (2013) using zooplankton as the primary consumer ($TL_{base}=2.3$, $\delta^{15}N_{base}=9$).

## 2.4 Data analysis

The different biometrics (colony length, wet weight, polyp density and diameter, and sclerite metrics) were analysed relative to the four colony sections, five geographic regions (Laurentian Channel-LC, Grand Banks-GB, Flemish Cap-FC, North Newfoundland-NNL and Labrador-LB; Figure 2-1), depth and depth ranges (100-m strata, to determine the presence of potential threshold) using linear regression, analysis of variance (one-way ANOVA or t-test) and analysis of covariance (ANCOVA) after verifying assumptions of normality and homogeneity of variances. Post-hoc pairwise analyses (Student-Newman tests) were conducted as appropriate. Normality and homogeneity of variance were verified using normal probability plots of data and residuals and predicted vs. residual scores. Data were transformed using log-10 and/or outliers removed if necessary. When assumptions were still not met after transformation, Spearman correlation, and Kruskal-Wallis or Mann-Whitney tests followed by Student-Newman or Dunn's tests were used. When the same trends were observed in all regions, statistical results of the pooled data are given. Two-way ANOVA on ranks and Kruskal-Wallis were used to compare the Mg/Ca ratio between species and among the different type of sclerites.

When considering variations with depth and region for biometrics of the rachis (polyp diameter and density, sclerites metrics), we used only measures taken in the middle colony section to avoid new polyps in the lower section and older polyps in the upper section. Due to varying sample sizes in the different geographic regions, some measures were restricted to specific regions. Macrometrics were analysed in all five regions for A. grandiflorum, in all regions except NNL for H. finmarchica and in three

regions (LC, GB and NNL) for P. aculeata. Micrometrics were analysed mostly in GB for H. finmarchica (with intra-colony variation analysed in LC, GB and LB), and only in GB and NNL for P. aculeata. The virtual absence of sclerites in A. grandiflorum precluded analyses of micrometrics in this species.

Principal component analyses (PCA) were used to determine which of the micrometric factors (length and stoutness of short sclerites in peduncle and rachis, and of long sclerites in rachis) best explained the differences between H. finmarchica and P. aculeata.

## 3. Results

This section outlines the most significant results for each of the metrics. The comprehensive results of the biometric analyses are available in the supplementary material (Appendices 2-B to 2-J).

### 3.1 Colony metrics

#### 3.1.1 Colony length, wet weight and weight/length ratio

Of the three species, H. finmarchica was the tallest (68.3±2.7 cm; Figure 2-3A) followed by A. grandiflorum (44.7±0.9 cm; Figure 2-2A) and P. aculeata (16.9±0.4 cm; Figure 2-4A). The central axis of A. grandiflorum and H. finmarchica occupied the entire length of the colonies. In the former, the skeleton was never exposed, whereas in the latter 45.1% of the colonies were missing tissues on the upper section (13.7±1.2% of the tissues were absent). The percent bare central axis remained constant with depth ($r=0.10$, $p=0.529$). Finally, the central axis occupied only the first 80.8±1.9% of the colony length in P. aculeata; bare skeleton sections never occurred in this species. Colony length and

colony height are positively correlated for the three species across regions (Appendix 2-B).

In A. grandiflorum, a decrease in colony length with increasing depth was detected in the GB region (214-1347 m; r=-0.51, p<0.001) while an inverse trend occurred in NNL (656-1285 m; r=0.54, p=0.033). Analyses by depth ranges showed the presence of longer colonies <800 m (51.3 ± 2.4 cm) compared to colonies >800 m (40.4 ± 2.8 cm) in GB ($F_{7,51}$=5.19, p<0.001), whereas colonies in NNL did not exhibit any clear zonation despite significant depth differences ($F_{2,10}$=13.95, p=0.001). H. finmarchica showed an increase in colony length with increasing depth in LB (r=0.65, p=0.012; Appendix 2-B). Analyses by depth ranges showed the presence of taller colonies in deeper locations (<700 m: 35.9 ± 2.9 cm, ≥700 m: 73.2 ± 7.2 cm; $F_{2,11}$=9.49, p=0.002); however, only 3 colonies were sampled at >700 m. Finally, P. aculeata showed a decrease in colony length with depth in both LC (r=0.28, p=0.033) and GB (r=0.28, p=0.003) regions (Appendix 2-B). However, no clear difference among depth ranges (100-m strata) was visible; therefore all depths were pooled together for the remaining analyses of colony length in this species. All three species generally exhibited a decreasing trend in colony length in the general south-north latitudinal axis except at deeper depths for H. finmarchica: A. grandiflorum, <800 m: $F_{3,142}$=8.11, p<0.001, >800 m: H=18.85, df=3, p<0.001 (Figure 2-5A); H. finmarchica, <700 m H=21.26, df=2, p<0.001, >700 m: $F_{2,42}$=1.44, p=0.248 (Figure 2-5B); P. aculeata, H=20.34, df=3, p<0.001 (Figure 2-5C).

There was a positive relationship between total wet weight and colony length in the three species: A. grandiflorum (r=0.81, p<0.001), H. finmarchica (r=0.90, p<0.001)

and P. aculeata (r=0.90, p<0.001). There was also a positive relationship between weight/length (W/L) ratio (g $cm^{-1}$) and colony length in all species and regions (A. grandiflorum: $r_s$=0.54, p<0.001, H. finmarchica: $r_s$=0.62, p<0.001, P. aculeata: $r_s$=0.65, p<0.001). Furthermore, a decrease in W/L ratio with increasing depth was recorded in A. grandiflorum and H. finmarchica in the GB region ($r_s$=-0.70, p<0.001, r=0.43, p=0.008, respectively) and in P. aculeata in the NNL region (r=0.42, p=0.046; Appendix 2-B). Specifically, a significantly higher ratio at shallower (<800 m) depths was detected in A. grandiflorum (H=34.22, df=5, p<0.001); whereas the visible decrease of the ratio at > 700 m was not statistically supported in H. finmarchica (H=10.13, df=5, p=0.072). Finally, P. aculeata showed significant differences in W/L ratio among depth ranges ($F_{1,17}$=3.24, p=0.048) but no threshold was detected.

The W/L ratio varied with months for A. grandiflorum in FC (Oct<Nov<Dec<Jun; H=8.99, df=3, p=0.029) and GB (Oct<May<Apr; H=19.22, df=2, p<0.001), and for P. aculeata in LC (Apr<May; U=216.0, p=0.021). Therefore, comparisons among regions were done in a specific month for both species while the absence of variation among months for H. finmarchica allowed us to pool all data. The W/L ratio of A. grandiflorum and H. finmarchica varied among regions (October, FC<GB<LB, H=10.35, df=2, p=0.006; pooled data, FC<LB<GB<LC, H=18.80, df=3, p<0.001, respectively) while no variation was observed for P. aculeata (April: t=-0.16, df=38, p=0.877 and May: t=-1.11, df=53, p=0.274).

### 3.1.2 Peduncle length

All species (across all regions) showed an increase in length of the peduncle with increasing colony length (Figure 2-6). Peduncle length varied between 1.1 (smallest colony studied) and 12 cm (longest colony studied) in A. grandiflorum, between 3.5 and 13.3 cm in H. finmarchica and between 1.2 and 13 cm in P. aculeata. The percent peduncle length (PPL) was significantly different among the three species ($H=331.80$, $df=2$, $p<0.001$); it was much greater in P. aculeata (42.7±0.4 %) then in A. grandiflorum (15.3±0.2 %) and H. finmarchica (13.2±0.4 %). The peduncle of P. aculeata was long and thin, and did not present any thickening, as seen in the two other species (Figures 2-2B, 2-3E and 2-4B). The maximum peduncle diameter was 1.9±0.1% of its length in P. aculeata, 8.8±0.3% in H. finmarchica and 13.2±0.4% in A. grandiflorum, with significant pairwise differences ($H=175.0$, $df=2$, $p<0.001$) indicating that the peduncle was proportionally longer and thinner in P. aculeata than in H. finmarchica and in A. grandiflorum.

Both A. grandiflorum and H. finmarchica showed a decrease in PPL with increasing colony length across regions ($r=-0.31$, $p<0.001$ and $r=-0.84$, $p<0.001$, respectively), while P. aculeata showed no variation of PPL with colony length ($r=0.10$, $p=0.249$). A significant influence of depth on PPL occurred only in A. grandiflorum sampled in two regions (GB: $r=-0.40$, $p=0.002$; FC: $r=-0.31$, $p=0.022$), but analyses did not reveal any specific threshold. A significant southward decrease in PPL was observed, from LB toward FC, and an increase westward from GB to LC ($H=49.03$, $df=4$, $p<0.001$). For H. finmarchica, colonies in LB showed a higher PPL than elsewhere

(LB>LC=GB=FC; H=16.96, df=3, p<0.001). Finally, P. aculeata showed significant but inconsistent differences in PPL among regions ($F_{3,142}$=8.78, p<0.001).

3.2 Polyp diameter and density

Mean polyp diameter varied significantly among the three species (H=569.13, df=2, p<0.001): A. grandiflorum (1.15±0.01 mm) > H. finmarchica (0.98±0.01 mm) > P. aculeata (0.76±0.01 mm). A. grandiflorum and H. finmarchica showed a significant increase in polyp diameter from the lower to the upper rachis sections (from 0.87±0.01 mm to 1.26±0.02 mm, H=389.83, df=2, p<0.001 and from 0.78±0.01 to 1.03±0.01 mm, H=378.71, df=2, p<0.001, respectively; Figure 2-7A and B, Appendix 2-C). In P. aculeata polyp diameter was maximal in the middle section (0.76±0.01 mm; H=72.13, df=2, p<0.001; Figure 2-7C). A. grandiflorum and H. finmarchica presented a general increased in polyp diameter with increasing colony length ($r_s$=0.32, p<0.001, $r_s$=0.33, p<0.001; respectively) while P. aculeata showed no clear patterns (Appendix 2-D). A. grandiflorum showed no variation in polyp diameter with depth except in GB where the diameter decreased with increasing depth ($r_s$=-0.36, p<0.001) and larger polyp diameters occurred in colonies <800 m (H=27.50, df=4, p<0.001). The largest polyps <800 m were found in GB (H=32.35, df=3, p<0.001), however polyp diameter >800 m increased northward (FC=GB<LB; H=12.01, df=2, p=0.002). Polyp diameter increased significantly with depth in FC and LB while a non-significant decrease was visible in GB (Appendix 2-D). Despite significant differences among depth ranges, no clear thresholds were visible in FC and LB (H=15.00, df=3, p=0.002, H=47.96, df=4, p<0.001, respectively), therefore all depths were grouped. Comparisons among regions for H. finmarchica showed a significant decrease in polyp diameter in a general northward

latitudinal trend (GB>LC>FC>LB; H=51.79, p<0.001). Finally, depth influenced polyp diameter in P. aculeata sampled in GB and NNL (GB: $r_s$=-0.20, p=0.010, NNL: r=-0.26, p<0.001); there was no clear depth threshold in GB, whereas larger polyps occurred at depths <1000 m in NNL ($F_{5,224}$=5.22, p<0.001). No significant regional trends in polyp diameter were found in P. aculeata (<1000 m: H=4.84, df=2, p=0.100, >1000m: H=1.66, df=2, p=0.320).

Overall, polyp density in H. finmarchica (18.0±0.6 polyp $cm^{-1}$) was significantly higher (U=4325.0, p<0.001) than in A. grandiflorum (12.1±0.3 polyp $cm^{-1}$). Polyp density in both species varied across rachis sections (lower<middle<upper: H=156.14, df=2, p<0.001; lower<middle=upper, H=20.15, df=2, p>0.001, respectively; Figure 2-7D and E, Appendix 2-C). In contrast, P. aculeata exhibited a significant increase in polyps density (per leaf) from the lower (5.9±0.1 polyp $leaf^{-1}$) to the middle (7.8±0.1 polyp $leaf^{-1}$) rachis section usually followed by a decrease in the upper section (7.2±0.2 polyp $leaf^{-1}$; H=95.99, df=2, p<0.001; Figure 2-7F). No variations in polyp density occurred with colony length in A. grandiflorum except an increase in the GB region (r=0.44, p=0.012). H. finmarchica showed no clear variation (Appendix 2-D) while P. aculeata showed an increase with colony length in two out of three regions (GB: r=0.47, p<0.001, NNL: r=0.72, p<0.001). A decrease in polyp density with increasing depth occurred in A. grandiflorum samples from two regions (GB: r=-0.59, p<0.001, FC: $r_s$=-0.30, p=0.011) with a significant depth threshold at 800 m (GB: H=28.01, df=3, p<0.001; FC: H=13.65, df=2, p=0.001). Similar trends were observed in two regions for P. aculeata (GB: $r_s$=-0.35, p=0.014, NNL: r=-0.53, p<0.001) with higher polyp density in colonies >1000 m. Finally polyp density of H. finmarchica was generally not influenced by depth, except in

FC (r=-0.56, p<0.001). No variation in polyp density among regions occurred in A. grandiflorum at shallower depth (<800m: H=4.67, df=3, p=0.197) and in any P. aculeata (<1000m: H=4.84, df=2, p=0.089, >1000m: H=1.66, df=2, p=0.320). At greater depth, A. grandiflorum showed a northward increase in polyp density (>800m: $F_{3,108}$=3.64, p=0.015, GB<FC<NNL<LB). H. finmarchica showed a significantly lower polyp density in FC than in the other regions (H=36.83, df=3, p<0.001).

3.3 Sclerite metrics

### 3.3.1 Description

Two different types of sclerites were present in the rachis of H. finmarchica; long sclerites around the column of the polyp (coined column sclerites: 0.40-2.35 mm, Figure 2-3B, C and F) and shorter sclerites in the tentacles (coined tentacle sclerites: 0.06-0.39 mm, Figure 2-3D and F). Both types were clear, three-flanged and rod-shaped, but the long sclerites had pointier ends. In the peduncle, the sclerites (coined peduncle sclerites: 0.05-0.32 mm) were short, clear, three-flanged and rod-shaped structures, resembling tentacle sclerites (Figure 2-3E and F), although the former were significantly smaller and stouter than the latter (length, U=94697.0, p<0.001; stoutness, U=134998.0, p<0.001; Appendix 2-E). All types of sclerites were found in colonies across geographic regions, except long sclerites and tentacle sclerites which were absent in colonies from LC. Sclerite concentration was significantly lower in the peduncle than in the three sections of the rachis (H=26.36, df=3, p<0.001) while sclerite abundance did not vary significantly between the peduncle and three sections of the rachis (H=1.08, df=3, p=0.782).

Three types of red, three-flanged, rod-shaped sclerites occurred in the rachis of P. aculeata: short tentacle sclerites (0.04-0.30 mm, Figure 2-4D), generally long sclerites in the leaves (coined leaf sclerites: 0.30-2.83 mm, Figure 2-4E, F and I) and on the dorsal track (coined dorsal sclerites: 0.30-1.67 mm, Figure 2-4G, H and I). The dorsal sclerites were wider, showing a significantly greater stoutness than those from the leaves ($U=21153$, $p<0.001$). Peduncle sclerites (0.08-0.49 mm) were pale pink to clear, significantly longer and stouter than the tentacle sclerites (length, $U=551394.5$, $p=0.014$; stoutness, $U=50977.5$, $p<0.001$; Appendix 2-F). Peduncle sclerites were rod-shaped or thick on both ends and thin in the middle (coined bi-lobed sclerites, Figure 2-4B, C and D). Sclerite concentration and abundance were both significantly lower in the peduncle than in the rachis (concentration: $H=32.31$, $df=3$, $p<0.001$; abundance: $F_{3,103}=125.41$, $p<0.001$; Appendix 2-G).

No sclerites were found in A. grandiflorum; therefore this species was excluded from the micrometric analyses. PCA on micrometrics of H. finmarchica and P. aculeata (length and stoutness of short sclerites in the peduncle and rachis, and of long sclerites in the rachis) showed that stoutness of the tentacle sclerites was the main contributor to PC1 (56.9%), while stoutness of the long sclerites in the rachis were the main contributors to PC2 (33.0%).

Inter-specific comparisons showed that the long rachis sclerites were significantly longer in H. finmarchica than in P. aculeata ($U=659165.0$, $p<0.001$), whereas the shorter sclerite types were longer in P. aculeata (tentacle sclerites: $U=111293.0$, $p<0.001$, peduncle sclerites: $U=179452.0$, $p<0.001$). Sclerite concentrations and abundance in all

colony sections were consistently higher in P. aculeata than in H. finmarchica (Table 2-1).

Elemental analysis of sclerites showed that they were all primarily composed of oxygen, calcium, magnesium and carbon (Table 2-2 for weight proportions). Significant differences in the Mg/Ca ratio were found between species ($F_{1,163}$=4.40, p=0.038) and among the types of sclerites in P. aculeata (H=8.08, df=2, p=0.018; Figure 2-8), whereas no significant difference was found within H. finmarchica (H=1.56, df=2, p=0.459). Finally no difference between species was found (two way ANOVA on ranks species sclerites: $F_{2,163}$= 0.286, p=0.752).

### 3.3.2 Influence of colony length, depth and region on sclerite metrics

Colonies of H. finmarchica sampled in GB were used to determine the influence of colony length and depth on sclerite metrics. Length of tentacle sclerites did not vary with colony length (r=0.11, p=0.301) or with depth (r=0.08, p=0.455; Appendix 2-H). Length of peduncle sclerites decreased with colony length ($r_s$=-0.24, p<0.001) but did not vary with depth (r=0.04, p=0.268). Column sclerites increased in length with colony length (r=0.41, p<0.001) and with depth (r=0.40, p<0.001). All short sclerites showed significant differences among geographic regions (in peduncle: $F_{2,138}$=9.67, p<0.001, in tentacles: $F_{2,254}$=4.87, p=0.008). Column sclerites were significantly shorter in FC than in GB and LB (H=36.41, df=2, p<0.001).

The stoutness of sclerites from the peduncle of H. finmarchica increased with colony length (r=0.28, p<0.001) and with depth ($r_s$=-0.19, p<0.001; Appendix 2-H) but no significant threshold was visible when comparing depth intervals. Tentacle sclerite

stoutness showed no variation with colony length ($r_s$=-0.14, p=0.203) or with depth (r=0.10, p=0.381). Column sclerites became stouter with increasing colony length ($r_s$=0.25, p<0.001) and increasing depth ($r_s$=0.26, p<0.001). Peduncle and tentacle sclerites were stouter in GB than other regions (H=64.16, df=2, p<0.001, H=15.16, df=2, p<0.001, respectively). Column sclerites were significantly stouter in FC (H=61.31, df=2, p<0.001). No variation of the concentration and abundance of sclerites with colony length or depth was visible apart from a decrease in peduncle sclerite concentration with colony length (r=-0.55, p=0.044, Appendix 2-H). Sclerite concentration and abundance were stable among regions in both the rachis and peduncle (concentration: $F_{3,22}$=2.19, p=0.118; abundance: H=2.94, df=3, p=0.400).

The length of all types of sclerites in P. aculeata showed no clear relationship with colony length (Appendix 2-I). Peduncle sclerite length decreased with depth ($r_s$=-0.29, p<0.001), while tentacle sclerite length increased with depth ($r_s$=0.17, p<0.001), with no depth threshold evident anywhere. The dorsal sclerites were not influenced by depth in any of the geographic regions (GB: $r_s$=0.008, p=0.899, NNL: $r_s$=0.04, p=0.584). Leaf sclerite length showed a decrease with depth in GB ($r_s$=-0.02, p<0.001) and NNL (r=-0.29, p<0.001), with a threshold at 1100 m. Tentacle sclerites showed significant differences among regions with an increase in length eastward (GB>LC) and southward (GB>FC=NNL; H=42.47, df=3, p<0.001). The same trends were observed for the peduncle sclerites (GB>LC, GB>FC>NNL; H=269.83, p<0.001). Despite significant regional differences in dorsal sclerite lengths (H=24.44, df=3, p<0.001), no clear pattern emerged. Long leaf sclerites were shorter in GB and FC than in LC and NNL at depths

<1100m (H=29.60, df=3, p<0.001); no difference between regions emerged for samples >1100 m (U=1616.0, p=0.652).

Sclerite stoutness in P. aculeata increased significantly with colony length in tentacles ($r_s$=-0.39, p<0.001; Appendix 2-I) and leaves ($r_s$=-0.08, p=0.034). Stoutness decreased in peduncle sclerites ($r_s$=0.47, p<0.001) and did not vary in dorsal sclerites ($r_s$=-0.05, p=0.351). Stoutness varied with depth for almost all types of sclerites (peduncle: $r_s$=-0.60, p<0.001, tentacle: $r_s$=0.39, p<0.001, leaves: $r_s$=0.16, p<0.001, dorsal: $r_s$=0.04, p=0.398), but only tentacle sclerites showed a threshold with stouter sclerites <1000m. Tentacle sclerites sampled <1000 m were stouter in NNL than elsewhere (H=91.16, df=3, p<0.001); whereas colonies >1000 m showed no significant regional variation (t=-0.58, df=136, p=0.564). Leaf sclerites showed a decrease in stoutness eastward (GB<LC) and then northward (GB<NNL; H=41.33, df=3, p<0.001). Dorsal sclerites were stouter in LC and FC than in NNL (H=24.35, df=3, p<0.001). Sclerite abundance and concentration in the rachis and peduncle did not vary with colony length, depth or region (Appendix 2-J).

3.4 Isotopic analysis

The three sea pens presented a similar $\delta^{15}$N signature ($F_{2,8}$=3.90, p=0.082): A. grandiflorum: 11.3±0.8 ‰ (TL: 2.9±0.2), H. finmarchica: 10.7±0.2 ‰ (TL: 2.8±0.1), P. aculeata: 9.9±0.2 ‰ (TL: 2.5±0.1; Figure 2-9A). However, the $\delta^{13}$C signature varied significantly with P. aculeata (-23.7±0.5 ‰) exhibiting significantly lower values ($F_{2,8}$=17.61, p=0.003) than A. grandiflorum (-20.9±0.8 ‰) and H. finmarchica (-20.9±0.1 ‰). The increase in $\delta^{13}$C between P. aculeata and the two other sea pens was correlated with an increase in polyp diameter (Figure 2-9B).

Stable isotope data for other species of deep-sea corals were not previously published for the LC region. However, data from a previous study on various deep-sea corals sampled along the continental slope of Newfoundland and Labrador (Sherwood et al. 2008) were used here to determine the influence of polyp size and colony height on the carbon and nitrogen signatures. A significant increase of the $\delta^{15}N$ value was visible with increasing polyp diameter (Figure 2-10A) while a non-significant decrease with colony height (Figure 2-10B) was observed. The $\delta^{13}C$ signature did not vary with polyp diameter (Figure 2-10C) or colony height (Figure 2-10D).

## 4. Discussion

The present study provides new data on the morphometry (Table 2-3) and elemental/isotopic composition of three of the most common sea pens in the Northwest Atlantic, providing strong support for the influence of environmental factors (e.g. food availability, currents, sediment type) on the morphological phenotype of sea pens and shedding some light on their trophic biology and ecology. It confirms some of the trends reported previously in some sea pens (e.g. decrease in polyp diameter and density with depth, addition of the new polyps at the base of the rachis, low variability in sclerite metrics) and challenges others (e.g. inverse relationship between carbon signature and colony length and between peduncle proportion and colony length). Further studies on deep-sea coral biometry are needed to expand the framework within which adaptations of corals to deep-water environments can be examined.

4.1 Variations and similarities in macrometrics

Soft tissues are present on the entire central axis of A. grandiflorum, whereas some colonies of H. finmarchica showed bare sections of the uppermost central axis, which can harbour epibionts such as sea anemones and hydrozoans (Chapter 5). Degeneration of the older polyps or predation might explain this absence of tissues. However, all sizes of colonies, including some of the smallest ones are affected, suggesting that predation from a pelagic origin, such as grazing by fish, is more probable than degeneration. Evidence of predation by fish was recorded in the shallow-water sea pen Stylatula elongata in the North Pacific (Davis et al. 1982). In contrast, the terminal section of the rachis in P. aculeata is devoid of a central axis. These differences suggest that A. grandiflorum and H. finmarchica depend on the central axis for support while P. aculeata might possess additional adaptations. For instance, P. aculeata is known to withdraw into the sediment (Langton et al. 1990), which may be facilitated by the absence of central axis in the upper rachis; whereas the two other species are not known to exhibit this behaviour.

Peduncle metrics also distinguished P. aculeata from both A. grandiflorum and H. finmarchica. Long colonies of the latter had proportionally smaller peduncles than shorter colonies, while peduncle length of P. aculeata remained constant within the size range tested. Interestingly, despite different species-specific maxima in colony length, the maximum length of the peduncle was ~13 cm in all three species. It has been suggested that sea pens with a longer rachis would need a longer peduncle to allow efficient anchorage in the sediment (Kastendiek 1976). However, here, longer colonies had proportionally smaller peduncles. A. grandiflorum and H. finmarchica appear to offset

their shorter peduncles with greater peduncle width (i.e. a bulge in its upper part) as previously suggested by Kastendiek (1976); long sea pens need wider peduncles to allow better anchorage in the sediment.

In all three species, the smallest polyps and lowest polyp density were found in the lower section of the rachis. The lower polyps were also characterised by the absence or under-development of oocytes, compared to polyps found higher along the rachis in A. grandiflorum and H. finmarchica (Baillon et al. 2014, Chapter 4). These findings support the idea that new polyps are added at the base of the rachis in those species, as previously suggested by Soong (2005) for Virgularia juncea.

4.2 Diet, trophic level and macrometrics

Previous studies have shown that sea pens are suspension feeders that ingest zooplankton (Edwards and Moore 2008) or the degraded fraction of particulate organic matter (POM) (Sherwood et al. 2008). Analysis of the stable isotopes provided further insight into the trophic ecology of the species under study. Lower nitrogen and carbon signatures set P. aculeata apart from the two other species, despite the overall non-significant differences in the nitrogen signatures of the three sea pen species. The trophic level of P. aculeata (TL=2.5) is close to that of a primary consumer like zooplankton (TL=2.3), feeding on fresh phytodetritus (low carbon signature) and microzooplankton. P. aculeata might complete its diet by occasionally capturing small invertebrates. As for A. grandiflorum and H. finmarchica, they sit at half a trophic level above P. aculeata indicating that both species likely feed on more degraded POM and more readily on small invertebrates (e.g. zooplankton, epibenthic copepods). A. grandiflorum has the highest nitrogen signature, closer to that of a secondary consumer (carnivorous).

The fact that P. aculeata has the lowest carbon signature and is the shortest species somewhat contradicts Sherwood et al. (2008) who proposed that small coral colonies (<30 cm, e.g. Anthomastus, Duva) fed on more degraded food (higher $\delta^{13}C$) than taller colonies (lower $\delta^{13}C$, e.g. Paragorgia, Primnoa). We suspect that factors other than colony height (e.g. tentacle morphology, colony posture, population density) are involved in selection/capture of food items, at least in sea pens. Because sea pens are anchored in mud, they might also encounter different environmental conditions than other deep-sea corals, which most commonly colonize hard substrates.

Putative differences in the diets of our focal species can further be explained by their different general morphologies. P. aculeata is the smallest species studied here with lateral extension (leaves) that may help intercept flowing particles (e.g. phytodetritus). The feather shape possibly generates turbulence around the polyps and facilitates occasional prey capture (small invertebrates), in a strategy highlighted by Williams (1997). In contrast, A. grandiflorum and H. finmarchica are slender and have a smaller lateral surface than P. aculeata, making them less effective at filtering flowing particles. The larger polyps could favour the capture of small invertebrates, especially in A. grandiflorum which has greater polyp extension than H. finmarchica (personal observation). In turn, H. finmarchica produces greater amounts of mucus, which is known to help trap phytodetritus or microorganisms in shallow-water corals (Lewis and Price 1975), confirming that it could rely more readily on phytodetritus than A. grandiflorum.

In the well-developed sections of the rachis, polyp diameters of the three species ranged from 0.76-1.26 mm. Correlation between size of polyp and size of prey was previously shown for some species (Sebens 1987) while other showed no correlation

(Palardy et al. 2005, 2006). Our analysis revealed that corals with larger polyps (Flabellum alabastrum) occupy higher trophic levels, suggesting the ability to catch larger prey, as evoked previously (Buhl-Mortensen et al. 2007; Sherwood et al. 2008). Of note is the fact that no correlation was visible in the lower size range of coral polyps (<5 mm) including the three sea pens studied here, and some soft corals, sea fans and a black coral from Sherwood et al. (2008). An analysis of the disposition, length, and width of polyp tentacles and pinnules might ultimately best explain variations in diet among species due to the link between these parameters and the feeding strategy (Sebens and Koehl 1984).

4.3 Spatial and temporal trends in macrometrics

Overall, macromorphology, including colony length, polyp diameter and density, varied less drastically across depths in the three species examined here than expected from previous studies in shallow-water coral species (West et al. 1993; Todd et al. 2001), in spite of the much greater depth and latitudinal gradients investigated here. For example, H. finmarchica showed a 29% decrease in polyp density over 410 m (790-1200 m depth), or a decrease of 0.7% every 10 m, whereas the gorgonian Briareum asbestinum in the Bahamas showed a 38% decrease in polyp density over 30 m (5-35 m depth) corresponding to 12.7% decrease every 10 m (West et al. 1993). Morphological variations among coral colonies are typically associated with variations in environmental parameters (Prada et al. 2008; Todd 2008), which tend to occur more quickly in the first meters of the ocean than in deeper strata below 200 m (Gage 1992) and could explained our results.

While weaker than expected, spatial variations in macrometrics (across depths and regions) were nevertheless evidenced in the species under study. The three sea pens were

sampled over comparable depth ranges in our study (A. grandiflorum: 98-1347 m; H. finmarchica: 214-1333 m; P. aculeata: 98-1415 m), although bathymetric ranges varied across regions. Overall, P. aculeata emerged as the species most clearly impacted by increasing depth. In two of the three regions where P. aculeata was collected, there was a decrease of colony length, weight-length ratio, polyp diameter and polyp density with depth. Similar bathymetric variations were visible in two and three regions out of five in A. grandiflorum and H. finmarchica, respectively. These differences might be explained by the known bathymetric distributions of the species (Baker et al. 2012). P. aculeata presents the narrowest range with maximum occurrence between 200 and 1000 m, while A. grandiflorum is present at deeper depths (to 1400 m) and H. finmarchica is common to 2000 m depth. As mentioned earlier, P. aculeata likely feeds principally on phytodetritus, which decreases in quantity and quality with depth (Pace et al. 1987; Gage 1992). It might therefore not obtain enough food for its development at greater depths, as reflected by a narrower bathymetric distribution. The two other species feed more readily on small invertebrates, allowing them to colonize greater depths. H. finmarchica seems to be the most adapted to deeper environments; it is the only species showing an increase in colony length with increasing depth. This peculiarity might be explained by the production of mucus at the surface of the colony that could create an efficient trap for particles (e.g. microorganisms, phytodetritus). The increase in polyp diameter and decrease in polyp density with increasing depth have been attributed to an adaptation of greater efficiency in food procurement in a few particular pennatulacean taxa such as Amphiacme, Chunella, and Umbellula (Williams 1992). Here, only H. finmarchica showed both adaptations (polyp density decrease and polyp diameter

increase), while A. grandiflorum and P. aculeata showed only a decrease in polyp density, supporting the better adaptation of H. finmarchica to life at depth.

P. aculeata showed very little morphological variation across regions at depths <1000 m, which may reflect sampling within its optimal latitudinal range distribution. The two other species showed a general decrease in colony length from South to North, possibly mirroring a latitudinal decrease in food availability. Interestingly, the plumper colonies of sea pens (all species) were found in LC (a wide channel) and the thinner ones in FC (a narrow pass). Stronger currents in FC may be less conducive to passive interception and prey capture than slower currents in LC.

Temporal variations in the weight-length ratio were noted in two of the three species (A. grandiflorum and P. aculeata). Determining trends in the weight-length ratio of the other species (H. finmarchica) was not possible due to the frequent and regionally inconsistent occurrence of colonies with partially denuded skeletons (devoid of flesh). The plumper colonies (higher weight-length ratio) of A. grandiflorum were found in the spring months (April to June) suggesting a potential link with seasonal food availability leading to somatic growth or development of reproductive tissues. The fact that the spring phytoplankton bloom occurs in those months in the Northwest Atlantic (Baillon et al. 2014, Chapter 3) supports this hypothesis. The downfall of phytodetritus (and marine snow) to the seafloor, which is at the base of many deep-water food chains, is generally correlated with the concentration of phytoplankton at the sea surface (Billett et al. 1983); A. grandiflorum might take advantage of the phytodetritus input during the spring months and feed seasonally more on phytodetritus than small invertebrates. Variations in

P. aculeata were less marked, possibly because of a less suitable sampling scheme across months.

All spatial and temporal variations in biometrics determined here (i.e. bathymetric and latitudinal decrease and seasonal variations) seem to share a connection with food availability as previously suggested for shallow-water marine invertebrate (Saunders et al. 2009; Saura et al. 2012). Other parameters, such as temperature, current, and oxygen concentration have also been shown to drive trends in body size of organisms with depth and latitude (Rice et al. 1992; Olabarria and Thurston 2003). Temperature is not a likely driver in our study since all colonies were sampled below the thermocline, which lies around 50 m and dissolves during winter in the Northwest Atlantic (de Vernal and Hillaire-Marcel 2000; Stein 2007). Nevertheless, monthly and geographic variations of bottom temperature exist (Colbourne 1994) that might impact biometrics; however, annual temperature data at the different stations studied here are not available. Data on current velocity and oxygen concentration are not available either. However, it can be assumed that these parameters can drive morphological adaptations and may explain some of the intra-species differences observed here at different scales.

### 4.4 Description and role of sclerites

Sclerites are commonly used as unique descriptors of octocoral species, particularly to identify gorgonians (Lewis and Von Wallis 1991; Carlo et al. 2011) in which they come in a variety of shapes. Here, the low inter-specific variability in sclerite shape, length, and stoutness confirms that they do not provide effective descriptors to discriminate pennatulacean species, as previously noted (Williams 1995, 2011). Long sclerites in the rachis and short sclerites in the tentacles and peduncle were found in

H. finmarchica and P. aculeata, while no sclerites were found in A. grandiflorum. This latter result agreed with previous accounts (Hecker et al. 1980; Williams 1990; López-González et al. 2001). However, López-González et al. (2001) described the presence of few minute oval sclerites in the peduncle of A. grandiflorum from the African Atlantic coast, a trait inconsistent among populations for reasons yet unknown (Gary Williams, personal communication).

Cnidarians present two different defense mechanisms: chemical defense afforded by toxins and physical defense through sclerites (Koh et al. 2000; Clavico et al. 2007). The lack of mineralization in A. grandiflorum suggests high concentrations of anti-predator chemicals since studies have generally found inverse relationships between the two modes of defense (Sammarco et al. 1987; Harvell and Fenical 1989). Contrary to A. grandiflorum, it can be assumed that P. aculeata does not rely strongly on chemical defenses due to high densities of sclerites which would render the tissues unpalatable. The long stouter sclerites aggregate that form points along the dorsal crest in P. aculeata likely act to defend the colony, while the long sclerites in the leaves presumably also offer support, running parallel to each other to form the leaf structure. The short sclerites of P. aculeata and H. finmarchica may complement defense and/or offer a minor structural support to the tentacles and peduncle. The longest sclerites in H. finmarchica form a calyce around the polyp that probably serves as a defense mechanism against predators. However, the common occurrence of naked areas of the central axis evokes relatively frequent predation events, questioning the efficiency of the long sclerites in protecting the colony from grazers.

Most sclerites found in H. finmarchica and P. aculeata have a three-flanged shape that increases surface area and potential for attachment to the tissues, reportedly rendering the sclerites more efficient in supporting the colony (Koehl 1982). Only sclerites in the peduncle of P. aculeata showed a different morphology (bi-lobed), which has occasionally been illustrated in sea pens (Kükenthal 1915; Williams 1990) but never described/discussed. Pennatula species are known to withdraw into the sediment (Langton et al. 1990), hence bi-lobed sclerites may be restricted to the peduncle of P. aculeata to ensure that it is strong enough to enable frequent withdrawing. The presence of only short sclerites in the peduncle of P. aculeata allows tissue flexibility to anchor and withdraw. Long sclerites may break or inhibit muscle contractions in the peduncle (Koehl 1982), which undergoes physical stress and frequent muscle contractions.

The elemental analysis showed that sea pen sclerites are composed of high-magnesium calcite (Mg content between 3 and 6%) as previously reported for octocorals (Rahman and Oomori 2008). The differences in Mg/Ca ratios among all the types of sclerites in P. aculeata (highest ratio in peduncle sclerites and lowest ratio in tentacle sclerites) suggest variations in their synthesis possibly linked to the different roles they play (support vs. defense). No differences in the Mg/Ca ratios were found in H. finmarchica in which sclerites are chiefly a means of defense.

### 4.5 Variations in sclerite metrics

In H. finmarchica and P. aculeata, the short sclerites in the peduncle and the tentacles presented a similarly wide range of lengths (0.03-0.39 mm). However those from the peduncle were stouter than those from the tentacles in both species, giving them

better resistance to friction against the sediment. The greater variability of sclerite metrics in the peduncle than in the tentacles may be attributed to a response to different types of sediment. For example, colonies of H. finmarchica have been found anchored in mud, sand, but also in gravelly substrates (Baker et al. 2012). Anchoring into harder sediments may require a more mineralized peduncle, and sediment types may also be a driver of sclerite abundance in the peduncle.

The leaf and peduncle sclerites of P. aculeata and peduncle sclerite of H. finmarchica presented variations with depth and region. These sclerites are the only ones in this study that are presumed to act primarily as support and they presented a higher variability in size and shape than other sclerite types presumed to play a primarily defensive role. Overall, variations in sclerite metrics are more important in P. aculeata than in H. finmarchica, with the latter apparently using sclerites more as a defense mechanism than the former. Support sclerites may exhibit greater plasticity than defense sclerites to allow phenotypic adjustment of the colony to the local environment in order to increase fitness.

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# Tables

Table 2-1 Comparison of sclerite concentration and abundance between (Hf) Halipteris finmarchica and (Pa) Pennatula aculeata in the peduncle and rachis.

| | Peduncle | Rachis |
|---|---|---|
| Concentration | U=2.0, p<0.001, Hf<Pa | t=-28.16, df=54, p<0.001, Hf<Pa |
| Abundance | U=15, p<0.001, Hf<Pa | U=0.0, p<0.001, Hf<Pa |

Table 2-2 Elemental composition (%) of the tentacle sclerites, long sclerites and peduncle sclerites of Halipetris finmarchica and Pennatula aculeata. Data shown as mean±SE.

| | Sclerites | Calcium | Magnesium | Carbon | Oxygen |
|---|---|---|---|---|---|
| Halipteris finmarchica | Tentacle | 39.2±3.3 | 5.8±0.7 | 2.4±0.3 | 52.0±2.9 |
| | Long | 35.5±2.9 | 6.4±0.7 | 1.6±0.2 | 56.5±2.3 |
| | Peduncle | 37.0±3.0 | 7.5±0.7 | 1.9±0.2 | 53.6±2.6 |
| | Average | 37.2±3.1 | 6.5±0.7 | 2.0±0.2 | 54.0±2.6 |
| Pennatula aculeata | Tentacle | 45.4±2.5 | 4.6±0.3 | 1.2±0.1 | 48.7±2.2 |
| | Long | 39.1±2.0 | 5.6±0.3 | 1.4±0.3 | 53.9±1.8 |
| | Peduncle | 40.7±3.0 | 6.4±0.5 | 1.4±0.2 | 51.6±2.5 |
| | Average | 41.7±2.5 | 5.5±0.4 | 1.3±0.2 | 51.4±2.2 |

Table 2-3 (Following page) Summary of the general biometric results for Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata. A dash indicates the absence of analysis.

| METRICS | VARIATIONS | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Anthoptilum grandiflorum | | | | Halipteris finmarchica | | | | Pennatula aculeata | | | |
| | In colony[a] | With colony length | With depth[b] | With region[c] | In colony | With colony length | With depth | With region | In colony | With colony length | With depth | With region |
| Macrometrics | | | | | | | | | | | | |
| Colony length | - | - | S>D in GB – threshold at 800 m<br>S<D in NNL | <800 m and > 800 m: LC=GB>FC=NNL=LB | - | - | S<D in LB – threshold at 700 m | <700 m: LC=GB>LB<br>>700 m: GB=FC=LB | | | S>D in LC and GB | LC*>GB*,**>FC*,**>NNL** |
| PPL | - | decrease | S>D in GB<br>S>D in FC | LC>GB>FC>NNL>LB | - | decrease | no variation | LC=GB=FC<LB | - | - No variation | No variation | GB>LC=NNL>FC |
| W/L ratio | - | increase | S>D in GB – threshold at 800 m | Oct: FC*<GB*,**<LB** | - | increase | S>D in GB | Pooled data: FC*<LB*,**<GB**<LC** | - | increase | S>D in NNL | Apr and May: GB<LC |
| Polyp diameter | L<M<U | increase | S>D in GB | <800 m: GB>LC=FC=LB<br>>800 m: LB>GB=FC | L<M<U | Increase | S<D in FC and LB – no threshold | GB*>LC*,**>FC**,***>LB*** | L<M>U | no variation | S>D in GB<br>S>D in NNL – threshold at 1000 m | no variation |
| Polyp density | L<M<U | decrease in GB | S>D in GB and FC – threshold at 800 m | <800 m: no variation<br>>800 m: GB<FC<NNL<LB | L<M<U | no variation | S>D in FC | LC=GB=LB>FC | L<M>U | no variation | S>D in GB and NNL – threshold at 1000 m | no variation |
| Micrometrics | | | | | | | | | | | | |
| Sclerite length | - | - | - | - | Ps<Ts | Ps: decrease<br>Cs: increase | Cs: S<D | Ps, Ts: GB*>LC*,**>LB**<br>Cs: FC<GB=LB | Ls>Ds<br>Ps>Ts | no variation | Ps: S>D<br>Ts: S<D<br>Ls: S>D in GB and NNL – threshold at 1100 m | Ps: LC<GB>FC>NNL<br>Ts: LC<GB>FC=NNL<br>Ds: GB*<LC*<FC*,**<NNL** Ls: <1100 m: GB=FC<LC=NNL; >1100 m: no variation |
| Sclerite stoutness | - | - | - | - | Ps>Ts | Ps: increase<br>Cs: increase | Cs: S<D<br>Ps S<D | Ps: GB<LB<LC<br>Ts: GB<LC=LB<br>Cs: FC<GB=LB | Ls>Ds<br>Ps>Ts | Ts, Ls: increase<br>Ps: decrease | Ps: S>D<br>Ts: S<D – threshold at 1000 m<br>Ls: S<D | Ts: <1000 m LC=GB=FC<NNL; >1000 m: no variation<br>Ls: LC>GB>NNL<br>Ds: LC=FC>NNL |
| Sclerite abundance | - | - | - | - | P=Rs | no variation | no variation | no variation | Ps<Rs | no variation | no variation | no variation |
| Sclerite concentration | - | - | - | - | P<Rs | no variation | no variation | no variation | Ps<Rs | no variation | no variation | no variation |

[a] P: peduncle, L: Lower rachis, M: middle rachis, U: upper rachis; [b] S: shallow, D: deep; [c] Where appropriate, regional analysis conducted separately on colonies from above/ below threshold depth determined to have an impact. Regions with corresponding letters are not significantly different. LC: Laurentian Channel, GB: Grand Bank, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador; [d] Ps: peduncle sclerites, Ts: tentacle sclerites, Cs: column sclerites, Ls: leaf sclerites, Ds: dorsal sclerites, Rs : rachis sclerites

# Figures

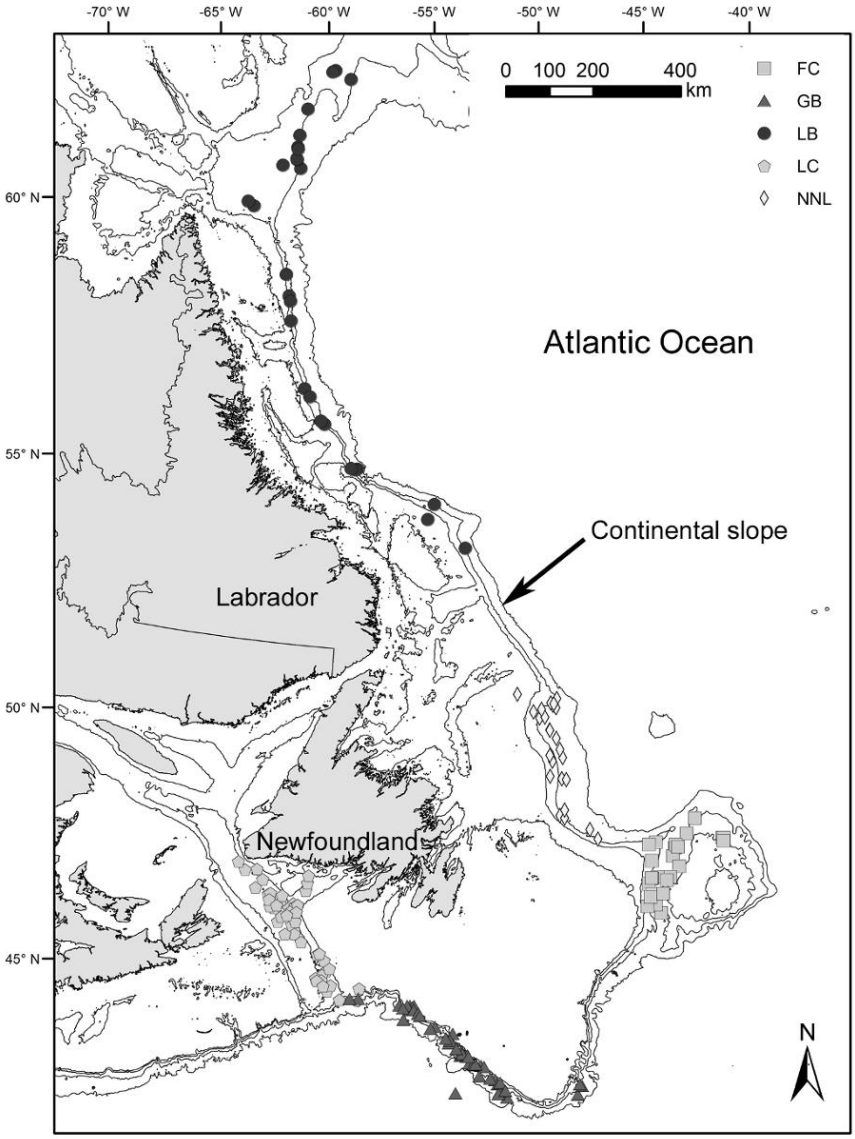


Figure 2-1 Map showing the five regions under study; LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador.

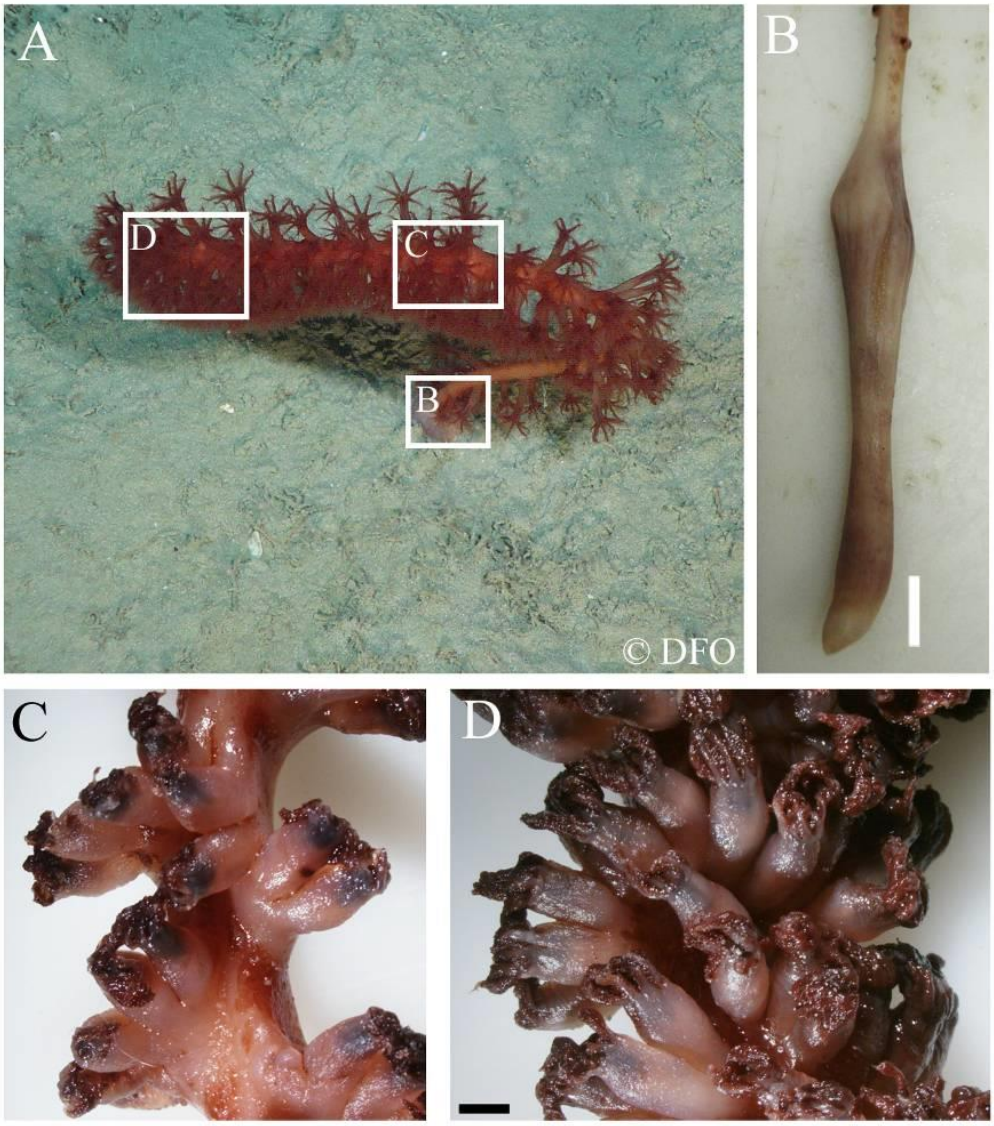


Figure 2-2 Anthoptilum grandiflorum. (A) In situ, (B) peduncle (C) middle section of rachis with low density of polyps, (D) upper rachis with higher density of polyps. No sclerites were observed. Scale bar in B: 1 cm, in C and D: 2 cm.

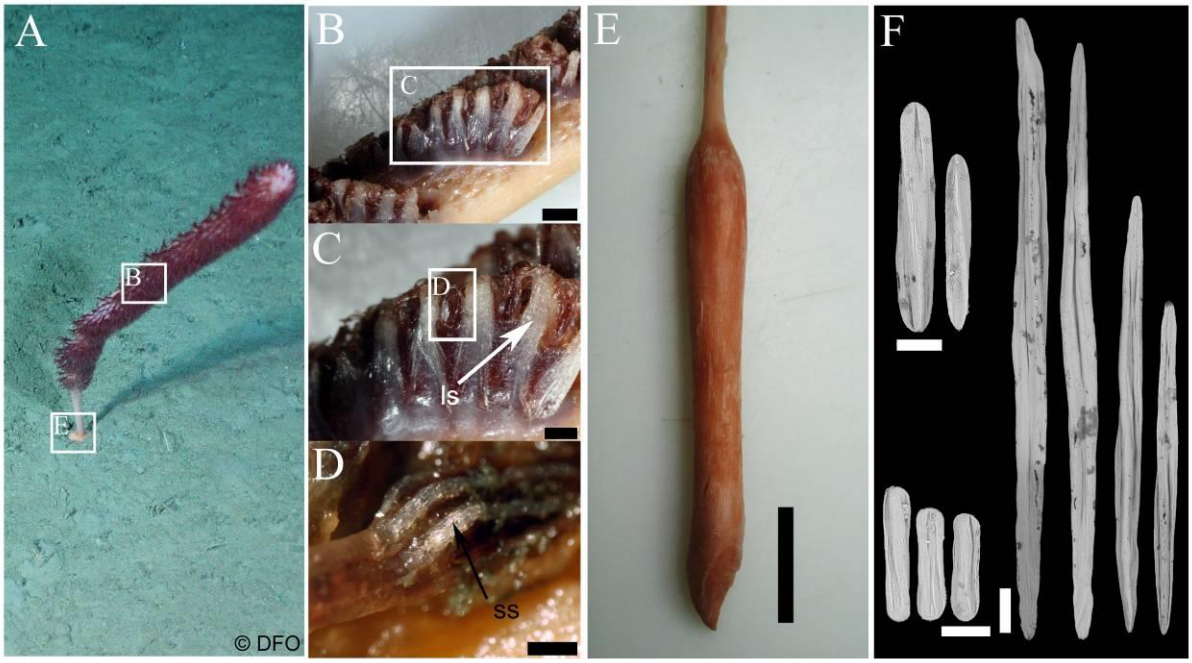


Figure 2-3 Halipteris finmarchica. (A) In situ, (B) rachis, (C) close up on a row of polyps in the rachis, with long sclerites (ls) in the middle section, (D) close up on the tentacles of the polyp, with short sclerites (ss), (E) peduncle, (F) SEM of peduncle sclerites (top left, scale bar: 40 µm), tentacle sclerites (bottom left, scale bar: 40 µm) and column sclerites (right, scale bar: 100 µm). Scale bar in B: 2 mm, in C: 1 mm, in D: 500 µm and in E: 2 cm.

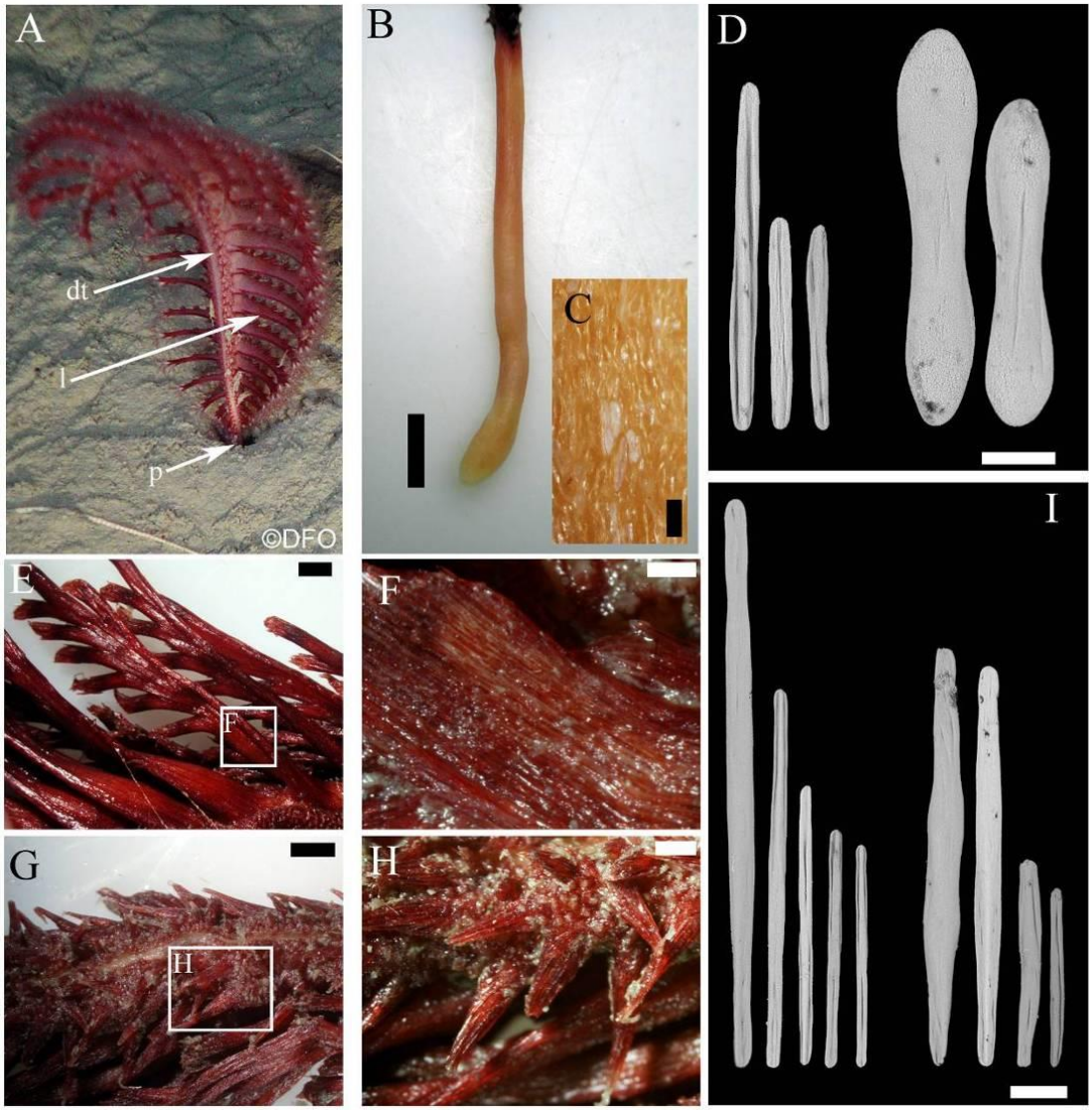


Figure 2-4 Pennatula aculeata. (A) In situ, showing the localisation of the peduncle (p) in the sediment, leaves (l) and dorsal track (dt), (B) peduncle, (C) close up on peduncle with visible sclerites, (D) SEM of tentacle sclerites (left) and peduncle sclerites (right), (E) leaves, (F) close up on a leaf with long sclerites, (G) dorsal track, (H) close up on dorsal track with sclerites forming peak, (I) SEM of leaf sclerites (left), and dorsal track sclerites (right). Scale bar in B: 1 cm, in C: 200 µm, in D: 45 µm, in E and G: 2 mm, in F: 500 µm, in H: 1 mm and in I: 110 µm.

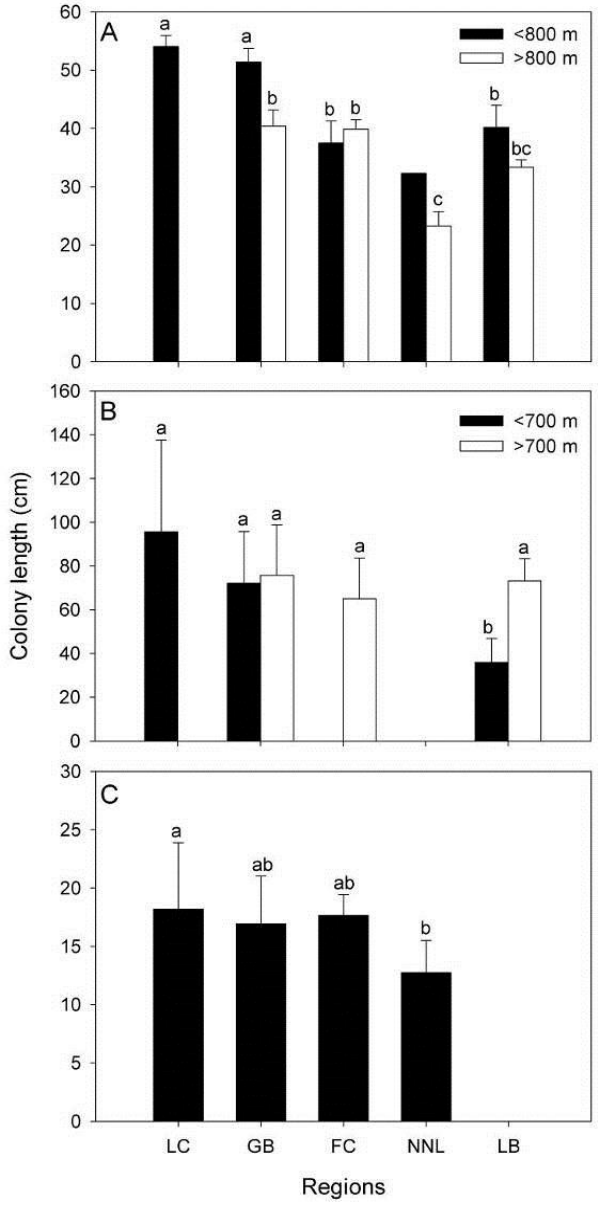


Figure 2-5 (A) Anthoptilum grandiflorum, (B) Halipteris finmarchica and (C) Pennatula aculeata: significant change in colony length across regions. LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cape, NNL: North Newfoundland, LB: Labrador. Data shown as regional mean ± standard error; values with corresponding letters are not significantly different, see text for statistical results.

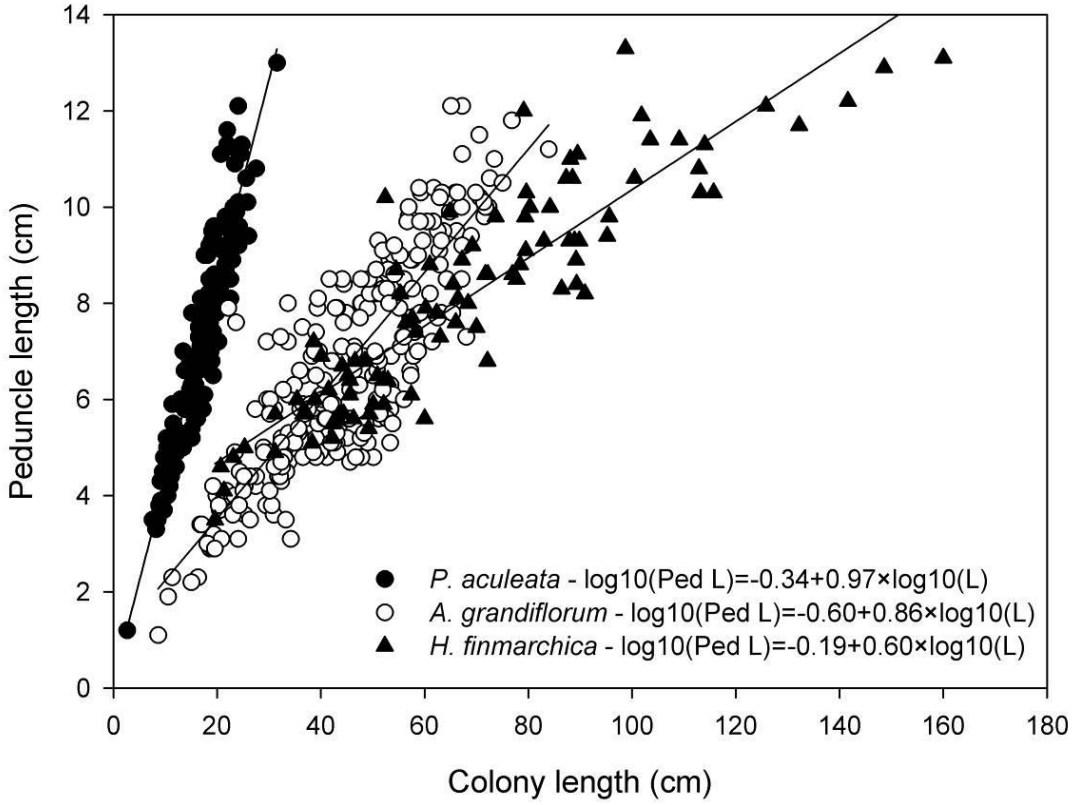


Figure 2-6 Linear regression between CL: colony length and Ped L: peduncle length for the three species (A. grandiflorum, r=0.89, $F_{1,249}$=941.24, p<0.001; H. finmarchica, r=0.92; $F_{1,93}$=484.28, p<0.001; P. aculeata, r=0.95, $F_{1,145}$=1463.92, p<0.001).

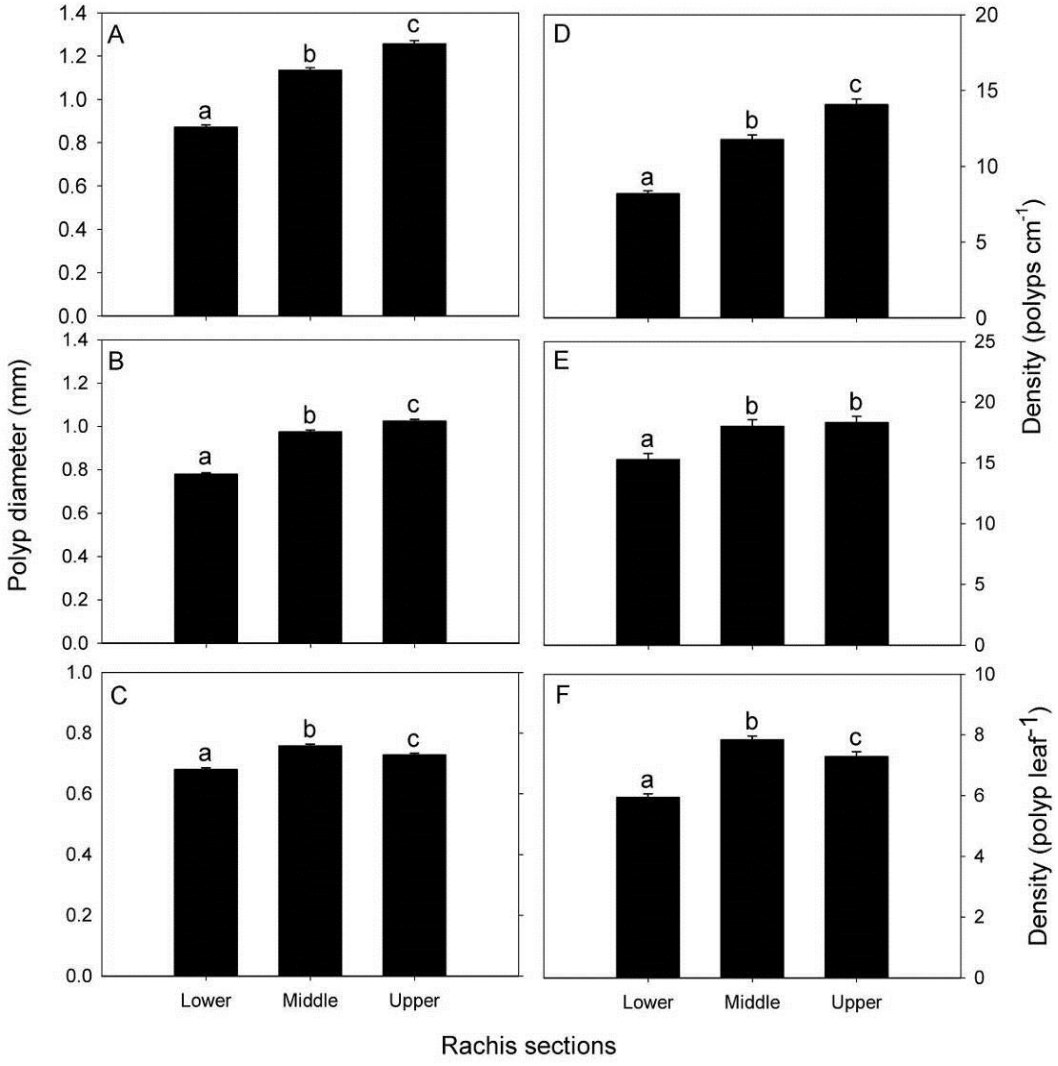


Figure 2-7 Variation in polyp diameter and density from lower to upper rachis sections in Anthoptilum grandiflorum: (A) diameter, (D) density. Halipteris finmarchica: (B) diameter, (E) density. Pennatula aculeata: (C) polyp diameter and (F) polyp density from lower to upper rachis sections. Values with corresponding letters are not significantly different, see text for statistical results.

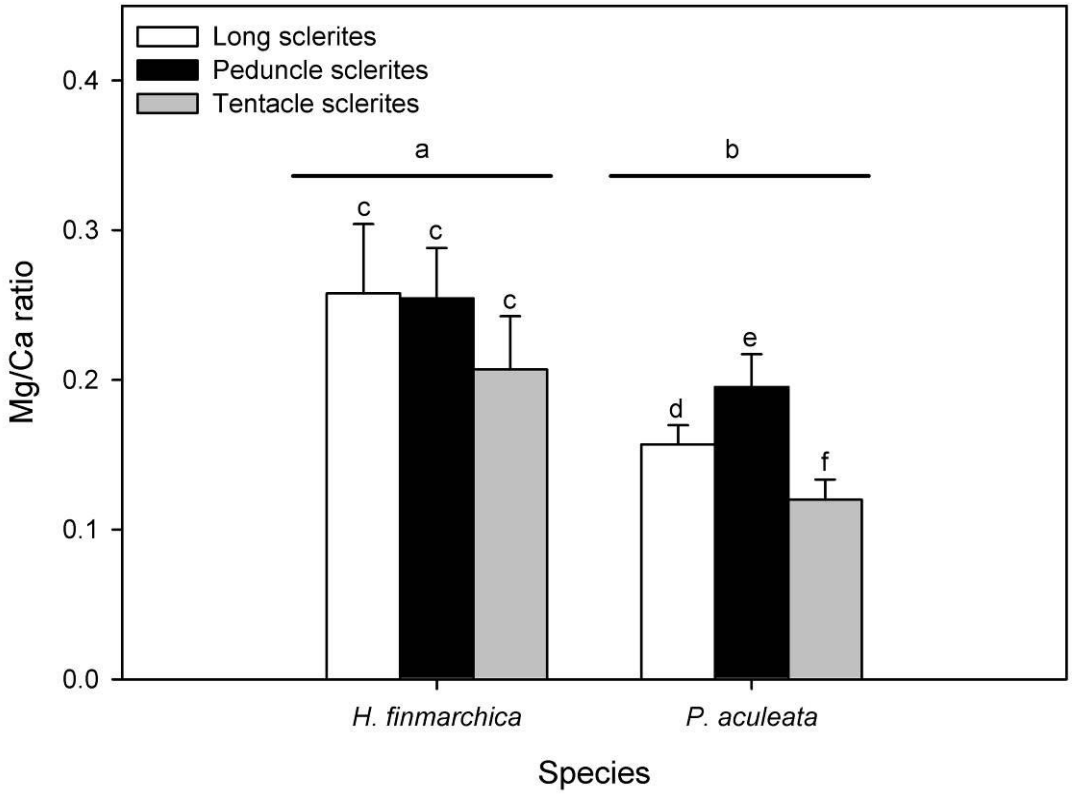


Figure 2-8 Mg/Ca ratio in the different types of sclerites in Halipteris finmarchica and Pennatula aculeata. Values with corresponding letters are not significantly different. See text for statistical results.

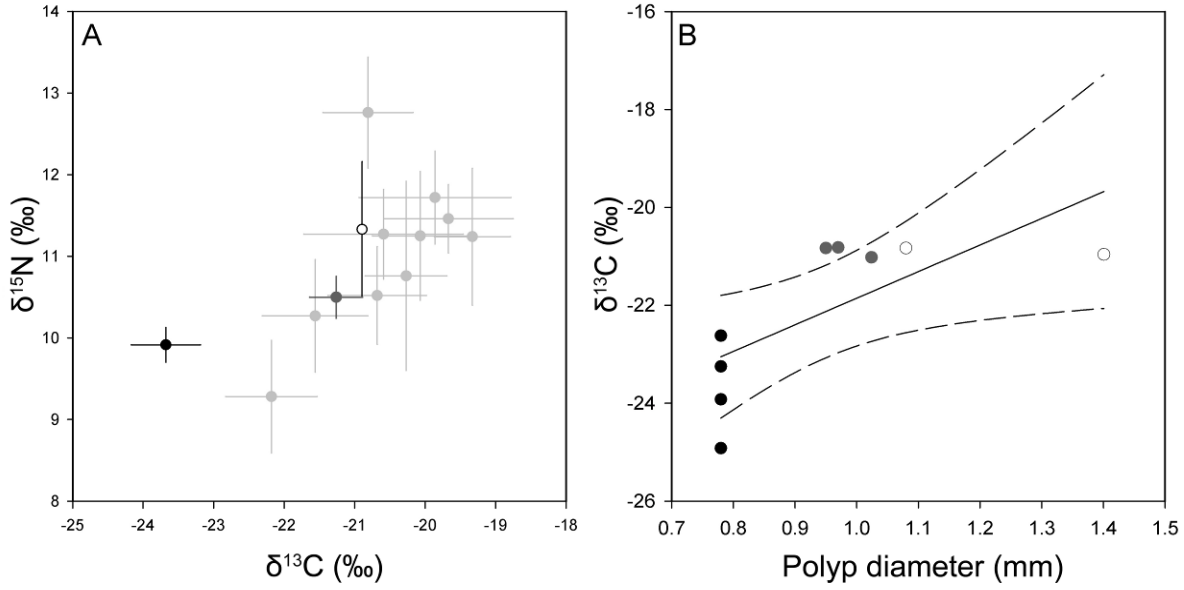


Figure 2-9 Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for sea pens (A) Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata. (B) Linear regression between polyp diameter and $\delta^{13}C$ for the three sea pens ($\delta^{13}C$=-27.29+5.434×polyp diameter, r=0.71, $F_{1,8}$=7.01, p=0.033). White: A. grandiflorum, dark grey: H. finmarchica, black: P. aculeata, light grey: other deep-sea coral from Sherwood et al. (2008). Result shown as mean ± SD (n=2-5).

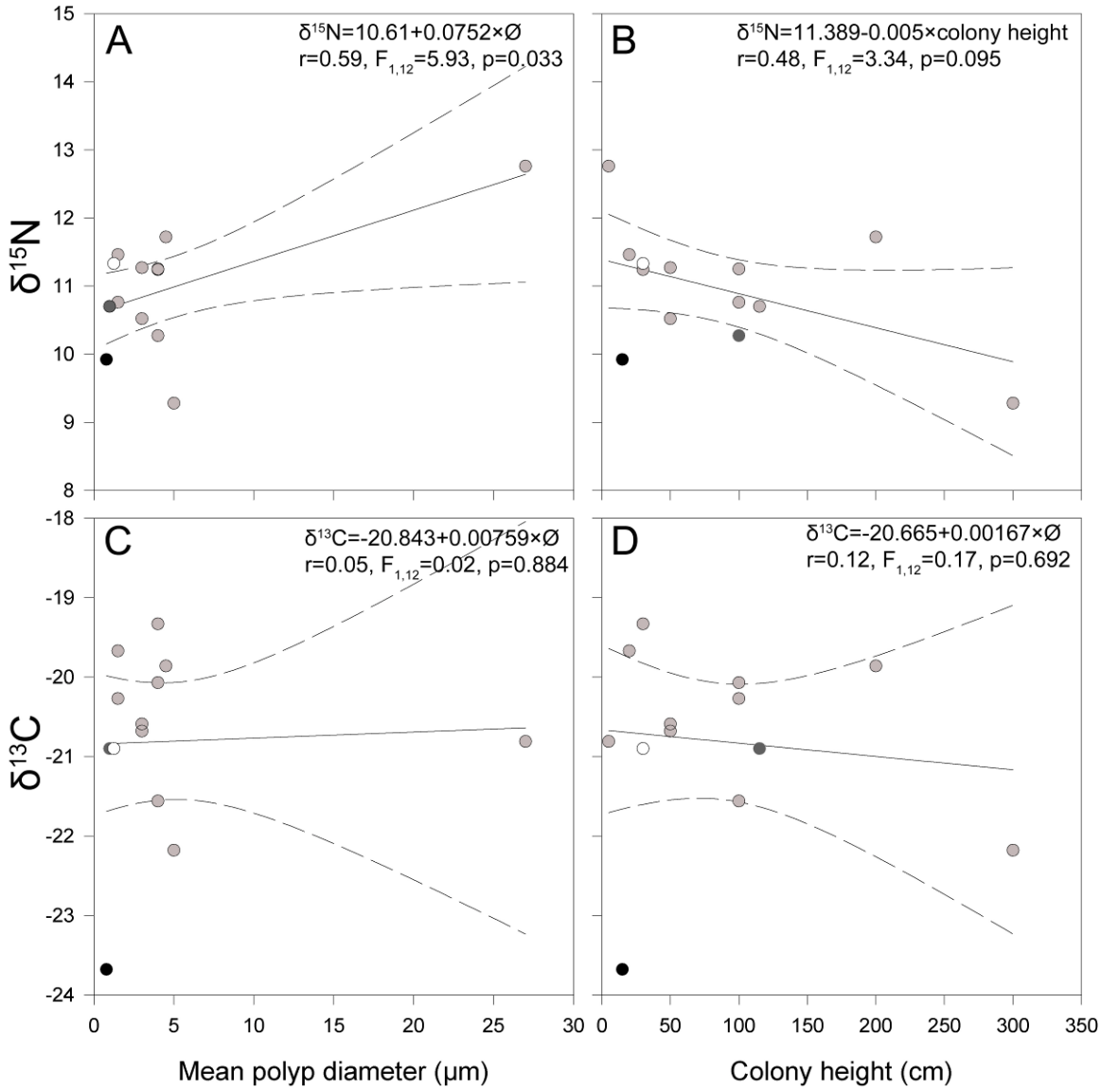


Figure 2-10 Variation in the nitrogen an carbon isotope signature with increasing polyp diameter and colony height of the three sea pens species and different deep-sea corals sampled along the continental slope of Newfoundland and Labrador from Sherwood et al. (2008). White: Anthoptilum grandiflorum, dark grey: Halipteris finmarchica, black: Pennatula aculeata and light grey: different deep-sea coral from Sherwood et al. (2008).

## Supplementary materials

Appendix 2-A Summary of samples of Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata examined from (LC) Laurentian Channel, (GB) Grand Banks, (FC) Flemish Cap, (NNL) North Newfoundland and (LB) Labrador with information on date, depth and latitude/longitude of collection

| Date | Depth | Region | Latitude | Longitude | Number of colonies collected | | |
|---|---|---|---|---|---|---|---|
| | | | | | A. grandiflorum | H. finmarchica | P. aculeata |
| 7/8/2004 | 942 | LB | 62°19'58.80"N | 60°37'60.00"W | 1 | | |
| 28/4/2005 | 464 | LC | 46°17'38.40"N | 57°42'16.20"W | 1 | | |
| 28/4/2005 | 448 | LC | 46°20'38.40"N | 58° 7'30.00"W | 2 | | |
| 30/4/2005 | 458 | LC | 46°49'6.60"N | 58°21'27.00"W | 1 | | |
| 25/8/2005 | 683 | LB | 63°44'33.00"N | 59°26'11.40"W | 1 | | |
| 8/10/2005 | 800 | GB | 44° 2'33.00"N | 52°58'3.00"W | 2 | | |
| 9/10/2005 | 953 | GB | 43°47'24.00"N | 52°37'33.60"W | | 5 | |
| 9/10/2005 | 600 | GB | 43°35'18.60"N | 52° 2'33.00"W | | | 4 |
| 10/10/2005 | 1124 | GB | 42°57'3.60"N | 51°18'5.40"W | 1 | | |
| 11/10/2005 | 785 | GB | 43°36'3.60"N | 52°13'12.00"W | | | 5 |
| 26/1/2006 | 1167 | NNL | 49°28'42.60"N | 49°35'42.00"W | 2 | | 2 |
| 27/1/2006 | 1070 | NNL | 51° 7'15.60"N | 49°42'43.20"W | 1 | | |
| 27/01/2006 | 918 | NNL | 50°59'16.80"N | 49°50'52.80"W | | 1 | 1 |
| 28/1/2006 | 1293 | NNL | 50°18'14.40"N | 49°45'0.00"W | | | 3 |
| 28/01/2006 | 1048 | NNL | 50°43'39.00"N | 50°17'6.00"W | | | 1 |
| 30/1/2006 | 821 | NNL | 48°43'22.80"N | 49°40'49.80"W | 3 | | 1 |
| 23/2/2006 | 1006 | NNL | 50°55'58.80"N | 49°45'0.00"W | | | 1 |
| 15/3/2006 | 864 | NNL | 51° 1'58.80"N | 49°55'1.20"W | 1 | | |
| 24/3/2006 | 821 | NNL | 51° 1'1.20"N | 49°49'1.20"W | 1 | | |
| 31/3/2006 | 891 | NNL | 51°13'1.20"N | 51° 0'0.00"W | 1 | | |
| 17/04/2006 | 317 | LC | 47° 1'24.60"N | 57°22'12.00"W | | | 1 |
| 18/4/2006 | 225 | LC | 47°10'44.40"N | 57°20'29.40"W | | | 1 |
| 18/04/2006 | 186 | LC | 47°19'57.00"N | 57°19'40.80"W | | | 1 |
| 14/06/2006 | 183 | GB | 44°46'58.80"N | 54° 0'0.00"W | | | 1 |
| 14/06/2006 | 237 | GB | 44°46'58.80"N | 54° 7'1.20"W | | | 1 |
| 14/06/2006 | 241 | GB | 44°45'0.00"N | 54° 2'60.00"W | | | 1 |
| 10/7/2006 | 1299 | LB | 55°41'56.40"N | 56°49'51.60"W | 1 | | |
| 14/7/2006 | 837 | LB | 62°52'60.00"N | 60°27'0.00"W | | 1 | |
| 27/7/2006 | 176 | LB | 61°40'1.20"N | 60°22'58.80"W | 2 | | |
| 28/7/2006 | 620 | LB | 58°33'21.60"N | 59°55'12.00"W | 4 | 2 | |
| 29/07/2006 | 452 | LB | 59° 3'37.80"N | 60° 7'42.60"W | | 1 | |
| 30/07/2006 | 528 | LB | 59°29'11.40"N | 60°21'9.00"W | | 1 | |
| 04/08/2006 | 563 | LB | 60°45'57.60"N | 62° 5'36.60"W | | 1 | |
| 04/08/2006 | 630 | LB | 60°50'29.40"N | 62°22'21.00"W | | 1 | |
| 5/8/2006 | 559 | LB | 61°40'42.60"N | 61° 9'34.20"W | | 3 | |
| 8/8/2006 | 724 | LB | 63°42'14.40"N | 59°33'5.40"W | | 1 | |
| 6/10/2006 | 618 | GB | 43°36'50.40"N | 52°11'15.00"W | | 2 | |
| 7/10/2006 | 626 | LB | 55°40'44.40"N | 56°54'21.60"W | 1 | | |
| 7/10/2006 | 466 | LB | 55°42'3.60"N | 57° 3'32.40"W | 1 | | |

Appendix 2-A continued

| Date | Depth | Region | Latitude | Longitude | Number of colonies collected | | |
|---|---|---|---|---|---|---|---|
| | | | | | A. grandiflorum | H. finmarchica | P. aculeata |
| 9/10/2006 | 803 | LB | 56°32'29.40"N | 58°11'6.00"W | 1 | | |
| 9/10/2006 | 424 | LB | 56°36'10.80"N | 58°19'39.00"W | | 1 | |
| 15/10/2006 | 548 | LB | 57°13'15.60"N | 59° 4'33.60"W | 3 | | |
| 17/10/2006 | 895 | LB | 57° 4'1.20"N | 58°50'20.40"W | 1 | | |
| 02/11/2006 | 256 | LB | 54°45'30.60"N | 54°11'16.80"W | | 1 | |
| 4/11/2006 | 1071 | LB | 54°12'3.60"N | 52°49'48.00"W | 1 | | |
| 19/11/2006 | 1286 | NNL | 49°54'30.60"N | 49°33'34.20"W | | | 2 |
| 24/11/2006 | 1018 | NNL | 48°13'30.00"N | 48°31'4.80"W | 1 | | |
| 24/11/2006 | 1415 | NNL | 48°25'17.40"N | 48°45'37.80"W | | | 1 |
| 27/11/2006 | 958 | FC | 48°15'32.40"N | 45°47'42.00"W | 2 | | |
| 28/11/2006 | 1125 | FC | 47°58'33.60"N | 46°11'9.60"W | 23 | 4 | |
| 28/11/2006 | 1134 | FC | 47°48'57.60"N | 46°15'30.60"W | 4 | | |
| 28/11/2006 | 788 | FC | 47°44'36.60"N | 46°54'39.60"W | | | 1 |
| 29/11/2006 | 810 | FC | 47°35'45.60"N | 46° 5'9.60"W | 2 | 1 | |
| 30/11/2006 | 780 | FC | 47°23'33.00"N | 46°22'57.00"W | | 2 | |
| 1/12/2006 | 1161 | FC | 47°24'18.00"N | 46°45'41.40"W | | 3 | |
| 1/12/2006 | 1075 | FC | 47°22'55.20"N | 46°56'0.60"W | | | 2 |
| 1/12/2006 | 1036 | FC | 47°16'30.00"N | 47° 2'25.80"W | 3 | | |
| 1/12/2006 | 1186 | FC | 46°57'21.60"N | 46°56'31.20"W | 2 | | |
| 01/12/2006 | 1162 | FC | 47° 4'8.40"N | 46°57'18.00"W | | | 1 |
| 2/12/2006 | 978 | FC | 46°39'27.00"N | 46°42'18.00"W | 1 | | |
| 14/4/2007 | 435 | LC | 47°20'11.40"N | 59°14'43.80"W | 8 | | |
| 15/4/2007 | 428 | LC | 47°10'26.40"N | 58°41'25.80"W | 4 | | |
| 15/4/2007 | 457 | LC | 46°43'48.00"N | 58° 6'27.00"W | 3 | | |
| 15/4/2007 | 456 | LC | 46°36'54.00"N | 58°16'21.00"W | 1 | | |
| 16/4/2007 | 457 | LC | 46°22'17.40"N | 57°36'41.40"W | 3 | | |
| 16/4/2007 | 352 | LC | 46°40'26.40"N | 57°42'57.60"W | 7 | | |
| 20/4/2007 | 377 | LC | 44°50'15.00"N | 56° 9'27.00"W | | 3 | |
| 28/4/2007 | 296 | LC | 45°36'54.00"N | 56°40'37.20"W | | | 18 |
| 28/4/2007 | 370 | LC | 45°43'53.40"N | 56°53'25.80"W | 3 | 3 | 12 |
| 29/4/2007 | 462 | LC | 46° 6'45.00"N | 57°36'14.40"W | 2 | | |
| 29/4/2007 | 366 | LC | 45°24'3.60"N | 56°36'18.00"W | | 5 | |
| 30/4/2007 | 389 | LC | 45°19'8.40"N | 56°42'45.00"W | | 2 | |
| 30/4/2007 | 422 | LC | 45°14'43.80"N | 56°53'24.00"W | 4 | 1 | |
| 30/4/2007 | 98 | LC | 45° 5'27.60"N | 55°36'14.40"W | 1 | | 1 |
| 4/5/2007 | 575 | GB | 44°47'2.40"N | 54°25'24.60"W | | 4 | |
| 16/5/2007 | 719 | GB | 44°41'45.60"N | 54° 6'27.00"W | | | 5 |
| 16/5/2007 | 167 | GB | 44°14'15.00"N | 52°51'37.80"W | | | 6 |
| 17/5/2007 | 600 | GB | 43°52'33.60"N | 52°35'38.40"W | 4 | | 1 |
| 17/5/2007 | 425 | GB | 43°48'3.60"N | 52°26'6.00"W | | 1 | |
| 17/5/2007 | 597 | GB | 43°37'8.40"N | 52°11'33.00"W | 3 | 3 | |
| 17/5/2007 | 214 | GB | 43°33'57.60"N | 51°57'1.80"W | 1 | 3 | |
| 18/5/2007 | 625 | GB | 43°18'36.00"N | 51°38'24.00"W | 1 | 1 | |
| 16/6/2007 | 465 | FC | 48° 7'1.20"N | 44°42'0.00"W | 2 | | |
| 16/6/2007 | 490 | FC | 48° 7'1.20"N | 44°40'1.20"W | 1 | | |
| 17/6/2007 | 430 | FC | 48° 2'60.00"N | 44°40'58.80"W | 1 | | |
| 18/6/2007 | 658 | GB | 46°49'44.40"N | 46°51'9.00"W | 2 | | |
| 30/06/2007 | 342 | GB | 44°46'58.80"N | 54°12'0.00"W | | | 1 |
| 03/07/2007 | 297 | GB | 44°42'0.00"N | 54° 0'0.00"W | | | 1 |
| 8/7/2007 | 701 | FC | 48° 6'45.00"N | 46°45'7.20"W | 1 | | |
| 17/7/2007 | 881 | LB | 63°36'0.00"N | 58°40'60.00"W | 3 | | |

Appendix 2-A continued

| Date | Depth | Region | Latitude | Longitude | Number of colonies collected | | |
|---|---|---|---|---|---|---|---|
| | | | | | A. grandiflorum | H. finmarchica | P. aculeata |
| 18/7/2007 | 871 | LB | 62° 4'58.80"N | 60°37'58.80"W | 3 | | |
| 20/7/2007 | 883 | LB | 61°49'58.80"N | 60°36'0.00"W | 2 | | |
| 21/7/2007 | 874 | LB | 61°51'0.00"N | 60°37'1.20"W | 2 | | |
| 28/7/2007 | 552 | LB | 58°57'21.60"N | 60° 2'20.40"W | 3 | 6 | |
| 7/10/2007 | 773 | GB | 43°51'18.00"N | 52°37'10.20"W | | 8 | 1 |
| 8/10/2007 | 1034 | GB | 43°38'27.60"N | 52°22'35.40"W | | 1 | |
| 8/10/2007 | 824 | GB | 43°37'3.00"N | 52°15'19.80"W | 6 | | |
| 8/10/2007 | 1333 | GB | 43°22'51.60"N | 52° 5'11.40"W | 5 | 1 | |
| 8/10/2007 | 796 | GB | 43°13'8.40"N | 51°34'3.00"W | | 1 | |
| 08/10/2007 | 823 | GB | 43°19'39.00"N | 51°46'44.40"W | | 2 | |
| 9/10/2007 | 1013 | GB | 43° 3'41.40"N | 51°30'21.60"W | 1 | | |
| 9/10/2007 | 1130 | GB | 43° 0'50.40"N | 51°32'29.40"W | 2 | | |
| 10/10/2007 | 1347 | GB | 42°59'29.40"N | 49°18'39.60"W | 2 | | |
| 10/10/2007 | 934 | GB | 43°10'8.40"N | 49°11'24.00"W | 6 | | |
| 10/10/2007 | 836 | GB | 43°12'21.60"N | 49°15'5.40"W | 3 | | |
| 16/10/2007 | 986 | LB | 62° 2'60.00"N | 60°37'1.20"W | 1 | | |
| 18/10/2007 | 1208 | FC | 46°49'44.40"N | 46°51'9.00"W | 4 | 3 | |
| 18/10/2007 | 1036 | FC | 46°48'21.60"N | 47° 6'7.20"W | | 3 | |
| 18/10/2007 | 1151 | FC | 46°59'56.40"N | 46°59'45.60"W | | 1 | |
| 18/10/2007 | 768 | FC | 47° 3'30.60"N | 46°36'57.60"W | | 1 | |
| 18/10/2007 | 1061 | FC | 47°23'18.60"N | 46°56'42.00"W | | 1 | 1 |
| 18/10/2007 | 896 | FC | 47°20'2.40"N | 46°29'2.40"W | 6 | | |
| 19/10/2007 | 743 | FC | 48° 2'51.00"N | 46° 8'60.00"W | | 2 | |
| 19/10/2007 | 1001 | FC | 47°59'52.80"N | 46° 5'15.00"W | 2 | | 1 |
| 28/10/2007 | 599 | GB | 44°22'17.40"N | 53°22'48.00"W | 4 | | |
| 28/10/2007 | 632 | GB | 44°43'37.20"N | 54°18'50.40"W | | | 2 |
| 29/10/2007 | 992 | FC | 48°33'41.40"N | 45°30'30.60"W | | 6 | |
| 29/10/2007 | 579 | GB | 44° 5'56.40"N | 52°56'24.00"W | 1 | 2 | |
| 30/10/2007 | 623 | GB | 43°37'12.00"N | 52°11'56.40"W | 4 | 5 | |
| 30/10/2007 | 593 | GB | 43°14'47.40"N | 51°29'42.00"W | 2 | 2 | |
| 31/10/2007 | 964 | NNL | 48°40'4.80"N | 49°32'6.00"W | | | 1 |
| 16/11/2007 | 1039 | LB | 55° 4'33.60"N | 53°58'58.80"W | 1 | | |
| 25/11/2007 | 651 | FC | 48° 4'39.00"N | 46°58'12.00"W | 1 | | |
| 8/12/2007 | 656 | NNL | 49°31'57.00"N | 49°57'45.00"W | 1 | | |
| 13/12/2007 | 1285 | NNL | 50°28'40.80"N | 49°57'36.00"W | | | 4 |
| 13/12/2007 | 883 | NNL | 50°51'27.00"N | 50°28'39.00"W | | | 1 |
| 13/12/2007 | 1226 | NNL | 50°28'21.00"N | 49°57'32.40"W | | | 1 |
| 14/12/2007 | 1285 | NNL | 50° 4'42.60"N | 49°38'22.20"W | 1 | | |
| 14/12/2007 | 851 | NNL | 49°50'29.40"N | 49°53'6.00"W | 1 | | 1 |
| 14/12/2007 | 844 | NNL | 49°57'27.00"N | 49°58'12.00"W | | | 1 |
| 8/5/2008 | 301 | LC | 46°43'48.00"N | 57°42'57.60"W | 1 | | 3 |
| 9/5/2008 | 462 | LC | 46°26'42.00"N | 58° 1'49.80"W | 1 | | |
| 13/5/2008 | 398 | LC | 45° 0'0.00"N | 56°32'7.80"W | | | 7 |
| 26/05/2008 | 195 | GB | 44°37'40.80"N | 53°52'40.80"W | | | 1 |
| 11/4/2009 | 494 | GB | 44°52'60.00"N | 55°37'0.00"W | 2 | | 1 |
| 11/4/2009 | 428 | GB | 44°52'0.00"N | 55°52'0.00"W | 1 | | |
| 17/4/2009 | 318 | LC | 47°22'24.60"N | 58°53'36.60"W | 4 | | |
| 18/4/2009 | 437 | LC | 46°59'47.40"N | 58°53'11.40"W | 2 | | |
| 18/4/2009 | 428 | LC | 46°52'57.00"N | 58°29'15.00"W | 1 | | |
| 18/4/2009 | 458 | LC | 46°54'45.00"N | 58°25'12.00"W | 2 | | |
| 19/4/2009 | 453 | LC | 46°38'25.80"N | 58°23'47.40"W | 2 | | |

Appendix 2-A continued

| Date | Depth | Region | Latitude | Longitude | Number of colonies collected | | |
|---|---|---|---|---|---|---|---|
| | | | | | A. grandiflorum | H. finmarchica | P. aculeata |
| 19/4/2009 | 445 | LC | 46°37'57.00"N | 57°50'15.00"W | 6 | | |
| 19/4/2009 | 317 | LC | 46°40'51.60"N | 57°39'37.80"W | 3 | | |
| 19/4/2009 | 299 | LC | 46°42'45.00"N | 57°34'31.80"W | 1 | | |
| 29/4/2009 | 451 | LC | 46°33'36.00"N | 57°47'22.20"W | 3 | | |
| 30/4/2009 | 348 | LC | 46°33'39.60"N | 57°36'5.40"W | 3 | | |
| 30/4/2009 | 488 | LC | 46° 5'54.60"N | 57°52'53.40"W | 1 | | |
| 1/5/2009 | 297 | GB | 44°34'55.20"N | 53°49'12.00"W | | | 3 |
| 3/5/2009 | 265 | LC | 45°27'25.20"N | 56°30'18.00"W | 1 | | 4 |
| 10/5/2009 | 404 | LC | 45°12'36.00"N | 56°42'46.80"W | 3 | | 1 |
| 11/5/2009 | 369 | LB | 45° 7'17.40"N | 56°26'6.00"W | | | 7 |
| 11/5/2009 | 422 | LC | 45°11'52.80"N | 56°51'10.80"W | 3 | | 1 |
| 11/5/2009 | 404 | LC | 45° 5'49.20"N | 56°39'18.00"W | 1 | | 3 |
| 13/5/2009 | 584 | GB | 44°29'42.00"N | 54°17'16.80"W | | | 1 |
| 14/5/2009 | 674 | GB | 44°19'33.60"N | 53°30'18.00"W | | | 8 |
| 24/5/2009 | 603 | GB | 43°49'31.80"N | 52°34'24.60"W | 3 | | |
| 24/5/2009 | 337 | GB | 43°46'8.40"N | 52°23'52.80"W | 2 | | |
| 26/5/2009 | 596 | GB | 43° 4'40.80"N | 51°20'33.00"W | 3 | | |
| 16/10/2009 | 978 | GB | 44° 8'21.00"N | 53° 3'27.00"W | | | 10 |
| 18/10/2009 | 887 | GB | 43°55'32.99"N | 52°45'6.01"W | | | 1 |
| 25/10/2009 | 1208 | GB | 43° 1'18.00"N | 52°45'6.01"W | | | 2 |
| 5/12/2009 | 1149 | NNL | 50°44'48.01"N | 50° 6'42.01"W | | | 2 |
| 5/12/2009 | 1030 | NNL | 50°54'27.00"N | 50°12'45.00"W | | | 3 |
| 10/12/2009 | 1334 | NNL | 49°27'27.00"N | 49°27'27.00"W | | | 5 |
| 13/12/2009 | 1316 | NNL | 48°50'12.00"N | 49°31'48.00"W | | | 1 |
| 15/4/2010 | 314 | LC | 45°45'7.20"N | 56°49'22.80"W | 2 | | 2 |
| 24/4/2010 | 456 | LC | 46°52'30.00"N | 58° 3'54.00"W | 1 | | 8 |
| 25/4/2010 | 449 | LC | 47°28'55.20"N | 59°28'15.60"W | 1 | | |
| 26/4/2010 | 433 | LC | 46°46'17.40"N | 58°26'45.60"W | 3 | | |
| 26/4/2010 | 455 | LC | 46°40'8.40"N | 58°12'1.80"W | 1 | | |
| 26/4/2010 | 468 | LC | 46°28'49.80"N | 57°52'17.40"W | 1 | | |
| 27/4/2010 | 447 | LC | 45°57'39.60"N | 57°23'11.40"W | 4 | | 8 |

Appendix 2-B. (Following page) Variations in macro-morphological traits (linear regression or spearman correlation) in Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata sampled in LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland and LB: Labrador

| | Regions | Colony length (L) | | | Colony weight/length ratio (W/L) | | Percent peduncle length (PPL) | |
|---|---|---|---|---|---|---|---|---|
| | | vs. depth | vs. weight (W) | vs. colony height (CH) | vs. depth | vs. colony length (L) | vs. depth | vs. colony length (L) |
| A. grandiflorum | LC | $r_s$=0.19, p=0.077 | W=-21.117+0.849×L r=0.65, $F_{1,88}$=65.806, p<0.001 | CH=1.278+0.559×L r=0.84, $F_{1,27}$=344.67, p<0.001 | $r_s$=0.02, p=0.864 | $r_s$=0.44, p<0.001 | $r_s$=0.17, p=0.109 | $r_s$=-0.31, p=0.003 |
| | GB | L=67.983-0.02×depth r=0.51, $F_{1,58}$=20.28, p<0.001 | $r_s$=0.84, p<0.001 | CH=2?406+0.475×L r=0.93, $F_{1,43}$=260.85, p<0.001 | $r_s$=-0.70, p<0.001 | $r_s$=0.73, p<0.001 | PPL=18.051-0.0038×depth r=0.40, $F_{1,58}$=10.83, p=0.002 | PPL=16.696-0.0336×L r=0.19, $F_{1,58}$=2.24, p=0.140 |
| | FC | $r_s$=0.12, p=0.380 | $r_s$=0.71, p<0.001 | CH=4.71+0.359×L r=0.90, $F_{1,46}$=200.49, p<0.001 | $r_s$=-0.09, p=0.528 | $r_s$=0.50, p<0.001 | log10(PPL)=1.265-0.00014×depth r=0.31, $F_{1,53}$=5.53, P=0.022 | log10(PPL)=1.326-0.00517×L r=0.68, $F_{1,54}$=45.47, P<0.001 |
| | NNL | L=-6.835+0.0333×depth r=0.54, $F_{1,14}$=56.04, p=0.033 | $r_s$=0.93, p<0.001 | CH=2.394+0.42×L r=0.97, $F_{1,13}$=171.90, p<0.001 | W/L=0.128-0.0000122×depth r=0.02, $F_{1,14}$=0.006, p=0.938 | $r_s$=0.89, p<0.001 | PPL=22.247-0.00665×depth r=0.43, $F_{1,14}$=3.11, p=0.100 | PPL=18.606-0.105×L r=0.42, $F_{1,14}$=3.02, p=0.104 |
| | LB | $r_s$=-0.19, p=0.295 | r=0.86, p<0.001 | CH=1.303+0.483×L r=0.96, $F_{1,27}$=344.67, p<0.001 | W/L=0.676-0.000743×depth r=0.06, $F_{1,30}$=0.10, p=0.758 | $r_s$=0.72, p<0.001 | PPL=18.567-0.00095×depth r=0.09, $F_{1,30}$=0.25, p=0.620 | r=-0.24, p=0.196 |
| H. finmarchica | LC | L=-130.649+0.601*depth r=0.22, $F_{1,13}$=0.61, p=0.450 | W=-9.789+0.488*L r=0.92, $F_{1,13}$=69.60, p<0.001 | CH=-4.885+0.942×L r=1.00, $F_{1,9}$=5280.51, p<0.001 | W/L=0.191+0.000431*depth r=0.05, $F_{1,12}$=0.03, p=0.847 | W/L=0.112+0.00224*L r=0.84, $F_{1,11}$=27.07, p<0.001 | PPL=22.896-0.0292*depth r=0.14, $F_{1,11}$=0.22, p=0.647 | PPL=18.812-0.0709*L r=0.93, $F_{1,11}$=69.24, p<0.001 |
| | GB | L=72.362+0.00177*depth r=0.02, $F_{1,37}$=0.01, p=0.919 | log10(W)=-2.202+1.898*log10(L) r=0.85, $F_{1,37}$=94.9, p<0.001 | CH=-4.766+0.944×L r=1.00, $F_{1,40}$=25759.94, p<0.001 | W/L=0.474-0.000243*depth r=0.43, $F_{1,36}$=7.93, p=0.008 | $r_s$=0.47, p=0.002 | $r_s$=0.0486, p=0.767 | PPL=18.347-0.0785*L r=0.77, $F_{1,38}$=59.62, p<0.001 |
| | FC | L=98.084-0.0324*depth r=0.27, $F_{1,25}$=1.95, p=0.175 | W=-18.946+0.51*L r=0.86, $F_{1,25}$=70.62, p<0.001 | CH=-2.974+0.931×L r=1.00, $F_{1,29}$=7371.17, p<0.001 | W/L=0.276-0.0000798*depth r=0.10, $F_{1,25}$=0.27, p=0.606 | W/L=-0.113+0.00458*L r=.83, $F_{1,24}$=52.03, p<0.001 | PPL=7.15+0.00435*depth r=0.39, $F_{1,24}$=4.19, p=0.052 | PPL=15.113-0.0533*L r=0.52, $F_{1,24}$=8.83, p=0.007 |
| | LB | L=-15.048+0.101*depth r=0.65, $F_{1,13}$=8.62, p=0.012 | $r_s$=0.70, p=0.002 | CH=-4.711+0.979×L r=1.00, $F_{1,16}$=1622.53, p<0.001 | log10(W/L)=--1.107+0.000617*depth r=0.19, $F_{1,15}$=0.51, p=0.486 | $r_s$=0.49, p=0.502 | PPL=21.623-0.0107*depth r=0.32, $F_{1,15}$=1.59, p=0.228 | PPL=21.834-0.157*L r=0.81, $F_{1,15}$=27.36, p<0.001 |
| P. aculeata | LC | L=27.46-0.0255*depth r=0.28, $F_{1,55}$=4.81, p=0.033 | $r_s$=0.84, p<0.001 | $r_s$=0.94, p<0.001 | r=0.14, p=0.291 | W/L=0.0352+0.00686*L r=0.67, $F_{1,53}$=42.56, p<0.001 | PPL=36.495+0.0129*depth r=0.20, $F_{1,55}$=2.36, p=0.130 | PPL=42.064-0.0568*L r=0.08, $F_{1,55}$=0.36, p=0.551 |
| | GB | L=19.63-0.00427*depth r=0.28, $F_{1,62}$=5.34, p=0.003 | log10(W)=-0.544+0.0572*L r=0.83, $F_{1,62}$=136.16, p<0.001 | CH=-0.422+0.581×L r=0.96, $F_{1,66}$=693.75, p<0.001 | W/L=0.206-0.0000448*depth r=0.17, $F_{1,62}$=1.82, p=0.182 | W/L=0.00282+0.0103*L r=0.59, $F_{1,62}$=33.16, p<0.001 | PPL=42.695+0.00303* depth r=0.18, $F_{1,62}$=1.99, p=0.163 | PPL=45.31-0.0413*L r=0.03, $F_{1,62}$=0.08, p=0.776 |
| | NNL | L=21.866-0.00738*depth r=0.38, $F_{1,21}$=3.58, p=0.072 | W=-1.171+0.2*L r=0.78, $F_{1,21}$=32.48, p<0.001 | CH=-0.538+0.629×L r=0.98, $F_{1,25}$=721.61, p<0.001 | W/L=0.251-0.00012*depth r=0.42, $F_{1,21}$=4.49, p=0.046 | W/L=-0.00406+0.00848*L r=0.57, $F_{1,21}$=10.30, p=0.004 | PPL=31.696+0.00821* depth r=0.36, $F_{1,21}$=3.25, p=0.086 | PPL=44.067-0.183*L r=0.16, $F_{1,21}$=0.54, p=0.471 |

Appendix 2-C. Intra-colony variations in polyp metrics of Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata in LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland and LB: Labrador. Data provided for L: lower section, M: middle section and U: upper section of the rachis

| | Species | Regions | Slope homogeneity section×log10(L) | ANOVA/ANCOVA - section |
|---|---|---|---|---|
| Polyp diameter | A. grandiflorum | LC | | $F_{2,417}$=38.46, p<0.001; L<M<U |
| | | GB | $F_{2,645}$=1.84, p=0.16 | $F_{2,647}$=213.48, p<0.001; L<M<U |
| | | FC | $F_{2,204}$=1.07, p=0.347 | $F_{2,206}$=56.02, p<0.001; L<M=U |
| | | LB | $F_{2,324}$=0.66, p=0.52 | $F_{2,326}$=33.58, p<0.001; L<M<U |
| | H. finmarchica | LC | | $F_{2,147}$=46.076, p<0.001; L<M=U |
| | | GB | | H=183.97, df=2, p<0.001; L<M<U |
| | | FC | | H=90.71, df=2, p<0.001; L<M<U |
| | | LB | $F_{2,359}$=10.30, p=0.358 | $F_{2,361}$=62.51, p<0.001; L<M<U |
| | P. aculeata | GB | | H=20.81, df=2, p<0.001; L=U<M |
| | | FC | | $F_{2,327}$=9.23, p<0.001; L<M=U |
| | | NNL | | $F_{2,685}$=26.72, p<0.001; L<M=U |
| Polyp density | A. grandiflorum | LC | | H=41.33, df=2, p<0.001; L<M<U |
| | | GB | | H=25.35, df=2, p<0.001; L<M=U |
| | | FC | | H=89.73, df=2, p<0.001; L<M<U |
| | | NNL | | H=17.36, df=2, p<0.001; L<M<U |
| | | LB | | H=40.78, df=2, p<0.001; L<M=U |
| | H. finmarchica | LC | | $F_{2,27}$=5.47, p=0.01; L<M=U |
| | | GB | | H=9.31, df=2, p=0.010; L=M, M=U |
| | | FC | $F_{2,97}$=2.24, p=0.112 | $F_{2,99}$=1.23, p=0.296 |
| | | LB | | H=18.30, df=2, p<0.001; L<M=U |
| | P. aculeata | GB | $F_{2,131}$=5.17, p=0.007 | $F_{2,131}$=12.41, p<0.001; L<M<U |
| | | FC | | $F_{2,96}$=25.43, p<0.001; L<M=U |
| | | NNL | $F_{2,195}$=2.26, p=0.107 | $F_{2,197}$=53.05, p<0.001; L<M<U |

Appendix 2-D. Inter-colony variations in polyp metrics (linear regression or spearman correlation) in Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata sampled in LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland and LB: Labrador

| | | Polyp diameter | | Polyp density | |
|---|---|---|---|---|---|
| | Regions | Colony length | Depth | Colony length | Depth |
| A. grandiflorum | LC | log10(Ø)=-0.0249+0.033×log10(L) r=0.02, $F_{1,138}$=0.08, p=0.777 | $r_s$=-0.16, p=0.065 | $r_s$=0.01, p=0.935 | log10(d)=1.446-0.00077×depth r=0.22, $F_{1,50}$=2.49, p=0.121 |
| | GB | log10(Ø)=-0.43+0.298×log10(L) r=0.36, $F_{1,216}$=31.97, p<0.001 | $r_s$=-0.36, p<0.001 | d=6.841+0.103×L r=0.44, $F_{1,30}$=7.10, p=0.012 | d=18.289-0.0067×depth r=0.59, $F_{1,30}$=15.91, p<0.001 |
| | FC | log10(Ø)=-0.801+0.531×log10(L) r=0.69, $F_{1,68}$=61.06, p<0.001 | $r_s$=-0.04, p=0.768 | d=8.259+0.0264×L r=0.14, $F_{1,60}$=1.21, p=0.276 | $r_s$=-0.30, p=0.011 |
| | NNL | | | d=8.923+0.056×L r=0.22, $F_{1,16}$=0.821, p=0.378 | $r_s$=-0.02, p=0.927 |
| | LB | log10(Ø)=-0.325+0.229×log10(L) r=0.30, $F_{1,108}$=10.57, p=0.002 | $r_s$=-0.05, p=0.583 | d=10.41+0.122×L r=0.30, $F_{1,24}$=2.38, p=0.136 | log10(d)=1.242-0.000116×depth r=0.27, $F_{1,24}$=1.94, p=0.176 |
| H. finmarchica | LC | log10(Ø)=-0.287+0.134×log10(L) r=0.15, $F_{1,48}$=1.13, p=0.293 | | d=20.633+0.0215×L r=0.08, $F_{1,8}$=0.05, p=0.828 | |
| | GB | log10(Ø)=-0.0557+0.0349×log10(L) r=0.05, $F_{1,228}$=0.52, p=0.473 | log10(Ø)=0.0587-0.0000694×depth r=0.11, $F_{1,228}$=2.79, p=0.096 | d=14.089+0.051×L r=0.21, $F_{1,38=1.74}$, p=0.195 | d=23.936-0.00743×depth r=0.21, $F_{1,39}$=1.73, p=0.197 |
| | FC | log10(Ø)=-0.677+0.359×log10(L) r=0.47, $F_{1,178}$=50.34, p<0.001 | log10(Ø)=-0.239+0.000179×depth r=0.31, $F_{1,164}$=17.13, p<0.001 | d=6.071+0.117×L r=0.68, $F_{1,33}$=27.98, p<0.001 | d=22.133-0.00891×depth r=0.56, $F_{1,30}$=13.51, p<0.001 |
| | LB | log10(Ø)=-0.428+0.234×log10(L) r=0.47, $F_{1,115}$=32.24, p<0.001 | log10(Ø)=-0.262+0.000368×depth r=0.50, $F_{1,115}$=37.64, p<000.1 | d=19.567+0.000568×L r=0.01, $F_{1,20}$=0.00007, p=0.993 | $r_s$=-0.24, p=0.280 |
| P. aculeata | GB | Ø=0.789-0.000611×L r=0.02, $F_{1,158}$=0.07, p=0.796 | $r_s$=-0.20, p=0.010 | d=6.493+0.143×L r=0.47, $F_{1,42}$=21.46, p<0.001 | $r_s$=-0.35, p=0.014 |
| | FC | Ø=0.941-0.00815×L r=0.13, $F_{1,108}$=1.93, p=0.167 | Ø=0.87-0.000109×depth r=0.13, $F_{1,108}$=1.902, p=0.171 | d=6.22+0.118×L r=0.19, $F_{1,31}$=1.19, p=0.284 | d=10.049-0.00215×depth r=0.26, $F_{1,31}$=2.25, p=0.144 |
| | NNL | $r_s$=0.19, p=0.005 | Ø=1.057-0.000265×depth r=0.26, $F_{1,228}$=16.55, p<0.001 | d=3.105+0.325×L r=0.72, $F_{1,61}$=65.71, p<0.001 | d=13.014-0.00468×depth r=0.53 $F_{1,61}$=23.87, p<0.001 |

Appendix 2-E. Comparison of short sclerite length and stoutness of sclerites from the (P) peduncle and the (T) tentacles of Halipteris finmarchica sampled in LC: Laurentian Channel, GB: Grand Banks and LB: Labrador

| | Regions | Short sclerites |
|---|---|---|
| Sclerite length | LC | U=7910.8, p<0.001; P<T |
| | GB | U=1199.5, p<0.001; P<T |
| | LB | U=8288.5, p<0.001; P<T |
| Sclerite stoutness | LC | U=12634.0, p<0.001; P<T |
| | GB | U=21081.0, p<0.001; P<T |
| | LB | U=12262.0, p<0.001; P<T |

Appendix 2-F. Comparison of short sclerite length and stoutness of sclerites from the (P) peduncle and the (T) tentacles of Pennatula aculeata sampled in LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap and NNL: North Newfoundland

| | Regions | Short sclerites |
|---|---|---|
| Sclerite lengths | LC | U=21802.5, p=0.176 |
| | GB | U=46694.5, p<0.001 – P>T |
| | FC | U=9451.0, p=0.553 |
| | NNL | U=21489.0, p<0.001 – P<T |
| Sclerite stoutness | LC | U=991.0, p<0.001 – P<T |
| | GB | U=15298.0, p<0.001 – P<T |
| | FC | U=1639.0, p<0.001 – P<T |
| | NNL | U=167.0, p<0.001 – P<T |

Appendix 2-G. Comparison of sclerite abundance and concentration among colony sections of Pennatula aculeata sampled in GB: Grand Banks and NNL: North Newfoundland

| | Regions | P. aculeata |
|---|---|---|
| Abundance | GB | H=16.68, df=3, p<0.001 |
| | NNL | $F_{3,32}$=3.14, p=0.039 |
| Concentration | GB | $F_{3,32}$=34.55, p<0.001 |
| | NNL | $F_{3,32}$=36.00, p<0.001 |

Appendix 2-H. Influence of (CL) colony length and depth on sclerite metrics, sclerite length: scl L, stoutness, abundance: Ab, and concentration: [scl], in Halipteris finmarchica sampled in the Grand Banks region

| Type | Metric | Variable | Peduncle | Rachis |
|---|---|---|---|---|
| Short sclerites | Sclerite length | Colony length | $r_s$=-0.24, p<0.001 | scl L=0.164+0.0000139×CL<br>r=0.11, $F_{1,85}$=1.09, p=0.301 |
| | | Depth | scl L=-0.892+0.0000257×depth<br>r=0.04, $F_{1,821}$=1.23, p=0.268 | scl L=0.156+0.000032×depth<br>r=0.08, $F_{1,85}$=0.56, p=0.455 |
| | Stoutness | Colony length | Log10(L/W)=0.786-0.136×log10(L)<br>r=0.28, $F_{1,816}$=67.40, p<0.001 | $r_s$=-0.135, p=0.203 |
| | | Depth | $r_s$=-0.19, p<0 .001 | L/W=3.706+0.000623×depth<br>r=0.10, $F_{1,86}$=0.78, p=0.381 |
| Column sclerites | Sclerite length | Colony length | | scl L=0.92+0.00497×L<br>r=0.41, $F_{1,206}$=42.80, p<0.001 |
| | | Depth | | scl L=0.648+0.000103×depth<br>r=0.40, $F_{1,206}$=39.81, p<0.001 |
| | Stoutness | Colony length | | $r_s$=0.25, p<0.001 |
| | | Depth | | $r_s$=0.26, p<0.001 |
| All sclerite | Abundance | Colony length | Log10(Ab)=5.473-1.951×log10(CL)<br>r=0.49, $F_{1,11}$=3.38, p=0.093 | Ab=84.695-0.348×CL<br>r=0.27, $F_{1,12}$=0.91, p=0.357 |
| | | Depth | $r_s$=0.19, p=0.516 | Ab=84.999-0.0408×depth<br>r=0.16, $F_{1,12}$=0.31, p=0.589 |
| | Concentration | Colony length | [scl]=5.484-0.0464×CL<br>r=0.55, $F_{1,12}$=5.08, p=0.044 | [scl]=13.84-0.0277×CL<br>r=0.15, $F_{1,12}$=0.28, p=0.605 |
| | | Depth | $r_s$=0.40, p=0 .148 | [scl]=17.581-0.00873×depth<br>r=0.24, $F_{1,12}$=0.75, p=0.494 |

Appendix 2-I. Influence of (CL) colony length and depth on sclerite metrics (sclerite length: scl L and stoutness) in Pennatula aculeata sampled in the GB: Grand Banks and NNL: North Newfoundland regions

| | | Sclerites | Regions | Peduncle | Rachis |
|---|---|---|---|---|---|
| Sclerite length | vs. Colony length | Short | GB | $r_s$=0.04, p=0.429 | $r_s$=0.01, p=0.836 |
| | | | NNL | $r_s$=0.15, p=0.098 | $r_s$=-0.17, p=0.013 |
| | | dorsal track | GB | | $r_s$=0.10, p=0.131 |
| | | | NNL | | $r_s$=0.07, p=0.323 |
| | | Leaf | GB | | $r_s$=0.19, p<0.001 |
| | | | NNL | | log10(scl L)=0.0122-0.14×log10(CL) r=0.14, $F_{1,358}$=7.37, p=0.007 |
| | vs. Depth | Short | GB | $r_s$=-0.29, p<0.001 | $r_s$=-0.17, p<0.001 |
| | | | NNL | $r_s$=-0.21, p<0.001 | $r_s$=-0.20, p=0.005 |
| | | dorsal track | GB | | $r_s$=0.01, p=0.899 |
| | | | NNL | | $r_s$=0.04, p=0.584 |
| | | Leaf | GB | | $r_s$=-0.02, p<0.001 |
| | | | NNL | | log10(scl L)=0.0562-0.000197×depth r=0.29, $F_{1,341}$=31.87, p<0.001 |
| Sclerite stoutness | vs. Colony length | Short | GB | log10(L/W)=-0.774+0.0343×log10(CL) r=0.04, $F_{1,410}$=0.56, p=0.455 | $r_s$=-0.21, p<0.001 |
| | | | NNL | $r_s$=0.23, p<0.001 | $r_s$=-0.33, p<0.001 |
| | | dorsal track | GB | | $r_s$=-0.04, p=0.521 |
| | | | NNL | | $r_s$=-0.06, p=0.463 |
| | | Leaf | GB | | $r_s$=-0.07, p=0.204 |
| | | | NNL | | $r_s$=-0.15, p=0.006 |
| | vs. Depth | Short | GB | $r_s$=-0.38, p<0.001 | $r_s$=0.29, p<0.001 |
| | | | NNL | $r_s$=-0.08, p=0.096 | $r_s$=0.23, p<0.001 |
| | | dorsal track | GB | | $r_s$=-0.04, p=0.548 |
| | | | NNL | | $r_s$=0.21, p=0.004 |
| | | Leaf | GB | | $r_s$=0.04, p=0.446 |
| | | | NNL | | $r_s$=-0.09, p=0.075 |

Appendix 2-J. Influence of depth and (CL) colony length on (Ab) abundance and ([scl]) concentration of sclerites in Pennatula aculeata sampled in GB: Grand Banks and NNL: North Newfoundland

| | Regions | Peduncle | Rachis |
|---|---|---|---|
| Abundance vs colony length | GB | Ab=5749.896-135.916×CL<br>r=0.29, $F_{1,8}$=0.63, p=0.453 | Ab=7943.883+42.947×CL<br>r=0.03, $F_{1,8}$=0.01, p=0.936 |
| | NNL | Ab=7590.562-354.411×CL<br>r=0.43, $F_{1,8}$=1.62, p=0.243 | Ab=7164.087-163.769×CL<br>r=0.31, $F_{1,8}$=0.79, p=0.404 |
| Abundcance vs depth | GB | Ab=2965.66+1.292×depth<br>r=0.17, $F_{1,8}$=0.21, p=0.663 | Ab=12374.041-9.455×depth<br>r=0.43, $F_{1,8}$=1.59, p=0.248 |
| | NNL | $r_s$=0.35, p=0.331 | Ab=4925.387+0.247×depth<br>r=0.03, $F_{1,8}$=0.006, p=0.941 |
| Concentration vs colony length | GB | $r_s$=-0.52, p=0.160 | [scl]=68.815-0.369×CL<br>r=0.24, $F_{1,8}$=0.43, p=0.532 |
| | NNL | [scl]=43.477-1.365×CL<br>r=0.31, $F_{1,8}$=0.76, p=0.411 | [scl]=86.309-1.73×CL<br>r=0.53, $F_{1,8}$=2.77, p=0.140 |
| Concentration vs depth | GB | [scl]=39.938-0.00952×depth<br>r=0.26, $F_{1,8}$=0.52, p=0.494 | [scl]=67.005-0.0111×depth<br>r=0.45, $F_{1,8}$=1.79, p=0.223 |
| | NNL | [scl]=32.728-0.00593×depth<br>r=0.08, $F_{1,8}$=0.05, p=0.834 | [scl]=75.308-0.0102×depth<br>r=0.18, $F_{1,8}$=0.26, p=0.629 |

# Chapter 3 : Seasonality in reproduction of the deep-water pennatulacean coral Anthoptilum grandiflorum

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## Abstract

The deep-sea pennatulacean coral Anthoptilum grandiflorum exhibits a cosmopolitan distribution and was recently determined to serve as habitat for other invertebrates and fish larvae in the Northwest Atlantic. Colonies collected at bathyal depths between 2006 and 2010 in eastern Canada were analyzed to determine their fecundity and characterise spatial and temporal trends in their reproductive cycle. Anthoptilum grandiflorum is a gonochoric broadcast spawner with a sex ratio that does not differ significantly from equality (although one hermaphrodite colony was observed). In male colonies, all the spermatocysts synthesized become mature over the annual cycle, while only ~21% of the initial production of oocytes reaches maturity in female colonies. Female potential fecundity based on mature oocytes just before spawning was on average 13 oocytes polyp$^{-1}$; male potential fecundity was 48 spermatocysts polyp$^{-1}$. The spawning period of A. grandiflorum differs among geographic regions, from April (in southern Newfoundland) to July (in Labrador), closely following regional spring phytoplankton blooms after accounting for the deposition of planktic detritus. Release of oocytes by a live colony held in the laboratory was recorded in April 2011, coincident with field data for similar latitudes. Seawater temperatures at the time of spawning were around 3.6-4.8ºC in all regions and in the laboratory. Early stages of gametogenesis were detected in colonies collected shortly after the spawning season and early and late growth stages occurred successively until December. Mature colonies were observed between April and July (depending on latitude). The diameter of mature oocytes (~1100 µm maximum diameter) is consistent with lecithotrophic larval development.

# 1. Introduction

Deep-water corals form important biogenic habitats and may host several species of invertebrates and fishes (Buhl-Mortensen and Mortensen 2004; Roberts et al. 2006; Baillon et al. 2012, Chapter 6). These biogenic habitats are often referred to as biodiversity hotspots, with a functional role comparable to that of tropical coral reefs (Buhl-Mortensen et al. 2010). Because of their slow growth (Sherwood and Edinger 2009), deep-sea corals are highly vulnerable to disturbances such as bottom trawling (Roberts 2002). Understanding their reproductive biology is important in our efforts to assess their resilience to these disturbances in the design of conservation plans. Reproductive studies in deep-sea corals initially centered on scleractinians (Waller et al. 2002; Burgess and Babcock 2005; Waller and Tyler 2005; Flint et al. 2007; Mercier et al. 2011a), but interest in octocorals has recently been amplified by their putative importance as essential habitat (Edinger et al. 2007; Baillon et al. 2012, Chapter 6), leading to several studies on their reproduction (reviewed by Watling et al. 2011).

A synthesis of octocoral reproduction (Kahng et al. 2011) across depths and habitats reveals a predominance of gonochorism, roughly equal occurrences of broadcast (free) spawners and brooders, and no clear pattern regarding spawning periodicity. Some species exhibit a seasonal/periodic cycle and others do not. When considering only deep-sea octocorals, two dominant strategies emerge: broadcast spawning and internal brooding (Watling et al. 2011). Aperiodic or indeterminate spawning was initially presumed to be most common in the deep sea (Gage and Tyler 1992), although the

occurrence of seasonal cycles has also been demonstrated (Orejas et al. 2007; Mercier and Hamel 2011).

Pennatulacean octocorals, commonly referred to as sea pens, are present in oceans worldwide, from the intertidal zone to the abyssal plain (Williams 2011) and have been identified as vulnerable species (NAFO 2008; Murillo et al. 2011). Sea pens are more abundant in the deep sea than in shallow waters (Daly et al. 2007; Kahng et al. 2011), but knowledge of their reproduction is mostly focused on shallow-water species (Fautin 2002; Soong 2005). There are at least 60 species of deep-sea corals (16 pennatulaceans) on the continental slope of Newfoundland and Labrador (Gilkinson and Edinger 2009; Murillo et al. 2011, Wareham unpubl. data). Sea pens can cover extensive areas in the deep sea, with large fields of Pennatula spp. being reported (Baker et al. 2012), suggesting that these communities, which occur on mud or sand flats, could provide an important structural habitat for other organisms (Tissot et al. 2006).

The reproductive biology of sea pens presents both similarities and differences with the general patterns reported in other octocorals. Of the species studied so far, most are gonochoric. The majority are broadcast spawners (12 species), with one putative internal brooder (Umbellula encrinus), which was questioned by Tyler et al. (1995) who suggested that the embryo observed in U. encrinus was an endoparasite. The reproductive patterns of shallow-water sea pens generally follow an annual cycle (Chia and Crawford 1973; Edwards and Moore 2008, 2009), whereas two of five deep-sea species studied to date (Anthoptilum murrayi and Pennatula aculeata) were determined to exhibit a "continuous" cycle (Eckelbarger et al. 1998; Pires et al. 2009) and one displayed a "non-seasonal" cycle (Kophobelemnon stelliferum, Rice et al. 1992).

Here we investigated the reproductive biology of Anthoptilum grandiflorum, which is one of the most common sea pens in the Northwest Atlantic (Wareham and Edinger 2007). This species is considered cosmopolitan, with confirmed occurrences in the North and South Atlantic, North and South Pacific, Indian and Antarctic Oceans (Williams 2011) from 100 to >2000 m (Baker et al. 2012). Recent findings have highlighted the role of A. grandiflorum as a nursery habitat for commercially harvested redfish (Sebastes spp.) in the deep waters of the Northwest Atlantic (Baillon et al. 2012, Chapter 6) stressing the importance of gaining knowledge on its reproductive biology to develop appropriate fish habitat management and conservation plans. Our major aims were to determine: (1) its fecundity and mode of reproduction; (2) patterns in its reproductive cycle and spawning periodicity; (3) the influence of depth, region and certain environmental factors (temperature, concentration of chlorophyll a) on its reproductive cycle and fecundity.

## 2. Material and methods

### 2.1 Sampling and handling

Samples of Anthoptilum grandiflorum were obtained as by-catch from annual research surveys (Multispecies Surveys and Northern Shrimp Research Surveys), and the At-Sea Observer Program, along the continental slope of eastern Canada (Fig. 3-1) which were all led by Fisheries and Oceans Canada (DFO). Anthoptilum grandiflorum colonies were collected in various months during these surveys in 2006 and 2007 and limited to the months of April and May in 2009-2010 (Appendix 3-B). The surveys followed a stratified random sampling design using a Campellen 1800 trawl with rockhopper footgear, towed

for 15 min at 3 knots with an average tow length ~1.4 km (gear opened and closed at depth). A total of 174 colonies of A. grandiflorum were sampled at 76 sites between 176 and 1347 m depth from the Laurentian Channel to Northern Labrador (Fig. 3-1, Appendix 3-A and 3-B) and frozen at -20°C upon collection. On a subset of surveys (November 2006 from FC), colonies were preserved in 4% formaldehyde. A dozen were kept alive on the ship in December 2010 and brought back to the Ocean Sciences Centre.

Live colonies were maintained in the laboratory from December 2010 to June 2011. They were kept in two dark 1-$m^3$ tanks provided with running unfiltered seawater (~1.5 l $min^{-1}$) at ambient temperature that fluctuated seasonally between -1 and 9°C according to a temperature-light logger HOBO Pendant (UA-002-64). This range is consistent with seasonal fluctuations in this area of the Canadian coast at depths down to 600–800 m (Stein 2007). Substrate was composed of ~15 cm of mud or fine sand to allow the anchorage of the colonies.

Primary production was used as an estimation of planktic detritus reaching the deep-sea floor, as shown by Billett et al. (1983). Maps of the concentration of chlorophyll a ([chl a]) at the sea surface in the regions under study were obtained from the Bedford Institute of Oceanography (website: http://www.bio.gc.ca/science/newtech-technouvelles/sensing-teledetection/composites-eng.php). Temperature data were obtained from Conductivity Temperature Depth (CTD) profiles collected at each site during sampling.

## 2.2 Sample processing

The length of each colony was measured from the base of the peduncle to the tip of the rachis. Three samples of tissue with polyps (~1−2 cm segment along the central axis) were collected in three different sections of each colony, coined lower, middle and upper rachis sections, similar to previous studies on sea pens (Soong 2005; Pires et al. 2009). These samples were preserved in 100% ethanol. Three polyps in each of these sections were isolated by micro-dissection under a stereomicroscope. They were opened longitudinally from the mouth to the base of the polyp (including the base of the tentacles when oocytes/spermatocysts were detected) and all oocytes/spermatocysts were carefully removed. The number of oocytes or spermatocysts per polyp, their Feret diameter, as well as their location in the polyp were established to determine size distributions, fecundity, gametogenic stage and maturity stage index as described in the following paragraphs. Complementary data were obtained from histological sections of polyps sub-sampled on three colonies preserved in 4% formaldehyde. These samples were prepared using standard histology protocols (Baillon et al. 2011). Samples were dehydrated in an ethanol series (70−100%), embedded in paraffin, sectioned (6 to 10 μm) and stained with haematoxylin and eosin. They were examined under a light microscope (Nikon Eclipse 80i) coupled to a digital camera (Nikon DXM1200F) and analyzed using the imaging software Simple PCI (v. 6.0).

## 2.3 Fecundity

Fecundity measured during oocyte and spermatocyst development (potential fecundity) can differ from true fecundity (realized fecundity) measured at spawning, as well as from actual fecundity which corresponds to the number of hatched larvae

(Ramirez Llodra 2002); only the latter determines the effective capacity of the species to produce offspring. In most studies on deep-sea corals, spawning cannot be observed, and investigators rarely take into consideration the time at which fecundity is measured, possibly leading to overestimations. Being mindful of this, we adapted our methodology and terminology (detailed in Mercier and Hamel 2011) using: potential relative fecundity (PRF) defined as the total number of oocytes/spermatocysts polyp$^{-1}$ irrespective of their maturity stage, and effective relative fecundity (ERF) as the number of mature entities (class IV; see below) polyp$^{-1}$. PRF can be measured in all samples whereas ERF has to be measured immediately before spawning and is a closer proxy for realized/actual fecundity. Comparison of PRF and ERF in mature colonies provides additional information on whether or not all oocytes/spermatocysts attain full maturity in a given reproductive cycle. We also determined effective colony fecundity (ECF = ERF × number of polyps) using an estimation of the number of polyps according to a linear regression between colony length and polyp density in each section (Appendix 3-C).

2.4 Gametogenic stages

Gametogenic development was divided into 5 different stages adapted from Mercier and Hamel (2011), based on oocyte and spermatocyst size structure and location in the polyp, using micro-dissection data. Histology was used to describe the morphology of the oocytes and spermatocysts. Oogenic stages were defined as: Early Growth (EG): large aggregation of pale whitish previtellogenic oocytes in size class I (<50 µm in diameter) at the base of the polyp (Fig. 3-2a, b); Growth (G): appearance of oocytes in size class II (51−300 µm) turning yellow (early vitellogenic oocytes) still found at the base of the polyps; Advanced Growth (AG): dominance of yellow vitellogenic oocytes in size class

III (301−700 μm) filling the gastrovascular cavity (Fig. 3-2c, d); Mature (M): small number of large yellow oocytes in size class IV (701−1100 μm) found generally close to the mouth or at the base of the tentacles (Fig. 3-2e), Post Spawning (PS): generally empty polyp sometimes containing a few large residual oocytes (Fig. 3-2f). Spermatogenic stages were defined as: Early Growth (EG): aggregation of small whitish-beige spermatocysts in size class I (<40 μm in diameter) found at the base of the polyp; lumen of spermatocysts empty with low density of spermatozoa (some flagella visible; Fig. 3-3a); Growth (G): appearance of spermatocysts in size class II (41−280 μm) and increased density of spermatozoa forming a dense layer, lumen empty (Fig. 3-3b); Advanced Growth (AG): presence of spermatocysts in size class III (281−520 μm), lumen of spermatocysts still empty; Mature (M): most spermatocysts in size class IV (521−760 μm), white in color and filling the entire cavity of the polyp, lumen filled with spermatozoa (Fig. 3-3c, d); Post Spawning (PS): presence of translucent spermatocysts 81−140 μm in diameter (containing pockets of residual unspawned spermatozoa, Fig. 3-3e). When at least 10% of oocytes or spermatocysts reached the size typical of a gametogenic stage, the stage was considered attained (e.g. in a mature female at least 10% of oocytes are in class IV).

2.5 Maturity stage index and oocyte and spermatocyst size distribution

The Maturity Stage Index (MSI) represents a quantitative measure of gametogenic maturity on a continuous scale that lends itself to statistical analysis (Doyle et al. 2012). It was determined for male and female colonies, using the equation: $\ln[(\text{PRF} \times \text{colony length}^{-1}) \times \text{total oocyte or spermatocyst volume} \times 0.01 + 1]$. Total oocyte or spermatocyst volume corresponded to the sum of individual oocyte or spermatocyst volumes in the

polyp; the volume was determined as: $\pi \times \text{diameter}^3/6$. For each gametogenic stage a corresponding range of MSI values can be attributed (Appendix 3-D): post-spawning females=7.4 ± 1.0 and males=6.3 ± 3.2, early-growth females=10.0 ± 0.5 and males=9.2 ± 0.5, growth females=13.3 ± 0.3 and males=12.9 ± 0.3, advanced-growth females=14.2 ± 0.2 and males=16.1 ± 0.1, mature females=15.1 ± 0.6 and males=17.7 ± 0.1.

Oocyte and spermatocyst size distributions were determined for each colony in the lower, middle and upper rachis sections based on counts and measures of all oocytes and spermatocysts present in the three polyps sub-sampled.

2.6 Data analysis

Sex ratios were analyzed according to depth (threshold at 800 m, see below) and region (Fig. 3-1). Chi-square analyses were used to determine whether the sex ratio was significantly different from equality (1:1). Changes in relative fecundity (PRF, ERF) and in MSI and oocyte/spermatocyst size distributions were analysed in samples from 2006–2007 according to different parameters: colony length, sampling month, geographic region (Laurentian Channel, Grand Banks, Flemish Cap, North Newfoundland and Labrador; Fig. 3-1) and depth. The latter variable was divided into > and < 800 m because colonies were found to be significantly smaller below this threshold depth (Chapter 1). A one-way ANOVA or t-test was used, after verifying assumptions of normality and homogeneity of variances. Post-hoc pairwise analysis (Student-Newman test) was conducted as appropriate. When an assumption of normality was not met even after transforming the data, Kruskal-Wallis or Mann-Whitney tests were used, followed by Dunn's tests as appropriate.

To assess variations in production of oocytes and spermatocysts with colony length and depth, PRF was used as a proxy. Variation in PRF and MSI with colony length and depth was examined in subsets of samples to avoid the potential influence of month and region. Therefore, for variation with colony length, samples collected in November 2006 in FC, and in April, July and October 2007 in LC, LB and GB, respectively, were used. Variation with depth was examined only in July 2007 in LB and October 2007 in GB. Inter-colony synchrony in gametogenesis was analyzed at all sites with > three females or three males by comparing values of MSI, using the previously described methods, and by comparing the gametogenic stages. For the latter, different levels of synchrony were defined according to Baillon et al. (2011): (1) totally synchronous when only one stage was found, (2) relatively synchronous when two consecutive stages were observed and (3) asynchronous when two non-consecutive stages or three or more consecutive stages were present. Quantitative analysis of synchrony between females and males was not possible due to the significant difference in MSI between corresponding stages in the two sexes (Appendix 3-D). All data are provided as mean ± SE.

## 3. Results

### 3.1 Sexuality and sex ratio

Anthoptilum grandiflorum is a gonochoric broadcast spawner, based on the observation of oocytes release in the laboratory and the absence of embryonic or larval stages in the polyps of colonies sampled year round. Of the 174 colonies analysed here, 81 were confidently identified as female and 72 as male; the others had no visible oocytes or spermatocysts. The sex ratio did not differ significantly from equality at all depths

(<800 m: $\chi^2$=0.39, p=0.530; >800 m: $\chi^2$=0.03, p=0.870) and regions examined (Table 3-1). A single case of hermaphroditism (at the polyp level in a dominantly male colony) was observed in November 2006 (colony from 998 m; Fig. 3-3f). The smallest colonies with visible oocytes or spermatocysts measured 15 cm. However, colonies under 24 cm (n = 13) presented either no oocytes/spermatocysts (n = 7) or fewer and/or smaller oocytes/spermatocysts (n = 6) than longer colonies at corresponding dates. Therefore, analyses of the reproductive cycle were restricted to colonies >24 cm (n = 160 colonies) which were unequivocally determined to produce fully grown (class IV) oocytes/spermatocysts.

3.2 Reproductive cycle

Gametogenesis followed an annual cycle culminating with spawning events spanning from April to July, depending on the geographic region (see below for detailed analysis). For the sake of clarity, only the pattern for 2007 is explained in detail, although the same trend was visible in 2006 (colonies showed the same range of MSI and gametogenic stages in the corresponding months, e.g. a MSI around 13 in October 2006 and 2007 for female colonies). Spawning events were determined based on the occurrence of male and female colonies in the post-spawning gametogenic stage, with partially or completely empty polyps (Fig. 3-2f, 3-3e, 3-4d, h). This assessment was corroborated by the oocyte and spermatocyst size distributions (Fig. 3-4d, h) and a dip in the MSI (Fig. 3-5). Female colonies in early-growth, growth and advanced-growth stages occurred successively from May to December (Fig. 3-2a-d), corresponding with a growing number of small and medium-sized oocytes (Fig. 3-4a, b) and with the increase in MSI between July and October (Fig. 3-5a). Males presented the same occurrence of stages but with the first

presence of mature colonies at the end of November. In both sexes, maturity was reached before the recorded spawning (Fig. 3-2e, 3-3d, e), as oocytes and spermatocysts reached their maximum size between fall (October) and early spring (April; Fig. 3-4c, g), also corroborated by high MSI values in the same interval (Fig. 3-5).

Colonies sampled in LC, GB and LB (where more samples were collected during April-July) provided a finer analysis of the MSI and gametogenic stages during the spawning period (April to July) and revealed regional trends that were similar in males and females (Fig. 3-6). In the Laurentian Channel (LC), a decrease in MSI, consistent with a spawning event, was visible from mid April to the beginning of May and was especially pronounced in females (Fig. 3-6a). The same pattern was observed in the Grand Banks (GB), but in mid June (Fig. 3-6b). The few colonies analysed in 2009 and 2010 during the months of April and May confirmed the spawning events at the beginning of spring in LC and GB through the successive presence of mature and post-spawning colonies. Moreover, on 21 April 2011 spawning was observed in a live colony kept in the laboratory (in St. John's, south-eastern Newfoundland) coincident with the spawning period determined from field samples in the two southern regions. Forty-three buoyant oocytes were released; they were similar in size to the mature oocytes measured in preserved colonies (1278 ± 22 µm). The MSI of colonies collected in Labrador (LB) showed low values in mid July and an increasing trend towards the end of the month, consistent with the initiation of gametogenesis following a putative spawning event (Fig. 3-6c). Samples were a mix of post-spawning colonies and colonies in the early-growth phase of gametogenesis, supporting this assumption. No clear reproductive pattern was

observed in colonies from FC and NNL regions due to the small number or absence of colonies sampled during the spring/summer months (April-July).

While the number of spermatocysts remained relatively constant as they grew in size throughout spermatogenesis, the number of oocytes decreased as oogenesis progressed with 21.5 ± 0.6% of the originally synthesized oocytes becoming mature (Fig. 3-4). This led to the transient appearance of two size-based cohorts of oocytes in mid-cycle (Fig. 3-4b). The smaller oocytes ultimately disappeared and only one cohort of large mature oocytes remained at the culmination of the cycle (Fig. 3-4c). Sex-specific patterns are further detailed in the fecundity section.

Analyses of inter-colony gametogenic synchrony were conducted using colonies of the same sex collected simultaneously (Fig. 3-7). In females, 67% of those samples were composed of colonies with significantly different MSI (Fig. 3-7); based on gametogenic stages, one third of the samples fell into one of three categories (totally synchronous, relatively asynchronous and asynchronous). In males, MSI values were significantly different in 75% of samplings (Fig. 3-7); half were asynchronous and half were relatively synchronous according to the gametogenic stages. There was no clear denominator (month or region) to explain synchronous versus asynchronous gametogenic development. For colonies collected on a specific month in a given geographic region, no significant correlation between MSI and colony length or sampling depth were found in either sex (Table 3-2), indicating that these factors cannot account for the disparities.

### 3.3 Fecundity

Potential relative fecundity (PRF) increased from the lower to the upper section of the rachis in all colonies examined (lower<middle<upper; data from all regions/depths

pooled: ♀ H = 286.53, df = 2, p > 0.001; ♂ H = 408.06, df = 2, p < 0.001). The lower section in both sexes was always empty of reproductive cells, whereas the middle and upper sections presented similar ranges of oocyte or spermatocyst sizes, except in April in the Laurentian Channel (where regular samples were collected), when the polyps of the middle sections of females showed potential signs of spawning (early April) before those of the upper section (late April). Because of this spatial organization, analyses of PRF, ERF, MSI and oocyte and spermatocyst size distributions were always carried out on polyp samples from the upper section of the rachis.

Measures of PRF taken at different stages of the reproductive cycle confirmed the sex-specific patterns of gametogenesis within A. grandiflorum colonies. In males, PRF (56.6 ± 1.8 spermatocysts polyp$^{-1}$) remained constant throughout the annual cycle (H = 12.72, df = 8, p = 0.122) and was similar to ERF (48.1 ± 3.7 spermatocysts polyp$^{-1}$; U = 104.0, p = 0.068). However, in females, PRF measured in the early-growth stage (oocytes <40µm: 60.6 ± 8.6 oocytes polyp$^{-1}$) differed significantly from PRF measured in mature colonies (U = 392.5, p = 0.007), which was not significantly different from ERF (oocytes >500 µm: 13.0 ± 1.8 oocytes polyp$^{-1}$; U = 138.5, p = 0.464). Thus mature colonies provide the most reliable measure of actual fecundity in females of this species. It was estimated that male colonies produced 18−70 mature spermatocysts polyp$^{-1}$ with a corresponding ECF of 5,500−14,601 spermatocysts colony$^{-1}$ in the smallest and largest colonies, respectively. Female colonies produced 6−31 mature oocytes polyp$^{-1}$, with an ECF of 998−4,331 oocytes colony$^{-1}$.

There was no detectable influence of colony length on the PRF (as a proxy of oocyte or spermatocyst density) in females or males. The only exception occurred in males in

November 2006, where more spermatocysts were found in larger colonies (Table 3-2). There was no influence of depth on PRF in females, whereas males showed a decrease in PRF with increasing depth in October 2007 (Table 3-2), indicating a lower production of spermatocysts in deeper colonies.

3.4 Relationship with chlorophyll and temperature

Surface [chl a] followed similar patterns in 2006 and 2007 relative to the spawning periods established in this study. Concentration of chlorophyll a was low at the beginning of the year (January-March; Appendix 3-Ea) and increased between late March and early April in LC and GB (Appendix 3-Eb) indicating the beginning of the spring bloom. The maximum [chl a] in both regions occurred at the end of April (Appendix 3-Ec) which corresponded to the spawning time in LC and to a few weeks before spawning in GB. Then the [chl a] decreased in the two regions. From May to June, a shift in the highest [chl a] occurred from South (GB) to North (LB) regions (Appendix 3-Ed, e). The putative spawning event in LB occurred a few weeks following the highest [chl a] in this region. A second increase in the [chl a] became visible in October-November (fall bloom) along the continental slope in the LC and GB regions (Appendix 3-Ef), corresponding with a rapid increase in size of the spermatocysts and with vitellogenesis.

Mature and post-spawning colonies were found when bottom temperature was 4.8 ± 0.1°C in LC, 4.5 ± 0.5°C in GB, 3.6 ± 0.1°C in FC and 4.0 ± 0.0°C in LB. Seawater temperature in the laboratory was 3.7 ± 0.1°C during the live spawning event and corresponded to the period when temperature starts to increase from minimum winter values.

## 4. Discussion

Anthoptilum grandiflorum is a gonochoric species, similar to all other known sea pens and, more generally, to most octocorals (Pires et al. 2009; Kahng et al. 2011; Watling et al. 2011). It relies on broadcast spawning and exhibits a relatively equal sex ratio, typical of other known sea pens (Kahng et al. 2011). A single hermaphroditic colony was detected during this study. While the occurrence of hermaphroditism in gonochoric species has been documented in other cold-water and tropical octocorals (Kahng et al. 2011), to our knowledge it is a first for sea pens. Hermaphroditism is considered to be an advantage in low density populations, as is often the case in deep-sea species, by allowing self-fertilization (Ghiselin 1969). This could be advantageous for A. grandiflorum which is usually found individually or in pairs, despite some locally high densities (Kenchington et al. 2011; Baker et al. 2012); however, evidence of self-fertilization in A. grandiflorum and other deep-sea corals has not yet been presented.

The presence of mature oocytes and spermatocysts provided an estimation of the size at first maturity around 24 cm, which corresponds to ~28% of maximum colony length recorded in Chapter 2 for A. grandiflorum off Newfoundland and Labrador. Given the notoriously slow growth of deep-water corals (Sherwood and Edinger 2009), colonies of A. grandiflorum likely take several years to reach this size. The only other data on size at first maturity in sea pens were provided by Edwards and Moore (2008) who estimated it between 5−9 cm axial rod length in Pennatula phosphorea (shallow water, Scotland), which was 35−65% of the longest axial rod measured (14.2 cm, Edwards, pers. comm.).

Mature oocytes (class IV) in A. grandiflorum were 700−1100 μm in diameter (frozen state; 1278 μm for live spawned oocytes), which is comparable to mature oocytes of the congeneric species A. murrayi (490−1200 μm; Pires et al. 2009) but larger than reported in all other species of sea pens studied to date (250−900 μm; see review by Kahng et al. 2011). The large oocyte size of A. grandiflorum together with data from other sea pens (Pires et al. 2009) strongly suggests that most of the known pennatulaceans (shallow and deep waters) exhibit a lecithotrophic, non-feeding, larval development. Other marine invertebrates (echinoderms) with large eggs (>300 μm, assumed to be lecithotrophic) were shown to exhibit the most widespread distributions in the deep sea (Young et al. 1997), presumably because low metabolic demand in cold waters increases the potential for dispersal without feeding (Shilling and Manahan 1994). This adaptation might partially explain the cosmopolitan distribution of A. grandiflorum.

Unlike three other deep-sea pennatulaceans studied to date that were determined to exhibit aperiodic reproduction, i.e. Anthoptilum murrayi (Pires et al. 2009), Pennatula aculeata (Eckelbarger et al. 1998), and Kophobelemnon stelliferum (Rice et al. 1992), evidence gathered here for A. grandiflorum strongly points to an annual cycle of gametogenic development that culminates in a spawning period which shifts geographically, starting earlier in southern regions and later towards the North. Mercier and Hamel (2011) showed a similar pattern in the reproductive cycle of a sympatric deep-sea octocoral, Anthomastus grandiflorus, which spawned in October off Newfoundland and from November to January in Labrador (northern locations), with corresponding shifts in the cycle of oocyte/spermatocyst development. Evidence of discrete spawning periods has so far been gathered for deep-sea corals (octocorals and scleractinians) from

several ocean basins (Orejas et al. 2002; Waller and Tyler 2005; Waller and Baco 2007; Mercier and Hamel 2011); for example the hard coral Lophelia pertusa spawns around January/February in the NE Atlantic (Waller and Tyler 2005) and the octocoral Keratoisis grayi (as K. ornata) spawns at the end of summer in the NW Atlantic (Mercier and Hamel 2011). However, spawning periodicity is not always linked to annual gametogenic cycles; oocytes were suggested to require more than a year to mature (Orejas et al. 2002; Edwards and Moore 2009; Beazley and Kenchington 2012; Brooke and Järnegren 2013).

Environmental factors have been shown to mediate the synchronization and coordination of gametogenesis and spawning in several marine invertebrates, including corals. In tropical and temperate octocorals, reproductive activity has been correlated with temperature, lunar cycles, and resource availability (Ben-David-Zaslow et al. 1999; Gori et al. 2007; Hwang and Song 2007; Ribes et al. 2007). Evidence for the influence of primary productivity (seasonal surface-derived downward flux of phytodetritus and remnants of secondary consumers) and lunar phases on peak planulation events also exists for brooding alcyonacean corals from the deep (Sun et al. 2010; Mercier and Hamel 2011; Mercier et al. 2011b), as well as for echinoderms and crustaceans (Gage 1992; Young 2003). Sedimentation rates, representing the accumulation of organic and inorganic material on the bottom of the tanks, which were measured in our laboratory in 2007, showed a sharp increase in March and maximal values in April and May (Mercier et al. 2011a). It is likely that a similar pattern occurs every year; therefore the spawning event observed in A. grandiflorum kept in the laboratory roughly coincided with maximum deposition of detritic matter. This observation is consistent with other deep-sea

corals, including the scleractinian Flabellum angulare (Mercier et al. 2011a), and the octocoral Gersemia fruticosa (Sun et al. 2011).

How the influx of fresh or detritic plankton influences reproduction is still unclear; it may act as a chemical cue or as a direct source of energy for final gamete maturation. Both hypotheses find support in the literature (Himmelman 1975; Starr et al. 1990; Plourde and Runge 1993; Benitez-Villalobos et al. 2007), and both could be at play here since deep-sea pennatulaceans are known to feed on degraded particulate organic matter (POM) and small invertebrates (Sherwood et al. 2008). The [chl a] at the sea surface places the maximum bloom in late April in LC and GB. This allows an approximation of the timing of phytodetritus and POM influx to the seafloor. Spawning of A. grandiflorum occurred in the days following the bloom in LC, while it occurred later in May/June in GB. The temporal difference between the two regions may be due to the greater sampling depths in GB. The sinking velocity of phytodetritus/POM has been estimated at 100−150 m $day^{-1}$ (Billett et al. 1983; Conte et al. 2001), therefore 3−4 days would be necessary for it to reach the seafloor in LC, and around 10 days in GB. Moreover, the quality of food decreases with depth (Gage 1992) suggesting that a greater quantity of food would be necessary to provide the energy required for final maturation of oocytes/spermatocysts, which could slightly delay spawning. The bloom of primary production occurs even later in May-June in Labrador, which could explain the later putative spawning in this region. In most locations studied, early gametogenesis occurred right after spawning and probably benefited from the energetic input gathered during/following the spring bloom. The fall bloom (October-November) coincided with an increase in the size of spermatocysts and with vitellogenesis. Temperature data in the present study revealed that

spawning occurred at 3.6−4.8°C in the field. Similar temperatures prevailed in the laboratory when the live colony spawned; coinciding with a spring increase in seawater temperature, which is also consistent with previous studies on deep-sea coral reproduction in Newfoundland and Labrador (Mercier and Hamel 2011; Sun et al. 2011). It suggests that spawning in A. grandiflorum could be influenced by the synergistic effects of nutrient input and temperature. More frequent sampling together with measurement of environmental factors in situ will be required to determine the exact spawning time and the respective roles played by phytodetritus/POM and temperature.

Regardless of how it is achieved, coordinated gamete release is crucial in broadcast spawners to insure high fertilization rates, especially in sessile and low-density species, which are common in the deep sea. Limited information is available on abundances of A. grandiflorum (Kenchington et al. 2011; Baker et al. 2012). Colonies of A. grandiflorum collected simultaneously, in various months and regions, were not consistently synchronous in their gametogenic development, similar to observations in the shallow-water sea pen Veretillum cynomorium (Lopes et al. 2012). Some asynchrony in oocyte/spermatocyst maturation of colonies could be adaptive by avoiding a single, short period of gamete release, thereby minimizing the potential impact of temporarily unfavourable conditions (Brooke and Young 2003). These findings may also be explained by the relatively large sampling scale in our study (1.4 km length); i.e. synchronous development at the local (meter) scale may be masked.

A further complexity of reproductive patterns in A. grandiflorum lies in the intra-colony variation. The polyps present in the three sections of the rachis (lower, middle and upper sections) showed significant differences in gametogenic development. The lower

section was always devoid of oocytes/spermatocysts; it also had fewer and smaller polyps than the other sections (Chapter 1). Presumably, new polyps are added to the base of the colony (personal observation in agreement with Soong's observations on Virgularia juncea (2005)). The middle and upper sections presented the same gametogenic stages and oocyte/spermatocyst size distributions (except in April), but the middle section showed a lower PRF than the upper section. Moreover, the middle section of colonies collected in April was generally empty, whereas the upper section still presented mature oocytes. Two hypotheses may explain this situation. First, spawning may start earlier in the middle section of the colony. This could have been overlooked in the field collections, due to low sample sizes in the months of February and March. Second, oogenesis may be initiated in the middle section without yielding fully mature oocytes. Instead, these oocytes may serve as a nutrient reserve for maturation of oocytes in the upper section, or as a general energy source for the whole colony. A gradient of contribution to reproduction along the rachis is plausible, with the polyps becoming progressively more fecund towards the tip. Evidence of increased polyp fecundity with increased polyp size has been found in other coral species (Sakai 1998). Therefore, the higher PRF in the upper polyps may be explained by their larger diameters. Two species, Anthoptilum murrayi (from 1051−1799 m depth, Pires et al. 2009) and Funiculina quadrangularis (from 19−24 m depth, Edwards and Moore 2009), showed the same trend as the present study. A third species, Virgularia juncea (from 0.5−1 m depth) presented a different trend, whereby the first 12% of the lower rachis and last 44% of the upper rachis were infertile (Soong 2005). The infertility of the lower part was explained by the presence of new polyps (as per the present study), whereas the presence of zooxanthellae in the tissue

emphasized the strict feeding role of polyps in the upper colony section (Soong 2005), a situation that is not relevant to azooxanthellate deep-sea corals.

Female colonies of A. grandiflorum consistently underwent a decrease in oocyte numbers as oogenesis progressed. Only ~21.5% of the early oocytes (class I) became mature (class IV) with an average production between ~1000–4000 mature oocytes per reproductive cycle in short (~30 cm) and long (~70 cm) colonies, respectively. Because of this oogenic pattern, PRF varied with time, with the highest PRF found during early-growth stages (July) and the lowest in mature stages (April-May). This strategy remains poorly understood but has been reported before in sea pens and other corals.10% of the oocytes become mature in the sea pen Funiculina quadrangularis (Edwards and Moore 2009) and between 14 and 27% in the sea pen Pennatula phosphorea (Edwards and Moore 2008). The gorgonian coral Keratoisis grayi (as K. ornata), sampled in the GB area at 302–958 m in 2005–2006, showed a maturation of ~15% of the oocytes (Mercier and Hamel 2011). The authors suggested the utilisation of the "supernumerary" oocytes as a source of energy to allow the maturation of fewer oocytes. Other corals show this potential use of supernumerary oocytes as a food source for maturation of the largest oocytes (Santangelo et al. 2003). This oogenic pattern serves to caution against measures of fecundity at indeterminate periods of the reproductive cycle in deep-sea corals, which may lead to an overestimation of fecundity and corresponding resilience.

Comparison of either colony or relative (polyp) fecundity with other sea pen species is not easy due to the different methods used to measure this parameter. For example, Edwards and Moore (2009) and Soong (2005) defined fecundity as the number of oocytes $cm^{-1}$ of tissue, while Duncan (1998) examined the number of oocytes $leaf^{-1}$ and other

studies looked at the number of oocytes polyp$^{-1}$ (Edwards and Moore 2008; Lopes et al. 2012). Some investigators noted that only a certain percentage of the oocytes became mature, but still measured fecundity as the total number of oocytes per polyp/leaf/colony (Edwards and Moore 2008, 2009). Only a few studies measured fecundity in mature females or restricted this measure to mature or vitellogenic oocytes. Two shallow-water sea pens, Ptilosarcus guerneyi (Chia and Crawford 1973) and Pteroides sp. (Duncan 1998), were shown to exhibit large mature oocyte (500−600 μm and 300−520 μm, respectively) and high colony fecundity (200,000 and 36,000 oocytes colony$^{-1}$, respectively). Our study suggests that fecundity is much lower in deep-sea species, presumably due to food limitation, but that oocytes are generally larger, which as stated by Levitan (2006) may favour fertilization success at low colony densities. Fecundity in the deep-sea pennatulacean Umbellula sp. was reportedly ~2000 oocytes colony$^{-1}$, with oocytes reaching 800 μm in diameter (Tyler et al. 1995). This fecundity is comparable to that of A. grandiflorum in our study (1000−4000 oocytes colony$^{-1}$). Interestingly, Umbellula sp. presents a significantly lower number of larger polyps (10 mm) compared to A. grandiflorum (1 mm, Chapter 1). Therefore, higher fecundity at the polyp level in Umbellula sp. can be attributed to the larger polyps that have more space available for oocyte/spermatocyst synthesis. Yet a similar fecundity at the colony level is achieved in A. grandiflorum through a greater number of smaller, less fecund polyps.

The use of by-catch corals has given us the opportunity to analyse a larger sample size and longer time series than most previous studies on deep-sea coral reproduction. It allowed us to determine that the reproductive biology of A. grandiflorum shares the general features of sea pens but also has particular characters such as seasonality

demonstrated here for the first time in a deep-sea pennatulacean. Sex-specific and intra-colony patterns were also found, which stresses the importance of methodological and temporal consistency in assessments of deep-sea coral fecundity, with consequences for assessments of resilience to disturbance. Finally, this study provides key information for the conservation of A. grandiflorum as an ecologically significant deep-sea biogenic habitat, given its use as nursery by fish larvae (Baillon et al. 2012, Chapter 6).

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## Tables

Table 3-1 Sex ratio analyses on colonies of Anthoptilum grandiflorum in the various regions under study

| Region | Females | Males | $\chi^2$ | p |
|---|---|---|---|---|
| Laurentian Channel (LC) | 24 | 17 | 1.20 | 0.274 |
| Grand Banks (GB) | 20 | 18 | 0.11 | 0.746 |
| Flemish Cap (FC) | 16 | 25 | 1.98 | 0.160 |
| North Newfoundland (NNL) | 6 | 2 | 2.00 | 0.157 |
| Labrador (LB) | 15 | 10 | 1.00 | 0.317 |

Table 3-2 Influence of colony length and depth of collection on the potential relative fecundity (PRF) and maturity stage index (MSI) of Anthoptilum grandiflorum (linear regression or spearman correlation); Flemish Cap (FC), Laurentian Channel (LC), Labrador (LB)

| | | MSI | | PRF | |
|---|---|---|---|---|---|
| | | Colony length | Depth | Colony length | Depth |
| Females | November 2006 - FC | $r_s$=0.22, p = 0.295 | -- | $r_s$=-0.17, p = 0.360 | -- |
| | April 2007 - LC | $r_s$=-0.12, p = 0.469 | -- | $r_s$=-0.11, p = 0.467 | -- |
| | July 2007 - LB | $r^2$=0.23, p = 0.287 | $r^2$=0.002, p = 0.992 | $r^2$=0.46, p = 0.015 | $r_s$=-0.24, p = 0.193 |
| | October 2007 - GB | $r_s$=-0.19, p = 0.295 | $r_s$=-0.26, p = 0.155 | $r^2$=0.06, p = 0.737 | $r^2$=0.32, p = 0.050 |
| Males | November 2006 - FC | $r_s$=-0.19, p = 0.295 | -- | $r_s$=0.45, p = 0.002 | -- |
| | April 2007 - LC | $r_s$=-0.27, p = 0.134 | -- | $r_s$=0.05, p = 0.798 | -- |
| | July 2007 - LB | -- | -- | -- | -- |
| | October 2007 - GB | $r_s$=-0.19, p = 0.295 | $r_s$=-0.26, p = 0.155 | $r^2$=0.001, p = 0.943 | $r^2$=-0.24, p = 0.004 |

## Figures

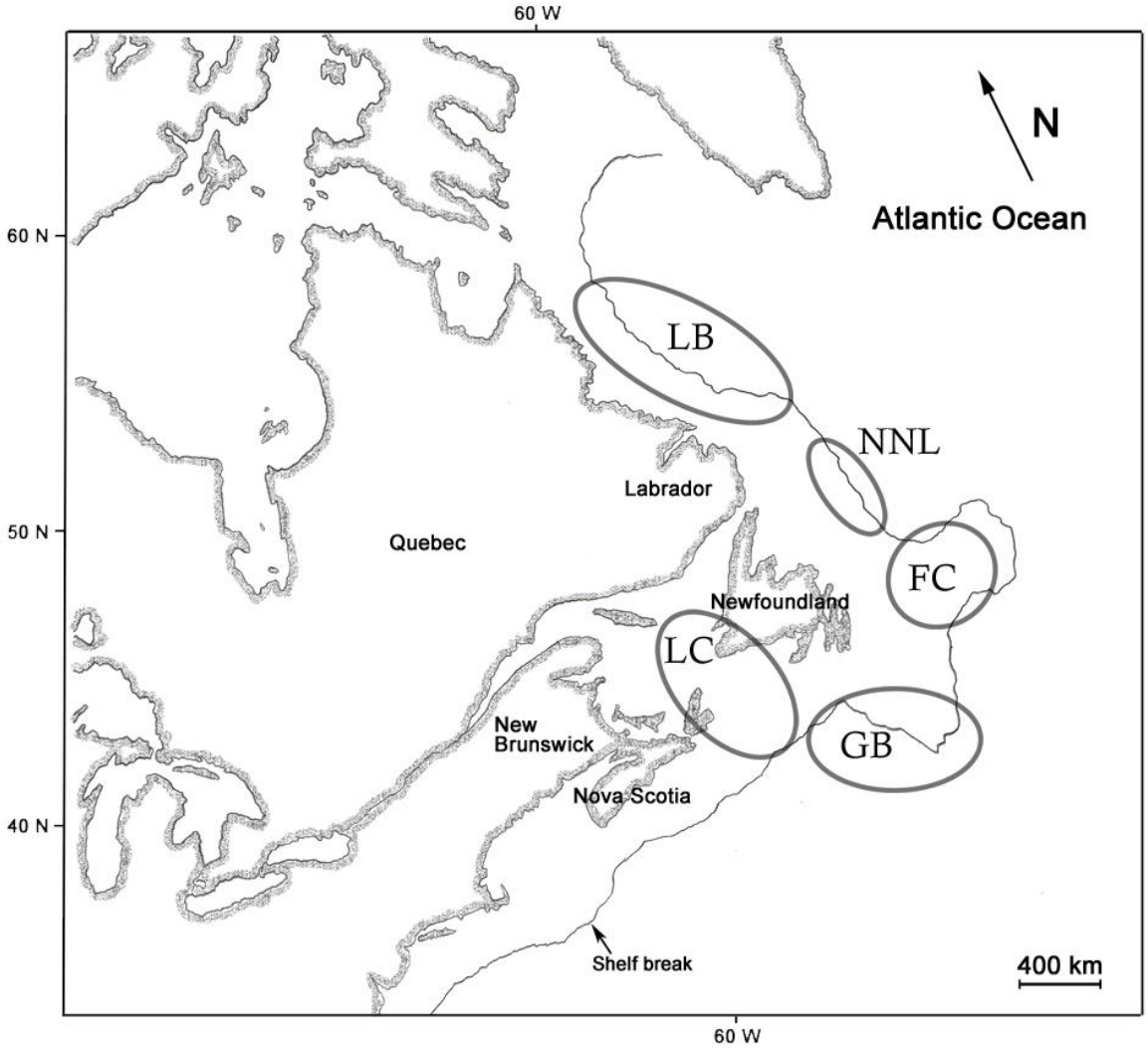



Figure 3-1 Map showing the five regions where colonies of Anthoptilum grandiflorum were collected: Laurentian Channel (LC), Grand Banks (GB), Flemish Cap (FC), North Newfoundland (NNL) and Labrador (LB)

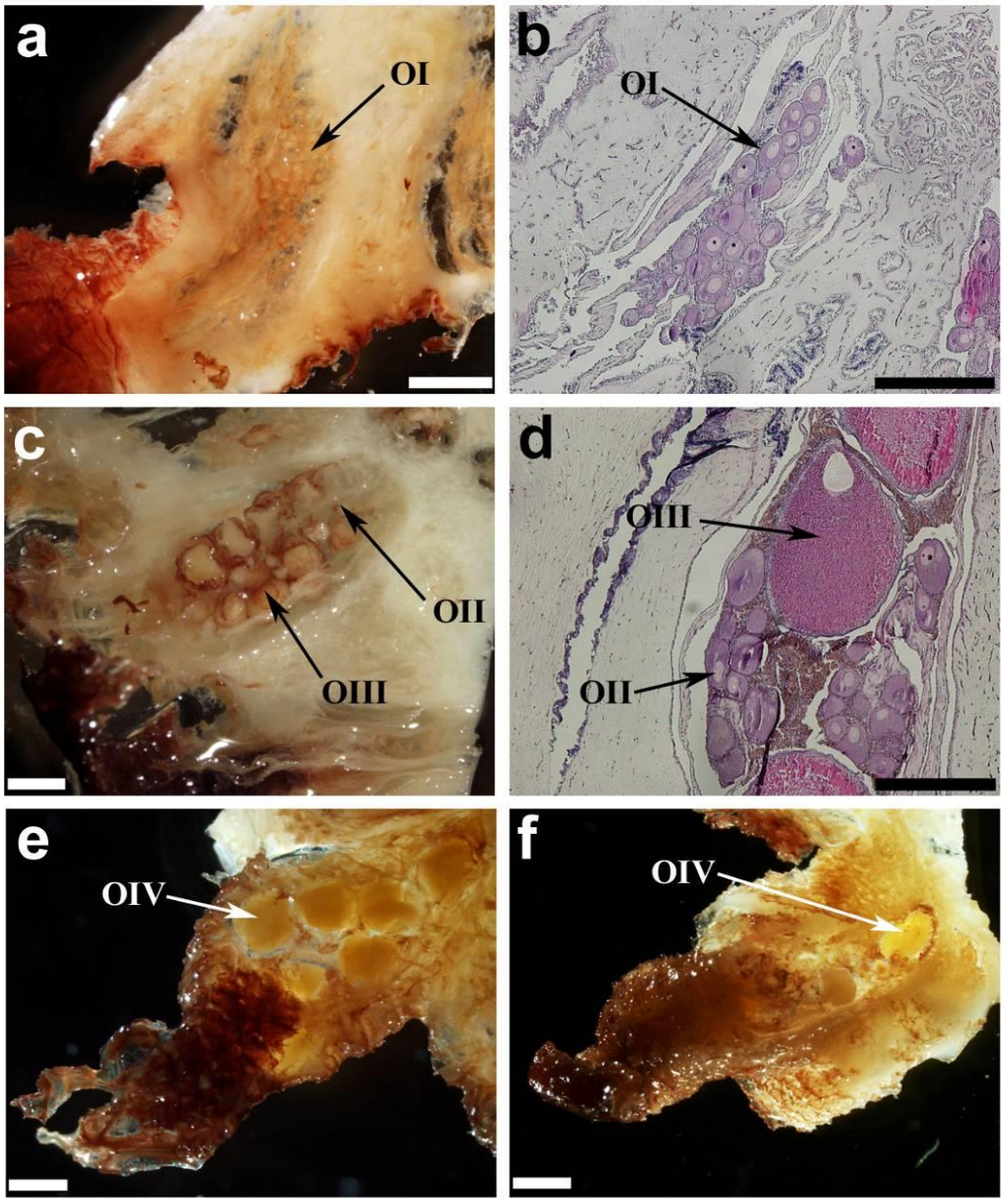



Figure 3-2 Micro-dissection and histology of female colony of Anthoptilum grandiflorum showing the different oogenic stages: a and b Early Growth, c and d Advanced Growth, e Mature, f Post Spawning. Scale bars represent 1 mm in a, b, c and f, 500 µm in d and e. OI: oocyte class I, OII: oocyte class II, OIII: oocyte class III, OIV: oocyte class IV

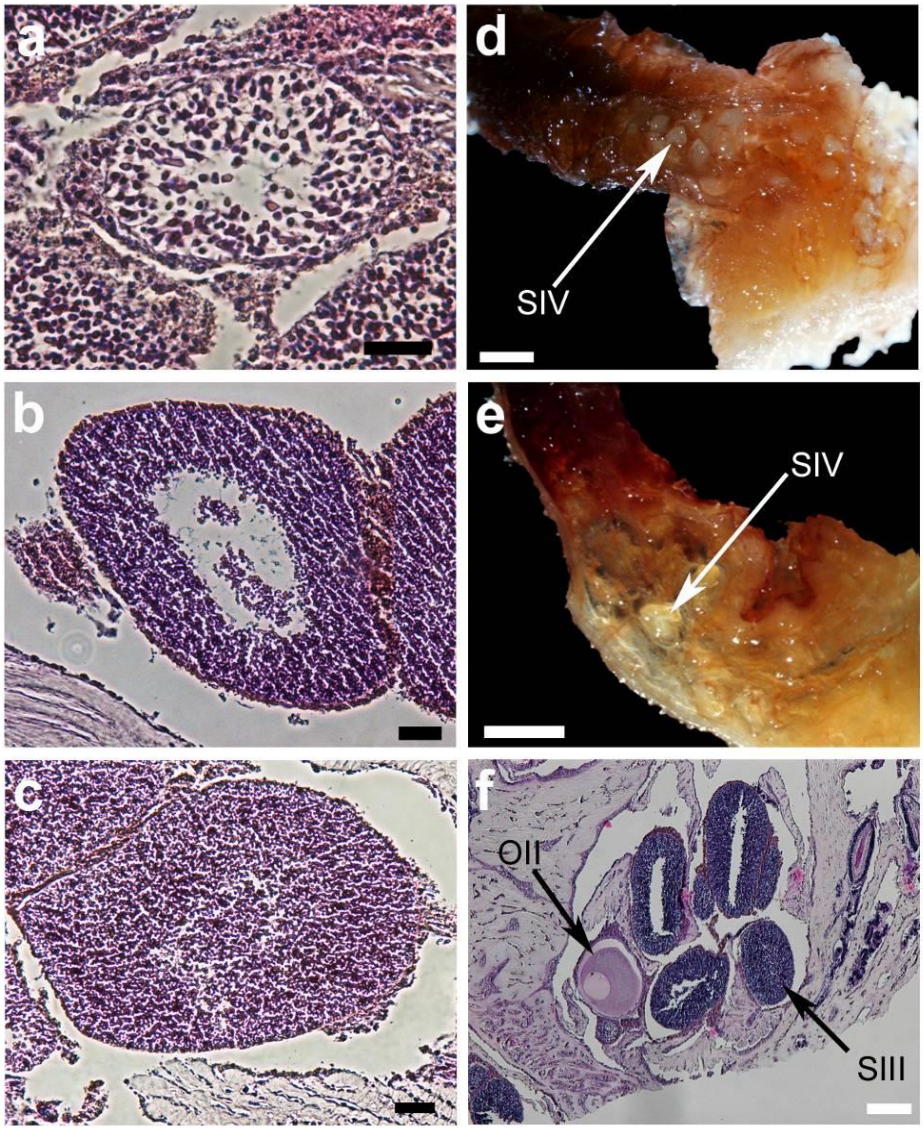
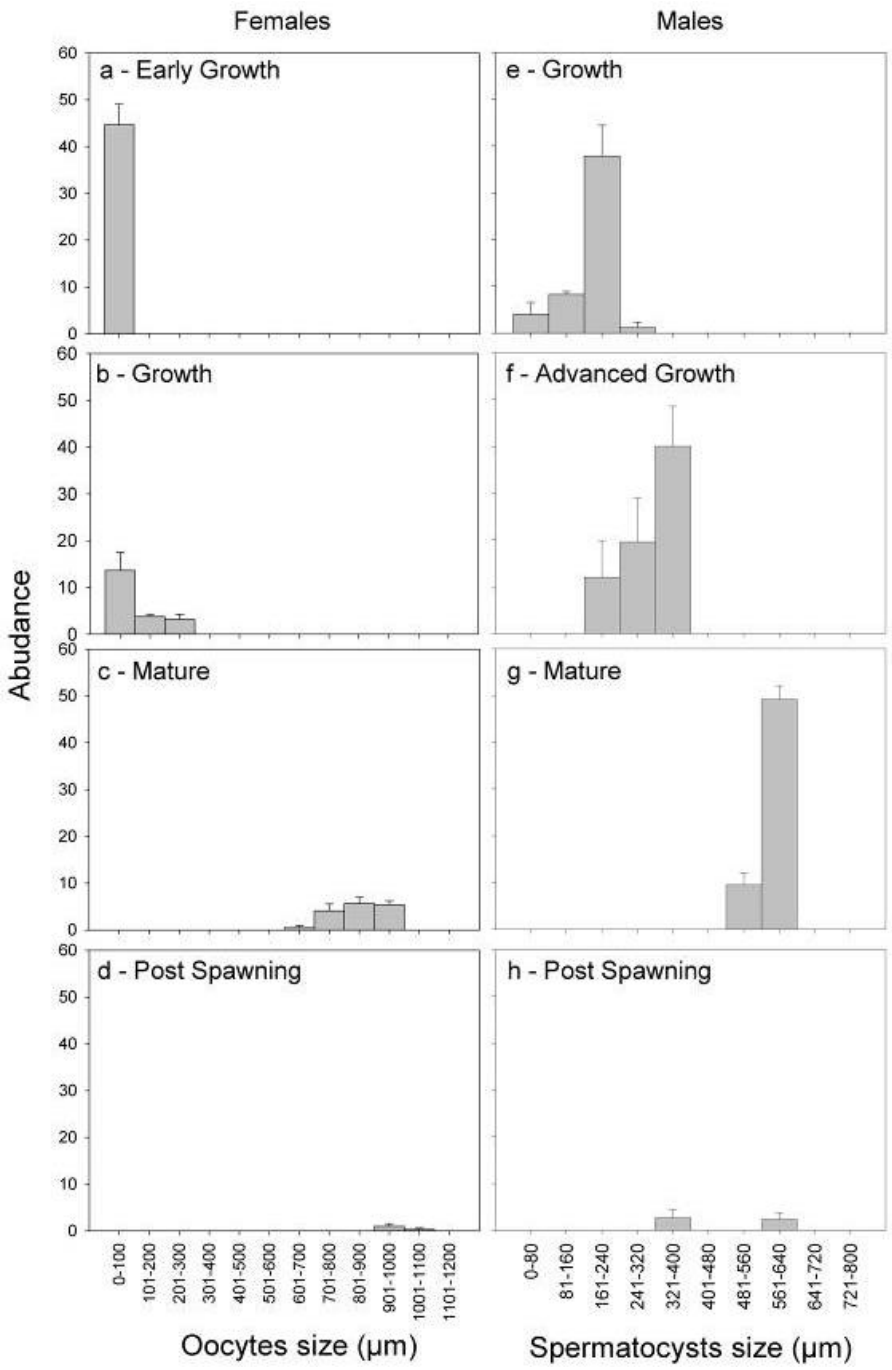



Figure 3-3 Micro-dissection and histology of male colony of Anthoptilum grandiflorum showing spermatocyst size classes and the different spermatogenic stages: a Spermatocyst class I, b Spermatocyst class II, c Spermatocyst class IV, d Mature stage, e Post-Spawning stage. Micrograph in f shows a hermaphroditic polyp. Scale bars represent 30 µm in a, 50 µm in b and c, 1 mm in d and e, 200 µm in f. SIII: spermatocyst class III, SIV: spermatocyst class IV, OII: oocyte class II

Figure 3-4 (Following page) Example of oocyte/spermatocyst size distributions from the upper section of the rachis of Anthoptilum grandiflorum during the major stages of the reproductive cycle (mean ± SE). Each graph corresponds to one characteristic colony representing the stage. Females: a Early Growth in October-November (n = 36−50 oocytes polyp$^{-1}$), b Growth in November-December (n = 12−29 oocytes polyps$^{-1}$), c Mature in April-July (n = 12−21 oocytes polyps$^{-1}$) and d Post Spawning in April-July (n = 0−3 oocytes polyps$^{-1}$). Males: e Growth in October-November (n = 39−60 spermatocysts polyp$^{-1}$), f Advanced Growth in November-December (n = 59−84 spermatocysts polyp$^{-1}$), g Mature in April-July (n = 56−61 spermatocysts polyp$^{-1}$) and h Post Spawning in April-July (n = 0−11 spermatocysts polyp$^{-1}$)


Females
Males
a - Early Growth
e - Growth
b - Growth
f - Advanced Growth
c - Mature
g - Mature
d - Post Spawning
h - Post Spawning
Abudance
0
10
20
30
40
50
60
0-100
101-200
201-300
301-400
401-500
501-600
601-700
701-800
801-900
901-1000
1001-1100
1101-1200
Oocytes size (µm)
0-80
81-160
161-240
241-320
321-400
401-480
481-560
561-640
641-720
721-800
Spermatocysts size (µm)

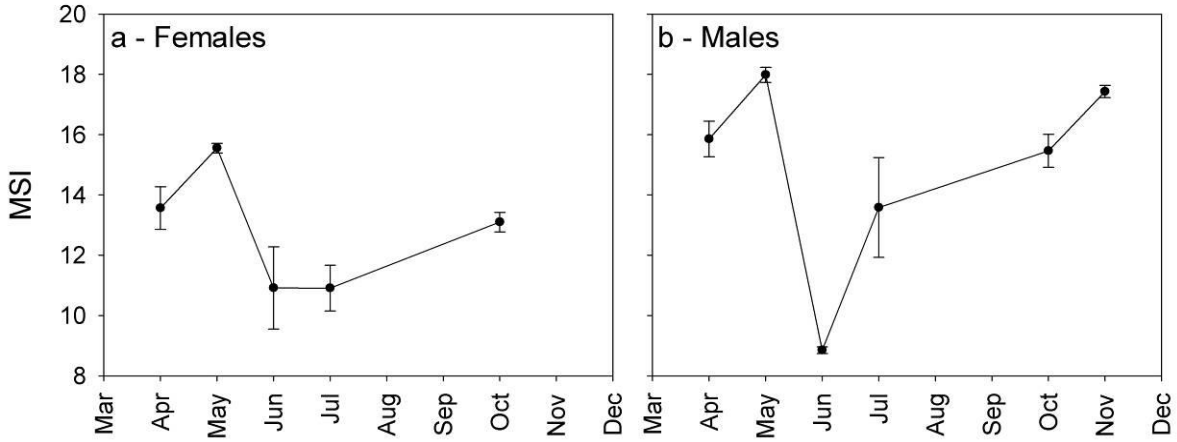



Figure 3-5 General annual (2007) variation in maturity stage index (MSI) of Anthoptilum grandiflorum in females and males (mean ± SE) when all samples from southern Newfoundland locations were pooled together

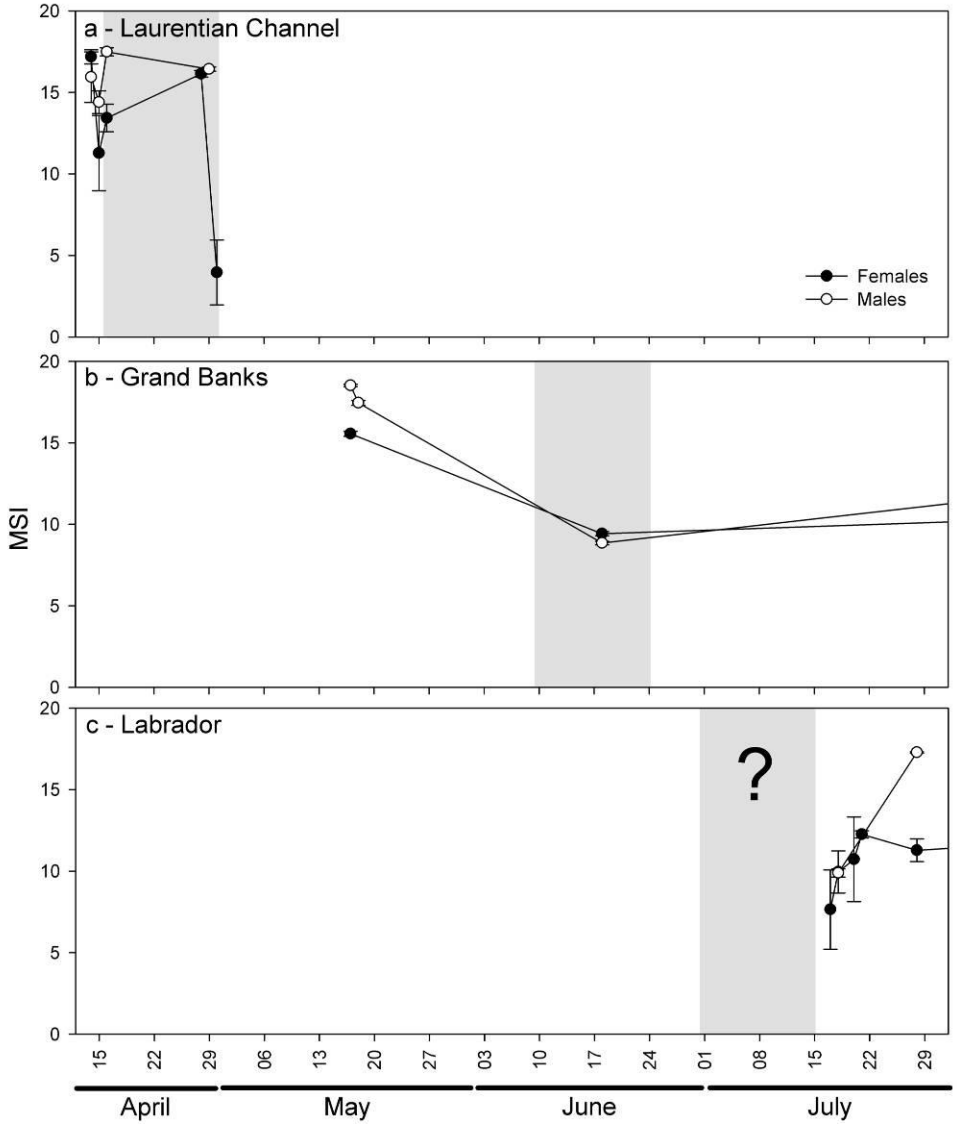



Figure 3-6 Variation in maturity stage index (MSI; mean ± SE) between April and July 2007 in females (black circles; n = 1−6 colonies) and males (white circles; n = 1−4 colonies) of Anthoptilum grandiflorum collected in Laurentian Channel, Grand Banks, Labrador. The grey areas correspond to spawning periods; the question mark indicates a suspected spawning period

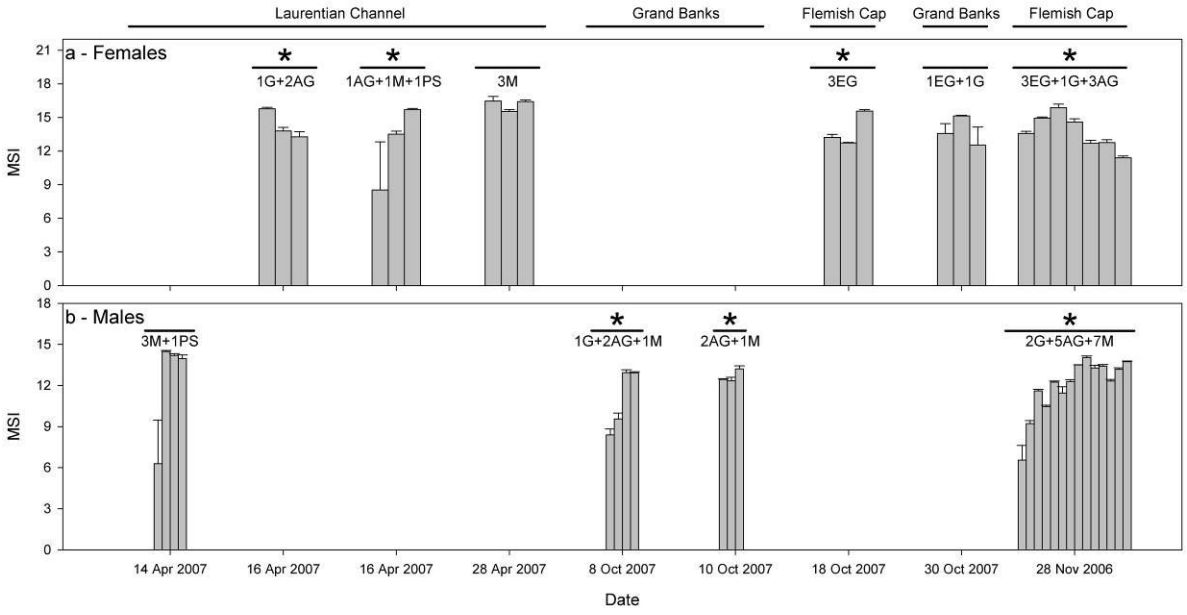



Figure 3-7 Inter-colony synchrony in gametogenic development based on the maturity stage index (MSI) in females and males of Anthoptilum grandiflorum. Each date corresponds to a specific site sampled in Laurentian Channel, Grand Banks and Flemish Cap. Significant differences among MSI values (asterisks) indicate asynchrony in the gametogenic development. Corresponding gametogenic stages are shown: Early Growth (EG), Growth (G), Advanced Growth (AG), Mature (M), Post Spawning (PS)

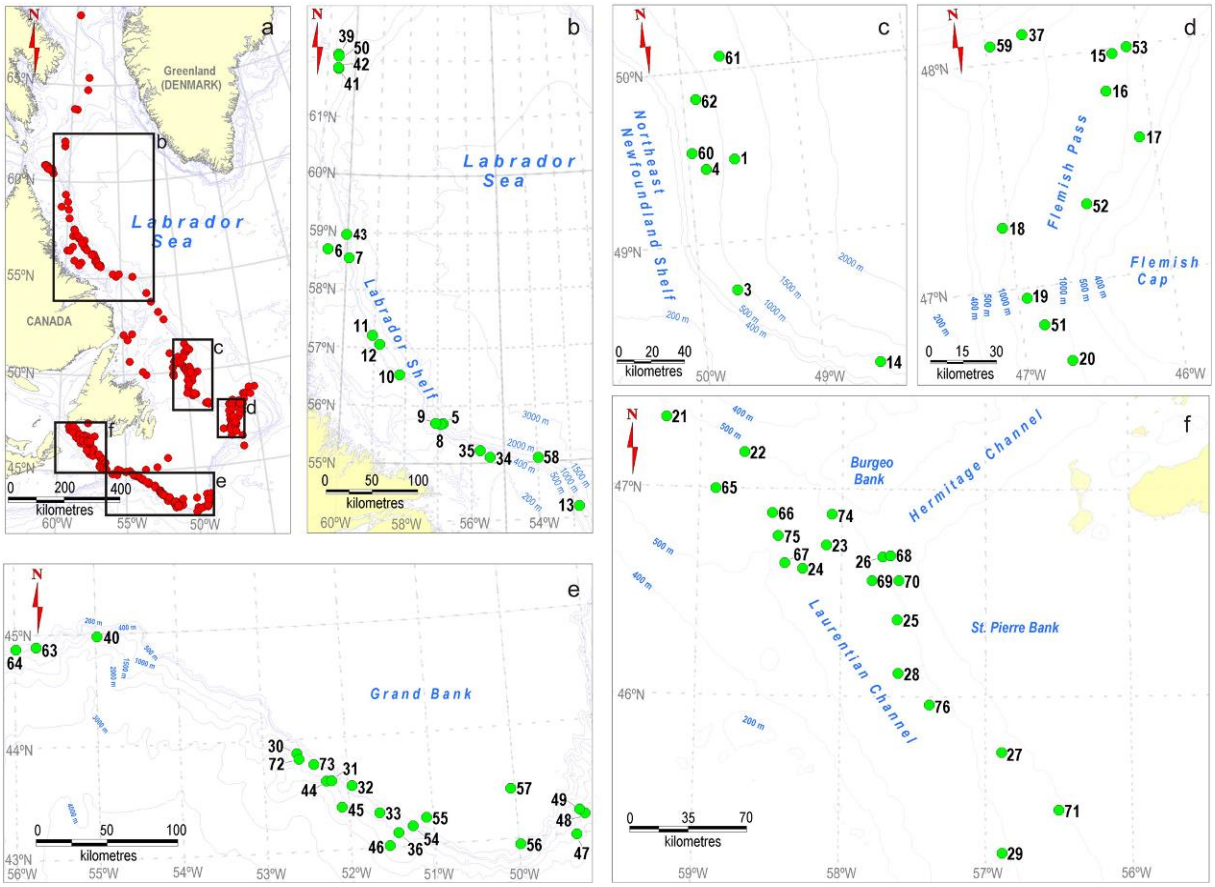
## Supplementary materials

Appendix 3-A Anthoptilum grandiflorum. Summary of sampling at 76 study sites (Appendix 3-B) from five regions (LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador). Sampling dates and depth with number of females, males and immature colonies analyzed

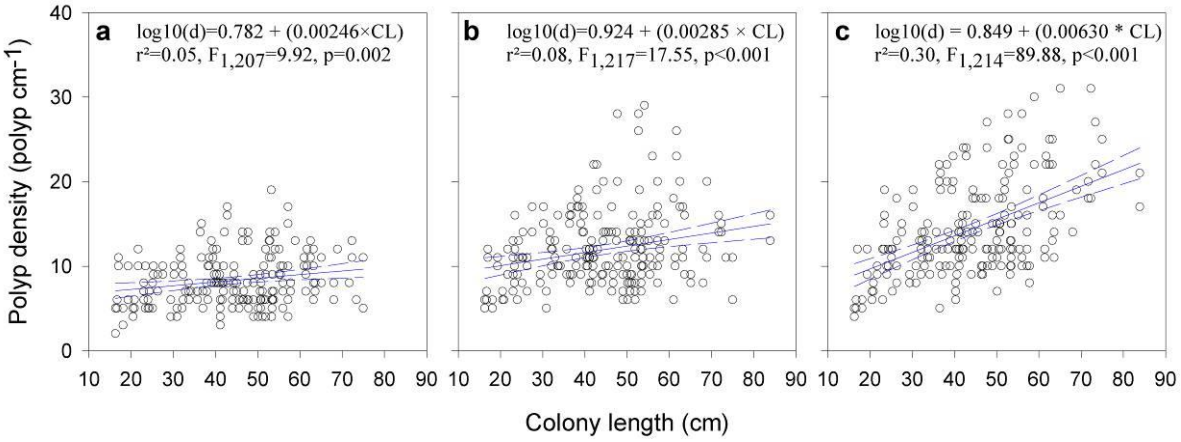
| ID# | Date | Depth | Regions | Latitude | Longitude | Females | Males | Non-sexed |
|---|---|---|---|---|---|---|---|---|
| 1 | 26/01/2006 | 1167 | NNL | 49°28'42.60"N | 49°35'42.00"W | 2 | 0 | 0 |
| 2 | 27/01/2006 | 1070 | NNL | 51° 7'15.60"N | 49°42'43.20"W | 1 | 0 | 0 |
| 3 | 30/01/2006 | 821 | NNL | 48°43'22.80"N | 49°40'49.80"W | 0 | 1 | 1 |
| 4 | 12/02/2006 | 921 | NNL | 49°25'60.00"N | 49°51'0.00"W | 1 | 1 | 0 |
| 5 | 10/07/2006 | 1299 | LB | 55°41'56.40"N | 56°49'51.60"W | 0 | 0 | 1 |
| 6 | 27/7/2006 | 176 | LB | 58°42'0.00"N | 60°37'58.80"W | 1 | 1 | 0 |
| 7 | 28/07/2006 | 620 | LB | 58°33'21.60"N | 59°55'12.00"W | 1 | 2 | 0 |
| 8 | 07/10/2006 | 626 | LB | 55°40'44.40"N | 56°54'21.60"W | 0 | 1 | 0 |
| 9 | 07/10/2006 | 466 | LB | 55°42'3.60"N | 57° 3'32.40"W | 0 | 0 | 1 |
| 10 | 09/10/2006 | 803 | LB | 56°32'29.40"N | 58°11'6.00"W | 0 | 0 | 1 |
| 11 | 15/10/2006 | 548 | LB | 57°13'15.60"N | 59° 4'33.60"W | 1 | 2 | 0 |
| 12 | 17/10/2006 | 895 | LB | 57° 4'1.20"N | 58°50'20.40"W | 0 | 1 | 0 |
| 13 | 04/11/2006 | 1071 | LB | 54°12'3.60"N | 52°49'48.00"W | 0 | 1 | 0 |
| 14 | 24/11/2006 | 1018 | NNL | 48°13'30.00"N | 48°31'4.80"W | 0 | 0 | 1 |
| 15 | 28/11/2006 | 1125 | FC | 47°58'33.60"N | 46°11'9.60"W | 7 | 14 | 0 |
| 16 | 28/11/2006 | 1134 | FC | 47°48'57.60"N | 46°15'30.60"W | 2 | 1 | 1 |
| 17 | 29/11/2006 | 810 | FC | 47°35'45.60"N | 46° 5'9.60"W | 1 | 1 | 0 |
| 18 | 01/12/2006 | 1036 | FC | 47°16'30.00"N | 47° 2'25.80"W | 0 | 1 | 0 |
| 19 | 01/12/2006 | 1186 | FC | 46°57'21.60"N | 46°56'31.20"W | 0 | 1 | 0 |
| 20 | 02/12/2006 | 978 | FC | 46°39'27.00"N | 46°42'18.00"W | 0 | 1 | 0 |
| 21 | 14/04/2007 | 435 | LC | 47°20'11.40"N | 59°14'43.80"W | 2 | 5 | 0 |
| 22 | 15/04/2007 | 428 | LC | 47°10'26.40"N | 58°41'25.80"W | 2 | 2 | 0 |
| 23 | 15/04/2007 | 457 | LC | 46°43'48.00"N | 58° 6'27.00"W | 1 | 1 | 1 |
| 24 | 15/04/2007 | 456 | LC | 46°36'54.00"N | 58°16'21.00"W | 0 | 0 | 1 |
| 25 | 16/04/2007 | 457 | LC | 46°22'12.00"N | 57°37'12.00"W | 3 | 0 | 0 |
| 26 | 16/04/2007 | 352 | LC | 46°40'26.40"N | 57°42'57.60"W | 3 | 4 | 0 |
| 27 | 28/04/2007 | 370 | LC | 45°43'53.40"N | 56°53'25.80"W | 3 | 0 | 0 |
| 28 | 29/04/2007 | 462 | LC | 46° 6'46.80"N | 57°35'42.00"W | 1 | 1 | 0 |
| 29 | 30/04/2007 | 422 | LC | 45°14'52.80"N | 56°52'55.20"W | 1 | 0 | 2 |
| 30 | 17/05/2007 | 600 | GB | 43°52'33.60"N | 52°35'38.40"W | 1 | 0 | 0 |
| 31 | 17/05/2007 | 597 | GB | 43°37'8.40"N | 52°11'33.00"W | 2 | 0 | 0 |
| 32 | 17/05/2007 | 214 | GB | 43°33'57.60"N | 51°57'1.80"W | 0 | 1 | 0 |
| 33 | 18/05/2007 | 625 | GB | 43°18'36.00"N | 51°38'24.00"W | 0 | 1 | 0 |

Appendix 3-A continued

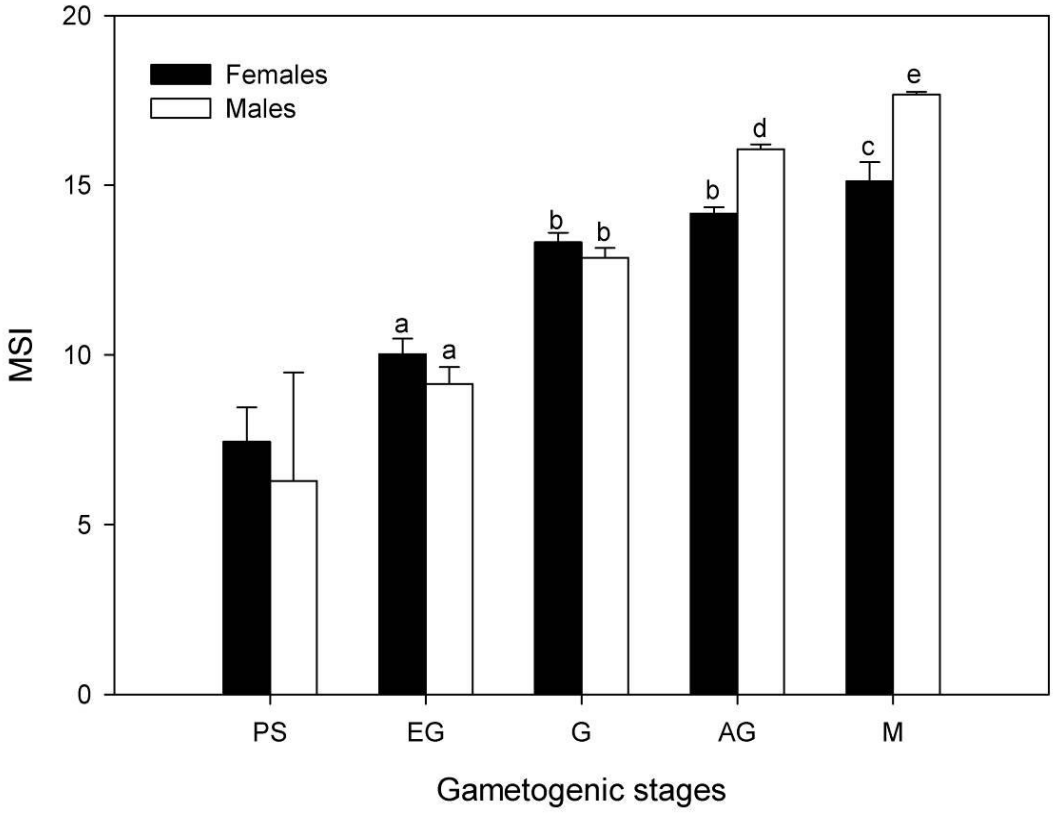
| ID# | Date | Depth | Regions | Latitude | Longitude | Females | Males | Non-sexed |
|---|---|---|---|---|---|---|---|---|
| 34 | 16/6/2007 | 465 | FC | 48° 7'1.20"N | 44°42'0.00"W | 1 | 0 | 1 |
| 35 | 16/6/2007 | 490 | FC | 48° 7'1.20"N | 44°40'1.20"W | 1 | 0 | 0 |
| 36 | 18/06/2007 | 658 | GB | 43° 7'33.60"N | 51°25'30.00"W | 1 | 1 | 0 |
| 37 | 08/07/2007 | 701 | FC | 48° 6'45.00"N | 46°45'7.20"W | 1 | 0 | 0 |
| 38 | 17/07/2007 | 881 | LB | 62° 4'60.00"N | 60°37'60.00"W | 3 | 0 | 0 |
| 39 | 18/07/2007 | 871 | LB | 62° 4'60.00"N | 60°37'60.00"W | 2 | 1 | 0 |
| 40 | 18/07/2007 | 501 | GB | 44°58'36.23"N | 54°58'50.52"W | 0 | 1 | 0 |
| 41 | 20/07/2007 | 883 | LB | 61°49'60.00"N | 60°36'0.00"W | 2 | 0 | 0 |
| 42 | 21/07/2007 | 874 | LB | 61°51'0.00"N | 60°37'0.00"W | 2 | 0 | 0 |
| 43 | 28/07/2007 | 552 | LB | 58°57'21.60"N | 60° 2'20.40"W | 2 | 1 | 0 |
| 44 | 08/10/2007 | 824 | GB | 43°37'3.00"N | 52°15'19.80"W | 2 | 4 | 0 |
| 45 | 08/10/2007 | 1333 | GB | 43°22'51.60"N | 52° 5'11.40"W | 2 | 0 | 1 |
| 46 | 09/10/2007 | 1130 | GB | 43° 0'50.40"N | 51°32'29.40"W | 2 | 0 | 0 |
| 47 | 10/10/2007 | 1347 | GB | 42°59'29.40"N | 49°18'39.60"W | 0 | 1 | 1 |
| 48 | 10/10/2007 | 934 | GB | 43°10'8.40"N | 49°11'24.00"W | 2 | 3 | 0 |
| 49 | 10/10/2007 | 836 | GB | 43°12'21.60"N | 49°15'5.40"W | 1 | 0 | 1 |
| 50 | 16/10/2007 | 986 | LB | 62° 2'60.00"N | 60°37'1.20"W | 1 | 0 | 0 |
| 51 | 18/10/2007 | 1208 | FC | 46°49'44.40"N | 46°51'9.00"W | 3 | 1 | 0 |
| 52 | 18/10/2007 | 896 | FC | 47°20'2.40"N | 46°29'2.40"W | 0 | 2 | 1 |
| 53 | 19/10/2007 | 743 | FC | 47°59'52.80"N | 46° 5'15.00"W | 0 | 2 | 0 |
| 54 | 28/10/2007 | 599 | GB | 44°22'17.40"N | 53°22'48.00"W | 2 | 1 | 0 |
| 55 | 29/10/2007 | 579 | GB | 44° 5'56.40"N | 52°56'24.00"W | 0 | 1 | 0 |
| 56 | 30/10/2007 | 623 | GB | 43°37'12.00"N | 52°11'56.40"W | 3 | 0 | 0 |
| 57 | 30/10/2007 | 593 | GB | 43°14'47.40"N | 51°29'42.00"W | 0 | 2 | 0 |
| 58 | 16/11/2007 | 1039 | LB | 55° 4'33.60"N | 53°58'58.80"W | 0 | 0 | 1 |
| 59 | 25/11/2007 | 651 | FC | 48° 4'39.00"N | 46°58'12.00"W | 0 | 1 | 0 |
| 60 | 08/12/2007 | 656 | NNL | 49°31'57.00"N | 49°57'45.00"W | 1 | 0 | 0 |
| 61 | 14/12/2007 | 1285 | NNL | 50° 4'42.60"N | 49°38'22.20"W | 0 | 0 | 1 |
| 62 | 14/12/2007 | 851 | NNL | 49°50'29.40"N | 49°53'6.00"W | 1 | 0 | 0 |
| 63 | 11/04/2009 | 494 | GB | 44°52'60.00"N | 55°37'0.00"W | 1 | 0 | 0 |
| 64 | 11/04/2009 | 428 | GB | 44°52'0.00"N | 55°52'0.00"W | 1 | 0 | 0 |
| 65 | 18/04/2009 | 437 | LC | 47° 0'0.00"N | 58°53'42.00"W | 0 | 0 | 1 |
| 66 | 18/04/2009 | 428 | LC | 46°52'55.20"N | 58°29'49.20"W | 1 | 0 | 0 |
| 67 | 19/04/2009 | 453 | LC | 46°38'34.80"N | 58°24'10.80"W | 0 | 1 | 0 |
| 68 | 19/04/2009 | 317 | LC | 46°40'30.00"N | 57°39'28.80"W | 2 | 0 | 0 |
| 69 | 29/04/2009 | 451 | LC | 46°33'43.20"N | 57°47'49.20"W | 0 | 1 | 0 |
| 70 | 30/04/2009 | 348 | LC | 46°33'54.00"N | 57°36'18.00"W | 1 | 0 | 1 |
| 71 | 03/05/2009 | 265 | LC | 45°27'25.20"N | 56°30'18.00"W | 0 | 1 | 0 |
| 72 | 24/05/2009 | 603 | GB | 43°49'22.80"N | 52°34'1.20"W | 0 | 1 | 0 |
| 73 | 24/05/2009 | 337 | GB | 43°45'46.80"N | 52°23'34.80"W | 0 | 1 | 0 |
| 74 | 24/04/2010 | 456 | LC | 46°52'30.00"N | 58° 3'54.00"W | 1 | 0 | 0 |
| 75 | 26/04/2010 | 433 | LC | 46°46'12.00"N | 58°27'18.00"W | 2 | 0 | 0 |
| 76 | 27/04/2010 | 447 | LC | 45°58'1.20"N | 57°23'6.00"W | 1 | 1 | 0 |



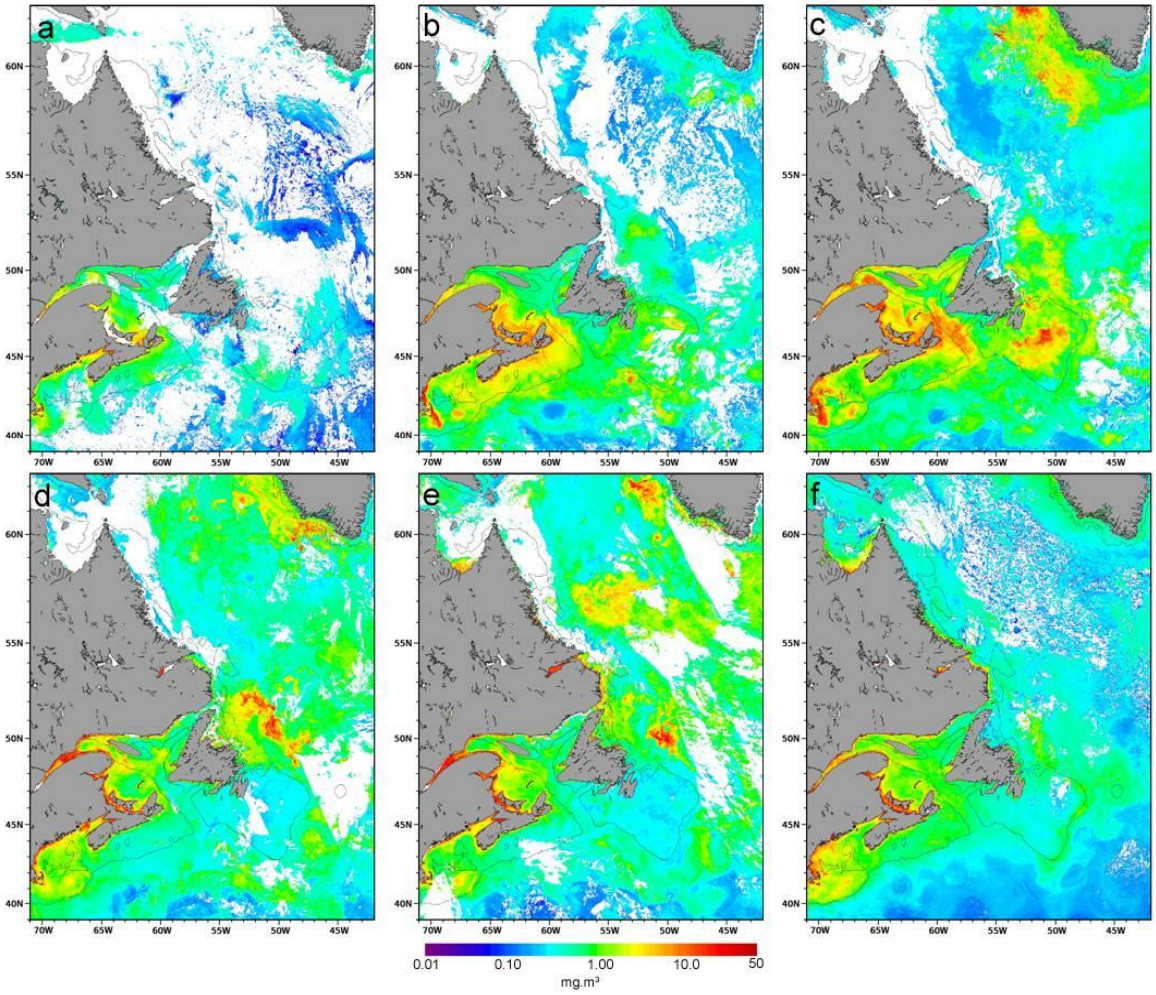

Appendix 3-B Known occurrences of Anthoptilum grandiflorum from DFO research surveys and distribution of the sampling sites among the different geographic regions: b Labrador, c North Newfoundland, d Flemish Cap, e Grand Banks and f Laurentian Channel




Appendix 3-C Anthoptilum grandiflorum. Linear regression of polyp density (d, polyp $cm^{-1}$) with colony length (CL, cm) for the three sections of the rachis, a lower section, b middle section, c upper section




Appendix 3-D Anthoptilum grandiflorum. A significant increase of the Maturity Stage Index (MSI) from Post-Spawning to Mature colonies was observed (♀, H = 115.49; df = 4, $p < 0.001$; ♂, H = 131.02, df = 4; $p < 0.001$) in both females and males, confirming the suitability of this quantitative tool to represent the reproductive cycle on a continuous scale. Only one male in Post-Spawning stage was found, therefore comparison of female and male stages were restricted to the 4 other stages. Females and males presented significantly different MSI values for the different stages (two-way ANOVA, sex×stage: $F_{3,357}=15.0$, $p < 0.001$). Values shown as mean ± SE (n = 3 per colony × 1-23 colonies). Bars with corresponding letters are not significantly different. Stages: Post Spawning (PS), Early Growth (EG), Growth (G), Advanced Growth (AG), Mature (M)




Appendix 3-E Surface concentration of chlorophyll a (mg $m^{-3}$) over the year in the Northwest Atlantic. a Late January, b early April, c late April, d late May, e early June and f late October. Colour scale located at the bottom of the Fig., with blue corresponding to low concentrations and red to high concentrations

# Chapter 4：Protracted oogenesis and annual reproductive periodicity in a deep-sea octocoral

## Abstract

Halipteris finmarchica is one of the most common species of deep-sea pennatulacean corals in the Northwest Atlantic; it was recently determined to act as biogenic substrate for other species and as nursery for fish larvae. Its reproductive cycle was investigated in colonies sampled in 2006 and 2007 along the continental slope of Newfoundland and Labrador (Canada). H. finmarchica exhibits large oocytes (maximum diameter of 1000 µm) which are consistent with lecithotrophic larval development. Female potential fecundity based on mature oocytes just before spawning was ~6 oocytes polyp$^{-1}$ (500–6,300 oocytes colony$^{-1}$); male potential fecundity was 16 spermatocysts polyp$^{-1}$ (5,500–57,400 spermatocysts colony$^{-1}$). Based on statistical analysis of size-probability frequency distribution, males presented one cohort of spermatocysts that matured inside 8–11 months, whereas females harboured two distinct cohorts of oocytes; a persistent pool of small ones (≤400 µm) and a small number (~20%) of larger ones (that grew from ~400 to >800 µm) over a year. Despite this difference in the tempo of oogenesis and spermatogenesis, a synchronic annual spawning was detected. A latitudinal shift in the spawning period occurred from South (April in the Laurentian Channel) to North (May in Grand Banks and July-August in Labrador/Lower Arctic), following the development of the phytoplankton bloom (i.e. sinking of phytodetritus). Prolonged oogenesis with the simultaneous presence of different oocyte stages in a given polyp is likely not uncommon in deep-sea octocorals and could hamper the detection of annual/seasonal reproduction when sample sizes are low and/or time series discontinued or brief.

# 1. Introduction

Pennatulaceans (Octocorallia: Pennatulacea), commonly called sea pens, are colonial corals that anchor themselves into soft sediment (mud, sand), allowing them to colonize large areas of the sea floor from the intertidal zone down to the abyssal plain (Williams 2011). Sea pens can occur sparsely or form large aggregations [e.g., large fields of deep-sea Pennatula spp.; (Baker et al. 2012)], suggesting that they may provide an important structural habitat to other organisms (Tissot et al. 2006; Baillon et al. 2012, Chapter 6; Baker et al. 2012) by increasing the complexity of the soft bottom seafloor. Despite this potentially important role and the existence of ~200 species (Williams 2011), sea pens are still poorly known, rendering their management and protection problematic. Sea pens have already been identified as vulnerable species (NAFO 2008; Murillo et al. 2011) due to their sensitivity to human disturbances such as trawl fisheries (Brodeur 2001).

Understanding the reproduction of a species is important to determine its resilience to disturbance. Despite the fact that sea pens are more abundant in the deep sea than in shallow waters (Daly et al. 2007; Kahng et al. 2011), knowledge about their reproduction is still limited and mostly focused on shallow-water species (Table 4-1,Soong 2005, Daly et al. 2007). All sea pens studied so far (in shallow and deep waters) were determined to be gonochoric broadcast spawners (Kahng et al. 2011; Lopes et al. 2012, Baillon et al. 2014, Chapter 3). Oogenesis was often inferred to take more than 12 months from the presence of two distinct cohorts of oocytes (Table 4-1). Protracted oogenesis has mainly been documented in taxa from Antarctica (Pearse and Giese 1966,

Pearse and Bosch 2002), or cold-water/deep-sea environments (van Praët et al. 1990, Orejas et al. 2002) although it has also been reported in shallow-water species of fishes (Hourigan and Radtke 1989, Mesa et al. 2006), echinoderms (Pearse and Giese 1966; Smiley 1988; Hamel et al. 1993; Pearse and Bosch 2002), bivalves (Richardson 1980), and cnidarians (Yamazato et al. 1981; Brazeau and Lasker 1989; van Praët et al. 1990). In most cases, protracted oogenesis was determined to be required to allow the development of large oocytes (Yamazato et al. 1981; Orejas et al. 2002). It has also been suggested that long oogenesis is associated with species exhibiting high fecundity, a synchronous maturation and a brief spawning period (Benayahu and Loya 1986). Typically, protracted oogenesis is characterized by the presence of two or more cohorts of oocytes; however, multiple cohorts are not always linked to prolonged oogenesis. For example, the fish Dicentrarchus labrax (sampled in the western English Channel) presents several cohorts which will be spawned during the same season, as the species is considered a "fractional spawner" (Mayer et al. 1990) or "multiple spawner" (West 1990).

While all the shallow-water sea pen species show annual/seasonal spawning, 3 of the 4 deep-sea species studied to date are considered to spawn "continuously" or non-seasonally (Table 4-1). However, recent evidence of seasonal spawning in the deep-sea pennatulacean Anthoptilum grandiflorum in the Northwest Atlantic (Baillon et al. 2014, Chapter 3) suggests that deep-water sea pens may not necessarily always show aperiodic reproductive cycles. While the so-called "continuous" reproduction was initially considered to be a common pattern in the deep sea (Gage and Tyler 1992; Eckelbarger and Watling 1995), seasonal spawning has now been reported in several phyla [e.g. mollusks (Rokop 1974; Lightfoot et al. 1979), sponges (Witte 1996), echinoderms

(Campos-Creasey et al. 1994; Mercier and Hamel 2009b)], as well as in scleractinian corals and octocorals (Mercier and Hamel 2011; Mercier et al. 2011a, Baillon et al. 2014, Chapter 3). This emphasizes the need to distinguish between the cycle of gamete synthesis and the timing of gamete release in assessments of reproductive rhythms. It has been shown that the range of depths and regions sampled can influence how accurately gametogenetic cycles and spawning periodicities can be evidenced (Mercier and Hamel 2009a; Baillon et al. 2011). Analyses of samples from different depths and regions can mask periodicities in reproduction and could hamper the detection of seasonal patterns in the deep sea (Mercier and Hamel 2009b).

The continental slope of Newfoundland and Labrador shelters at least 60 species of deep-sea coral, of which 16 are pennatulaceans (Gilkinson and Edinger 2009; Murillo et al. 2011, Wareham unpublished data). Here, we investigated the reproductive biology of Halipteris finmarchica, one of the most common species of sea pen in the Northwest Atlantic (Wareham and Edinger 2007), which can be found between 200 and >2000 m (Baker et al. 2012). H. finmarchica is a long whip-like sea pen that can form large fields, creating structural habitat on the uniform muddy seafloor (Tissot et al. 2006). The ecological importance of H. finmarchica as essential fish habitat was shown recently from the associated presence of larvae of the commercial fish Sebastes spp. (Baillon et al. 2012, Chapter 6); this species also harbours several symbionts (Chapter 5). Moreover, studies in the North Pacific (142−248 m) showed a high abundance of adult Pacific ocean perch Sebastes alutus in fields of the congeneric sea pen H. willemoesi (Brodeur 2001), emphasising the ecological importance of this genus and stressing the need to increase our knowledge of their biology. The major aims of the present study were to determine

the fecundity and mode of reproduction of H. finmarchica, and to assess the influence of depth, region and certain environmental factors on these life-history traits. We also strove to draw parallels with patterns documented in sympatric and distant species of sea pens and to critically assess our current understanding of gametogenic cycles and spawning periodicities in deep-water octocorals.

## 2. Material and Methods

### 2.1 Sampling

Samples of Halipteris finmarchica were obtained as by-catch from annual research surveys (Multispecies Surveys and Northern Shrimp Research Surveys), and the At-Sea Observer Program, led by Fisheries and Oceans Canada (DFO) along the continental slope of eastern Canada from southern Newfoundland to the lower Arctic (Fig. 4-1). The surveys followed a stratified random sampling design using a Campellen 1800 trawl with rockhopper footgear, towed for 15 min at 3 knots with an average tow length ~1.4 km (gear opened and closed at depth). A total of 63 colonies of H. finmarchica were sampled at 31 sites between 256 and 1161 m depth in 2006 and 2007 (Fig. 4-1, Appendix 4-A) and frozen at -20°C upon collection.

Temperature data were obtained from Conductivity Temperature Depth (CTD) profiles collected at the different sites during sampling.

### 2.2 Processing

The length of each colony was measured from the base of the peduncle to the tip of the rachis. Three samples of tissue with polyps (~1-2 cm segment along the central axis) were collected in three different sections of each colony, coined lower, middle and upper rachis

sections, similar to previous studies on sea pens (Soong 2005; Pires et al. 2009; Baillon et al. 2014, Chapter 3). These tissues were preserved in 100% ethanol for microdissection or 4% formaldehyde for histology. Three polyps in each of these sections were isolated by microdissection under a stereomicroscope. They were opened longitudinally from the mouth to the base of the polyp and all oocytes/spermatocysts were carefully removed. The number of oocytes or spermatocysts per polyp, their Feret diameter, as well as their colour and location in the polyp were established to determine their size distributions, fecundity, gametogenic development, and maturity stage index as described in the following paragraphs. Complementary data were obtained from histological sections using standard histology protocols (Baillon et al. 2011). Samples were dehydrated in an alcohol series (70-100%), embedded in paraffin, sectioned (6 to 10 µm) and stained with haematoxylin and eosin. They were examined under a light microscope (Nikon Eclipse 80i) coupled to a digital camera (Nikon DXM1200F) and analysed using the imaging software Simple PCI (v. 6.0).

2.3 Gametogenic development

Oocytes and spermatocysts were divided into 4 classes according to their size and location in the polyp. Oocyte class I: small whitish oocytes (<100µm) found at the base of the polyp (Fig. 4-2A), class II: oocytes (101−400 µm) turning yellow still found at the base of the polyp, class III (Fig. 4-2B): yellow oocytes (401−700 µm) filling the gastrovascular cavity (Fig. 4-2D) and class IV: mature oocytes (701−1000 µm) found close to the mouth or at the base of the tentacles (Fig. 4-2C-E). Spermatocyst class I: small whitish translucid spermatocysts (<80 µm) found at the base of the polyp, class II: slightly opaque and whitish spermatocysts (81−200 µm) still found at the base of the

polyp (Fig. 4-3A), class III: larger white spermatocysts (201−400 μm, Fig. 3C) found in the gastro-vascular cavity with their lumen full of spermatozoa (Fig. 4-3B), and class IV: large white mature spermatocysts (>401 μm) filling the entire cavity of the polyp (Fig. 4-3D).

2.4 Fecundity

For the sake of clarity, as outlined in Baillon et al. (2014, Chapter 3) and Mercier and Hamel (2011), we defined fecundity as follows: potential relative fecundity (PRF) defined as the total number of oocytes/spermatocysts per polyp irrespective of their maturity stage, and effective relative fecundity (ERF) as the number of mature entities (stage IV; see above) per polyp. PRF can be measured in all samples whereas ERF has to be measured immediately before spawning (i.e. in mature colonies) and is a closer proxy of realized/actual fecundity. Comparison of PRF and ERF in mature colonies provides additional information on whether all oocytes/spermatocysts attain full maturity in a given reproductive cycle. We also determined effective colony fecundity (ECF = ERF × number of polyps) using an estimation of the number of polyps according to the linear regression between colony length and polyp density in each section (Appendix 4-B).

2.5 Maturity stage index and oocyte/spermatocyst size distributions

The maturity stage index (MSI) represents a quantitative measure of gametogenic maturity on a continuous scale that lends itself to statistical analysis (Doyle et al. 2012; Baillon et al. 2014, Chapter 3). It was determined for male and female colonies, using the equation adapted from Baillon et al. (2014, Chapter 3): (PRF × colony length$^{-1}$) × total oocyte or spermatocyst volume × $10^{-6}$. Total oocyte or spermatocyst volume corresponded to the sum of individual oocyte or spermatocyst volumes in the polyp; the

volume was determined as: $\pi \times \text{diameter}^3/6$. For the females, MSI was determined considering all the oocytes present in the polyps and for each cohort separately.

Oocyte and spermatocyst size-abundance distributions were determined for each colony in each of the lower, middle and upper rachis sections based on the Ferret diameter of all the oocytes and spermatocysts present in the three polyps sub-sampled.

2.6 Data analysis

Sex ratios were analyzed according to depth (threshold at 700 m, see below) and region (Fig. 4-1). Chi-square analyses were used to determine whether the sex ratio was significantly different from equality (1:1) in regions with a minimum of 10 colonies to meet the requirements for sample size (McDonald 2008).

Gamete diameter-frequency histograms were computed and analyzed using mixture distribution analyses (Lango-Reynoso et al. 2006) and the mixdist package in the statistical software R. This was initially done to model the different cohorts of oocyte/spermatocyst in the polyps of each colony. The mixdist procedure was used following Du (2002). The precision of the modelled distributions was determined using the ANOVA function included in the mixdist package. As a second step, the mean oocyte or spermatocyst diameter (corresponding to the peak of the curve for each modeled cohort) of each colony where pooled per month to determine annual variations for the different cohorts. Due to sample sizes, we combined months in 2006 (January, July, August, November and December) and 2007 (April, May, October) to obtain a full annual cycle. These two consecutive years had previously been shown to elicit consistent reproductive cycles in a sympatric pennatulacean coral (Baillon et al. 2014, Chapter 3).

Changes in relative fecundity (PRF, ERF), in MSI and in mean oocyte/spermatocyst size were analysed according to different parameters: colony length, sampling month, geographic region (Laurentian Channel, Grand Banks, Flemish Cap, North Newfoundland, Labrador and lower Arctic) and depth. Analyses were divided into below and above 700 m because colonies were found to be significantly longer below this threshold depth (Chapter 2). One-way ANOVA or t-test were used, after verifying assumptions of normality and homogeneity of variances, to compare the different parameters among months, regions and depths. Post-hoc pairwise analysis (Student-Newman test) was conducted as appropriate. When the assumption of normality was not met even after transforming the data, spearman correlations and Kruskal-Wallis or Mann-Whitney tests were used, followed by Dunn's tests as appropriate.

To assess variations in production of oocytes and spermatocysts with colony length and depth, PRF was used as a proxy. Variation in PRF and MSI with colony length and depth was examined in subsets of samples to avoid the potential influence of month and region. Therefore female samples collected in October 2007 in GB and male samples collected in October 2007 in FC were used. All data are provided as mean ± SE.

## 3. Results

### 3.1 Sexuality and sex ratio

*Halipteris finmarchica* is gonochoric. Oocytes and spermatocysts were visible in all the colonies examined except two (21.3–25.3 cm colony length, sampled in July and November 2006). Thirty-two colonies were identified as female (18.8–119.9 cm), 26 as male (23.1–148.6 cm) and 5 were unsexable (19.6–38.3 cm). Sex ratios varied markedly

in the regional samples, with inconsistent inequalities that were not statistically significant (Table 4-2). Overall, sex ratios did not differ significantly from equality at the two main depths (below 700 m: $\chi^2$ = 2.13, P = 0.144; above 700 m: $\chi^2$ = 0.14, P = 0.705) or when the samples were pooled together (Table 4-2).

3.2 Fecundity

Potential relative fecundity (PRF) increased from the lower to the middle section of the rachis in all colonies examined (lower<middle=upper; data from all regions/depths pooled: ♀ H = 82.51, df = 2, P < 0.001; ♂ H= 63.23, df = 2, P < 0.001). Oocytes and spermatocysts were found in almost all the polyps examined in the middle and upper sections; in the lower section, 33.3% of female polyps and 23.1% of male polyps examined were empty. Because of this pattern and the absence of flesh in the upper section of some of the colonies, analyses of PRF, ERF, MSI and oocyte and spermatocyst size distributions were always carried out on polyps sampled from the middle section of the rachis.

Female and male PRF showed inter-colony variability at each sampling date. For female colonies sampled in October 2007 in GB (n = 9 colonies), PRF ranged from 4 to 79 oocytes polyp$^{-1}$. Neither colony length ($F_{1,26}$ = 1.99, P = 0.171) nor depth (F1,26 = 0.17, P = 0.687) were significant drivers of this variation. Male colonies sampled in October 2007 in FC (n = 7 colonies) showed the same variation in PRF (4 to 81 spermatocysts polyp$^{-1}$) with no influence of colony length ($F_{1,19}$ = 0.002, P = 0.964) or depth ($F_{1,19}$ = 0.45, P = 0.511). Male colonies showed an ERF (spermatocysts >400 µm only) varying between 2 and 31 mature spermatocysts polyp$^{-1}$ with a corresponding ECF between 1,900 and 19,100 spermatocysts colony$^{-1}$ in the smallest and largest colonies,

respectively. Female colonies produced between 3 and 14 mature oocytes polyp$^{-1}$ (oocytes >700 µm only), with an ECF between 500 and 6,300 oocytes colony$^{-1}$.

In pooled samples, female PRF varied significantly among months ($F_{6,95}$ = 4.88, P < 0.001) with the highest values (41.9 ± 2.8 oocytes polyp$^{-1}$) occurring in November, while the lowest values (22.1 ± 4.1 oocytes polyp$^{-1}$) occurred during the spawning events identified between April and August coincided with the spawning events (see below).

3.3 Ooogenesis, spermatogenesis and reproductive cycles

The mixture distribution analysis of oocytes and spermatocysts revealed bimodal oogenic and unimodal spermatogenic development in H. finmarchica over the annual cycle (Fig. 4-4). A persistent cohort of small oocytes (≤400 µm, coined C1) was a dominant component of the oocyte pool (encompassing 81.0 ± 3.7 % of the oocytes). This cohort included oocytes in classes I and II and was present year-round (Fig. 4-4). A second cohort encompassed a smaller portion of the oocyte pool (19.0 ± 3.7% of the oocytes) and started to be visible in October (Fig.4-4) suggesting initiation of final oogenic maturation in August-September. This group (coined C2) was composed of larger oocytes (in classes III and IV, >400 µm; Fig. 4-4, S2 and S3, Table 4-3) and was transient. Despite overlap in October and November, the peak of C1 (mean size of oocytes forming the cohort) was always significantly lower than the peak of C2 (two-way ANOVA, month×peak: $F_{5,47}$ = 27.38, P < 0.001, Fig. 4-5A). A unimodal size distribution of spermatocysts was evident when colonies were pooled per month (Fig. 4-4, Table 4-3). However, at the colony level, the analysis revealed the presence of two potential cohorts in all colonies sampled immediately before or during the spawning months (April to August, see below;

Appendix 4-E), while most of the colonies sampled from October to January showed only one cohort (Appendix 4-F).

The peak of C1 of female colonies remained stable year-round (171.2 ± 6.3 µm; $F_{6,27} = 2.29$, $P = 0.074$; Fig. 4-5A). The peak of C2 varied annually; it was maximum from May to August (786.6 ± 27.2 µm) followed by a drop and the appearance of significantly smaller oocytes between October and December (468.4 ± 12.4 µm, $F_{4,20} = 42.66$, $P < 0.001$; Fig. 4-5A). For male colonies, mean spermatocyst size peaked in April, May and August (431.2 ± 16.6 µm), followed by a drop and appearance of significantly smaller spermatocysts from October to January (156.9 ± 7.7 µm; $F_{3,21} = 81.40$, $P < 0.001$; Fig. 4-6A). The drop in size was coincident in oocytes and spermatocysts, indicating a spawning event (between August and October, Fig. 4-5A and 4-6B) followed by initiation of the final gamete maturation at the beginning of the fall (September). This result was corroborated by a drop in the MSI between August and September for both sexes (Fig. 4-5B and 4-6B). The MSI values calculated either in pooled oocytes or separately in the two cohorts showed the same annual pattern (Fig. 4-5B and C), i.e. a steady increase from April to August, then a sharp drop followed by relatively stable values from October to December.

The pattern outlined above was observed in the middle and upper section of the rachis. When the lower section of the rachis of female colonies was considered, only one cohort of small oocytes was present year-round except for two colonies sampled in October that exhibited 2 cohorts (C1:123.8 ± 13.7 µm; C2: 346.9 ± 47.5 µm) and three colonies with empty polyps sampled in October, July and August. The peak of C1 remained stable year round (129.9 ± 6.3 µm, $F_{5,24} = 2.68$, $P = 0.054$). The peak of C1 in

the lower section was significantly lower than the peak of C1 in the middle section ($t = -5.79$, $df = 57$, $P < 0.001$).

Male PRF (i.e. spermatocyst density) remained stable year-round (33.0 ± 2.5 spermatocysts polyp$^{-1}$, $H = 3.48$, $df = 6$, $P = 0.746$; Fig. 4-6C). In female colonies, the number of oocytes found in C2 was stable between October and December (9.8 ± 1.0 oocytes polyp$^{-1}$, $H = 0.27$, $df = 2$, $P = 0.874$) while it varied significantly among spawning months (April to August); some colonies spawned before others (3.5±0.8 oocytes polyp$^{-1}$; $H = 22.20$, $df = 3$, $P < 0.001$; Fig. 4-5D). Finally, C1 showed a general but non-significant increase in oocyte (class I and II) number from May to October ($F_{6,89} = 1.84$, $P = 0.101$; Fig. 4-5D). The proportion of oocytes in class I (least developed oocytes) in C1 remained stable year-round (11.2 ± 2.0 %, $F_{6,30} = 1.07$, $P = 0.407$).

Female and male colonies sampled in 4 different regions (LC, GB, LB, LA; see Figure 4-1) and presented signs of spawning that spanned between April and August. A first spawning event was identified at the end of April in LC by the presence of colonies either completely or largely devoid of large oocytes (C2). The same characteristic (complete or near total absence of oocytes in C2) allowed the identification of a spawning event in July in LB. Finally the mixed presence of colonies with C2 comprised of numerous mature oocytes (class IV) or few/zero mature oocytes suggested two other spawning events: one in May in GB and another in August in LA. All these periods also coincided with male colonies harbouring mature spermatocysts.

### 3.4 Influence of colony length and depth on MSI

Values of MSI in female (October 2007 in GB) and male (October 2007 in FCcolonies were not influenced by colony length (♀: rs = 0.04, P = 0.861; ♂: $r_s$ = 0.04, P = 0.863) or by depth (♀: $r_s$ =0.32, P = 0.107; ♂: $r_s$ = 0.13, P = 0.558).

### 3.5 Relationship with seawater temperature

Presence of colonies with mature oocytes and spermatocysts or showing signs of recent spawning was found when bottom temperature was 4.7 ± 0.1°C in LC, 4.6 ± 0.2°C in GB, 4.1 ± 0.1°C in LB and 4.0 ± 0.1°C in LA.

## 4. Discussion

Halipteris finmarchica is a gonochoric broadcast spawner, based on the absence of embryonic or larval stages in the polyps of colonies sampled year round, similar to the other sea pens studied so far (Watling et al. 2011; Lopes et al. 2012; Servetto et al. 2013; Baillon et al. 2014, Chapter 3). Mature oocytes (stage IV) measure between 700 and 1000 µm, suggesting that H. finmarchica exhibits a lecithotrophic, non-feeding, larval development also consistent with other deep-water sea pens (reviewed in Table 4-1). The fact that nearly all colonies studied (size range: 18.8-148.6 cm) presented mature gametes or gametes undergoing maturation in the current cycle, suggested that size at first maturity is reached at or before 18 cm colony length. This size corresponds to ~12 % (or less) of the maximum length which is a smaller proportion than recorded in the sympatric species Anthoptilum grandiflorum (28%, Baillon et al. 2014, Chapter 3) and the shallow-water Pennatula phosphorea in Scotland (35-65%, Edwards and Moore, 2008), indicating that H. finmarchica matures at a proportionally earlier stage. Growth rate of the

congeneric species H. willemoesi sampled in the Bering Sea (142−248 m depth) showed that small colonies (25−29 cm) have a growth rate of 3.9 cm $yr^{-1}$ (Wilson et al. 2002). Considering that the water temperature in deep waters around Newfoundland and Labrador is similar to that in the Bering Sea (http://www.afsc.noaa.gov/RACE/groundfish/survey_data/ebswater.htm), both species might have similar growth rates, suggesting that H. finmarchica may become fertilee at about 5 years of age.

Oocytes and spermatocysts were present in all the sections of the rachis (lower, middle and upper sections). However, polyps sampled in the lower section were often empty (in ~33% of female and ~23% of male polyps examined). The non-empty female polyps in the lower section contained only immature oocytes, smaller than in the other sections, suggesting that this section is not involved in reproductive output. The polyps from the lower section might participate in reproduction in the following years, as the colony grows. The fact that new polyps are added to the base of the colony (in agreement with Soong, 2005) implies a gradual contribution of the new polyps in female reproduction, as hypothesized for the sympatric A. grandiflorum (Baillon et al. 2014, Chapter 3). In contrast, males present the same spermatocyst size-distribution in the three sections of the colony suggesting that spermatogenesis does not depend as directly on polyp size and age.

The significant increase in gamete density (PRF) from the lower to the middle sections of female and male colonies might be explained by the concurrent increase in polyp diameter across these sections (24.3 ± 1.6% increase, Chapter 2). A larger polyp diameter allows higher fecundity, as observed in the sea pen A. grandiflorum (Baillon et

al. 2014, Chapter 3) and in other coral species (Sakai 1998). For the most developed polyps, H. finmarchica showed a lower fecundity at the polyp level (6.2 ± 1.0 mature oocytes polyp$^{-1}$) than the sympatric A. grandiflorum (13.0 ± 1.8 mature oocytes polyp$^{-1}$; Baillon et al. 2014, Chapter 3), which might be explained by the smaller polyp diameter of H. finmarchica (1.03 ± 0.01 mm compared to 1.26±0.02 mm in A. grandiflorum; Chapter 1). However, whole colony fecundities exhibit a comparable range (500-6,300 oocytes colony$^{-1}$ in H. finmarchica vs. 1,500-4,000 oocytes colony$^{-1}$ in A. grandiflorum) also similar to that reported in the deep-sea Umbellula sp. in the Northeast Atlantic [~2,000 oocytes colony$^{-1}$; (Tyler et al. 1995)]. This finding supports the assumption of lower fecundity in deep-sea than in shallow-water species; e.g. Ptilosarcus guerneyi in the Northeast Pacific produces 200,000 oocytes colony$^{-1}$ (Chia and Crawford 1973) and Pteroides sp. in the Southwest Pacific 36,000 oocytes colony$^{-1}$ (Duncan 1998). This might be explained by lower food availability and temperature at greater depth.

The disappearance of larger oocytes (>400 µm) from samples collected between April and August, the presence of mature male colonies during the same months, as well as the drop in mean oocytes/spermatocysts diameter and in the MSI between August and October suggest seasonal spawning between April and August. A shift in the spawning period from the South to the North was detected; colonies spawn in April in LC, in May in GB, in July in LB and in August in LA. Different environmental factors, such as the lunar cycle (Mercier et al. 2011b) and phytodetritus production (Gage 1992), have been shown to influence spawning periodicity in deep-sea invertebrates. Here, variation in production and downfall of phytodetritus is likely involved, as observed for A. grandiflorum (Baillon et al. 2014, Chapter 3) in the same geographic area. Both species

spawned in the different regions following the spring bloom of phytoplankton, which started in LC in March/April and shifted to the North in the following months, supporting the late (August) spawning in the lower Arctic. Moreover, in all locations studied spawning occurred when bottom temperature was around 4°C, as previously observed (Baillon et al., 2014), evoking a synergy between these two parameters in the determination of pennatulacean reproductive season along the continental slope of eastern Canada.

The presence in H. finmarchica of two cohorts of oocytes and the persistence of the smaller oocytes after spawning suggests that oogenesis takes >12 months, while spermatogenesis starts in September (presence of spermatocysts II) and is completed in 8-10 months. Prolonged oogenesis (>12 months) has previously been observed in three shallow-water sea pens (Table 4-1). Oogenesis occurs in 12 to 14 months in Virgularia juncea with all the oocytes of a specific cohort maturing and being spawned together (Soong 2005). Oogenesis in Funiculina quadrangularis (Edwards and Moore 2009) and Pennatula phoshorea (Edwards and Moore 2008) is also >12 months, with the maintenance of a pool of small oocytes year-round and release of a cohort of large oocytes annually. The latter matures from the pool of small oocytes and represents 10% of the oocytes in the polyp in F. quadrangularis and 30% in P. phosphorea. Similarly, the largest cohort of oocytes in H. finmarchica represented ~20% of the oocytes and became distinct from the pool of small oocytes (C1) in October, suggesting the initiation of final maturation in August-September (during a secondary peak of primary productivity). The bimodal pattern precludes us from assessing when new oocytes are added to the cohort of small oocytes. They may be added regularly following a continuous or quasi-continuous

pattern of oogenesis. Alternatively, oogenesis may follow the pattern described in the Antarctic sea star Odontaster validus. The sea star showed a slow oocyte growth rate during the first year (0-75 µm) and then a faster rate during the second summer (September to April, 75-150 µm) just before spawning (Pearse 1965). Therefore, the protracted oogenesis of H. finmarchica might indicate slow oocyte growth to ~400 µm diameter (roughly half the size of mature oocytes) during the first year, then a faster growth over the following 8-11 months (September to April-August, >800 µm).

Prolonged oogenesis has previously been observed in both warm/shallow-water (Benayahu and Loya 1986; Coma et al. 1995) and cold/deep-water octocorals (Orejas et al. 2002; Orejas et al. 2007; Mercier and Hamel 2011) as well as in other taxa (Hamel et al. 1993; Pearse and Bosch 2002; Kennedy et al. 2010). It has been suggested to support the development of large oocytes (Yamazato et al. 1981); however, the existence of species able to produce similarly large or larger oocytes in one year or less challenges this assumption (Benayahu and Loya 1986). For example, oocytes of the sea pen Anthoptilum grandiflorum reach 1100 µm in one year (Baillon et al. 2014, Chapter 3), while oocytes of H. finmarchica need >12 months to reach 1000 µm. These species are sympatric and presumably have access to the same resources, minimizing the role of food limitation as an explanatory factor. Protracted oogenesis was also associated with highly fecund species, exhibiting synchronous maturation and a brief spawning period (Benayahu and Loya 1986). However, H. finmarchica exhibits low fecundity (~6 mature oocytes polyp$^{-1}$).

Different strategies in the allocation of energy for reproduction might best explain contrasting oogenic patterns among species. H. finmarchica exhibits low polyp fecundity

(ERF) with the maintenance of a pool of small oocytes that will mature in the following year. Part of its energy is thus allocated to final oogenic maturation while another part is allocated to the synthesis of oocytes forming the reserve pool. In contrast, A. grandiflorum favours higher fecundity (ERF) at the polyp level with the maturation of ~21% of the initial oocytes over the annual cycle (Baillon et al. 2014, Chapter 3), consistent with the investment of all available energy in oogenic maturation. Three different processes have been described for yolk synthesis (i.e. storage product in the oocytes): autosynthetic (yolk is synthesized by the oocyte itself), heterosynthetic (yolk is synthesized by other cells around the oocytes) and a combination of autosynthetic and heterosynthetic routes (Wourms 1987). During heterosynthetic synthesis, the material can be stored in advance, leading to a more rapid oocyte maturation than for oocytes depending on autosynthetic production (Jaeckle 1995). So far, only the shallow-water sea pen Pennatula aculeata was described as heterosynthetic (Eckelbarger et al. 1998); however no other study of this nature exists on corals. Therefore, differences in the tempo of oocyte maturation might be due to differences in the way yolk is synthesised; they might also be due to differences in energy stored in the oocytes. Correlation between energy content and oocyte size exists over broad scales (inter-specific differences); however, at smaller scales (e.g. intra-species comparison of egg energy content) this correlation is not as clear and the energy content can vary among oocytes of similar size (Moran and McAlister 2009). For example, the oocytes of the shallow-water green sea urchin Strongylocentrotus droebachiensis showed different energy contents depending on different food rations to adults, while oocyte sizes did not differ (Thompson 1983).

Precise analyses of oogenic pathways will be necessary to determine the influence of these parameters on oocyte maturation.

The co-occurrence of post-spawning and mature female colonies suggests a delay in oogenesis among colonies, as suggested in previous studies (Lopes et al. 2012; Baillon et al. 2014). While uniform spermatocyst growth occurs over the annual cycle, the presence of two temporary cohorts of spermatocysts was detected just before or during the spawning months, most likely due to a slight asynchrony in reaching full spermatocyst size. Some spermatocysts might become mature slightly earlier than others and release spermatozoa as the first females spawn, while other batches continue to grow to be released slightly later, ensuring fertilization throughout the few weeks of spawning (slight asynchrony among females) in most geographic regions studied.

The present study supports the occurrence of seasonal reproduction in deep-sea pennatulaceans as a function of phytodetritus production and temperature (Baillon et al. 2014, Chapter 3). Other studies on deep-sea pennatulaceans, including Anthoptilum murrayi (Pires et al. 2009), Pennatula aculeata (Eckelbarger et al. 1998) and Kophobelemnon stelliferum (Rice et al. 1992),have inferred "continuous" spawning or non-seasonal reproduction (Table 4-1) from the presence of different oocyte stages in the same polyp. Originally, the term "continuous" was used to describe non-seasonal reproduction due to the absence of synchrony among populations or individuals in a population (Tyler and Young, 1992). However, retention of mature oocytes until release at a specific time is also possible (Eckelbarger and Watling, 1995). Here we showed that the simultaneous presence of distinct oocyte cohorts (or multiple oogenic stage) did not prevent the identification of discrete spawning period(s) in H. finmarchica. This was

partly made possible by the fairly large sampling effort in different months of the year, compared with previous studies on deep-water sea pens, which were based on <40 colonies, from a mix of depth and regions, and on a limited period of time [4 months for A. murrayi (Pires et al., 2009), 1 week for P. aculeata (Edwards and Moore, 2008)] or a combination of several years [between 1977 and 1986 for K. stelliferum(Rice et al., 1992)]. All these parameters are known to influence the reproductive cycle (Giese 1959; Mercier and Hamel 2009b; Baillon et al. 2011). We therefore advocate that, when continuous or quasi-continuous gametogenesis is detected, spawning periodicity should be assessed separately (e.g. from a drop in MSI, or disappearance of mature oocytes), or otherwise characterized as undetermined.

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## Tables

Table 4-1 Review of oogenic patterns, spawning period, sexuality, reproductive strategies and oocyte size in pennatulacean corals from shallow and deep waters. Empty cases indicate the absence of information.

| Species | Region | n | Depth range | Oogenesis | Spawning period | Sexuality | Sex ratio (M:F) | Reproductive strategy | Maximum oocyte | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Anthoptilum grandiflorum | Northwest Atlantic (Canada) | 174 | 176-1347 m | ~12 months | Annual (Apr to Jul – according to region) | Gonochoric (observation of 1 polyp hermaphrodite) | 1:1 | Broadcast spawner | 1100 µm | (Baillon et al. 2014, Chapter 3) |
| Anthoptilum murrayi | Southwestern Atlantic (Brazil) | 24 | 1300-1799 m | (2 cohorts) | Continuous (?) | Gonochoric | 1:1 | Broadcast spawner | 1200 µm | Pires et al. (2009) |
| Funiculina quadrangularis | West Scotland (UK) | ~180 | 18.9-24.3 m | > 12 months (2 cohorts) | Annual (Oct-Jan) | Gonochoric | 1:1 | Broadcast spawner | 900 µm | Edwards and Moore (2009) |
| Halipteris finmarchica | Northwest Atlantic (Canada) | 63 | 256 - 1161 m | > 12 months | Annual (Apr to Aug – according to region) | Gonochoric | 1:1 | Broadcast spawner | 1000 µm | Present study |
| Kophobelemnon stelliferum | Northeast Atlantic (Porcupine Seabight) | 40 | 400-1000 m | Asynchronous | Non-seasonal (?) | Gonochoric | 1:1 | Broadcast spawner | 800 µm | Rice et al. (1992) |
| Malacobelemnon daytoni | Antartica | | 15-22 m | < 12 months | | Gonochoric | 1:1 | Broadcast spawner | 350 µm | Servetto et al. (2013) |
| Pennatula aculeata | Northwest Atlantic (Gulf of Maine, USA) | 22 | 113-231 m | | Continuous (?) | Gonochoric | 1:1 | Broadcast spawner | 880 µm | Eckelbarger et al. (1998) |
| Pennatula phosphoarea | West Scotland (UK) | ~180 | 18.2-19.9 m | > 12 months (2 cohorts) | Annual (Jul-Aug) | Gonochoric | 1:1 | Broadcast spawner | 580 µm | Edwards and Moore (2008) |

Table 4-1 continued

| Species | Region | n | Depth range | Oogenesis | Spawning period | Sexuality | Sex ratio (M:F) | Reproductive strategy | Maximum oocyte | References |
|---|---|---|---|---|---|---|---|---|---|---|
| Ptilosarcus guerneyi | Seattle (USA) | 15 | shallow water | | Annual (Mar) | Gonochoric | | Broadcast spawner | 600 µm | Chia and Crawford (1973) |
| Renilla koellikeri | Southern California (USA) | | subtidal | | Annual (May-Jul) | Gonochoric | | Broadcast spawner | 400 µm | Tremblay et al. (2004) |
| Renilla reniformis | Northwest Atlantic (North Carolina, USA) | | shallow water | | Annual (May-Jul) | Gonochoric | | Broadcast spawner | 350 µm | Wilson (1883) |
| Vetetillum cynomorium | Atlantic Northeast (Portugal) | 210 | intertidal zone | | Annual (Jul) | Gonochoric | 1:1.7 | Broadcast spawner | 967 µm | Lopes et al. (2012) |
| Virgularia juncea | Tawain Strait (China) | ~240 | intertidal zone | 12-14 months | Annual (Aug-Sep) | Gonochoric | | Broadcast spawner | 250 µm | Soong (2005) |

Table 4-2 Sex ratio analyses on colonies of Halipteris finmarchica collected from the various regions under study.

| Region | Females | Males | $\chi^2$ | p |
|---|---|---|---|---|
| Laurentian Channel (LC) | 2 | 2 | -- | -- |
| Grand Banks (GB) | 13 | 8 | 1.19 | 0.275 |
| Flemish Cap (FC) | 7 | 13 | 1.80 | 0.180 |
| North Newfoundland (NNL) | 0 | 1 | -- | -- |
| Labrador (LB) | 5 | 1 | -- | -- |
| Lower Arctic (LA) | 4 | 1 | -- | -- |
| Total | 31 | 26 | 0.44 | 0.508 |

Table 4-3 Halipteris finmarchica. Mean gamete size (μm) corresponding to peaks of cohorts determined by the mixture distribution analysis. n: number of colony, π: proportion occupied by cohort, SD: standard deviation, df: degree of freedom, $\chi^2$: chi-square, p: p-value.

| | | 1st peak | | | 2nd peak | | | Goodness-of-fit | | |
|---|---|---|---|---|---|---|---|---|---|---|
| sex | n | π | mean ± SE (μm) | SD | π | mean ± SE (μm) | SD | df | $\chi^2$ | p |
| January | | | | | | | | | | |
| F | | | | | | | | | | |
| M | 1 | 1.00 | 182.3 ± 3.8 | 36.4 | | | | 6 | 0.53 | 0.997 |
| April | | | | | | | | | | |
| F | 2 | 0.91 | 211.3 ± 8.5 | 79.4 | 0.09 | 675.1 ± 13.5 | 37 | 1 | 15.3 | 0.169 |
| M | 2 | 1.00 | 344.4 ± 9.4 | 93.3 | | | | 13 | 3.75 | 0.994 |
| May | | | | | | | | | | |
| F | 3 | 0.76 | 193.3 ± 9.4 | 78.27 | 0.24 | 727.0 ± 18.2 | 85 | 16 | 9.94 | 0.870 |
| M | 4 | 1.00 | 384.3 ± 10.5 | 104.3 | | | | 12 | 10.1 | 0.611 |
| July | | | | | | | | | | |
| F | 5 | 0.98 | 143.6 ± 5.9 | 56.58 | 0.02 | 851.6 ± 38.3 | 56 | 14 | 8.41 | 0.867 |
| M | | | | | | | | | | |
| August | | | | | | | | | | |
| F | 4 | 0.80 | 219.7 ± 11.3 | 93.9 | 0.20 | 788.4 ± 22.9 | 94 | 16 | 6.73 | 0.978 |
| M | 1 | 1.00 | 414 ± 10.9 | 108.4 | | | | 12 | 14.8 | 0.252 |
| October | | | | | | | | | | |
| F | 12 | 0.74 | 153.7 ± 9.0 | 53.1 | 0.26 | 448.9 ± 29.1 | 98 | 14 | 1.98 | 1.000 |
| M | 11 | 1 | 150.6 ± 4.6 | 44.5 | | | | 8 | 2.32 | 0.970 |
| November | | | | | | | | | | |
| F | 3 | 0.74 | 162.4±10.4 | 60.1 | 0.26 | 467 ± 24.9 | 84 | 12 | 3.58 | 0.990 |
| M | 6 | 1 | 162.7 ± 6.4 | 62.5 | | | | 12 | 7.31 | 0.837 |
| December | | | | | | | | | | |
| F | 2 | 0.74 | 160.3 ± 8.5 | 60.9 | 0.26 | 499.5 ± 5.6 | 65 | 11 | 5.24 | 0.919 |
| M | 1 | 1 | 149.8 ± 3.6 | 33.7 | | | | 5 | 3.44 | 0.63 |

## Figures

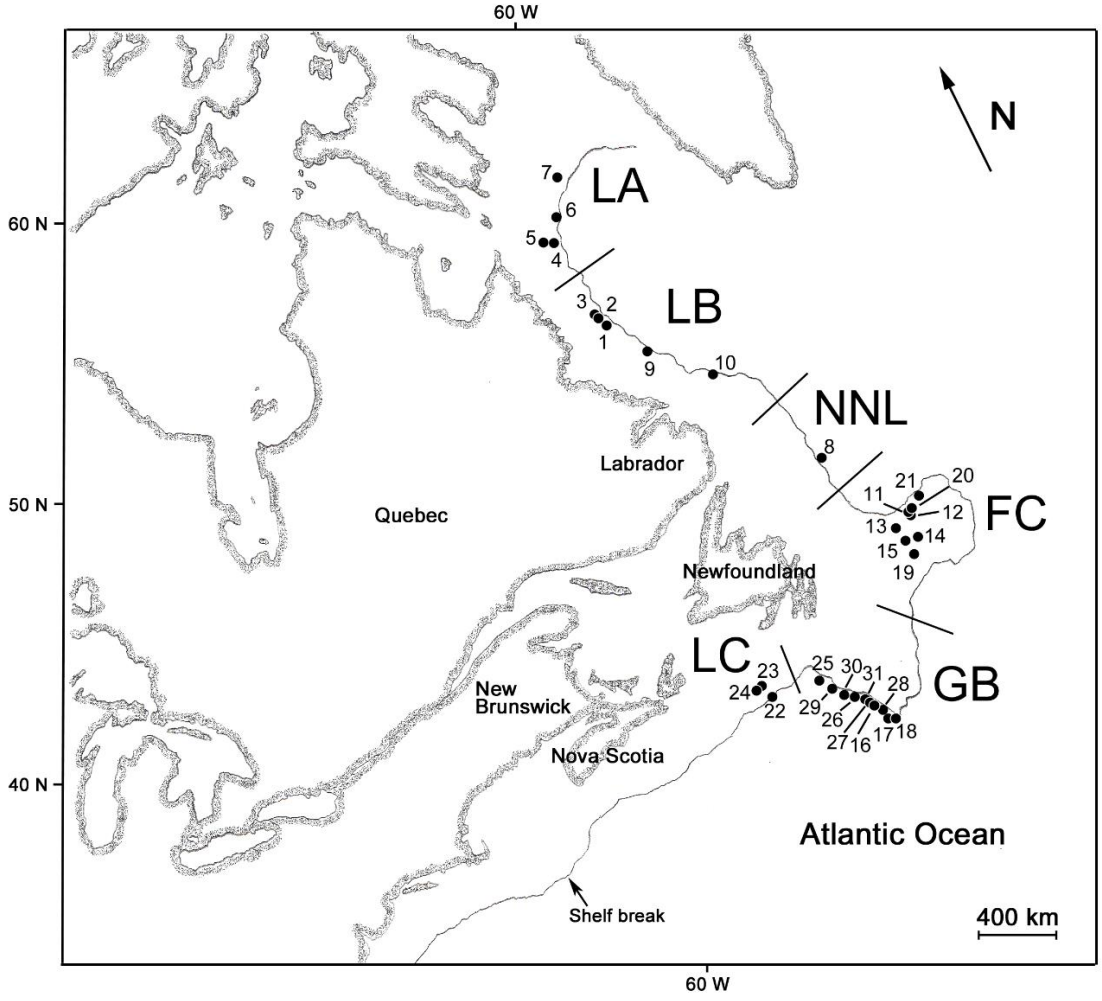



Figure 4-1 Map showing the different sites where colonies of Halipteris finmarchica were collected (sample numbers detailed in Appendix 4-A). LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador and LA: Lower Arctic.

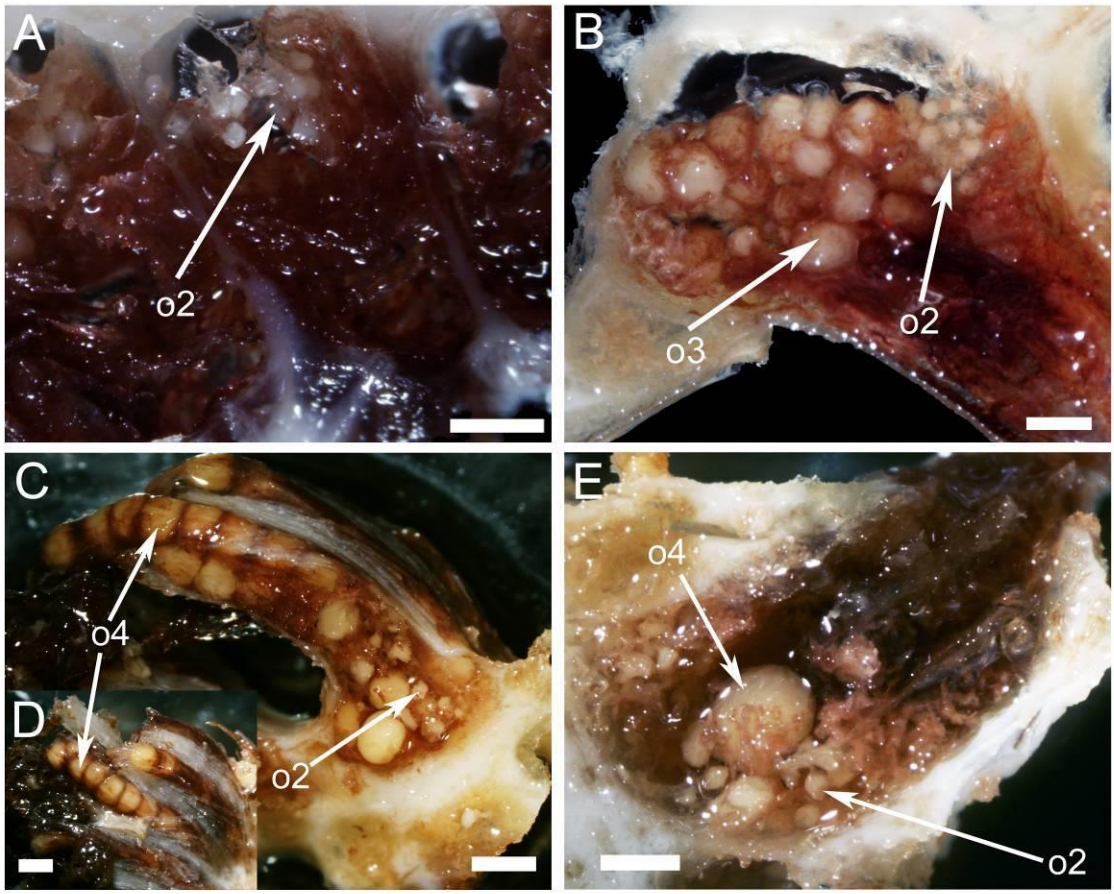



Figure 4-2 Micro-dissection of female colony of Halipteris finmarchica showing the different oogenic stages in the polyp: (A) oocytes II at the base of the polyp, (B) oocytes III starting to fill the gastro-vascular cavity with oocytes II at the base of the polyp, (C) oocytes IV filling the gastro-vascular cavity with the presence of oocytes II at the base of the polyp, (D) external view of the oocytes IV filling the gastro-vascular cavity, (E) residual oocyte IV after spawning with the presence of oocytes II at the base of the polyp. Scale bars represent 500 µm in A, B, 1 mm in C, D and E. o2: oocyte stage II, o3: oocyte stage III, o4: oocyte stage IV.

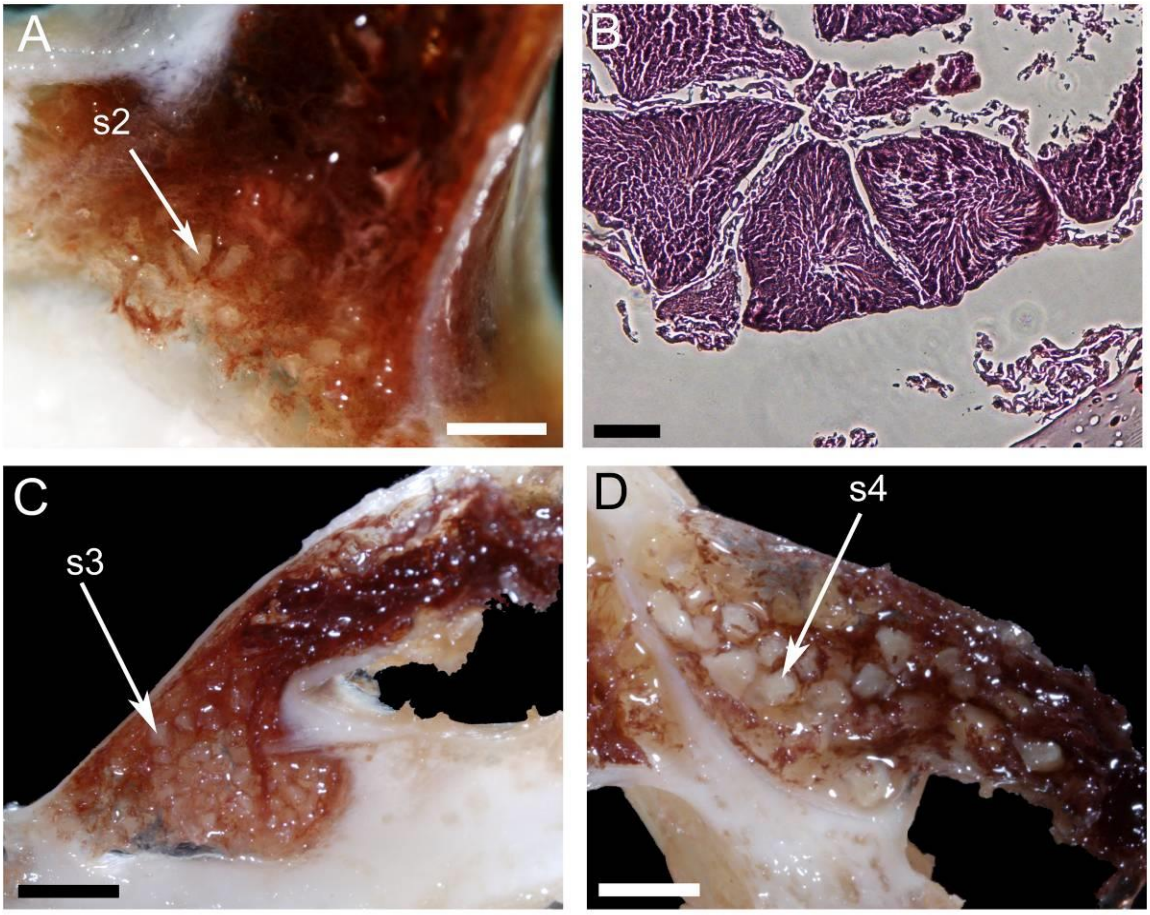



Figure 4-3 Micro-dissection and histology of male colony of Halipteris finmarchica showing the different spermatogenic stages in the polyp: (A) spermatocyst II at the base of the polyp, (B) histology of spermatocysts II, (C) spermatocyst III starting to fill the gastro-vascular cavity, (D) spermatocyst IV filling the gastro-vascular cavity. Scale bars represent 500 µm in A, 100 µm in B, 1 mm in, C and D. s2: spermatocyst stage II, s3: spermatocyst stage III, s4: spermatocyst stage IV.

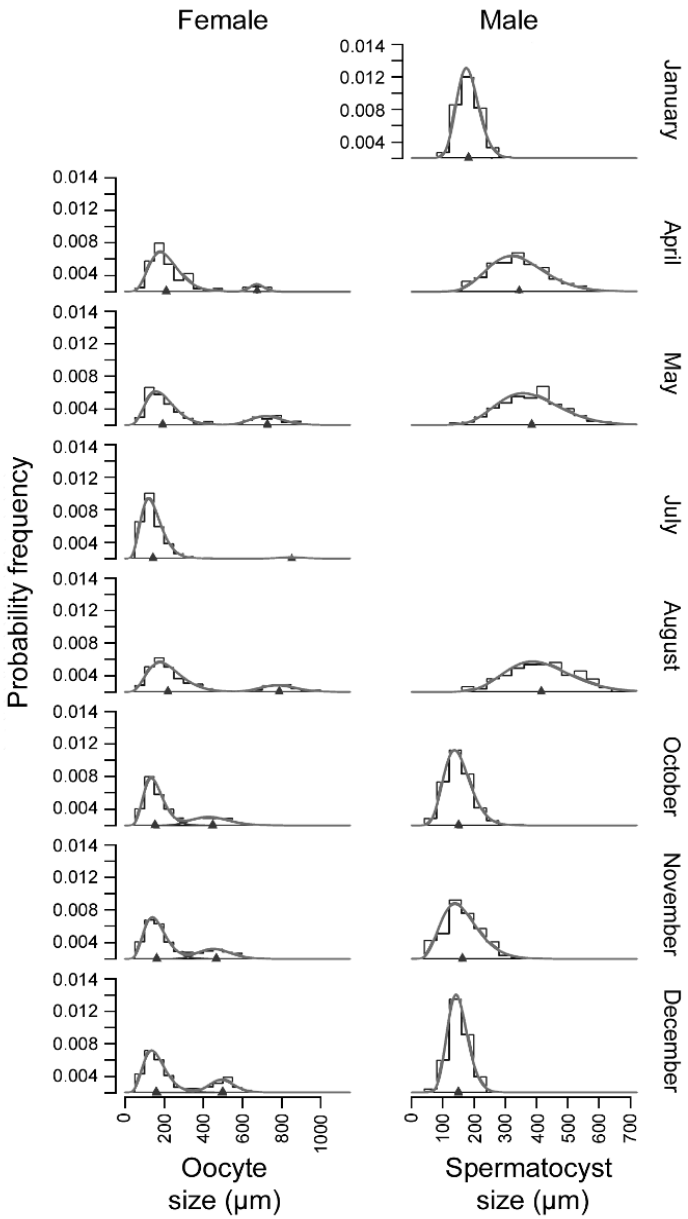
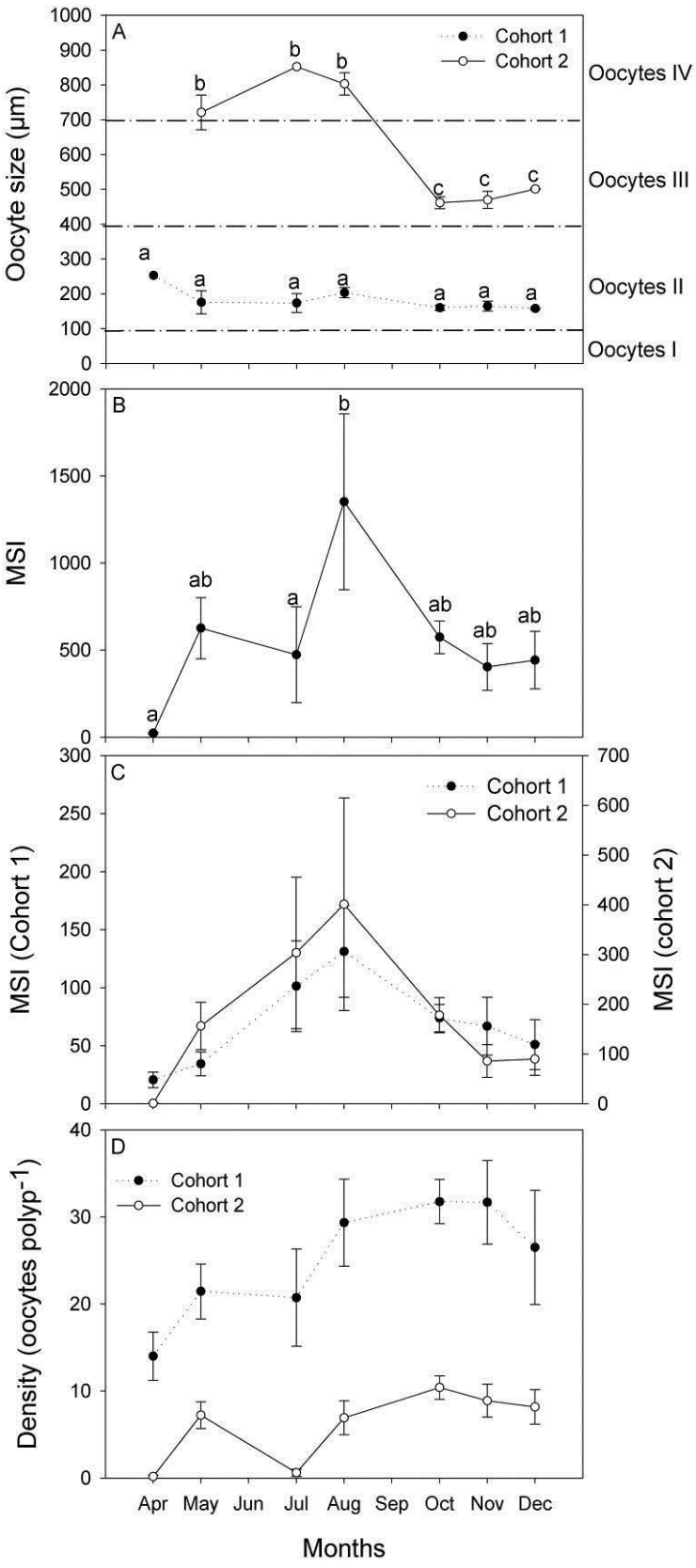



Figure 4-4 Oocyte and spermatocyst size-probability frequency distributions of Halipteris finmarchica coupled with the modeled cohort structure determined using the mixture distribution analyses. Colonies were pooled per months. The triangles indicate the mean size of each cohort.

Figure 4-5 (Following page) Halipteris finmarchica. Annual variation in (A) oocyte size at peak of modeled cohorts 1 and 2 with the corresponding oocyte stages (right axis), (B) maturity stage index (MSI) measured using all oocytes, (C) MSI measured separately in cohorts 1 (left axis) and 2 (right axis) and (D) density of oocytes in female colonies (mean±SE). Values with corresponding letters are not significantly different (see statistical result in the text).


A
Cohort 1
Cohort 2
Oocytes IV
Oocytes III
Oocytes II
Oocytes I
Oocyte size (µm)
B
MSI
C
MSI (Cohort 1)
MSI (cohort 2)
D
Density (oocytes polyp-1)
Apr
May
Jun
Jul
Aug
Sep
Oct
Nov
Dec
Months

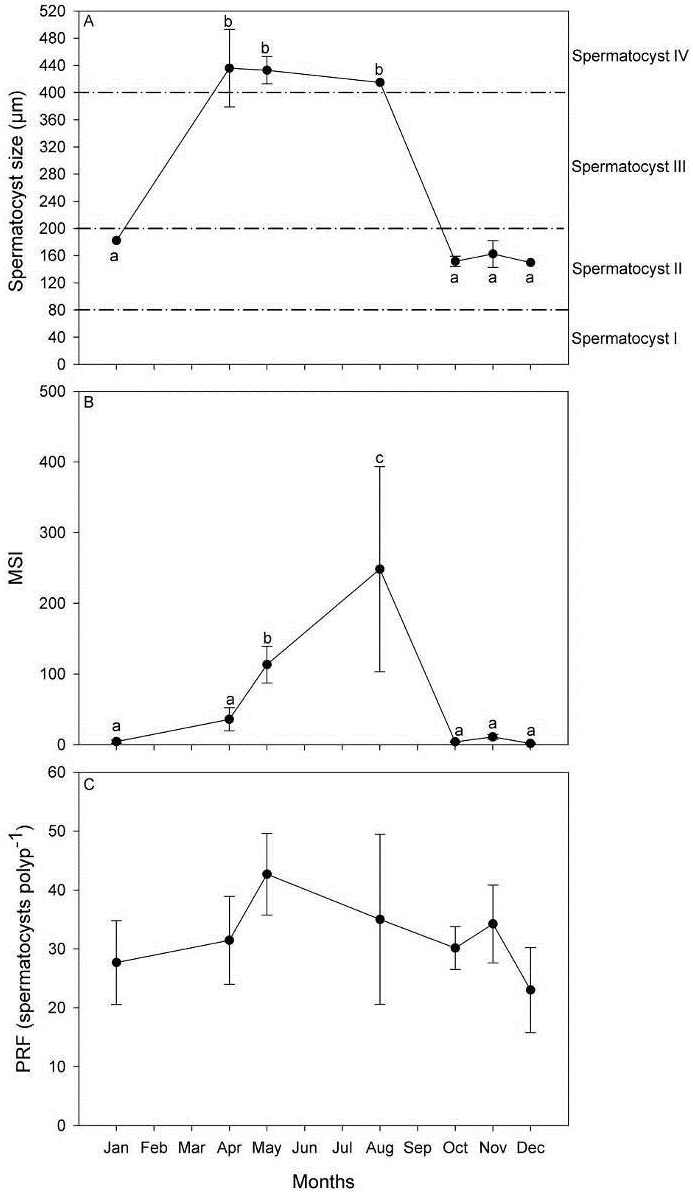



Figure 4-6 Halipteris finmarchica. Annual variation in (A) spermatocyst size at peak of modeled cohort with the corresponding spermatocyst stages (right axis), (B) maturity stage index (MSI) measured using all spermatocysts and (C) potential relative fecundity (PRF) colonies (mean±SE). Values with corresponding letters are not significantly different (see statistical result in the text).

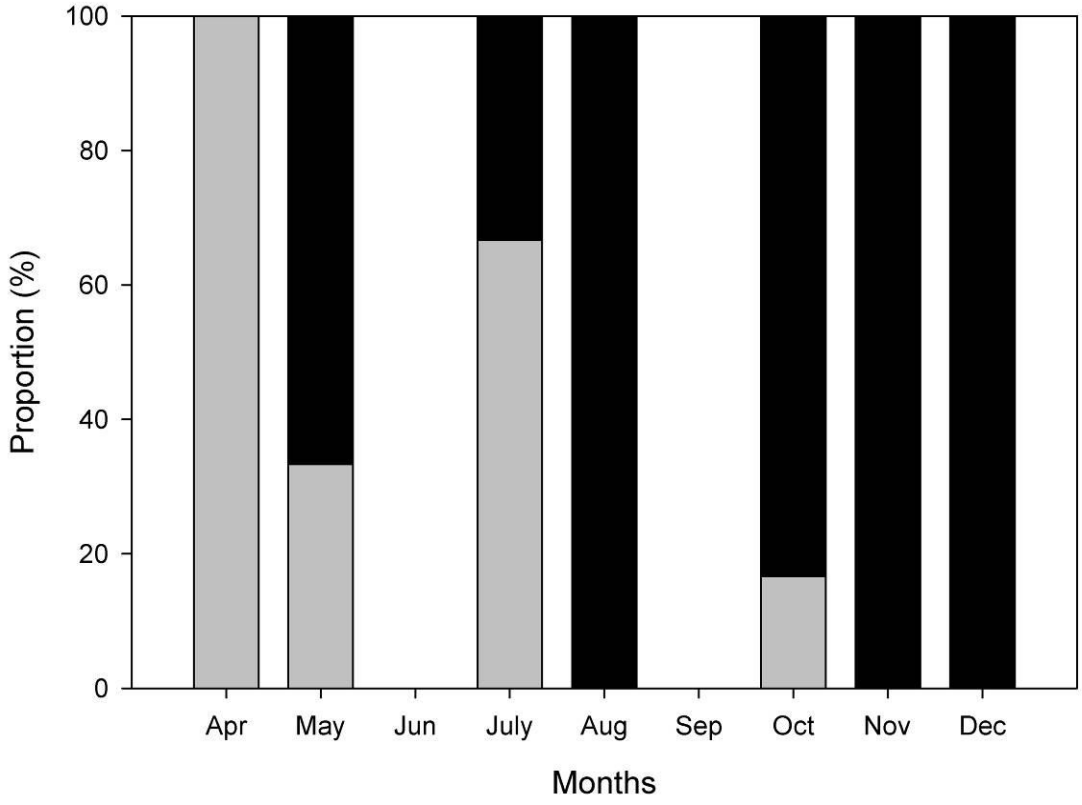
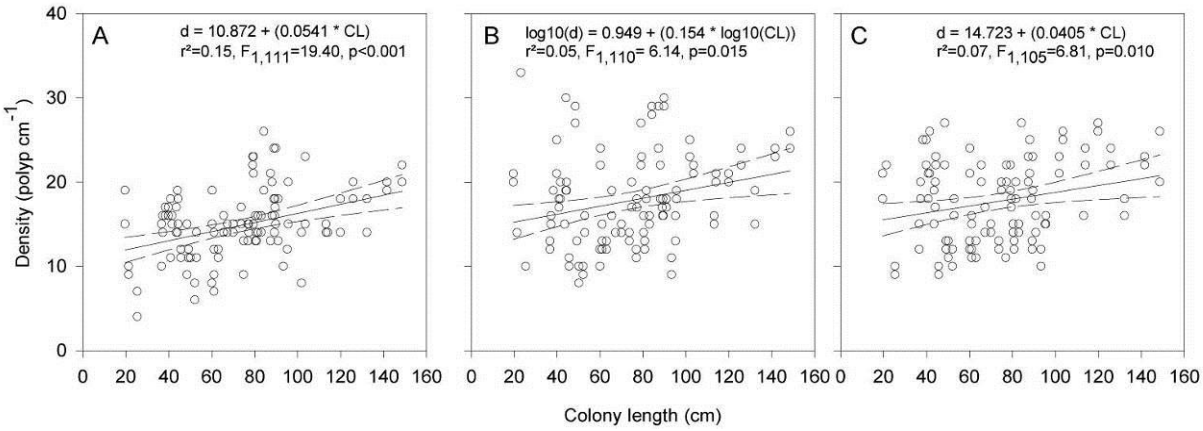



Figure 4-7 Proportion of female colonies of Halipteris finmarchica harbouring a single cohort (C1) of oocytes (grey bars) or harbouring two cohorts of oocytes (C1 and C2; black bars) in the different sampling months.

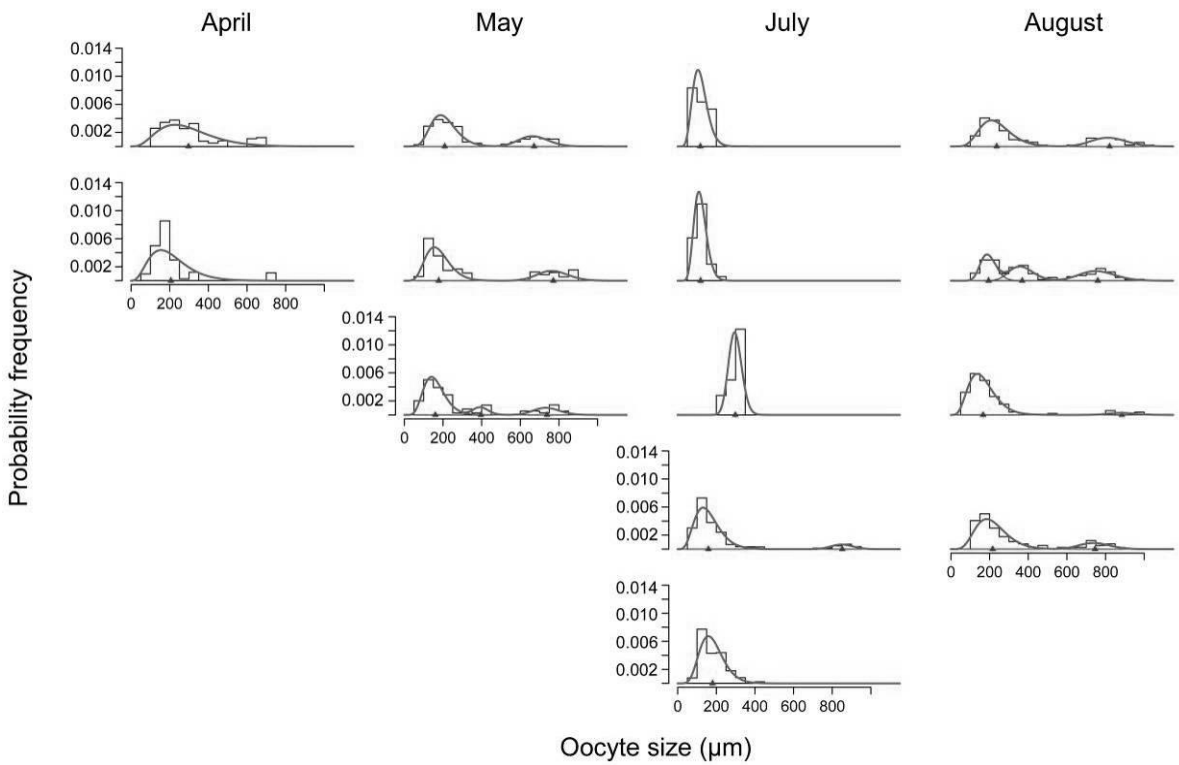
## Supplementary materials

Appendix 4-A Halipteris finmarchica. Summary of the sampling design with information on the number of female, male or non-sexed colonies analyzed. LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador, LA: Lower Arctic

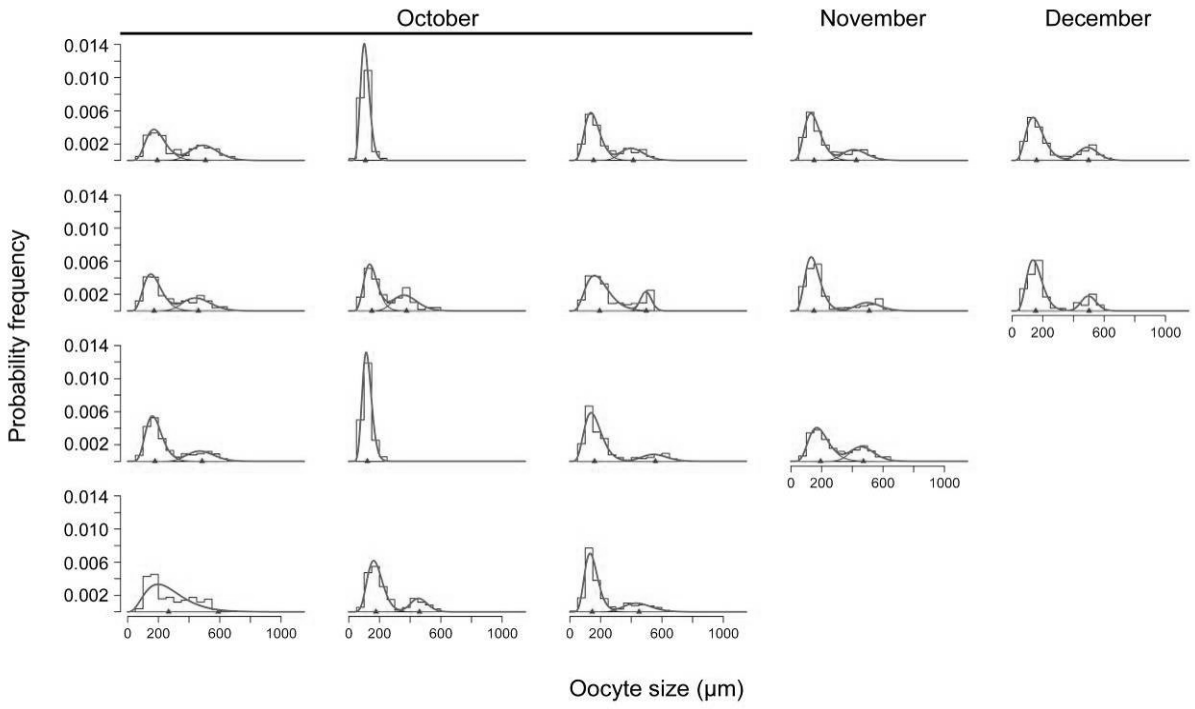
| Station | Region | Date | Depth | Latitude | Longitude | Females | Males | Non-sexed |
|---|---|---|---|---|---|---|---|---|
| 1 | LB | 28/7/2006 | 620 | 8°33'21.60"N | 59°55'12.00"W | 1 | | 1 |
| 2 | LB | 28/7/2006 | 552 | 58°57'21.60"N | 60° 2'20.40"W | 4 | | 2 |
| 3 | LB | 29/7/2006 | 452 | 59° 3'37.80"N | 60° 7'42.60"W | 1 | | |
| 4 | LA | 4/8/2006 | 563 | 60°45'57.60"N | 62° 5'36.60"W | | 1 | |
| 5 | LA | 4/8/2006 | 630 | 60°50'29.40"N | 62°22'21.00"W | 2 | | |
| 6 | LA | 5/8/2006 | 559 | 61°40'42.60"N | 61° 9'34.20"W | 1 | | |
| 7 | LA | 8/8/2006 | 724 | 63°42'14.40"N | 59°33'5.40"W | 1 | | |
| 8 | NNL | 27/1/2006 | 918 | 50°59'16.80"N | 49°50'52.80"W | | 1 | |
| 9 | LB | 09/10/2006 | 424 | 56°36'10.80"N | 58°19'39.00"W | | | 1 |
| 10 | LB | 2/11/2006 | 256 | 54°45'30.60"N | 54°11'16.80"W | | 1 | |
| 11 | FC | 28/11/2006 | 1125 | 47°58'33.60"N | 46°11'9.60"W | | 3 | 1 |
| 12 | FC | 28/11/2006 | 1073 | 47°51'32.40"N | 46°10'42.60"W | 1 | 1 | |
| 13 | FC | 28/11/2006 | 788 | 47°44'36.60"N | 46°54'39.60"W | 1 | | |
| 14 | FC | 30/11/2006 | 780 | 47°23'33.00"N | 46°22'57.00"W | 1 | 1 | |
| 15 | FC | 1/12/2006 | 1161 | 47°24'18.00"N | 46°45'41.40"W | 2 | 1 | |
| 16 | GB | 8/10/2007 | 823 | 43°19'39.00"N | 51°46'44.40"W | 4 | | |
| 17 | GB | 8/10/2007 | 1094 | 43°12'39.60"N | 51°39'39.60"W | 1 | | |
| 18 | GB | 8/10/2007 | 796 | 43°13'8.40"N | 51°34'3.00"W | | 1 | |
| 19 | FC | 18/10/2007 | 1036 | 46°48'21.60"N | 47° 6'7.20"W | | 2 | |
| 20 | FC | 19/10/2007 | 743 | 48° 2'51.00"N | 46° 8'60.00"W | | 1 | |
| 21 | FC | 29/10/2007 | 992 | 48°33'41.40"N | 45°30'30.60"W | 2 | 4 | |
| 22 | LC | 20/4/2007 | 377 | 44°50'15.00"N | 56° 9'27.00"W | | 1 | |
| 23 | LC | 29/4/2007 | 366 | 45°24'3.60"N | 56°36'18.00"W | 1 | 1 | |
| 24 | LC | 30/4/2007 | 389 | 45°19'8.40"N | 56°42'45.00"W | 1 | 1 | |
| 25 | GB | 4/5/2007 | 575 | 44°47'2.40"N | 54°25'24.60"W | 2 | 2 | |
| 26 | GB | 17/5/2007 | 425 | 43°48'3.60"N | 52°26'6.00"W | 1 | | |
| 27 | GB | 17/5/2007 | 597 | 43°37'8.40"N | 52°11'33.00"W | 1 | 1 | |
| 28 | GB | 18/5/2007 | 625 | 43°18'36.00"N | 51°38'24.00"W | | 1 | |
| 29 | GB | 28/10/2007 | 599 | 44°22'17.40"N | 53°22'48.00"W | | 1 | |
| 30 | GB | 29/10/2007 | 579 | 44° 5'56.40"N | 52°56'24.00"W | 2 | | |
| 31 | GB | 30/10/2007 | 623 | 43°37'12.00"N | 52°11'56.40"W | 2 | 1 | |



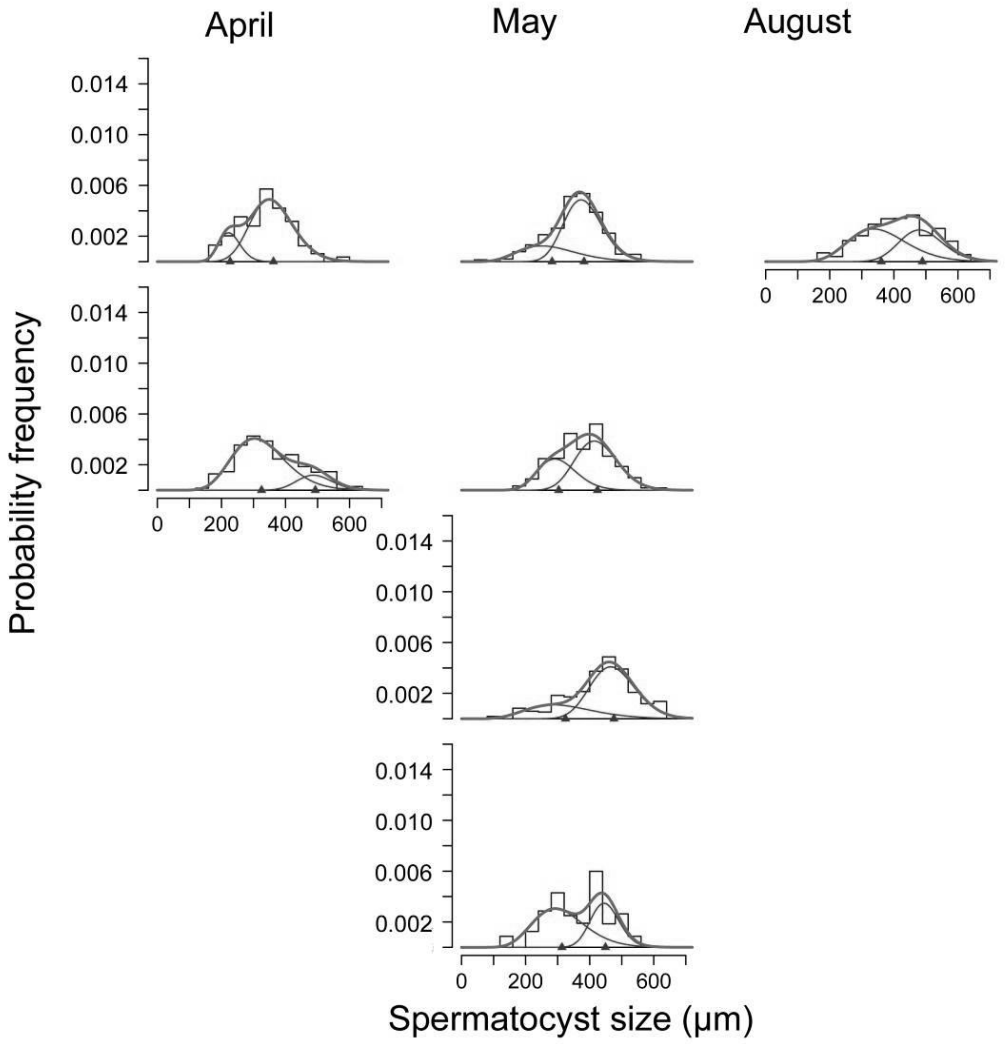

Appendix 4-B Halipteris finmarchica. Linear regression of polyp density (d, polyp $cm^{-1}$) with colony length (CL, cm) for the three sections of the rachis, (A) lower section, (B) middle section, (C) upper section



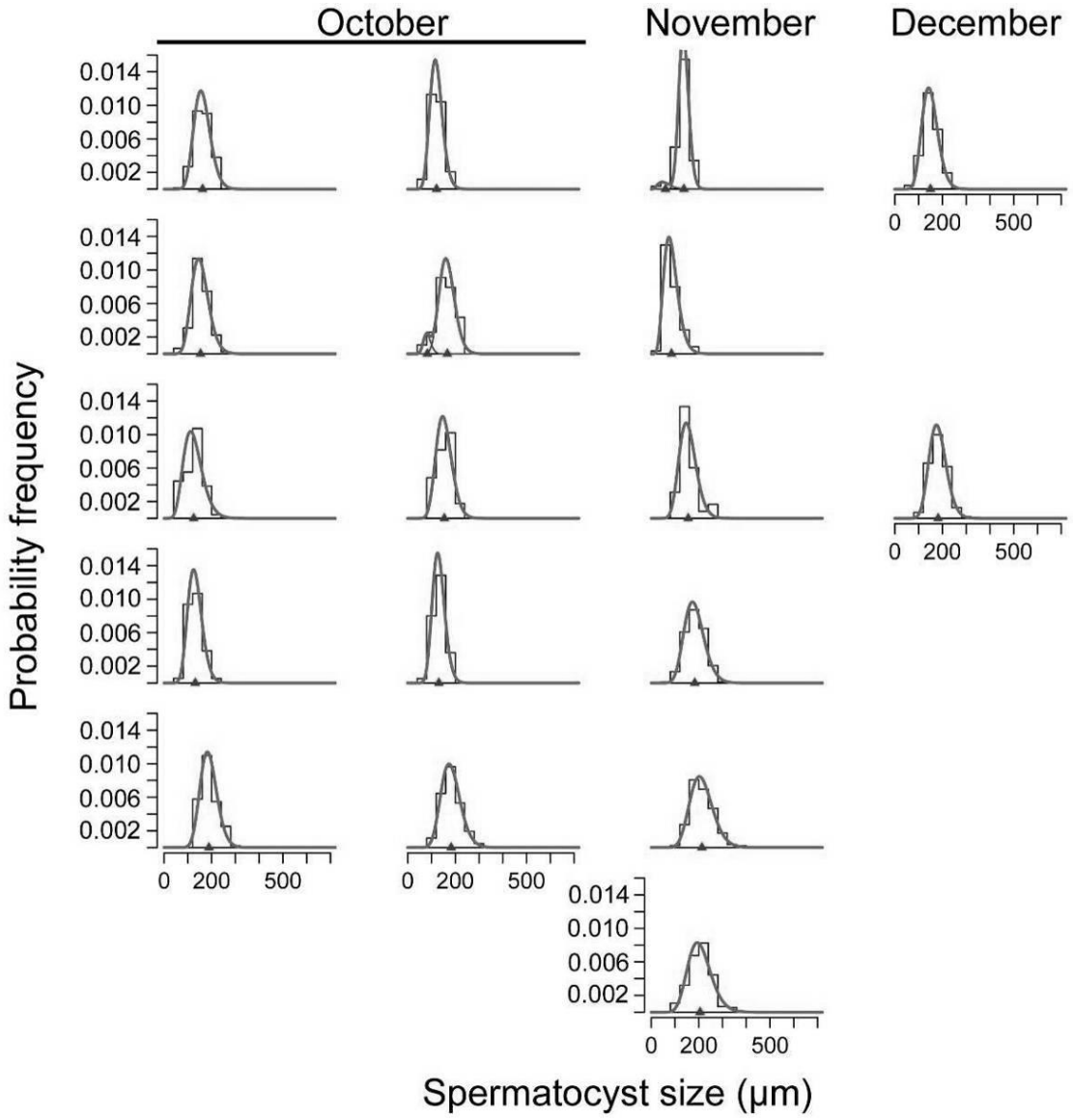

Appendix 4-C Oocyte size-probability frequency distribution of each colony Halipteris finmarchica sampled between April and August coupled with the modeled cohort structure determined using mixture distribution analyses. The triangles indicate the mean size of each cohort.




Appendix 4-D Oocyte size-probability frequency distribution of each colony Halipteris finmarchica sampled between October and December coupled with the modeled cohort structure determined using the mixture distribution analyses. The triangles indicate the mean size of each cohort.




Appendix 4-E Spermatocyst diameter size-probability frequency distribution of each colony of Halipteris finmarchica sampled between April and August coupled with the modeled cohort structure determined using the mixture distribution analyses. The triangles indicate the mean size of each cohort.




Appendix 4-F Spermatocyst diameter size-probability frequency distribution of each colony of Halipteris finmarchica sampled between October and December coupled with the modeled cohort structure determined using the mixture distribution analyses. The triangles indicate the mean size of each cohort.

# Chapter 5 : Diversity and distribution of species associated with deep-sea pennatulacean corals in the Northwest Atlantic

## Abstract

Anthoptilum grandiflorum and Halipteris finmarchica are two deep-sea corals (Octocorallia: Pennatulacea) common on soft bottoms in the North Atlantic where they are believed to act as biogenic habitat. To assist conservation efforts, this study examines spatial and temporal patterns in the abundance, diversity, and nature of their associates in eastern Canada. A total of 16 species were found in association with (in, on or close to) A. grandiflorum and 6 species in association with H. finmarchica. Rarefaction analyses suggest that more species might be found in association with A. grandiflorum while most species interacting with H. finmarchica have been sampled. Biodiversity of associates increases from northern to southern locations but does not vary with depth. Seasonal patterns show higher biodiversity during spring/summer due to the transient presence early life stages of fishes and shrimps. Two distinct endoparasitic species of highly modified copepods (families Lamippidae and Corallovexiidae) commonly occur in the polyps of A. grandiflorum and H. finmarchica, and a commensal sea anemone frequently associates with H. finmarchica. Stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) indicate that A. grandiflorum and H. finmarchica, together with the ectobiotic sea anemone, likely share a diet of small invertebrates. The trophic level of the parasitic copepods suggests that they feed on host tissue and/or compete for their host's food. Overall, the diversity of obligate/permanent associates of sea pens is moderate; however the presence of mobile/transient associates highlights an ecological role that has yet to be fully elucidated and supports their key contribution to the enhancement of biodiversity in the Northwest Atlantic.

# 1 Introduction

Corals form one of the most complex biological habitats of the deep sea, offering a variety of microhabitats that serve as feeding, shelter, foraging and spawning sites to other species (Buhl-Mortensen and Mortensen 2005; Longo 2005; Murillo et al. 2011; Baillon et al. 2012). Deep-sea corals occur as unitary forms (i.e. composed of a single polyp) or colonial forms (i.e. composed of many polyps), and can be sparsely distributed or form fields, large thickets and even reefs that may stretch 300 m high and several kilometres wide (Buhl-Mortensen and Mortensen 2005; Longo 2005; Roberts et al. 2006). A good understanding of deep-sea corals and their associated fauna, i.e. the organisms that live in or on the corals (Buhl-Mortensen and Mortensen 2005), is essential to evaluate the importance of these unique deep-sea ecosystems and to implement adequate measures for their conservation (Buhl-Mortensen and Mortensen 2004b).

Studies of the associated fauna of deep-sea corals showed that biodiversity around deep-sea corals can be comparable to that of tropical coral reefs and that main associates include crustaceans, molluscs, echinoderms, cnidarians, sponges, polychaetes and fishes (Krieger and Wing 2002; Buhl-Mortensen and Mortensen 2004a; Buhl-Mortensen et al. 2010; Baillon et al. 2012). Buhl-Mortensen and Mortensen (2004a) catalogued 983 invertebrate species associated with 74 species of deep-sea soft and hard corals; 114 of the associates were characterized as symbionts (living in a close relationship with the coral host) of which 53% were parasites (detrimental to the host) and 47% were commensals (having no consequence for the host). Deep-sea coral feed on zooplankton and phytodetritus based on analyses of $\delta^{13}C$ and $\delta^{15}N$ (Sherwood et al. 2008; Carlier et al.

2009) as indicators of food sources and trophic levels, respectively (Fry 1988). However, to our knowledge, trophic relationships between deep-sea corals and their associated species have not been explicitly studied.

When considering all deep-sea octocorals (excluding Pennatulacea and Helioporacea), a total of 59 intimate associates (symbionts) have been described so far: 49 commensals and 10 parasites in association with 42 coral species (Watling et al. 2011). Only one study that we are aware of has analyzed the associates of deep-sea octocorals in the Northwest Atlantic (in gorgonians). A total of 114 associates (47 species on Paragorgia arborea and 97 species on Primnoa resedaeformis) were found with 2 characterized as parasitic species (Buhl-Mortensen and Mortensen, 2005). The older sections of gorgonian corals, corresponding to the skeletons devoid of tissues, exhibited the highest macrofaunal biodiversity (Buhl-Mortensen and Mortensen 2005). Sea pens (order Pennatulacea) are typically not afforded the attention of other deep-sea corals (Buhl-Mortensen and Mortensen 2004a; Watling et al. 2011) even though they are very common and have been identified as vulnerable species in both shallow and deep environments (NAFO 2008; Donaldson et al. 2010; Murillo et al. 2011).Moreover, sea pens can be collected whole, allowing precise determination/quantification of faunal species living in, on or around them, which is not always the case with larger or more fragile corals (e.g. gorgonians) for which analyses of colony fragments is often the rule.

Sea pens are "structural" species due to their extension above the seafloor (Troffe et al. 2005) and have been suggested to create complex biohabitat (Buhl-Mortensen et al., 2010). However, except few studies showing the presence of adult fish in large field of sea pens (Brodeur 2001), no clear evidence showed that they form a biogenic habitat.

According to Etnoyer et al. (2005), the majority of the species forming biogenic habitat exhibit complex morphology (e.g. branches) and a sufficient size to provide substrate or refuge for other species. Sea pens do not correspond to this definition but they have already been shown to serve as biogenic substrate for different species (Dalyell 1848, Buhl-Mortensen et al. 2010, Nygren and Pleijel 2010) and serve as nursery habitat for fish larvae (Baillon et al. 2012). Moreover, sea pens can cover extensive areas in the deep sea, and are sometimes found in high densities (Baker et al. 2012), occurring on mud or sand flats, where they could provide an important structural biohabitat to other organisms (Tissot et al. 2006) in relatively featureless environments.

Buhl-Mortensen et al. (2010) noted that there seemed to be few species associated with sea pens, but that was plausibly due to a lack of data. In their review, they only mentioned the association between the ophiuroid Asteronyx loveni and the sea pen Funiculina quadrangularis. Other associates were described including a copepod parasite in Anthoptilum grandiflorum (Laubier 1972) in the Labrador Sea (1210 m depth) and a polychaete living between the polyps of Funiculina quadrangularis (Nygren and Pleijel 2010) along the Swedish west coast (300 m depth). More associated species have been found in, on or around shallow-water sea pens, including different parasitic copepods on various host species (Bouligand 1965; Laubier 1972; Humes 1978, 1985), the gametophyte of an algae living inside the tissues of Ptilosarcus guerneyi (Dube and Ball 1971), and the hydrozoan Eudendrium ramosum on Virgularia mirabilis (Dalyell 1848). At least 5 symbionts were reported on Ptilosarcus guerneyi (Johnstone 1969) and a porcellanid crab was found between the leaves of Pteroeides esperi (Sankarankutty 1961).

Pennatulacean corals are common on the continental slope of eastern Canada (16 species of sea pen, Gilkinson and Edinger 2009; Murillo et al. 2011). The present study focuses on two of the most common ones: Anthoptilum grandiflorum (Anthoptilidae) and Halipteris finmarchica (Halipteridae) whichwere recently found to act as essential larval fish habitat (Baillon et al. 2012). A. grandiflorum exhibits virtually a cosmopolitan distribution, with confirmed occurrences in the North and South Atlantic, North and South Pacific, Indian and Antarctic Oceans (Williams 2011) while H. finmarchica is restricted to the North Atlantic (Williams 2013). Both species are present from 100 to >2000 m (Baker et al. 2012). The main goal of this study was to better define their role and importance as biogenic substrate or habitat with the following objectives: (1) determine the diversity and abundance of their associated species, with an emphasis on spatial and temporal patterns; (2) characterize the dominant symbiotic relationships; and (3) elucidate trophic interactions between the most common associates and their hosts.

## 2 Material and Methods

### 2.1 Collection

Samples of Anthoptilum grandiflorum (from 98-1347 m) and Halipteris finmarchica (from 256-1333 m) were obtained in 2006 and 2007 as by-catch from annual research surveys (Multispecies Surveys and Northern Shrimp Research Surveys), and the At-Sea Observer Program, along the continental slope of eastern Canada (Fig. 1, Appendix 5-A and 5-B) all led by Fisheries and Oceans Canada (DFO). Additional samples collected in April and May of 2009 and 2010 were used to determine the consistent presence of some associated suspected to be particularly abundant during these

months. Colonies of A. grandiflorum and of H. finmarchica were frozen at -20 °C on board the vessels. A dozen colonies of A. grandiflorum were also maintained alive in two 1-$m^3$ tanks, kept in the dark and provided with running unfiltered seawater at a temperature between -1 and 9 °C on a substrate composed of ~15 cm of mud or fine sand.

2.2 Processing of samples

When less than three colonies were sampled at a site, all the colonies were analysed. When more than three colonies were available, a minimum of three colonies were analysed, more if needed, in order to reach a minimum of 20 % of the colonies sampled at each site. Few exceptions occurred when samples were unavailable or damaged. Overall, samples of A. grandiflorum examined included 185 colonies (meaduring 15-83.9 cm) in 2006-2007 (Appendix 5-A) and 60 colonies (19.8-76.8 cm) in 2009-2010 (Appendix 5-B). Samples of H. finmarchica consisted of 92 colonies (17.2-148.6 cm) in 2006-2007 (Appendix 5-A) and 12 colonies (15.8-94.0 cm) in 2009-2010 (Appendix 5-B). Colonies were thawed in filtered seawater before measuring colony length (from the peduncle to the tip of the sea pen), polyp diameter (n = 10) and density in the three rachis sections, coined lower, middle and upper section as in previous studies on sea pens (Soong 2005; Pires et al. 2009; Baillon et al. 2014). Colonies were subsequently inspected under a stereomicroscope (Nikon SMZ1500) coupled to a digital camera (Nikon DXM1200F) to isolate and identify associated species. The position of each associate along the central axis was recorded (peduncle, lower, middle and upper sections of the rachis). After extraction from the sea pens, samples of associated species were preserved in 100% ethanol for DNA analyses or dried for 48h at 60 °C for isotopic analyses.

2.3 Identification of the associated species

Associated species were grouped according to their morphology and identified to the lowest possible taxonomic level. They were divided into three categories: (1) endobiont (living inside the tissues of the sea pen), (2) ectobiont (or epibiont, living attached to the surface of the sea pen) and (3) free-living (found unattached to the sea pen). For the main associates, measures of length (e.g. copepod) or basal diameter (e.g. sea anemone) were recorded.

A total of 93 samples of associates were processed by the Canadian Centre for DNA Barcoding (University of Guelph, Canada) for genetic identification. They were analyzed using standard polymerase chain reaction (PCR) and DNA sequencing protocols (Ivanova et al. 2006; DeWaard et al. 2008). Partial COI sequences with all meta-data are registered in the Barcode of Life Data Systems (Ratnasingham and Hebert 2007), project SBDSC, and deposited in GenBank (accession numbers KF930998-KF931047). Identifications were made by running the sequences against the BOLD and BLAST databases.

2.4 Distribution of the associated species

The prevalence of associates (percentage of sea pen colonies harbouring a given species) was determined for pooled associates and for the three categories separately (endobiont, ectobiont, free-living; described above). The mean yield (MY) was defined as the mean number of associates per colony (ind colony$^{-1}$) considering all sea pens examined, and the mean exact yield (MEY) was defined as the mean number of associates colony$^{-1}$ considering only sea pens harbouring this associated species. Both measures were extrapolated to obtain total yields for the associates (MYtot and MEYtot),

both overall and within each category of associate. The MY for a site (site mean yield [SMY] or site mean exact yield [SMEY]) was defined as the number of associates found in that site divided by the number of sea pen colonies examined for that site (as individuals colony$^{-1}$). All parameters, i.e. prevalence, MY, MEY were also separately determined for the most common (key) associated species.

2.5 Specificity of the Lamippidae and Corallovexiidae

Complementary data were obtained from histological sections of polyps of A. grandiflorum colonies infested by L. bouligandi that were preserved in 4% formaldehyde (n=12). Polyp samples were prepared using standard histology protocols (Baillon et al. 2011). They were dehydrated in an ethanol series (70−100%), embedded in paraffin, sectioned (6 to 10 µm) and stained with haematoxylin and eosin. They were examined under a light microscope (Nikon Eclipse 80i) coupled to a digital camera (Nikon DXM1200F) and analyzed using the imaging software Simple PCI (v. 6.0).

To determine the impact of Lamippe bouligandi on A. grandiflorum, the density and Ferret diameter of oocytes were determined in 5 polyps harbouring a copepod and 5 polyps without copepods sampled in a given colony. The measures were limited to the upper section of the colony to avoid the variation of fecundity along the rachis (increase of the fecundity from the lower to the upper section, Baillon et al. 2014, Chapter 3).

2.6 Trophic interactions

Due to putative regional variations in carbon and nitrogen signatures of pennatulaceans (Sherwood et al. 2008), only samples from the Laurentian Channel sampled in 2007 were used for isotopic analysis; this location/date yielded several colonies with enough copepods to allow comparisons. Analyses of stable isotopes were

conducted according to Sherwood et al. (2008) on 17 samples of associates (2 L. bouligandi, 3 undescribed Corallovexiidae and 5 S. nexilis) and on their hosts (2 A. grandiflorum and 4 H. finmarchica). Briefly, dried samples were ground to powder and treated with 5% (v/v) HCl to remove carbonates, then rinsed three times with de-ionised water and dried again for 24h at 60 °C. Between 0.6 and 2.3 mg of sample was placed into 10×10 mm ultralight Sn capsules. Due to the small size of the copepods, specimens from a given colony were pooled to obtain the minimum weight necessary. The analyses were carried out using a Carlo Erba 1500 elemental analyser connected via a ConFlo-II interface to a FinniganTM MAT 252 isotope ratio mass spectrometer in the Department of Earth Sciences at Memorial University. The carbon and nitrogen isotopic values are provided using the standard δ-notation: $\delta X=[(R_{sample}/R_{standard})-1]\times10^3$, where X corresponds to $^{13}C$ or $^{15}N$ and R is $^{13}C/^{12}C$ and $^{15}N/^{14}N$, respectively.

As per Sherwood et al. (2008) a proxy for particulate organic matter (POM) was used in the form of sedimentary organic matter (SOM) from the LC (sampled at 268-531 m) between October and December 1990 (Muzuka and Hillaire-Marcel 1999). Data for pelagic and benthic invertebrates were not available for LC. However, previous data from offshore NNL were used (Sherwood and Rose 2005) including amphipods and euphasiids for the pelagic invertebrates, and shrimps (Pandalus borealis and Pasiphae multidentata) and snow crab for benthic invertebrates to situate the sea pens in the food web.

Trophic level (TL) was estimated from the $\delta^{15}N$ values using the following equation (Nilsen et al. 2008): $TL_{consumer}= [(\delta^{15}N_{consumer}-\delta^{15}N_{base})/\Delta\delta^{15}N]+TL_{base}$ where $\delta^{15}N_{consumer}$ corresponds to the $\delta^{15}N$ of the taxa considered, while $\delta^{15}N_{base}$ and $TL_{base}$ correspond to the value of the baseline of the trophic web considered, and $\Delta\delta^{15}N$ is the

trophic fractionation for $\delta^{15}N$ (average 3.8‰ for polar and deep-sea studies (Iken et al. 2005)). Here the base value was determined as per Gale et al. (2013) using zooplankton as the primary consumer ($TL_{base}$=2.3, $\delta^{15}N_{base}$=9).

In addition, the gastro-vascular content of the sea anemone Stephanauge nexilis (an associate of H. finmarchica, see results) were extracted and preserved in 100% ethanol for DNA analyses. Eight samples were processed for DNA identification as outlined above.

2.7 Data analysis

Rarefaction curves (Hurlbert 1971) were used to compare species richness of faunal associates between sea pen host species using BioDiversity Pro software (©Natural History Museum, London/Scottish Association of Marine Sciences). Rarefaction analysis allows an estimation of the number of species expected ($E_{(Sn)}$) for a specific number of individuals observed (n) removing the influence of the sample effort (Clarke and Warwick 2001). The evenness (or equitability, indicating whether or not species are represented by a similar number of individuals) of the assemblage of species was determined for both sea pens using the Shannon –Wiener diversity index: $H' = -\sum_{i=1}^{S} (N_i / N) \times \log(N_i / N)$ where S is the total number of taxa, N the total number of individuals, $N_i$ the number of individuals of the $i^{th}$ taxa.

Principal component analyses (PCA) were used to determine the influence of season and region on the species distribution at the studied sites. Data were pooled per site and a log(x+1) transformation was applied to the faunal abundance values (Clarke 2001). This transformation allows the consideration of both the most abundant and rarer

species. The general repartition of the associated species, their diversity and the repartition of the most common associates were analysed according to sea pen colony length, colony section, depth, region (Figure 5-1; Laurentian Channel, Grand Banks, Flemish Cape, North Newfoundland, Labrador) and season. Additionally, sea pen morphometry (polyp density and polyp diameter) was also used to analyze the fine scale distribution of the most common associated species. According to the parameter considered, linear regression and one-way ANOVA or t-test were used, after verifying assumptions of normality and homogeneity of variances. Post-hoc pairwise analysis (Student-Newman test) was conducted as appropriate. When assumptions were not met even after transforming the data, Spearman correlation and Kruskal-Wallis or Mann-Whitney tests were used, followed by Dunn's tests as appropriate. Due to the sample size, analysis of the influence of depth on the yield was carried out only when more than 10 colonies with associated species were sampled in the same region for a specific season. Therefore analyses were limited for A. grandiflorum to LC-spring (n = 28), FC-fall/winter (n = 39), GB-spring (n = 12), GB-fall (n=24) and LB-summer (n = 20); while only GB-fall (n = 11) was used for H. finmarchica. For the influence of depth on biodiversity, all data irrespective of region and depth were use and data were pooled per range of depth (100-m interval) to determine $E_{(S15)}$.

# 3 Results

## 3.1 Species identification and diversity

A total of 1652 individuals belonging to 16 species were found on the 175 colonies of A. grandiflorum examined and a total of 189 individuals belonging to 6

species occurred on the 43 colonies of H. finmarchica (Table 5-1, Figure 5-2). Nine species associated with A. grandiflorum were classified as free-living, 1 as ectobiont and 6 as endobionts, whereas 1 free-living associate, 2 ectobionts and 3 endobionts were found on H. finmarchica. The free-living species included fish larvae (Sebastes spp. and Benthosema glaciale from Baillon et al., 2012), shrimp larvae (Acanthephyra pelagica, Pandalus montagui), amphipoda, copepoda, chaetognata (Pseudosagitta maxima), nematoda and one unidentified species. The ectobionts included one occurrence of one egg of the fish Lycodes esmarkii attached to the tissues of one colony of A. grandiflorum (from Baillon et al., 2012), several sea anemones Stephanauge nexilis and a hydrozoan colony found on the naked upper section of colonies of H. finmarchica. Finally the endobionts included parasitic copepods (Lamippe bouligandi on A. grandiflorum, an undescribed Corallovexiidae and an unidentified Lamippidae both found on H. finmarchica) and 6 unidentified species (including 4 putative egg masses on A. grandiflorum; Table 5-1).

Values of $E_{(S50)}$, when all species were considered, were higher for H. finmarchica (5.2 expected species) than A. grandiflorum (3.3 expected species). However, values of $E_{(S180)}$ were comparable (~6 expected species). The rarefaction curve for A. grandiflorum did not reach an asymptote (Figure 5-3A) while the curve for H. finmarchica showed a steeper increase of the number of species towards an asymptote (Figure 5-3B). However, when the rarest species (with only one observation) were removed, the rarefaction curve of both species reached the asymptote (Figure 5-3). Evenness was lower for A. grandiflorum ($H'$ = 0.48) than for H. finmarchica ($H'$ = 1.04).

Overall, 96.2% of the individuals found on the two sea pens belonged to 4 species. The most common (78.7% of the associates) occurred on A. grandiflorum and was identified as Lamippe bouligandi (Figure 5-4A), a parasitic copepod living inside the tissues of the polyp column (Figure 5-4A and C). The next two most common species were found on H. finmarchica: a sea anemone (representing 1.6% of the associates) found attached to the central axis, showing 96% DNA similarity with Hormathiidae and identified as Stephanauge nexilis (Figure 5-5A), and a parasitic copepod (6.8% of the associates; Figure 5-6A) living inside the polyp, in the space typically hosting reproductive cells. The latter was identified as a copepod (from the family Corallovexiidae) based on the presence of nauplii (characteristic of crustacean) and its general morphology. A genetic similarity of ~85.5% was obtained between the undescribed Corallovexiidae and L. bouligandi (family: Lamippidae) suggesting that the two species likely belong to different families despite their similarities (including in the shape and size of the male). Given the localisation of these copepods in their hosts, they were considered endobionts. The fourth common species was present on both sea pens (representing 9.1% of associates): larvae of redfish, Sebastes spp. (for more details see Baillon et al., 2012, Chapter 6). In addition to the fish larvae, 12 shrimp larvae were found in April 2006 and April 2007 on A. grandiflorum; they were identified as Acanthephyra pelagica (DNA: 99% certainty) and Pandalus montagui (DNA: 100% certainty). Six shrimp larvae were also found on four colonies of A. grandiflorum in April 2009, one of them identified as Pasiphaea multidentata (DNA: 100% certainty).

3.2 Species distribution on the hosts

All faunal associates were found on the rachis section of the host colonies. At least one of the associates was found on 75.9% of A. grandiflorum and 46.6% of H. finmarchica colonies. Across regions, prevalence proportion varied between 58.3% (NNL) and 96.8% (LB) for A. grandiflorum and between 23.8% (FC) and 90.0% (LC) for H. finmarchica (Table 5-2). For both species the endobionts were the most common (prevalence on A. grandiflorum = 72.3%, on H. finmarchica = 38.6%) across geographic regions. They were principally represented by L. bouligandi (98.9%) in A. grandiflorum and by the undescribed Corallovexiidae (87.5%) in H. finmarchica.

The yield of associates (as MEY) on A. grandiflorum was significantly greater for endobionts (9.3±0.9 ind colony$^{-1}$) than for ectobionts (1.0±0.0 ind colony$^{-1}$) and for free-living species (3.2±0.7 ind colony$^{-1}$; H = 42.83, df = 2, P < 0.001). No significant differences were found in the MEY of each category of associate on H. finmarchica (endobiont: 4.0±0.9 ind colony$^{-1}$; ectobiont: 1.9±0.5 ind colony$^{-1}$; free-living: 4.3±2.3 ind colony$^{-1}$; H = 4.64, df = 2, P = 0.099). Comparisons between the two sea pens showed that they harboured the same number of ectobionts (U = 30.0, P = 0.121) and free-living associates (U = 84.5, P = 0.401) whereas A. grandiflorum hosted a significantly higher number of endobionts than H. finmarchica (U = 1707.0, P < 0.001).

Endobionts were present in all the sections of the rachis in both sea pen species. A significant increase of the endobiont MEY occurred from the lower to the upper section of A. grandiflorum colony (H = 95.50, df = 2, P < 0.001), while the endobionts in H. finmarchica showed a significantly higher MEY in the middle section than in the two other sections (middle >lower=upper; H = 12.39, df = 2, P = 0.002). For both sea pens,

when removing the most common associate (i.e. L. bouligandi and the undescribed Corallovexiidae), no significant differences were found among sections for other associates (A. grandiflorum: H = 3.10, df = 2, P = 0.212; H. finmarchica: $F_{2.6} = 1.5$, P = 0.296). In H. finmarchica the sea anemone S. nexilis and a hydrozoan (ectobionts) were always attached directly to the central axis in the upper section of the colonies. A. grandiflorum showed a significant increase of the MEYtot with colony length ($r_s = 0.16$, P = 0.036) while no variation was noted for H. finmarchica ($r_s = 0.05$, P = 0.735). Analyses per category of associate showed an increase of the MEY with colony length for free-living associates ($r_s = 0.36$, P = 0.007) of A. grandiflorum while no variation occurred for other categories in either sea pen species.

3.3 Variations with seasons, regions and depths

Principal component analysis (PCA) on the associated species of A. grandiflorum revealed that the copepod L. bouligandi was the main contributor to the first principal component (PC1: 67.4%) and the fish larvae to the second principal component (PC2: 22.0%). Two groupings were visible (Figure 5-7A) corresponding, for the first, to the colonies harbouring fish larvae (April-May in the LC region, Fig. 5-1) and, for the second group, to all other samples in various regions/months. PCA on the associated species of H. finmarchica showed that the undescribed Corallovexiidae was the main contributor to the first principal component (PC1: 56.0%) and fish larvae and the sea anemone S. nexilis to the second principal component (PC2: 24.1%). However, no specific groupings emerged (Figure 5-7B). Therefore, to account for the influence of fish larvae on the repartition of the study sites, the remaining analyses were conducted considering both regions and seasons (spring/summer vs. fall/winter). The number and distribution of

ectobionts and free-living associates among seasons depths and regions precluded the statistical analysis for these associates alone. Therefore, the analyses of seasonal, bathymetric and regional variations were carried out using MEYtot and biodiversity index.

Seasonal analyses inside specific regions showed that the diversity of species associated with A. grandiflorum was higher in spring/summer than in fall in GB ($E_{(S120)spring}$ = 7.0> $E_{(S120)fall}$ = 3.7) and LB ($E_{(S150)spring}$ = 4.3> $E_{(S120)fall}$ = 2.0). However, the MEYtot did not show any significant seasonal variations at any site (GB: U = 139.0, P = 0.596; LB: U = 95.0, P = 0.843). The associates of H. finmarchica showed a lower diversity in spring than fall in GB ($E_{(S20)spring}$ = 2.0< $E_{(S20)fall}$ = 3.8) but no significant difference in MEYtot was observed in GB (U = 27.0, P = 0.565).

Regional analyses within the various seasons revealed that the associated biodiversity of A. grandiflorum exhibited a general northward decrease in fall and spring/summer (Figure 5-8A and B) while the MEYtot showed no significant variation among regions (spring/summer: $F_{2,91}$ = 2.90, P = 0.060; fall: H = 4.72, df = 3, P = 0.193). In fall, H. finmarchica showed the same biodiversity of associates in FC and GB ($E_{(S15)}$ = 3.94 and 3.48, respectively; Figure 5-8C) as well as the same MEYtot (U = 37.0, P = 0.925), while in summer colonies showed a higher biodiversity of associates in LC than FC and GB (Figure 5-8D) but no regional differences in MEYtot ($F_{2,22}$ = 1.49, P = 0;247).

No significant influence of depth was found on the biodiversity of associates for either sea pen host (A. grandiflorum: $r^2$ = 0.28, $F_{1,9}$ = 3.55, P = 0.092, H. finmarchica: $r^2$ = 0.03, $F_{1,5}$ = 0.14, P = 0.721). No bathymetric variation in MEYtot was found either,

except a decrease of MEYtot with depth in GB during the fall for A. grandiflorum (Table 5-3).

3.4 Relationship between hosts and dominant associates

### 3.4.1 Lamippe bouligandi in Anthoptilum grandiflorum

A total of 1126 females and 23 males of the copepod L. bouligandi (MEYtot= 9.4±0.9 copepods colony$^{-1}$) were recorded from 118 colonies (15-84 cm) of A. grandiflorum (prevalence of 71.1%) from all five geographic regions under study. Eggs and nauplius larvae of L. bouligandi were found in association with 36 females (3.2%) in 18 sea pen colonies (10.8%) sampled year-round. Females mainly occurred singly in a polyp; whereas males were always paired with a female. The female copepods measured 5.06±0.07 mm (Figure 5-4B) while the males were smaller at 1.39±0.17 mm (Figure 5-4D). Two females occurred in the same polyp on 25 occasions (in 18 sea pen colonies) while larger groups of 3-4 females were found in only 4 polyps distributed on 3 colonies sampled year-round. No seasonal pattern emerged to explain the pairings/groupings. Infestation was between 0.1 and 19.1% of the polyps in an affected colony (i.e. 1-50 polyps). Overall, most (57.6%) of the colonies had less than 2% of polyps infested and only 3.4% of the colonies had >10% of polyps infested. An average of 44.0±4.7 white/yellowish oocytes was present in non-infested polyps and measured 429.1±11.7 µm. The infested polyps showed a significantly lower fecundity (19.6±5.1 oocytes polyp$^{-1}$, representing a 45±6.9% decrease in relative fecundity) than the non-infested polyps ($t = 3.51$, $df = 8$, $P = 0.008$), and they were translucent and significantly larger (520.5±18.8 µm, $U = 7591.5$, $P < 0.001$).

No influence of colony length on the yield (MEY) of copepods was found ($r_s$ = 0.17, P = 0.057). A significant increase in the abundance of female copepods occurred from the lower to the upper section of the rachis (H = 77.71, df = 2, P < 0.001), with 60.3% of females occupying the upper section. Positive correlations were found between the abundance of copepod and both polyp density ($r_s$ = 0.34, P < 0.001) and polyp diameter ($r_s$ = 0.31, P = 0.005). Infestation with L. bouligandi occurred at all sampling depths. No correlation of MEY with depth ($r_s$ = -0.16, P = 0.075) and no influence of season (H = 4.06, df = 3, P = 0.255) were detected. However, significant regional differences in MEY were evidenced (H = 13.49, df = 4, P = 0.009) between LB (16.07±0.95 copepods colony$^{-1}$) and GB (5.04±0.95 copepods colony$^{-1}$).

### 3.4.2 Undescribed Corallovexiidae in Halipteris finmarchica

A total of 112 females and 2 males copepods belonging to the Corallovexiidae (MEYtot= 4.7±1.0 copepods colony$^{-1}$) were recorded inside the polyps (Figure 5-6A) of 28 colonies (21-132 cm) of H. finmarchica (prevalence of 29.8 %) from all five geographic regions under study. When a male was found, it was always paired with a female. Females measured 4.52±0.51 mm (Figure 5-6B) and males were smaller at 0.73±0.05 mm (Figure 5-6C). Females occurred at the base of the polyp where reproductive cells typically grow (Figure 5-6A). No oocytes or spermatocysts were observed in the infested polyps while the surrounding non-infested polyps harboured oocytes or spermatocysts. Overall 61.6% of the female copepods were found in association with eggs/nauplii (Figure 5-6D) at various times of the year. Contrarily to L. bouligandi in A. grandiflorum, a polyp never hosted more than one female

Corallovexiidae. Infestation rates varied between 0.1 and 1.6% (1-20 infested polyps) in an affected colony with only five colonies (17%) harbouring more than 5 copepods.

MEY was not influenced by colony length ($r^2 = 0.07$, $F_{1,22} = 1.56$, $P = 0.225$). The middle section of the rachis showed greater infestation than the upper and lower sections ($H = 13.46$, $df = 2$, $P < 0.001$), with 50% of the Corallovexiids occurring there. This copepod was present at all depths sampled. Despite a significant decrease of the MEY with depth ($r_s = 0.52$, $P = 0.010$), no clear threshold was detected; i.e. there was no significant difference among 100-m depth intervals ($H = 7.24$, $df = 7$, $P = 0.404$). Comparison among seasons showed a higher MEY in spring than in fall ($H = 7.98$, $df = 2$, $P = 0.019$). No significant regional differences were evidenced ($F_{2,20} = 2.39$, $P = 0.117$).

### 3.4.3 Stephanauge nexilis on Halipteris finmarchica

A total of 28 sea anemones S. nexilis were found attached to the central axis of H. finmarchica, usually in the upper section of the rachis that was devoid of soft tissues (Figure 5-5A and C). However, three small individuals were found surrounded by polyps (Figure 5-5B). Sea anemones had a basal diameter ranging from 0.4 to 9.9 cm (3.4±0.5 cm). Between 1 and 8 sea anemones (MEYtot= 4.7±1.0 ind colony$^{-1}$) were found on 14 colonies of H. finmarchica (prevalence of 15.4%).

Stephanauge nexilis was present on H. finmarchica colonies from all sampling depths studied (366-1125 m) with no influence of depth on the MEY ($r_s = -038$, $P = 0.178$). However, this association was restricted to the southern regions (85.7% in LC and GB, and 14.3% in FC). No significant seasonal difference in MEY was found ($U = 12$, $P = 0.142$).

### 3.4.4 Trophic interactions between hosts and dominant associates

Analysis of isotopic ratios in tissues of the two sea pen species collected from LC showed they had similar $\delta^{13}C$ and $\delta^{15}N$ signatures (Table 5-4; $\delta^{13}C$: U = 3.5, P = 0.800; $\delta^{15}N$: U = 2.0, P = 0.533). No significant differences were detected between the sea anemone S. nexilis and its host H. finmarchica despite the fact that the sea anemone had a higher $\delta^{13}C$ (~1 ‰, t = -1.36, df = 6, P = 0.224) and $\delta^{15}N$ (~1 ‰, t = -2.42, df = 6, P = 0.052). Both sea pens and the sea anemone showed the same TL (Table 5-4). The two associated copepods had similar $\delta^{13}C$ and $\delta^{15}N$ signatures (Table 5-4; $\delta^{13}C$: t = -1.12, df = 3, P = 0.344; $\delta^{15}N$: t = -1.40, df = 3, P = 0.255). They had a significantly lower $\delta^{13}C$ (~2 ‰, $F_{4,14}$ = 22.16, P < 0.001) and a significant higher $\delta^{15}N$ (~2 ‰, $F_{4,14}$ = 10.12, P = 0.002) than their sea pen hosts (Figure 5-9). On average, copepods were a little over half a trophic level (0.4-0.6) above their hosts.

Gastro-vascular contents found in 8 (28.5%) of the sea anemones comprised small pelagic invertebrates: amphipods, copepods and halocyprids (based on DNA; Figure 5-5D-F).

## 4 Discussion

Different measures of biodiversity exist and its estimation depends on the number of species and the respective abundance of those species (Pielou 1966). Biodiversity expressed as $E_{(S180)}$ was similar between faunal associates of Anthoptilum grandiflorum and Halipteris finmarchica; however, the rarefaction curves showed that increasing sample size would yield a greater numbers of associates for A. grandiflorum probably due to the higher number of free-living species found in association with this host (see

below). When removing the rarest species (single occurrences), the rarefaction curves reached an asymptote, suggesting that the most common associates of both sea pens have been sampled. The Shannon-Weiner index ascribed more even abundances to the associates of H. finmarchica than to those of A. grandiflorum. Associates of A. grandiflorum are clearly dominated by one species, i.e. Lamippe bouligandi. Associates of H. finmarchica are dominated by two species, an undescribed Corallovexiidae and Stephanauge nexilis, resulting in a slightly more even distribution. Overall, specialized copepods emerge as the predominant associates of sea pens. Variations in richness of faunal associates were not observed across depths, while a northward decrease was visible for both sea pens, which is in accordance with previous studies reporting a general decrease of biodiversity with increasing latitude (Rex et al. 2000; Rex and Etter 2010). Variation in primary productivity over large spatial scales has been proposed to generate this trend (Rex et al. 1997).

Sea pen colonies, studied here, only yielded associated species on the rachis, and none on the peduncle. This is not unexpected since the peduncle is almost completely buried in the sediment in both A. grandiflorum and H. finmarchica. However, a polychaete (Ophryotrocha sp.) was recorded in association with the peduncle of sea pen colonies that had been maintained alive in the laboratory for a few weeks (including A. grandiflorum and H. finmarchica); the polychaete appears to be a new deep-sea species that feeds on sea pen flesh and decaying matter (Mercier et al., unpubl. data). An earlier report by Johnstone (1969) described the presence of a parasitic copepod living in/on the half-buried peduncle of the shallow-water sea pen Ptilosarcus guerneyi from the North Pacific.

The number of associates species identified in A. grandiflorum and H. finmarchica is similar to that reported in the shallow-water sea pen P. guerneyi (Johnstone 1969) supporting the idea that sea pens attract a moderate diversity of associated species. The biodiversity associated with both sea pens is lower than that reported in order Gorgonacea (sea fans). Two gorgonians, from the Northwest Atlantic, Paragorgia arborea and Primnoa resedaeformis, showed an $E_{(S100)}$ around 12 and 35 expected species, respectively (Buhl-Mortensen and Mortensen 2005), which is 3-7 times higher than $E_{(S100)}$ in A. grandiflorum and H. finmarchica from the same geographic region. This difference likely results from the type of habitat offered by sea pens vs. sea fans. Two different microhabitats characterize gorgonians: (1) living tissues in the young body parts of the colony and (2) exposed skeleton in the older body part of the colony. The former harboured a lower biodiversity but the highest abundance of specialized associates. The greater biodiversity in the older/dead section is due to the capacity of sessile species to settle there, as also observed in dead sections of deep-sea scleractinians (Mortensen et al. 1995; Mortensen 2001). The relatively low biodiversity associated with sea pens might therefore be due to the less frequent availability of exposed skeleton for other species to colonize. The central axis of sea pens is formed of collagen and calcite (Wilson et al. 2002), and provides support to the colony; however, it apparently does not survive the colony's death for long, since no dead skeletons were sampled here (personal observations) or reported previously. Some colonies of H. finmarchica showed no tissue on the older upper section where two ectobiotic species were found (sea anemone S. nexilis and a hydrozoan). The biodiversity in this older section was not consistently higher than elsewhere along the colony, which can be due to its small diameter and the

smooth surface of the central axis, less favorable to settlement, as well as its susceptibility to erosion or grazing predators, as suspected by Baillon et al. (Chapter 2). Few ectobiotic species are reported on the living tissue of gorgonians and all are highly specialised symbionts (Buhl-Mortensen and Mortensen 2005). A similar rarity of ectobiotic species were identified on sea pens, none of which were found on the living tissues of H. finmarchica and only one on the soft tissues of A. grandiflorum: an egg mass of the eelpout Lycodes esmarkii. Ectobiotic species are probably rare because soft corals, including sea pens, produce toxic chemicals acting as antifouling agents (Coll 1992; Krug 2006; Changyun et al. 2008). A study on the shallow-water sea pen Renilla octodentata confirmed the negative effect of those agents on the settlement of barnacle (García-Matucheski et al. 2012). Chemicals, if present, seem to have a limited impact on colonisation by endobiontic species, which represent 87.7% of the associates recorded. The ability of endobionts to colonize sea pen tissues might be explained by the fact that most of them are parasitic and have developed adaptations to thwart their host's defenses (Kaltz and Shykoff 1998). Overall, 38.6% of the colonies of H. finmarchica harboured endobionts compared to 66.7% of A. grandiflorum, suggesting that the former may be better protected against infestations. The rachis of H. finmarchica produces a larger quantity of mucus than that of A. grandiflorum (personal observation), which might create a barrier against settlement and mitigate infestation.

While chemical deterrents produced by corals may influence colonisation by ectobionts and endobionts, they are also known to deter predators (Mackie 1987; Changyun et al. 2008). Hence, corals may offer protective shelter to free-living associates. We found a clear difference in the number of free-living associates between

the two sea pens. All 9 free-living associates were found on A. grandiflorum and only one (larvae of Sebastes spp.) on H. finmarchica. It is presumed that A. grandiflorum relies only on chemical defenses while H. finmarchica also harbours sclerites forming a calyce around the polyps (physical defense, Chapter 2). However, the common observation of bare central axis in H. finmarchica suggests that this species is more often grazed than A. grandiflorum, possibly explaining why free-living associates might favour A. grandiflorum, even though it can be predated by slow-moving species such as the sea star Hippasteria phrygiana (Gale 2013). Alternatively, the morphology of the two sea pens might explain this discrepancy. The elongated polyps of A. grandiflorum occur singly, while the polyp rows on H. finmarchica are fused at their base, forming ridges, as described by Williams (1995). Thus, A. grandiflorum is more "bushy" than H. finmarchica, which probably allows small invertebrates (e.g. shrimp larvae, copepods) and small vertebrates (e.g. fish larvae) to use A. grandiflorum for shelter and protection. The shallow-water sea pen P. guerneyi provided anchorage to various species against the tidal flow and a hiding place for small invertebrates, e.g. amphipods, caprellids and shrimps (Johnstone 1969), emphasising the importance of sea pens as shelter and structural habitat. While H. finmarchica is a less likely shelter for free-living organisms, stomach contents of its ectobiont, the sea anemone Stephanauge nexilis, showed the presence of small invertebrates (free-living amphipods, copepods and halocypriods), suggesting their presence around colonies of H. finmarchica. The whip morphology of H. finmarchica may be less likely to retain small associates during sampling and lead to an underestimation of this type of association. Buhl-Mortensen and Mortensen (2005) indicated that sampling of the associated species of deep-sea gorgonians by trawl led to

the loss of most of the mobile crustaceans, which were sampled when using suction devices with a ROV. An additional challenge is that some free-living associates of sea pens are present only during a specific life stage and/or a specific season: three different species of shrimp larvae (Acanthephyra pelagica, Pandalus montagui and Pasiphaea multidentata) were found here in April/May (spring) exclusively. Previously, fish larvae of Sebastes spp. were also found on both species of sea pens in April and May, prompting the suggestion that sea pens act as essential fish habitat (Baillon et al. 2012, Chapter 6). The additional presence of shrimp larvae underscores the importance of sea pens for the early life history of other species, including commercially harvested ones.

While transient free-living associates are important, the three most common associates found on both sea pens (L. bouligandi on A. grandiflorum, S. nexilis and undescribed Corallovexiidae on H. finmarchica) can be considered symbionts. L. bouligandi and the corallovexiid are endoparasitic that spend most of their life history inside the polyp. While A. grandiflorum and H. finmarchica are sympatric species, their respective endoparasitic copepods are distinct. Lamippidae are adapted to their coral host (Bouligand 1966) supporting the assumption that L. bouligandi is highly specific to A. grandiflorum. In contrast, Corallovexiidae are either monospecific or found in 2 or 3 closely related coral hosts (Stock 1975), suggesting that the corallovexiid in H. finmarchica might yet be found in other sea pens. Copepod in A. grandiflorum predominated in the upper rachis section, whereas those in H. finmarchica occurred mostly in the middle section. This trend can be explained by the greater density and larger diameter of polyps in these sections, which correspond to older polyps (Chapter 2), and thus provide greater opportunity for infestation.

Both copepods had an impact on the polyps they infested: a total absence of oocytes/spermatocysts suggesting an inhibition of gametogenesis in H. finmarchica, and ~45% decrease in relative fecundity in A. grandiflorum. Parasitic copepods disrupt vitellogenesis (yolk deposition) either because they interfere with feeding or increase energy expenditure by the polyp (e.g. immune reaction). At the colony level, few polyps are infested limiting the effect on total fecundity of the colony Lamippidae were previously shown to increase mortality rates of sea pen hosts under stress (e.g. anoxic condition) despite their healthy appearance in optimal conditions (Johnstone 1969). Overall, copepods are the most common parasites identified in deep-sea octocorals (Watling et al. 2011). Here, in addition to the two species discussed above, 7 individuals of an unidentified Lamippidae were recorded in the polyps of H. finmarachica. Furthermore, a copepod of the genus Linaresia was recently found in the polyps of a deep-sea gorgonian, Paramuricea sp., in the Northwest Atlantic (de Moura Neves et al. 2013).The sea anemone S. nexilis found on H. finmarchicus is commonly reported from the Northwest Atlantic, from the Gulf of Mexico (Ammons and Daly 2008) to Labrador (Fautin 2013). S. nexilis emerges as a facultative ectobiont with a low specificity for H. finmarchica. It is found attached to rocks, empty shells and sponges in the Gulf of Mexico (Ammons and Daly 2008). The life history of this species is not known, but it can be hypothesised that it settles at the larval stage on the central axis of the sea pen and remains there due to the general absence of other suitable substrata where muddy seafloor dominates. Whether the absence of polyps around the sea anemones is a prerequisite to their settlement on sea pens, or an outcome of it, remains unclear. Some colonies of H. finmarchica exhibited a naked central axis without any visible ectobionts, suggesting that

loss of living tissue may precede colonization and supporting the assumption that the sea anemone is a commensal symbiont. On the other hand, a small number of sea anemones (probably newly settled) were observed to be closely surrounded by healthy tissues/polyps. Perhaps they initially settled on a small naked section of the colony and grew toward living tissues. It is not impossible that they are able to dislodge the polyps, which would correspond to a previously unreported case of parasitism.

The present study attempted to elucidate trophic relationships among sea pens and their principal associates. Previous work showed an increase of ~3.8‰ in $\delta^{15}N$ between prey and predator in polar and deep-sea environments (Iken et al. 2005). Here, the endoparasitic copepods fell about half a trophic level above their sea pen hosts. Parasites are presumed to feed on a single source during a specific life stage (Lafferty et al. 2008), indicating that feeding on the host tissues should elicit a full trophic increase in $\delta^{15}N$, whereas feeding on the same food as the host should result in no difference between $\delta^{15}N$ of parasite and host (Iken et al. 2001). The intermediate values recorded here suggest that copepods might use a mix of feeding strategies. This hypothesis is supported by the location of the parasitic copepods inside the polyps, which suggests that they can both feed directly on sea pen tissues and feed on items ingested by the polyp. Johnstone (1969) proposed a similar hypothesis for the diet of Lamippe sp. associated with the shallow-water P. guerneyi based on its location and on the observation of orange material in its digestive tract (the color of the sea pen's tissues). Our isotopic results also confirm that the sea anemone and both sea pens feed on sedimentary organic matter in addition to small pelagic invertebrates (Edwards and Moore 2008). However, the sea anemone is potentially targeting different prey based on small invertebrates found in their gastro-

vascular cavity, which were not observed in the sea pen polyps when open to look at the presence of associated species, suggesting that the sea anemone is not competing with its host for food.

Overall, sea pens appear to have a moderate number of associated species, as previously hypothesized (Buhl-Mortensen et al. 2010). Nevertheless, sea pens play important roles in the life history of their associates. Some, such as parasitic copepods spend most of, possibly all, their life in association with sea pens and depend on them to survive and reproduce. The presence of the sea anemone on H. finmarchica confirms that sea pens offer a suitable biogenic substrate for other species. Sea pens are also important for mobile species such as fishes and shrimps that use them transiently as shelter during early life stages, indicating that sea pens can be considered as biogenic habitat. However, the seasonality in these associations as well as the distribution of the sea pens (patchy occurrence of sea pen fields) emphasizes the difficulty in gaining a comprehensive understanding of their role as biogenic habitats. The sampling method used in this study (trawl by-catch) does not allow precise determination of functional interactions with free-living associates or a quantitative analysis, as these associates might be lost during sampling. However, this method is advantageous by permitting a large spatial and temporal coverage, as well as a large sample size, allowing the identification of spatial and temporal patterns which would not be possible with other sampling methods (e.g. ROV). Furthermore, co-occurrences were not investigated here; only close (physical) associations. Recent studies have shown that the sea star Mediaster bairdi is usually found in sea pen fields in the Northwest Atlantic (Gale 2013), and that lobsters often occur in association with sea pens in Norway fjords (Buhl-Mortensen and Buhl-

Mortensen 2014) suggesting that the contribution of pennatulacean corals to deep-sea biodiversity has yet to be fully elucidated.

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## Tables

Table 5-1 Species found on the sea pens Anthoptilum grandiflorum and Halipteris finmarchica during the present study (2006-2007).

| Associated species | Number of individuals | Prevalence (%) | Type of association | Life stage | Link to pictures |
|---|---|---|---|---|---|
| On Anthoptilum grandiflorum | | | | | |
| Actinopterygii | | | | | |
| Scorpaeniformes | | | | | |
| Sebastes spp. | 150 | 17.1 | Free-living | Larvae | Baillon et al. (2012) |
| Myctophiformes | | | | | |
| Benthosema glaciale | 1 | 0.4 | Free-living | Larvae | Baillon et al. (2012) |
| Perciformes | | | | | |
| Lycodes esmarkii | 1 | 0.4 | Ectobiont | Egg | Baillon et al. (2012) |
| Chaetognatha | | | | | |
| Aphragmophora | | | | | |
| Pseudosagitta maxima | 4 | 0.9 | Free-living | Adult | Figure 5-2G |
| Crustacea | | | | | |
| Copepoda | | | | | |
| Lamippe bouligandi | 1458 | 66.2 | Endobiont | Adult | Figure 5-4 |
| Unidentified copepoda | 4 | 1.7 | Free-living | Adult | Figure 5-2H |
| Decapoda* | | | | | |
| Acanthephyra pelagica | 2 | 0.4 | Free-living | Larvae | Figure 5-2B |
| Pandalus montagui | 3 | 0.9 | Free-living | Larvae | Figure 5-2B |
| Unidentified decapoda | 7 | 1.7 | Free-living | Larvae | Figure 5-2B |
| Amphipoda | | | | | |
| Unidentified amphipoda | 3 | 1.3 | Free-living | Adult | -- |
| Nematoda | | | | | |
| Unidentified nematoda | 2 | 0.4 | Free-living | Adult | -- |
| Unidentified species | | | | | |
| Unidentified sp. 1 | 6 | 2.6 | Endobiont | Egg | Figure 5-2D |
| Unidentified sp. 2 | 2 | 0.9 | Endobiont | Egg | Figure 5-2F |
| Unidentified sp. 3 | 6 | 2.1 | Endobiont | Egg | Figure 5-2C |
| Unidentified sp. 4 | 1 | 0.4 | Endobiont | Egg | Figure 5-2E |
| Unidentified sp. 5 | 1 | 0.4 | Endobiont | ? | -- |
| Unidentified sp. 6 | 1 | 0.4 | Free-living | ? | Figure 5-2A |
| On Halipteris finmarchica | | | | | |
| Actinopterygii | | | | | |
| Scorpaeniformes | | | | | |
| Sebastes spp. | 17 | 4.3 | Free-living | Larvae | Baillon et al. (2012) |
| Cnidaria | | | | | |
| Actinaria | | | | | |

Table 5-1 continued

| Associated species | Number of individuals | Prevalence (%) | Type of association | Life stage | Link to pictures |
|---|---|---|---|---|---|
| Stephanauge nexilis | 28 | 16.0 | Ectobiont | Adult | Figure 5-5 |
| Hydrozoa | | | | | |
| Unidentified Hydrozoa | 1 | 1.1 | Ectobiont | Adult | -- |
| Crustacea | | | | | |
| Copepoda | | | | | |
| Undescribed Corallovexiidae | 112 | 29.8 | Endobiont | Adult | Figure 5-6 |
| Unidentified Lamippidae | 7 | 7.5 | Endobiont | Adult | Figure 5-2I |
| Unidentified species | | | | | |
| Unidentified sp 7 | 10 | 4.3 | Endobiont | ? | Figure 5-2J and K |

* 6 more larvae were found in April 2009 with a third species identified as Pasiphaea multidentata.

Table 5-2 Prevalence of associates on colonies of A. grandiflorum and H. finmarchica in the different geographic regions (as percent colonies harbouring them). Data also shown separately for endobionts, ectobionts and free-living associates. LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cape, NNL: North Newfoundland, LB: Labrador.

| | | All regions combined | LC | GB | FC | NNL | LB |
|---|---|---|---|---|---|---|---|
| A. grandiflorum | All associates | 75.9 | 67.5 | 74.0 | 70.0 | 58.3 | 96.8 |
| | Endobionts | 72.3 | 52.9 | 72.2 | 70.0 | 58.3 | 96.8 |
| | Ectobionts | 0.6 | 0.0 | 0.0 | 3.3 | 0.0 | 0.0 |
| | Free-living | 26.0 | 52.9 | 8.3 | 0.0 | 0.0 | 16.1 |
| H. finmarchica | All associates | 44.7 | 90.9 | 44.7 | 23.8 | -- | 55.6 |
| | Endobionts | 37.2 | 45.5 | 36.8 | 23.8 | -- | 55.6 |
| | Ectobionts | 20.0 | 63.6 | 18.4 | 4.8 | -- | 0.0 |
| | Free-living | 4.3 | 27.3 | 0.0 | 4.8 | -- | 5.6 |

Table 5-3 Influence of increasing depth on total mean yield (MEYtot) of all associates on colonies of Anthoptilum grandiflorum and Halipteris finmarchica in the different geographic regions (only sites with more than 10 colonies harbouring associated species are shown). LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cape, LB: Labrador, n: number of sea pen colonies analysed. Empty cells correspond to regions without enough data available for analyses.

| Species | Region | Depth range (m) | n | Spring/summer | Fall/winter |
|---|---|---|---|---|---|
| Anthoptilum grandiflorum | LC | 301-488 | 28 | $r^2$=0.07, $_{F1,27}$=1.81, p=0.190 | |
| | GB | 98-603 | 12 | $r^2$=0.03, $F_{1,11}$=0.34, p=0.570 | $r^2$=0.41, $F_{1,23}$=15.06, p<0.001 |
| | FC | 273-1208 | 39 | | $r_s$=-0.06, p=0.734 |
| | LB | 176-883 | 20 | $r^2$=0.02, $F_{1,19}$=0.34, p=0.569 | |
| Halipteris finmarchica | GB | 579-1333 | 11 | | $r^2$=0.01, $F_{1,10}$=0.05, p=0.825 |

Table 5-4 Carbon and nitrogen stable isotope signatures ($\delta^{13}C$ and $\delta^{15}N$), and trophic level (TL) of Anthoptilum grandiflorum and Halipteris finmarchica and their dominant associates. n: number of samples analysed (mean±SE).

| | n | $\delta^{13}C$ (‰) | $\delta^{15}N$ (‰) | TL |
|---|---|---|---|---|
| Anthoptilum grandiflorum | 2 | -20.9±0.1 | 11.3±0.8 | 3.0 |
| Lamippe bouligandi | 2 | -22.7±0.2 | 13.4±0.1 | 3.4 |
| Halipteris finmarchica | 4 | -21.3±0.4 | 10.5±0.3 | 2.7 |
| Stephanauge nexilis | 5 | -20.4±0.3 | 11.5±0.2 | 2.9 |
| Undescribed Corallovexiidae | 3 | -23.3±0.3 | 12.8±0.3 | 3.3 |

## Figures

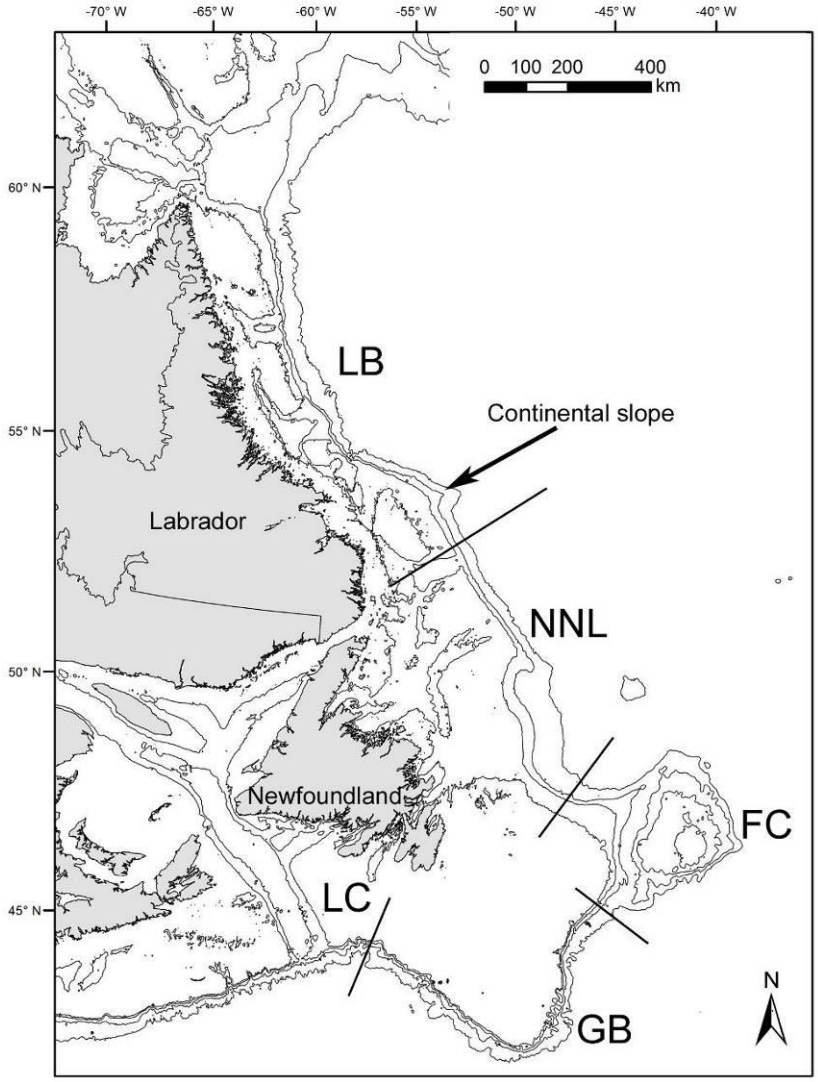


Figure 5-1 Map showing the five geographic regions where colonies of the sea pens Anthoptilum grandiflorum and Halipteris finmarchica were collected along the continental slope. LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador.

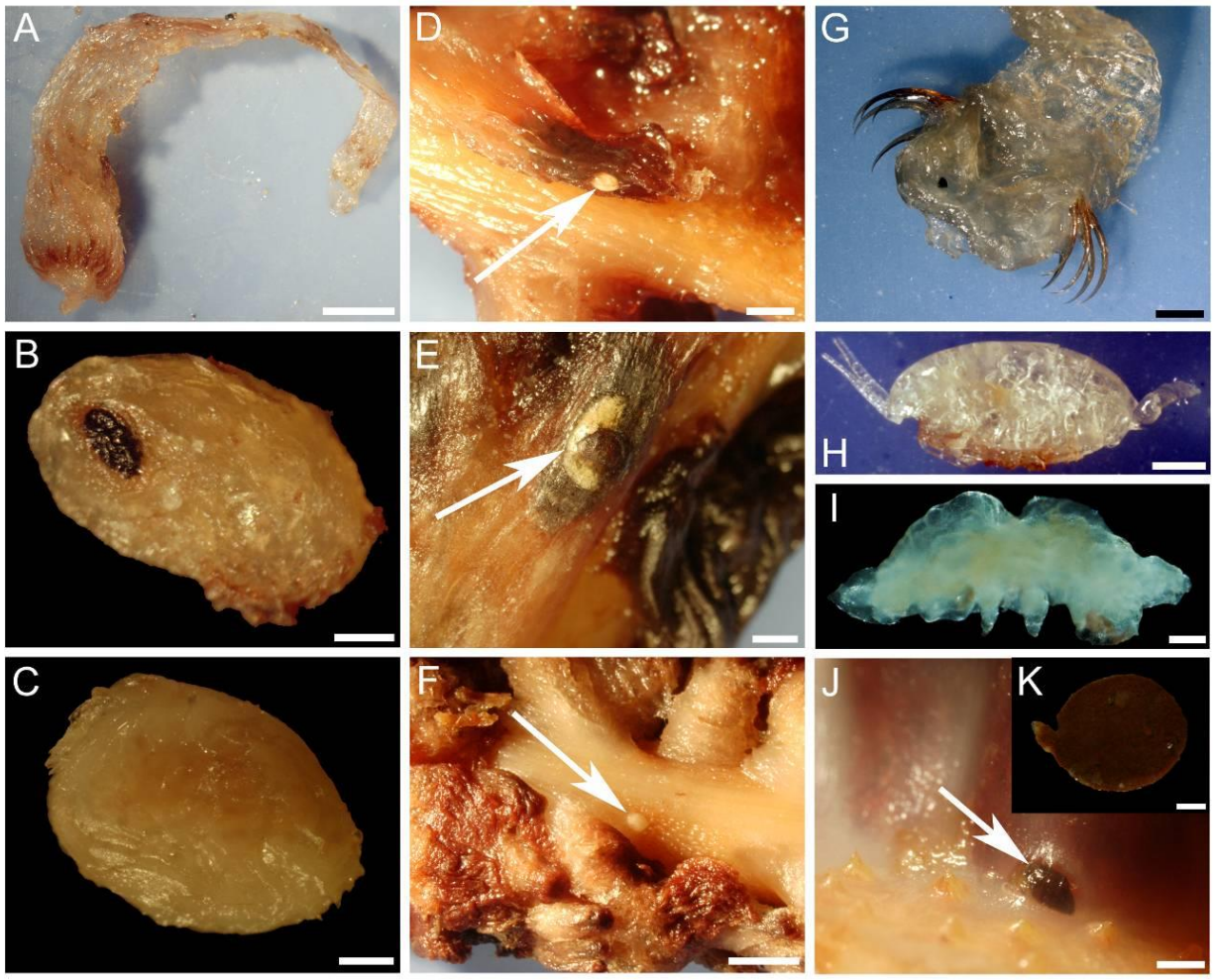


Figure 5-2 Associates of Anthoptilum grandiflorum: (A) unidentified sp. 6, (B) decapoda larva, (C) unidentified sp. 3, (D) unidentified sp. 1, (E) unidentified sp. 4, (F) unidentified sp. 2, (G) Sagitta maxima, (H) unidentified copepoda. Unidentified sp. 1 to 4 correspond to potential egg mass. Associates of Halipteris finmarchica: (I) unidentified Lamippidae, (J and K) unidentified sp. 7. Scale bar in A and F=4 mm, B=200 µm, C, H and J=500 µm, D and G= 2 mm, E=2 mm, I and K= 100 µm. Species numbers linked to Table 5-1.

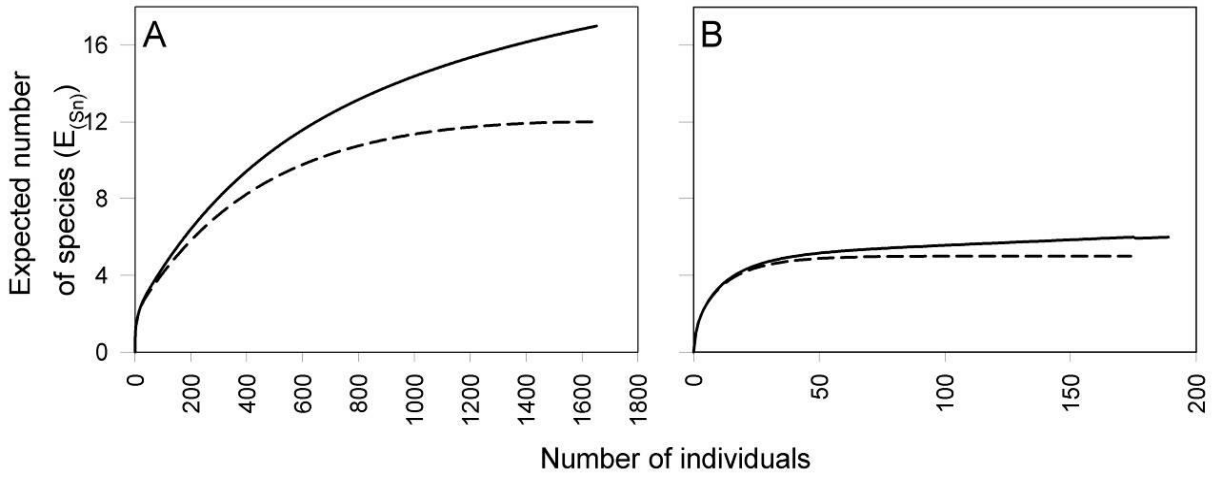


Figure 5-3 Rarefaction curves for the associated fauna of (A) Anthoptilum grandiflorum and (B) Halipteris finmarchica considering all the species (plain line) or with the rarest species (1 occurrence) removed (dash line)

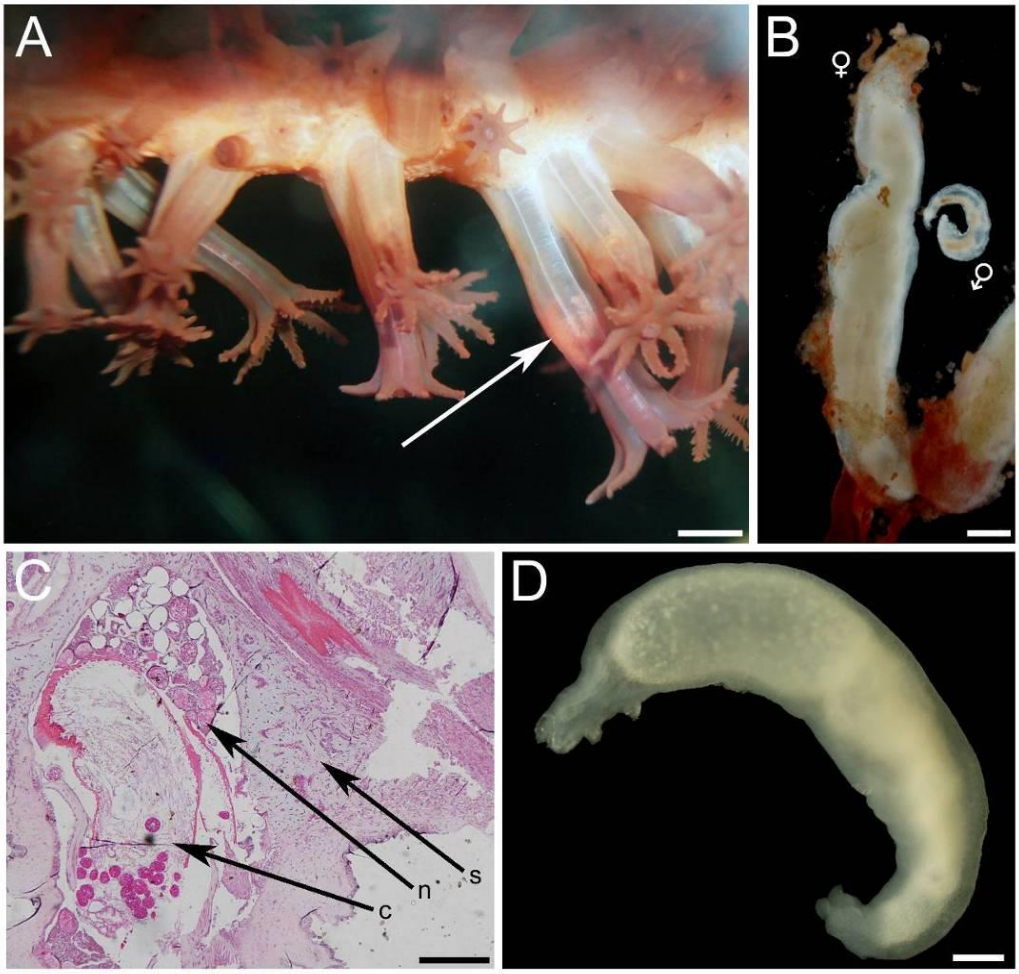


Figure 5-4 Lamippe bouligandi, a parasitic copepod living inside the polyps of Anthoptilum grandiflorum: (A) in situ view of the copepod (arrow) through the transparent polyp wall, (B) male and female copepods illustrating the size dimorphism, (C) histology of a female copepod surrounded by polyp tissues, with nauplii larvae visible, (D) a male. C: copepod, s: polyp tissue and n: nauplii larvae. Scale bar in A=1 mm, in B and C=500 µm and in D=200 µm.

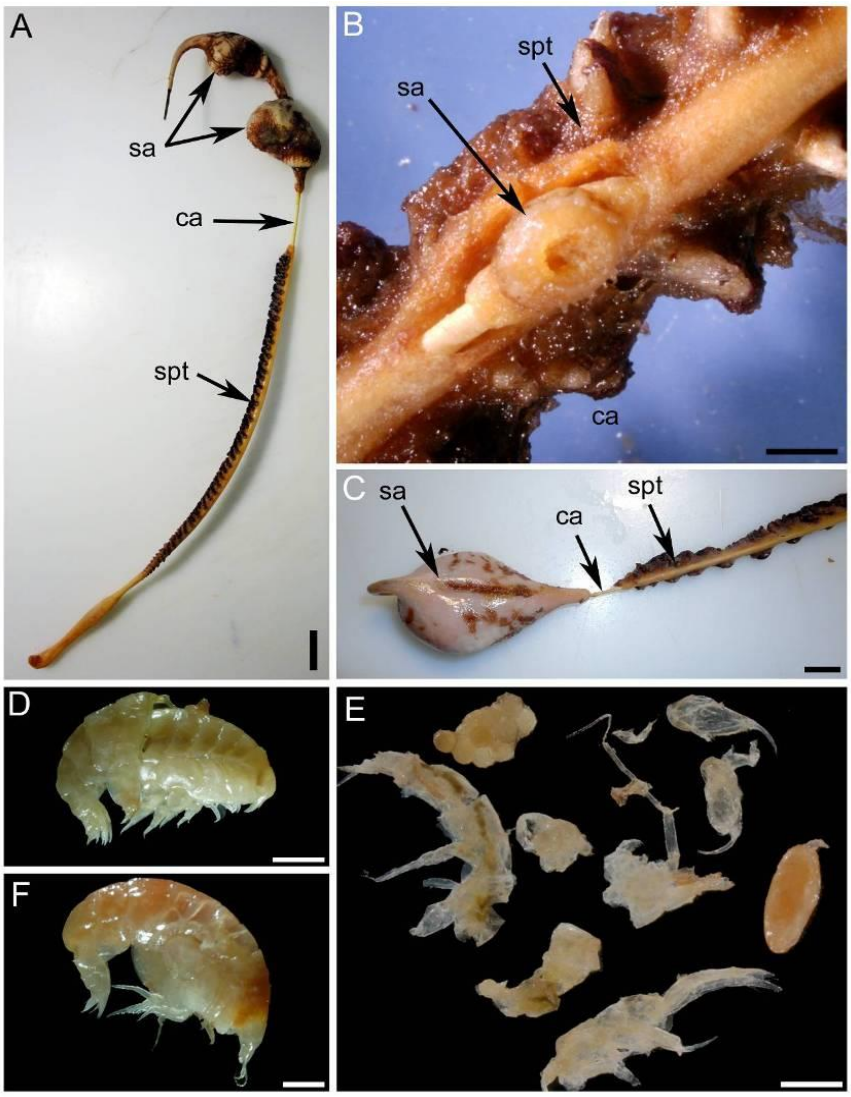


Figure 5-5 The sea anemone Stephanauge nexilis using the central axis of Halipteris finmarchica as a substrate: (A) general view of a colony of H. finmarchica harbouring two sea anemones in the upper section, (B) a small sea anemone surrounded by sea pen tissues, (C) dorsal view of the sea anemone found on the upper section of the sea pen colony. Gastro-vascular contents were found: (D and F) amphipoda, (E) mix of prey including amphipoda, halocyprids, egg mass, and unidentified piece of prey extracted from one sea anemone. sa: sea anemone, ca: central axis, spt: sea pen soft tissues. Scale bar in A=2 cm, in B and D=2 mm and C and F=1 cm, E=1 mm.

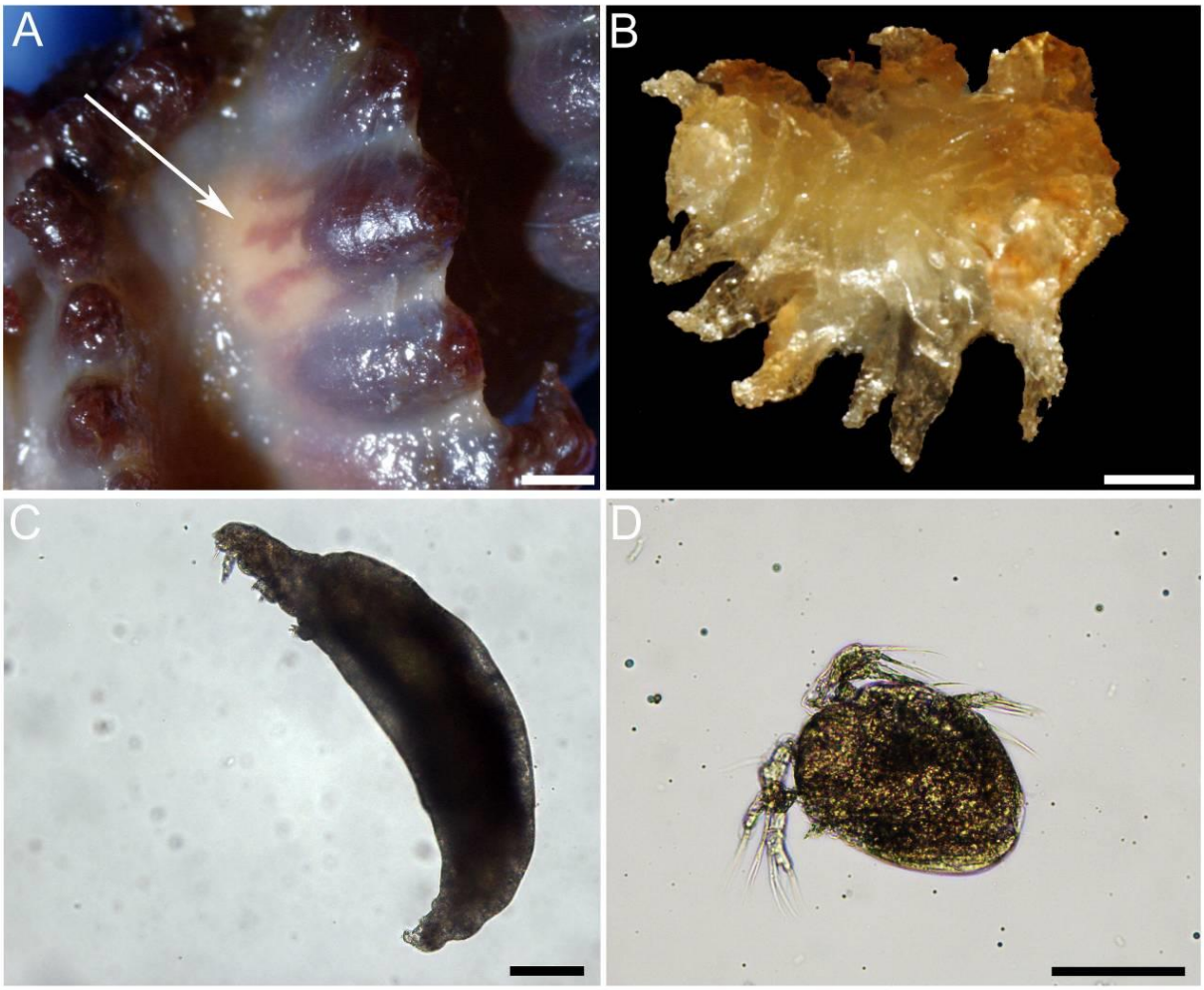


Figure 5-6 Undescribed copepod species belonging to Corallovexiidae living inside the polyps of Halipteris finmarchica: (A) row of polyps including a polyp infested with a copepod (arrow), (B) female copepod, (C) male copepod and (D) nauplius larvae. Scale in A=1 mm, in B=500 µm, in C and D=100 µm.

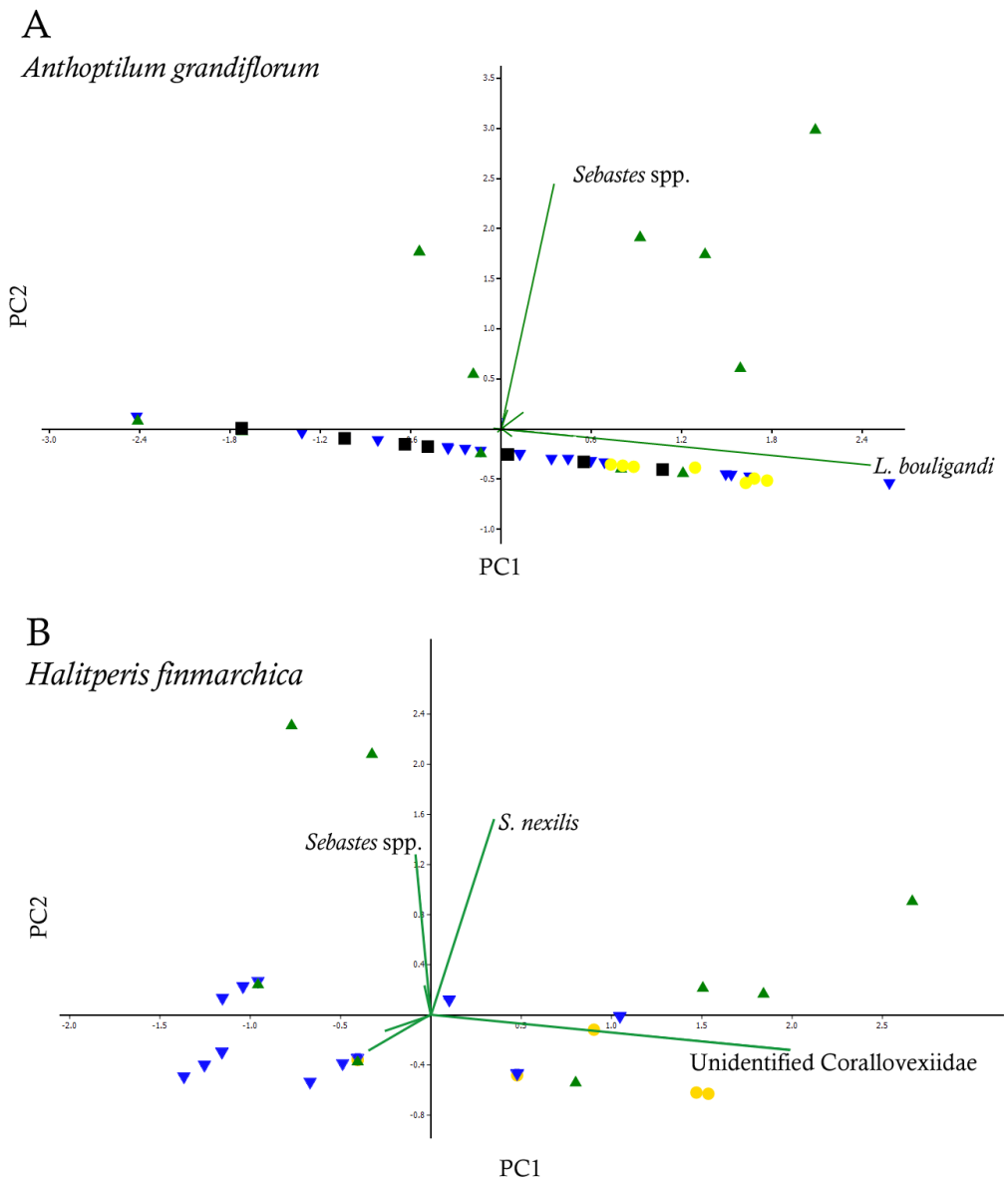


Figure 5-7 Principal component analyses and biplots for Anthoptilum grandiflorum (A, B) and Halipteris finmarchica (C, D). ▲: spring; ●: summer; ▼: fall; ■: winter.

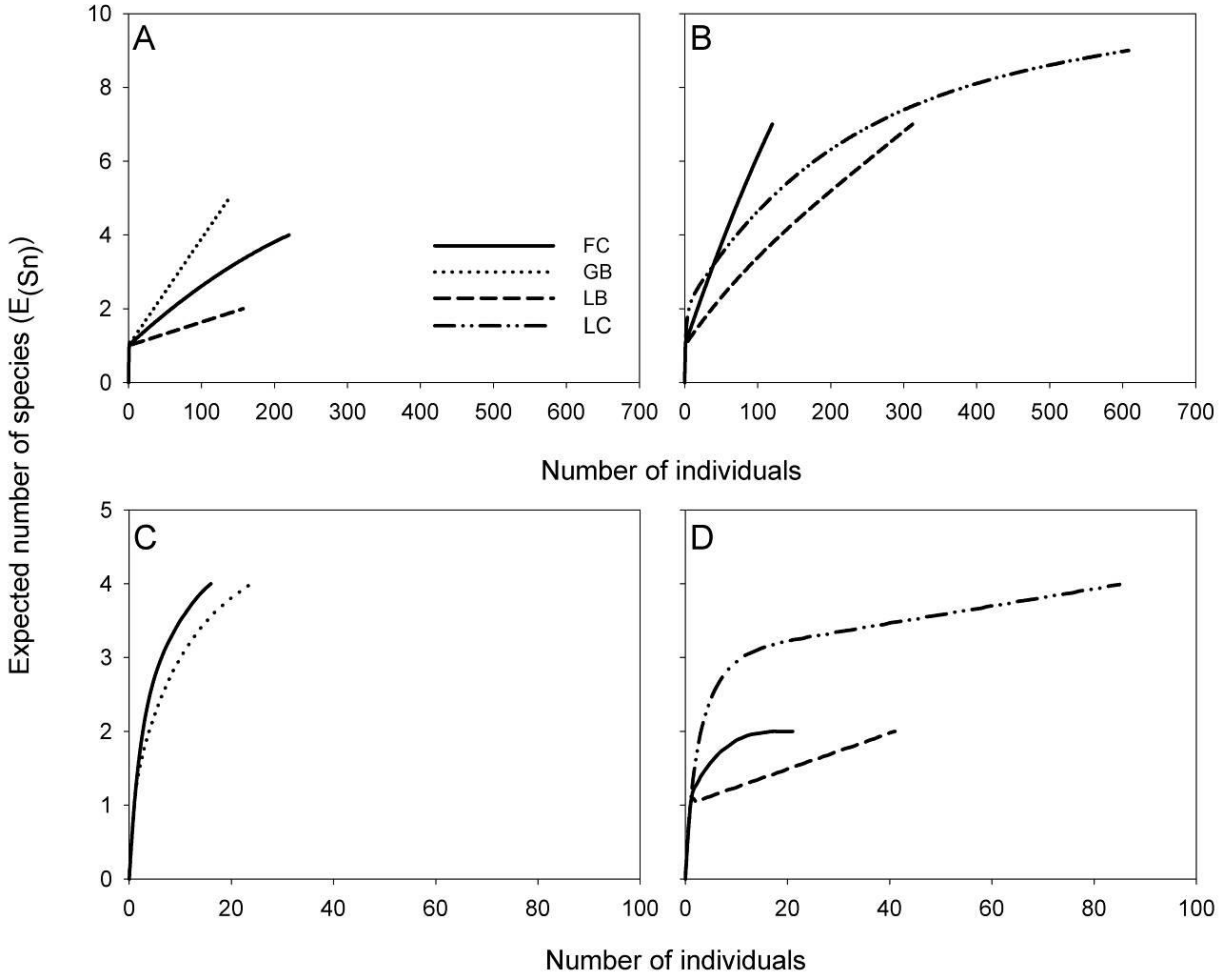


Figure 5-8 Rarefaction curves according to sampling regions for the associated fauna of Anthoptilum grandiflorum in the (A) fall and (B) in spring/summer and for Halipteris finmarchica in the (C) fall and (D) summer.

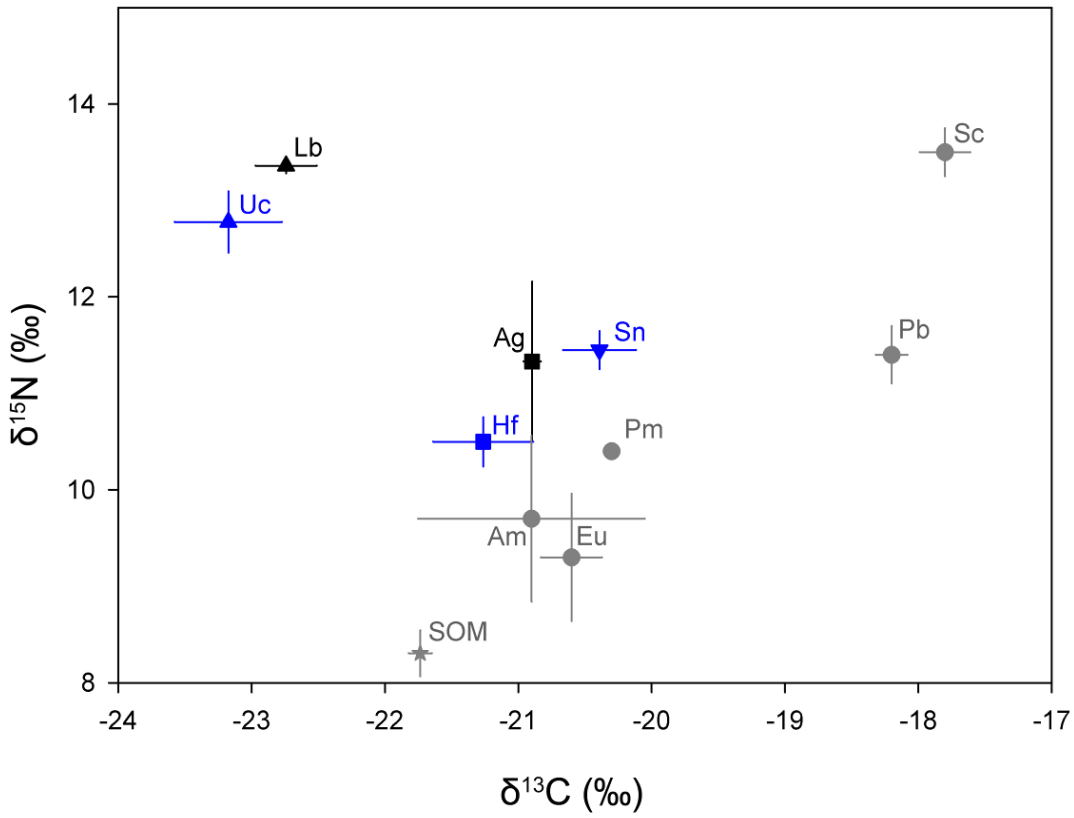


Figure 5-9 Stable isotope values ($\delta^{15}N$ and $\delta^{13}C$) for sea pens (Ag: Anthoptilum grandiflorum and Hf: Halipteris finmarchica) and their associated species (Lb: Lamippe bouligandi, Sn: Stephanauge nexilis and Uc: Undescribed Corallovexiidae). To locate and compare the signature of the sea pens, values for other invertebrates are shown, Am: Amphipods, Eu: Euphausiids, Pm: Pasiphae multidentata, Pb: Pandalus borealis and Sc: snow crab from Sherwood & Ross (2005), as well as sedimentary organic matter (SOM) from Muzuka & Hillaire-Marcel (1999). Result shown as mean ± SE (n=2-5). Black: A. grandiflorum and its associates, Blue: H. finmarchica and this associates, Grey: other invertebrates and SOM.

## Supplementary materials

Appendix 5-A List of the sites analysed in 2006-2007. The symbol -- indicates that no samples were analysed.

| | | | | Number of colonies examined | | SMEY/SMY[b] (individual colony$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Region[a]** | **Date** | **Depth (m)** | ***A. grandiflorum*** | ***H. finmarchica*** | ***A. grandiflorum*** | | ***H. finmarchica*** | |
| 1 | NNL | 26/01/2006 | 1167 | 2 | -- | 11 | 5.5 | -- | -- |
| 2 | NNL | 27/01/2006 | 918 | -- | 1 | -- | -- | 0 | 0 |
| 3 | NNL | 27/01/2006 | 1070 | 1 | -- | 6 | 6 | -- | -- |
| 4 | NNL | 30/01/2006 | 821 | 2 | -- | 11 | 5.5 | -- | -- |
| 5 | NNL | 12/02/2006 | 921 | 2 | -- | 6 | 3 | -- | -- |
| 6 | NNL | 15/03/2006 | 864 | 1 | -- | 1 | 1 | -- | -- |
| 7 | NNL | 31/03/2006 | 891 | 1 | -- | 1 | 1 | -- | -- |
| 8 | LB | 10/07/2006 | 1299 | 1 | -- | 0 | 0 | -- | -- |
| 9 | LB | 14/07/2006 | 837 | -- | 1 | -- | -- | 14 | 14 |
| 10 | LB | 27/7/2006 | 176 | 2 | -- | 21 | 21 | -- | -- |
| 11 | LB | 28/07/2006 | 552 | 3 | 5 | 7.7 | 7.7 | 3.3 | 2.6 |
| 12 | LB | 28/07/2006 | 620 | 5 | 2 | 13.6 | 13.6 | 4 | 4 |
| 13 | LB | 29/07/2006 | 452 | -- | 1 | -- | -- | 0 | 0 |
| 14 | LB | 30/07/2006 | 528 | -- | 1 | -- | -- | 1 | 1 |
| 15 | LB | 04/08/2006 | 563 | -- | 1 | -- | -- | 1 | 0.5 |
| 16 | LB | 04/08/2006 | 630 | -- | 2 | -- | -- | 1 | 0.5 |
| 17 | LB | 05/08/2006 | 559 | -- | 1 | -- | -- | 0 | 0 |
| 18 | LB | 08/08/2006 | 724 | -- | 1 | -- | -- | 4 | 4 |
| 19 | GB | 06/10/2006 | 618 | -- | 2 | -- | -- | 1 | 0.5 |
| 20 | LB | 07/10/2006 | 626 | 1 | -- | 53 | 53 | -- | -- |
| 21 | LB | 07/10/2006 | 466 | 1 | -- | 3 | 3 | -- | -- |
| 22 | LB | 09/10/2006 | 424 | -- | 1 | -- | -- | 0 | 0 |
| 23 | LB | 09/10/2006 | 803 | 1 | -- | 17 | 17 | -- | -- |
| 24 | LB | 15/10/2006 | 548 | 3 | -- | 17 | 17 | -- | -- |
| 25 | LB | 17/10/2006 | 895 | 1 | -- | 8 | 8 | -- | -- |
| 26 | LB | 02/11/2006 | 256 | -- | 1 | -- | -- | 0 | 0 |
| 27 | LB | 04/11/2006 | 1071 | 1 | 0 | 1 | 1 | -- | -- |
| 28 | FC | 24/11/2006 | 1018 | 1 | -- | 1 | 1 | -- | -- |
| 29 | FC | 27/11/2006 | 958 | 2 | -- | 6 | 6 | -- | -- |
| 30 | FC | 28/11/2006 | 1073 | -- | 2 | -- | -- | 0 | 0 |
| 31 | FC | 28/11/2006 | 788 | -- | 1 | -- | -- | 1 | 1 |
| 32 | FC | 28/11/2006 | 1134 | 4 | -- | 3.3 | 2.5 | -- | -- |
| 33 | FC | 28/11/2006 | 1125 | 20 | 4 | 7.8 | 7.4 | 1.5 | 0.8 |
| 34 | FC | 30/11/2006 | 780 | -- | 2 | -- | -- | 0 | 0 |
| 35 | FC | 01/12/2006 | 1161 | -- | 3 | -- | -- | 0 | 0 |
| 36 | FC | 01/12/2006 | 1186 | 2 | -- | 9.5 | 9.5 | -- | -- |
| 37 | FC | 01/12/2006 | 1036 | 3 | -- | 11 | 11 | -- | -- |
| 38 | FC | 02/12/2006 | 978 | 1 | -- | 3 | 3 | -- | -- |
| 39 | LC | 14/04/2007 | 435 | 8 | -- | 3.3 | 1.3 | -- | -- |
| 40 | LC | 15/04/2007 | 457 | 3 | -- | 38 | 12.7 | -- | -- |

Appendix 5-A continued

| | | | | Number of colonies examined | | SMEY/SMY[b] (individual colony$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Region[a]** | **Date** | **Depth (m)** | ***A. grandiflorum*** | ***H. finmarchica*** | ***A. grandiflorum*** | | ***H. finmarchica*** | |
| 41 | LC | 15/04/2007 | 456 | 1 | -- | 9 | 9 | -- | -- |
| 42 | LC | 15/04/2007 | 428 | 4 | -- | 16.7 | 12.5 | -- | -- |
| 43 | LC | 16/04/2007 | 457 | 3 | -- | 13.3 | 13.3 | -- | -- |
| 44 | LC | 16/04/2007 | 352 | 6 | -- | 5.8 | 4.8 | -- | -- |
| 45 | GB | 20/04/2007 | 377 | -- | 2 | -- | -- | 0 | 0 |
| 46 | LC | 28/04/2007 | 370 | 3 | 3 | 17 | 9.3 | 13.3 | 13.3 |
| 47 | LC | 29/04/2007 | 366 | -- | 4 | -- | -- | 6 | 4.5 |
| 48 | LC | 29/04/2007 | 462 | 2 | -- | 5 | 5 | -- | -- |
| 49 | LC | 30/04/2007 | 389 | -- | 2 | -- | -- | 8 | 8 |
| 50 | GB | 30/04/2007 | 98 | 1 | -- | 1 | 1 | -- | -- |
| 51 | LC | 30/04/2007 | 422 | 4 | 2 | 21.5 | 21.5 | 5.5 | 5.5 |
| 52 | GB | 04/05/2007 | 575 | -- | 4 | -- | -- | 1 | 0.3 |
| 53 | GB | 17/05/2007 | 425 | -- | 1 | -- | -- | 6 | 6 |
| 54 | GB | 17/05/2007 | 214 | 1 | -- | 0 | 0 | -- | -- |
| 55 | GB | 17/05/2007 | 597 | 3 | 2 | 3.5 | 2.3 | 1 | 0.5 |
| 56 | GB | 17/05/2007 | 600 | 4 | -- | 2.8 | 2.8 | -- | -- |
| 57 | GB | 18/05/2007 | 625 | -- | 1 | -- | -- | 0 | 0 |
| 58 | FC | 18/06/2007 | 658 | 2 | -- | 0 | 0 | -- | -- |
| 59 | FC | 08/07/2007 | 701 | 1 | -- | 0 | 0 | -- | -- |
| 60 | LB | 17/07/2007 | 881 | 3 | -- | 9 | 9 | -- | -- |
| 61 | LB | 18/07/2007 | 871 | 3 | -- | 8.3 | 8.3 | -- | -- |
| 62 | LB | 20/07/2007 | 883 | 2 | -- | 30 | 30 | -- | -- |
| 63 | LB | 21/07/2007 | 874 | 2 | -- | 31 | 31 | -- | -- |
| 64 | FC | 27/07/2007 | 267 | 1 | -- | 1 | 1 | -- | -- |
| 65 | GB | 07/10/2007 | 773 | -- | 5 | -- | -- | 2 | 0.4 |
| 66 | GB | 08/10/2007 | 1034 | -- | 1 | -- | -- | 0 | 0 |
| 67 | GB | 08/10/2007 | 823 | -- | 4 | -- | -- | 1 | 0.3 |
| 68 | GB | 08/10/2007 | 1094 | -- | 1 | -- | -- | 0 | 0 |
| 69 | GB | 08/10/2007 | 796 | -- | 1 | -- | -- | 0 | 0 |
| 70 | GB | 08/10/2007 | 1333 | 2 | 1 | 1 | 0.5 | 2 | 2 |
| 71 | GB | 08/10/2007 | 824 | 3 | -- | 3.7 | 3.7 | -- | -- |
| 72 | GB | 09/10/2007 | 1130 | 2 | -- | 1 | 0.5 | -- | -- |
| 73 | GB | 10/10/2007 | 1347 | 1 | -- | 5 | 5 | -- | -- |
| 74 | GB | 10/10/2007 | 836 | 2 | -- | 6 | 6 | -- | -- |
| 75 | GB | 10/10/2007 | 934 | 6 | -- | 3.6 | 3 | -- | -- |
| 76 | LB | 16/10/2007 | 986 | 1 | -- | 1 | 1 | -- | -- |
| 77 | FC | 18/10/2007 | 1036 | -- | 2 | -- | -- | 4 | 2 |
| 78 | FC | 18/10/2007 | 1151 | -- | 1 | -- | -- | 0 | 0 |
| 79 | FC | 18/10/2007 | 768 | -- | 1 | -- | -- | 5 | 5 |
| 80 | FC | 18/10/2007 | 1061 | -- | 1 | -- | -- | 0 | 0 |
| 81 | FC | 18/10/2007 | 896 | 6 | -- | 4 | 0.7 | -- | -- |
| 82 | FC | 18/10/2007 | 1208 | 4 | 3 | 4 | 3 | 0 | 0 |
| 83 | FC | 19/10/2007 | 743 | 2 | -- | 5 | 2.5 | -- | -- |
| 84 | FC | 19/10/2007 | 743 | 2 | -- | 1 | 0.5 | -- | -- |
| 85 | GB | 28/10/2007 | 599 | 3 | 1 | 5 | 3.3 | 4 | 4 |
| 86 | FC | 29/10/2007 | 992 | -- | 4 | -- | -- | 2 | 0.5 |
| 87 | GB | 29/10/2007 | 579 | 1 | 2 | 0 | 0 | 3 | 1.5 |
| 88 | GB | 30/10/2007 | 593 | 2 | 2 | 8 | 4 | 1.5 | 1.5 |
| 89 | GB | 30/10/2007 | 623 | 4 | 3 | 12.5 | 12.5 | 2.7 | 2.7 |

Appendix 5-A continued

| | | | | Number of colonies examined | | SMEY/SMY[b] (individual colony$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Region[a]** | **Date** | **Depth (m)** | ***A. grandiflorum*** | ***H. finmarchica*** | ***A. grandiflorum*** | | ***H. finmarchica*** | |
| 90 | LB | 14/11/2007 | 1041 | -- | 1 | -- | -- | 0 | 0 |
| 91 | LB | 16/11/2007 | 1039 | 1 | -- | 22 | 22 | -- | -- |
| 92 | FC | 25/11/2007 | 651 | 1 | -- | 7 | 7 | -- | -- |
| 93 | FC | 26/11/2007 | 273 | 2 | -- | 20 | 10 | -- | -- |
| 94 | FC | 29/11/2007 | 810 | 2 | 1 | 1 | 0.5 | 0 | 0 |
| 95 | NNL | 08/12/2007 | 656 | 1 | -- | 0 | 0 | -- | -- |
| 96 | NNL | 14/12/2007 | 851 | 1 | -- | 0 | 0 | -- | -- |
| 97 | NNL | 14/12/2007 | 1285 | 1 | -- | 5 | 5 | -- | -- |

[a] LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador

b SMEY: Site Mean Exact Yield, SMY: Site Mean Yield

## Appendix 5-B List of sites analysed in 2009-2010

| | | | | Number of colonies examined | |
|---|---|---|---|---|---|
| **Site** | **Region*** | **Date** | **Depth (m)** | ***A. grandiflorum*** | ***H. finmarchica*** |
| 98 | LC | 08/05/2008 | 301 | 1 | 0 |
| 99 | LC | 09/05/2008 | 462 | 1 | 0 |
| 100 | GB | 11/04/2009 | 494 | 2 | 0 |
| 101 | GB | 11/04/2009 | 428 | 1 | 0 |
| 102 | LC | 17/04/2009 | 318 | 4 | 0 |
| 103 | LC | 18/04/2009 | 437 | 2 | 0 |
| 104 | LC | 18/04/2009 | 428 | 1 | 0 |
| 105 | LC | 19/04/2009 | 453 | 2 | 0 |
| 106 | LC | 19/04/2009 | 299 | 1 | 0 |
| 107 | LC | 19/04/2009 | 317 | 3 | 0 |
| 108 | LC | 19/04/2009 | 445 | 6 | 0 |
| 109 | LC | 29/04/2009 | 451 | 3 | 0 |
| 110 | LC | 30/04/2009 | 488 | 1 | 0 |
| 111 | LC | 30/04/2009 | 348 | 3 | 0 |
| 112 | LC | 03/05/2009 | 265 | 1 | 0 |
| 113 | LC | 10/05/2009 | 404 | 3 | 0 |
| 114 | LC | 11/05/2009 | 404 | 1 | 0 |
| 115 | LC | 11/05/2009 | 422 | 3 | 2 |
| 116 | LC | 12/05/2009 | 410 | 0 | 1 |
| 117 | GB | 24/05/2009 | 337 | 2 | 2 |
| 118 | GB | 24/05/2009 | 603 | 3 | 4 |
| 119 | GB | 26/05/2009 | 596 | 3 | 1 |
| 120 | LC | 14/04/2010 | 384 | 0 | 2 |
| 121 | LC | 15/04/2010 | 314 | 2 | 0 |
| 122 | LC | 24/04/2010 | 456 | 1 | 0 |
| 123 | LC | 25/04/2010 | 449 | 1 | 0 |
| 124 | LC | 26/04/2010 | 455 | 1 | 0 |
| 125 | LC | 26/04/2010 | 468 | 1 | 0 |
| 126 | LC | 26/04/2010 | 433 | 3 | 0 |
| 127 | LC | 27/04/2010 | 447 | 3 | 0 |
| 128 | LC | 11/05/2010 | 673 | 1 | 0 |

*LC: Laurentian Channel, GB: Grand Banks, FC: Flemish Cap, NNL: North Newfoundland, LB: Labrador

# Chapter 6 : Deep cold-water corals as nurseries for fish larvae

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## Abstract

As a consequence of the decline of numerous commercial fish populations, an ecosystem-based approach to fisheries management, which includes the protection of essential fish habitat (EFH), has emerged. Although cold-water coral (CWC) sites are recognized as biodiversity hotspots, CWC destruction and degradation by anthropogenic activities are well documented. However, although functional connections between CWCs and fish stocks are suspected, based on correlative evidence, proof of any close or direct relationship identifying CWCs as EFH is still lacking. Here, we provide evidence of the utilization of CWCs by fish larvae, mainly those of redfish (Sebastes spp). In multiyear surveys, fish larvae were consistently found closely associated with five species of sea pen (Octocorallia: Pennatulacea) in April and May. Prevalence and/or yields of fish larvae varied with coral host species, depth, location, and colony size. Evidence of the role of CWCs in the early life history of some fish species provides the strongest argument yet for the categorization of CWCs as EFH in the design of management programs.

# 1. Introduction

In light of the global decline and poor recovery of many fish populations, including commercial stocks, as a result of historical and anthropogenic factors (Pauly et al. 2002; Devine et al. 2006), the need for a holistic ecosystem-based approach to fisheries management is increasingly being recognized. Greater emphasis is being placed on habitat health and productivity, notably through the designation of essential fish habitat (EFH), which can be defined as "those waters and substrates necessary to fish for spawning, breeding, feeding, or growth to maturity" (Rosenberg et al. 2000). Concomitantly, cold-water coral (CWC) ecosystems are emerging as systems of ecological and economic value, raising concern over their rapid destruction (Fosså et al. 2002; Foley et al. 2010a). CWCs form one of the most complex biological habitats on continental slopes, where they act as biogenic substrates by offering a variety of microhabitats presumed to serve as feeding and spawning sites for other species (Buhl-Mortensen et al. 2010; Watling et al. 2011). Studies of their associated fauna have shown that biodiversity in deep-sea CWCs is comparable to that of tropical coral reefs (Buhl-Mortensen et al. 2010; Watling et al. 2011). However, contrary to the association between shallow coral reefs and fish, the exact relationship between CWCs and fish is not clear (Auster 2005), and CWCs are mostly considered to be facultative habitat (important but non-essential for the survival of a species). Evidence of a functional role that would qualify CWCs as EFH in support of their protection (Foley et al. 2010a) is still lacking.

The putative ecological importance of CWCs is magnified by their widespread distribution on continental slopes, canyons, and seamounts worldwide, in water depths ranging from 39 m to more than 3000 m (Foley et al. 2010a). It has often been suggested that CWCs may be used as nursery grounds by fish (Etnoyer and Warrenchuk 2007; Buhl-Mortensen et al. 2010), based on the assumption that corals offer protection against predators (Auster 2005). It has been suggested that CWCs fit the definition of essential habitat for redfish (Sebastes spp) in Norway, on the basis of habitat–fish models (Foley et al. 2010b). However, to date, no direct evidence of the presence of juvenile or larval fish in CWCs has been presented (Husebø et al. 2002; Foley et al. 2010a). Correlative studies and predictive models have shown increasing adult fish densities and sizes around deep-water corals compared with non-coral areas (Husebø et al. 2002; Auster 2005). Spring aggregations of swollen (presumably gravid) redfish females were detected around the scleractinian coral Lophelia pertusa in Norway (Fosså et al. 2002), and catshark egg cases were found attached on the gorgonian coral Callogorgia sp in the Mississippi Canyon, Gulf of Mexico (Etnoyer and Warrenchuk 2007). Nevertheless, the potential importance of CWCs remains unclear because studies do not cover the period when fish might use this habitat (ie for spawning or as juveniles; Auster 2007).

To date, most of the limited studies on the distribution and biology of CWCs have focused on the subclass Hexacorallia, order Scleractinia (stony corals), and to a lesser degree on some species of the subclass Octocorallia, particularly gorgonians (sea fans) and other members of the order Alcyonacea (soft corals) (Buhl-Mortensen et al. 2010). Members of the order Pennatulacea (sea pens, each of which is a colony of polyps) are comparatively overlooked despite being common throughout the world's oceans and

considered as habitat-forming vulnerable species in both shallow and deep environments (Langton et al. 1990; Murillo et al. 2011; Williams 2011). Distributional data have hinted at the possible importance of soft corals, small gorgonians, and sea pens as potential fish habitat (Edinger et al. 2007). Here, we provide unprecedented evidence of the use of CWCs by larvae of commercial fish species, which were found in deep-water sea pens (Figure 6-1a) collected as bycatch from epibenthic surveys conducted from 2005 to 2010 off the east coast of Canada.

## 2. Materials and methods

### 2.1 Sampling

This study took advantage of routine multispecies research surveys conducted by Fisheries and Oceans Canada (DFO) from 2005 to 2010 in the Laurentian Channel and southern Grand Banks, located off the coast of the Atlantic Provinces of eastern Canada (Appendix 6-B). Surveys followed a stratified random sampling design with a Campellen 1800 trawl towed for 15 minutes on approximately 1.4 km (gear opened and closed at depth). Bycatch was examined for corals by trained technicians. Different species of sea pens were sampled at 58 stations between 98 m and 719 m depth (Appendix 6-A); samples were immediately frozen at -20°C. An analysis of the sea pen Anthoptilum grandiflorum was first conducted on all samples collected in 2006 and 2007 (spanning 7-8 mo for each year) in a comprehensive study of their biology and species that are associated with them. This investigation revealed the presence of fish larvae in April and May. Consequently, available samples of all sea pen species collected in April and May between 2005 and 2010 were examined for the present study. A total of 288 sea pen

colonies belonging to five species – Anthoptilum grandiflorum, Halipteris finmarchica, Funiculina quadrangularis, Pennatula aculeata, and Pennatula grandis – were studied (Table 6-1). A complementary analysis of other soft corals in the order Alcyonacea (Duva florida, Drifa glomerata, and Gersemia fruticosa) was conducted for the 23 sites where fish larvae were present on sea pens.

2.2 Sample analysis

For A. grandiflorum and P aculeata, ascertaining the number of colonies to be examined was based on (1) the quantity of samples available for a given site and (2) the presence or absence of fish larvae. A minimum of three haphazardly chosen colonies per site were initially inspected (however, if there were fewer than three colonies at a given site, all colonies were inspected). When fish larvae were found on at least one of the three colonies examined in a site with more than three colonies, the number of subsamples examined was increased to account for 20% of the samples available. For H. finmarchica, F. quadrangularis, and P. grandis, all colonies were inspected, except for F. quadrangularis at three sites where fish larvae were absent in the first three samples. There were also several sites where only one colony had apparently been preserved by DFO, although the presence of additional colonies was indicated in survey records. Incomplete or damaged colonies of sea pens were excluded from the analysis.

The frozen colonies were thawed in filtered seawater before examination. We measured the size (from base to tip) and damp weight of each colony, and determined the presence, quantity, and size of fish larvae under a stereomicroscope (Nikon SMZ1500). Polyps were carefully manipulated to expose and uncover fish larvae. A separate study of polyp digestive tract did not find any evidence of fish larvae, ruling out a prey–predator

relationship. Fish tissues (whole or half larvae) preserved in 100% ethanol were shipped to the Canadian Centre for DNA Barcoding (University of Guelph, Canada) for genetic analysis. We analyzed a subsample of 196 specimens using standard polymerase chain reaction and DNA sequencing protocols (Ivanova et al. 2006; DeWaard et al. 2008). Partial sequences of the cytochrome c oxidase subunit I gene with all metadata are registered in the Barcode of Life Data System (BOLD; Ratnasingham and Hebert 2007), project FLSB, and submit to Genbank (n# JX008540-JX008735). Identifications were made by running the sequences against the BOLD and Basic Local Alignment Search Tool (BLAST) databases (99.5–100% certainty).

The mean yield (MY) was defined as the mean number of fish larvae per colony (fish larvae colony$^{-1}$) considering all sea pens examined, and the mean exact yield (MEY) was defined as the mean number of fish larvae colony$^{-1}$ considering only sea pens with fish larvae. The MY for a site (site mean yield [SMY] or site mean exact yield [SMEY]) was defined as the number of fish larvae found in that site divided by the number of sea pen colonies inspected for that site (as fish larvae colony$^{-1}$). The number of fish larvae at a site was extrapolated from the total number of colonies sampled there, multiplied by MY. Concentrations were recorded as the number of fish larvae per 100 grams of coral (100-g$^{-1}$).

2.3 Data analysis

Parametric tests were conducted when assumptions of normality and equal variance were met, otherwise non-parametric counterparts were used. Kruskall-Wallis tests were conducted to assess differences in fish larvae yields at various locations and depths, followed by Dunn's post-hoc tests. A Mann-Whitney test was used to compare yields in

day and night samplings. Two-way analyses of variance (no interactions) on the abundance of A. grandiflorum colonies per site showed a significant influence of year ($F_{4,42}$ = 3.57, P = 0.014) but not month (April or May, P = 0.137). Similarly, colony size was significantly affected by year ($F_{4,102}$ = 2.76, P = 0.032) but not month (P = 0.379). Months were therefore pooled for the analysis of annual trends. Regression analyses and correlations (Pearson or Spearman) were used to examine relationships between variables. All data are expressed as mean ± standard error (SE).

Principal component analyses (PCAs) were used to explore which of the biotic (various sea pen species, adult redfish at different maturity stages) or abiotic (depth, latitude, month, temperature, day/night) factors best explained the presence of fish larvae. Normalization for the abiotic data and log(x + 1) transformation for the biotic data were applied before all PCAs. The log(x + 1) transformation allows for the consideration of both the most abundant and more rare species. A correlation matrix for the abiotic data and a variance–covariance matrix for the biotic data (Quinn and Keough 2002) were used.

## 3. Results and discussion

DNA analysis confirmed that most of the fish larvae detected in sea pens belonged to two redfish species (Sebastes fasciatus and Sebastes mentella), both of which are commercially exploited and listed as endangered or threatened in Canada (COSEWIC 2010). The two species cannot accurately be distinguished through DNA analysis and are suspected to hybridize; we therefore grouped them together as Sebastes spp in this study. Eggs or larvae of the lantern fish (Benthosema glaciale) and the eelpout (Lycodes

esmarkii) were also found. The MEY for redfish was 5.4 ± 0.8 larvae colony$^{-1}$. No evidence of a predatory relationship was found.

Occurrences of redfish larvae were consistently concentrated between April and May in the Laurentian Channel, which features one of the highest known densities of sea pens (Kenchington et al. 2010), perhaps because the high primary and secondary production at the surface generates planktonic material that sinks to the seafloor, providing food for suspension-feeding azooxanthellate corals (Sherwood et al. 2008). The intimacy of the association (Figure 6-1, d and e) and the small size of the fish larvae (< 8 mm with/without yolk sac; Figure 6-1, b and c) suggest that the viviparous redfish release their progeny near corals, where they remain during early ontogeny. The calcified parts (sclerites) of, and/or toxic chemicals produced by soft corals deter potential predators (Changyun et al. 2008). Therefore, fish may possibly release larvae deliberately among corals to provide them with shelter and protection. The rapid retraction of sea pen polyps and branches upon disturbance (observed during capture) probably trapped the fish larvae within the colonies. Whether sea pens and other CWCs provide nourishment to larval fish in the form of mucus and/or associated particles remains to be determined.

Prevalence of the association between sea pens and redfish larvae varied between 11.5% and 100% among five host species (Table 6-1). Anthoptilum grandiflorum had the second highest fish larvae prevalence (35.8%), had the highest maximum yield (36 fish larvae colony$^{-1}$), and was the most abundant sea pen species (present at 79% of all sites). Fifty-three percent of the 23 sites where fish larvae occurred were monospecific for sea pens; of those monospecific sites, 84% featured only A. grandiflorum. Fish larvae were absent at the remaining monospecific sites. Of the multispecific sites excluding A.

grandiflorum, only 20% were associated with fish larvae, suggesting the importance of this sea pen species, which has a worldwide distribution (Williams 2011). Sea pen colony size and the number of fish larvae they harbored were positively correlated (Figure 6-2a), a finding that raises some concern, given (1) the longevity and slow growth of sea pens and other CWCs (Andrews et al. 2002; Wilson et al. 2002) and (2) the apparent decline in both number and size of A. grandiflorum colonies (Figure 6-2, b and c). Prevalence of fish larvae in A. grandiflorum colonies collected in April also dropped between 2005 and 2010 (Figure 6-2d). Other soft corals in the order Alcyonacea (D. florida, D. glomerata, and G. fruticosa) exhibited a MEY of 2.6 ± 0.7 fish larvae colony$^{-1}$ (n = 11) at sites where sea pens hosted fish larvae (excluding 244 larvae found in one D. florida colony).

PCAs on abiotic factors revealed that month and latitude were the main contributors to the first principal component (PC1; 39.5%), temperature was the main contributor to the second principal component (PC2; 26.2%), and depth had a moderate influence in both components (Figure 6-3a). Most sites (92%) where fish larvae occurred on sea pens were characterized by bottom temperatures of 4–6°C and had greater yields at 400–600 m than at other depths (Kruskal-Wallis H-test; H = 34.33, degrees of freedom [df] = 3, P < 0.001; Figure 6-2e). Nearly all sites where sea pens harbored larvae were in the Laurentian Channel (Appendix 6-B) where greater yields occurred (Kruskal-Wallis H-test; H = 37.73, df = 2, P < 0.001; Figure 6-2e). Day or night sampling did not influence yields (Mann-Whitney U-test; U = 146.5, P = 0.197).

The PCA integrating abundance of coral species and female redfish (immature, maturing [with some ripe eggs], mature [presence of larvae], and spent [having spawned]) in research surveys did not clearly explain the occurrence of fish larvae (Figure 6-3b),

suggesting an ontogenetic shift in habitat use. Eigenvectors indicated that 42.8% of the site variations could be accounted for by PC1, with both immature and spent females as positive contributors. In PC2 (accounting for 21.4% of site variations), mature females made the most significant contribution. Unlike fish larvae, adult redfish were found at 57 of the 58 sites; mature and spent females were present at 78% and 66% of sites, respectively; and there was no link between the number of sea pens and the number of adult ($P = 0.983$) or female ($P = 0.162$) redfish at the sites. The number of adult redfish did not correlate with the yield of fish larvae colony$^{-1}$ or the abundance of larvae extrapolated for each site ($P = 0.210$ and $P = 0.432$, respectively). The extrapolated abundance of larvae followed that of mature ($r_s = 0.303$, $P = 0.023$) but not spent ($P = 0.115$) females. Finally, the largest research-based catches of redfish in April and May occurred in the Grand Banks region, where few fish larvae were found (Appendix 6-Ca). A similar trend was observed in concurrent commercial catches (Appendix 6-Cb).

## 4. Conclusions

Evidence for fish–CWC associations has, to date, largely been limited to habitat–fishery or distributional studies (Stone 2006; Edinger et al. 2007; Foley et al. 2010b) that suggest co-occurrences but provide no direct functional links (Auster 2007). Now, with evidence that fish larvae shelter around soft corals, we believe there is a strong argument for classifying those CWCs as EFH and as vulnerable marine ecosystems. Annual occurrences restricted to specific months confirm that key associations may be transient and easily overlooked, as suggested previously (Auster 2007). Furthermore, barring natural spatial variation, bycatch data for 2005–2010 reveal decreasing numbers and sizes

of sea pens, and a decline in the prevalence of colonies hosting fish larvae. Loss of CWCs has previously been documented and a diversity of current and future threats identified (Roberts et al. 2006; Watling et al. 2011). When combined with this knowledge, the findings reported here underscore the need for fisheries managers to assess available data on sea pens and other soft corals and to take appropriate action under the range of legal regimes across the globe.

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# Table

Table 6-1 Distribution of fish larvae in the various sea pen species

| | Sea pen species | | | | |
|---|---|---|---|---|---|
| | Anthoptilum grandiflorum | Pennatula aculeata | Funiculina quadrangularis | Halipteris finmarchica | Pennatula grandis |
| Number of colonies | 109 | 96 | 43 | 35 | 5 |
| Prevalence of association (% colonies with larvae) | 35.8 | 11.5 | 27.9 | 17.1 | 100 |
| Total number of fish larvae | 148 | 58 | 98 | 69 | 20 |
| Mean yield (fish larvae colony$^{-1}$) | 1.4 | 0.6 | 2.3 | 1.9 | 4 |
| Mean exact yield (fish larvae colony$^{-1}$) | 3.8 | 5.3 | 8.2 | 11.5 | 4 |
| Maximum yield (fish larvae colony$^{-1}$) | 36 | 16 | 22 | 26 | 4 |
| Mean concentration (fish larvae 100-g$^{-1}$) | 12.5 | 106 | 405 | 19.1 | 6.3 |
| Maximum concentration (fish larvae 100-g$^{-1}$) | 91.6 | 222.2 | 540.7 | 35.33 | 14.1 |

## Figures

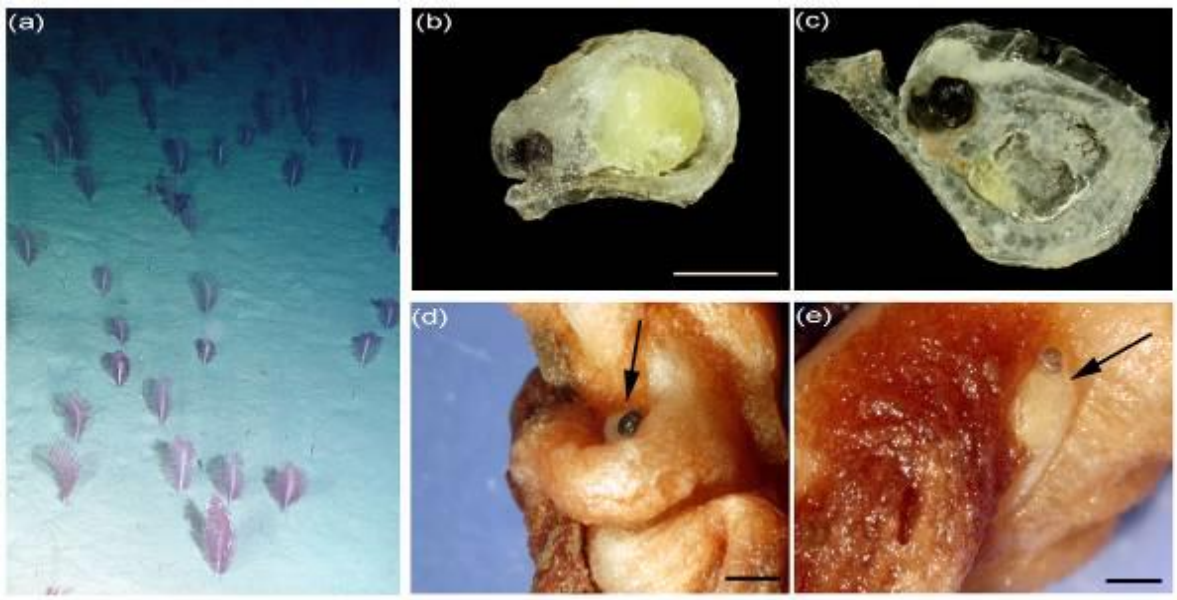



Figure 6-1 (a) Field of sea pens (©DFO). Larvae of redfish were found with yolk sac either present (b) or nearly resorbed (c). Scale bar (1 mm) applies to both panels. (d, e) Fish larvae (arrows) tucked among polyps of A. grandiflorum; scale bars = 1 mm.

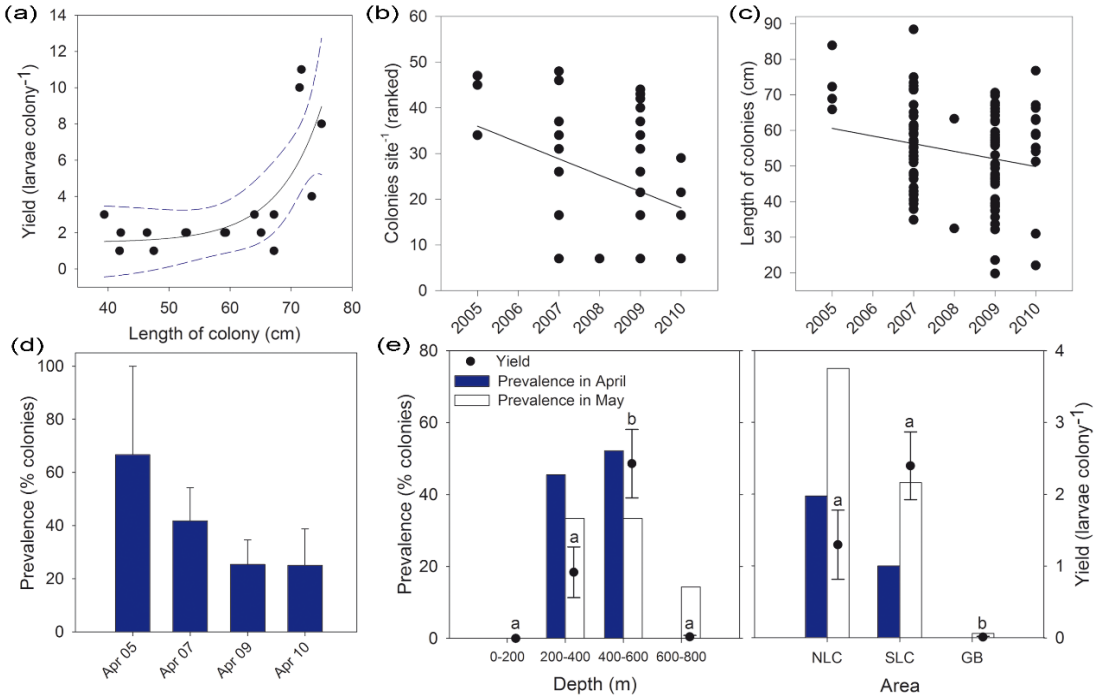



Figure 6-2 (a) Relationship between colony length and yield in A. grandiflorum collected in April 2007 (regression, $y=y_0+a^{bx}$; $F_{2,14}=9.06$, P=0.003, r=0.751); dashed lines, 95% CI. Downward trends in (b) number colonies per site (P=0.012, $r_s=0.362$) and (c) colony size (P=0.027, r=0.213) in A. grandiflorum from 2005 to 2010. (d) Prevalence of the association (% colonies with fish larvae) in A. grandiflorum collected in April of 2005, 2007, 2009 and 2010. (e) Prevalence of colonies with fish larvae (bars) according to depth (left panel) and area (right panel), with corresponding mean yields (closed circles; ±SE). Different letters indicate significant differences (P<0.001).

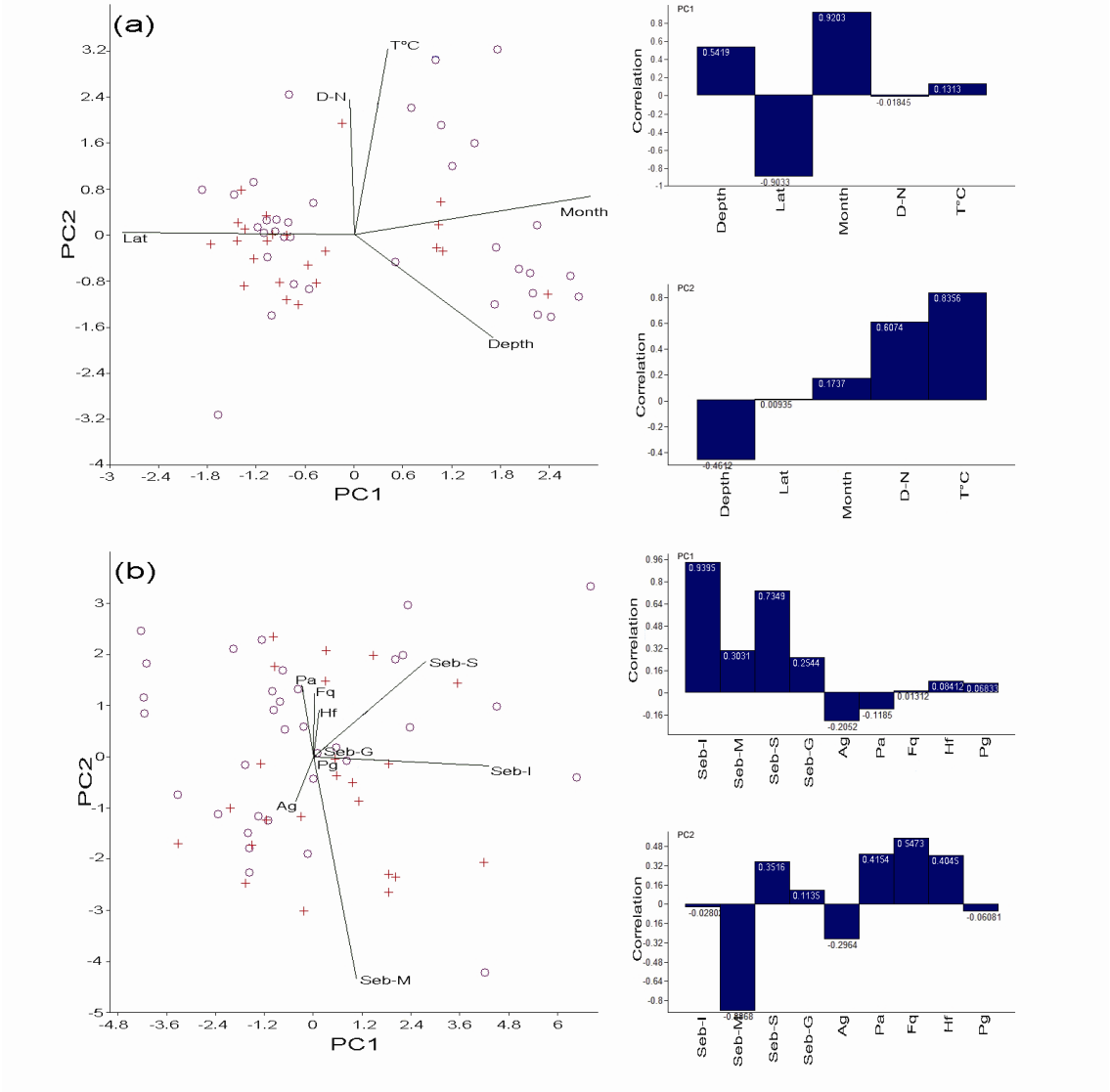



Figure 6-3 PCA biplots and corresponding loadings for (a) abiotic and (b) biotic parameters at sites with the presence (+) or absence (○) of fish larvae on sea pens. Lat.: Latitude, D-N: Day-Night, T°C: temperature, Seb-I: Sebastes immature, Seb-M: Sebastes mature, Seb-S: Sebastes spent, Seb-G: Sebastes maturing, Ag: Anthoptilum grandiflorum, Pa: Pennatula aculeate, Fq: Funiculina quadrangularis, Hf: Halipteris finmarchica, Pg: Pennatula grandis.

## Supplementary material

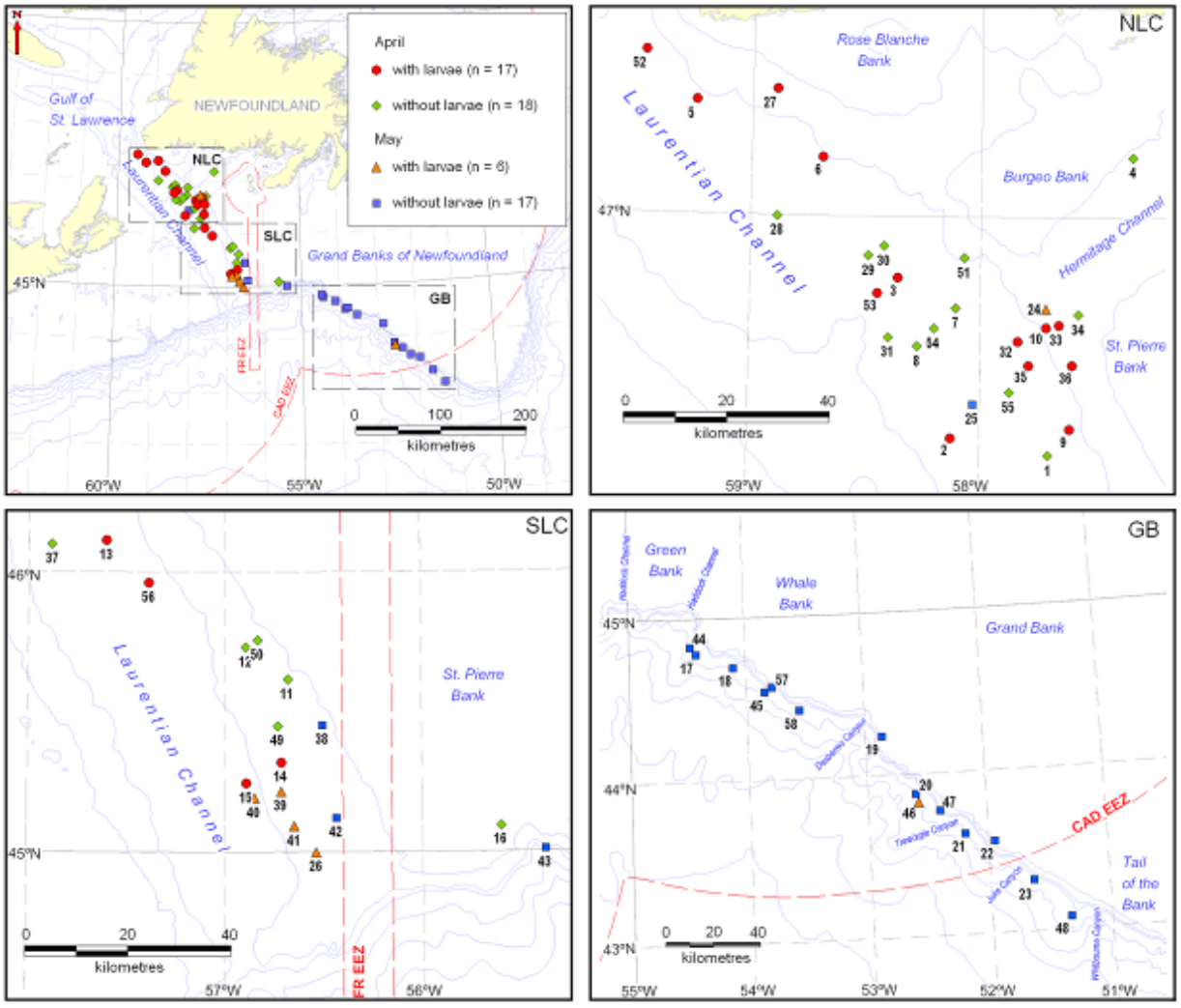
### Appendix 6-A Summary of sea pen samples collected and examined

| Parameters | | | | | | | Number of colonies examined/collected* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Date | Depth (m) | Latitude | T (°C) | SMY/SMEY (larvae colony$^{-1}$) | | A. grandiflorum | P. aculeata | F. quadrangularis | H. finmarchica | P. grandis |
| 1 | 28/04/2005 | 464 | 46°17'38.40"N | 4.9 | 0 | 0.0 | 1/35 | | | | |
| 2 | 28/04/2005 | 448 | 46°20'38.40"N | 4.9 | 1.5 | 1.5 | 2/7 | | | | |
| 3 | 30/04/2005 | 458 | 46°49'6.60"N | 4.8 | 36.0 | 36.0 | 1/30 | | | | |
| 4 | 18/04/2006 | 224 | 47°10'44.40"N | 6.1 | 0 | 0.0 | | 1/2 | | | |
| 5 | 14/04/2007 | 435 | 47°20'11.40"N | 4.7 | 0.9 | 2.0 | 8/34 | | | | 1/1 |
| 6 | 15/04/2007 | 428 | 47°10'26.40"N | 4.7 | 0.5 | 2.0 | 4/7 | | | | |
| 7 | 15/04/2007 | 457 | 46°43'48.00"N | 4.4 | 0 | 0.0 | 3/9 | | | | |
| 8 | 15/04/2007 | 456 | 46°36'54.00"N | 4.1 | 0 | 0.0 | 1/4 | | | | |
| 9 | 16/04/2007 | 457 | 46°22'17.40"N | 4.5 | 2.0 | 2.0 | 3/40 | | | | |
| 10 | 16/04/2007 | 352 | 46°40'26.40"N | 4.6 | 1.0 | 1.8 | 7/14 | | | | |
| 11 | 28/04/2007 | 296 | 45°37'12.00"N | 5.3 | 0 | 0.0 | 1/1 | 17/87 | | 1/1 | |
| 12 | 28/04/2007 | 370 | 45°43'53.40"N | 4.5 | 0 | 0.0 | 3/7 | 11/60 | 6/6 | 3/8 | |
| 13 | 29/04/2007 | 462 | 46° 6'43.20"N | 4.4 | 3.0 | 3.0 | 2/2 | | | | |
| 14 | 30/04/2007 | 389 | 45°18'54.00"N | 4.6 | 13.6 | 13.6 | | | 1/1 | 4/4 | |
| 15 | 30/04/2007 | 422 | 45°14'34.80"N | 4.4 | 5.6 | 7.5 | 4/6 | | 1/1 | 2/4 | 1/1 |
| 16 | 30/04/2007 | 98 | 45° 5'27.60"N | 0.0 | 0 | 0.0 | 1/2 | 1/1 | 1/1 | | |
| 17 | 04/05/2007 | 575 | 44°47'2.40"N | 4.5 | 0 | 0.0 | | | 4/5 | 3/4 | |
| 18 | 16/05/2007 | 719 | 44°41'45.60"N | 4.3 | 0 | 0.0 | | 5/5 | 2/2 | | |
| 19 | 16/05/2007 | 167 | 44°14'24.00"N | 7.5 | 0 | 0.0 | | 6/6 | | | |
| 20 | 17/05/2007 | 600 | 43°52'33.60"N | 4.3 | 0 | 0.0 | 4/4 | 1/1 | 1/1 | 1/2 | |
| 21 | 17/05/2007 | 597 | 43°37'8.40"N | 4.3 | 0 | 0.0 | 3/5 | | | 3/17 | |
| 22 | 17/05/2007 | 214 | 43°33'57.60"N | 6.8 | 0 | 0.0 | 1/1 | | | 3/4 | |
| 23 | 18/05/2007 | 625 | 43°18'36.00"N | 4.3 | 0 | 0.0 | 1/1 | | | 1/1 | |
| 24 | 08/05/2008 | 301 | 46°43'48.00"N | 5.4 | 1.5 | 2.0 | 1/1 | 3/3 | | | |
| 25 | 09/05/2008 | 462 | 46°26'16.80"N | 5.0 | 0 | 0.0 | 1/1 | | | | |
| 26 | 13/05/2008 | 398 | 46°26'42.00"N | 4.5 | 2.0 | 2.3 | | 6/6 | 1/1 | | |
| 27 | 17/04/2009 | 318 | 47°22'12.00"N | 5.2 | 0.5 | 1.0 | 4/7 | | | | |
| 28 | 18/04/2009 | 437 | 46°59'34.80"N | 5.0 | 0 | 0.0 | 2/2 | | | | |
| 29 | 18/04/2009 | 428 | 46°52'58.80"N | 5.0 | 0 | 0.0 | 1/1 | | | | |
| 30 | 18/04/2009 | 458 | 46°54'43.20"N | 5.0 | 0 | 0.0 | 2/2 | | | | |
| 31 | 19/04/2009 | 453 | 46°38'16.80"N | 5.0 | 0 | 0.0 | 2/3 | | | | |
| 32 | 19/04/2009 | 445 | 46°38'16.80"N | 4.9 | 0.6 | 1.0 | 6/29 | 2/2 | | | |
| 33 | 19/04/2009 | 317 | 46°41'13.20"N | 5.4 | 1.0 | 1.5 | 3/10 | | | | |
| 34 | 19/04/2009 | 299 | 46°42'36.00"N | 5.2 | 0 | 0.0 | 1/2 | | | | |

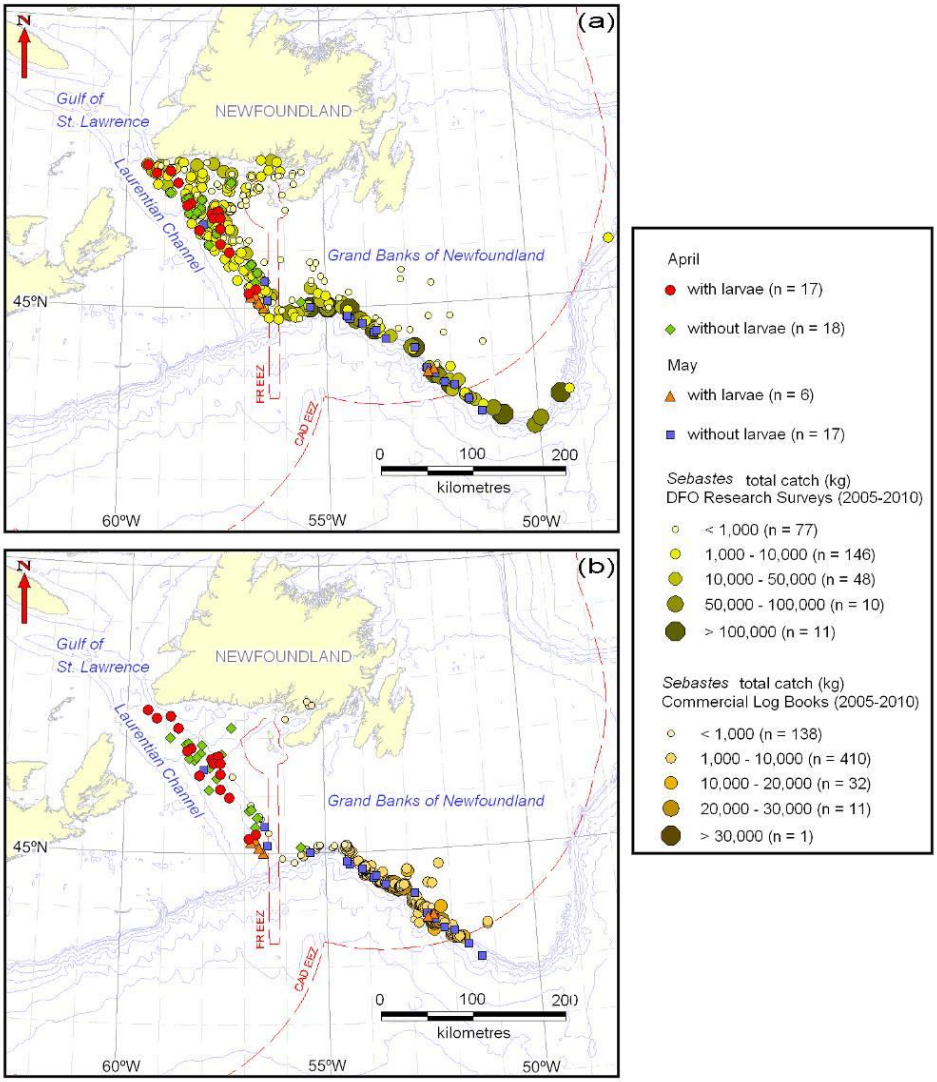
Appendix 6-A continued

| Parameters | | | | | | | Number of colonies examined/collected* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Site | Date | Depth (m) | Latitude | T (°C) | SMY/SMEY (larvae colony$^{-1}$) | | A. grandiflorum | P. aculeata | F. quadrangularis | H. finmarchica | P. grandis |
| 35 | 29/04/2009 | 451 | 46°33'28.80"N | 4.9 | 2.0 | 3.0 | 3/6 | | | | |
| 36 | 30/04/2009 | 348 | 46°33'25.20"N | 5.0 | 0.3 | 1.0 | 3/11 | | | | |
| 37 | 30/04/2009 | 488 | 46° 5'42.00"N | 4.9 | 0 | 0.0 | 1/10 | | | | |
| 38 | 03/05/2009 | 265 | 45°26'52.80"N | 7.9 | 0 | 0.0 | 1/1 | 4/4 | | | |
| 39 | 10/05/2009 | 404 | 45°13'22.80"N | 5.1 | 0.8 | 1.3 | 3/12 | 1/1 | | | 1/1 |
| 40 | 11/05/2009 | 422 | 45°11'13.20"N | 5.1 | 0.8 | 2.3 | 3/10 | 1/1 | 1/2 | 2/2 | 2/2 |
| 41 | 11/05/2009 | 404 | 45° 5'24.00"N | 5.1 | 10.9 | 10.9 | 1/3 | 3/3 | 9/10 | | |
| 42 | 11/05/2009 | 396 | 45° 6'54.00"N | 5.8 | 0 | 0.0 | | 8/14 | 3/18 | | |
| 43 | 12/05/2009 | 410 | 45° 0'28.80"N | 6.0 | 0 | 0.0 | | | | 1/1 | |
| 44 | 13/05/2009 | 584 | 44°49'55.20"N | 4.8 | 0 | 0.0 | | 2/2 | | | |
| 45 | 14/05/2009 | 674 | 44°32'13.20"N | 4.6 | 0 | 0.0 | | 7/26 | 1/1 | | |
| 46 | 24/05/2009 | 603 | 43°49'40.80"N | 4.9 | 0.1 | 1.0 | 3/8 | | 4/14 | 4/6 | |
| 47 | 24/05/2009 | 337 | 43°46'30.00"N | 7.4 | 0 | 0.0 | 2/4 | | 3/5 | 2/4 | |
| 48 | 26/05/2009 | 596 | 43° 4'58.80"N | 4.8 | 0 | 0.0 | 3/3 | | 3/3 | 1/4 | |
| 49 | 14/04/2010 | 384 | 45°26'34.80"N | 5.4 | 0 | 0.0 | | | | 2/2 | |
| 50 | 15/04/2010 | 314 | 45°45'36.00"N | 6.6 | 0 | 0.0 | 2/2 | 2/2 | | | |
| 51 | 24/04/2010 | 456 | 46°52'58.80"N | 5.3 | 0 | 0.0 | 1/1 | 8/22 | | | |
| 52 | 25/04/2010 | 449 | 47°29'13.20"N | 5.2 | 15.0 | 15.0 | 1/1 | | | | |
| 53 | 26/04/2010 | 433 | 46°46'22.80"N | 5.3 | 1.7 | 2.5 | 3/5 | | | | |
| 54 | 26/04/2010 | 455 | 46°39'54.00"N | 5.3 | 0 | 0.0 | 1/1 | | | | |
| 55 | 26/04/2010 | 468 | 46°28'40.80"N | 5.3 | 0 | 0.0 | 1/1 | | | | |
| 56 | 27/04/2010 | 447 | 45°57'18.00"N | 5.3 | 0.3 | 1.0 | 3/3 | | | | |
| 57 | 10/05/2010 | 603 | 44°34'4.80"N | 5.5 | 0 | 0.0 | | | | 2/5 | |
| 58 | 11/05/2010 | 673 | 44°25'4.80"N | 4.7 | 0 | 0.0 | 1/1 | 8/59 | 2/4 | | |

Notes: *Less than three colonies or 20% of the available colonies were examined due to damaged or missing samples.




Appendix 6-B Location of sampling areas in eastern Canada (2005–2010; 98–719 m): North Laurentian Channel (NLC), South Laurentian Channel (SLC), slope of South Grand Banks (GB).




Appendix 6-C Catches of redfish in the study area (2005–2010) based on (a) research surveys and (b) commercial landings.

# Chapter 7 : General conclusions

## 1. Summary

The abundance and widespread distribution of Pennatulacea in oceans worldwide indicate that sea pens have the potential to play an important ecological role by increasing the complexity of muddy/sandy sea floors (Tissot et al. 2006). However, limited information is available on either shallow or deep-water sea pens (Buhl-Mortensen et al. 2010). My thesis provides novel results and insights on the biology and role of three of the most common sea pens in the Northwest Atlantic, Anthoptilum grandiflorum, Halipteris finmarchica and Pennatula aculeata. The main findings are summarized in Tables 7-1, 7-2 and 7-3.

The three species studied here have different general morphologies. A. grandiflorum is of medium size (~45 cm on average); it has a question-mark shape and polyps that occur singly. H. finmarchica can reach total lengths of 160 cm (~70 cm on average) and has a whip-like shape with polyps that are fused at their base to form a ridge. P. aculeata is the smallest (~17 cm on average) and has a quill shape with polyps arranged in leaves. The investigation of their biometry (colony length, peduncle length and width, polyp density and diameter, sclerite length and stoutness, sclerite abundance and concentration) highlighted different adaptations of each species to environmental conditions (Chapter 1). I identified shifts in feeding strategies based on their isotopic signature and morphology (polyp diameter, colony length, general shape). I concluded that P. aculeata feeds mostly on phytodetritus while the two other species are more predatory and feed on a combination of phytodetritus and small invertebrates (e.g.

zooplankton, benthic copepods). Variations in their morphologies were visible along depth and latitude gradients and are likely tied with food availability, which tends to decrease northward and with increasing depth. Biometrics varied more clearly along the depth gradient in P. aculeata than in the two other species, confirming the shallower depth tolerance of this species (200-1000 m). On the other hand, H. finmarchica is clearly the most depth adapted of the three species, being the only one to show a decrease in polyp density and an increase in polyp diameter with increasing depth, variations typically associated with adaptations to lower food availability (Pasternak 1989; Williams 1992). A. grandiflorum is an intermediate species in its depth distribution, probably due to its greater predatory attributes than P. aculeata, allowing it to colonize greater depths. Different defense strategies were also identified according to the presence and localisation of sclerites in the tissues. A. grandiflorum likely depends on chemical defense given the absence of sclerites, while P. aculeata exhibits the highest sclerite abundance and concentration suggesting that it depends mostly on sclerites for defense. Finally H. finmarchica is less mineralized than P. aculeata and is therefore presumed to depend on a mix of chemical and physical defenses.

Reproductive strategies were determined for A. grandiflorum (Chapter 2) and H. finmarchica (Chapter 3). Both species are gonochoric broadcast spawners. My thesis presents the first accounts of seasonal reproduction in deep-sea pennatulaceans; both species were determined to spawn annually between April (Southern Newfoundland) and July/August (Labrador/Lower Arctic). The latitudinal shift in spawning followed the development of the surface phytoplankton bloom (i.e. sinking of phytodetritus) indicating a link with food availability. Phytodetritus (or marine snow) might allow the final

maturation of gametes and/or serve as a chemical clue to induce spawning. A low fecundity was detected at the polyp level (6 and 13 oocytes polyp$^{-1}$ for A. grandiflorum and H. finmarchica, respectively) while similar whole-colony fecundity was found (500-6000 oocytes colony$^{-1}$). I highlighted the importance of using a suitable and consistent measure of fecundity (i.e. determine the number mature oocyte just before spawning) to avoid an overestimation of fecundity and to allow comparisons among species. Only ~20% of the oocyte matured in both species. The remaining oocytes in A. grandiflorum disappeared, indicating that oogenesis develops and culminates over 12 months. In H. finmarchica there was a persistent pool of small oocytes, consistent with oogenesis lasting >12 months. Commonly, the presence of a protracted oogenesis and different cohorts of oocytes in a polyp has been interpreted as proof of "continuous" spawning; however, my study showed clear evidence of seasonal spawning. Therefore, I point out that continuous gametogenesis can be coupled with seasonal spawning, and stress the importance of finding other evidence of spawning (drop in number of oocytes, drop in maturity stage index or direct observation of spawning in situ or in the lab) before making any conclusions on spawning patterns and periodicities.

The analysis of their associated biodiversity (Chapter 4) showed that sea pens have relatively few associated species, compared to gorgonians and hard corals, but that they play an important role in the life history of their associates. A. grandiflorum harboured a higher number of associated species, probably due to its more "bushy" morphology, allowing it to hold more free-living species than the whip-shaped H. finmarchica, which might not retain free-living species as readily during sampling (trawl by-catch). Very few ectobionts (3 species) were found, suggesting antifouling

protection in sea pens. Most of the ectobionts (2/3) were found on the bare central axis of H. finmarchica confirming the importance of coral skeleton as substrate for sessile species in the deep sea. Associates of both sea pens were dominated in number by endobionts, especially two parasitic copepods which probably have developed adaptation (co-evolved) to thwart the defense of the sea pens. Four species were common, representing 96.6% of all the associates. Some were obligate symbionts (the parasitic copepods, i.e. Lamippe bouligandi and the undescribed Corallovexiidae), others were facultative symbionts (the sea anemone Stephanauge nexilis). The fourth most common association involved redfish larvae (Sebastes spp.), which were present only during the spring months (April-May). Their presence identified sea pens as a nursery habitat, and provided a major argument to classify them as an essential fish habitat (Chapter 5). This role as nursery was emphasized by the presence of different shrimp larvae (Pandalus borealis, Pasiphae multidentata and Acanthephyra pelagica) during the spring months.

## 2. Future directions

Overall, my PhD thesis provides novel knowledge on sea pens as major components of benthic communities on the continental slope of eastern Canada. While the biometric study highlighted important aspects of the ecology of the species, it is important now to confirm these findings in situ or via laboratory studies of sea pen behaviour, especially their feeding modes and diets. Moreover, sampling the different components (phytodetritus, microzooplankton, zooplankton, sea pens, other corals, fishes) from the same site would help to fully understand the position of sea pens in the food web.

The presence of several cohorts of oocytes and long oogenesis have often been interpreted as evidence of continuous reproduction (Eckelbarger et al. 1998; Pires et al. 2009). Here I demonstrated that it is not necessarily the case and that it can still be combined with seasonal spawning (Chapter 3). Additional studies are necessary to fully understand the reasons underlying this type of oogenesis. Moreover, future analysis of the reproductive strategy of deep-sea species will have to take into consideration this aspect and bring clear evidence to support a proposed "continuous" spawning or gametogenesis.

This thesis brings the first direct evidence of the use of deep-sea coral as nursery by fish and shrimp, finally confirming what had previously been suggested from indirect evidence (Fosså et al. 2002; Husebo 2002; Roberts et al. 2009). This discovery emphasizes the importance of understanding the biology of sea pens, and other deep-sea soft corals, and their capacity to recover from disturbances. However, sampling methods were not specifically designed to sample sea pens and their associated species. The utilisation of trawl by-catch gave me access to a large quantity of colonies sampled year-round, therefore allowing the identification of seasonal and regional changes. This information cannot be obtained with the use of ROVs due to the high cost and limited sampling opportunities (centered on summer months) afforded by this tool. However, the use of ROV technology is necessary to better characterize and quantify associations between free-living species and sea pens. Therefore, it would be interesting to target specific sampling locations and seasons for an ROV to get a clear image of the free-living species that use the biogenic habitat created by sea pens.

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## Tables

Table 7-1 Summary of findings on the sea pen biometry from Chapter 2. N/A indicates a non applicable parameter due to its absence in the species considered.

| |  Anthoptilum grandiflorum |  Halipteris finmarchica |  Pennatula aculeata |
|---|---|---|---|
| Average colony length | ~45 cm | ~70 cm | ~17 cm |
| Peduncle | Maximum 13 cm | Maximum 13 cm | Maximum 13 cm |
| Addition of new polyps | At the base of the rachis | At the base of the rachis | At the base of the rachis |
| Depth influence on morphology | Yes | Yes – observed the weakest modification with depth | Yes – observed the strongest modification with depth |
| Depth optimal distribution | 200-1400 m200 | -2000 m (better adaptation with a decrease in polyp density and an increase in polyp diameter) | 200-1000 m |
| Latitude influence on morphology | Yes | Yes | Yes |
| Diet | Feeds principally on zooplankton and complements with phytodetritus | Feeds principally on zooplankton and complements with phytodetritus | Feeds principally on fresh phytodetritus and occasionally on zooplankton |
| Feeding strategy | Large carnivorous polyps | Long colony with predatory/carnivorous polyps and mucus to trap phytodetritus | Leaves form a trap (net), favouring interception of flowing particles |
| Presence of sclerites | No | Yes (forming calyce around the polyp and in the tentacles) | Yes (everywhere and in high abundance) |
| Putative defense mechanism | Chemical | Mix of chemical and physical (sclerites) | Mostly physical (sclerites) |
| Sclerite variation with depth and latitude | N/A | Yes | Yes - Defense sclerite varied the most compare to structural sclerite |
| Sclerite composition | N/A | High magnesium calcite – no difference among sclerite types | High magnesium calcite – difference among sclerite types |

Table 7-2 Summary of findings on the reproduction of sea pens from Chapters 3 and 4.

| |  Anthoptilum grandiflorum |  Halipteris finmarchica |
|---|---|---|
| Sexuality | Gonochoric (observation of 1 hermaphrodite polyp) | Gonochoric |
| Reproductive strategy | Broadcast spawner | Broadcast spawner |
| Spermatogenesis | < 12 months | < 12 months |
| Oogenesis | < 12 months | > 12 months (2 cohorts) |
| Maximum oocyte diameter | 1100 µm | 1000 µm |
| Percentage of oocyte maturing every year | ~21% - the remaining oocytes disappear | ~20% - the remaining oocytes form a pool of small oocytes maturing in the following year(s) (2 cohorts) |
| Spawning | Seasonal (April to July) - latitudinal shift from the South to the North following the phytoplankton bloom | Seasonal (April to August) - latitudinal shift from the South to the North following the phytoplankton bloom |
| Sea temperature during spawning | 3.6-4.8°C | 4.0-4.7°C |
| Polyp fecundity | ~13 oocytes polyps$^{-1}$ | ~6 oocytes polyp$^{-1}$ |
| Colony fecundity | 1000-4300 oocyte colony$^{-1}$ | 500-6300 oocytes colony$^{-1}$ |

Table 7-3 Summary of findings on the associated species of sea pens from Chapters 5 and 6.

| |  Anthoptilum grandiflorum |  Halipteris finmarchica |
|---|---|---|
| Number of associated species identified | 16 species | 6 species |
| Biodiversity | $E_{(S180)}$= ~6 expected species<br>H'=0.48 | $E_{(S180)}$= ~6 expected species<br>H'=1.04 |
| Biodiversity and depth | No variation | No variation |
| Biodiversity and latitude | Northward decrease | Northward decrease |
| Associated species | Numerically dominated by endobionts | Numerically dominated by endobionts |
| Major associates | Lamippe bouligandi (parasitic copepod)<br>Sebastes spp. (fish larvae) | Stephanauge nexilis (sea anemone)<br>Undescribed Corallovexiidae (parasitic copepod)<br>Sebastes spp. (fish larvae) |
| **Major associates'** diet | Lamippe bouligandi: feeds on host tissue and/or compete for host food | Stephanauge nexilis: feeds on zooplankton (larger than prey of host sea pen)<br>Undescribed Corallovexiidae: feeds on host tissue and/or compete for host food |
| Seasonal associates | Sebastes spp. (fish larvae)<br>Pandalus borealis, Pasiphae multidentata and Acanthephyra pelagica (shrimp larvae) | Sebastes spp. (fish larvae) |