REPRODUCTIVE BIOLOGY OF THE DEEP-WATER GORGONIAN CORAL *ACANELLA ARBUSCULA* FROM THE NORTHWEST ATLANTIC

by

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for the degree of Master of Science

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DEPARTMENT OF BIOLOGY

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Abstract

This thesis examined the reproductive biology of the poorly-known deep-water gorgonian *Acanella arbuscula* from the Northwest Atlantic. Colonies were collected from The Gully in 2007 and 2010 between 914 and 1860 m depth, and the Flemish Cap in 2009 between 671 and 1264 m. Mean polyp fecundity was relatively high for both females and males, and the large oocyte size suggests that *A. arbuscula* produces lecithotrophic larvae. This species may have overlapping periodic or seasonal cycles of gametogenesis, and the absence of planulae suggests that *A. arbuscula* is a broadcast spawner. No spatial variation in the reproductive characteristics of this species was found, suggesting that environmental conditions are similar between the two sites. Female polyp fecundity decreased with increasing depth, which may be due to the high cost of producing oocytes versus sperm. The relatively high mean polyp fecundity, probable lecithotrophic larval development, and broadcast spawning may allow for the wide dispersal and settlement of *A. arbuscula* across the North Atlantic.

List of Abbreviations and Symbols Used

α	Significance Level
AIC	Akaike Information Criterion
ANOVA	Analysis of Variance
χ^2	Chi-Square Statistic
CTD	Conductivity, Temperature, and Depth Sensor
df	Degrees of Freedom
EDTA	Ethylenediaminetetraacetic acid
EEZ	Exclusive Economic Zone
ERMS	European Register of Marine Species
ε	Experimental Error
F	F Statistic
β	Effect due to i-th level of Fixed Factor
b	Effect due to j-th level of Random Factor
μ	Grand Mean
GMT	Greenwich Mean Time
HSD	Tukey's Honestly Significant Difference
µm	Micrometre
MAM	Minimum Adequate Model
mg m^{-3}	Milligrams per Cubic Metre
MPA	Marine Protected Area
n	Sample Size
NAFO	Northwest Atlantic Fisheries Organization

P	P Value
R^2	Coefficient of Determination
RFMO	Regional Fisheries Management Organization
ROPOS	Remotely Operated Platform for Ocean Science
ROV	Remotely Operated Vehicle
SD	Standard Deviation of the Mean
SE	Standard Error of the Mean
SeaWiFS	Sea-Viewing Wide Field-of-View Sensor
t	T Statistic
UNGA	United Nations General Assembly
VME	Vulnerable Marine Ecosystem
y	Response (Polyp Fecundity or Mean Gamete Diameter)

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Chapter 1. Introduction

1.1. General Introduction

The existence of corals in the deep ocean has been known since the mid 1700's. Originally discovered by fishermen, deep-living corals were documented scientifically in 1752 upon the discovery of reef-forming species off the coast of Norway (Hovland 2008). Despite knowledge of their existence for over 250 years, much of what we know about deep-water corals has been acquired within the last two decades (Roberts and Hirshfield 2004). Recent advancements in marine technology, including the use of ROVs and submersibles, have allowed scientists to not only observe these organisms in their natural habitat, but to sample and study them.

The terms 'deep-water coral', 'deep-sea coral', and 'cold-water coral' are all used in the literature to identify and distinguish corals living at depth from their shallow-water, tropical counterparts. However, these terms are ambiguous, and are often not true descriptors of the geographic and depth range of these organisms. For instance, the depth range of several corals spans from shallow waters (i.e. less than 200 m) to the deep-sea, but are still referred to as "deep-water" corals (Krieger and Wing 2002; Försterra et al. 2005; Stone 2006). Similarly, the term 'cold-water' coral is often mistaken for coral species distributed in high latitude regions, despite the fact that waters in the deep ocean are generally cold no matter what latitude. In all cases, these terms refer to corals that lack symbiotic zooxanthellae found in shallow, tropical corals, and thus they derive their energy by suspension feeding from the water column. This thesis adopts the term 'deep-water' coral to indicate corals that are azooxanthellate, and occur in, but may not be restricted to, depths below the continental shelf (~200 m) at any latitude.

Deep-water corals are organized into several orders and families within the classes Anthozoa and Hydrozoa (Phylum Cnidaria; European Register of Marine Species (ERMS): http://www.marbef.org/data/erms.php). They occur as solitary, colonial, and reef-building forms. Under the Class Anthozoa deep-water corals are organized in two different subclasses and one superorder: 1) the Octocorallia, which includes orders Alcyonacea (soft corals), Gorgonacea (sea fans), and Pennatulacea (sea pens), and 2) the Hexacorallia, which includes the Scleractinia (stony corals) and Zoanthidea (zoanthids), and 3) the superorder Ceriantipatharia, which includes the Order Antipatharia (black corals). Deep-water corals under the Class Hydrozoa include members of the families Stylasteridae and Hydractiniidae (Bouillon et al. 1997; Cairns 2007). Deep-water corals are widely distributed in the world's oceans, commonly between depths of 200 to 1500 m (Mortensen and Buhl-Mortensen 2005), but have been recorded down to 6000 m (Baco 2007). They are found in aggregations along the edge of the continental shelf, banks, and seamounts, and within deep channels and canyons which concentrate their food source (Breeze et al. 1997; MacIsaac et al. 2001; Rogers 2004).

Little information exists on the functional role of corals in the deep ocean. In some cases they provide structural habitat for a diversity of organisms, from commercially important fish species to invertebrates (Rogers 1999; Husebø et al. 2002; Krieger and Wing 2002; Roberts and Hirshfield 2004). Many studies have documented a high diversity of associated species, comparable to that of shallow-water coral reefs. For instance, Rogers (1999) recorded over 850 associated sponge, crustacean, mollusc and fish species on or in *Lophelia pertusa* reefs in the Northeast Atlantic. Krieger and Wing (2002) identified 10 megafauna taxa associated with *Primnoa* spp. in the Gulf of Alaska that were using the corals for either food, to enhance suspension feeding by elevating

their position in the water column, or for protection. Deep-water corals may be important in the early life stage of some commercially important fish species. Deep-water *Oculina* reefs off the coast of Florida provide breeding grounds for gag and grouper species, and nursery grounds for the juvenile snowy grouper (Reed 2002). Consequently, their association with managed fish species makes deep-water coral communities a target for destructive commercial fishing.

Global concern has been raised over the status and protection of deep-water corals around the world. As shallow water fisheries are declining, fishing effort is being displaced into deeper waters, placing corals at an even greater risk of destruction from bottom fishing gear such as trawls and dredges. Their often slow growth and delicate morphology makes these organisms highly susceptible to mechanical damage. Similarly, oil and gas exploration and extraction also pose threats to deep-water coral ecosystems (Rogers 1999). Evidence of their destruction by anthropogenic activities such as bottom fishing has led to the creation of legislation to protect these remote organisms. In 2004, the United Nations General Assembly (UNGA) drew attention to the state of deep-water coral habitat and their destruction by commercial fishing gear. The subsequent 2006 UNGA Sustainable Fisheries Resolution 61/105 called for all member countries to take immediate action individually and through Regional Fisheries Management Organizations (RFMOs), which are responsible for fisheries management on the high seas, to sustainably manage fish stocks and protect vulnerable marine ecosystems (VMEs). Vulnerable marine ecosystems are defined as those which are highly susceptible to disturbance and are slow to recover. Included under this definition are seamount regions, hydrothermal vents, and deep-water coral habitat (Fuller et al. 2008). The Northwest Atlantic Fisheries Organization (NAFO), the RMFO responsible for fisheries

management in the Northwest Atlantic, uses the following criteria to identify coral VME components: size, ability to form dense aggregations, structural complexity, rarity, vulnerability to damage, role in ecosystem, international status, and longevity (NAFO 2008). However, not all corals are vulnerable or form ecosystems, and thus, NAFO only considers the following taxonomic groups as indicators and key components of VMEs: antipatharians, gorgonians, cerianthid anemone fields, *Lophelia* and other reef forming species, and pennatulacean fields (Fuller et al. 2008; NAFO 2008). In particular, antipatharians are included in part because of their expected low growth rates, low fecundity and recruitment, and high mortality, and gorgonians because of their long life spans, low growth rates, and episodic recruitment (Fuller et al. 2008). Apart from this, reproduction and recruitment are not considered in the criteria used by NAFO to identify VME indicators and components. Reproduction and recruitment, and their consequences for recovery potential remain largely unexplored for all groups of deep-water coral.

Many studies on deep-water corals are dedicated not to basic life history characteristics such as growth and reproduction, but to distribution (Wilson 1979; Langton et al. 1990; Breeze et al. 1997; Hovland et al. 2002; Etnoyer and Morgan 2005; Watling and Auster 2005; Murillo et al. 2011), taxonomy (Williams 1995; Cairns and Bayer 2005; Sánchez 2005; Cairns 2007), phylogeny (Le Goff-Vitry et al. 2004; Strychar et al. 2005), and associated species (Rogers 1999; Krieger and Wing 2002; Buhl-Mortensen and Mortensen 2004; Auster et al. 2005). Knowledge of growth, reproduction, and recruitment of deep-water corals is important for our understanding of population dynamics, persistence, and resilience (Hughes and Tanner 2000; Hourigan et al. 2007; Knittweis et al. 2009). In particular, reproduction is important as it may have consequences for biogeography, the amount of genetic exchange between populations,

and it may determine the ability to re-colonize an area after disturbance. Whether a coral reproduces through sexual or asexual methods may determine the ability to recover from local or regional damage. For instance, high levels of connectivity and colonization of large areas are achieved only through larval recruitment, and thus sexual reproduction. However, reproduction through asexual methods, such as fragmentation, may allow a coral to maintain its population at the local scale, as fragments which break off the coral colony often do not settle far from the parent. In sexually-reproducing shallow-water corals, the mode of sexual reproduction (i.e. brooding or broadcasting) and larval development type (i.e. lecithotrophic or planktotrophic) may also have important consequences for dispersal potential and colonization of distant habitat (Nishikawa et al. 2003; Harrison and Wallace 1990), however, it remains unknown how these characteristics influence the dispersal of corals in the deep-sea.

In this study I describe the reproductive biology of the azooxanthellate gorgonian coral *Acanella arbuscula* collected from deep waters in two areas of the Northwest Atlantic: The Gully MPA on the Scotian Shelf, and the Flemish Cap area off Newfoundland, Canada. This thesis is divided into four chapters. Chapter 1 gives a general introduction to the study. Chapter 2, which is written in the form of a manuscript, investigates the major reproductive characteristics of *A. arbuscula*, including colony sexuality, mode of sexual reproduction, and aspects of gametogenesis. In this chapter I also investigate whether there is intra-colony variation in polyp fecundity (i.e. the number of oocytes or spermatic cysts per polyp) and gamete size, colony size at first reproduction, and the influence of colony size on fecundity and gamete size. Chapter 3, also in the form of a manuscript, investigates whether some of the reproductive characteristics of *A. arbuscula* investigated in Chapter 2 differ between The Gully and Flemish Cap, and

whether these characteristics are influenced by depth. Chapter 4 provides a general conclusion to the study. Sections of the materials and methods are repeated between Chapters 2 and 3.

This thesis is the first study to describe the reproductive biology of a deep-water gorgonian coral from the Northwest Atlantic, and to my knowledge, is the first study aimed at determining whether differences in the reproductive characteristics within a species of deep-water coral exist between two geographically distant locations and along a depth gradient. The overall goal of this thesis was to increase our general knowledge of the reproductive biology of deep-water corals, especially members of the understudied subclass Octocorallia.

Chapter 2. Reproductive Biology of *Acanella arbuscula*

2.1. Introduction

Over the past few decades there has been substantial research dedicated to the reproductive processes of shallow-water, tropical anthozoans. Egg size, colony sex, polyp-level fecundity, mode of reproduction, and gametogenic cycles have been well documented in this group (Rinkevich and Loya 1987; Harrison and Wallace 1990; Eckelbarger et al. 1998; Kruger et al. 1998; Fan et al. 2005). Much less is known, however, of the reproductive biology of anthozoans found below the photic zone and in deep waters. This is likely due in part to the logistical difficulties of collecting specimens from the deep ocean and/or subsequent culturing in the laboratory. Within the last decade there has been some effort to describe reproduction of deep-water corals, however this effort has been focused on reef-building and solitary corals of the Order Scleractinia (Harrison and Wallace 1990; Waller et al. 2002; Brooke and Young 2003; Waller and Tyler 2005; Waller et al. 2005; Flint et al. 2007). What these studies have revealed is that, as in shallow-water corals, deep-water species also exhibit of wide variety of reproductive traits. Diversity in fecundity, egg size, reproductive mode, and timing of reproduction has been observed in this group of corals.

Despite their diversity and ecological significance in deep waters, reproductive studies of members of the subclass Octocorallia remain scarce. Much of our current knowledge of deep-water octocoral reproduction is based on a few studies of Antarctic species (Orejas et al. 2002; Orejas et al. 2007) and members of the Order Pennatulacea (Rice et al. 1992; Tyler et al. 1995; Pires et al. 2009). Octocorals, and anthozoans in general exhibit two modes of sexual reproduction: internal fertilization and brooding of

planula larvae, and broadcast spawning with external fertilization of gametes. Brooding may occur in one of two ways: internally in the gastrovascular cavity, or siphonozooids of some species (*Anthomastus ritteri*, Cordes et al. 2001; *Corallium secundum* and *C. lauuense*, Waller and Baco 2007), or on the surface of the colony (*Parerythropodjum fulvum fulvum*, Benayahu and Loya 1983; *Briareum asbestinum*, Brazeau and Lasker 1990; *Paramuricea clavata*, Coma et al. 1995b; *Pseudopterogorgia elisabethae*, Gutiérrez-Rodríguez and Lasker 2004). Larvae of many brooding shallow-water corals have short competency periods and often settle and metamorphose into an adult approximately 1-2 days after release, whereas broadcast spawning species tend to settle 4-6 days after larval development, thus increasing their potential for long distance dispersal (Harrison and Wallace 1990). For instance, Nishikawa et al. (2003) found that the pre-competency periods of planulae were shorter for the brooder *Stylophora pistillata* than for broadcast spawner *Acropora tenuis*, the settlement peak after spawning occurred earlier for *S. pistillata* than for *A. tenuis*, and the competency period was longer for *A. tenuis* than for *S. pistillata*, suggesting that broader dispersal is more likely for broadcaster *A. tenuis* than for brooder *S. pistillata*. Large differences in settlement times between brooders and broadcasters have been recorded in deep-water species. For instance, planulae of the deep-water brooding coral *Anthomastus ritteri* settled 2-3 days after release (Cordes *et al*. 2001), whereas larvae of the broadcaster *Oculina varicosa* settled 21 days after spawning (Brooke and Young 2003). In octocorals, the frequency of brooding versus broadcast spawning appears to be taxon-specific. For instance, brooding appears to be a common mode of reproduction in the Alcyonacea (Benayahu and Loya 1983; Brazeau and Lasker 1990; Cordes et al. 2001; Hwang and Song 2007; Sun et al. 2010). In contrast, all members of the Pennatulacea appear to broadcast their gametes

(Chia and Crawford 1973; Eckelbarger et al. 1998; Edwards and Moore 2008; Pires et al. 2009). Of the deep-water octocorals studied to date (summarized in Table 2.1), the Pennatulacea and Alcyonacea appear to follow this pattern, however, in the Gorgonacea a clear pattern can not be determined as some species brood, and the reproductive mode remains unconfirmed or undetermined for others. Shallow-water gorgonians display similar proportions of internal brooders, external brooders, and broadcast spawners (Ribes et al. 2007). Both hermaphroditism and gonochorism have been reported in shallow-water gorgonians, however, gonochorism is more prevalent. To date, no hermaphroditic deep-water gorgonian, or octocoral in general, has been discovered.

In Atlantic Canada there are at least 45 species of deep-water coral, and a large portion are members of the subclass Octocorallia (Cogswell et al. 2009). Three areas on the Scotian shelf contain high concentrations of these organisms and are designated as either conservation areas or marine protected areas (MPAs): the Gully MPA, the Northeast Channel Coral Conservation Area, and the *Lophelia* Coral Conservation Area, commonly known as the Stone Fence. Despite the high diversity and abundance of octocorals in Atlantic Canada, the reproductive biology of only one octocoral from this region has been documented in the primary literature. This is the work of Sun et al. (2010) on the alcyonacean *Drifa glomerata*, which revealed that this species is a gonochoristic brooder. Of these 45 species, approximately 10 are gorgonian corals, and yet, there is no knowledge of their reproductive biology beyond anecdotal observations.

The overall goal of my study was to increase our general knowledge of the reproductive biology of the poorly known subclass Octocorallia, and in particular, of gorgonian corals from the Northwest Atlantic. My study focused on the reproductive biology of the small branching coral *Acanella arbuscula* (Johnston 1862) of the family

Table 2.1 Summary of major reproductive characteristics of deep-water octocoral studies in the primary literature

Order	Species	Sexuality	Reproductive Mode	Reference
Pennatulacea	*Kophobelemnon stelliferum*	Gonochoristic	Predicted broadcaster	Rice et al. 1992
	Umbellula lindahli, *U. thomsonii*, *U. durissima*, *U. monocephalus*	Gonochoristic	Broadcaster	Tyler et al. 1995
	Pennatula aculeata	Gonochoristic	Broadcaster	Eckelbarger et al. 1998
	Anthoptilum murrayi	Gonochoristic	Probable broadcaster	Pires et al. 2009
Alcyonacea	*Anthomastus ritteri*	Gonochoristic	Brooder	Cordes et al. 2001
	Drifa glomerata	Gonochoristic	Brooder	Sun et al. 2010
Gorgonacea	*Acanella arbuscula*	Gonochoristic	Predicted brooder	Lawson 1991
	Thouarella variabilis	Gonochoristic	Brooder	Brito et al. 1997
	Ainigmaptilon antarcticum	Gonochoristic	Probable broadcaster	Orejas et al. 2002
	Dasystenella acanthina	Gonochoristic	Undetermined	Orejas et al. 2007
	Fannyella rossii, *F. spinosa*	Gonochoristic	Brooder	Orejas et al. 2007
	Thouarella sp.	Gonochoristic	Brooder	Orejas et al. 2007
	Corallium lauuense, *C. secundum*	*C. lauuense* likely gonochoristic *C. secundum* gonochoristic	Undetermined	Waller and Baco 2007

Isididae, Order Gorgonacea (ERMS). *A. arbuscula* is distributed in the Northwest Atlantic from the Davis Strait (Gass and Willison 2005) and Greenland (Deichmann 1936; Grasshoff 1981), and down the eastern seaboard of North America and the Gulf of Mexico (Watling and Auster 2005; Brooke and Schroeder 2007). It is also found in the Northeast Atlantic (Laubier and Sibuet 1979; Lawson 1991; Bronsdon et al. 1997; Roberts et al. 2000; Watling and Auster 2005) from Iceland to the Mid Atlantic Ridge (Grasshoff 1981) and Morocco (Molodtsova et al. 2008). This species anchors in soft sediments and has an overall depth range of 150 to 4800 m (Molodtsova et al. 2008; Kenchington et al. 2009). *A. arbuscula* represented an ideal candidate for a reproductive study as it has a high local abundance in many areas of the Northwest Atlantic and because very little is known of its reproduction.

Lawson (1991) described the reproductive biology of *A. arbuscula* from Station 'M' (57°18´N, 10°11´W), located in the northern region of the Rockall Trough, Northeast Atlantic (Gage and Tyler 1982). Lawson (1991) predicted that *A. arbuscula* was a brooder based on its large egg size and gamete developmental cycles. Lawson (1990 in 1991) also suggested there was no variability in the reproductive output from different areas of the same colony, which contrasts the findings of many studies which have examined intra-colony variation in reproduction in octocorals (Benayahu and Loya 1986; Brazeau and Lasker 1989; Coma et al. 1995a; Brito et al. 1997; Kapela and Lasker 1999; Orejas et al. 2002; Santangelo et al. 2003; Gutiérrez-Rodríguez and Lasker 2004; Orejas et al. 2007; Pires et al. 2009).

The objectives of my study were to 1) describe the general features of *A. arbuscula's* reproduction, such as colony sexuality, mode of sexual reproduction, and aspects of gametogenesis, 2) investigate intra-colony variation in polyp-level fecundity

(the number of gametes per polyp) and gamete diameter, and 3) determine a minimum size at first reproduction and the influence of colony size on polyp-level fecundity and gamete diameter. The results of the present study were compared to the findings and predictions by Lawson (1991) on *A. arbuscula* from the Northeast Atlantic.

Knowledge of reproduction is essential for understanding population dynamics and therefore is useful for conservation and management efforts. For instance, a species with late first reproduction, low fecundity, short or infrequent spawning periods, and low dispersal potential and recruitment is unlikely to re-colonize an area rapidly after a disturbance. This study addresses some of these biological parameters, and should be considered in conservation measures for this species. This is the first study to examine the reproductive biology of a gorgonian coral from Atlantic Canada and the Northwest Atlantic in general.

2.2. Materials and Methods

2.21. Study Areas and Sample Collection

A. arbuscula (Fig. 2.1a) colonies were collected from two different areas within the Northwest Atlantic, The Gully Marine Protected Area (MPA) on the Scotian Shelf, Atlantic Canada, and the Flemish Cap area in international waters off Newfoundland, Canada. The Gully (Fig. 2.2) is the deepest submarine canyon on the eastern coast of North America. Located near Sable Island on the Scotian Shelf, the Gully's high-sloped regions and unique hydrographical conditions make it a hotspot for large, branching deep-water corals. In 2004 The Gully was designated as a MPA to protect its high

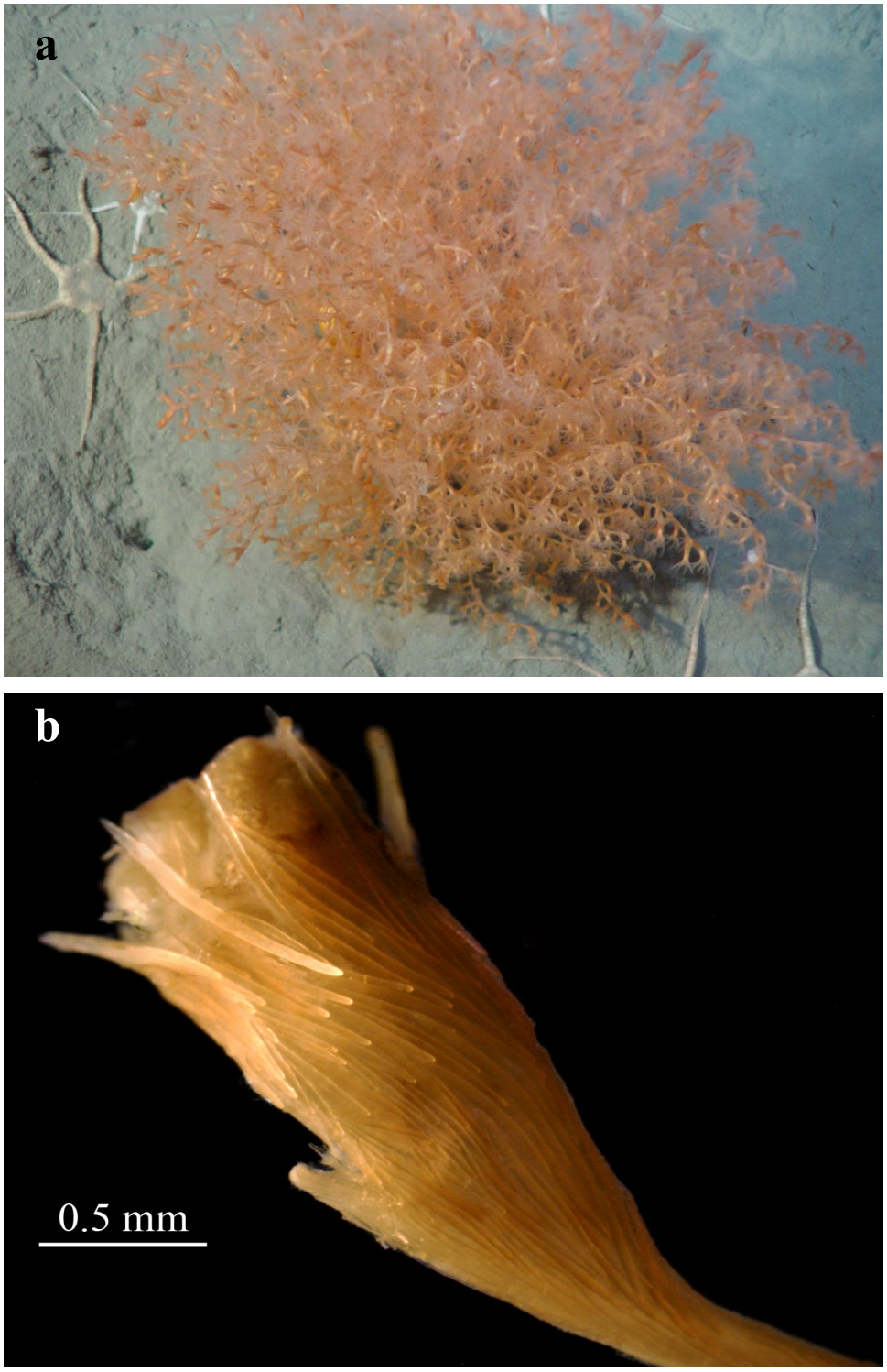

Fig. 2.1 **a** *A. arbuscula* colony in Desbarres Canyon, southwest Grand Banks, at 824 m depth. **b** Polyp of *A. arbuscula* showing elongate spicules extended towards the retracted tentacles

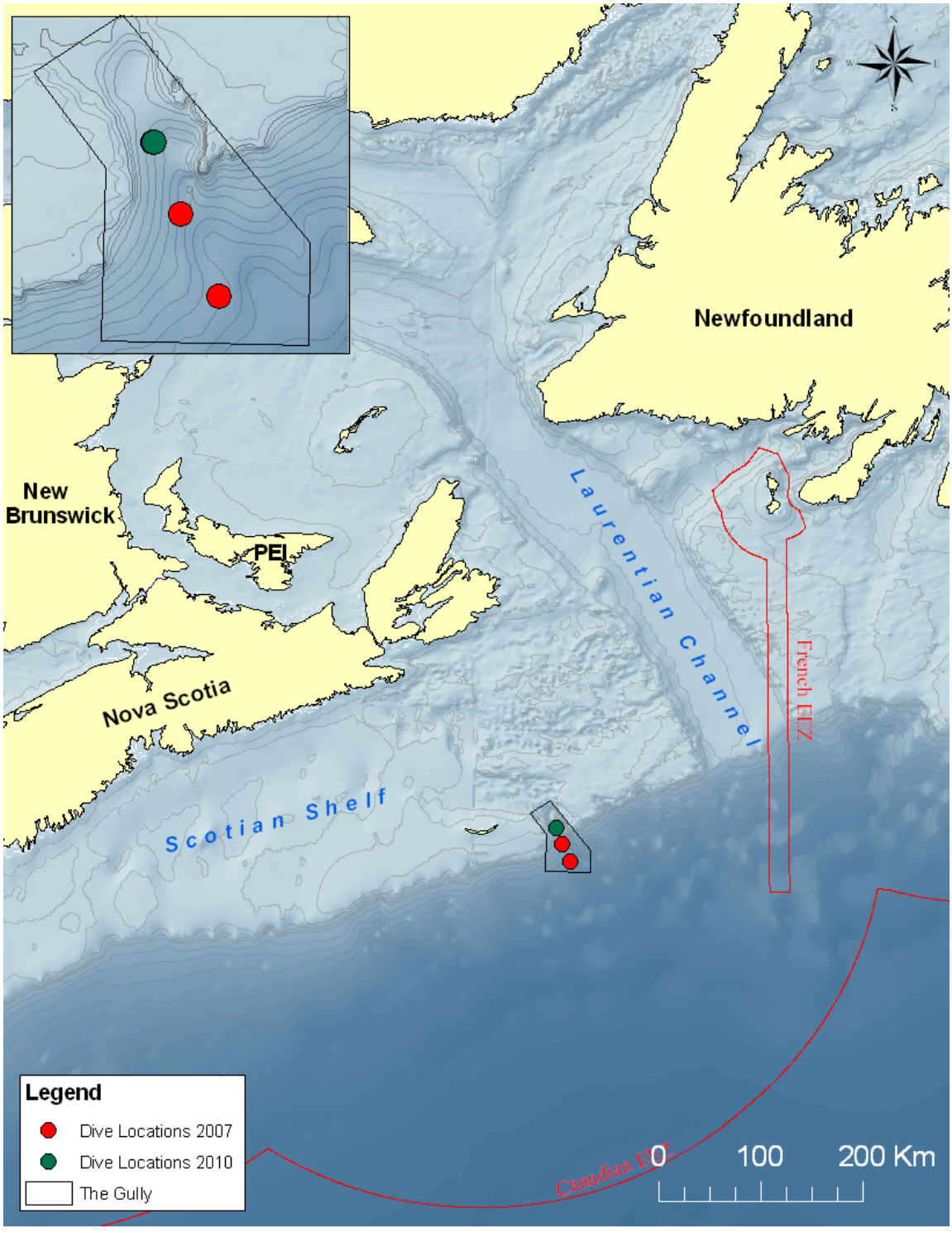

Fig. 2.2 The Gully MPA on the Scotian Shelf showing the locations of each dive where collections of *A. arbuscula* were made using ROPOS in 2007 and 2010. Insert shows close up of Gully and sampling locations. Red line indicates Canadian exclusive economic zone (EEZ)

concentration of deep-water corals and an endangered population of northern bottlenose whales that resides there.

The Flemish Cap (Fig. 2.3) is a shallow region located 600 kilometres east of Newfoundland. It is separated from the Grand Banks by a rift zone called the Flemish Pass. Depth ranges from 125 to 700 m on the Cap (Stein 2007). A steep slope exists at the southern tip of the Cap, and the slope off the western part of the cap near the Flemish Pass reaches depths upwards of 1100 m (Stein 2007). Many parts of the Flemish Cap and surrounding regions are bottom trawled for a variety of species, including northern shrimp (Gianni 2004), redfish (Avila de Melo et al. 2000), and Greenland halibut (Igashov 2001), threatening the high concentrations of deep-water corals and sponge found there. Several regions within the vicinity of the Cap have been closed to fishing by NAFO and are designated as vulnerable marine ecosystems (VMEs) in order to protect the deep-water corals and sponge species residing there.

Colonies of *A. arbuscula* were collected from The Gully MPA between depths of 1630 and 1861 m during a research cruise on the C.C.G.S. Hudson in July 2007 (Table 2.2) using the mechanical arm of the remotely operated vehicle ROPOS (Remotely Operated Platform for Ocean Science). In May, June, and August 2009 *A. arbuscula* was collected through a series of benthic surveys conducted by Spain on the eastern and south-western slope of the Flemish Cap. There, colonies were collected on the Miguel Oliver between depths of 671 and 1264 m using both a rock dredge and box corer. In July 2010 The Gully was revisited, and *A. arbuscula* was collected using ROPOS between depths of 914 to 1112 m. In 2010 samples were individually collected using a customized plankton mesh collection bag (250 μm mesh size; Fig. 2.4) to capture any larvae that may be spontaneously spawned out due to collection stress and/or surfacing.

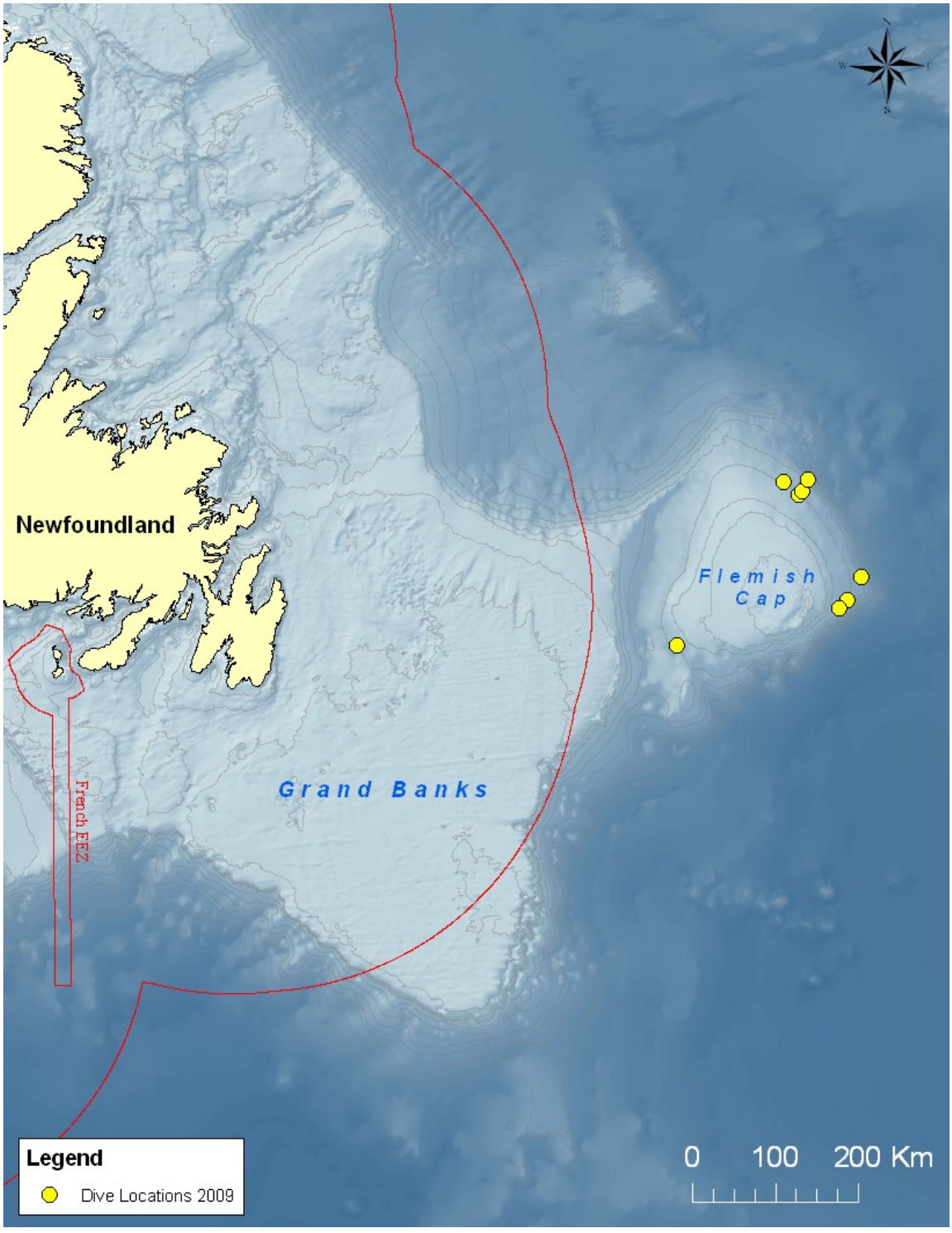

Fig. 2.3 The Flemish Cap and Grand Banks area showing the locations of each dive where collections of *A. arbuscula* were made during the Spain surveys in 2009. Red line indicates Canadian exclusive economic zone (EEZ); French EEZ represents Saint-Pierre et Miquelon

Table 2.2 Collection details and sex of *A. arbuscula* colonies collected between 2007 and 2010 from The Gully and Flemish Cap and used for analysis in this study. Start and end depth for dredges in 2009

Number of colonies	Sex	Cruise/Dive ID	Area	Gear	Depth (m)	Coordinates	Date collected
1	Unknown	HUD025/R1056	The Gully	ROPOS	1861	43˚ 40′ 30.2″ N -58˚ 49′ 20.6″ W	09/07/2007
1	Female	HUD025/R1060	The Gully	ROPOS	1630	43˚ 49′ 49.9″ N -58˚ 55′ 33.1″W	12/07/2007
1	Female	HUD025/R1060	The Gully	ROPOS	1630	43˚ 49′ 49.9″ N -58˚ 55′ 33.1″ W	12/07/2007
1	Male	Miguel Oliver/DR2	Flemish Cap	Dredge	671-739	48˚ 13′ 13.4″ N -44˚ 25′ 15.9″W	29/05/2009
3	2 Male 1 Female	Miguel Oliver/DR8	Flemish Cap	Dredge	700-701	48˚ 3′ 27.0″ N -44˚ 12′ 0.6″ W	03/06/2009
1	Male	Miguel Oliver/DR9	Flemish Cap	Dredge	864-861	48˚ 5′ 41.3″ N -44˚ 8′ 45.8″ W	04/06/2009
4	1 Male 3 Females	Miguel Oliver/DR20	Flemish Cap	Dredge	1122-1113	47˚ 4′ 20.4″ N -43˚ 26′ 56.9″ W	15/06/2009
3	1 Male 2 Females	Miguel Oliver/DR21	Flemish Cap	Dredge	870	46˚ 50′ 45.8″ N -43˚ 43′ 3.5″ W	16/06/2009
3	1 Males 2 Females	Miguel Oliver/DR23	Flemish Cap	Dredge	1127-1108	46˚ 46′ 29.5″ N -43˚ 51′ 54.4″ W	18/06/2009

Number of colonies	Sex	Cruise/Dive ID	Area	Gear	Depth (m)	Coordinates	Date collected
1	Male	Miguel Oliver/DR56	Flemish Cap	Dredge	795-712	46˚ 38′ 49.4″ N -46˚ 28′ 39.9″ W	18/08/2009
1	Male	Miguel Oliver/BC17	Flemish Cap	Box corer	1264	48˚ 12′ 31.9″ N -44˚ 0′ 29.9″ W	04/06/2009
1	Male	HUD029/R1347	The Gully	ROPOS	1112	43˚ 58′ 5.7″ N -59˚ 0′ 13.2″ W	27/07/2010
1	Female	HUD029/R1347	The Gully	ROPOS	914	43˚ 58′ 10.0″ N -59˚ 0′ 27.8″ W	27/07/2010
1	Female	HUD029/R1347	The Gully	ROPOS	914	43˚ 58′ 9.9″ N -59˚ 0′ 27.9″ W	27/07/2010
1	Male	HUD029/R1347	The Gully	ROPOS	1099	43˚ 58′ 5.9″ N -59˚ 0′ 14.1″ W	27/07/2010

Fig. 2.4 ROPOS manipulator arms placing *A. arbuscula* colony into mesh collection bag used in 2010. Depth= 1853 m

Upon surfacing the collection bags were examined for the presence of larvae. Each bag was washed with seawater which was passed through a series of sieves ranging in size from 1000 to 200 μm. Any material left on the sieves was examined under a dissecting microscope.

Colonies collected during the 2007 ROPOS mission to The Gully were fixed in 10% formalin in seawater for several months and were later transferred to 70% ethanol for long-term storage. Colonies collected from all other missions were fixed in 10% formalin in seawater for 24 to 48 hours, and were transferred to 70% ethanol.

2.22. Histological Preparation and Examination

Reproductive tissue was prepared for examination using standard histological techniques (Kiernan 1999; Etnoyer et al. 2006). Polyps of *A. arbuscula* (Fig. 2.1b) were dissected from the colony and decalcified using a solution of 10% hydrochloric acid and EDTA for approximately 2 to 3 hours, or until no calcareous material remained. Tissues were then dehydrated through a series of graded alcohol concentrations and cleared using xylene. Polyps were embedded in paraffin wax and longitudinally-sectioned 5 μm thick using a rotary microtome. Ribbons were mounted on slides and stained using Harris' hematoxylin and eosin. Slides were examined using a Nikon E-800 Eclipse microscope and oocytes and spermatic cysts were followed through their serial sections and photographed using mounted Nikon Digital Eclipse DXM 1200 and Nikon DS-Ri1 cameras when they were at their largest size, which, in oocytes, may or may not have corresponded to when the nucleus was bisected. The number of gametes per polyp was counted, and the maximum diameter of each gamete was measured using Image Pro Plus, version 5.1. Based on the literature (Farrant 1986, Fan and Dai 1995; Kruger et al. 1998;

Hwang and Song 2007), each oocyte and spermatic cyst was staged according to their morphological and histological characteristics.

2.23. Intra-Colony Variation in Polyp Fecundity and Gamete Size

The branching morphology of gorgonian corals can be classified following a system described by Brazeau and Lasker (1988). In this classification system, the most distal branches are usually first order branches (1°), and secondary (2°) and tertiary (3°) branches arise when two first order or two second order branches join, respectively (Fig. 2.5). This system also distinguishes between source and tributary branches, where source branches are any two branches that join to form secondary branches, and tributary branches are branches which join a branch of higher order, but do not increase the order of the system. In the current study, branches were only chosen if no other branch originated from it, which corresponds to first order source and tributary branches of the branching system described by Brazeau and Lasker (1988).

To determine whether there was variability in the number and size of oocytes and spermatic cysts in polyps selected from different areas of the same colony, each colony was divided into three 'zones' of equal length based on its height (height= length between the tips of the uppermost branches to the lowest branches): the basal (lowest), medial (middle), and apical (highest) zones. Five randomly-chosen polyps were dissected from a randomly-chosen first order source or tributary branch that originated in each zone, giving a total of 15 polyps per colony. A second effect was evaluated by dividing a source or tributary first order branch into three segments of equal length based on the total length of the branch: proximal (inner), central (middle), and distal (outer) segments (Fig. 2.6). Five polyps were randomly-chosen and dissected from each branch segment of randomly-

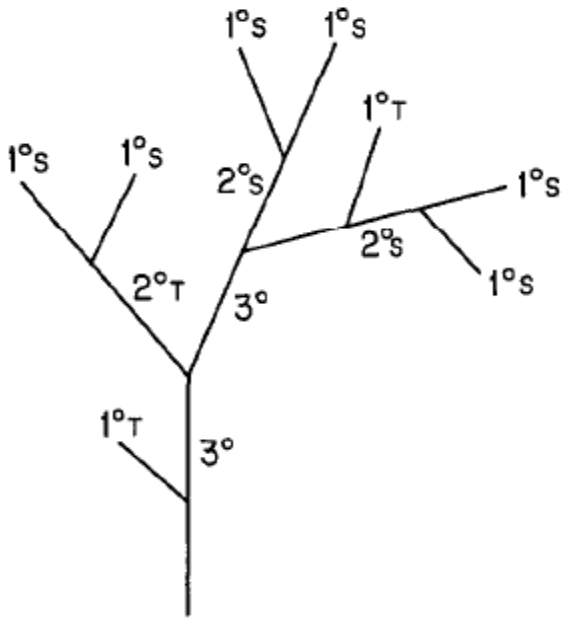

Fig. 2.5 Branching classification system of a gorgonian coral showing first (1°), second (2°), and third order (3°) branches, and source (S) and tributary (T) branches. From Brazeau and Lasker (1988)

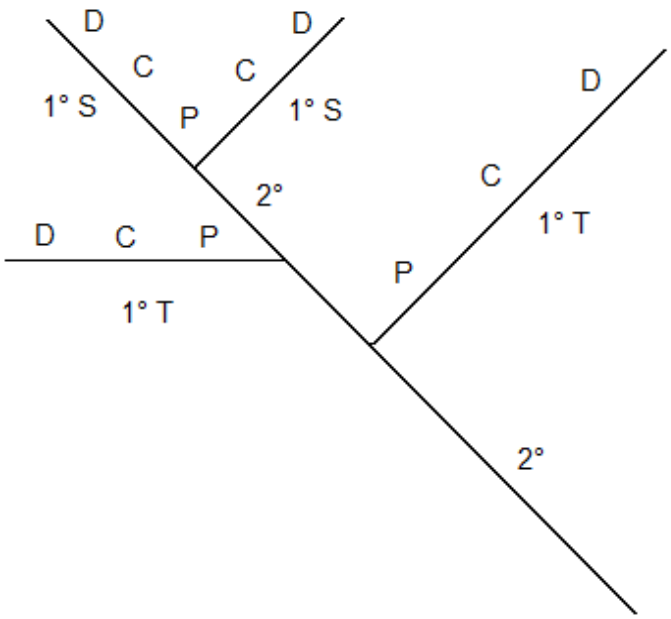

Fig. 2.6 Division of source (S) and tributary (T) first order (1°) branches into three segments: proximal (P), central (C), and distal (D)

chosen branches from the colony, without respect to which zone the branch originated in, giving a total of 15 polyps per colony. In all cases, no broken branches were used in this study.

Although the majority of *A. arbuscula* colonies have two to three orders of branching (personal observation), only polyps from source and tributary first-order branches were used to study both the colony 'zone' and 'branch segment' effects. This may impose a limitation to the study, especially when making inferences of whole-colony fecundity. However, Beiring and Lasker (2000) and Santangelo et al. (2003) found no significant differences in fecundity and fertility between first and second order polyps in the gorgonians *Plexaura flexuosa* and *Corallium rubrum*, respectively.

Preliminary analysis of three male and four female colonies revealed no significant differences between zone and polyp fecundity (Figs. 1, 2, Tables 1, 2, Appendix A) (ANOVA: oocytes (square root transformed): $F_{(2,54)}$= 0.280, P= 0.757; spermatic cysts (not transformed): $F_{(2,40)}$= 0.953, P= 0.394), or mean gamete diameter (ANOVA: oocytes (not transformed): $F_{(2,47)}$= 0.098, P= 0.906; spermatic cysts (not transformed): $F_{(2,39)}$= 0.036, P= 0.965). Consequently, factor zone was removed from the study, and only the branch segment effect was examined in subsequent colonies.

2.24. Statistical Analyses

A chi-square (χ^2) test was used to determine whether the sex ratio (ratio of males to females) of the Flemish Cap colonies was significantly different from 1:1. Although it is not ideal to calculate deviance from a 1:1 sex ratio using samples collected over wide spatial (Gori et al. 2007) and depth ranges (Benayahu and Loya 1983), sample sizes were

too small at any particular location and depth, and therefore all samples collected across the Flemish Cap area were combined. Due to the small sample size of The Gully collections (2 females in 2007, 2 males and 2 females in 2010) the sex ratio was not tested for deviance from 1:1.

The percent frequency of each of the five stages of oogenesis and four stages of spermatogenesis between collection months and branch segments was determined. A Log likelihood ratio (G-test) test of independence was used to test the null hypothesis of equality of frequencies between months and branch segments. If any cells contained zero values, the William's correction of continuity was applied (Gotelli and Ellison 2004).

Intra-colony variation in fecundity and gamete size was investigated in a replicated blocked design. In the analysis for differences in polyp fecundity between branch segments, branch segment was included as a fixed effect, and to incorporate any between-colony variability in polyp fecundity and increase the generalizability of the results, colony was included as a random (block) effect:

Model 1: $y_{ijk} = \mu + \beta_i + b_j + \varepsilon_{ijk}$,

where y_{ijk} is the response (polyp fecundity), μ is the grand mean, β_i is the effect of branch segment (fixed), b_j is the effect of colony (random), ε_{ijk} is the experimental error, i= 1, 2, 3, j= 1,... 12, k= 1,...5 for females, and i= 1, 2, 3, j= 1,...11, k= 1,...5 for males. Replication in the number of polyps allowed for investigation of the presence interactions (see Pinheiro and Bates 2000) between colony and branch segment (i.e. to assess whether differences between branch segments was different for different colonies). Within each colony, polyp fecundity was averaged between the 5 polyps per branch segment, giving 3 fecundity values, one for each branch segment. Mean fecundity per branch segment was

plotted for each colony, and interactions between branch segment and colony were deemed present if the lines were not parallel (Appendix B, Fig. 1). When possible interactions were present (i.e. in all cases), a second model was fit with branch segment as a fixed effect, and 'branch segment nested within colony' as a random interaction term:

Model 2: $y_{ijk} = \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$

where y_{ijk} is the response (polyp fecundity), μ is the grand mean, β_i is the effect of branch segment (fixed), b_j is the effect of colony (random), b_{ij} is the interaction term (random), ε_{ijk} is the experimental error, i= 1, 2, 3, j= 1,... 12, k= 1,...5 for females, and i= 1, 2, 3, j= 1,...11, k= 1,...5 for males. The Akaike Information Criterion (AIC) was used to compare and select between the two models. The model with the lowest AIC value gives the best fit to the data (Pinheiro and Bates 2000; Zuur et al. 2009). If the difference in AIC values between the two models was less than two, the models were deemed to have approximately equal weight in the data (Burnham and Anderson 2002; Schwarz 2010). As the difference in AIC values between Model 1 and Model 2 was less than two, the simpler Model 1 was chosen as the minimum adequate model for the analysis of polyp fecundity by branch segment for both female and male colonies (Table 2.3). In the test for differences in gamete diameter between branch segments, all gamete measurements per polyp were averaged to avoid pseudo-replication (Underwood 1997), and a linear mixed model was fit according to the procedure above (see Appendix B, Fig. 2). Model 1 was also chosen as the AIC values were within two values of Model 2 (Table 2.4) for both female and male colonies. The same model fitting procedure was applied to determine differences in polyp fecundity and gamete diameter between the three colony zones (see Materials and Methods; Appendix A).

Table 2.3 Results of the minimum adequate model based on a linear mixed-effects model testing the effect of branch segment (fixed) on polyp fecundity in female and male colonies (random) of *A. arbuscula*. AIC=Akaike Information Criterion. Based on the AIC values, Model 1 was chosen for both female and male datasets

Females		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	695.071
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	696.988
Males		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	573.738
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	572.315

Table 2.4 Results of the minimum adequate model based on a linear mixed-effects model testing the effect of branch segment (fixed) on mean gamete diameter per polyp in female and male colonies (random) of *A. arbuscula*. AIC=Akaike Information Criterion. Based on the AIC values, Model 1 was chosen for both female and male datasets

Females		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	738.753
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	739.902
Males		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	1431.336
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	1433.336

An analysis of variance (ANOVA) was used to assess the overall effect of branch segment on mean polyp fecundity and mean gamete diameter. If significance was detected, Tukey's Honestly Significant Difference (HSD) test was used post-hoc to determine which pairs were significantly different from one another. All dependent variables were tested for the ANOVA assumptions of normality and homogeneity of variances using the Shapiro-Wilk test for normality and the Levene's test, respectively. If non-normality and heterogeneity were detected, the dependent variables were square-root transformed to meet the assumptions. Regression models were used to examine the influence of colony height on polyp fecundity, mean gamete diameter per polyp, and the percentage of mature (stage IV and V) oocytes per colony. Significance of the relationship was determined using Pearson's product-moment correlation once both the dependent and independent variables were examined for normality using the Shapiro-Wilk test of normality. Any non-normal variable was subsequently square root transformed closer to normality and the relationship tested for significance. All statistical analyses were carried out in R version 2.10.0 (R Development Core Team, 2009, http://www.R-project.org; package nlme for mixed model analysis).

2.3. Results

2.31. General Reproductive Characteristics

A total of 26 *A. arbuscula* colonies were collected from the Gully and Flemish Cap areas. All examined colonies were gonochoristic at both the polyp and colony level. Of the 26 colonies, 13 were female, 12 male, and 1 contained no gametes and so sex was indeterminable. The sex ratio of the Flemish Cap (9 female and 10 male) population was

not significantly different from 1:1 (χ^2_1= 0.053, *P*= 0.819). Two colonies from the Flemish Cap, 1 male and 1 female, were not used for fecundity estimates or for measurements of gamete size due to their poor histological preservation, giving 24 usable colonies (12 female and 11 male).

On average, female polyps contained 18.8 ± 16.2 (mean ± SD) oocytes, with fecundity reaching a maximum of 75 oocytes in one polyp. Mean oocyte diameter was 136.1 ± 125.1 µm, with the largest oocyte 702.4 µm in diameter. In males, polyps contained an average of 14.0 ± 14.4 spermatic cysts per polyp, with a maximum of 92 cysts in one polyp. Mean spermatic cyst diameter was 135.3 ± 97.9 µm, with the largest spermatic cyst reaching 462.1 µm in diameter.

2.32. Gametogenesis

Oogenesis

Oogenesis can be divided into five stages in *A. arbuscula*. Oocytes of all stages were often observed simultaneously within the same polyp.

Stage I: Oogonia (Fig. 2.7a, 19.9 ± 5.1 µm, (Mean Diameter ± SD), n= 282)

The earliest female gametes (oogonia) were observed in clusters embedded in the mesoglea of the mesenteries. These oogonia had a high nucleus:ooplasm ratio, with a translucent nucleus and often visible, single nucleolus. The ooplasm was basophilic and translucent.

Stage II: Pre-vitellogenic oocytes (Fig. 2.7b, 114.9 ± 64.9 µm, n= 2837)

Stage II oocytes were observed within the gastrovascular cavity but connected to the mesenteries via a pedicel (Cordes et al. 2001; Gutiérrez-Rodríguez and Lasker 2004), and often occurred in bundles. The nucleus:ooplasm ratio decreased in stage II oocytes, and the ooplasm stained basophilic. Often, more than one darkly-stained nucleolus was visible in the nucleus. The ooplasm of later stage II oocytes contained multiple vacuoles. The development of a follicle cell layer began at this stage.

Stage III: Onset of vitellogenesis (Fig. 2.7c, 311.0 ± 75.2 µm, n= 67)

By stage III, vitellogenesis had begun. The ooplasm was heavily granulated and stained slightly eosinophilic. These oocytes were observed free within the gastrovascular cavity. Occasionally, stage III spermatic cysts were observed within the pharynx or above it near the tentacles. The nucleus was large and often resided at the periphery of the ooplasm, and more than one nucleolus was often visible. A follicle cell layer was often observed around stage III oocytes.

Stage IV: Vitellogenic oocytes (Fig. 2.7d, 538.9 ± 66.6 µm, n= 118)

Stage IV oocytes were heavily granulated due to the presence of numerous yolk droplets, and consequently the ooplasm stained a conspicuous pink colour (highly eosinophilic). The nucleus was often located near the periphery of the ooplasm, and may have contained more than one darkly-stained nucleolus. A thick follicle cell layer surrounded stage IV oocytes.

Stage V: Late-vitellogenic oocytes (Fig. 2.7e, 526.3 ± 71.9 µm, n= 89)

The large yolk droplets observed in Stage IV oocytes became flattened in stage V. If visible, the nucleus was crescent-shaped, however, often the nuclear envelope had

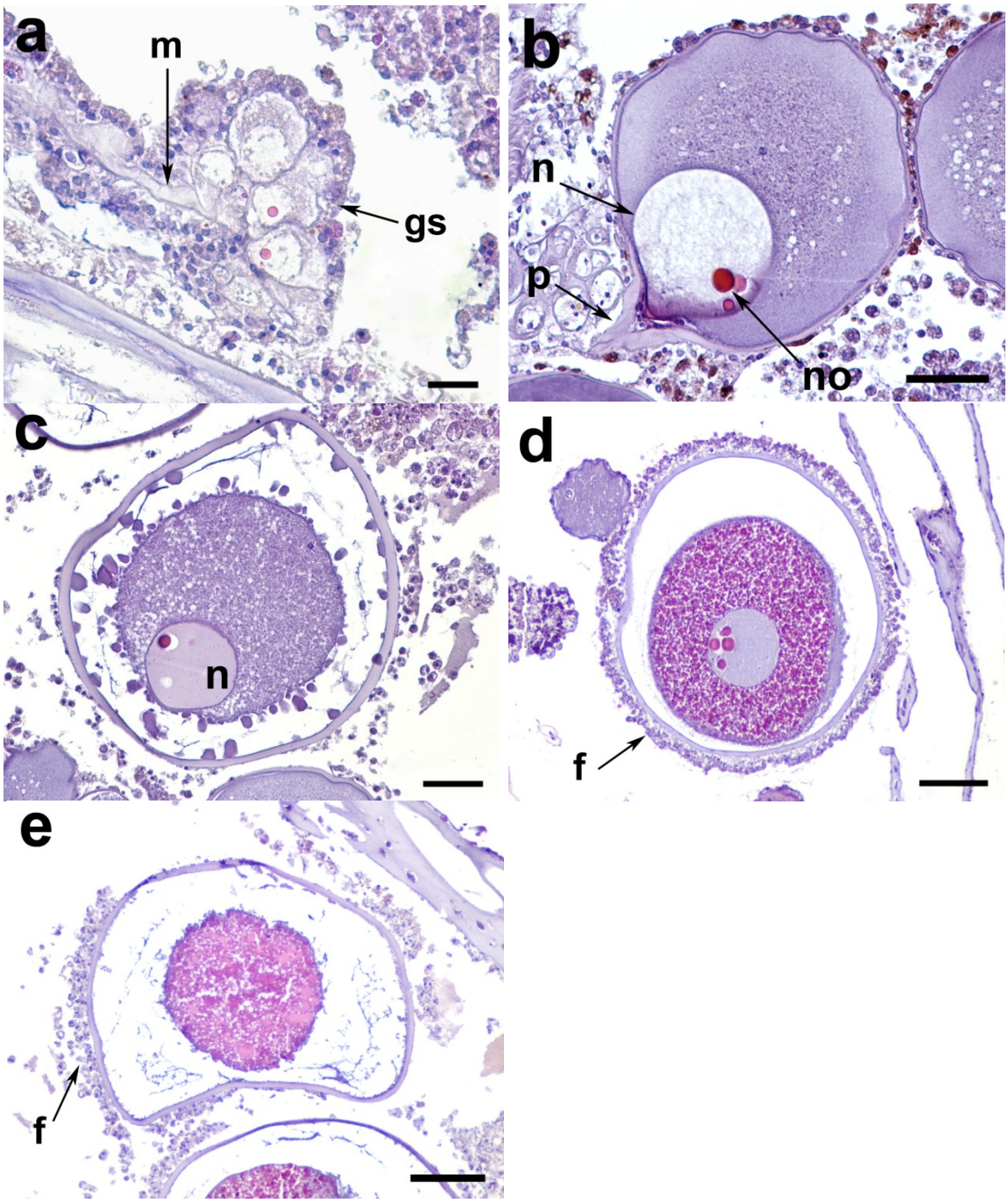

Fig. 2.7 Stages of oogenesis in *A. arbuscula*. **a** Cluster of stage I oogonia embedded within mesenterial (m) tissue and surrounded by gastroderm (gs), **b** stage II oocytes with nucleus (n), nucleolus (no), and pedicel (p), **c** stage III oocyte with peripheral nucleus and ooplasm stained slightly eosinophillic, **d** stage IV vitellogenic oocyte with thick follicle cell layer (f), heavily granulated ooplasm, and multiple nucleoli, and **e** stage V late vitellogenic oocyte with follicle layer layer slightly sloughed off. Scale bars: **a**= 20 µm; **b**, **c**, **d** and **e**= 50 µm

begun to break down and the nucleus was barely visible or not visible at all. Stage V oocytes were surrounded by a thick follicle cell layer and were often irregularly-shaped due to tight packing within the polyp.

No embryos or planula larvae were observed in any of the histological slides from any colony. Similarly, inspection of the mesh collection bags used in 2010 to collect *A. arbuscula* revealed no embryos or planula larvae, suggesting that they were not aborted during the collection process.

Spermatogenesis

Spermatogenesis can be divided into four stages. Each spermatic cyst contained many spermatogenic cells which differentiated relatively synchronously. Spermatic cysts of all stages were often observed simultaneously within a single polyp, however, only one stage IV spermatic cyst was observed out of all the samples.

Stage I: Spermatogonia (Fig. 2.8a, 24.8 ± 17.4 µm (Mean Diameter ± SD), n= 205)

Stage I consisted of loosely packed aggregations of spermatogonia either embedded within the mesoglea of the mesenteries, or attached to the mesenteries via a pedicel. The mesogleal layer surrounding stage I spermatic cysts was not distinct.

Stage II: Spermatic cyst with spermatocytes (Fig. 2.8b, 73.7 ± 39.6 µm, n= 1194)

Stage II spermatic cysts consisted of aggregations of loosely packed spermatocytes and occasional spermatogonia. A follicle cell layer began to develop at this stage. Stage II spermatic cysts were observed either attached to the mesenteries via a pedicel or free within the gastrovascular cavity.

Stage III: Maturing spermatic cyst with spermatocytes (Fig. 2.8c, 244.3 ± 51.7 μm, n= 892)

Stage III spermatic cysts consisted of darkly-stained spermatocytes densely packed and arranged around a distinct lumen in the centre of the cyst. Tails were occasionally observed in the centre of the lumen. A thick follicle cell layer was present, and these cysts were found floating freely in the gastrovascular cavity.

Stage IV: Late-stage spermatic cyst with spermatids and spermatozoa (Fig. 2.8d and e, n= 1, Measurement=297.8 μm)

Only one stage IV spermatic cyst was found in a proximally-located polyp from a sample collected in May 2009. The spermatic cyst consisted of spermatids and spermatozoa with heads located near the periphery, and pink-stained spermatozoa tails projecting towards the centre of the lumen (Fig. 2.8e). A thick follicle cell layer was not observed around this cyst, however, it may have been sloughed off due to collection or histological stress.

2.33. Gamete Size-Frequency Distributions

The oocyte size-frequency distributions of individual colonies showed variable patterns within and between collection months and years. The majority of oocyte size-frequency distributions (Fig. 2.9) were similar in shape, possessing a right-skewed/bimodal pattern with a large mode of smaller oocytes (approximately ≤400 μm) and a small mode of larger oocytes (approximately >400 μm). Exceptions to this pattern included four colonies collected on July 12 2007, June 15 2009, and June 15 and 16, 2009 which all lacked a second mode. The majority of male colonies exhibited a bimodal distribution pattern, with one mode of cysts ≤100 to 150 μm and one >100 to 150 μm

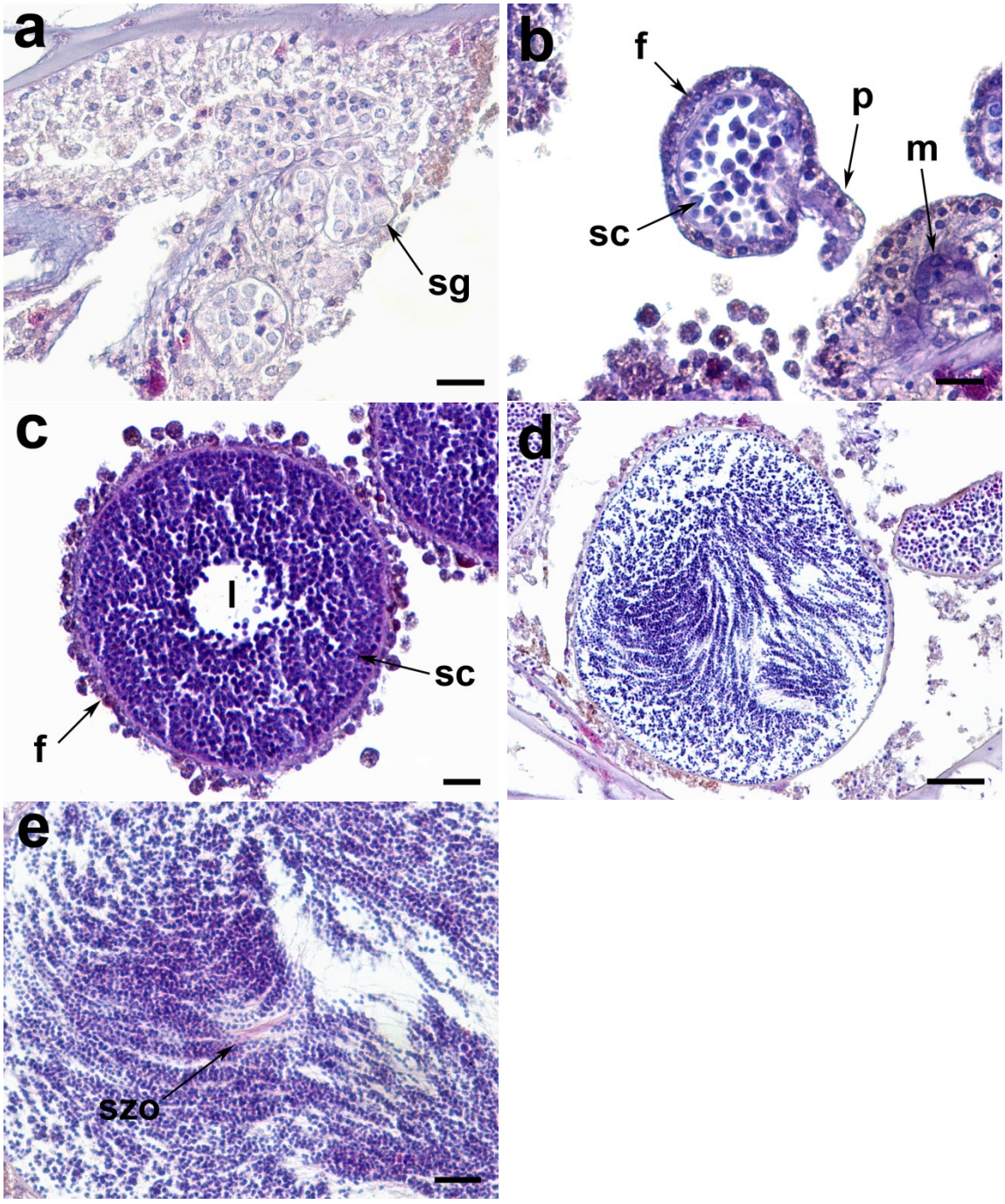

Fig. 2.8 Stages of spermatogenesis in *A. arbuscula*. **a** Stage I spermatic cyst with clusters of spermatogonia (sg), **b** stage II spermatic cyst containing spermatocytes (sc), surrounded by a follicle cell layer (f) and attached to mesentery via a pedicel (p), **c** stage III cyst with spermatocytes and lumen (l), **d** stage IV late-stage spermatic cyst with spermatids and mature spermatozoa (szo), and **e** stage IV cyst with pink tails projecting towards the centre of the cyst. Scale bars: **a**, **b** and **e**= 20 µm, **c**= 20 µm, **d**= 50 µm

(Fig. 2.10). These modes were present in equal proportions in most colonies. A colony collected on July 27 2010 showed evidence of bimodality, suggested by the small peak at 150 μm, however, a colony collected in August completely lacked a second peak of spermatic cysts, and displayed a right-skewed pattern.

The percent frequency of the five stages of oogenesis significantly differed between colonies collected in June and July (Table 2.5). Colonies collected in June had a higher percent frequency of stage I oogonia compared to colonies collected in July. Both months had a similar frequency of stage II oocytes, whereas colonies collected in June had a higher frequency of maturing (stage III and IV) oocytes. Samples collected in July had a higher frequency of late-stage mature oocytes (stage V) than samples collected in June.

In male colonies, the percent frequency of the four stages of spermatogenesis differed significantly between colonies collected in May to August (Table 2.6). The percent frequency of stage I spermatic cysts increased from May to July, and was absent in August. Similar frequencies of stage II cysts occurred between May and June, with a decrease in July. In August, 100% of the spermatic cysts were stage II. Stage III spermatic cysts occurred in similar frequencies between May and July, but were absent in August. Only one stage IV spermatic cyst was found in one colony collected in May 2009.

2.34. Intra-Colony Variation in Polyp Fecundity and Gamete Size

Polyp fecundity significantly differed between the three branch segments in female colonies (Table 2.7). Tukey's HSD post-hoc test revealed that fecundity was

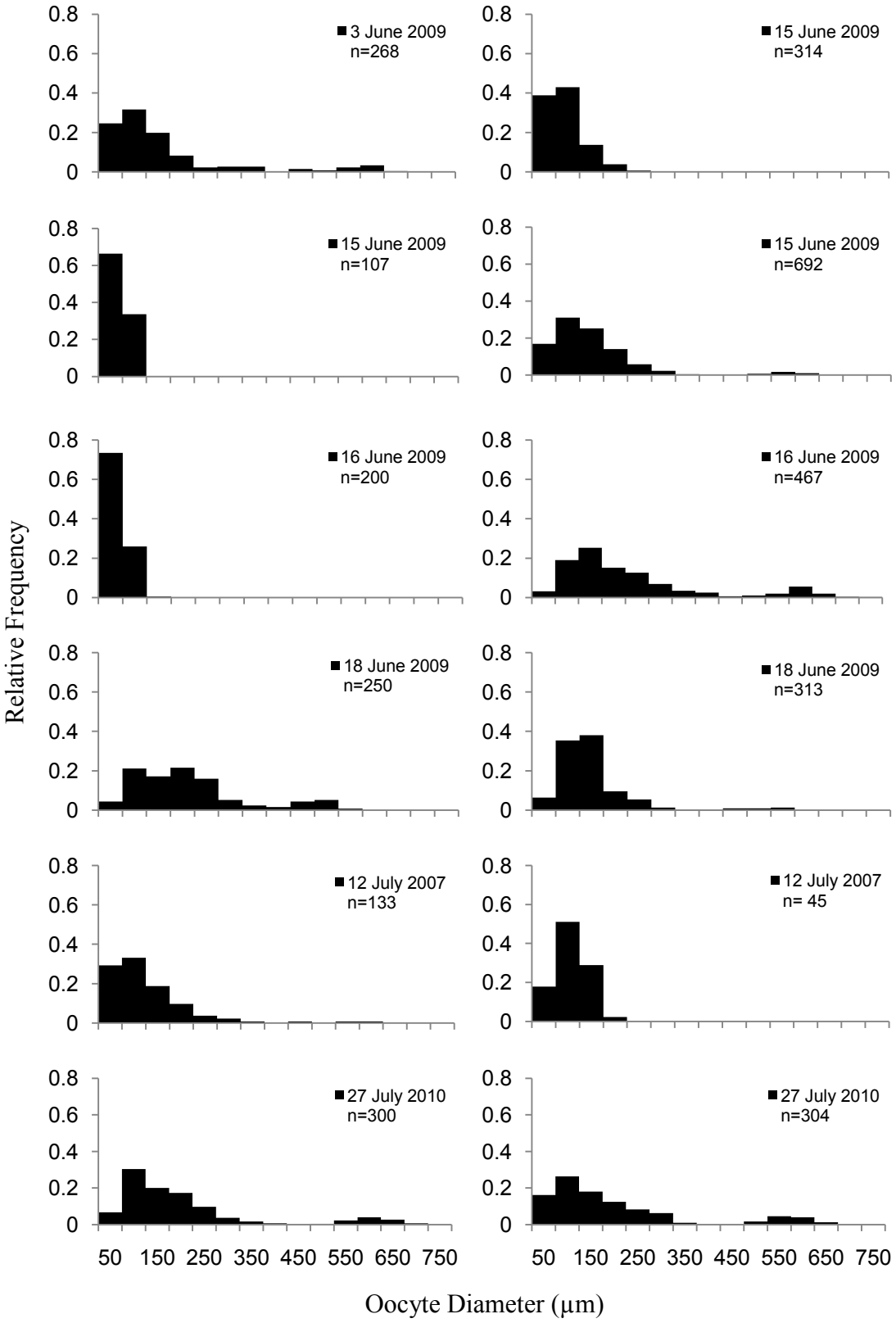

Fig. 2.9 Oocyte size-frequency distributions of individual *A. arbuscula* colonies collected in July 2007, June 2009, and July 2010. Date is date of collection. n= number of oocytes.

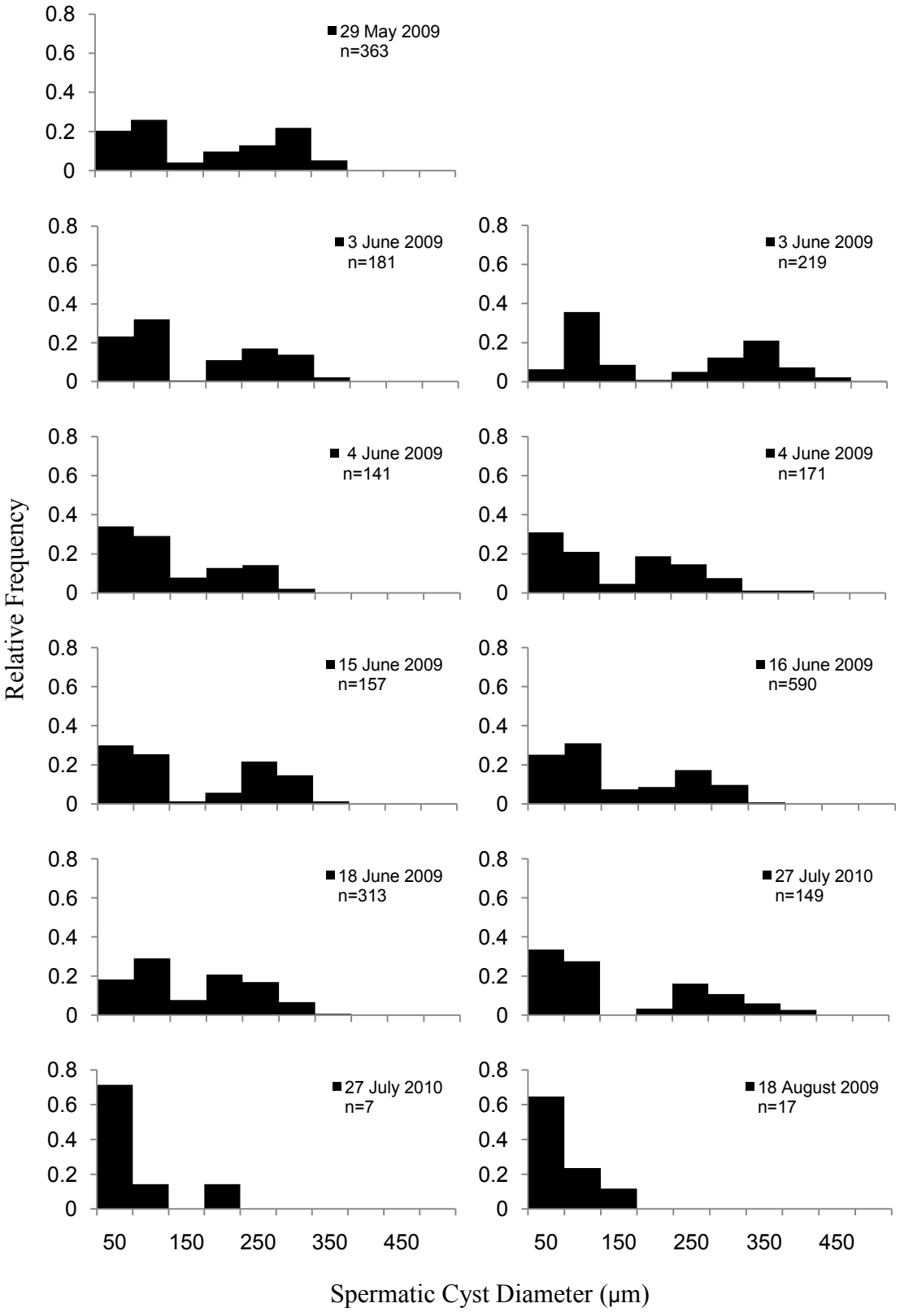

Fig. 2.10 Spermatic cyst size-frequency distributions of individual *A. arbuscula* colonies collected in May through August 2009, and July 2010. Date is date of collection. n= number of cysts.

Table 2.5 Percent (%) frequency of the five stages of oogenesis in female colonies of *A. arbuscula* collected in June and July. n= number of oocytes. Log likelihood ratio (G-test) test of independence testing null hypothesis of equality of proportions between months. Asterisk (*) indicates significance at α=0.05

	Percent (%) Frequency					
Month	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**Total n**
June	9.41	82.86	2.31	4.07	1.35	2611
July	4.48	86.19	0.90	1.53	6.91	782
G statistic	**df**	**P value**				
98.878	4	$<2.200*10^{-16}$*				

Table 2.6 Percent (%) frequency of the four stages of spermatogenesis in male colonies of *A. arbuscula* collected in May through August. n= number of sperm cysts. Log likelihood ratio (G-test) test of independence with Williams' correction of continuity testing null hypothesis of equality of proportions between months. Asterisk (*) indicates significance at α=0.05

	Percent (%) Frequency				
Month	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Total n**
May	1.65	53.44	44.63	0.28	363
June	8.41	53.61	37.98	0	1772
July	42.31	21.15	36.54	0	156
August	0	100	0	0	17
G statistic	**df**	**P value**			
51.036	9	$6.874*10^{-8}$*			

highest in distal polyps compared to proximal and central polyps (Fig. 2.11a and Table 2.7). Polyp fecundity also significantly differed between branch segments in male colonies (Table 2.8 and Fig. 2.11a), and post- hoc analysis revealed proximal polyps had significantly lower fecundity than central and distal polyps. Mean oocyte diameter per polyp did not significantly differ between branch segments (Table 2.9 and Fig. 2.11b), however, mean spermatic cyst diameter significantly differed between branch segments (Table 2.10 and Fig. 2.11b), with proximal polyps having smaller mean gamete diameters per polyp than central and distal polyps (Table 2.10 and Fig. 2.11b).

The percent frequency of the five stages of oogenesis significantly differed between the three branch segments (Table 2.11). Stage I oogonia were present in a higher percentage in central polyps than in proximal and distal polyps. Stage II, stage III, and stage IV oocytes were present in similar proportions between central and distal polyps, but were higher in proximal polyps. Stage V oocytes were present in the highest proportion in distal polyps, and were found in lowest proportion in proximal polyps.

The percent frequency of the four stages of spermatogenesis was significantly different between branch segments (Table 2.12). Stage I spermatic cysts and stage II spermatic cysts were present in higher percentages in proximal polyps than in central and distal polyps. Stage III spermatic cysts were most abundant in distal polyps, and were lowest in proximal polyps. Only one stage IV spermatic cyst was found out of all the branch segments, and was found in a proximal polyp.

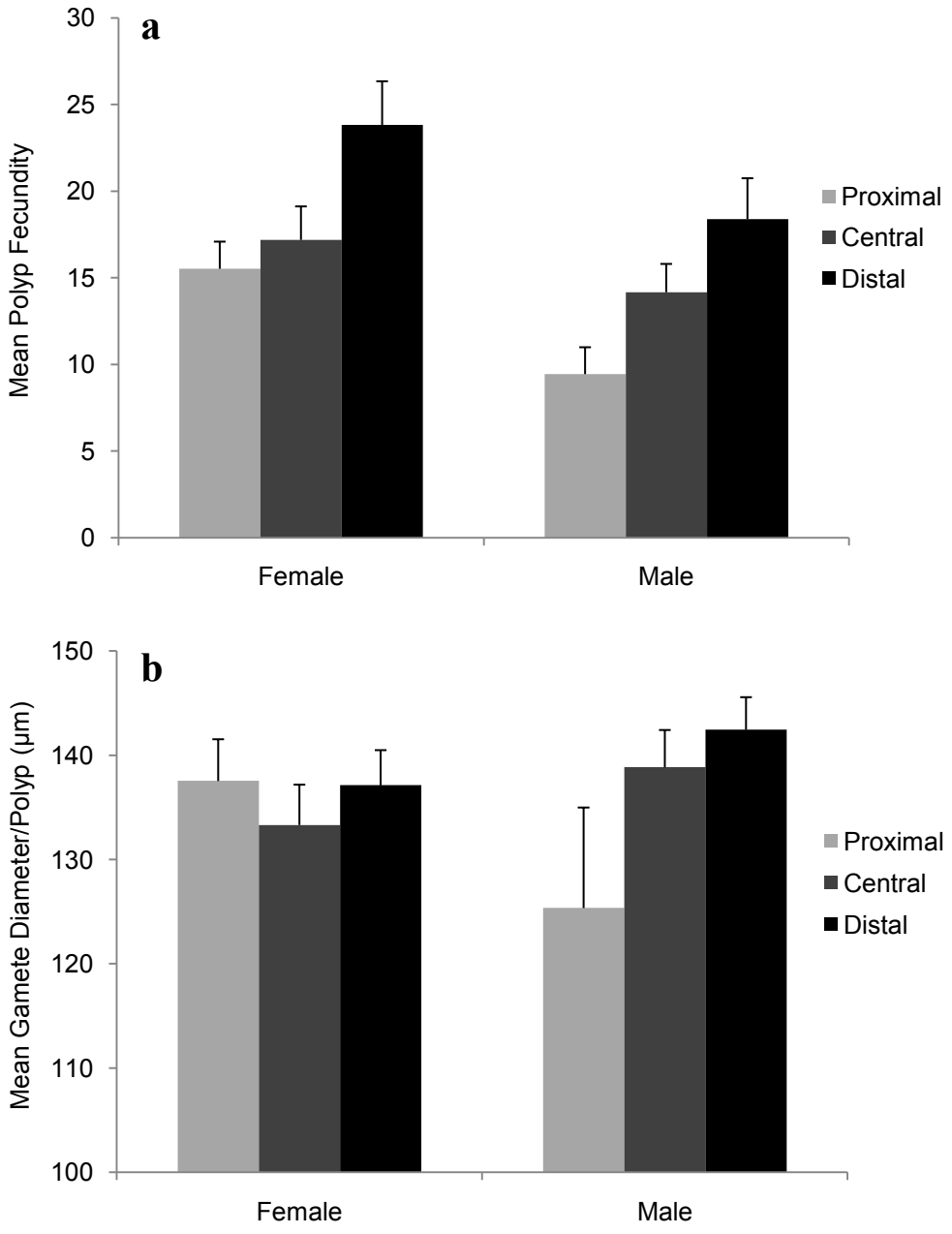

Fig. 2.11 **a** Mean number of gametes per polyp between the proximal (light grey), central (dark grey), and distal (black) branch segments for female and male *A. arbuscula* colonies. **b** Mean gamete diameter per polyp between the proximal (light grey), central (dark grey), and distal (black) branch segments for female and male colonies. Error bars are ± 1 SE

Table 2.7 ANOVA for a mixed-effects model testing differences in female polyp fecundity (square root transformed) between branch segments of *A. arbuscula*. Tukey's Honestly Significant Difference (HSD) post hoc test shows comparison of means and relationship where P=proximal, C=central, D=distal. Asterisk (*) indicates significance at α=0.05

Source of Variation	**df**	**F value**	**P value**
Branch segment	2	7.439	<0.001*
Tukey's HSD post-hoc test	**P value**	**Relationship of means**	
Proximal-Central	0.843	P=C	
Proximal-Distal	<0.001*	P<D	
Central-Distal	0.007*	C<D	

Table 2.8 ANOVA for a mixed-effects model testing differences in male polyp fecundity (square root transformed) between branch segments of *A. arbuscula*. Tukey's Honestly Significant Difference (HSD) post hoc test shows comparison of means and relationship where P=proximal, C=central, D=distal. Asterisk (*) indicates significance at α=0.05

Source of variation	**df**	**F value**	**P value**
Branch segment	2	15.527	<0.001*
Tukey's HSD post-hoc test	**P value**	**Relationship of means**	
Proximal-Central	<0.001*	P<C	
Proximal-Distal	<0.001*	P<D	
Central-Distal	0.149	C=D	

Table 2.9 ANOVA for a mixed-effects model testing differences in mean oocyte diameter (μm) (square root transformed) between branch segments of *A. arbuscula*. Asterisk (*) indicates significance at α=0.05

Source of Variation	**df**	**F value**	**P value**
Branch segment	2	0.844	0.432

Table 2.10 ANOVA for a mixed-effects model testing differences in mean spermatic cyst diameter (μm) (not transformed) between branch segments of *A. arbuscula*. Tukey's Honestly Significant Difference (HSD) post hoc test shows comparison of means and relationship, where P=proximal, C=central, D=distal. Asterisk (*) indicates significance at α=0.05

Source of Variation	**df**	**F value**	**P value**
Branch segment	2	6.741	0.002*
Tukey's HSD post hoc test	**P value**	**Relationship of means**	
Proximal-Central	0.017*	P<C	
Proximal-Distal	0.002*	P<D	
Central-Distal	0.760	C=D	

Table 2.11 Percent (%) frequency of the five stages of oogenesis across the proximal, central, and distal branch segments. n= number of oocytes. Log likelihood ratio (G-test) test of independence testing null hypothesis of equality of proportions between branch segments. Asterisk (*) indicates significance at α=0.05

	Percent (%) Frequency					
Branch Segment	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Stage 5**	**Total n**
Proximal	4.2	87.3	2.9	3.9	1.7	931
Central	10.1	81.6	1.8	3.3	2.3	1030
Distal	9.2	82.2	1.6	3.4	3.5	1385
G statistic	**df**	**P value**				
48.487	8	$8.000*10^{-8}$*				

Table 2.12 Percent (%) frequency of the four stages of spermatogenesis across the proximal, central, and distal branch segments. n= number of sperm cysts. Log likelihood ratio (G-test) test of independence with Williams' correction of continuity testing null hypothesis of equality of proportions between branch segments. Asterisk (*) indicates significance at α=0.05

	Percent (%) Frequency				
Branch Segment	**Stage 1**	**Stage 2**	**Stage 3**	**Stage 4**	**Total n**
Proximal	11.6	54.8	33.4	0.2	518
Central	8.6	52.5	38.9	0	779
Distal	7.8	50.4	41.8	0	995
G statistic	**df**	**P value**			
12.775	6	0.047*			

2.35. Size at First Reproduction and Influence of Colony Height on Polyp Fecundity and Gamete Size

Size at first reproduction could not be determined for *A. arbuscula*. The smallest female was 3.4 cm in height and contained 200 oocytes (of stage I and stage II), whereas the smallest male was 3.0 cm and contained 17 spermatic cysts (of stage II). One colony contained no gametes, and was 7.0 cm in height. Colony height had a significant effect on the mean polyp fecundity in females (t_{10}= 2.676, P= 0.023), but not in males (t_9= 1.135, P= 0.286). Colony height explained approximately 42% of the variability in mean polyp fecundity in female colonies, and approximately 12% of the variability in mean polyp fecundity in males (Fig. 2.12). Mean gamete diameter per polyp for female and male colonies was also positively correlated with colony height, but neither relationship was significant (females: t_{10}= 2.050, P= 0.068; males: t_9= 2.150, P= 0.060). Colony height explained approximately 30% of the variability in mean gamete diameter per polyp in females, and approximately 34% of the variability in mean gamete diameter per polyp in males (Fig. 2.12b). The relationship between colony height and the percent frequency of mature (stage IV and V) oocytes was not significant (t_{10}= 2.080, P= 0.064) (Fig. 2.13). Colony height explained approximately 30% of the variation in the frequency of mature oocytes per colony.

2.4. Discussion and Conclusion

2.41. General Features of Reproduction

The general features of reproduction in *A. arbuscula* are similar to those observed in shallow- and other deep-water gorgonians. *A. arbuscula* is gonochoristic at both the

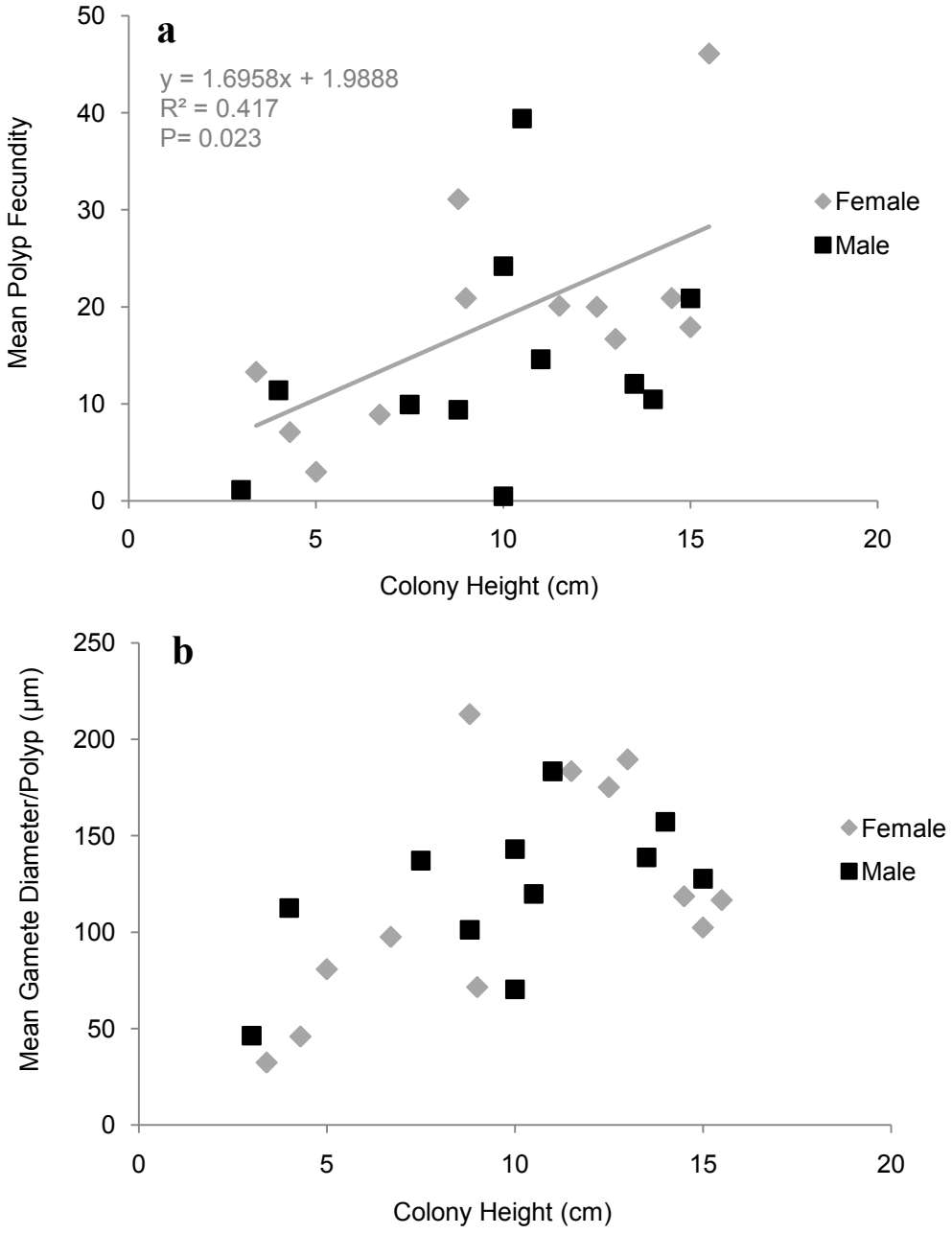

Fig. 2.12 **a** Mean polyp fecundity per colony for female (grey) and male (black) *A. arbuscula* colonies as a function of colony height (cm). **b** Mean gamete diameter (µm) per polyp per colony for female (grey) and male (black) *A. arbuscula* colonies as a function of colony height (cm). Colour of regression line, equation, R^2 and P value indicates sex

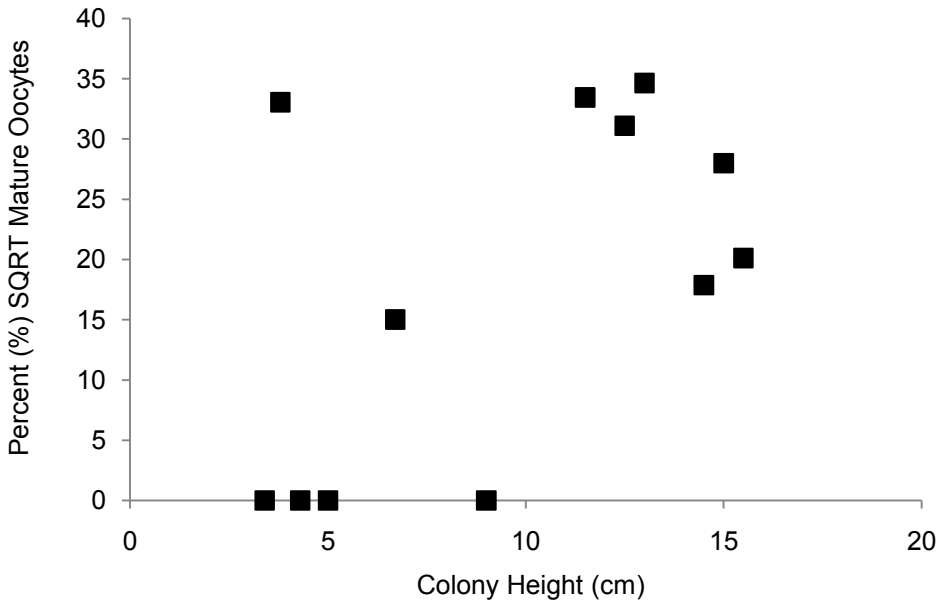

Fig. 2.13 Percent (%) of mature (Stage IV and Stage V) oocytes (square root transformed) per colony as a function of colony height (cm)

polyp and colony level, which is the dominant pattern of sexuality in members of the Octocorallia (Brazeau and Lasker 1990; Kruger et al. 1998; Ben-Yosef and Benayahu 1999; Orejas et al. 2002; Santengelo et al. 2003; Gutiérrez-Rodríguez and Lasker 2004; Hwang and Song 2007; Orejas et al. 2007; Edwards and Moore 2008; Pires et al. 2009). Sequential hermaphroditism is unlikely as colonies of a wide size range were examined. Lawson (1991) also reported separate male and female colonies of *A. arbuscula* from Station 'M' in the Rockall Trough, Northeast Atlantic. The sex ratio of the Flemish Cap samples of *A. arbuscula* was not significantly different from 1:1. Deviations in the sex ratio of octocorals are most commonly caused by a higher proportion of females to males (Brazeau and Lasker 1989; Babcock 1990; Ben-Yosef and Benayahu 1999; Santangelo et al. 2003). The only octocorals known to have a male-biased sex ratio are the gorgonians *Briareum asbestinum* from the Caribbean (Brazeau and Lasker 1990) and *Paramuricea clavata* from the Mediterranean (Gori et al. 2007), and the tropical soft corals *Xenia macrospiculata* from the Gulf of Eilat (Benayahu and Loya 1984) and *Capnella gaboensis* from Australia (Farrant 1986). Cerrano et al. (2005) noted a shift in the sex ratio from parity to male-biased in the Mediterranean gorgonian *Paramuricea clavata* after a thermal anomaly in 1999 that caused mass mortality of the benthic community. The authors attributed the biased sex ratio to differential responses of each sex to the perturbation. Greater contribution of one sex to the population via asexual reproduction may also produce a skewed sex ratio (Benayahu and Loya 1984; Coffroth and Lasker 1998). The 1:1 sex ratio observed in *A. arbuscula* is common among octocorals, and in terms of the division of resources allocated to sexual reproduction, this sex ratio represents the predicted optimal resource allocation in populations with random mating (Pianka 1978; Leigh et al. 1985; Edwards and Moore 2008; West 2009).

Many shallow-water tropical gorgonians studied to date have low polyp fecundity compared to members of the Alcyonacea and Pennatulacea (Simpson 2009). In general, these interspecific differences are thought to be a function of the smaller length of gorgonian polyps (Brazeau and Lasker 1989). This pattern of lower polyp fecundity in the Gorgonacea compared to the Alcyonacea and Pennatulacea also holds true for some deep-water octocorals. For instance, the Antarctic deep-water gorgonian *Thouarella variabilis* produces only one mature oocyte per polyp at a time (Brito et al. 1997). Similarly, the average number of oocytes per polyp was 1.2 ± 0.1 (± SE) and 1.1 ± 0.1 for the Antarctic deep-water gorgonians *Dasystenella acanthina* and *Thouarella* sp., respectively, and 1.5 ± 0.1 and 1.4 ± 0.1 for *Fannyella rossii* and *F. spinosa,* respectively. In comparison, the deep-water pennatulacean *Anthoptilum murrayi* had an average of 47 ± 12.4 (± SD) oocytes and 36.6 ± 3.6 spermatic cysts per polyp and 31465 ± 5080 oocytes and 19871 ± 5793 spermatic cysts per colony (Pires et al. 2009). A large colony of the deep-water alcyonacean *Anthomastus ritteri* could contain in excess of 4000 oocytes and larvae at any one time (Cordes et al. 2001). Polyps of *A. arbuscula* contained high numbers of oocytes (18.8 ± 16.2; mean ± SD) and spermatic cysts (14.0 ± 14.4) per polyp compared to other deep-water gorgonians, although, it remains unknown whether all of these oocytes will reach maturity as some immature oocytes may be resorbed to provide nutrients for mature oocytes (Harrison and Wallace 1990; Sier and Olive 1994; Loya et al. 2004; Mercier et al. 2010). Despite the common belief that many deep-sea benthos exhibit low fecundity (Gage and Tyler 1991), the results of the current study and those on other deep-water corals (Waller et al. 2008) show that fecundity of these organisms is comparable to that of their tropical, zooxanthellate counterparts.

The maximum oocyte size observed in *A. arbuscula* (702.4 μm) is comparable to that of some shallow- (zooxanthellate and azooxanthellate) and deep-water gorgonians (Table 2.13). However, much larger oocyte diameters have been recorded for the deep-water Antarctic gorgonian *Dasystenella acanthina* (1200 μm, Orejas et al. 2007) and for other deep-water octocorals (1200 μm in *Anthoptilum murrayi*, Pires et al. 2009). Oocyte diameters ranging from ~4800 to ~5200 μm have been recorded for deep-water scleractinian cup corals of the genus *Flabellum* (Waller et al. 2008). In *A. arbuscula* from the Northeast Atlantic, the maximum oocyte diameter recorded was 730 μm (Lawson 1991), which is comparable to the findings of the present study. Both shallow and deep-water corals with large oocytes often have non-feeding, lecithotrophic larvae (Chia and Crawford 1973; Hartnoll 1975; Cordes et al. 2001; Mercier et al. 2010). It was previously believed that species with lecithotrophic larvae spend less time in the plankton and therefore have limited dispersal capabilities (Young 2003), however, a lecithotrophic strategy may better allow for long-distance dispersal in the oligotrophic regions of the deep-sea (Young et al 1997). The large oocyte diameters observed in *A. arbuscula* suggest that this species has a lecithotrophic larval type, which could explain its wide distribution range within the North Atlantic. Nonetheless, further study on the larval development mode is required to confirm this.

Lawson (1991) predicted that *A. arbuscula* from the Northeast Atlantic was a brooder, based on the gonad developmental cycles and large oocyte diameters observed. However, no planula larvae were found in any of the colonies in this study. We now know that large oocyte diameters do not necessarily indicate a brooding strategy (Eckelbarger et al. 1998; Kruger et al. 1998; Orejas et al. 2007; Edwards and Moore 2009; Mercier et al. 2010). Levitan (2006) suggested that a large egg size increases the

Table 2.13 Maximum oocyte diameters (µm) comparable to *A. arbuscula* of some gorgonian corals from shallow- (i.e. <200 m) and deep-water (i.e. >200 m) habitats

Species	Habitat	Maximum Oocyte Diameter (µm)	Reference
Plexaura kuna	Shallow	600	Brazeau and Lasker 1989
Briareum asbestinum	Shallow	900	Brazeau and Lasker 1990
Paramuricea clavata	Shallow	500	Coma et al. 1995b
Pseudoplexaura porosa	Shallow	750	Kapela and Lasker 1999
Thouarella variabilis	Deep	750	Brito et al. 1997
Ainigmaptilon antarcticum	Deep	900	Orejas et al. 2002
Corallium secundum	Deep	650	Waller and Baco 2007

chance of fertilization, and would be advantageous for broadcast spawners. In the present study, no embryos or planula larvae were observed in the histological slides, suggesting that *A. arbuscula* is a broadcast spawner. It is possible that embryos or larvae were extruded through the mouth of the polyp during collection stress and/or surfacing from the deep-sea. This is supported by the discovery of immature male spermatic cysts (stage III) in the pharynx or above it near the tentacles in the histology sections. However, no embryos or planulae were found in the mesh collection bags used in 2010. Also, the chance of observing planula larvae within the polyps is greater if the polyps contain oocytes in various stages of maturity (Waller et al. 2002), which provides further support that *A. arbuscula* is a broadcast spawner.

2.42. Cycle of Gametogenesis

The presence of oocytes and spermatic cysts in different stages of development in the same polyp suggests that *A. arbuscula* has a continuous cycle of gametogenesis (Brito et al. 1997; Cordes et al. 2001; Waller and Baco 2007; Pires et al. 2009). Alternatively, this pattern may represent overlapping periodic or prolonged, seasonal cycles of gametogenesis (Kruger et al. 1998; Orejas et al. 2007). Polyps with oocytes in various stages of development were also observed in the Antarctic deep-water gorgonian *Thouarella variabilis*, and it was suggested that this species has a continuous or two year cycle of oogenesis (Brito et al. 1997). The majority of oocyte size-frequency distributions (Fig. 2.9) of individual *A. arbuscula* colonies displayed two modes, which may correspond to two different generations of oocytes (Pires et al. 2009), suggesting overlapping periodic or seasonal gametogenic cycles over continuous gametogenesis. The majority of spermatic cyst size-frequency distributions also showed a bimodal pattern

(Fig. 2.10). Orejas et al. (2007) observed bimodality in the oocyte size-frequency distributions of the deep-water gorgonians *Dasystenella acanthina* and *Thouarella* sp., and suggested these species have overlapping and long (1-2 year) cycles of oocyte development, possibly with undetermined seasonal spawning events. A gametogenic cycle of 2 years was also documented in the deep-water Antarctic primnoid *Ainigmaptilon antarcticum* (Orejas et al. 2002). The cause of extended gametogenic cycles in corals remains uncertain (Edwards and Moore 2009). Previous studies have suggested that an extended oogenic cycle is required to produce large oocytes, however, a prolonged oogenic cycle has been reported in a azooxanthellate coral with relatively small oocytes (*Balanophyllia elegans*, Fadlallah and Pearse 1982). It is possible that in the deep ocean where resources are limited, two successive spring/summer periods when food abundance is highest may be required to complete gametogenesis (Orejas et al. 2007). However, many shallow, zooxanthellate corals also have prolonged cycles of oogenesis (Benayahu and Loya 1986; Benayahu 1989; Brazeau and Lasker 1989; Coma et al. 1995b; Kruger et al. 1998; Ribes et al. 2007; Edwards and Moore 2008). Alternatively, Benayahu and Loya (1986) suggested that extended cycles of oogenesis are found in species with high fecundity, synchronized oocyte maturation, and brief spawning periods. Further investigation into the driving forces of the duration of the gametogenic cycle in both shallow- and deep-water corals is warranted.

Bimodality of oocyte size-frequency distributions and seasonal development and spawning of oocytes has been reported for several deep-water anemones (Van-Praet 1990; Van-Praet et al. 1990). Van-Praet et al. (1990) observed bimodality in two species of deep-water anemone, *Phelliactis hertwigi* and *P. robusta*, and related the variability in the number of the larger size class of oocytes to their seasonal disappearance. This

pattern is similar to that observed in colonies of *A. arbuscula*. In *A. arbuscula*, periodicity or seasonality is suggested from the percent frequency of the developmental stages of oocytes and spermatic cysts, but is not evident from the individual gamete size-frequency distributions. For example, the percent frequency of stage IV oocytes decreased from ~4 to ~1.5% from June to July (Table 2.5), and the percent frequency of stage V oocytes increased from ~1.4 to 7% from June to July, suggesting a shift towards more late-vitellogenic oocytes in July. Also, stage 1 oogonia decreased in frequency from June to July (4.5% compared to 9.5% in June). These results suggest that a spawning event might have occurred during or after July. In males however, a spawning event may have occurred earlier than July. Only one spermatic cyst with mature spermatozoa was observed in a sample collected in May (Table 2.6). In male colonies, spawning is usually close when spermatozoa tails are present in the spermatic cysts (Brazeau and Lasker 1989). It is possible that a spawning event occurred during or just before samples were collected in May, which would explain why only one mature spermatic cyst with spermatozoa tails was present. Alternatively, stage III spermatic cysts may mature quickly into stage IV spermatic cysts (Harrison and Wallace 1990) and are released, which could explain why no stage IV cysts were present past May.

A. arbuscula from the Northeast Atlantic exhibited seasonal cycles of gametogenesis and spawning. Lawson (1991) observed an increase in mean spermatic cyst and oocyte diameters throughout the year, with the largest spermatic cysts occurring in September. In females, a cohort of smaller oocytes was present year-round, with medium and large oocytes increasing in frequency throughout the year. The largest numbers of large (>0.45 μm) oocytes occurred in October, the time at which Lawson (1991) hypothesized that spermatic cysts were spawned. Lawson (1991) related this

seasonal cycle of gametogenesis to the sinking of the spring phytoplankton bloom in late May to August, and hypothesized that *A. arbuscula* may use this food source as a cue to initiate vitellogenesis. Alternatively, Lawson (1991) suggested that the timing of release of larvae may be linked with the arrival of the bloom to the sea floor, suggesting larvae or newly-settled polyps would benefit from the increased resources. In the present study, the pattern of gametogenesis and spawning of *A. arbuscula* remains unclear. The presence of two cohorts of oocytes suggests it is possible that *A. arbuscula* maintains a pool of pre-vitellogenic oocytes throughout the year, with maturation and spawning of only a small portion of the cohort occurring periodically, or seasonally as in *A. arbuscula* from the Northeast Atlantic. Monthly sampling throughout the year combined with laboratory experiments are required to confirm the duration and cycle of gametogenesis and spawning in this species.

2.43. Intra-Colony Variation in Polyp Fecundity and Gamete Size

Intra-colony variation in reproduction has been documented both in tropical zooxanthellate and deep-water corals (Benayahu and Loya 1986; Brazeau and Lasker 1989; Coma et al. 1995a; Brito et al. 1997; Sakai 1998; Kapela and Lasker 1999; Orejas et al. 2002; Santangelo et al. 2003; Gutiérrez-Rodríguez and Lasker 2004; Orejas et al. 2007; Pires et al. 2009). However, polyp fecundity and mean gamete diameters per polyp in both females and males did not significantly differ between the three colony zones (i.e. apical, medial, and basal) in *A. arbuscula*. In constrast, Orejas et al. (2002) found that polyps from the apical and medial zones had significantly higher fecundities than basally-located polyps in the deep-water primnoid coral *Ainigmaptilon antarcticum*, and related this pattern to possible elevated prey capture rates in more apically-located polyps, and/or

differential investment of energy to growth instead of reproduction in basal polyps. Pires et al. (2009) found that basal polyps of the deep-water sea pen *Anthoptilum murrayi* had the highest frequency of small oocytes compared to medial and apical polyps. In the deep-water gorgonians *Fanyella rossii* and *F. spinosa*, no significant differences in polyp fecundity between colony zones was observed, however mean spermatic cyst diameter was significantly different between zones in *F. rossii*, with the smallest diameters occurring in the basal zone (Orejas et al. 2007). In *A. arbuscula*, it is possible that there are no differences in prey capture rates in different areas of the same colony (Coma et al. 1995a), or transport of resources from apical polyps, which may acquire more food than medial or basal polyps, occurs equally to other areas of the same colony.

Despite a lack of variation in polyp fecundity and mean gamete diameters between colony zones, intra-colony variation was observed along individual branches in *A. arbuscula*. Polyp fecundity differed significantly depending on where the polyp was located along a branch in both females and males. In females, fecundity was highest in distal polyps, with no difference between proximal and central polyps (Table 2.7), and was lowest in proximal polyps, with no difference between central and distal polyps (Table 2.8) in male colonies. Distal polyps also had slightly higher numbers of mature oocytes and spermatic cysts (Tables 2.11 and 2.12), however, mean oocyte diameter did not significantly differ between branch segments (Table 2.9). Overall these findings suggest that polyps further out along a branch have higher fecundity and larger spermatic cysts than polyps closest to the branch origin. This pattern is in contrast with other studies on intra-colony variation in fecundity in tropical and deep-water gorgonians, the majority of which have reported significantly lower fecundity in distal polyps than in proximal or central polyps (Brazeau and Lasker 1988; Brito et al. 1997; Santangelo et al. 2003; Orejas

et al. 2007). In tropical octocorals, distal polyps are typically young, fast growing, and sexually immature (Connell 1973; Benayahu and Loya 1986; Kapela and Lasker 1999), although they may display an adult size (Brito et al. 1997). Thus, polyps close to the growth tips of branches may be allocating energy to growth instead of reproduction (Brazeau and Lasker 1988). Alternatively, polyps within the same colony may differ in their function. For example, Brito et al. (1997) hypothesized that the lower fecundity observed in the distal polyps of the Antarctic deep-water gorgonian *Thouarella variabilis* was due to differences in polyp functionality, and suggested that reproducing polyps enter a quiescent phase and do not feed, receiving food from the peripheral polyps which have greater access to resources in the water column.

Few studies have documented higher fecundity in peripheral polyps as observed in the present study. For instance, in the azooxanthellate gorgonian *Paramuricea clavata*, a decrease in fecundity and fertility (number of gravid polyps) with increasing branch order was observed (Coma et al. 1995a). Coma et al. (1995a) suggested that higher fecundity in polyps of first order branches may be due to higher prey capture rates of those polyps, the resources of which would in turn be allocated towards reproduction. Similarly, the deep-water gorgonian *Thouarella* sp. had a higher number of oocytes in the central and distal polyps than in proximal polyps (Orejas et al. 2007). In *A. arbuscula*, the higher fecundity observed in polyps closer to the tips may be the result of higher prey capture rates and thus greater resources for reproduction as suggested by Coma et al. (1995a). Alternatively, it may represent an adaptation to release more gametes further and higher into the water column. Colony morphology may also have an impact on polyp fecundity in gorgonian corals. For instance, higher fecundity in proximal compared to distal polyps is often observed in gorgonians with fan-type morphology (e.g. Orejas et al. 2007). *A.*

arbuscula is radially bushy with many internal branches. Hosting the largest number of gametes in peripherally-located polyps may aid in their release further into the water column and reduce passive deflection and inhibition by other branches on the colony.

Predation on gorgonian polyps is common in tropical waters (Harmelin-Vivien and Bouchon-Navaro 1983; Ruesink and Harvell 1990; Goh et al. 1999), and may negatively affect the reproductive output of a coral (Lasker 1985; Rotjan and Lewis 2009). For instance, the butterflyfish *Chaetodon capistratus* concentrates its feeding on *Plexaura kuna* colonies when the polyps contained visible ripe gonads (Lasker 1985). Rotjan and Lewis (2009) observed that Caribbean parrotfish selectively grazed on polyps of the gorgonian *Montastraea annularis* with high total reproductive effort. Shallow, tropical corals may host the largest numbers of gametes closer to the interior of the colony to avoid predation on the most gravid polyps. In the deep-sea, predation pressure on coral polyps is likely not as significant as in shallow waters, and thus, there may not be the selective pressure to host large numbers of gametes near the interior of the colony as in tropical gorgonians. Nonetheless, not all deep-water corals host more gametes in distal polyps than in proximal polyps (see Orejas et al. 2007). A combination of these factors likely caused the unique pattern of intra-colony variation in reproduction observed in this species.

2.44. Size at First Reproduction and Influence of Colony Height on Polyp Fecundity and Gamete Size

It is well documented that shallow-water corals delay reproduction until the colony reaches a minimum size (Brazeau and Lasker 1989; Coma et al. 1995a; Sakai 1998; Kapela and Lasker 1999; Beiring and Lasker 2000; Santangelo et al. 2003;

Gutiérrez-Rodríguez and Lasker 2004; Tsounis et al. 2006). Newly-settled corals are susceptible to high mortality rates (Babcock 1991; Lasker et al. 1998), and are believed to allocate their resources to growth instead of reproduction in order to grow rapidly out of the size classes that are most vulnerable. In *A. arbuscula*, all colonies collected were reproductive, so a minimum size at first reproduction could not be determined. The smallest female collected was 3.4 cm in height and contained 200 oocytes, whereas the smallest male was 3.0 cm in height and contained 17 spermatic cysts, suggesting that sexual maturity in *A. arbuscula* occurs when colony size is less than ~3 cm. However, these two colonies did not contain mature oocytes or spermatic cysts, suggesting they may have just reached sexual maturity. A colony that contained no gametes was 7.0 cm in height, however, it was collected from the deepest site (1861 m), which may explain its infertility (see Chapter 3). Deep-water corals may not have the selective pressures to grow quickly out of the smaller size classes, which could explain why the smallest *A. arbuscula* colonies were reproductive. The few studies which have examined growth in deep-water corals have generally documented slow growth rates in comparison to shallow, zooxanthellate corals (Gladfelter et al. 1978; Bak 1983; Huston 1985; Yoshioka and Yoshioka 1991; Andrews et al. 2002; Roark et al. 2006; Sherwood and Edinger 2009; Hamel et al. 2010). This study did not sample the full size range of *A. arbuscula*, and colonies smaller than 3 cm must be examined in order to determine their reproductive status and actual size at first reproduction.

The positive relationship between polyp fecundity and colony height, and gamete diameter and colony height observed in both female and male *A. arbuscula* (Fig. 2.12) colonies is common among shallow-water corals (Coma et al. 1995a; Kapela and Lasker 1999; Beiring and Lasker 2000; Tsounis et al. 2006), although the relationship was

statistically significant only for female polyp fecundity by height (Fig. 2.12a) in the current study. Increasing fecundity with colony size is thought to occur in one of two ways. First, as a coral colony grows more branches and polyps are formed, increasing the total fecundity of the colony. Second, gamete production per polyp increases with growth in some corals (Babcock 1991; Coma et al. 1995a), which is thought to be caused by a shift in resource allocation from growth to reproduction as the colony matures (Connell 1973; Kojis and Quinn 1981). If the organism is highly branched, reproductive output may increase exponentially with growth (Coma et al. 1995a; Beiring and Lasker 2000; Tsounis et al. 2006), causing a disproportionate contribution of the largest colonies to gamete production in a population (Coma et al. 1995a; Beiring and Lasker 2000).

Some corals experience reproductive senescence with age, as indicated by decreased polyp fecundity among the largest colonies (Kapela and Lasker 1999). As a colony grows, the number of interior branches greatly increases, which have less access to the water column. Kim and Lasker (1998) noted a decrease in resource availability to inner modules due to active feeding and/or passive deflection by modules on the periphery of colonial corals, which may be responsible for the reproductive senescence observed in some corals. Whether reproductive senescence occurs in *A. arbuscula* remains unclear. Flow would be extensively reduced to inner modules of large colonies of this species (Kim and Lasker 1998), which are highly branched with little space between branches. The majority of female colonies 10 cm and greater have similar mean polyp fecundity levels (Fig. 2.12a), suggesting that polyp fecundity stabilizes at this height.

2.45. Conclusion

In conclusion, the reproductive traits of the deep-water gorgonian *A. arbuscula* are similar to those observed in tropical, shallow- and deep-water octocorals. *A. arbuscula* is gonochoristic with a sex ratio not significantly different from 1:1. This species appears to have overlapping periodic or seasonal cycles of gametogenesis, and the absence of embryos and planulae within the polyps suggests that *A. arbuscula* is a broadcast spawner. In contrast to many tropical gorgonians, polyp fecundity was highest among the distal polyps, which may be caused by a combination of factors, such as colony morphology and low predation pressure compared to shallow water habitats.

Although height at first reproduction is small (<3 cm), the axial growth rate of *A. arbuscula* is low (>0.30 cm/year^{-1}, Sherwood and Edinger 2009), suggesting it may take many years of growth to reach reproductive maturity. This study has shown that the relationship between colony height and mean polyp fecundity is positive and significant, with larger colonies producing more oocytes than smaller ones. Thus, it may take many years to reach a size that ensures high reproductive success (Torrents et al. 2005). These characteristics, along with possible infrequent spawning events, suggest that *A. arbuscula* may have a low potential to recover from disturbance. However, the mean polyp fecundity of this species is quite high compared to other deep-water gorgonians, suggesting that this species may experience high reproductive success in general. Dense patches of this coral have been observed in certain areas of the Northwest Atlantic (Beazley 2008), which may also enhance the fertilization success of a sessile gonochoristic species with a 1:1 sex ratio (Pires et al. 2009). These features, along with the probable lecithotrophic larval development and broadcast spawning reproductive

mode, may allow for the wide dispersal and settlement of *A. arbuscula* across the North Atlantic.

Chapter 3. Spatial and Depth Variability in Reproduction of *Acanella arbuscula*

3.1. Introduction

The development and spawning of gametes or planulae in corals occurs either continually, periodically, or seasonally, and may or may not be synchronized between members of the same population. In sessile gonochoristic corals, synchronous spawning of gametes into the water column is important to maximize fertilization success (Oliver and Babcock 1992), and brief, synchronous spawning events are common among shallow-living, reef-building species (Kojis and Quinn 1982; Harrison et al. 1984; Babcock et al. 1986; Harrison and Wallace 1990).

In shallow waters, environmental factors can be responsible for the synchronous development and spawning of gametes in corals. For example, temperature, photoperiod, lunar phase, wind intensity, tidal cycle, rainfall, and food supply have been linked to the timing of gametogenesis and/or spawning of shallow-water corals (Kojis and Quinn 1982; Shelsinger and Loya 1985; Babcock et al. 1986; Farrant 1986; Harrison and Wallace 1990; Richmond and Hunter 1990; Mendes and Woodley 2002; van Woesik 2010). These factors may control reproduction on various temporal scales. Babcock et al. (1986) suggested that sea surface temperature controls the time of year when corals spawn on the Great Barrier Reef, the lunar and tidal cycles control the time of month, and the diurnal light cycle controls the time of day. Although these factors are well established as proximate cues to reproduction (Oliver et al. 1988), much less is known of the ultimate evolutionary forces driving synchronous gametogenesis and spawning (van Woesik 2010).

Many studies on the reproduction of shallow, tropical corals have documented variability in the reproductive traits of the same species located in different latitudinal and geographic locations (Hartnoll 1975; Kojis and Quinn 1984; Kojis 1986; Rinkevich and Loya 1987; Babcock et al. 1994; Fan and Dai 1995; Tsounis et al. 2006; Gori et al. 2007). Commonly, populations of the same species display shifts in the sex ratio (Soong 1991; Santangelo et al. 2003; Tsounis et al. 2006; Gori et al. 2007), timing of gamete or planula release, and fecundity (Richmond and Hunter 1990; Benayahu 1991; Sier and Olive 1994; Fan and Dai 1995; Kruger et al. 1998) between different locations, however, oocyte size (Sier and Olive 1994; Fan and Dai 1995; Tsounis et al. 2006) and colony size at sexual maturity (Hartnoll 1975; Fan and Dai 1995) may also differ between localities. For instance, Gori et al. (2007) noted differences in the timing of gamete release in the gorgonians *Paramuricea clavata* and *Eunicella singularis* between the Medes Islands and Cape of Palos populations, which coincided with the increase in sea water temperature and food supply in each area. Fan and Dai (1995) noted greater oocyte diameters in the scleractinian *Echinopora lamellosa* from Yenliao Bay compared to Nanwan Bay in northern and southern Taiwan, respectively, and attributed this to an increased investment in larval survivorship in response to unfavourable environmental conditions at Yenliao Bay. Thus, differences in the reproductive traits within the same species may represent adaptations to the environmental conditions at a particular location.

Besides a spatial influence, reproductive characteristics within a species of coral may also differ between different depths in the same location (Grigg 1977; Benayahu and Loya 1983; Kojis and Quinn 1984; Rinkevich and Loya 1987; Tsounis et al. 2006). For instance, Rinkevich and Loya (1987) found that shallow-living colonies (5 m) of the scleractinian *Stylophora pistillata* produced up to 5 times more female gonads per polyp

and released 5 to 20 times more planula larvae than colonies of the same species living in deeper waters (25 to 45 m). Similarly, Tsounis et al. (2006) found significantly larger gonad diameters in a shallow-living (18 m) population of the red coral *Corallium rubrum* in the Mediterranean compared to deeper-living (40 m) colonies, and attributed this pattern to depth-staggered spawning induced by high temperature gradients that occur in the summer. Grigg (1977) also reported a time delay in the spawning of the gorgonians *Muricea californica* and *M. fruticosa* in deeper waters of California due to differences in the timing of peak temperatures at depth. Grigg (1977) suggested that several months of warming are required to complete gametogenesis, which was experienced later in the year in deeper waters.

The decrease in fecundity and planulation with increasing depth observed in some tropical corals may be caused by decreased light at increasing depths and a reduction of the amount of energy available for reproduction. Energy derived from symbiotic zooxanthellae during photosynthesis is allocated to reproduction in shallow corals (Rinkevich and Loya 1983), and a significant amount of the carbon fixed by zooxanthellae may be lost during planulation in brooding species (Rinkevich 1989). McCloskey and Muscatine (1984) found that zooxanthellae of the Red Sea coral *Stylophora pistillata* fixed less carbon at 35 m depth compared to 3 m, and also transferred less carbon to the host coral tissues at the greater depth. These results highlight the importance of light and the amount energy transfer to the reproductive processes of shallow corals.

With the exception of hydrothermal vent areas and the Red and Mediterranean Seas, temperature and salinity are thought to vary little in bathyal and abyssal environments (Tyler 1988; Gage and Tyler 1991), and areas below 1000 m are unlikely to

experience any light (Tyler 1988). The seemingly stable environment of the deep-sea has previously led to many hypotheses regarding the reproductive processes of its inhabitants. For instance, Orton (1920) predicted that temperature controlled reproduction, and that reproduction of deep-sea species would be continuous due to the lack of seasonal variation in temperature as experienced in shallow waters (coined as Orton's rule by Thorson (1946)). Another prediction of deep-sea reproduction was known as Thorson's rule (Mileikovsky 1971), which predicted that deep-sea species will have low fecundity and a brooding reproductive strategy. However, these hypotheses have since been disproven by subsequent studies which have documented seasonality in reproduction (Lightfoot et al. 1979; Tyler et al. 1982; Tyler et al. 1990; Van-Praet 1990; Van-Praet et al. 1990; Mercier and Hamel 2009), high fecundity (Gage and Tyler 1982; Waller et al. 2008), broadcast spawning (Mercier and Hamel 2009), and pelagic larval development (Gage and Tyler 1982; Tyler et al. 1990; Pearse 1994) in deep-sea organisms. We now know that the deep-sea environment is more complex than previously believed and is subjected to various disturbances such as diurnal tidal variation, seasonal variation in ocean currents, turbidity currents and benthic storms (Billett et al. 1983; Tyler 1988, Gage and Tyler 1991; Weaver and Thomson 1993; Scheltema 1994), which may affect the reproductive processes of the benthic community living there.

Although the majority of deep-sea benthic invertebrates are reported to have continuous reproductive cycles (Tyler 1988; Young 2003), seasonality of reproduction has been noted in some deep-sea benthic organisms, the timing of which is often related to the seasonal sinking of the spring phytoplankton bloom (Tyler et al. 1982; Tyler et al. 1990; Van-Praet 1990; Van-Praet et al. 1990; Lawson 1991; Scheltema 1994; Tyler et al. 1994; Waller and Tyler 2005; Mercier and Hamel 2009; Sun et al. 2010b; Mercier et al.

2010). In deep-water anthozoans, maximum phytoplankton or phytodetritus abundance has been linked to the onset of gametogenesis (Lawson 1991; Waller and Tyler 2005) and the timing of spawning or planula release (Lawson 1991; Mercier and Hamel 2009; Sun et al. 2010a; Sun et al. 2010b; Mercier et al. 2010; Waller and Tyler 2010). For instance, Lawson (1991) observed a seasonal pattern of gamete development in the gorgonian coral *Acanella arbuscula* from the Rockall Trough, NE Atlantic, and related this to the sinking of organic material from the surface. Lawson (1991) believed that the timing of rapid spermary growth and presence of large oocytes in June was related to the arrival of the spring phytoplankton bloom to the deep-sea (late May through August). Lawson (1991) suggested that planulae or newly-settled polyps (planula release thought to occur in May, but not confirmed) may benefit from the seasonal input of material from the surface. Nonetheless, the link between seasonal reproduction and the sinking of surface-derived organic material is highly speculative, and currently there is no evidence directly relating seasonal reproduction of the mega- and macrobenthos in the deep-sea to the seasonal sinking of organic material (Eckelbarger and Watling 1995). Eckelbarger and Watling (1995) stated that the seasonal sinking of phytodetritus may represent the proximate cue to seasonal reproduction in the deep-sea benthos, however, because the reproductive capability of a given species is phylogenetically constrained, its response to that cue will reflect its phylogenetic history. This hypothesis explains why all organisms do not exhibit a seasonal pattern despite similar environmental cues. However, the pattern observed in shallow corals where the reproductive characteristics within a species differ between localities (Hartnoll 1975; Kojis and Quinn 1984; Kojis 1986; Rinkevich and Loya 1987; Babcock et al. 1994; Fan and Dai 1995; Tsounis et al. 2006; Gori et al. 2007) suggests a strong response to local environmental conditions.

The goals of my study were to 1) determine whether spatial variability existed in the main features of reproduction (i.e. sexuality, sex ratio, mode of reproduction, polyp fecundity, gamete size, colony size at first reproduction) of the deep-water gorgonian coral *Acanella arbuscula* collected from two areas in the Northwest Atlantic, and 2) determine whether polyp fecundity, gamete size, and the percentage of mature oocytes differed along a depth gradient, as shown in other shallow- and deep-water corals (Rinkevich and Loya 1987; Waller et al. 2002; Tsounis et al. 2006; Flint et al. 2007; Mercier et al. 2010; Waller and Tyler 2010). Characteristics of *A. arbuscula*'s reproductive biology examined in Chapter 2 are re-examined and compared between sampling locations in this chapter. Clues to the timing and duration of gametogenesis and potential spawning in relation to certain environmental conditions were only briefly discussed due to the poor temporal resolution of the collections in this study.

This study is important for the management and conservation of deep-water corals. Corals that show spatial variability in their reproductive characteristics may require individual management strategies based on their location. Destructive fishing practices such as trawling and dredging are moving into deeper habitats and threatening the deep-water corals living there, a consequence which is not fully understood. Species that show variability in their reproductive characteristics with depth, especially fecundity, are at greater risk, as shallower populations may be removed by human activities, leaving deeper populations without the capacity to re-populate. This is the first study aiming to directly compare the reproduction of a deep-water gorgonian coral between two geographically distant locations and along a depth gradient deeper than 200 m.

3.2. Materials and Methods

3.21. Study Areas and Sample Collection

A. arbuscula colonies were collected from two areas in the Northwest Atlantic: The Gully Marine Protected Area (MPA) located on the Scotian shelf, and the Flemish Cap region off Newfoundland, Canada (Table 3.1). The Gully is located approximately 40 km east of Sable Island on the edge of the Scotian shelf. It is the largest submarine canyon on the eastern seaboard of North America, being more than 70 km long and 20 km wide, with depths reaching upwards of 2700 m in the main canyon (Fader and Strang 2002). Nine feeder canyons and channels extend from the Sable Island Bank into the western flank of The Gully. The seabed consists of both hard and soft sediments, with shallow areas composed of sediments ranging from silty sand to gravel and winnowed till (Gordon and Fenton 2001), with the deepest portion, the thalweg, composed of sand and mud sediments (Fader and Strang 2002). Water currents and their interaction within The Gully are greatly influenced by its unique formation and steep topography. The Labrador Current reaches and mixes with the Nova Scotia Current flowing out of the Gulf of St. Lawrence at the Laurentian Channel, moving cooler waters in a south westerly direction along the Scotian shelf. Part of these waters are veered into The Gully, and flow in along the eastern side and out along the western side, creating a partial gyre near the surface that is present in the summer, fall, and winter (Rutherford and Breeze 2002). This circulation pattern is thought to aid in the local retention of nutrients, fuelling greater primary productivity in The Gully than on the adjacent shelf (Strain and Yeats 2005). Near-bottom currents are thought to carry weakly suspended material, such as plankton

and marine snow, from the surrounding banks and trough of The Gully down into deeper regions of the canyon. Despite the apparent retention of material in The Gully, the submarine canyon does not appear to have much greater phytoplankton biomass (as measured by chlorophyll concentration) than other areas on the Scotian Shelf as a whole (Head and Harrison 1998; Kepkay et al. 2001), which, as suggested by Strain and Yeats (2005), may be due to a reduction in phytoplankton biomass caused by the greater abundance of organisms in high trophic levels.

The Flemish Cap is a shallow region located 600 kilometres east of Newfoundland. It is separated from the Grand Banks by a rift zone called the Flemish Pass. On the Cap, depth ranges from approximately 125 to 700 (Stein 2007). A steep slope exists at the southern tip of the Cap, and the slope off the western part of the Cap near the Flemish Pass reaches depths upwards of 1100 m (Stein 2007). Circulation on and around the Flemish Cap is dominated by two major currents: the Labrador Current and the North Atlantic Current. The Labrador Current brings cold (3-4°C) and low salinity (34-35) waters from the north to the south through the Flemish Pass and to the east and southeast around the northern and eastern slopes of the Cap (Colbourne and Foote 2000). The North Atlantic Current transports relatively warm (>4°C), high salinity (>34.8) waters along the southeast slope of the Grand Banks and Flemish Cap and to the northeast (Colbourne and Foote 2000). These two water masses create an anticyclonic gyre directly on the Cap, trapping water with elevated temperatures and dissolved inorganic nutrients, creating the potential to host elevated primary and secondary production on the Cap (Maillet et al. 2005). Despite the higher concentrations of nutrients on the Cap, phytoplankton biomass is largely confined to the adjacent shelf during the spring, however, biomass is higher in the summer and autumn months on the Flemish Cap

Table 3.1 Collection details of *A. arbuscula* colonies collected between 2007 and 2010 from The Gully and Flemish Cap

Number of colonies	Cruise/Dive ID	Area	Gear	Depth (m)	Temperature (°C)	Coordinates	Date collected
1	HUD025/R1056	The Gully	ROPOS	1861	4.52	43° 40′ 30.2″ N -58° 49′ 20.6″ W	09/07/2007
1	HUD025/R1060	The Gully	ROPOS	1630	4.83	43° 49′ 49.9″ N -58° 55′ 33.1″W	12/07/2007
1	HUD025/R1060	The Gully	ROPOS	1630	4.83	43° 49′ 49.9″ N -58° 55′ 33.1″ W	12/07/2007
1	Miguel Oliver/DR2	Flemish Cap	Dredge	671-739	3.76 (at 753 m depth)	48° 13′ 13.4″ N -44° 25′ 15.9″W	29/05/2009
3	Miguel Oliver /DR8	Flemish Cap	Dredge	700-701	3.75 (at 691 m depth)	48° 3′ 27.0″ N -44° 12′ 0.6″ W	03/06/2009
1	Miguel Oliver /DR9	Flemish Cap	Dredge	864-861	3.62 (at 915 m depth)	48° 5′ 41.3″ N -44° 8′ 45.8″ W	04/06/2009
4	Miguel Oliver /DR20	Flemish Cap	Dredge	1122-1113	3.59 (at 1120 m depth)	47° 4′ 20.4″ N -43° 26′ 56.9″ W	15/06/2009
3	Miguel Oliver /DR21	Flemish Cap	Dredge	870	3.74 (at 849 m depth)	46° 50′ 45.8″ N -43° 43′ 3.5″ W	16/06/2009

Number of colonies	Cruise/Dive ID	Area	Gear	Depth (m)	Temperature (°C)	Coordinates	Date collected
3	Miguel Oliver /DR23	Flemish Cap	Dredge	1127-1108	3.77 (at 1150 m depth)	46˚ 46′ 29.5″ N -43˚ 51′ 54.4″ W	18/06/2009
1	Miguel Oliver /DR56	Flemish Cap	Dredge	795-712	4.21 (at 682 m depth)	46˚ 38′ 49.4″ N -46˚ 28′ 39.9″ W	18/08/2009
1	Miguel Oliver /BC17	Flemish Cap	Corer	1264	3.53 (at 1246 m depth)	48˚ 12′ 31.9″ N -44˚ 0′ 29.9″ W	04/06/2009
1	HUD029/R1347	The Gully	ROPOS	1112	4.35	43˚ 58′ 5.7″ N -59˚ 0′ 13.2″ W	27/07/2010
1	HUD029/R1347	The Gully	ROPOS	914	4.40	43˚ 58′ 10.0″ N -59˚ 0′ 27.8″ W	27/07/2010
1	HUD029/R1347	The Gully	ROPOS	914	4.39	43˚ 58′ 9.9″ N -59˚ 0′ 27.9″ W	27/07/2010
1	HUD029/R1347	The Gully	ROPOS	1099	4.17	43˚ 58′ 5.9″ N -59˚ 0′ 14.1″ W	27/07/2010

and slope waters than on the Grand Banks (Maillet et al. 2005). Surface blooms on the Flemish Cap and slope waters generally form in early March and extend well into June and July (Maillet et al. 2005).

Colonies of *A. arbuscula* were collected from The Gully MPA during a research cruise on the C.C.G.S. Hudson in July 2007. The remotely operated vehicle ROPOS was deployed, and colonies were collected using the mechanical arm of the ROV at depths between 1630 and 1861 m. In May through August 2009 *A. arbuscula* colonies were collected through a series of benthic surveys conducted by Spain on the eastern and southwestern slope of the Flemish Cap. There, colonies were collected between depths of 670 and 1264 m using both a rock dredge and box corer. In July 2010 The Gully was revisited, and colonies were collected using ROPOS between depths of 914 to 1112 m. In order to acquire information on *A. arbuscula*'s reproduction from shallower depths where it was not collected in 2007, a specific depth range (between 500 and 1500 m) was targeted to collect *A. arbuscula* from The Gully in 2010.

Colonies collected during the 2007 ROPOS mission to The Gully were fixed in 10% seawater-buffered formalin for several months and were later transferred to 70% ethanol for long-term storage. Colonies collected from all other missions were fixed in 10% seawater-buffered formalin for 24 to 48 hours, and were then transferred to 70% ethanol.

3.22. Histological Preparation and Examination

Reproductive tissue was prepared for examination using standard histological techniques (Kiernan 1999; Etnoyer et al. 2006). Fifteen randomly-chosen *A. arbuscula* polyps were dissected from randomly-chosen branches of a colony and decalcified using

a solution of 10% hydrochloric acid and EDTA for approximately 2 to 3 hours, or until no calcareous material remained. Tissues were then dehydrated through a series of graded alcohol concentrations and cleared using xylene. Polyps were embedded in paraffin wax and longitudinally-sectioned 5 µm thick using a rotary microtome. Ribbons were mounted on slides and stained using Harris' hematoxylin and eosin. Slides were examined using a Nikon E-800 Eclipse microscope and oocytes and spermatic cysts were followed through their serial sections and photographed using mounted Nikon Digital Eclipse DXM 1200 and Nikon DS-Ri1 cameras when they were at their largest size, which may or may not have corresponded to when the nucleus was bisected in the oocytes. The number of gametes per polyp was counted, and the maximum diameter of each gamete was measured using Image Pro Plus software, version 5.1.

3.23. Environmental Characteristics of each Study Area

Temperature data were collected every second using three temperature probes attached to ROPOS in 2007. The average temperature of the three probes at the GMT (Greenwich Mean Time) when *A. arbuscula* was collected was calculated. In 2010, temperature data were collected every second by ROPOS using a pumping CTD (Conductivity, Temperature, Depth) sensor (SBE 19plus, Sea-Bird) attached to the ROV. The temperature corresponding to the GMT time when a sample was collected was extracted. During the Spain surveys in 2009, temperature data were collected 8 times/second using a Sea-Bird CTD (SBE 25) at the end of each dredge or box core. Temperature for the deepest depth of each CTD cast was used.

Sea surface phytoplankton biomass, as measured by chlorophyll *a* concentration, was estimated from January to December from years 1998 to 2004 for both The Gully

and Flemish Cap regions using the Ocean Colour Database (OCDB) provided by The Department of Fisheries and Oceans, Canada: http://www2.mar.dfo-mpo.gc.ca/science/ocean/database/data_query.html. This database maintains SeaWiFS Local Area Coverage 1.5 x 1.5 km-resolution semi-monthly composites for the North Atlantic from September 2007 to December 2004. The semi-monthly composites (taken on the 15th and last day of each month) were averaged, giving a single chlorophyll *a* concentration for each month. For The Gully, chlorophyll *a* concentration was estimated using the system polygon provided by the database for that region (Scotian Shelf polygon 8). For the Flemish Cap, instead of estimating chlorophyll *a* for the entire NAFO 3 M area, this area was divided into a smaller rectangle to encompass only the area occupied by the Flemish Cap (46.0 to 50.0 N, 47.0 to 42.0 W).

3.24. Statistical Analyses

Deviance from parity in the sex ratio (ratio of males to females) of The Gully and Flemish Cap populations of *A. arbuscula* was individually tested using a chi-square (χ^2) test. Polyp-fecundity values for each of the 15 polyps examined per colony were averaged to get a single polyp fecundity estimate per colony. All gamete diameters were averaged for each polyp to avoid pseudo-replication, and averaged across the 15 polyps per colony, giving a single gamete diameter value per colony. Differences in mean polyp fecundity per colony and mean gamete diameter per polyp per colony between The Gully and Flemish Cap were examined using the following linear model:

$y_{ij} = \mu + \beta_i + \varepsilon_{ij}$,

where y_{ij} is the response (either mean polyp fecundity or mean diameter per polyp per colony), μ is the grand mean, β_i is the effect of sampling location (either The Gully or Flemish Cap) (fixed), ε_{ij} is the experimental error, i= 1, 2, j= 1,...12, for females, and i= 1, 2, j= 1,...11, for males. The overall effect of sampling location on mean polyp fecundity and mean gamete diameter per polyp per colony was assessed using a one-way ANOVA. All datasets were tested for the assumptions of normality and homogeneity of variances using the Shapiro-Wilk analysis of variance test and the Levene's test, respectively. The mean number of fertile and unfertile polyps per colony for female and male colonies was determined for each area, were compared between areas using a one-way ANOVA.

In order to avoid binning colonies into arbitrarily-chosen depth bins, regression models were used to examine the influence of depth on mean polyp fecundity per colony and mean gamete diameter per polyp per colony for females and males. Depth for the dredge collections made on the Flemish Cap in 2009 was calculated by taking the average depth between the beginning and end depth for each dredge. For both female and male colonies, mean polyp fecundity per colony was Log X+1 transformed in order to bring a single outlier closer to the group mean. A regression model was also used to examine the influence of depth on the percentage of mature (stage IV and V) oocytes per colony. Significance for all relationships was determined using Pearson's product-moment correlation once both the dependent and independent variables were examined for normality using the Shapiro-Wilk test. Any non-normal variable was subsequently transformed closer to normality as specified and the relationship tested for significance.

Statistical analyses were conducted using R version 2.10.0 (R Development Core Team, 2009, http://www.R-project.org).

3.3. Results

3.31. Spatial Variability in Reproduction

No differences in the basic reproductive characteristics were observed between the two different *A. arbuscula* populations. Colonies collected from both The Gully and Flemish Cap were gonochoristic at both the polyp and colony level. The sex ratio of the Flemish Cap population was not significantly different from 1:1 (χ^2_1= 0.053, P= 0.819). The sex ratio of The Gully population (2 females from 2007, 2 females and 2 males from 2010) could not be tested for deviance from parity due the small sample size, however, judging from the collections made in 2010, the sex ratio of The Gully population is likely not different from 1:1.

No embryos or planula larvae were observed in colonies collected from either The Gully or Flemish Cap. The mesh collection bags used for the collection of *A. arbuscula* from The Gully in 2010 also did not contain any embryos or planula larvae.

Size at first reproduction could not be determined for either The Gully or Flemish Cap populations of *A. arbuscula*, as all colonies sampled contained gametes. The smallest female and male colonies sampled from The Gully were 5.0 and 7.5 cm in height, respectively, and contained 82 oocytes and 149 spermatic cysts, respectively. The smallest female and male colonies from The Flemish Cap were 3.4 and 3 cm in height, respectively, and contained 200 oocytes and 17 spermatic cysts, respectively.

Mean polyp fecundity per female colony did not significantly differ between The Gully and Flemish Cap (Fig. 3.1a, Table 3.2). Fig. 3.2 shows box plots of the mean polyp fecundity per colony for female colonies collected from The Gully in 2007 and 2010, and the Flemish Cap in 2009. Mean polyp fecundity per colony is similar between colonies collected from The Gully in 2010 and Flemish Cap in 2009, however, mean fecundity is significantly different between colonies collected in 2007 and 2010 from The Gully (One-way ANOVA: $F_{(1,2)}$= 22.839, P= 0.041). However, mean polyp fecundity did not differ significantly among the three years (One-way ANOVA: $F_{(2,9)}$= 1.768, P= 0.225). For male colonies, mean polyp fecundity also did not differ significantly between The Gully and Flemish Cap (Fig. 3.1a, Table 3.3).

Mean oocyte and spermatic cyst diameters per polyp per colony also did not differ significantly between The Gully and Flemish Cap (Tables 3.4 and 3.5, Fig. 3.1b). Fig. 3.3 shows box plots of the mean oocyte diameter per polyp per colony for females collected from The Gully in 2007 and 2010, and the Flemish Cap in 2009. The Gully 2010 collections had the highest mean oocyte diameter per polyp (Fig. 3.3), and colonies from The Gully collected in 2007 had the lowest mean oocyte diameter per polyp. Mean oocyte diameter was significantly different between colonies collected in 2007 and 2010 from The Gully (One-way ANOVA: $F_{(1,2)}$= 92.581, P= 0.011). However, mean oocyte diameter per polyp per colony did not significantly differ among the three years (One-way ANOVA: $F_{(2,9)}$= 1.488, P= 0.277).

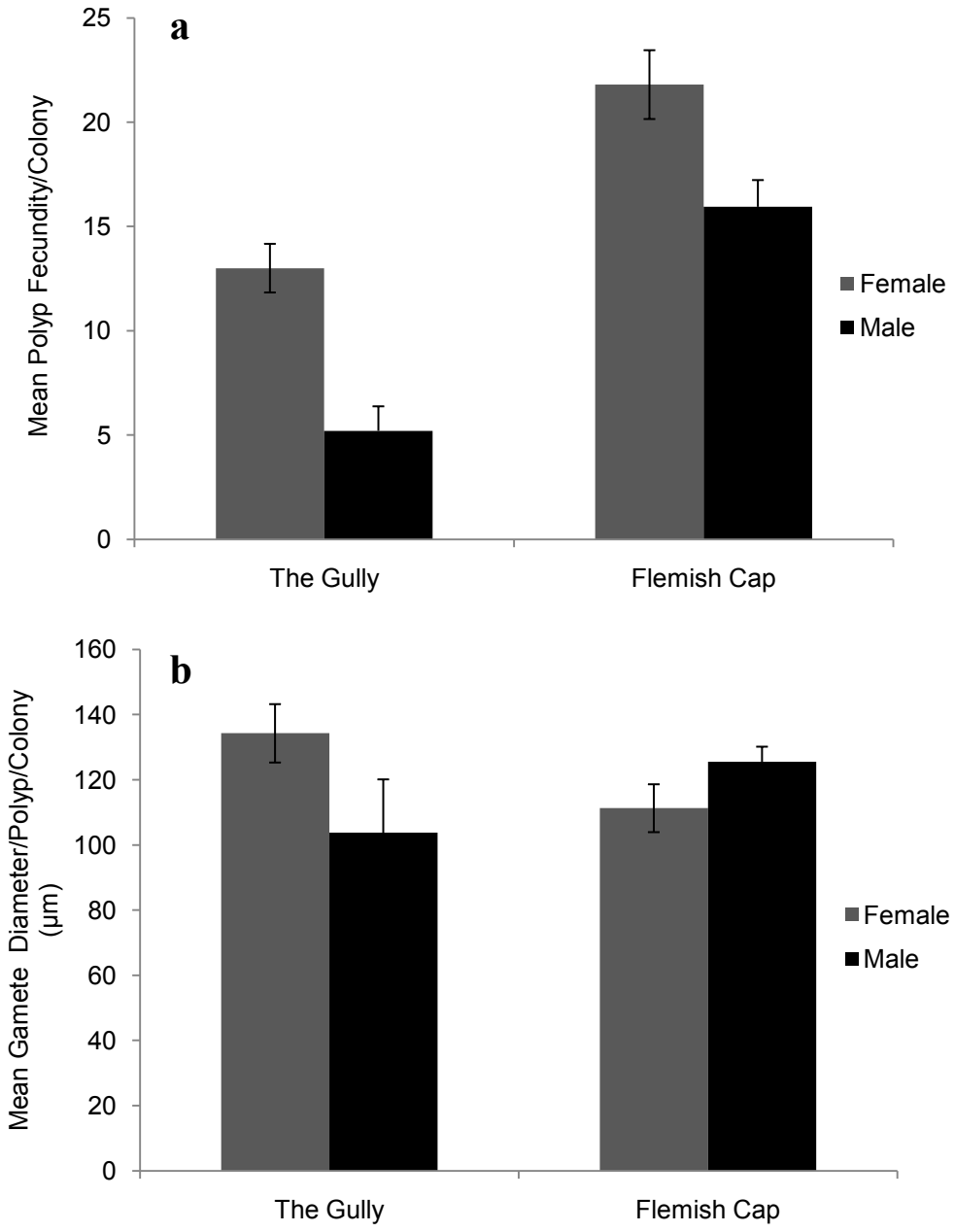

Fig. 3.1 **a** Mean polyp fecundity per colony for female and male *A. arbuscula* colonies collected in The Gully (both 2007 and 2010), and in the Flemish Cap. **b** Mean gamete diameter (µm) per colony for female and male *A. arbuscula* colonies collected in The Gully (both 2007 and 2010) and in the Flemish Cap. Error bars are ± 1 SE

Table 3.2 ANOVA for one-way model testing differences in mean polyp fecundity (not transformed) per polyp per female colony between The Gully and Flemish Cap

Source of Variation	df	Sums of Square	Mean Square	F value	P value
Area	1	204.170	204.17	1.668	0.226
Residual	10	1224.360	122.44		

Table 3.3 ANOVA for one-way model testing differences in mean polyp fecundity (not transformed) per polyp per male colony between The Gully and Flemish Cap

Source of Variation	df	Sums of Square	Mean Square	F value	P value
Area	1	192.440	192.440	1.743	0.219
Residual	9	993.920	110.440		

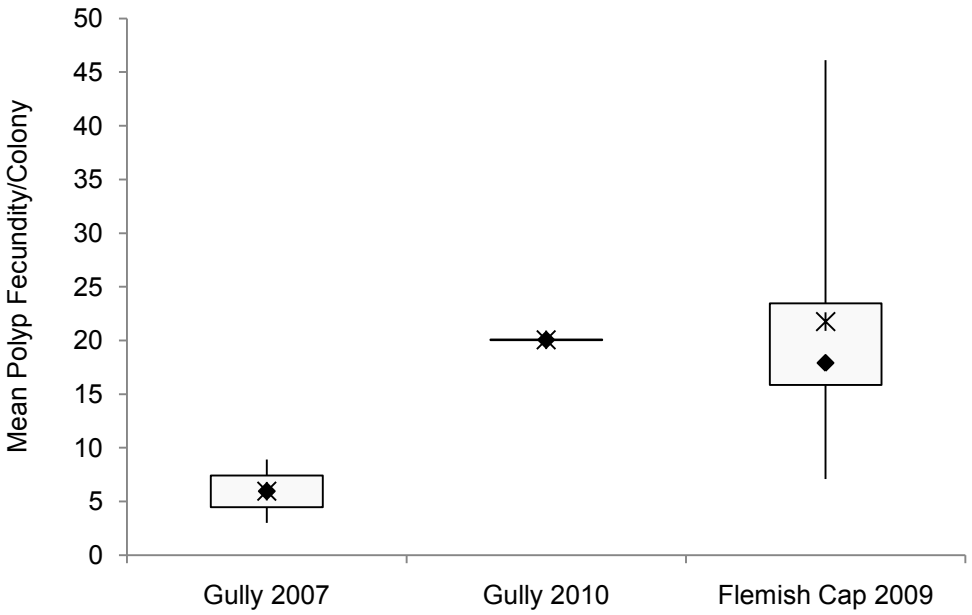

Fig. 3.2 Box plots of mean polyp fecundity per colony for female colonies of *A. arbuscula* collected from The Gully in 2007 (2 colonies) and 2010 (2 colonies), and the Flemish Cap (8 colonies) in 2009. Black diamonds represent the median and stars the mean

Table 3.4 ANOVA for one-way model testing differences in mean oocyte diameter (μm) (not transformed) per polyp per colony between The Gully and Flemish Cap

Source of Variation	df	Sums of Square	Mean Square	F value	P value
Area	1	1409.000	1409.100	0.381	0.551
Residual	10	36972.000	3697.200		

Table 3.5 ANOVA for one-way model testing differences in mean spermatic cyst diameter (μm) (not transformed) per polyp per colony between The Gully and Flemish Cap

Source of Variation	df	Sums of Square	Mean Square	F value	P value
Area	1	778.900	778.860	0.496	0.500
Residual	9	14122.000	1569.110		

Fig. 3.3 Box plots of mean oocyte diameter (µm) per polyp per colony for female colonies of *A. arbuscula* collected from The Gully in 2007 (2 colonies) and 2010 (2 colonies), and the Flemish Cap (8 colonies) in 2009. Black diamonds represent the median and stars the mean

The mean number of fertile polyps per female colony was high for both The Gully and Flemish Cap (Table 3.6), and was not significantly different between the two areas ($F_{(1,11)}$= 0.233, P= 0.639). Male colonies collected from The Gully had similar mean fertile and unfertile polyps per colony, whereas the Flemish Cap colonies had a high mean number of fertile polyps per colony. However, no significant differences were detected between the mean number of fertile polyps in male colonies between The Gully and Flemish Cap (One-way ANOVA: $F_{(1,9)}$= 2.363, P= 0.159).

The oocyte size-frequency distribution of colonies collected from the Flemish Cap in June displayed a bimodal pattern, with a single large mode of oocytes ≤400 μm in diameter, and small mode of oocytes ranging from 401 to 750 μm in diameter (Fig. 3.4). Colonies collected from The Gully in July also displayed a bimodal pattern, with a single large mode of oocytes ≤400 μm in diameter, and a smaller mode of oocytes ranging in size from 500 to 650 μm (Fig. 3.4). The frequency of the larger mode of oocytes collected from The Gully was greater than that of the larger mode from the Flemish Cap, however, the largest oocyte was from a colony collected from the Flemish Cap.

Male colonies collected from the Flemish Cap in May and June also displayed a bimodal pattern in their spermatic cyst size-frequency distributions, with one mode of small (≤100 μm) spermatic cysts and one mode of larger cysts ranging from 101 to 350 μm in May, and 101 to 500 μm in June (Fig. 3.5). In the June samples the single mode of larger spermatic cysts displayed a prominent right-skewed pattern with larger spermatic cysts compared to the single large mode in the May samples. The size-frequency distribution of colonies collected from The Gully in July also displayed a bimodal pattern, with a large mode of small (≤100 μm) cysts and a small mode of larger cysts ranging from 150 to 400 μm in diameter (Fig. 3.5). The smaller mode of large cysts had a more

Table 3.6 Mean number of fertile and unfertile polyps per colony for female and male colonies collected in The Gully (both 2007 and 2010) and Flemish Cap

	Mean Number of Fertile Polyps per Colony			
	Female		Male	
Area	**Fertile**	**Infertile**	**Fertile**	**Infertile**
The Gully	14	1	9.5	5.5
Flemish Cap	13	2	13.78	1.22

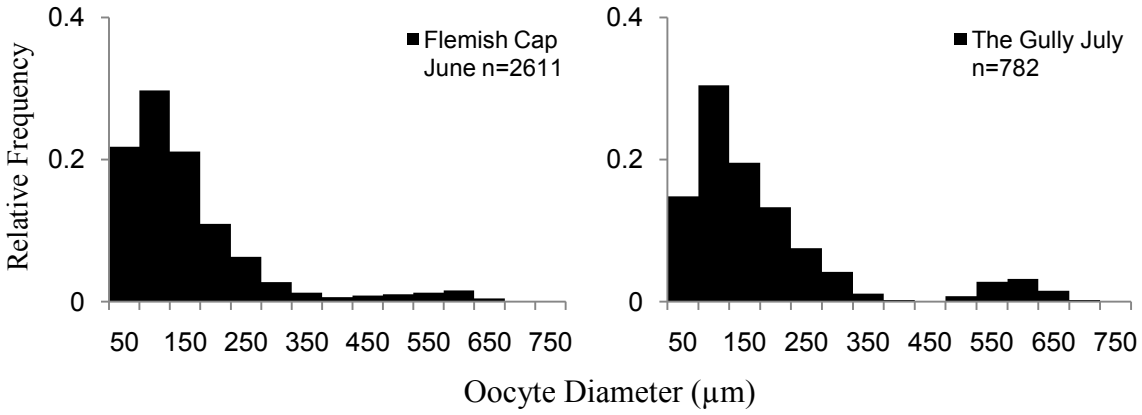

Fig. 3.4 Oocyte size-frequency distributions of *A. arbuscula* colonies collected in the Flemish Cap (June) and The Gully (both July 2007 and 2010). n= number of oocytes

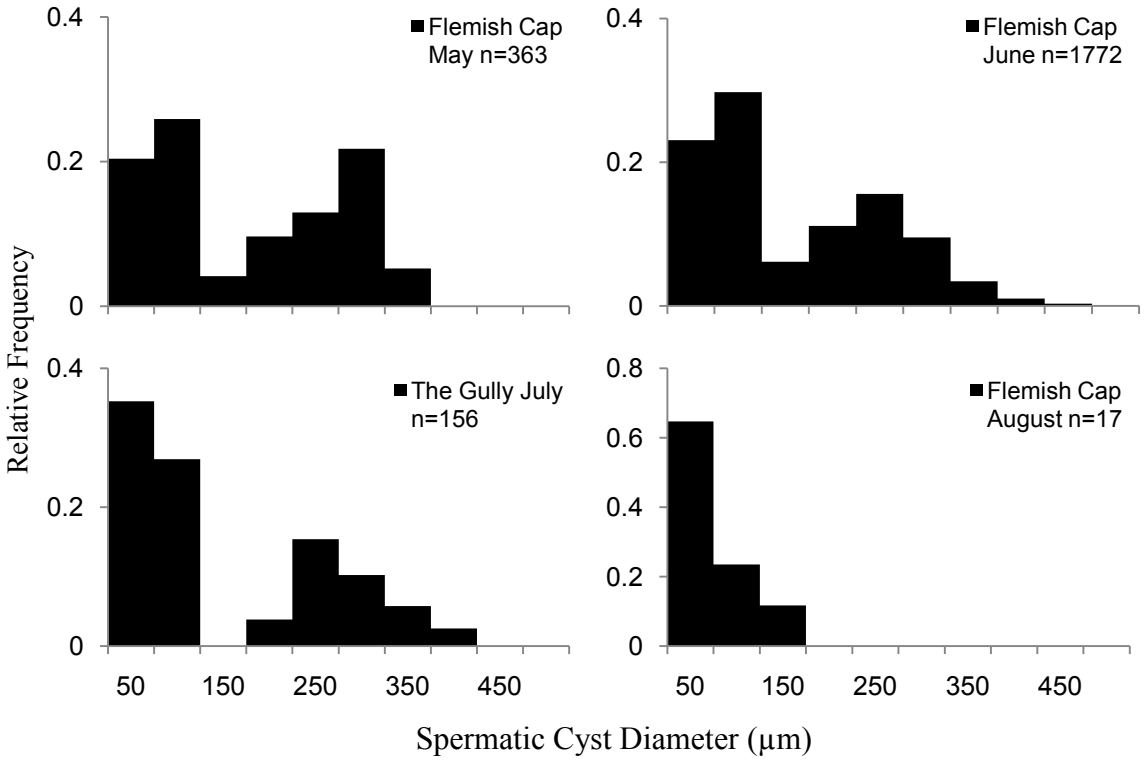

Fig. 3.5 Spermatic cyst size-frequency distributions of *A. arbuscula* colonies collected in the Flemish Cap (May, June, August) and The Gully (July 2010). n= number of spermatic cysts

prominent right-skewed pattern compared to the large mode of smaller cysts in the same distribution. A single August collection from the Flemish Cap revealed a single mode of small (≤150 μm) spermatic cysts (Fig. 3.5).

3.32. Depth Variability in Reproduction

Depth had a significant effect on the mean polyp fecundity per colony in female colonies (Fig. 3.6a, t_{10}= -2.509, P= 0.031). In females, depth explained approximately 39% of the variation in mean polyp fecundity per colony. At 1118 m depth, colonies from the Flemish Cap displayed great variability in mean polyp fecundity, however, colonies collected from The Gully in 2010 at the same depth (two from 914 m) had similar mean polyp fecundities. At depths of 1118 m and above, mean polyp fecundity per colony was similar between The Gully 2010 and Flemish Cap 2009 at similar depths.

In male colonies, depth did not have a significant effect on mean polyp fecundity per colony (Fig. 3.6b, t_9= -4.021, P= 0.697; R^2=0.018). Colonies collected from The Gully in 2010 showed great variability in mean polyp fecundity despite being collected from similar depths (1099 and 1112 m). At similar depths, mean polyp fecundity per colony was similar between colonies collected from The Gully and Flemish Cap, with the exception of two colonies, one from The Gully (1112 m) and one from Flemish Cap (754 m), which showed much lower mean polyp fecundity values compared to other colonies collected from similar depths. The colony with the highest mean polyp fecundity was collected at 870 m depth from the Flemish Cap. Depth did not have a significant effect on mean oocyte diameter per polyp per colony in females (Fig. 3.7a, t_{10}= -0.887, P= 0.396; R^2= 0.073). Colonies collected in the same year and at the same depth from The Gully

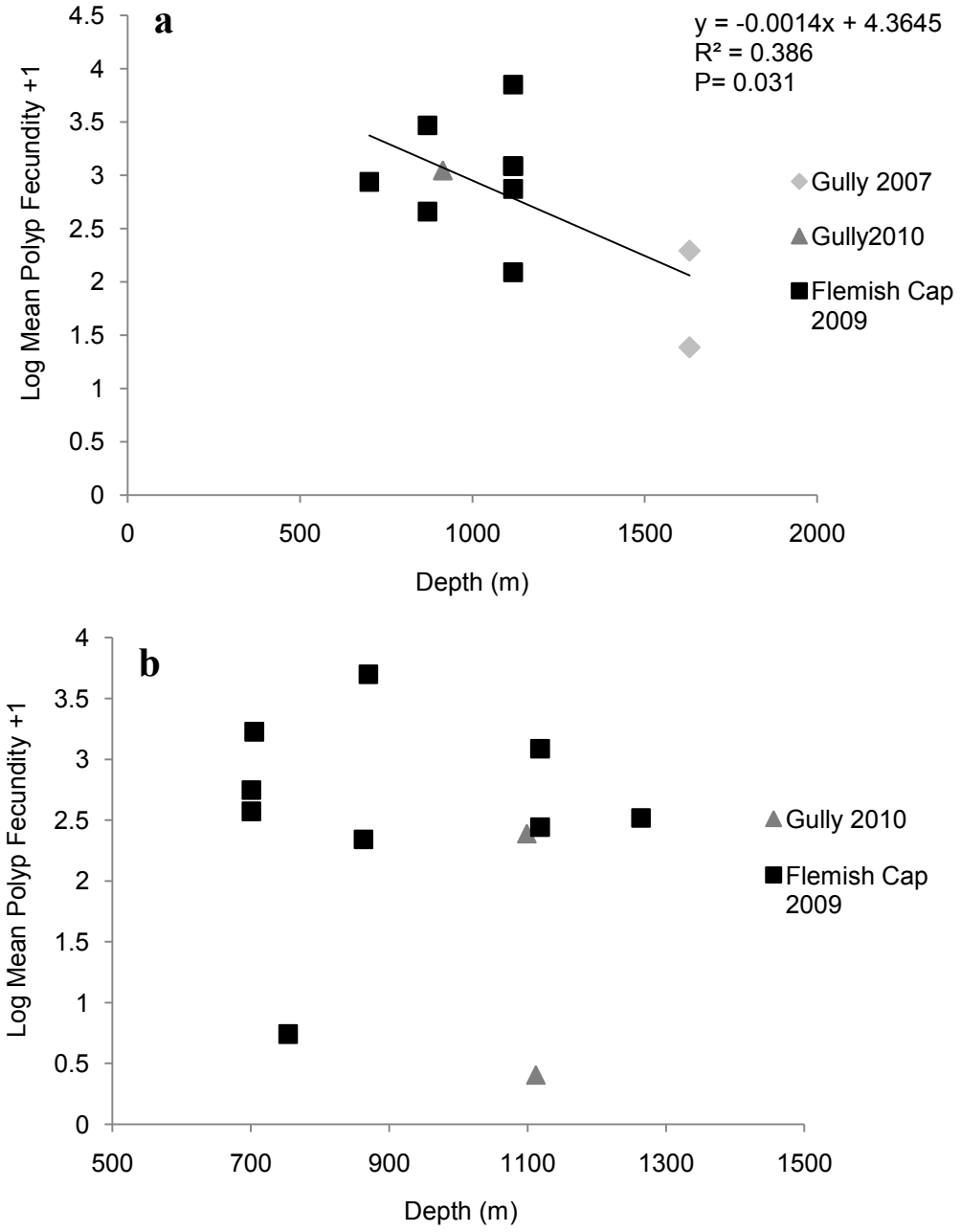

Fig. 3.6 **a** Log +1 mean polyp fecundity per colony for female *A. arbuscula* colonies collected in The Gully in 2007 and 2010, and in the Flemish Cap. **b** Log +1 mean polyp fecundity for male *A. arbuscula* colonies collected in The Gully in 2007 and Flemish Cap in 2009

had very similar mean oocyte diameters per polyp, however, colonies collected from the Flemish Cap at similar depths showed great variability in mean oocyte diameters.

Depth also did not have a significant effect on the mean spermatic cyst diameter per polyp per colony (Fig. 3.7b, t_9= -0.379, P= 0.714; R^2= 0.016). Unlike in females, mean spermatic cyst diameter per polyp was variable between the two colonies collected at the same depth from The Gully. Mean spermatic cyst diameter per polyp per colony was also variable at similar depths in colonies collected from the Flemish Cap. The largest mean spermatic cyst diameter per polyp was observed from a colony collected at 701 m depth from the Flemish Cap.

Depth did not have a significant effect on the percentage of mature oocytes (stage IV and V) (Fig. 3.8, t_{10}= -0.714, P= 0.492; R^2= 0.049). Colonies collected from 1118 and 1630 m showed great variability in the percentage of mature oocytes per colony. Three colonies collected from a wide depth range (870, 1118, and 1630 m) did not contain mature oocytes. A colony collected from 1118 m depth contained the highest percentage of mature oocytes, although multiple colonies collected between 870 and 914 m contained high and similar percentages of mature oocytes.

3.33. Comparison of Sea Surface Chlorophyll *a* Concentration between Areas

In The Gully, surface chlorophyll *a* concentration began to increase in February and reached a maximum (2.2 mg m^{-3}) during the month of April (Fig. 3.9). A smaller peak (1.2 mg m^{-3}) occurred again during the month of October. In the Flemish Cap area, surface chlorophyll *a* also began to increase in March, although the increase was more gradual than in The Gully. Chlorophyll *a* concentration reached a peak during the month

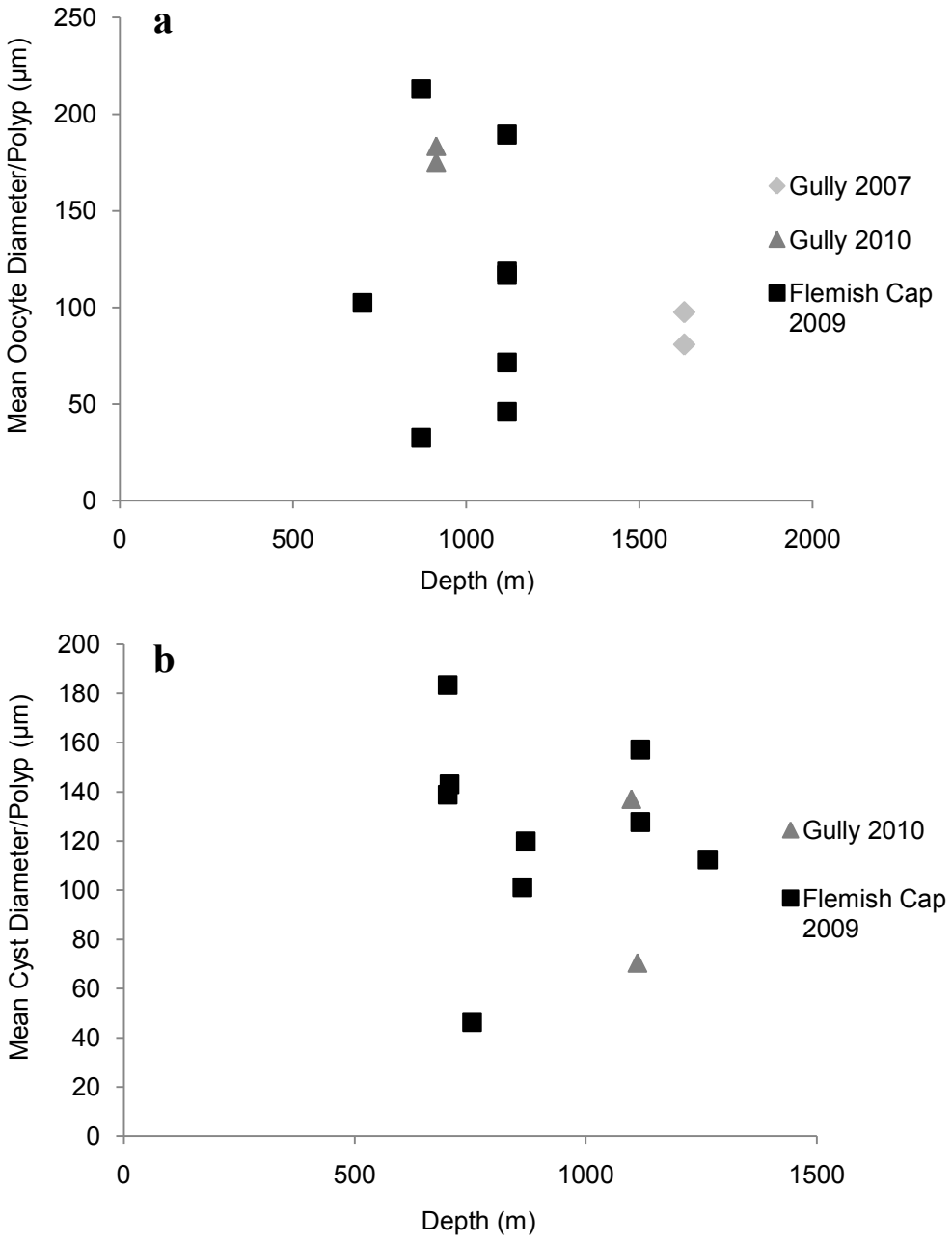

Fig. 3.7 **a** Mean oocyte diameter (µm) per polyp per colony for female *A. arbuscula* colonies collected in The Gully in 2007 and 2010, and in the Flemish Cap as a function of depth (m). **b** Mean sperm cyst diameter (µm) per polyp per colony for male *A. arbuscula* colonies collected in The Gully and Flemish Cap as a function of depth (m)

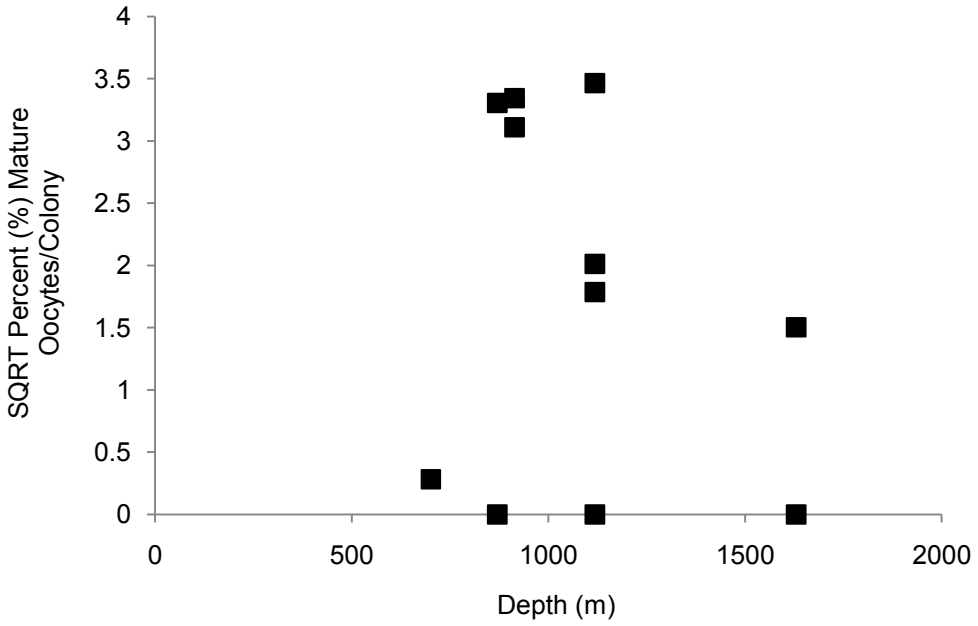

Fig. 3.8 Percent (%) of mature (Stage 4 and Stage 5) oocytes (square root transformed) per colony as a function of depth (m)

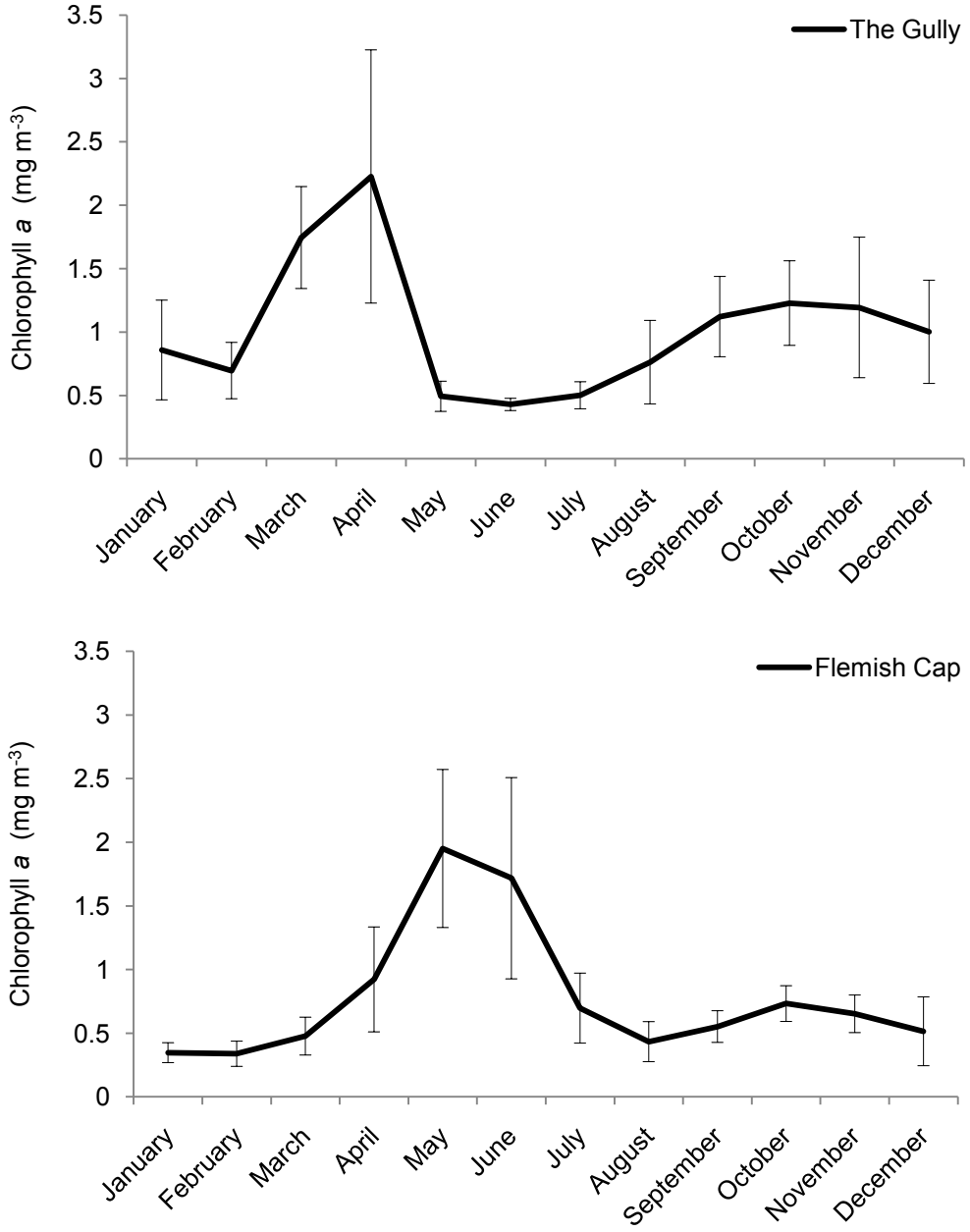

Fig. 3.9 Mean monthly surface chlorophyll *a* (mg m^{-3}) concentration in The Gully and Flemish Cap from 1998 to 2004. Error bars are ± 1 SD

of May (2.0 mg m^{-3}), and remained high for the month of June (1.7 mg m^{-3}). A second peak (0.7 mg m^{-3}) occurred during the month of October in this area.

3.4. Discussion and Conclusion

3.41. Spatial Variability in Reproduction

Reproductive characteristics of shallow-water corals, such as the sex ratio, timing of gametogenesis, the release of gametes or planulae, polyp fecundity, and gamete size have been shown to vary within the same species located in different areas, and this variability is most often related to differences in local environmental conditions. The results of this study did not show any variability in the reproductive characteristics examined between populations of *A. arbuscula* from The Gully MPA on the Scotian Shelf and the Flemish Cap area off Newfoundland, two areas located approximately 1200 km apart.

In corals, sexuality (i.e. hermaphroditism or gonochorism) is commonly reported to vary in members of the same genus (see Hartnoll 1977, Soong 1991) located in different geographic areas, but rarely within the same species (see Benayahu et al. 1990). In the present study, no variability in the sexuality of *A. arbuscula* was found between colonies from The Gully and the Flemish Cap, and colonies from both areas were gonochoristic at both the polyp and colony level. It is possible that corals which have shown different patterns of sexuality in different locations are sequential hermaphrodites that appear gonochoristic (Harrison and Wallace 1990). With the large total sample size and wide size range of the colonies examined, sequential hermaphroditism is unlikely in *A. arbuscula* from these two areas.

The 1:1 sex ratio observed in the Flemish Cap population of *A. arbuscula* represents the stable sex ratio of sessile gonochoristic organisms (Ribes et al. 2007) and is common among both tropical and deep-water octocorals (Brazeau and Lasker 1990; Kruger et al. 1998; Ben-Yosef and Benayahu 1999; Orejas et al. 2002; Santengelo et al. 2003; Gutiérrez-Rodríguez and Lasker 2004; Hwang and Song 2007; Orejas et al. 2007; Edwards and Moore 2008; Pires et al. 2009). Despite the inability to calculate deviance from parity in the sex ratio of The Gully population, the collections made in this area in 2010 (2 females and 2 males) suggest that the sex ratio is also not different from 1:1. Tsounis et al. (2006) suggested that differences in the sex ratio of *Corallium rubrum* between Costa Brava, Spain (1:1) and the Calafuria coast, Italy (1:1.37, from Santangelo et al. 2003) may be explained by different population densities and thus larval recruitment strategies between the areas. Based on this hypothesis, population density and larval recruitment may be similar between populations from The Gully and Flemish Cap.

In the Scleractinia, both brooding and broadcast spawning have been documented within the same population and in distantly-located populations of the same species (Harrison and Wallace 1990; Sakai 1997; Nishikawa and Sakai 2003). However, Harrison and Wallace (1990) suggested that some reports of both brooding and broadcast spawning within a species may be incorrect and have likely resulted from taxonomic misidentification, abnormal conditions, or misinterpretation. Ward (1992) reported both asexual brooding via parthenogenesis and broadcast spawning in individuals of *Pocillopora damicornis* in Western Australia, but noted that brooding was more prevalent than broadcasting on reefs that experienced greater disturbance. Alternatively, Glynn et al. (1991) found that this species only broadcasts gametes in the Eastern Pacific. Both brooding and broadcast spawning have been reported in deep-water corals (Cordes et al.

2001; Brooke and Young 2003; Orejas et al. 2007; Mercier et al. 2010). However, the few studies which have examined the reproductive biology of the same species in different localities have not documented different modes of reproduction (Waller et al. 2002; Flint et al. 2007; Mercier et al. 2010; Waller and Tyler 2010). In the present study, no embryos or planula larvae were found in any polyps and colonies collected from either The Gully or Flemish Cap, which suggests *A. arbuscula* has a broadcast spawning mode of reproduction in both locations. Lawson (1991) predicted that *A. arbuscula* from Station 'M' in the Northeast Atlantic brooded planula larvae, however, no larvae were observed in any of the colonies. If *A. arbuscula* is in fact a brooder in the Northeast Atlantic, perhaps the levels of disturbance are greater in this location than in The Gully and Flemish Cap, and therefore brooding young would be advantageous for larval survivorship (Harrison and Wallace 1990).

All colonies collected from both The Gully and Flemish Cap were fertile, and so size at first reproduction for each population could not be determined. Smaller colonies were collected from the Flemish Cap compared to The Gully, which was likely due to the different collection gear used in each area and the greater amount of samples collected from the Flemish Cap. For instance, the rock dredge used in the Flemish Cap region in 2009 likely collected everything in its path, including small colonies of *A. arbuscula*, however, small colonies of this species are hard to detect and target for collection when watching video of the seabed (as in 2007 and 2010 in The Gully using ROPOS; personal observation). Fan and Dai (1995) found that colony size at sexual maturity in the scleractinian *Echinopora lamellosa* was greater (8.8 cm in height) in the Yenliao Bay population in Northern Taiwan compared to Nanwan Bay in Southern Taiwan (3.5 cm). The authors also noted a slower growth rate in small colonies from Yenliao Bay, and

hypothesized that the larger size at maturity of this population may be due to the unfavourable environmental conditions in the area, such as lower sea temperature and light intensity, and lack of suitable substrate, causing this population to allocate resources to growth instead of reproduction in early years. In contrast, Hartnoll (1975) noted spatial variability in the size at maturity in the alcyonacean *Alcyonium digitatum* between two areas near the Isle of Man, Irish Sea, and attributed the greater size at first reproduction in one population to the more favourable conditions and higher growth rates at that site.

Despite the larger sample size and wider size range of colonies collected from the Flemish Cap, mean polyp fecundity and mean gamete diameter per polyp for both female and male colonies of *A. arbuscula* did not differ between the two locations (Tables 3.2 through 3.5). The mean number of fertile polyps per colony also did not differ for females and males between the two areas (Table 3.6). Tsounis et al. (2006) also reported no differences in both female and male gonad numbers and sperm sac diameters in *Corallium rubrum* between sampling stations in the Costa Brava, and concluded that reproductive variability was negligible in populations of one geographic region that have similar colony size structure and population density. However, Tsounis et al. (2006) predicted that on a larger geographic scale, colony growth rate, which may vary on larger geographic scales due to natural or anthropogenic influences, may differentially affect the reproductive output of different populations.

Photosynthetically-derived energy is allocated to reproduction in tropical, zooxanthellate corals (Rinkevich and Loya 1983), and in shallow habitats, corals may produce more gametes or planula larvae compared to those in deeper waters (Rinkevich 1989; Harland et al. 1992). In the deep-sea, corals completely lack symbiotic zooxanthellae and must rely solely on heterotrophic feeding. In this environment,

differential reproductive output in the same species from different locations may be due to differences in the nutritional state of the coral, which in turn is related to the amount of resources available at a particular location (Gori et al. 2007). The partial counter-clockwise gyre that is present near the surface of The Gully in the summer, fall and winter traps nutrients in the surface waters, increasing the primary productivity there. A large phytoplankton bloom occurs in the spring months in this area, and reaches its peak concentration in April (Fig. 3.9). As the phytoplankton deplete nutrients in the surface layers the bloom sinks to deeper, more nutrient-rich waters in late spring, and is replaced by smaller species of phytoplankton at the surface in the summer (Rutherford and Breeze 2002). The sinking of the phytoplankton bloom, along with the downward flow of bottom waters from The Gully trough and surrounding banks deep into the canyon bring food resources to the benthic community. The Flemish Cap has a comparable current regime to The Gully in that the Labrador and North Atlantic Currents create an anticyclonic gyre on the Cap, entraining waters with elevated temperatures and inorganic nutrients, which are thought to increase primary and secondary production on the Cap compared to the surrounding Grand Banks (Maillet et al. 2005). Phytoplankton begin increasing in abundance in February and reach peak abundance later in the year compared to The Gully (in May; Fig. 3.9). The maximum concentration of surface chlorophyll *a* is similar between The Gully and Flemish Cap (Fig. 3.9). The elevated nutrients that occur in both areas year-round, and the similar concentrations of phytoplankton (as indicated by surface chlorophyll *a*) may provide the benthic community with similar levels of resources at comparable depths, which would explain why no differences in mean polyp fecundity and gamete diameters were observed between the two populations of *A. arbuscula*. It would be interesting to compare the reproductive traits of *A. arbuscula* from this study to

populations located in shelf areas in the Northwest Atlantic which may not experience high primary and secondary production as in The Gully and Flemish Cap.

Both temperature (Mercier and Hamel 2009; Mercier et al. 2010; Sun et al. 2010b) and the sinking of the spring phytoplankton bloom (Van-Praet 1990; Van-Praet et al. 1990; Lawson 1991; Waller and Tyler 2005) have been linked to the onset of gametogenesis and spawning of deep-water anthozoans. For example, the release of oocytes in the deep-water scleractinian *Flabellum angulare* from the Northwest Atlantic coincided with rising seawater temperatures and peak phytoplankton/phytodetritus abundance, and initiation of gametogenesis corresponded to a smaller peak in productivity that occurred during August to September (Mercier et al. 2010). Sun et al. (2010b) suggested that the rise in temperature may synchronize the reproductive cycle of the deep-water alcyonacean *Drifa glomerata*, and observed peak planulation during times of elevated food availability. The timing of initiation and the cycle of gametogenesis and spawning of *A. arbuscula* from both The Gully and Flemish Cap remains unclear. The bimodal pattern of the oocyte size-frequency distributions of colonies from both the Flemish Cap (in June) and The Gully (in July) (Fig. 3.4) suggests overlapping periodic or prolonged seasonal cycles of oogenesis (Benayahu and Loya 1986; Benayahu 1989; Brazeau and Lasker 1989; Van-Praet 1990; Van-Praet et al. 1990; Coma et al. 1995b; Kruger et al. 1998; Orejas et al. 2007; Ribes et al. 2007; Edwards and Moore 2008). Unfortunately, samples were not collected in the same months from both areas, making it difficult to compare the cycle of oogenesis between them. *A. arbuscula* from the Northeast Atlantic maintains a pool of pre-vitellogenic oocytes throughout the year, with maturation and brooding of only a small portion of that pool (Lawson 1991). The increase in large oocytes in colonies collected from July in The Gully may indicate that maturation

of the second mode has occurred from June to July. The spring phytoplankton bloom occurs later in the Flemish Cap area than in The Gully, and would likely reach the bottom in the summer months as opposed to late spring in The Gully (Rutherford and Breeze 2002). No female colonies were collected from this area in July or August, and so it is unknown whether the larger cohort is still present during those months (absence may indicate a spawning event).

Male colonies collected during May and June from the Flemish Cap, and from The Gully in July also displayed a bimodal pattern. The single cohort of spermatic cysts in the male colony collected in August from the Flemish Cap suggests that a spawning event may have occurred between June and August, which would approximately coincide with when the phytoplankton bloom reaches the seabed. However, this colony is small (3 cm in height), which may also explain why the second mode of larger cysts was absent from the colony (see Chapter 2).

Only one mature spermatic cyst with spermatozoa was observed in a colony collected in May (from Chapter 2), not in later months, which may indicate that spawning occurred between May and June. However, maturation and spawning of spermatic cysts may have occurred rapidly (Harrison and Wallace 1990) in *A. arbuscula*, which could explain why no mature cysts were observed in colonies collected past May.

Without laboratory experiments it is impossible to determine the effects of temperature on the reproductive traits and timing of reproduction in the two *A. arbuscula* populations. Bottom temperature close to the point of collection was similar for colonies collected from The Gully and Flemish Cap (Table 3.1), however, temperatures were slightly higher in The Gully overall, even at deeper depths. Consistent, monthly sampling coupled with laboratory experiments on live colonies is required to determine the cycle of

gametogenesis and spawning of *A. arbuscula*, and whether these cycles are influenced by environmental factors such as temperature.

3.42. Depth Variability in Reproduction

Approximately 1% of open ocean primary production reaches the seabed between 4000 and 5000 m depth, compared to the nearly 50% of coastal production that reaches the seabed in coastal/continental shelf areas (Walsh et al. 1981). As corals experience trade-offs between reproduction and growth/regeneration (Rinkevich 1996), it is expected that as resources decrease with increasing depth, the reproductive output of a species may also decrease. Decreasing fecundity with increasing depth has been noted in several studies of deep-water corals (Waller et al. 2002; Flint et al. 2007; Mercier et al. 2010; Waller and Tyler 2010), however, comparisons were made not only between different depths, but also between different geographic locations. For instance, Flint et al. (2007) noted that the scleractinian *Fungiacyanthus marenzelleri* from Station 'M' in the Northeast Pacific at 4100 m depth had a mean potential fecundity approximately half of that noted in the same species from 2100 m depth from Station 'M' in the Northeast Atlantic (Waller et al. 2002). Flint et al. (2007) hypothesized that the lower fecundity at 4100 m may have been caused by reduced food availability at the greater depth. However, the spatial scale at which these two studies were conducted was large, and the different fecundity values may be caused by different environmental characteristics experienced at each site.

In the present study, polyp fecundity showed a significant decreasing trend with increasing depth in female colonies of *A. arbuscula*, but not in male colonies (Fig. 3.6a, b). A colony collected from The Gully in 2007 at 1861 m depth did not contain any

gametes, which suggests a recent spawning event may have occurred, or that gametogenesis was not occurring during the time of collection at these depths. Gamete diameters of both sexes, and the percentage of mature oocytes (Fig. 3.8) also did not show trends with depth (Fig. 3.7a, b). The differential response of polyp fecundity between the sexes to changes in depth may be explained by different energy requirements to produce oocytes and sperm. For instance, the production of lipid-rich oocytes is thought to be more energetically costly than the production of sperm in marine invertebrates (Hughes and Hall 1996; Ramirez Llodra 2002). Thus, females may be more sensitive to decreasing food availability with depth than males.

Within The Gully, female colonies collected in 2007 and 2010 displayed significant differences in mean polyp fecundity and oocyte diameters (Fig. 3.2 and 3.3). However, colonies collected in 2007 were collected from deeper depths than those in 2010 (Fig. 3.6a), which may be responsible for the lower fecundity and smaller oocyte diameters compared to 2010.

Variability in mean polyp fecundity and gamete diameters in colonies collected at similar depths from the Flemish Cap may be explained by variability in the size of the colonies. Colony height explained 42% and 12% of the variability in mean polyp fecundity in females and males, respectively in this species (Chapter 2). Height was similar between colonies collected in the same year from The Gully (5.0 and 6.7 from 2007, and 11.5 and 12.5 cm in 2010), whereas height was more variable between colonies collected from the Flemish Cap at 1118 m (ranging from 4.3 to 15.5 cm). The heights of two male colonies collected from The Gully which displayed variability in mean polyp fecundity were 7.5 and 10.0 cm. For where data existed, there was little variation in mean polyp fecundity and oocyte diameters per polyp in females between The Gully and

Flemish Cap collected from similar depths (Fig. 3.6a and 3.7a), which further emphasizes no differences in the reproductive biology between the two populations of *A. arbuscula* at these depths.

3.43. Conclusion

This study did not find any spatial variability in the reproductive characteristics between The Gully and Flemish Cap populations of the deep-water gorgonian *A. arbuscula*. Similar environmental conditions between The Gully and Flemish Cap may be responsible for the similar reproductive traits between the two populations. However, this study does not suggest that the reproductive biology of this species is the same in all geographic locations. Future studies should examine the reproductive biology of this species on shelf areas in the Northwest Atlantic which are thought to experience different levels of inorganic nutrients and thus phytoplankton levels than The Gully and Flemish Cap, as the availability of resources may significantly impact female reproductive output (as suggested in the depth analysis).

The significant, decreasing relationship between polyp fecundity and depth in female colonies suggests that this species may be more vulnerable to destructive fishing practices in shallower waters. However, the relationship between depth and reproduction must be further examined with more samples collected over a large depth gradient. With the depletion of shallow fish stocks and increasing fishing effort being displaced into deeper waters, bottom fishing may be removing colonies that have the highest fecundities and are thus contributing the most to the population. Thus, this species may have a reduced capacity to re-populate shallow populations removed by fishing gear.

Chapter 4. Conclusion

4.1. General Conclusion

The seemingly stable environment of the deep-sea encouraged many hypotheses regarding the life-history characteristics of its inhabitants. One theory predicted that the deep-sea benthos would exhibit life history traits closely resembling K-selected species from shallow waters (Young 2003). However, life history traits of deep-sea organisms are diverse and span across the r/K selection continuum; some species show long life expectancy, low adult mortality, and low rates of fecundity and recruitment, or high rates of growth, high fecundity and recruitment, and short life spans, while others display attributes characteristic of both r- and K-selection (Gage 1994; Levin et al. 1994; Eckelbarger and Watling 1995). However, where comparisons of closely related taxa are made, those found in the deeper environment tend to have lower fecundities, slower rates of growth and longer life spans (Gage and Tyler 1991; Gage 1994), and the majority of organisms are reported to have asynchronous or continuous cycles of reproduction (Tyler 1988; Young 2003), suggesting adaptations to the deep-sea environment.

Since the formulation of Orton's and Thorson's Rules, both of which predicted uniformity of reproduction in the deep-sea benthos, many studies have documented a diversity of reproductive strategies in the deep-sea, as varied as those observed in shallow waters. Also surprising, was the discovery that the reproductive strategies (e.g., mode of sexual reproduction, gonochorism or hermaphroditism) observed in shallow-water organisms were also employed by deep-water species. As in other deep-sea organisms, no one reproductive strategy is characteristic of deep-water anthozoans; combinations of gonochorism, hermaphroditism, planktotrophy, lecithotrophy, brooding, and broadcast

spawning have all been documented in this group. However, it remains unknown to which degree phylogeny shapes the reproductive strategies of these organisms. For instance, the mode of reproduction (i.e. brooding or broadcast spawning) may vary among members of the same order, as in the Gorgonacea (Table 2.1), however all members of the Order Pennatulacea from both shallow and deep habitats, broadcast spawn their gametes. Within some species, the mode of reproduction has been shown to vary in different locations (Harrison and Wallace 1990), indicating adaptations to local environmental conditions. Characteristics of the reproductive biology of the deep-water gorgonian *A. arbuscula* examined in this thesis are similar to those of other deep-water (see Table 2.1) and shallow, zooxanthellate octocorals. Like all other deep-water octocorals and the majority of deep-water scleractinians studied to date, *A. arbuscula* has separate male and female colonies. Similarly, sex ratios not different from 1:1, and probable broadcast spawning have also been reported in other deep-water octocorals. The relatively high polyp fecundity and small size (<3 cm) at first reproduction are indicative of opportunistic r-selected species, however, this species has a slow growth rate (Sherwood and Edinger 2009) and likely long life span, characteristic of K-selection. Thus, the results of this thesis show that certain life history attributes of *A. arbuscula* are indicative of both r- and K-selected organisms, a feature also observed in some shallow-water gorgonians (Grigg 1977). However, the decreasing pattern of polyp fecundity with increasing depth in females suggests constraints on reproduction due to decreased resource availability experienced with increasing depth.

The paucity of information on the life history characteristics of deep-water gorgonians in this region calls for an increase in their research, however, care must be taken when sampling polyps from colonies. In order to avoid removing entire colonies

from the seabed, studies of both shallow- (Ribes et al. 2007) and deep-water (personal observation) gorgonians attempt to remove only a portion of the colony, often the distal tips of branches. Other methods of collection include trawling, however, the majority of branching corals become fragmented from the gear. Many habitat-forming branching corals are abundant in certain areas of the Northwest Atlantic, including *Paragorgia arborea* and *Primnoa resedaeformis*. These species are included by NAFO as indicators and key components of vulnerable marine ecosystems, yet there is little information on their reproductive biology. The depth range of *P. arborea* and *P. resedaeformis* is relatively shallow, ranging from 200 to 1300 m and 150 to 1150 m for each species, respectively (Kenchington et al. 2009), and consequently, both have been caught as bycatch by deep-water fisheries (Breeze et al. 1997; Edinger et al. 2007). I suggest that future studies on gorgonian reproduction should focus on poorly known species such as *P. arborea* and *P. resedaeformis*, and when possible (i.e. using ROVs for collection), test for differences in fecundity along branches and between branch orders. Comparisons between the different colony morphologies (fan-shaped *P. arborea* colonies versus bushy colonies of *A. arbuscula* and *P. resedaeformis*) may provide clues as to whether the pattern of intra-colony variation in fecundity observed in *A. arbuscula* is due to colony morphology or represents an adaptation to the deep-sea environment that is present in some deep-water gorgonians. If only fragments of these corals are analyzed, care must be taken when making inferences of whole colony fecundity.

The biggest limitation to this study was the small temporal resolution of the collections. Samples were collected in the late spring/summer months, which made it impossible to fully describe the cycle of gametogenesis, and to predict when this species releases its gametes. Also, samples were not collected during the same months from both

The Gully and Flemish Cap, making it difficult to compare certain aspects of *A. arbuscula*'s reproductive biology between the areas. Often, the cost of conducting research cruises is expensive, making it difficult to conduct multiple surveys throughout the year. Also, the majority of cruises in Atlantic Canada only operate in the summer months when the weather conditions permit the usage of ROVs and other benthic survey equipment. In Atlantic Canada, benthic trawl surveys are conducted outside of the summer months (usually in March and October), and studies which have successfully collected deep-water corals during these surveys (see Mercier et al. 2010; Sun et al. 2010b) collected species that may not be as susceptible to damage from the equipment (e.g. soft corals and scleractinian cup corals). In the present study, some *A. arbuscula* colonies were collected via trawling in March, however, the samples were too damaged to examine using histology. With all the logistical difficulties in collecting organisms from this environment, any collections that can be made, and information that can be acquired from those collections, will greatly contribute to the overall knowledge of these organisms.

Overall, the results of this study suggest that *A. arbuscula* has high polyp fecundity compared to other deep-water gorgonians, and may have the potential to rapidly re-colonize an area after disturbance via larval dispersal. Currently it remains unknown whether colonies of *A. arbuscula* reproduce via asexual reproduction, but this could represent a means of local recovery from anthropogenic disturbance. Trawling has been shown to damage colonies of the deep-water reef forming coral *Lophelia pertusa*, and fragment them down to a size that is no longer capable of reproducing sexually, but is able to re-populate via asexual fragmentation (Le Goff-Vitry and Rogers 2005; Waller and Tyler 2005). As colonies of *A. arbuscula* are small and somewhat flexible, trawling is

likely to affect them differently than reef-building species, by completely removing them from their habitat or burying them under sediment, instead of creating multiple fragments that are able to regenerate whole colonies.

It is not known how far larvae of *A. arbuscula* can disperse, and whether populations of this species are connected via larval dispersal. Investigation into the genetic connectivity between populations may provide insight into the recoverability of populations of *A. arbuscula* from anthropogenic events. For instance, if genetic exchange exists between populations, then loss of some populations may not compromise the overall genetic diversity of the species (Le Goff-Vitry et al. 2004). Nonetheless, recent studies have shown high levels of inbreeding within subpopulations of other deep-water corals (Le Goff-Vitry et al. 2004; Baco and Shank 2005; Le Goff-Vitry and Rogers 2005), suggesting the prevalence of self-recruitment and restricted gene flow over connectivity. The gyre circulation that exists in both The Gully and Flemish Cap may help retain larvae and prevent dispersal to other areas, promoting self-sustaining populations of *A. arbuscula*. I suggest that future studies on *A. arbuscula* and other deep-water corals should examine the dispersal capabilities, population structure, and level of differentiation between populations in order to create effective policy for their conservation management.

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Appendix A. Interaction Plots and Model Comparison for Factors Colony and Zone

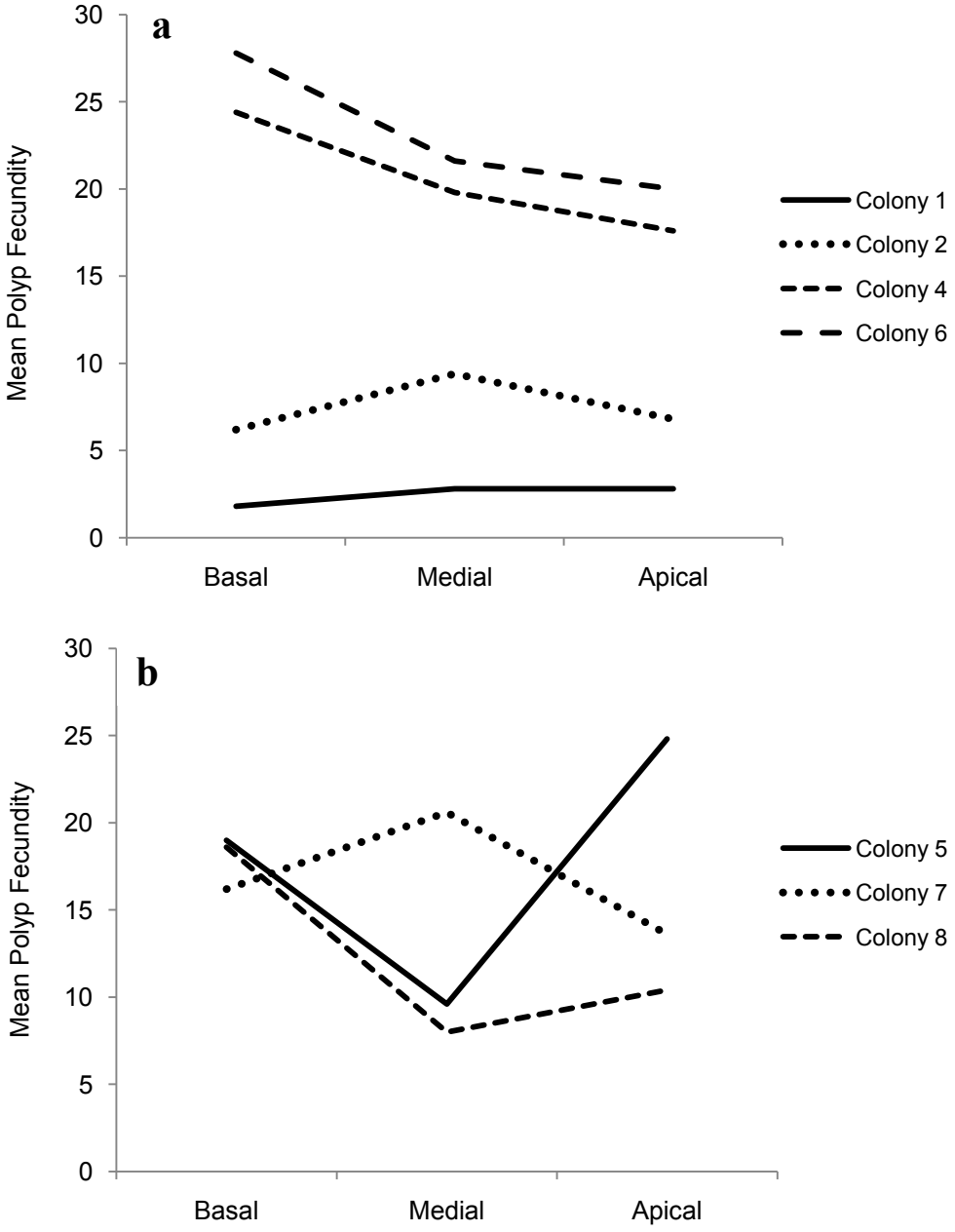

Fig. 1 **a** Interaction plot between factors colony and zone on mean polyp fecundity in female colonies of *Acanella arbuscula*. **b** Interaction plot between factors colony and zone on mean polyp fecundity in male colonies of *Acanella arbuscula*. Potential interactions were deemed present in both **a** and **b** as lines were not parallel

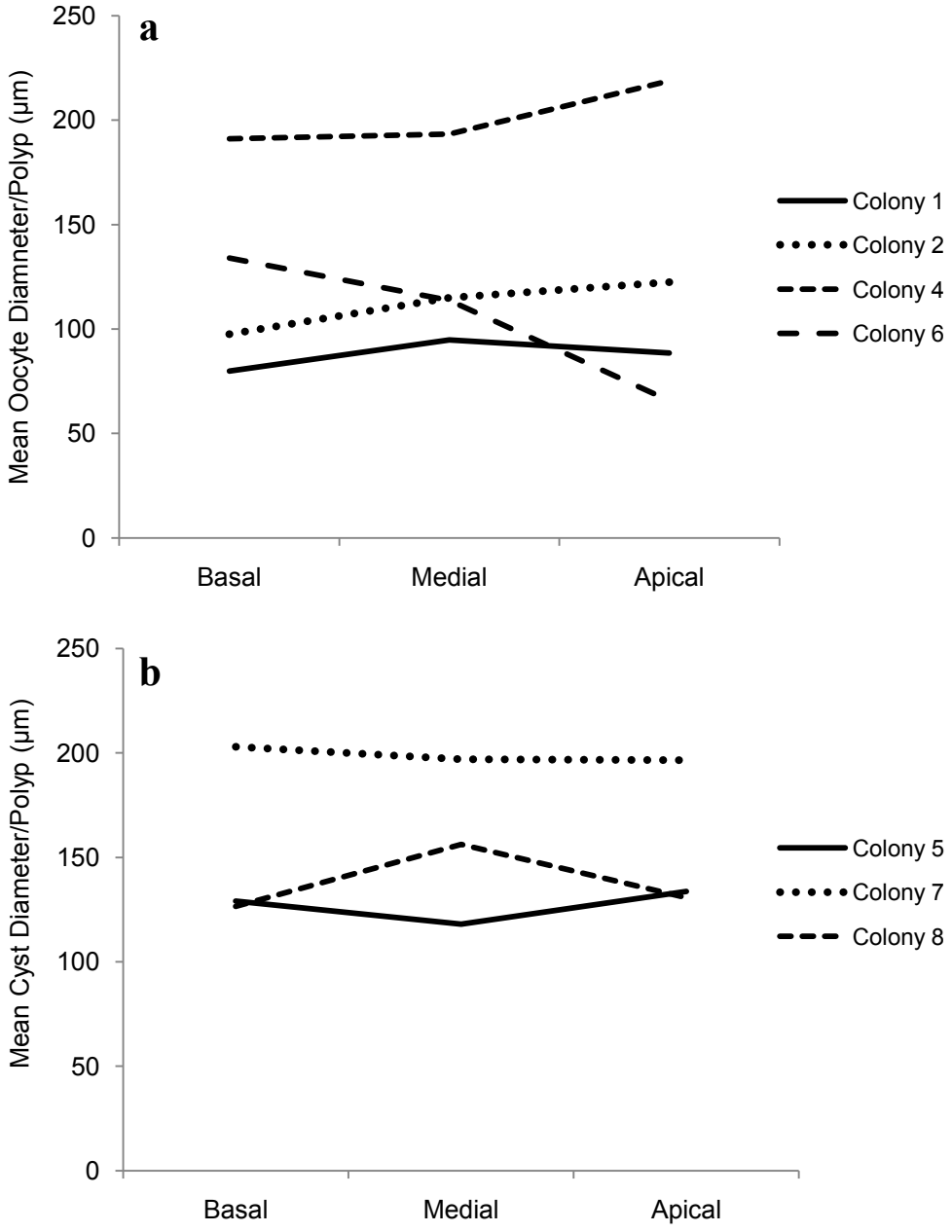

Fig. 2 **a** Interaction plot between factors colony and zone on mean oocyte diameter per polyp in female colonies of *Acanella arbuscula*. **b** Interaction plot between factors colony and zone on mean spermatic cyst diameter in male colonies of *Acanella arbuscula*. Potential interactions were deemed present in both **a** and **b** as lines were not parallel

Appendix A continued

Table 1. Results of the minimum adequate model (MAM) based on a linear mixed model testing the effect of zone on polyp fecundity in female and male colonies of *Acanella arbuscula*. y_{ijk}= response, polyp fecundity, β_i= effect of zone (fixed), b_j= effect of colony (random), b_{ij}= interaction term (random), i= 3, j= 4, k= 5 for females, i= 3, j= 3, k= 5 for males. AIC=Akaike Information Criterion. Based on the AIC values, Model 1 was chosen for both female and male datasets

Females		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	205.698
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	207.698
Males		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	335.356
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	336.431

Table 2. Results of the minimum adequate model (MAM) based on a linear mixed model testing the effect of zone on mean gamete diameter per polyp in female and male colonies of *Acanella arbuscula*. y_{ijk}= response, mean gamete diameter per polyp, β_i= effect of zone (fixed), b_j= effect of colony (random), b_{ij}= interaction term (random), i= 3, j= 4, k= 5 for females, i= 3, j= 3, k= 5 for males. AIC=Akaike Information Criterion. Based on the AIC values, Model 1 was chosen for both female and male datasets

Females		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	528.337
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	527.024
Males		
Model		**AIC**
1	$y_{ijk}= \mu + \beta_i + b_j + \varepsilon_{ijk}$	463.813
2	$y_{ijk}= \mu + \beta_i + b_j + b_{ij} + \varepsilon_{ijk}$	465.813

Appendix B. Interaction Plots for Factors Colony and Branch Segment

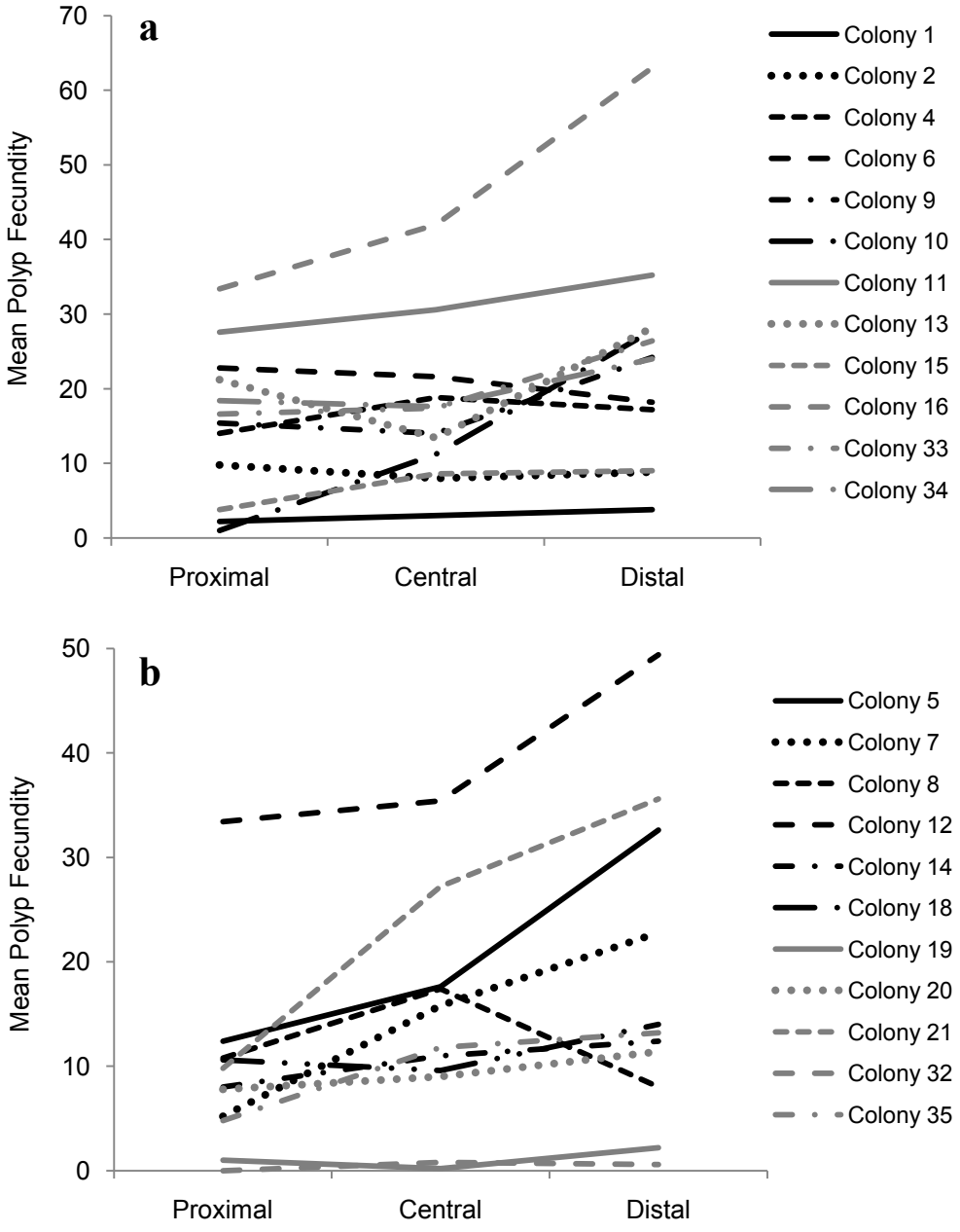

Fig. 1 **a** Interaction plot between factors colony and branch segment on mean polyp fecundity in female colonies of *Acanella arbuscula*. **b** Interaction plot between factors colony and branch segment on mean polyp fecundity in male colonies of *Acanella arbuscula*. Potential interactions were deemed present in both **a** and **b** as lines were not parallel

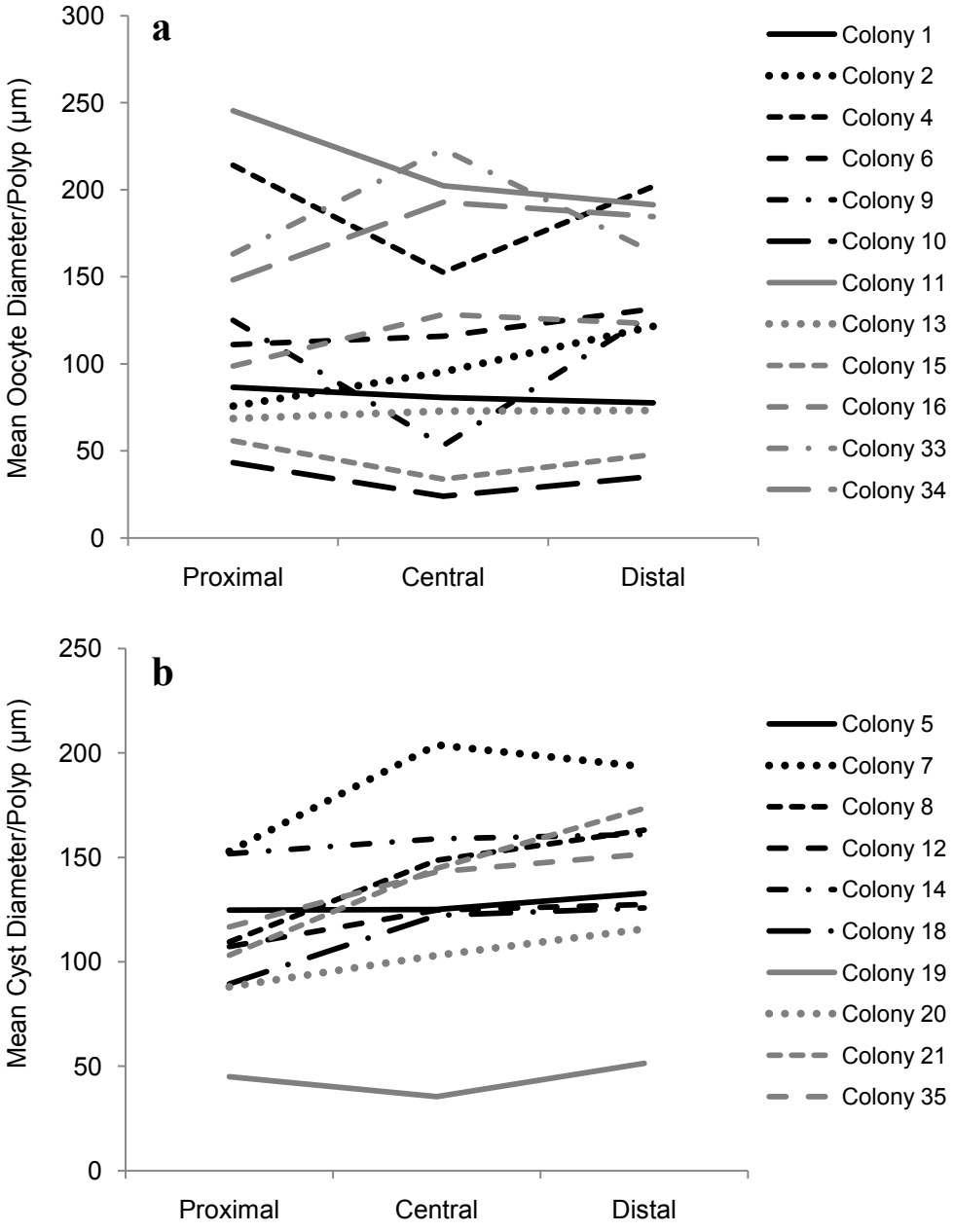

Fig. 2 **a** Interaction plot between factors colony and branch segment on mean oocyte diameter per polyp in female colonies of *Acanella arbuscula*. **b** Interaction plot between factors colony and branch segment on mean spermatic cyst diameter in male colonies of *Acanella arbuscula*. Potential interactions were deemed present in both **a** and **b** as the lines were not parallel