

REPRODUCTIVE BIOLOGY OF DEEP-SEA SOFT CORALS
IN THE NEWFOUNDLAND AND LABRADOR REGION

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**REPRODUCTIVE BIOLOGY OF
DEEP-SEA SOFT CORALS IN THE
NEWFOUNDLAND AND LABRADOR REGION**

by

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ABSTRACT

This research integrates processing of preserved samples and, for the first time, long-term monitoring of live colonies and the study of planula behaviour and settlement preferences in four deep-sea brooding octocorals (Alcyonacea: Nephtheidae). Results indicate that reproduction can be correlated to bottom temperature, photoperiod, wind speed and fluctuations in phytoplankton abundance. Large planula larvae are polymorphic, exhibit substratum selectivity and can fuse together or with a parent colony. Planulae of two *Drifa* species are also able to metamorphose in the water column before settlement. This research thus brings evidence of both the resilience (i.e., extended breeding period, demersal larvae with a long competency period) and vulnerability (i.e., substratum selectivity, slow growth) of deep-water corals; and open up new perspectives on experimental studies of deep-sea organisms.

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我亲爱的父亲母亲

慈爱的师长

和一路同行的朋友们

感谢你们为我撑开一片天空

让我能够无忧无虑

充满力量的奔跑

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CO-AUTHORSHIP STATEMENT

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

Authorship for publication arising from **Chapter 2** will be Z. Sun, J.-F. Hamel, E. Edinger and A. Mercier.

Authorship for publications arising from **Chapter 3** and **Chapter 4** will be Z. Sun, J.-F. Hamel and A. Mercier.

CHAPTER 1 : INTRODUCTION



ROV ROPOS

1.1 Brief history of deep-sea research

Two thirds of the Earth's surface is covered by oceans, and most of it is more than 2000 m deep (Gage and Tyler 1991). Life probably started to appear in the earliest seas four billion years ago, and some scientists suggested that this long evolutionary process could favour a high biodiversity in the deep oceans (Herring 2007).

In spite of their importance, deep-sea habitats are still poorly studied and understood. This is mainly due to the fact that deep-sea research requires advanced technologies and sophisticated and very expensive sampling equipment. Nonetheless, over the years, the exploration of the deep ocean has painted a beautiful and increasingly detailed picture of this hidden world (Gage and Tyler 1991).

The early investigation of the deep-sea environment started with the voyage of the "Challenger" from 1872 to 1876 (Gage and Tyler 1991). Not only did this voyage arouse people's curiosity toward the unknown deep-water world, but it also encouraged further exploration of the deep. In the 1950s, Russian biologists launched further exploration of the ocean and provided some of the first quantitative surveys. Later in the 1960s and 1970s, scientists from the United States of America joined the efforts toward the study of deep-sea ecology. Over the 1970s and 1980s, deep-sea research has expanded and is now the focus of worldwide attention (Gage and Tyler 1991).

1.2 Importance of deep-sea coral ecosystems

People used to think that corals grew only in the shallow regions of tropical oceans and seas despite the earliest records of deep-sea corals provided in 1755 by Reverend Erik Pontoppidan, Bishop of Bergen (Wilson 2001; Koslow 2007). These early reports referred to deep-sea corals as “sea trees”. In those days, cold-water and deep-sea corals collected by fishermen and divers were used as food or medicine (Wilson 2001). Extensive research on deep-sea corals only began in the last few decades.

Deep-sea corals are “loosely defined paraphyletic assemblages of hexacorals, octocorals, and hydrocorals belonging to the phylum Cnidaria” (Etnoyer et al. 2006). They occur between 50 and 4000 m (Risk et al. 2002) in ocean strata that are typically cold, dark and under immense pressures.

In contrast to shallow-water species that have the support of symbiotic algae, deep-sea corals depend largely on environmental nutrition and their own capture ability to sustain their metabolic needs (Freiwald et al. 2004). Deep-sea corals are often long-lived; many gorgonian species have been shown to live several hundred years (Risk et al. 2002; Roark et al. 2006; Sherwood et al. 2006). Furthermore, some species of deep-sea corals, such as *Desmophyllum cristigalli* and *Primnoa resedaeformis*, are excellent indicators of climate change (Smith et al. 1997; Heikoop et al. 2002).

Deep-sea corals are also important for the maintenance of biodiversity since they provide crucial habitats for fish and other marine creatures such as sponges, echinoderms and crustaceans (Buhl-Mortensen and Mortensen 2005; Costello et al. 2005). With the development of advanced technologies that allowed the exploration of deep-sea environments, opportunities increased to study the distribution, biology and vulnerability of deep-sea corals to human disturbances (Freiwald et al. 2004; Gass and Willison 2005; Guinotte et al. 2006). Most of the research has shown deep-sea corals to be extremely slow-growing and susceptible to physical disturbance, indicating that human activities, including bottom trawling, mineral extraction, and oil and gas exploitation, are the most severe threats to deep-water coral ecosystems (Gass and Willison 2005; Mortensen and Buhl-Mortensen 2005).

Although the reproduction of deep-sea corals is a key element in determining the level of their vulnerability or resilience to disturbances, very little information exists on the sexual and asexual proliferation of deep-sea species worldwide. The life histories of deep-sea corals remain largely unresolved, thereby limiting our understanding of cold-water reef ecology.

1.3 Complexity of reproductive biology in corals

Reproductive patterns and behaviours and the role played by environmental factors in gametogenesis and gamete/larval release have been widely studied in shallow-water coral

species. Investigations have revealed that corals can reproduce both sexually and asexually and that individual colonies may use both reproductive modes within their life time (Lasker 1990).

Sexual reproduction in corals can be subdivided into two main types: (1) broadcast spawning with fertilization and development in the water column; (2) fertilization inside or on the parent colony and subsequent internal or external brooding of embryos. After embryogenesis, which includes early cell cleavages, blastulation and gastrulation, embryos develop into a characteristic coral larva called the planula.

In reproductive research of shallow-water species, corals are often classified by sexuality (hermaphroditic or gonochoric) and reproductive mode (broadcast spawning or brooding). Szmant (1986) described four reproductive patterns in 11 Caribbean reef coral species: (a) hermaphroditic broadcasters; (b) gonochoric broadcasters; (c) hermaphroditic brooders; (d) gonochoric brooders. However, the reproductive strategy of corals is much more complex. For instance, some species are known to change sex during their life history (Rinkevich and Loya 1987; Loya and Sakai 2008). Cyclical hermaphroditism was documented in the study of three deep-sea *Caryophyllia* species (Scleractinia) in the North-East Atlantic Ocean (Waller et al. 2005); gametes of both sexes exist in the same mesentery, but only one kind of gametes is functional at any given time.

Reproductive modes are highly variable even in the same family (Fautin 1991). It is hard to say which is more basic or general between broadcast-spawning and brooding or to predict the reproductive pattern of an unknown species. However, brooding is viewed as a strategy to compensate for the relatively small number of eggs produced (Benayahu and Loya 1983), because it decreases the mortality during larval development.

Several factors can influence reproduction: (1) physical or environmental conditions (i.e. time of year, depth, location, tidal and lunar cycles, abundance of food, etc.) and (2) biological factors (i.e. differences between species, colony size, etc.). In summary, it is difficult to say whether any one factor is critical in determining the mode, periodicity, and success of reproduction. The reproductive process of most corals is multi-faceted and very complex; it is even more difficult to understand in deep-sea species which have often never been examined alive.

Planula behaviour, settlement preferences, recruitment and growth have been studied in shallow-water coral species. For many of them, chemical or biological cues are essential to induce settlement. For instance, some crustose coralline algae and bacteria have been shown to induce larval metamorphosis and selection of suitable habitats, such as parental habitats (Heyward and Negri 1999; Baird et al. 2003; Harrington et al. 2004).

Environmental factors, such as seawater temperature, depth, current, physical texture and orientation of substrata also influence the choice of substratum and time to settlement and

metamorphosis (Atoda 1951; Hodgson 1985; Rogers 1990; Fabricius 1997; Heyward and Negri 1999; Baird et al. 2003; Harrington et al. 2004). However, research on settlement behaviour and preferences has rarely been carried out in deep-sea corals. Brooke and Young (2003) studied the deep-sea scleractinian coral *Oculina varicosa* from southeast Florida shelf at depths of 80-100 m, and they found that planulae could swim for 1-2 weeks before actively probing substrata. The paucity of data on deep-sea planulae behaviour and development is likely related to the difficulties associated with maintaining and spawning live animals in the laboratory. For example, Buhl-Mortensen et al. (2007), kept the cup coral *Flabellum alabastrum* in captivity for 21 months, but observed no reproductive activity.

1.4 Challenges of deep-sea coral research

Today, thousands of deep-sea species are described and probably millions remain unidentified and will be discovered and described in the coming years. This huge biodiversity is one of the greatest challenges in deep-sea research (Herring 2007). True soft corals (subclass Octocorallia) are among the three major groups of reef-building and habitat-forming corals in cold waters and they are widely distributed (Freiwald et al. 2004; Lumsden et al. 2007; Wareham and Edinger 2007). However, little research has been carried out on their reproductive biology. This is partially due to their morphological plasticity, which makes their identification particularly difficult (Watling and Auster 2005). In the present study, Catherine McFadden, an expert on soft corals,

helped me to identify the coral samples. However, she informed me that the illustrations in the original publications of certain *Drifa* species that were described in the early 20th century are too poor to allow comparison. Hence, the only way to confirm identification would be to examine preserved European museum specimens to do a comparison of the sclerites and other morphological characters. Despite my efforts to do such a comparison, this had not been possible so far.

Besides the difficulty in identification, two problems were faced during the course of the present research: (1) the limited number of samples and (2) maintaining healthy deep-sea specimens alive for long periods in the laboratory. It is difficult to obtain deep-sea coral specimens, let alone live ones. Two techniques were used to collect samples of deep-sea corals: bottom trawling and remotely operated vehicles (ROVs). A large proportion of samples in this project were collected within the Department of Fisheries and Oceans (DFO) multi-species surveys by bottom trawling, and either frozen immediately at -20 °C or kept alive whenever possible. However, most deep-sea corals collected by bottom trawling are badly damaged, and cannot survive. Although ROVs provide fewer samples than bottom trawling, this technique is a good alternative to obtain specimens that are healthy enough to maintain alive in the laboratory for reproductive research.

Like all deep-sea animals, corals are very sensitive to environmental conditions, such as water temperature, light, and water quality. Apart from the present study, there are few published examples where deep-sea corals have been successfully maintained in the

laboratory for scientific purposes. Spawning and larval rearing have rarely been documented. The unique location and characteristics of our laboratory facilities made it possible to maintain deep-sea soft corals alive for experimental studies: (1) the relatively low year round seawater temperatures reduced the need for mechanical chilling to only a few months per year; (2) the laboratory is supplied with natural unfiltered running seawater, i.e. rich in oxygen, nutrients and food; (3) samples were collected on the CCGS *Teleost* and *Hudson*; both vessels were equipped with cold seawater supply and brought samples back to the St. John's harbour which is close to our laboratory.

1.5 Objectives and outline of the present study

Thirty species of deep-sea corals have so far been recorded in Newfoundland and Labrador, eastern Canada (Wareham and Edinger 2007), including thirteen alcyonaceans, two antipatharians, four solitary scleractinians, and eleven pennatulaceans; however, deep-sea corals research in this region is still in an exploratory phase, especially with respect to their reproductive biology. Unfortunately, the continental slope of Newfoundland and Labrador has experienced intense bottom trawling, which can significantly influence deep-sea ecosystems and damage deep-sea corals; no concrete conservation measures have been taken so far to protect this huge geographical area (Wareham and Edinger 2007).

Within the Octocorallia, larger gorgonians, or sea fans, have so far attracted the greatest conservation concern in Newfoundland and Labrador. However, the present study focused on alcyonaceans of the family Nephtheidae, because: (1) the number of samples was larger and quality was better for nephtheids; (2) live nephtheids survived better in the laboratory than other species tested. Furthermore, soft corals are often overlooked despite their prevalence and importance in deep-sea habitats (Dinesen 1983; Freiwald et al. 2004; Watling and Auster 2005). The main goal of this study was to elucidate the mode and timing of reproduction, settlement, recruitment and early growth of four deep-sea Nephtheidae species in an effort to gather pertinent information for the conservation of deep-sea corals in this area and to significantly expand knowledge of deep-sea coral biology.

The study combined: (1) histological analysis, micro-dissection and image analysis of frozen samples to assess patterns of development and maturation of gametes, embryos and planulae, (2) collection, maintenance and observation of live animals in the laboratory to gather data on reproductive behaviour and periodicity, (3) in-lab rearing of planulae and post-settled polyps to ascertain substrate selectivity, development and growth.

The following chapters of the thesis include: the mode and timing of reproduction of *Drifa glomerata*, comparing the reproductive strategy at different depth ranges and latitudes, and suggesting environmental factors that may influence the reproductive cycle

(Chapter 2); the gamete development, planulation, metamorphosis, settlement preferences and growth of *Drifa glomerata* and *Drifa* sp. (Chapter 3); and the planulation and metamorphosis of *Gersemia fruticosa* and *Duva florida* (Chapter 4). Finally, I present a summary of the main conclusions and their significance and identify areas in which future research is particularly needed (Chapter 5).

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**CHAPTER 2 : REPRODUCTIVE BIOLOGY OF THE DEEP-SEA
OCTOCORAL *DRIFA GLOMERATA* IN THE NORTH-WEST
ATLANTIC**



Deep-water soft corals of the NW Atlantic

2.1 Abstract

This study of the mode and timing of reproduction in bathyal corals was undertaken in an effort to gather information on poorly understood deep-water corals. Samples of the alcyonacean *Drifa glomerata* were collected between 103 and 400 m along the coast of Newfoundland (eastern Canada) from 2004 to 2007. The ratio of fertile colonies was >50% year round. Among the fertile colonies, the number of planulae within a single fertile polyp varied between 1 and 10. The size of oocytes and/or planulae was consistently greater in the polyps than in the branchlets across all dates and depths studied, indicating that the development pathway of oocytes to planulae is from the branchlets to the polyps. Although larval production seemed to persist year round, the onset of major planulation events was determined to occur in December or January of two consecutive years, when large mature planulae ones were released. Larval release continued until June, during the progressive increase in photoperiod, although peak planulation occurred after the phytoplankton bloom as seawater temperature was steadily increasing, between March and early June. This seasonal trend was supported by spawning of a live colony in the laboratory between early January and June in 2008. The large planulae (ca. 4-5 mm long on average) actively crawled on and probed the substratum immediately after release.

2.2 Introduction

Deep-sea corals are important components of marine ecosystems because of their role in the maintenance of biodiversity. They provide crucial habitats for fish and other marine organisms such as sponges, echinoderms and crustaceans (Buhl-Mortensen and Mortensen 2005; Costello et al. 2005). They are usually long-lived, but extremely slow-growing and susceptible to physical disturbance (Freiwald et al. 2004). With the development of new equipment and technologies, opportunities are increasing to study the distribution, biology and vulnerability of deep-sea corals (Freiwald et al. 2004; Gass and Willison 2005; Guinotte et al. 2006). Up to now, the study of reproduction in octocorals has traditionally focused on shallow-water Alcyoniidae, Xeniidae, and Gorgoniidae from the tropical Pacific, Red Sea and the Caribbean (Richmond and Hunter 1990; Benayahu 1991), whereas very limited research has been carried out on the reproductive biology of deep-sea species, with a focus on scleractinians (Waller 2005; Waller and Tyler 2005; Waller et al. 2005; Flint et al. 2007).

Although octocorals in the family Nephtheidae are widely distributed around the world (Dinesen 1983; Roberts et al. 2006; Lumsden et al. 2007; Wareham and Edinger 2007), information on the reproduction and development of this group is very limited (Farrant 1986; Benayahu et al. 1992; Dahan and Benayahu 1997; Hwang and Song 2007; Sun et al 2009; Chapters 3 and 4). This paucity of information may be a result of their taxonomical

diversity and morphological similarity, which make identification very difficult (Watling and Auster 2005).

Generally, the sexuality of shallow-water corals is divided into hermaphroditic or gonochoric. However, research in temperate or deep-sea corals has revealed much more complex arrays of reproductive strategies. For instance, the work of Waller et al. (2005) described the occurrence of cyclical hermaphroditism in three deep-sea scleractinian corals (*Caryophyllia*). They demonstrated that gametes of both sexes existed in the same mesentery; however, only one type of gametes was functional at any given time. Gametogenesis was continuous within colonies, and was not synchronous within coral colonies or at the population level, which made fertilization possible all year round.

In shallow-water octocorals, gametogenesis especially oogenesis, can span from several months (Farrant 1986) to two years (Yamazato et al. 1981); and different cohorts of gametes may exist simultaneously in a single colony when development takes more than one year (Brazeau and Lasker 1990). Furthermore, gonad development is not always synchronous within a coral colony or at the population level. Farrant (1986) mentioned that the Nephtheidae coral *Capnella gaboensis* exhibited major gonad abundance differences between branches within the same colony, and between colonies, which could explain the prolonged spawning period observed in this species at the population level.

Corals mainly have two reproduction modes: broadcast spawning and brooding. Broadcasters usually produce large numbers of oocytes, which are released simultaneously to be fertilized and develop in the water column with a high dispersal capability. In contrast, brooders release mature planulae into the water column, usually corresponding to more limited dispersal scales (Sebens 1983a; 1983b; Harrison and Wallace 1990; Richmond and Hunter 1990). In addition, brooding has been proposed in deep-sea organisms as an adaptation to the harsh environment (Gage and Tyler 1991).

Several factors can affect reproductive strategies and cycles, including depth, geographical location and environmental factors. Depth has been shown to delay breeding, and also influence the sex ratio in corals (Benayahu and Loya 1983). Geographical locations also have an influence on reproductive patterns and strategies, i.e., *Heteroxenia coheni* (Xeniidae) is hermaphroditic in the Red Sea but gonochoric on the Great Barrier Reef (Benayahu et al. 1990). Furthermore, environmental factors, such as temperature, food supply, light and lunar cycle were described as having an impact on gamete synthesis and synchronicity of spawning (Shlesinger and Loya 1985; Farrant 1986; Richmond and Hunter 1990; Ben-David-Zaslow et al. 1999).

This work focuses on the deep-water coral *Drifa glomerata* (Alcyonacea: Nephtheidae), a soft coral species which has been previously recorded in the western Atlantic Ocean (Verseveldt 1967; Lumsden et al. 2007). The goal of the present study was to examine the reproductive features of *D. glomerata* using series of preserved samples and a limited

number of live specimens. By using micro-dissection and histological procedures, and monitoring live specimens in the laboratory, I aimed to elucidate the mode and timing of reproduction, compare the reproductive strategy at different depth ranges, suggest environmental factors that may influence the reproductive cycle, and finally record planulation in the laboratory.

2.3 Materials and Methods

Sample collection and maintenance

Samples of *Drifa glomerata* were collected by bottom trawls during the multi-species survey conducted by the Department of Fisheries and Oceans Canada (DFO) on board of the CCGS *Templeman* and CCGS *Teleost* and during the Fisheries Observer Project (FOP). All samples were collected between November 2004 and December 2007 off Newfoundland and Labrador, eastern Canada (Table 2-1, Figure 2-1) at depths ranging from 103 to 334 m and were immediately frozen at -20 °C. In addition, two live colonies of *D. glomerata* were collected in July 2007 at ca. 350-400 m on the continental slope of the SW Grand Banks of Newfoundland (44°21'38"N-53°15'57"W), using the remotely operated vehicle ROPOS on board of the CCGS *Hudson*. On the ship, the specimens were maintained in chilled seawater at 2-3 °C in darkness. In the laboratory, the two colonies were kept together in a 20-L tank provided with running seawater (ca. 1.5 L min⁻¹) in total darkness. The tank was supplied with unfiltered seawater at ambient

Table 2-1. Date, location and depth of collection of *Drifa glomerata*. The Location column indicates whether a given sample was collected in the northern (N) or southern (S) region of the geographical range (either sides of the dotted line shown in Figure 2-1).

Sample*	Date	Number of colonies	Depth (m)	Coordinates	Location
1	Nov-04	2	147	51°49'26"N-53°49'00"W	N
2		2	203	52°01'22"N-53°31'26"W	N
3		6	186	51°29'06"N-53°02'16"W	N
4	Dec-04	1	259	50°43'48"N-52°26'24"W	N
5		2	283	50°51'18"N-52°10'40"W	N
6		6	222	51°11'20"N-52°44'38"W	N
7		15	170	50°01'30"N-51°45'14"W	S
8	Jun-05	1	191	48°02'06"N-49°33'46"W	S
9		1	116	47°31'19"N-51°15'00"W	S
10		1	117	47°25'58"N-51°20'45"W	S
11		1	146	46°08'56"N-53°32'20"W	S
12		3	191	48°02'06"N-49°33'46"W	S
13		3	320	48°46'22"N-52°09'36"W	S
14		4	151	47°16'04"N-51°38'45"W	S
15		7	295	48°03'03"N-48°42'00"W	S
16	Nov-05	1	180	52°04'12"N-54°35'49"W	N
17		1	204	47°15'14"N-47°41'34"W	S
18		2	197	52°53'24"N-54°11'52"W	N
19		4	143	52°11'20"N-55°03'32"W	N
20		4	219	51°44'24"N-54°15'03"W	N
21		6	268	51°45'14"N-53°59'02"W	N
22		5	122	46°03'46"N-53°18'25"W	S
23		7	227	51°54'07"N-54°17'27"W	N
24		11	227	51°47'16"N-54°36'00"W	N
25		12	208	52°03'50"N-54°55'22"W	N
26		15	143	46°11'16"N-53°39'18"W	S
27	21	146	46°08'56"N-53°35'31"W	S	
28	Jan-06	3	231	50°59'49"N-54°27'14"W	N
29		4	198	50°01'22"N-53°24'03"W	S
30		7	258	49°55'48"N-53°14'56"W	S
31	Jun-06	1	127	47°26'02"N-49°26'13"W	S
32		1	290	48°49'30"N-50°04'33"W	S

33		1	313	48°52'33"N-49°50'16"W	S
34		4	200	48°40'04"N-50°09'50"W	S
35		10	134	49°25'58"N-49°29'24"W	S
36	Nov-06	1	152	52°52'33"N-55°14'42"W	N
37		1	182	54°33'43"N-55°02'56"W	N
38		1	153	53°31'08"N-55°26'56"W	N
39		1	188	53°38'24"N-54°25'40"W	N
40		1	260	53°28'08"N-54°52'44"W	N
41		1	268	54°28'33"N-55°32'56"W	N
42		1	230	52°24'03"N-53°08'31"W	N
43		1	333	54°35'13"N-55°55'15"W	N
44		2	152	52°52'33"N-55°14'42"W	N
45		3	272	55°02'16"N-57°16'19"W	N
46		4	127	55°14'24"N-57°41'09"W	N
47	Jul-07	1	146	46°19'26"N-52°37'48"W	S
48		1	158	46°14'06"N-52°53'27"W	S
49		2	103	46°23'09"N-53°43'33"W	S
50		2	148	46°09'32"N-53°12'21"W	S
51		3	148	46°10'44"N-53°20'34"W	S
52		10	128	46°13'08"N-54°08'45"W	S
53		14	143	46°06'57"N-53°24'07"W	S
54	Dec-07	3	148	47°12'03"N-52°03'57"W	S
55		6	148	52°09'57"N-55°04'48"W	N
56		1	145	46°20'06"N-52°29'20"W	S
57		2	128	46°18'46"N-53°28'26"W	S
58		2	118	46°09'54"N-52°37'22"W	S

* Corresponding to the samples identified in Figure 2-1.

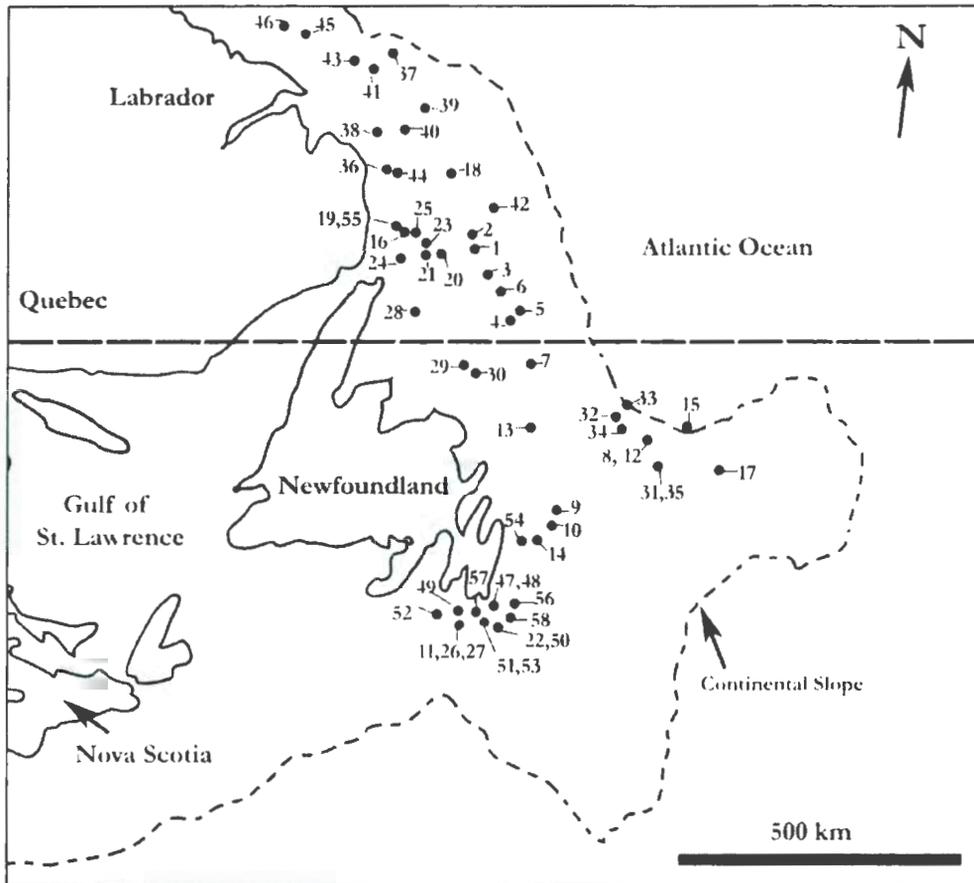


Figure 2-1. Locations where samples of *Drifa glomerata* were collected in the northwestern Atlantic. The dotted line ($51^{\circ}40' N$) shows subdivision of samples into northern and southern groups. Information on corresponding number of colonies can be found in Table 2-1.

temperature (between -1 and 9 °C); however, when coastal temperatures were higher than deep-water conditions, the seawater was chilled to maintain it below 10 °C.

Correlations with depth, latitude and environmental factors

In order to characterize the samples and detect natural habitat boundaries, bottom temperatures of all the trawls containing soft corals were analyzed. The average bottom temperature was collected by a CTD recorder on the trawl headgear for each 15 minute trawl. Bottom temperature data from 2002 to 2007 (Figure 2-2) in the sampling area (roughly 46°10' N to 55°10' N) were used to assess the possible occurrence of latitudinal and depths variations. Bottom temperatures were initially divided into two groups according to their location, roughly north and south of 51°40' N (dotted line in Figure 2-1), and each location group was further subdivided into two subgroups: from 100 to 200 m and from 200 to 300 m for analysis. Bottom temperatures were significantly different at the two depth ranges in both the southern ($\chi^2_{1, 237}=133.02$, $p<0.001$) and northern ($\chi^2_{1, 170}=27.35$, $p<0.001$) locations. Thus, considering the limited number of samples and the bottom temperature differences at the two depth ranges in both locations, samples from the same depth ranges (from 103 to 203 m and from 204 to 334 m, Figure 2-2) were grouped together, regardless of the site of collection.

Information on annual temperature fluctuation and phytoplankton abundance (using chlorophyll fluorescence as an indicator) were obtained from DFO for station 27 (<http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/azmp-pmza/hydro/index-eng.html>;

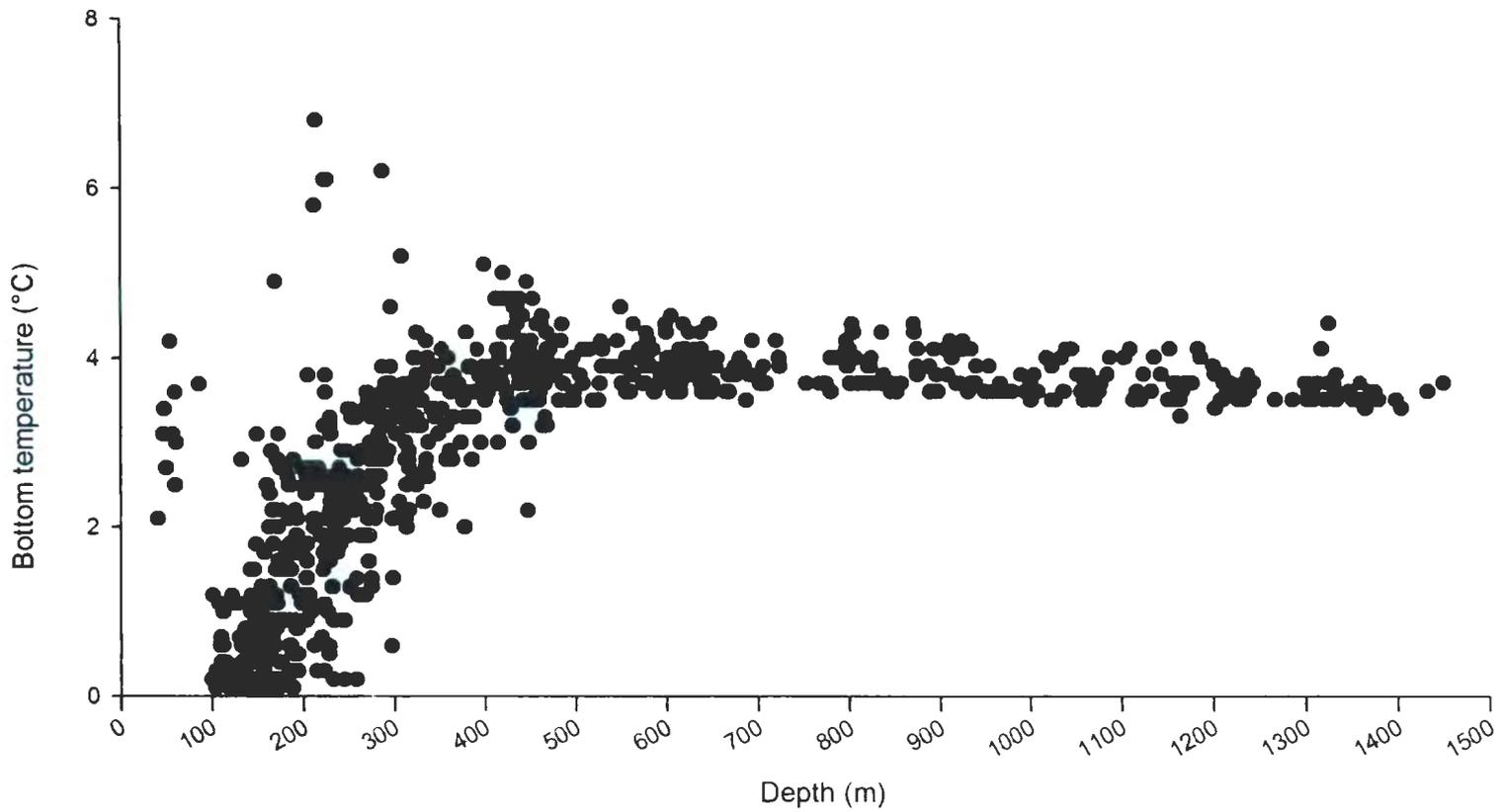


Figure 2-2. Combined bottom temperature from all the trawls containing soft corals (including *Drifa glomerata* and all other alcyonacean species) between December 2002 and December 2007 in Newfoundland and Labrador.

fixed zonal monitoring program; 47°32'04"N 52°35'06"W) at a depth of ca. 150 m from 2004 to 2007 and from January to July 2008. Information on maximum wind speed was gathered from Environment Canada, using data collected at St John's Airport (47°22'19"N 52°26'38"W) 140.5 m above sea level from 2004 to 2007 (http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html). Wind speed may be an important factor as it has been observed to correspond to large amount of resuspended organic and inorganic materials that can influence the feeding rate and reproductive cycle of certain marine invertebrates (Mercier and Hamel 2008). Lunar cycle data were obtained from the StarDate Online website (<http://stardate.org/nightsky/moon/>) and photoperiod from Environment Canada. In the laboratory, seawater temperatures in 2008 were monitored continuously by a temperature-light logger HOBO Pendant (UA-002-XX) placed in the tank.

Ratio of fertile colonies

All intact frozen colonies (i.e., excluding fragments) were briefly rinsed under running seawater, gently wiped with paper towel to remove ice and excess water, and weighted. Colonies that possessed large pinkish polyps containing oocytes/planulae were referred to as colonies with fertile polyps or fertile colonies. Based on size and colour, the fertile polyps in a given colony were easy to distinguish and count with the naked eye. All samples were examined to establish the ratio of colonies with and without fertile polyps at each sampling date and depth range (previously described). Those individuals without fertile polyps were further studied to determine whether they were male colonies or

non-reproductive female colonies by using histology (see description of histological technique used below).

Reproductive cycle and fecundity

As described in the previous section, for each colony, the total weight and the number of fertile polyps were established. The wet weight of all colonies varied from 1.6 g to 42.1 g. To avoid any bias due to the presence of immature colonies, only colonies with more than ten fertile polyps (considered as putative sexually mature colonies) were used to establish maturity and fecundity (number of planulae g^{-1}). All sexually mature colonies were further divided into three subgroups according to weight (<5 g, 5-20 g, and >20 g) in order to estimate the influence of size of the colony on the various measures and indices.

A fertile polyp index (FPI) was calculated as the number of fertile polyps per gram of wet colony weight. The average FPI was calculated for each sampling date and depth range. After counting the number of fertile polyps to establish the FPI, all sexually mature colonies (with more than ten fertile polyps) were examined under a Nikon SMZ1500 stereomicroscope. For each colony ($n=87$), ten haphazardly chosen fertile polyps were used to establish the number of planulae they contained and to estimate the maximum reproductive output within a single fertile polyp and per colony. Each of the ten fertile polyps was dissected under a stereomicroscope by cutting the wall of polyp open with thin forceps, and the visible planulae inside were counted and measured: the maximum

diameter and orthogonal diameter of planulae/oocytes were recorded using a graduated ocular eyepiece. The following formula was used to calculate the surface area:

$$(1) \text{ Surface area} = \pi AB,$$

Where A = maximum diameter, and B = orthogonal diameter

In addition, oocytes were measured in three haphazardly chosen branchlets in each colony using the technique described for the fertile polyps. Average oocyte surface area data were pooled for each sampling date and depth range.

To determine the precise arrangement of gametes within the various sections of the branchlets and polyps, and to assess the status of non fertile specimens, eighteen frozen colonies (four with fertile polyps and fourteen without) were prepared for histological analysis. One branch was sampled from each colony and preserved in 4 % formaldehyde. To improve the quality of the sections, the samples were first embedded in Histo-Gel™ before standard histological preparation. Samples were dehydrated in a graded alcohol series (Flex™ 80-95 and 100 % ethyl alcohol). Thereafter, the samples were cleared in two stations of Clear Rite 3™, followed by paraffin infiltration and embedding. Sections (20 μm) were stained with hematoxylin and eosin to distinguish the nuclear material from the cytoplasm. Because of the variability in the morphology of the colonies, pieces of tissue were also preserved in 70 % ethyl alcohol for the study of sclerites (skeletal elements) to make sure that all samples were *Drifa glomerata*. Histological sections and

sclerites were examined under a Nikon SMZ1500 stereomicroscope and Nikon Eclipse 80i microscope both attached to a Nikon DXM1200F digital camera. Normally, oocytes are divided into four stages based on the location in the coelenteron, and size and proportion of nucleus versus cytoplasm/yolk (Cordes et al. 2001). However, the freezing process sometimes made it difficult to distinguish the nucleus from the cytoplasm/yolk. Hence, oocytes were divided into two groups: early-stage oocytes and late-stage oocytes. Early-stage oocytes were defined as those either embedded in or connected to the mesenteries, and late-stage oocytes were defined as those that had migrated to the coelenteron.

Planulation and post-larval development

To support the microdissection work, the number of planulae emitted by the live colony of *Drifa glomerata* was recorded daily. Planulae were collected on the bottom of the tank within 24 h of their release and placed into culture vessels.

Statistical analyses

The generalized linear model procedure (GENMOD) of SAS (SAS 1988) was used for analyses. Proportional data were tested using logistic regression (binomial distribution, logit link); logistic regression was used to test the influence of depth range (103-203 m or 204-334 m), sampling year (2004, 2005, 2006, 2007), and sampling period (January, June, July, November, December) on the proportion of fertile colonies. Regression models were used for all other analyses. The three factors listed above were also tested

(regression) on the fertile polyp index, the number of planulae per fertile polyp, the number of planulae per colony, fecundity and the surface area of planulae and oocytes. In addition, the influence of location inside the colony (polyp or branchlet) was tested on the size of planulae and oocytes. Poisson regressions were used to test the relation between colony weight and number of fertile polyps (logit link) and the influence of lunar phases on the planulation of *Drifa glomerata*. For all statistical analyses, residuals were examined to evaluate the assumptions of homogeneity, independence and normality. Interactions were tested when there was enough degree of freedom, and normal distribution and identity link were used unless otherwise stated. If a normal distribution was not appropriate, gamma or Poisson error distributions were used (McCullagh and Nelder 1989). Tolerance of type I error was set at $\alpha=0.05$.

2.4 Results

Drifa glomerata is an internal brooder. Female sexual cells and planulae are pinkish in colour. The early stages of female gametes are attached to or embedded in the mesenteries, and develop into planulae that are retained in fertile polyps (feeding polyps hosting maturing or mature planulae) until released into the water column from the mouth of fertile polyp (Figure 2-3).

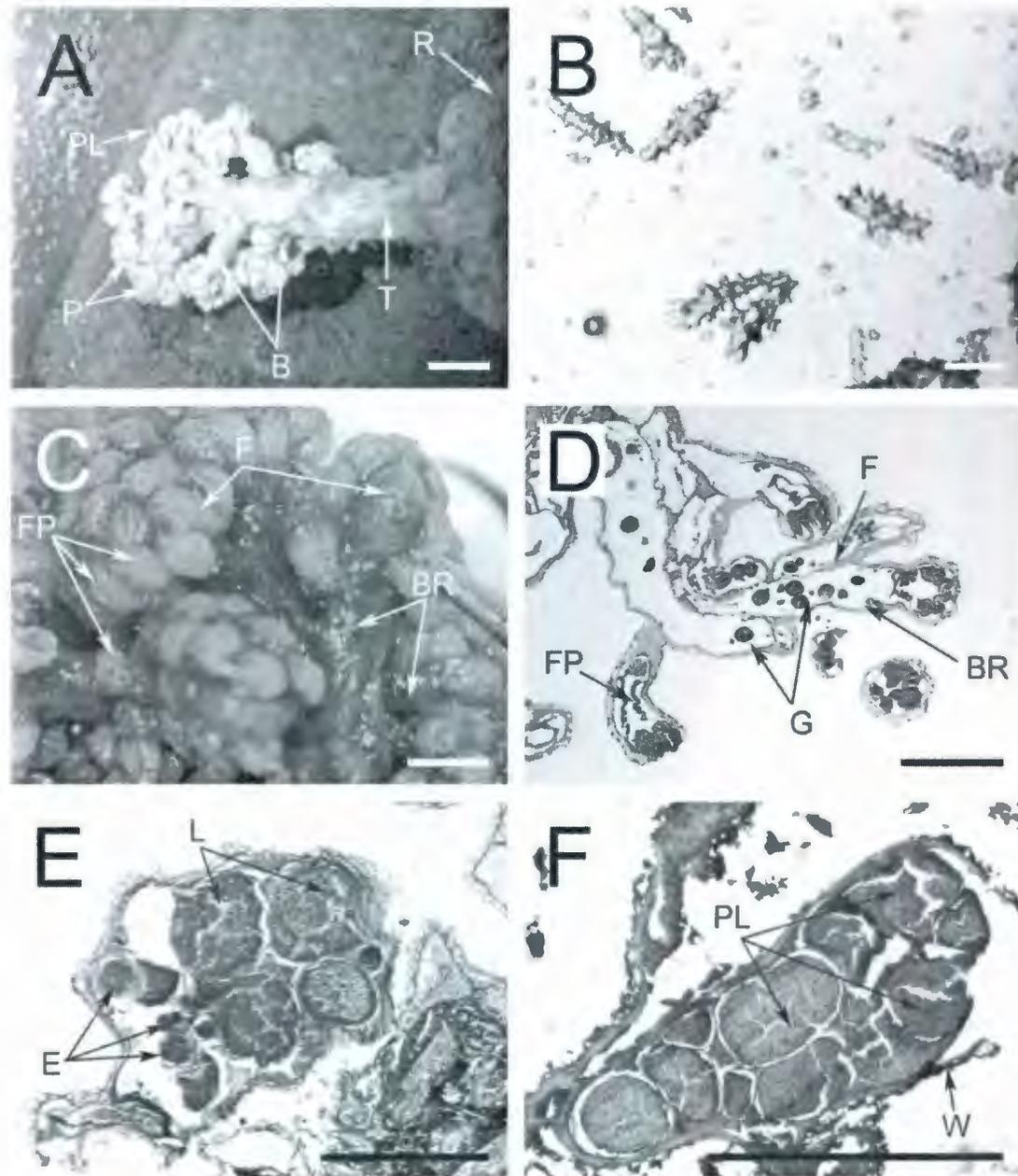


Figure 2-3. *Drifa glomerata*. A) Live reproductive colony measuring 7x4x3 cm collected at 350 m and photographed in the laboratory in January 2008. B) Spicules extracted from the body wall of the colony. C) Close-up on the surface of the colony showing fertile polyps containing planulae, and branchlets reddish in color containing different size classes of oocytes. D) Histological section of fertile polyps and branchlets, showing gametes in branchlets. E) Arrangement of different stages of oocytes in the branchlet. F) Arrangement of planulae in the fertile polyp. R: rock, P: polyp, B: branch, PL: planula, T: trunk, F: fertile polyp, FP: feeding polyp, BR: branchlet, G: gamete, E: early-stage oocyte, L: late-stage oocyte, W: polyp wall. The scale bar in A represents 1 cm; the scale bar in B represents 0.1 mm; all other scale bars represent 1 mm.

Ratio of fertile colonies

Overall the proportion of colonies possessing fertile polyps varied between 0 and 100 % in different trawls, although the average proportion of fertile colonies for all sampling dates was never less than 50 % at both depth ranges (103-203 and 204-334 m) (Figure 2-4). The proportion of fertile colonies did not differ between depth ranges ($\chi^2_{1, 57}=0.05$, $p=0.825$), sampling years ($\chi^2_{3, 57}=1.82$, $p=0.611$), and sampling periods ($\chi^2_{4, 57}=5.25$, $p=0.263$). No male gonads were observed from microdissection and histological section; in addition, some fertile colonies with less than 10 fertile polyps contained a few planulae/oocytes, which might indicate that non-fertile colonies were spent or that they were immature.

Reproductive cycle and fecundity

The number of fertile polyps within a single colony varied from 2 to 1030. The number of fertile polyps in sexually mature colonies (i.e. those colonies that contained more than 10 fertile polyps) increased with the weight of the colony ($\chi^2_{1, 86}=2355.81$, $p<0.001$, Figure 2-5). Larger colonies generally had more fertile polyps at both depth ranges and all sampled dates. However, some large colonies (up to 36 g) were found to be devoid of fertile polyps (Figure 2-5).

Although sample size of the third weight class (> 20 g) was too small to draw any solid conclusion (Figure 2-6), the fertile polyp index (FPI) showed a similar seasonal change in the other two weight classes of colonies (<5 g and 5-20 g): most November and June/July samples had low FPI values, and some of the December and January samples had higher

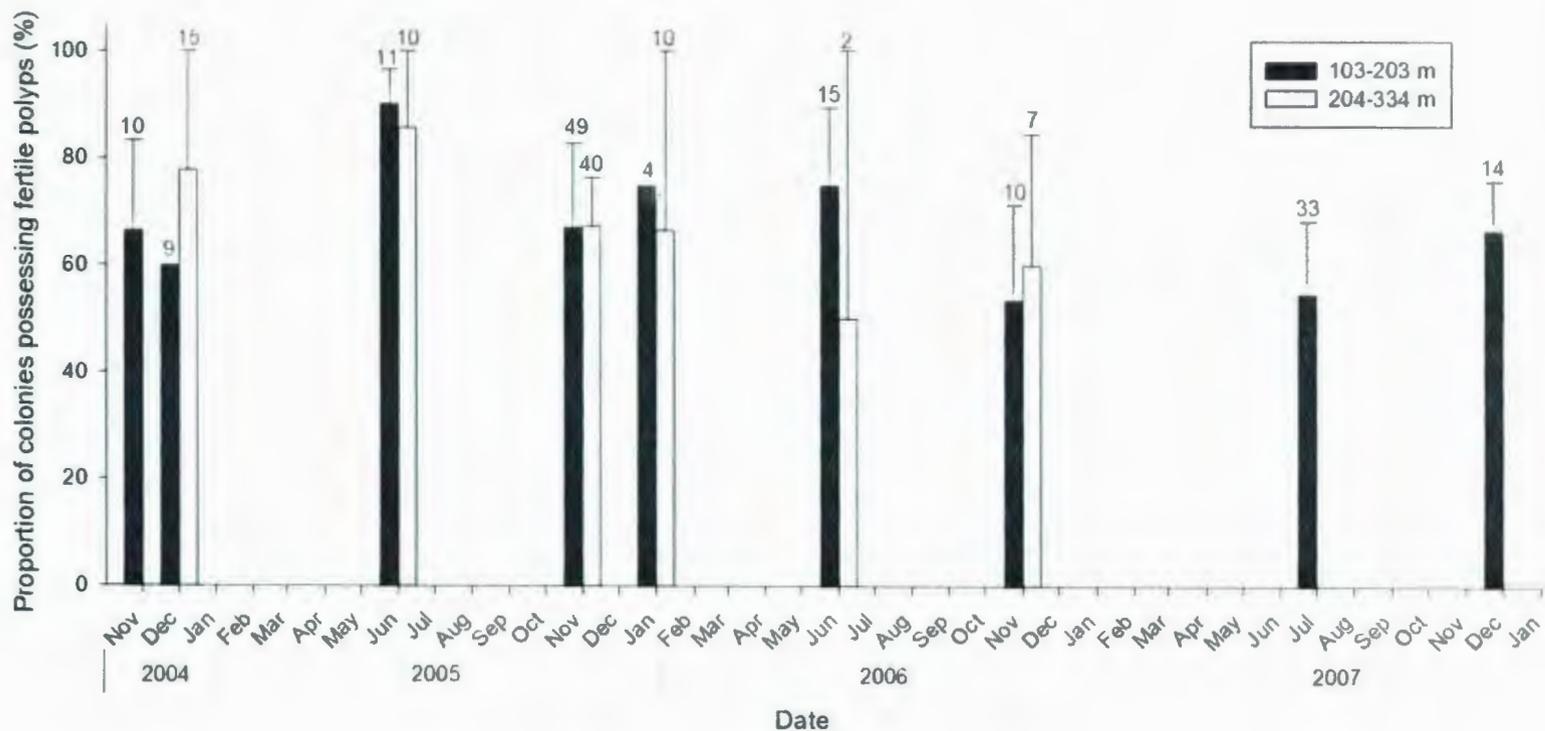


Figure 2-4. *Drifa glomerata*. Proportion of colonies possessing fertile polyps (%) in samples from 103-203 m and 204-334 m between November 2004 and December 2007. All colonies from the same trawl were examined and data were pooled (Mean \pm SE). The n value (total number of colonies at the given sampling date) is shown above each bar.

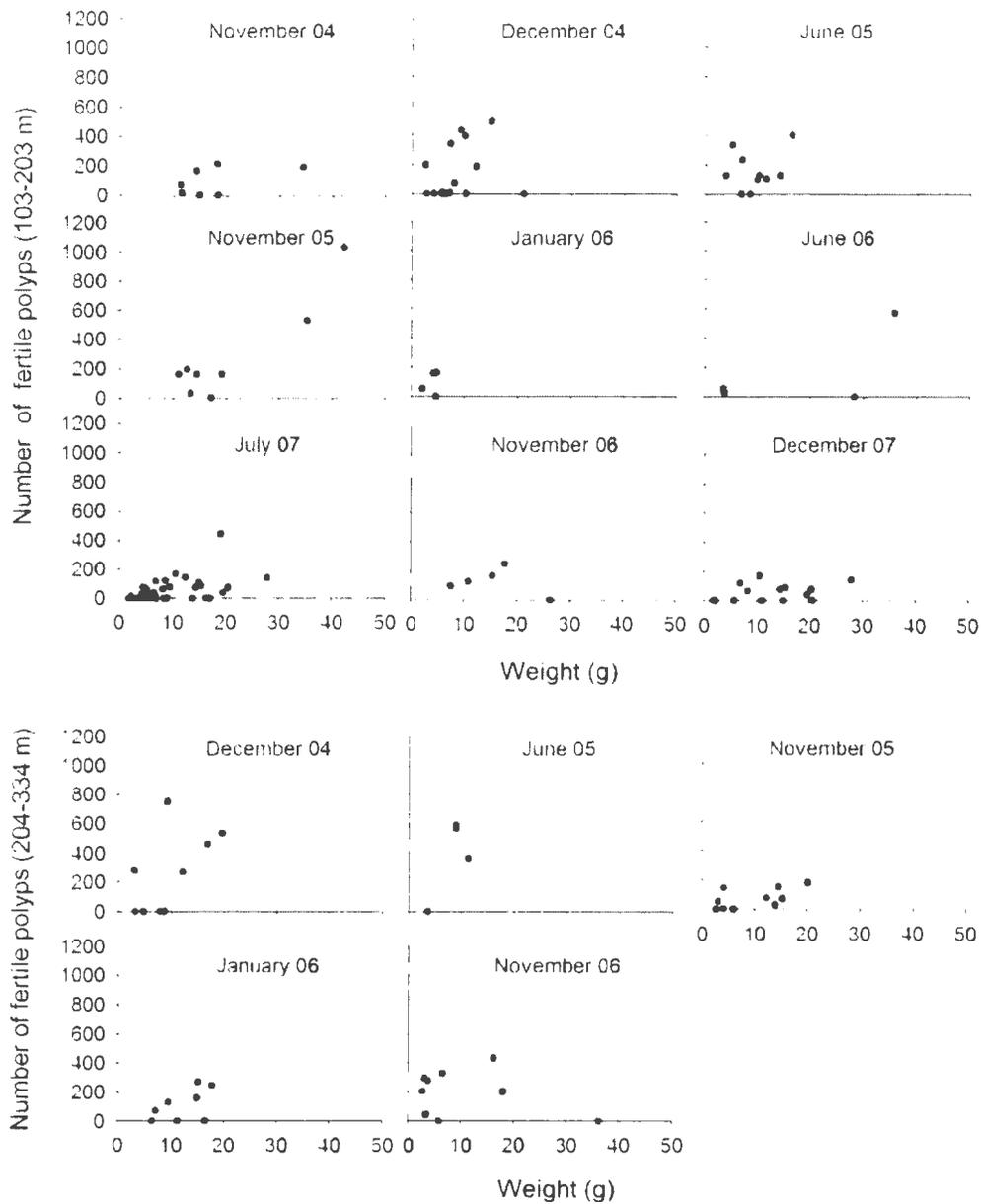


Figure 2-5. *Drifa glomerata*. Number of fertile polyps relative to the weight of the colony (g) at each sampling date and both depth ranges (103-203 m, 204-334 m) between November 2004 and December 2007.

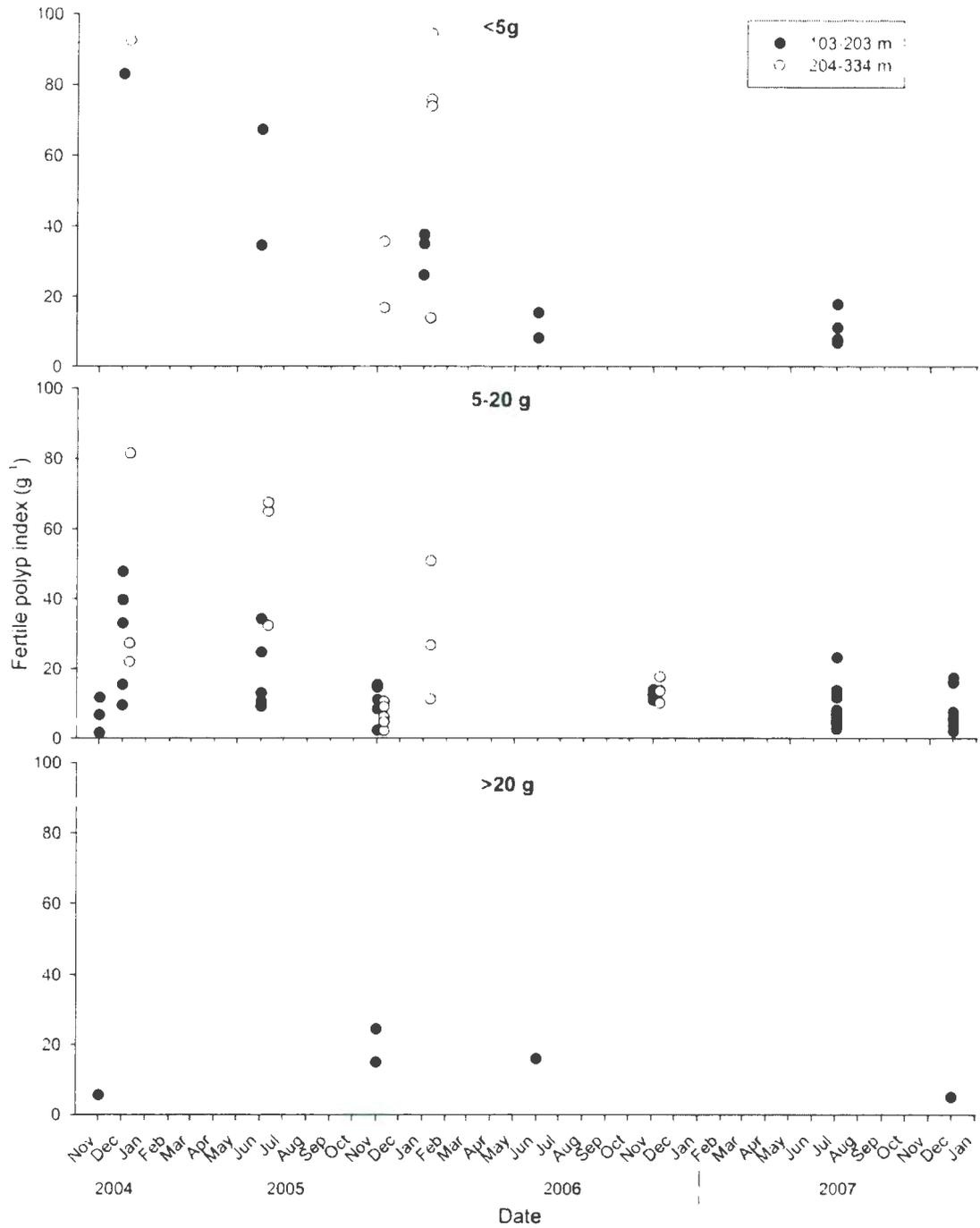


Figure 2-6. *Drifa glomerata*. Fertile polyp index (g⁻¹) at each sampling date and both depth ranges (103-203 m, 204-334 m) for three weight classes of colonies: <5 g, 5-20 g and >20 g.

values. Based on data pooled for the three weight classes, the FPI was higher in the samples from 204-334 m than those from 103-203 m ($\chi^2_{1, 86}=5.99$, $p=0.014$, gamma distribution, log link, Figure 2-7). Significant differences in FPI were also detected in different sampling years ($\chi^2_{3, 86}=1.48$, $p<0.001$, gamma distribution, log link). Significant interactions were detected between depth ranges and sampling periods ($\chi^2_{3, 86}=8.86$, $p=0.031$). More precisely, FPI varied significantly between different sampling periods, both at 103-203 m ($\chi^2_{4, 59}=13.22$, $p=0.010$) and 204-334 m ($\chi^2_{3, 26}=15.44$, $p=0.002$); however, the trends were different at the two depths studied. From 2004-2005 and 2005-2006, samples from shallow waters had lower values and a clear seasonal trend: the average FPI increased from November to December/January, and decreased from December/January to June/July in a given year (Figure 2-7). Samples from deeper waters had a similar trend with low values in November; however, they had consistently higher FPI values from January to June/July in a given year (Figure 2-7). Data from December 2006 to December 2007 did not follow the pattern in the previous two years, because of the lack of data in December 2006 and January 2007.

Based on the size differences of sexual cells, planulae seem confined to polyps and oocytes to branchlets, although this could not always be confirmed with histology, because of the poor quality of frozen samples. Among sexually mature colonies, the number of planulae within a single fertile polyp varied from 1 to 10. The average number of planulae in these fertile polyps varied between 2 and 4 (Figure 2-8). For all the samples processed, the number of planulae per fertile polyp did not show any significant

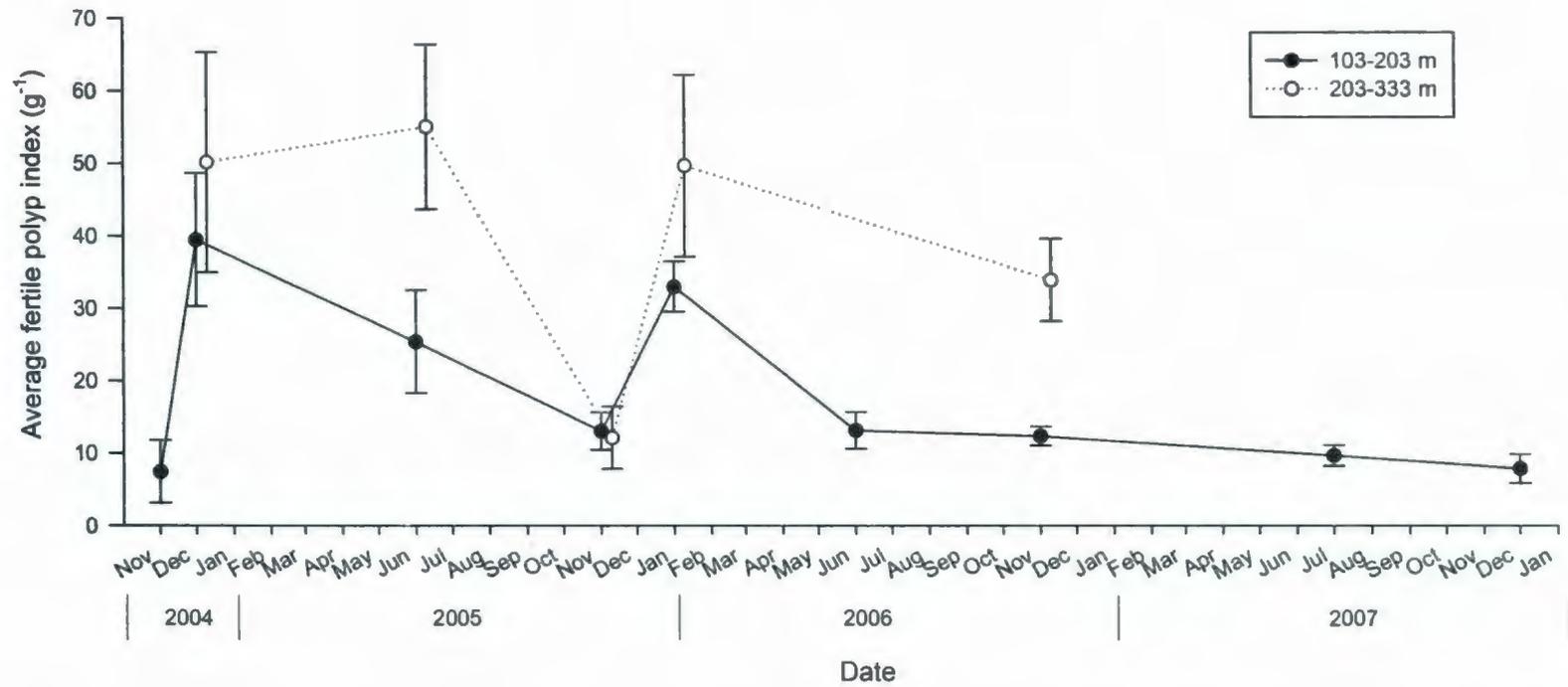


Figure 2-7. *Drifa glomerata*. Average fertile polyp index (g^{-1}) at each sampling date and both depth ranges (103-203 m, 204-334 m) from November 2004 to December 2007. Colonies from the same sampling date and depth range were combined together (Mean \pm SE, $n=3-15$).

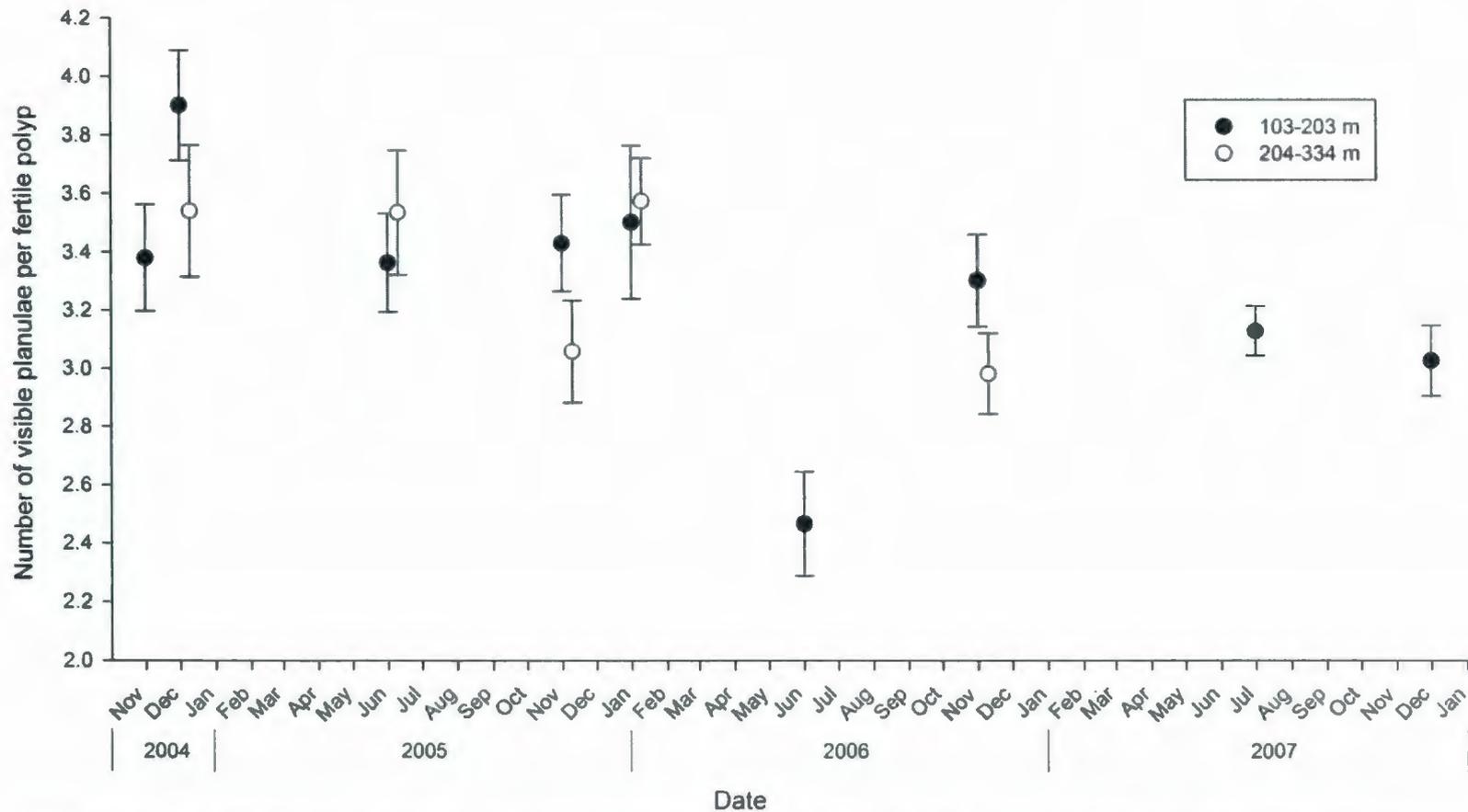


Figure 2-8. *Drifa glomerata*. Number of visible planulae per fertile polyp at each sampling date for both depth ranges (103-203 m, 204-334 m) from November 2004 to December 2007. Measures were taken in 10 fertile polyps for each colony and data from the same sampling date and depth were combined together and expressed as Mean \pm SE (n=30-150).

variation between different depth ranges ($\chi^2_{1, 86}=0.04$, $p=0.841$), sampling years ($\chi^2_{3, 86}=6.96$, $p=0.073$) and sampling periods ($\chi^2_{4, 86}=3.77$, $p=0.438$).

The total number of planulae per colony was estimated using the number of fertile polyps multiplied by the number of planulae in each of the ten haphazardly-chosen fertile polyps. For all of the colonies examined, the total number of planulae per colony varied from 42 to 2987. A larger proportion of samples from deeper waters had more planulae per colony than those from shallow waters ($\chi^2_{1, 86}=4.25$, $p=0.039$, Figure 2-9). In addition, the total number of planulae per colony was significantly different between sampling years ($\chi^2_{3, 86}=16.71$, $p=0.001$). Significant interactions were detected between depth range and sampling period ($\chi^2_{3, 86}=18.36$, $p<0.001$). More precisely, at 103-204 m, the total number of planulae per colony did not differ significantly between sampling periods ($\chi^2_{4, 59}=6.24$, $p=0.181$); however, at 204-334 m, the total number of planulae per colony was significantly different between sampling periods ($\chi^2_{3, 26}=29.26$, $p<0.001$).

Fecundity was calculated as the total number of planulae per gram of wet colony weight. It was higher in samples from 204-334 m than those from 103-203 m ($\chi^2_{1, 86}=12.87$, $p<0.001$, Figure 2-10). Significant differences in fecundity were also detected between sampling years ($\chi^2_{3, 86}=16.53$, $p=0.001$). Furthermore, significant interactions were detected between depth range and sampling period ($\chi^2_{3, 86}=8.94$, $p=0.030$). More precisely, fecundity significantly differed between sampling periods at both depth ranges: 103-203 m ($\chi^2_{4, 59}=12.50$, $p=0.014$) and 204-334 m ($\chi^2_{3, 26}=16.99$, $p=0.001$); although the

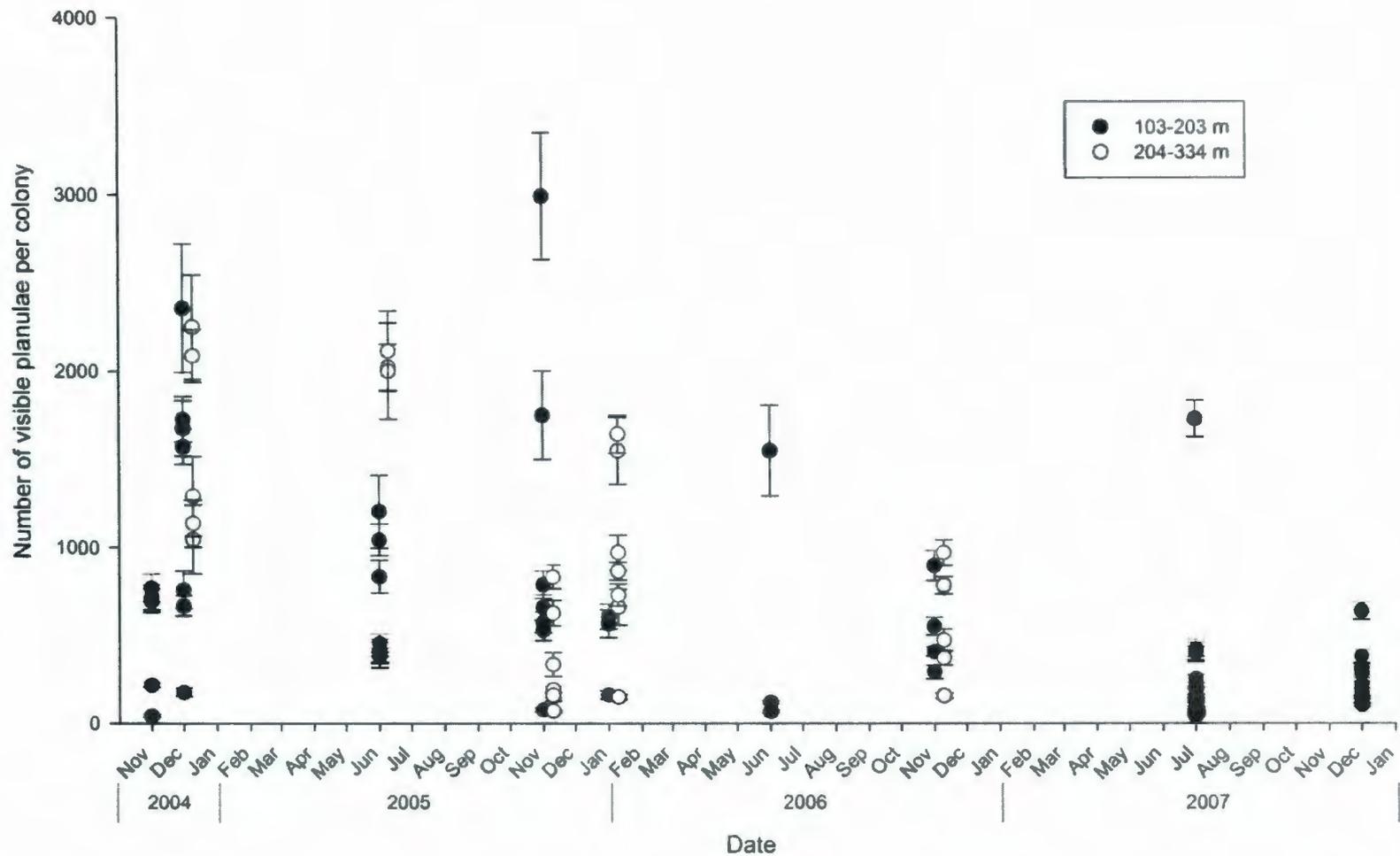


Figure 2-9. *Drifa glomerata*. Number of visible planulae per colony at each sampling date for both depth ranges (103-203 m, 204-334 m) from November 2004 to December 2007. All colonies with more than 10 fertile polyps were dissected to count the number of planulae. Data are expressed as Mean \pm SE (n=10).

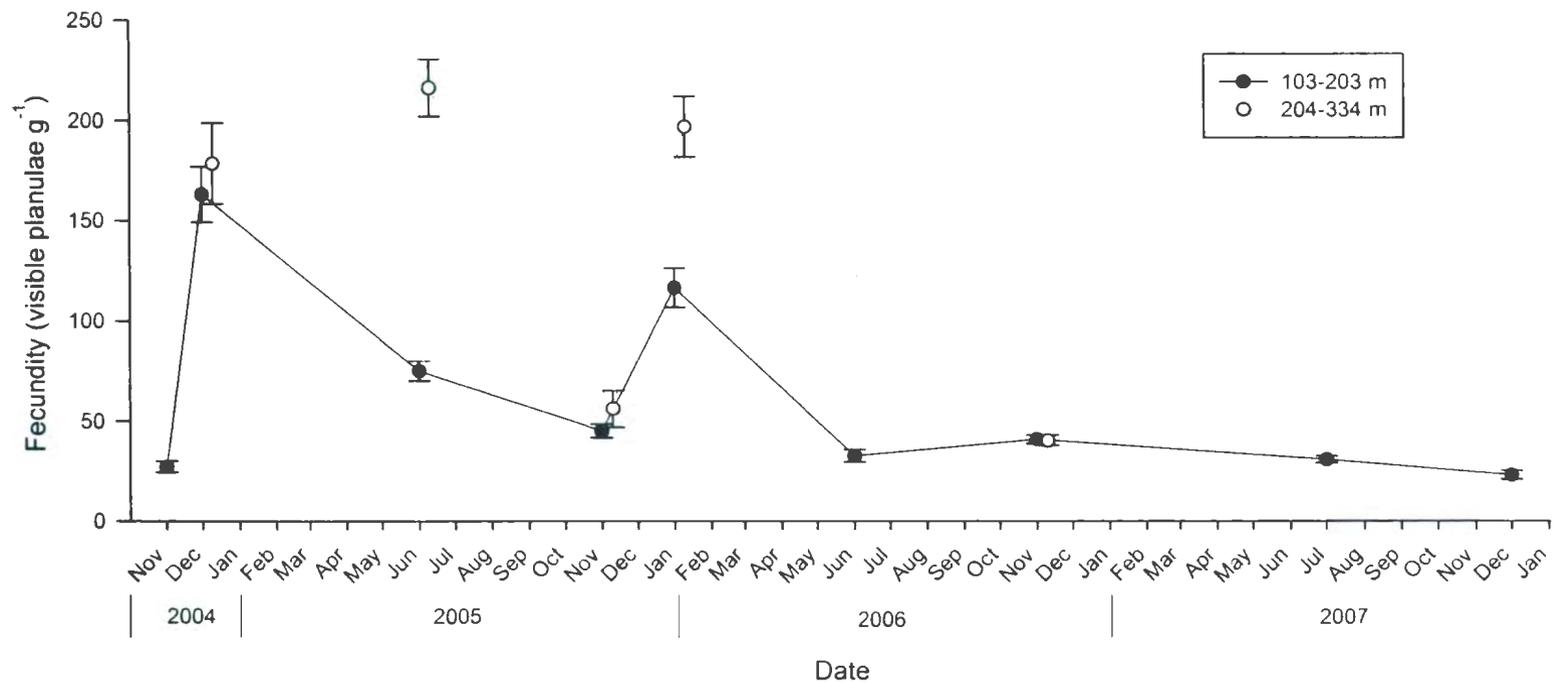


Figure 2-10. *Drifa glomerata*. Fecundity (planulae g⁻¹) at each sampling date and both depth ranges (103-203 m, 204-334 m) from November 2004 to December 2007. Data are expressed as the average of planulae in ten fertile polyps multiplied by the number of fertile polyps divided by the wet weight of the colony. Colonies from the same sampling date and depth range were pooled together (Mean ± SE, n=30-150).

trends were different. At 103-203 m, fecundity showed a seasonal trend in trend in 2004-2005 and 2005-2006, with an increase between November and December/January and lower values in June of a given year; on the other hand, at 204-334 m, fecundity was high in December/January and June, and low values were recorded in November. However, data from 2006-2007 did not follow the pattern in 2004-2005, 2005-2006: data were consistently low in November 2006, July and December 2007.

The average size of oocytes and planulae was similar at both depth ranges ($\chi^2_{1, 167}=0.07$, $p=0.793$), and in different sampling years ($\chi^2_{3, 167}=7.73$, $p=0.052$). Significant interactions were detected between the location of oocytes/planulae (branchlets and polyps) and sampling period ($\chi^2_{4, 167}=11.13$, $p=0.025$, Figure 2-11). More precisely, the surface area of oocytes in branchlets was not significantly different between sampling months ($\chi^2_{4, 80}=8.80$, $p=0.066$); however, the surface area of planulae in fertile polyps significantly differed between sampling periods ($\chi^2_{4, 86}=30.83$, $p<0.001$). The average size of planulae in fertile polyps decreased from November to June/July, and increased from June/July to November in a given year (Figure 2-11).

Environmental factors in the field from 2004 to 2007

From 2004 to 2007 at a depth of 150 m, temperature increased from July/August, peaked in January, and decreased from January to July (Figure 2-12). Phytoplankton abundance (indicated by chlorophyll fluorescence) was low in December/January and relatively high from April to July (Figure 2-12). Inversely, wind speed exhibited low values in June/July,

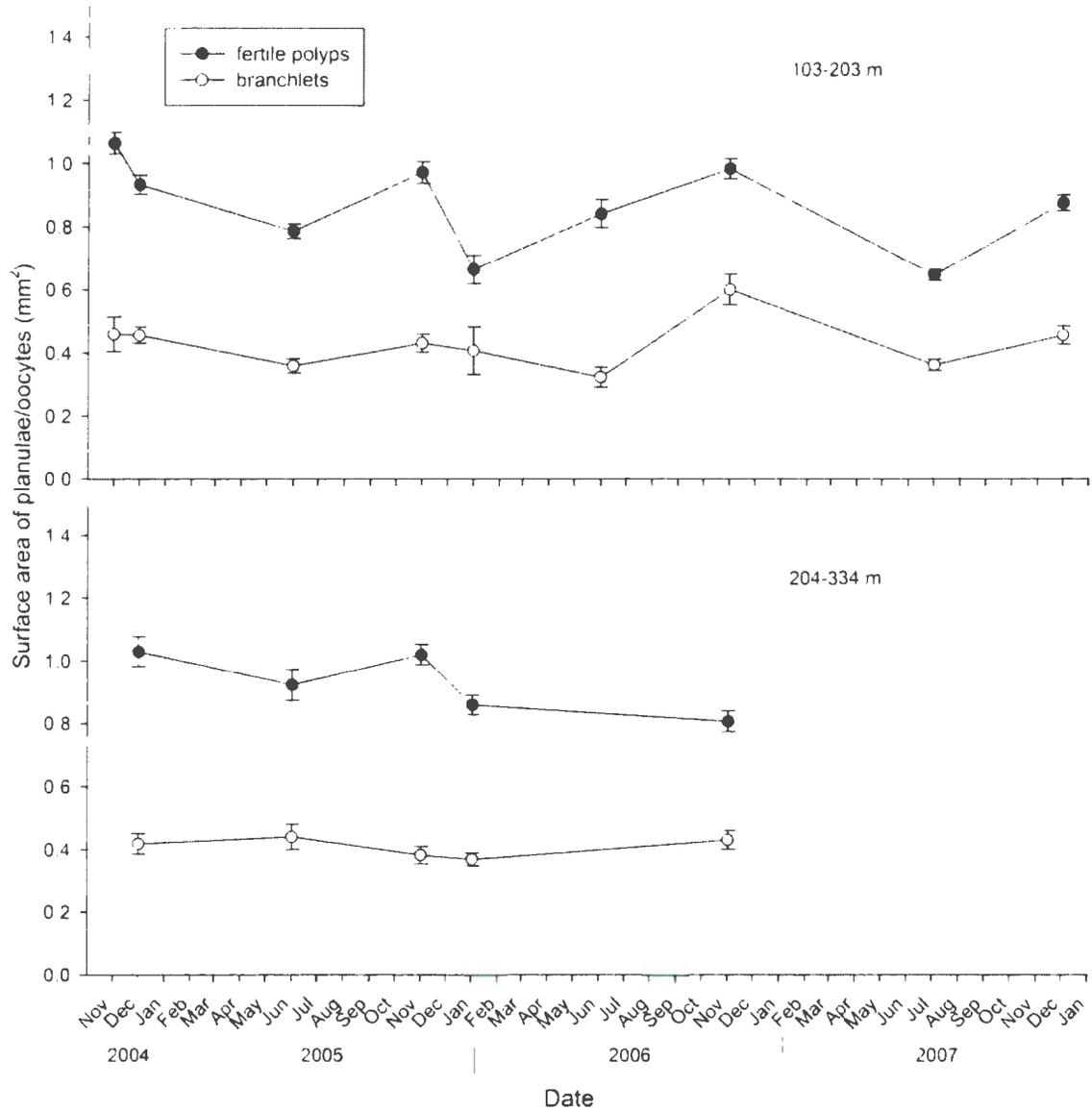


Figure 2-11. *Drifa glomerata*. Surface area of planulae/oocytes in fertile polyps and branchlets of all colonies at each sampling date and both depth ranges (103-203 m, 204-334 m) from November 2004 to December 2007. Data from the same sampling date and depth range were pooled together and expressed as Mean \pm SE, in fertile polyps (n=74-458), and in branchlets (n=6-114).

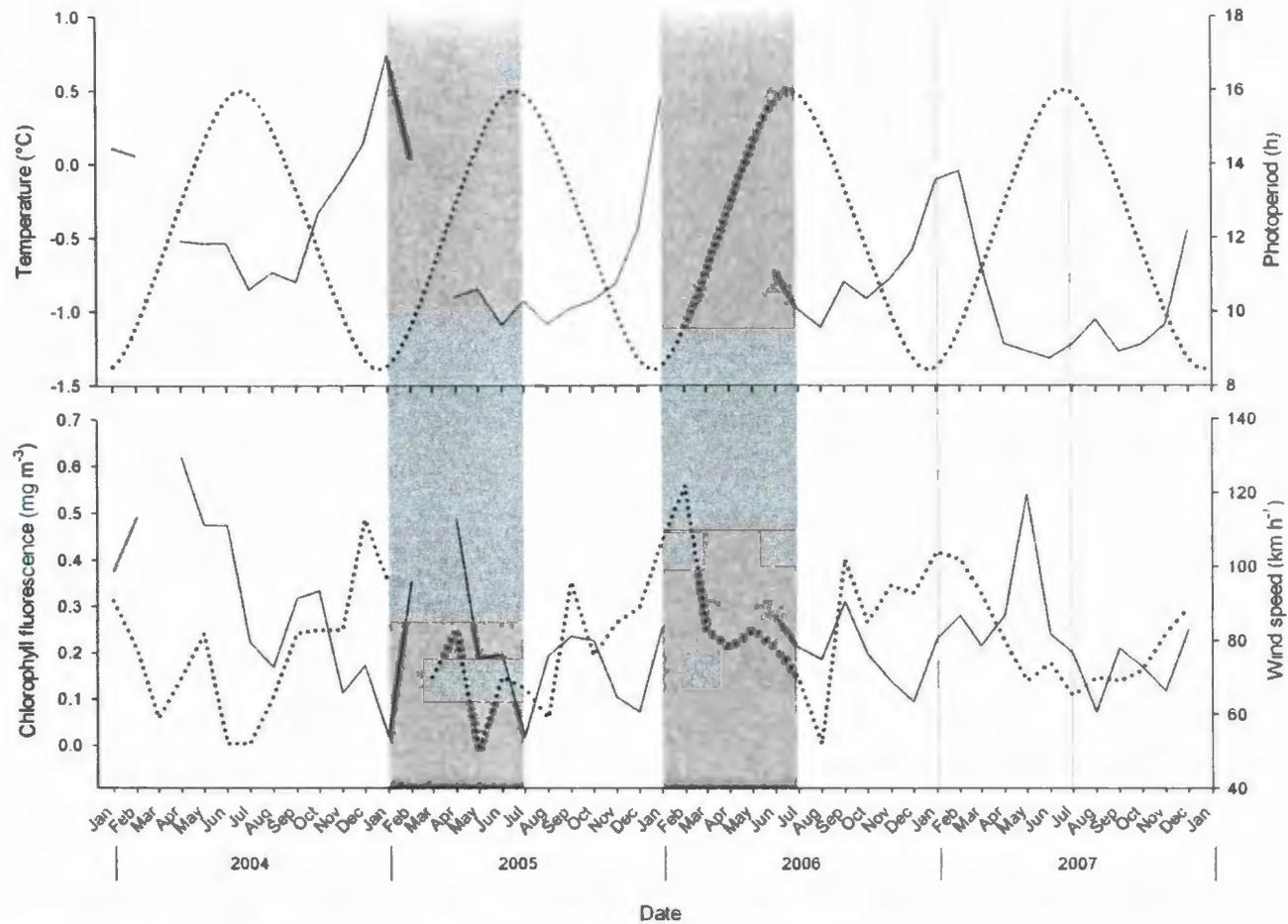


Figure 2-12. Environmental factors from January 2004 to December 2007 at a depth of 150 m, including temperature (solid line), photoperiod (dotted line), chlorophyll fluorescence (an indicator of phytoplankton abundance, solid line) and maximum wind speed (dotted line). The light grey areas represent the three peak planulation periods in two consecutive years 2004-2005 and 2005-2006. The dashed area represents the putative peak planulation period in the year 2006-2007.

a progressive increase in fall and the highest values of the year in January (Figure 2-12). Photoperiod started to increase in January to a maximum in the third week of June, followed by a progressive decrease until the third week of December.

Planulation and related environmental factors in the laboratory

Two live colonies of similar size were maintained under laboratory conditions for 11 months, and only one possessed fertile polyps. The first release of planula in the laboratory occurred on January 4, 2008, six months after collection, when a single planula was expelled through the mouth of a fertile polyp. The average length of fully extended planulae was ca. 4-5 mm, and the maximum length recorded was ca. 6 mm (Figure 2-13). Planulation lasted through June 2008, with a total of 74 planulae released by the single colony (Figure 2-14). Planulae were released at any time of the day without any detectable diel patterns. The colony released 1 to 4 planulae in a single day, generally from different polyps. The simultaneous release of more than one planula from the same polyp was rarely observed.

Planulation started as day length began to increase in January 2008. This period corresponded to low seawater temperatures of ca. 2 °C. More planulation events were noted in March/April after the period of yearly maximum wind speeds, as temperature started to increase after its yearly minimum (Figure 2-14). Major increases in spring phytoplankton bloom and temperature in mid-May coincided with maximum planulation recorded during my study. No clear lunar pattern was noted ($\chi^2_{3, 135}=5.59, p=0.232$).

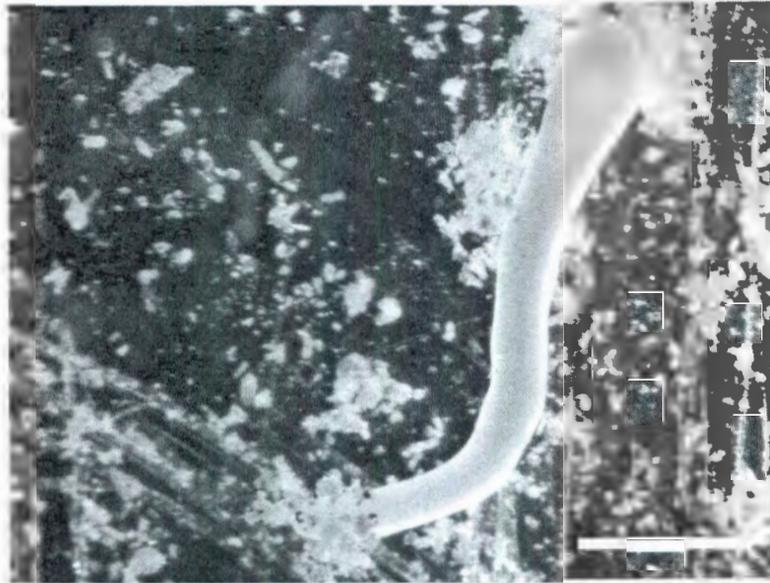


Figure 2-13. *Drifa glomerata*. Newly released planula. The scale bar represents 1 mm.

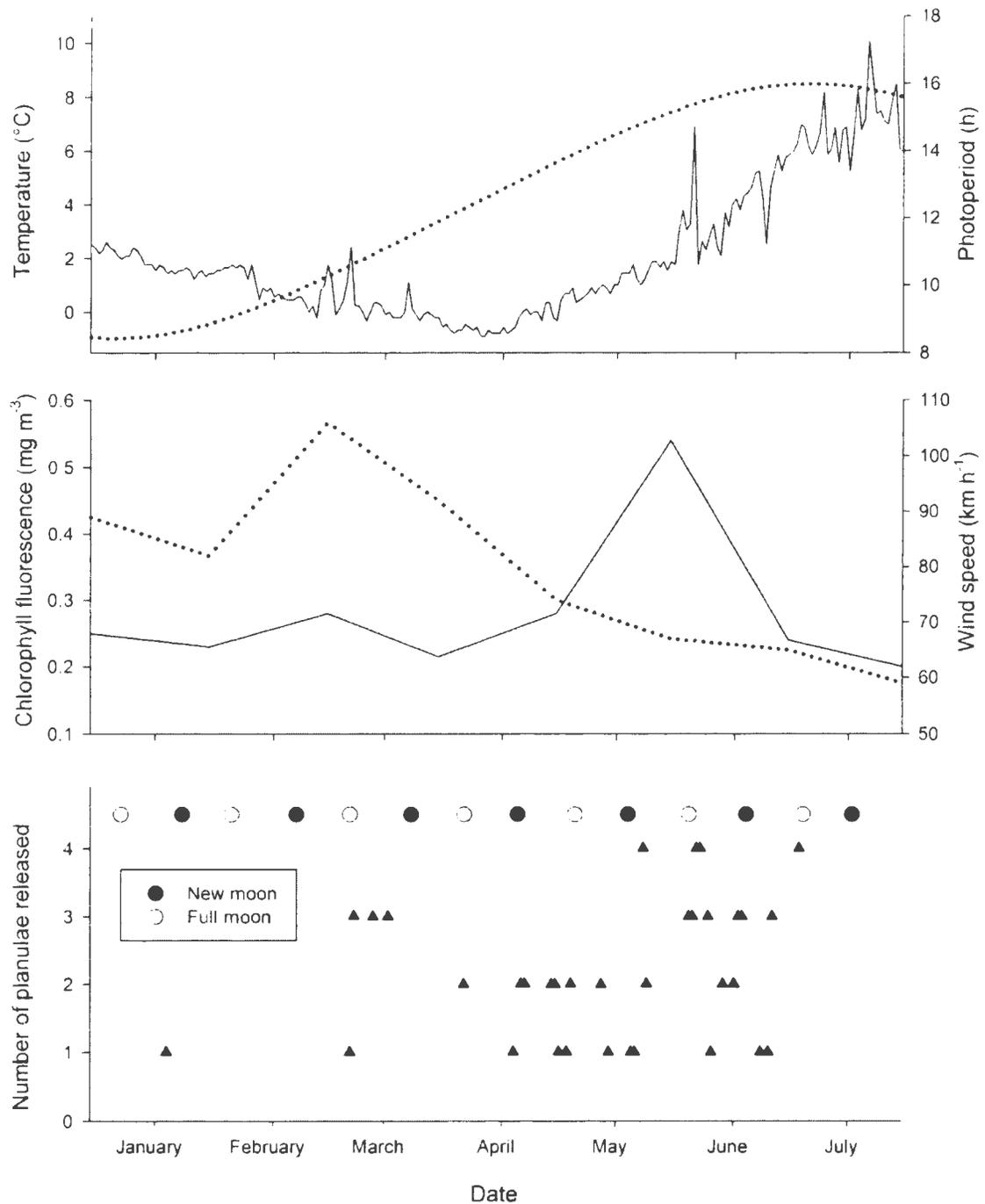


Figure 2-14. *Drifa glomerata*. Planulation events observed in a live colony between January and June 2008 in correlation with temperature (solid line), photoperiod (dotted line), chlorophyll fluorescence (an indicator of phytoplankton abundance, solid line), wind speed (dotted line) and lunar cycle.

2.5 Discussion

The present study elucidates for the first time certain aspects of the reproductive cycle and strategies of a deep-sea nephtheid and suggests the importance of specific environmental factors in the observed timing. It also provides a rare account of reproduction of live deep-sea coral colonies, thus allowing the study of planulation periodicity and behaviour under controlled laboratory conditions.

Marine invertebrates can usually be categorized as either hermaphroditic or gonochoric; however, sexual reproduction in corals has been shown to be very complex (Fadlallah 1983; Benayahu 1991). Sometimes only individuals of one sex are observed. For example, no male or hermaphroditic colonies were found during monthly samplings over three years in an undescribed Caribbean gorgonian species at San Blas Point, Panama (Brazeau and Lasker 1989). Similarly, no male gametes were observed in the 135 colonies of *Drifa glomerata* dissected during my investigation. Three possible explanations are proposed: (1) Freezing may have deteriorated any male tissues beyond recognition. (2) The concentration of sampling dates in January, June, July, November and December may have missed spermatogenesis, if the process is short-lived. The research of Waller and Tyler (2005) on the deep-sea scleractinian *Lophelia pertusa* lends support to this assumption. Samples of *L. pertusa* were collected in March, July, August, September and October; however, male colonies were only found in October. In addition, spermatogenesis has been reported to be shorter than oogenesis in several scleractinians and octocorals (Yamazato et al. 1981; Benayahu and Loya 1986; Szmant 1986; Brazeau

and Lasker 1990). (3) *D. glomerata* is a parthenogenetic or female-dominated hermaphroditic species. Parthenogenesis has been reported in the soft coral *Alcyonium hibernicum* from Great Britain (Hartnoll 1977) and in an undetermined gorgonian species from the Caribbean (Brazeau and Lasker 1989). In addition to parthenogenesis the latter investigators suggested a skewed sex ratio (extremely rare males) as another explanation for the absence of male colonies.

The occurrence of fertile colonies all year round suggests prolonged/continuous development of larvae at the population level, which is common in both shallow and deep-water brooders (Fadlallah 1983; Benayahu and Loya 1984; Ben-David-Zaslow et al. 1999) and some deep-sea broadcasters (Waller et al. 2002; Flint et al. 2007). In the present study, the live colony of *D. glomerata* spawned in the laboratory from January to June 2008 which was consistent with the analysis of preserved samples from 2004-2007. It remains unclear whether planulation could be continuous, but with a seasonal peak at the population level. However, year long planulation with seasonal peak was observed in *Drifa* sp. (Chapter 3), an as yet unidentified species that was collected in the same geographical area.

The consistently smaller size of sexual cells in branchlets than in fertile polyps and the evidence of planulae occurring only in the fertile polyps indicates that the development pathway is from branchlets to polyps. In addition, the number of planulae inside a single fertile polyp was not influenced by season, which suggests that migration of oocytes to

fertile polyps and the release of mature planulae occur at the same time at the level of individual polyps.

On the other hand, fecundity (i.e. number of planulae g^{-1}) was directly linked to the FPI (i.e. number of fertile polyps g^{-1}). Thus, the decrease (from December/January to June/July) observed in fecundity and FPI at the colony level were probably coincident with the major release of mature planulae. This interpretation was confirmed by the decrease in the size of planulae in fertile polyps from November to June/July in a given year, possibly indicating the generation of small-sized planulae and also the major release of mature planulae in December or early January. Furthermore, the increase in the size of planulae from June/July to November possibly indicates the growth of new oocytes and/or planulae. In summary, the seasonal trends recorded in some of the parameters suggest major planulation events occurred between January and June/July from 2004 to 2006. The seasonal pattern observed was consistent with planula release in the laboratory from January to June 2008. However, the seasonal pattern was only detected in samples from ca. 100-200 m; and reproductive activities appeared to be more constant throughout the year in colonies from ca. 200-300 m depth.

Benayahu (1997) observed that most soft corals reproduced during summer, coinciding with high water temperature in the Gulf of Eilat (Red Sea). Similarly, Glassom et al. (2004) found that coral recruitment was high in summer and low in autumn-winter in shallow waters along the coastline of Eilat (northern Red Sea). The influence of a

seasonal flux in food availability (i.e. phytodetritus) on reproduction has been documented for several species of benthic invertebrates (Tyler et al. 1982; Wigham et al. 2003). Recently, a biannual pattern of spawning related to water temperature fluctuations was observed in a deep-water asteroid from the same general geographical area (Mercier and Hamel 2008). Similar driving forces may help to synchronise the reproductive cycle of *D. glomerata* in eastern Canada. From December to January, the increasing temperature might initiate the breeding period, given that maximum planulation coincided with the rapid increase in seawater temperature. On the other hand, the low abundance of phytoplankton in December and January probably had no influence on the already well-developed planulae of *D. glomerata*. Nevertheless, the strong winds in late fall and winter could enhance the downward mixing of particulate food and compensate for low phytoplankton abundance in winter. The increase in bottom temperature and food availability may benefit the growth of new cohorts of oocytes/planulae and favour the major planulation from December/January to June/July of the following year. In addition, higher phytoplankton abundance from April to May/June could be beneficial for the growth of newly settled polyps of *D. glomerata*, which, according to my laboratory observations begin to feed a few days after settlement.

Consistent with the FPI patterns observed in preserved samples, planulation in the laboratory started in January 2008 and continued until June 2008 in parallel with the increase of day length, suggesting that photoperiod may be an important mediating factor. Although it is usually deemed irrelevant in the deep sea (Young 1994; Young 2003),

photoperiod should perhaps not be dismissed in trying to explain the patterns observed at the depths studied (100-300 m). The limit of downwelling illumination (i.e. twilight zone) is commonly set at ca. 1000 m (Schiebel et al. 2007), suggesting that some light could reach down to 300 m in mildly productive waters such as the ones around Newfoundland. The deep-water soft coral populations studied here could thus receive faint light cues associated with seasonal day length fluctuations. Deep-sea crustaceans have already been shown to respond to light intensities occurring at depths of 500-600 m (Frank and Widder 1994; Frank and Widder 1996). Moreover, the influence of light cycles might explain why seasonal breeders in the bathyal zone are apparently more common below clear tropical seas than temperate ones (Young 2003).

Depths may also have significant influence on the reproduction pattern of deep-sea corals. For example, most of the high values of FPI ($>50 \text{ g}^{-1}$) were observed in the deeper water population below 200 m. However, colonies heavier than 20 g were only found in shallower water. A possible explanation is that colonies from the deeper habitat reach reproductive maturity at a smaller size. Flint et al. (2007) found that fecundity decreased with depth as a result of lowered food availability, however, the opposite pattern was observed in the research of Waller et al. (2005) on three hermaphroditic deep-sea scleractinian corals. In this study, the smaller size at sexual maturity and the increased FPI and fecundity with depth are possibly due to the warmer water temperatures at depths of 200-300 m compared to 100-200 m.

On a broader scale, certain aspects of reproductive strategies in corals can vary according to geographical locations. For example, the soft coral *Heteroxenia coheni* is hermaphroditic in the Red Sea but gonochoric on the Great Barrier Reef (Benayahu et al. 1990). In this study, the sampling area was initially divided into two geographical locations; however, data were pooled together because of the limited number of samples and similar bottom temperature differences detected at 100-200 m and 200-300 m at both locations. Further studies comparing the influence of geographical location would be valuable.

Most of the research on deep-sea corals has been restricted by limited sampling opportunities, low number of samples and spatially scattered sampling locations. Because the reproductive features of deep-sea corals appear to be extremely complex and variable, more complete sampling series and better preserved samples would be needed to strengthen my conclusions. This study has shown that complementary investigations of live organisms are also instrumental in pursuing this goal.

2.6 Acknowledgement

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**CHAPTER 3 : PLANULA RELEASE, SETTLEMENT,
METAMORPHOSIS AND GROWTH IN TWO *DRIFA* SPECIES
OF DEEP-SEA SOFT CORALS**



Planula release in *Drifa* sp.

3.1 Abstract

The life history of deep-sea octocorals has rarely been studied, mostly because of the difficulty in collecting and maintaining live specimens under laboratory conditions. Here I present data on the reproductive biology, timing of larval release, settlement, and early growth of *Drifa glomerata* and a second species of *Drifa*, as yet unidentified. Specimens collected off the SW Grand Banks (eastern Canada) were monitored from July 2007 to April 2009. Planulae of *Drifa* sp. were released throughout the year with a peak season in October and November 2007. Planulation of *Drifa glomerata* was observed in a single colony from January to June 2008. Both species had large planulae (ca. 3-5 mm long) and the largest observed planula was ca. 6 mm for *D. glomerata*. In *Drifa* sp, planulae exhibited cycles of contraction and expansion that allowed them to alternately sink to the bottom and float in the water column. In *D. glomerata*, the demersal planulae displayed complex crawling behaviours. Settlement of *Drifa* sp. usually occurred 1 to 30 d after release, though a small portion of planulae took >3 mo to settle; for *D. glomerata*, all planulae settled within one month and a few planulae metamorphosed prior to settlement. Settlement trials showed that planulae of both *Drifa* spp. settled more readily on hard irregular substrata (i.e. shells and rough artificial surfaces) than on smooth surfaces. After settlement, the eight primary mesenteries appeared within 24 h, and polyps developed small pinnules and reached the maximum size after two to three months post settlement. The first branching polyp was observed after ca. 9 months of growth in *D. glomerata*, whereas no evidence of branching was detected in *Drifa* sp. over the period of study.

3.2 Introduction

Although deep-sea corals are important constituents of marine ecosystems (Freiwald et al. 2004) and there is increasing concern over their destruction worldwide (Gass and Willison 2005; Roberts et al. 2006), research on the reproductive biology of deep-water and cold-water corals remains scarce. Most of the limited information gathered to date has largely focused on stony corals (order Scleractinia) and a few horny corals (order Gorgonacea), whereas little attention has been given to soft corals (order Alcyonacea), despite their prevalence and importance in deep-sea habitats (Dinesen 1983; Freiwald et al. 2004; Watling and Auster 2005).

Soft corals in the family Nephtheidae are widely distributed in temperate and cold waters around the world, including the deep ocean: they have been mainly recorded in the North West Atlantic, North East and West Pacific and Indian Ocean. However, little research has been conducted on their reproduction and development (Farrant 1986; Benayahu et al. 1992; Dahan and Benayahu 1997; Hwang and Song 2007; Sun et al 2009; and also Chapters 2 and 4). This gap might be attributed to the morphological diversity of members of this family, which makes identification very problematic (Watling and Auster 2005). In contrast, much knowledge has been gathered from the study of shallow reef corals, especially scleractinian species. The reproduction of octocorals has mainly been investigated in shallow-water Alcyoniidae, Xeniidae, and Gorgoniidae from the tropical Pacific, Red Sea, and the Caribbean (Benayahu 1991; Shlesinger et al. 1998).

Shallow-water corals are primarily classified as broadcasters or brooders. Broadcasters generally have synchronous cycles of gametogenesis; while brooders have protracted and asynchronous gametogenesis and larval release (i.e., planulation). Furthermore, spawning patterns in corals are very diverse. For instance, synchronous or asynchronous spawning, seasonal or (so-called) continuous reproduction, lunar or shifted lunar spawning have been reported. In addition, the patterns of sexual reproduction have been correlated to several factors, such as temperature, tidal, lunar and solar cycles (Jokiel and Guinther 1978; Stimson 1978; Benayahu and Loya 1983; Benayahu and Loya 1984; Benayahu 1997; Ben-David-Zaslow et al. 1999).

The free swimming larval stage is an important life history phase that increases the chance of finding suitable substrata (Müller and Leitz 2002), and settlement and recruitment are major processes in the life history of most benthic marine invertebrates. Rodriguez et al. (1993), defined settlement as the stage where larvae search for suitable substrata and undergo metamorphosis. Chemical or biological cues are usually essential to induce settlement. For instance, some crustose coralline algae and bacteria have been shown to induce larval metamorphosis and selection of suitable habitats (Heyward and Negri 1999; Baird et al. 2003; Harrington et al. 2004). Environmental factors, such as seawater temperature, depth, current, physical texture and orientation of substrata also influence the time to settlement and metamorphosis (Atoda 1951; Jokiel and Guinther 1978; Hodgson 1985; Rogers 1990; Abelson 1997; Fabricius 1997; Heyward and Negri 1999; Baird et al. 2003; Harrington et al. 2004). Most of the settlement preference

research was performed on shallow-water tropical corals; however, research has rarely been carried out in deep-sea corals, most likely as a result of the difficulties associated with maintaining live animals and having them spawn in the laboratory.

Recruitment is the post-settlement stage during which newly settled individuals reach a certain size (Rodriguez et al. 1993); it is essential in the maintenance and recovery of coral reef systems (Glassom et al. 2006). The type of larva and its competency period are important indications of the probability of local recruitment and long-range dispersal (Richmond 1987). In shallow-water coral species, several factors have been demonstrated to influence the competency period and settlement/metamorphosis rates of coral larvae (i.e., planulae). For example, broadcasters usually release gametes into the water column where fertilization occurs and is followed by a long planktonic larval phase, thereby favouring long-range dispersal. Conversely, brooders rear the larvae internally or externally, releasing mature planulae, which are ready to settle within the parental habitat, typically resulting in more restricted dispersal (Sebens 1983a and b; Harrison and Wallace 1990; Richmond and Hunter 1990). Local dispersal and aggregation ensures that planulae settle within appropriate habitats and are helpful in maintaining the locally-adapted populations; however, it decreases colonizing ability (Sebens 1983b), and affects population genetic structure (Ayre and Miller 2004).

Very limited information exists on the reproductive biology of deep-sea corals, particularly with respect to larval release, settlement, and dispersal potential in brooding

species. This work focused on two deep-sea nephtheids, *Drifa* sp. and *D. glomerata*, with the goal of examining various aspects of their life histories and expanding our understanding of deep-water coral biology. By using histological procedures, transmission electron microscopy (TEM) and monitoring live specimens for a year in the laboratory, the development and arrangement of oocytes/planulae, larval type and behaviour, planulation periodicities, time to metamorphosis and settlement, substratum selection and growth were elucidated. In addition, the environmental factors that may influence the larval competency period and the recruitment rates were suggested.

3.3 Materials and Methods

Information from the field

Still images and video footage from the expedition (see details below) were analyzed to evaluate typical substrata utilization, orientation and aggregation of soft coral colonies. Particular attention was given to images and videos captured at depths of ca. 500 m and 1200 m in areas where the live specimens were collected.

Collection and maintenance of adult colonies

Twenty-six adult colonies of *Drifa* sp. were collected in July 2007 at two target depths of ca. 500 and ca. 1200 m on the continental slope of the SW Grand Banks, using the remotely operated vehicle ROPOS on board the CCGS *Hudson* (Table 3-1 and Figure 3-1). Samples attached to small rocks or tiny pieces of firm substratum sparsely

Table 3-1. Depth and coordinates of collection sites of *Drifa* sp. (1-4) and *D. glomerata* (5 and 6) in July 2007.

Sample*	Depth (m)	Latitude (N)	Longitude (W)	Number of reproductive colonies /Number of colonies
1	495	44°58'36"	54°58'51"	1/1
2	526	44°49'18"	54°28'23 "	10/10
3	744	44°49'45"	55°33'41"	2/2
4	1238	44°13'04"	53°07'14"	11 /13
5	358	44°21'38"	53°15'57"	1/1
6	358	44°21'38"	53°15'58"	1/1

* Corresponding to the samples identified in Figure 3-1.

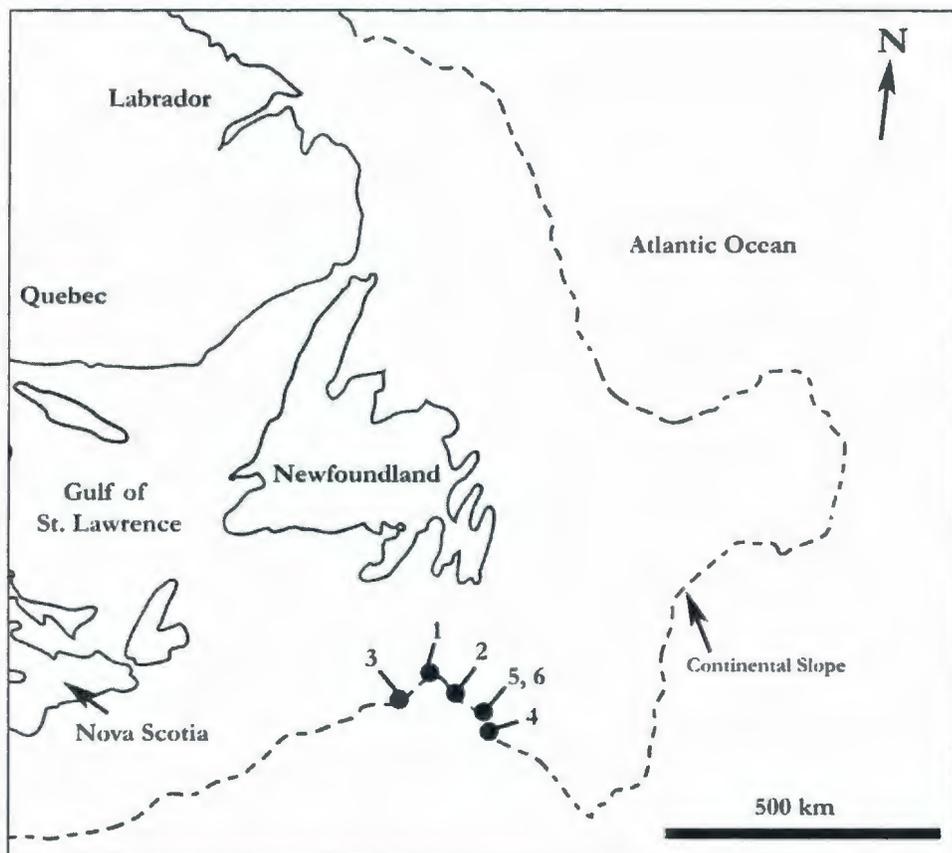


Figure 3-1. Locations where samples of *Drifa* sp. and *Drifa glomerata* were collected in the northwestern Atlantic. Information on corresponding sample numbers can be found in Table 3-1.

distributed on mud were the best candidates for collection, and were obtained using the articulated claw or a specially designed scooping device fitted to the claw. Aboard the ship, specimens were maintained in tanks provided with slowly flowing chilled seawater (2-3 °C) placed in a dark refrigerated compartment. In the laboratory, colonies were sorted according to sampling depth. Individuals from similar locations (Table 3-1) were maintained together in one or two 20-L tanks based on the number of samples (2-9 individuals per tank), and were provided with running seawater (ca. 1.5 L min⁻¹) in total darkness. All tanks were supplied with natural unfiltered seawater at ambient temperature (between -1 and 9 °C); however, from July to November 2007, when surface temperatures are higher than deep-water temperatures, the seawater was chilled to maintain it below 10 °C. Similarly, two adult colonies of *D. glomerata* were collected at a depth of ca. 350 m on the continental slope of the SW Grand Banks, and maintained under the same conditions as described above for *Drifa* sp. (Table 3-1 and Figure 3-1).

The number of colonies that possessed gametes and planulae was recorded for both *Drifa* species from all depth ranges (Table 3-1). Except for seven colonies of *Drifa* sp. That were set aside for histological analysis (see below), all colonies of both species were observed daily to monitor the behaviour, with a particular focus on natural planulation.

Collection and culture of planulae

Daily monitoring of reproductive behaviour extended from July 2007 to June 2008.

Planulation episodes were directly observed on multiple occasions, allowing a detailed

description of natural planula release. Otherwise, planulae of both *Drifa* species were routinely collected within 24 h of their release, either on the bottom of the tank or in the water column. The free-swimming planulae were reared in culture plates in semi-static conditions with half of the seawater changed every day until settlement 2-98 days post release. Developing larvae were lecithotrophic and subsequently not fed during this period. Some individuals were used in settlement preference trials as detailed below. Newly-settled primary polyps were maintained under flow-through conditions similar to those used for adult colonies and fed a mixture of algae (*Isochrysis* sp., *Tetraselmis* sp., *Nannochloropsis* sp.) and rotifers on a continuous basis via a peristaltic pump (ca. 40 ml min⁻¹). A few planulae of *Drifa* sp. were able to settle within 24 h on the rocks or tank walls before the daily collection; thus, the total number of polyps settled inside the holding tanks was counted in June 2008, when this experiment ended.

In addition, planulae of *Drifa* sp. were surgically extracted from seven colonies to determine whether natural release was a prerequisite to successful development to settlement, and whether surgical extraction would affect planula behaviour and metamorphosis. The colonies used for this experiment were collected on four different dives at depths of 495, 525, 744, and 1238 m; and colony size ranged from 2.3-4.0 cm in length and 1.5-2.5 cm in width when extended (Table 3-2). The reproductive polyps were cut open and planulae were gently pushed out into the surrounding seawater. Planulae were cultured in 50-ml beakers at a density of ca. 30 individuals per beaker, in running chilled seawater, at a temperature of 5-8 °C, under darkened conditions. Cultures were

Table 3-2. Settlement of planulae extracted surgically from colonies of *Drifa* sp. The depth at which the adult colony was collected, the size of adult colony, the number of large (elongated) and small (round) planulae extracted and the number of settled planulae after one month (August 2007) are presented.

Colony	Depth (m)	Length X width (cm)	Number of large planulae	Number of small planulae	Number of settled planulae
1	495	3.5 X 1.6	33	34	15
2	526	2.3 X 1.5	23	65	15
3	526	2.8 X 1.5	0	10	0
4	744	3.5 X 1.5	0	44	0
5	744	5.0 X 2.5	20	33	15
6	1238	4.0 X 2.5	9	72	9
7	1238	3.0 X 2.5	40	142	23

monitored daily to record larval behaviour, the occurrence of settlement, and subsequent growth in order to compare patterns to those in naturally-released larvae.

Histology and transmission electronic microscopy (TEM)

During the extraction of planulae, one branch with visible reproductive polyps of each *Drifa* sp. colony was preserved in 4 % formaldehyde for standard histological analysis. Another piece of tissue was preserved in 70 % ethyl alcohol for the study of sclerites (skeletal elements), and two branches with reproductive polyps were preserved in 3 % glutaraldehyde for histological preparation using standard techniques, except that the embedding medium was methacrylate instead of paraffin. Using a terminology adapted from Cordes et al. (2001), oocytes were divided into four stages: Stage I, oogonia budding from the mesenteries; Stage II, previtellogenic oocytes containing a large nucleus; Stage III, vitellogenic oocytes, characterized by the rapid accumulation of yolk; Stage IV, late vitellogenic oocytes that are mature and filled with yolk droplets, which stain a conspicuous pink colour. Sclerite and histological analysis of *Drifa glomerata* was performed on serially collected samples in the course of a related study (Chapter 2).

Additional planulae of *Drifa* sp. were collected and fixed in 3 % glutaraldehyde for processing using transmission electron microscopy (TEM) techniques. Tissues were placed in Karnovsky fixative and transferred to 1 M sodium cacodylate buffer, then dehydrated and infiltrated in 1 % osmium tetroxide, 1 M Na cacodylate buffer, successive ethanol baths (70-100 %), followed by absolute acetone, 50:50 acetone and TAAB 812

resin. Samples were embedded in flat moulds in order to ensure correct orientation, and were then polymerized at 80 °C overnight. Polymerized blocks were trimmed and then cut at 0.5 µm sections on a Leica Ultra Cut E ultramicrotome using a diamond knife. Sections were stained with 1 % toluidine blue in 1 % aqueous sodium borate. Light microscopy observations were made in under a Nikon SMZ1500 stereomicroscope and Nikon Eclipse 80i microscope, both of which were attached to a Nikon DXM1200F digital camera.

Settlement preferences

Given the difficulty of obtaining planulae, the experiments outlined here were conducted on an opportunity basis and were limited by the small number of available planulae (i.e. planulae were only released a few at a time). Furthermore, because of protracted planula competency, which sometimes extended to several months, this series of experiments was particularly challenging and required unexpected time and space to gather a modest amount of data.

In *Drifa* sp., naturally-released planulae were studied in order to determine the time to settlement and metamorphosis on different substrata. Planulae released at approximately the same time by colonies from similar depths were used to test the following independent treatments: (1) NP: microbially-conditioned culture plates (i.e., placed in running seawater for at least 7 days to allow a biofilm to develop); (2) NS: conditioned culture plates with shell fragments that had been conditioned in running seawater for

several months; (3) NPS: conditioned culture plates that had been sanded to create an irregular surface; (4) CS: culture plates with shell fragments that were cleaned in freshwater every day; (5) CP: culture plates which were cleaned in freshwater every day. Each condition was tested using 1-9 planulae at a time and replicated independently as many times as possible, depending on the availability of significant batches of planulae; the total number of planulae exposed to each treatment varied from 8 to 75.

To investigate the settlement preferences and assess whether the larvae exhibit some type of substratum selectivity, I also performed pair-wise experiments where I offered the planulae a choice between substrata. I compared natural versus artificial substrata, rough versus smooth surfaces, and naturally conditioned versus clean surfaces. More precisely, three pairs of substrata were tested, as permitted by the limited number of planulae available, typically using 12-27 planulae for each trial, with 4-8 trials per condition: (1) NS (natural shell fragments) versus NPS (sanded and conditioned Petri dish surface): the surface area of shells covered half of the surface area of the Petri dish; (2) R (rough) versus S (smooth) surface: half the side of a Petri dish was sanded, then conditioned in running seawater for several days prior to use; (3) N (natural) versus C (clean) surface: conditioned Petri dish with half of the surface cleaned every other day (prior to use, each Petri dish was conditioned in running seawater for several days, and half of its surface was cleaned every day). Planulae were monitored daily for one month after their release to record the time to settlement and the number of settled planulae on each substratum.

In *Drifa glomerata*, because planulation occurred in one single colony, the limited numbers of naturally-released planulae were used to perform only three pair-wise trials. The first two treatments are the same as for *Drifa* sp.: (1) NS (natural shell fragments) versus NPS (sanded and conditioned Petri dish surface); (2) R (rough) versus S (smooth) surface. However, in the third treatment with N (natural) versus C (clean) surface, the Petri dishes were sanded prior to use, based on preliminary indication of a preference for rough substrata. The number of planulae tested for each trial varied from 12 to 27, with 4-8 trials per condition.

Environmental factors

Information on seawater temperatures in the laboratory during the rearing and experimental period in 2007 was gathered using a temperature-light logger HOBO Pendant (UA-002-XX) placed in one of the holding tanks. Information on annual phytoplankton abundance (using chlorophyll fluorescence as an indicator) was obtained from the Department of Fisheries and Oceans Canada for station 27 (<http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/azmp-pmza/hydro/index-eng.html>, fixed zonal monitoring program; 47°32'04"N 52°35'06"W) at a depth of ca. 150 m from July 2007 to July 2008. Information on maximum wind speed (as an indicator of the amount of resuspended organic and inorganic materials) was gathered from Environment Canada, using data collected at St John's Airport (47°22'19"N 52°26'38"W) 140.5 m above sea level from July 2007 to July 2008

(http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html). Lunar cycle data were obtained from the StarDate Online website (<http://stardate.org/nightsky/moon/>).

Statistical analyses

The generalized linear model procedure (GENMOD) of SAS (SAS 1988) was used for testing. Poisson regressions were used to test the influence of depth range (500 m or 1200 m) and lunar phase [new moon (three days before and after new moon), first quarter moon (week between new moon and full moon), full moon (three days before and after full moon), third quarter moon (week between full moon and new moon)] on the planulation of *Drifa* sp. Data from independent treatments were analyzed with logistic regression (binomial distribution, logit link) to test the influences of depth range (500 m or 1200 m) and substratum categories (NP, NS, NPS, CS, CP) on the proportion of settled planulae; and regressions were used to test the same factors on time to settlement. In addition, data from pair-wise treatments were analyzed with logistic regression (binomial distribution, logit link) to test the influence of different substratum types (NS versus NPS, R versus S, N versus C) on the proportion of settled planulae of *Drifa* sp. and *D. glomerata*. Residuals were examined to evaluate the assumptions of homogeneity, independence and normality. If a normal error distribution was not appropriate, gamma or Poisson error distributions were used (McCullagh and Nelder, 1987). Tolerance of type I error was set at $\alpha=0.05$.

3.4 Results

Information from the field

At depths shallower than 500 m, most of the colonies occurred on cobbles, rocks or rocky cliffs, both horizontally and vertically. On all photographs from the ROV, the analyses suggested gregarious distribution with few individuals occurring in isolation. At 1200 m, the substratum was primarily mud, with very few occurrences of cobbles or rock surfaces. Colonies were sparse on mud and occurred in clusters where appropriate hard substrata were available, such as bedrock, pebbles, granules, sponges, empty shells, tube worms, etc. (Figure 3-2, Table 3-3).

Reproductive features

In *Drifa* sp., female sexual cells (oocytes) and planulae tend to be pinkish in colour, whereas the male sexual organs (spermaries) are white and larger in size than the planulae (Figure 3-3). All stages of female gametes co-existed within a single individual based on the histological sections of August 2007 colonies. Oocytes at stage I or II were attached to/embedded in the mesenteries (Figure 3-4) and the maturing oocytes (stages III and IV) migrated to the coelenteron. Stage I oocytes were $38.1 \pm 2.8 \mu\text{m}$ (Mean \pm SE, n=30) in diameter, stage II were $111.1 \pm 6.6 \mu\text{m}$ (n=23), stage III were $242.0 \pm 17.0 \mu\text{m}$ (n=15), and stage IV $490.1 \pm 23.8 \mu\text{m}$ (n=15). No embryos were observed in any of the colonies and planulae were brooded inside the polyps.

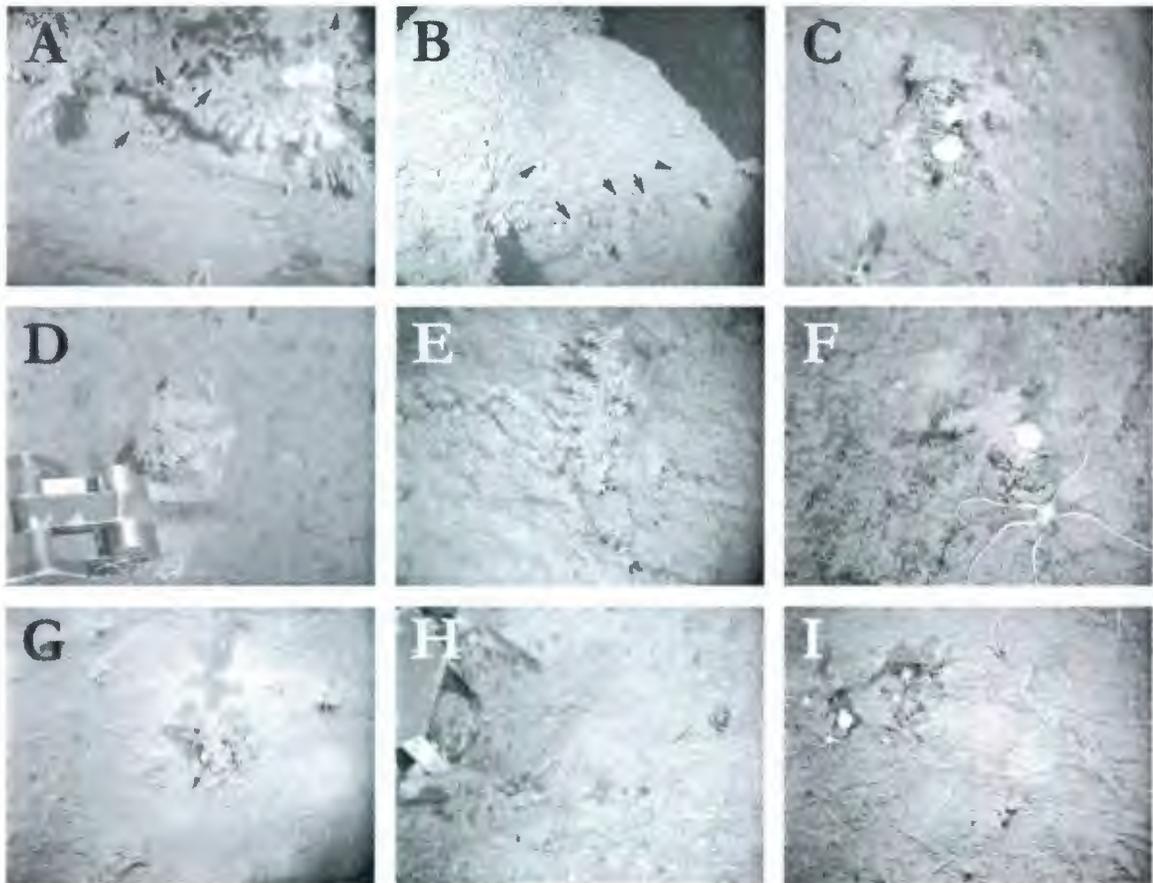


Figure 3-2. Field observations of *Drifa* sp. A-B) Colonies settled on cliff, arrows indicate *Drifa* sp. colonies. C-D) Colonies settled on rock. E) Colony settled on mud without any apparent firm substratum. F-I) Colonies settled on sponges or other organisms. The depths and coordinates associated with these observations are summarized in Table 3-3. Photos © Department of Fisheries and Oceans, Canada.

Table 3-3. Depth and coordinates of soft coral colonies observed in the field (labels refer to Figure 3-2).

Label	Depth (m)	Latitude (N)	Longitude (W)
A	1052	57°10'24"	44°25'41"
B	1046	51°29'19"	44°25'16"
C	1390	57°09'15"	44°23'38"
D	525	54°28'23"	44°49'18"
E	1399	57°09'27"	44°23'58"
F	744	55°33'41"	44°49'45"
F	1218	53°07'01"	44°13'18"
H	528	52°55'10"	44°05'15"
I	1238	53°07'14"	44°13'04"

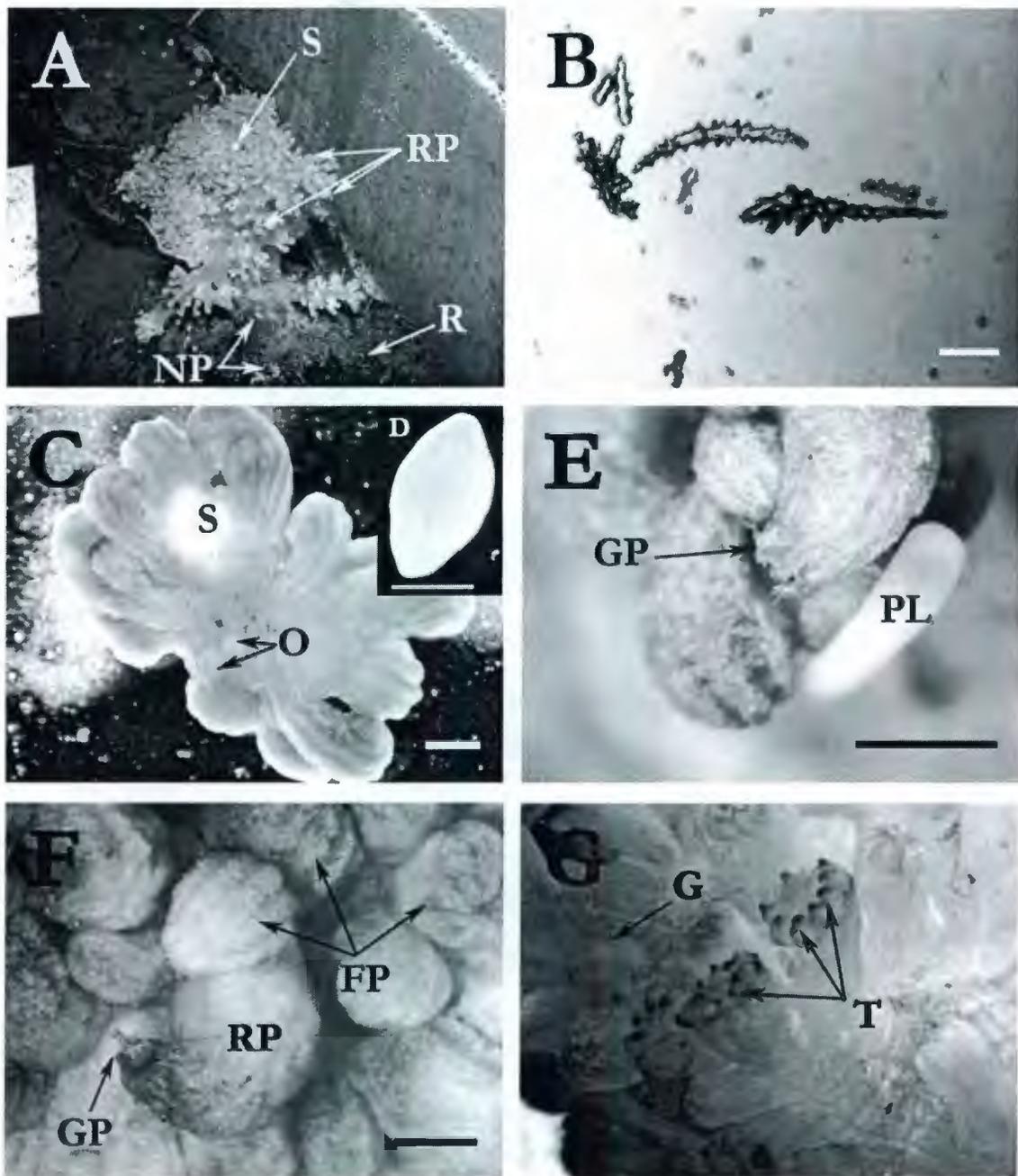


Figure 3-3. *Drifa* sp. A) A live colony collected at 500 m that spawned in the laboratory from August 2007 to April 2008 (extended colony size: 11 x 7 x 7 cm; contracted colony size: 5 x 3 x 2 cm). B) Sclerites. C) Branch with visible male and female gametes with close-up on a surgically extracted D) Spermary. E) Planula released from the opening of a reproductive polyp. F) Reproductive polyps and G) Feeding polyps of a colony relaxed in MgCl₂. S: spermary, RP: reproductive polyps, NP: newly settled polyp, R: rock, O: oocyte, GP: genital pore of reproductive polyp, PL: planula, FP: feeding polyp, T: tentacle. The scale bar in B represents 0.1 mm; all other scale bars represent 1 mm. The scale bar in C also applies to G.

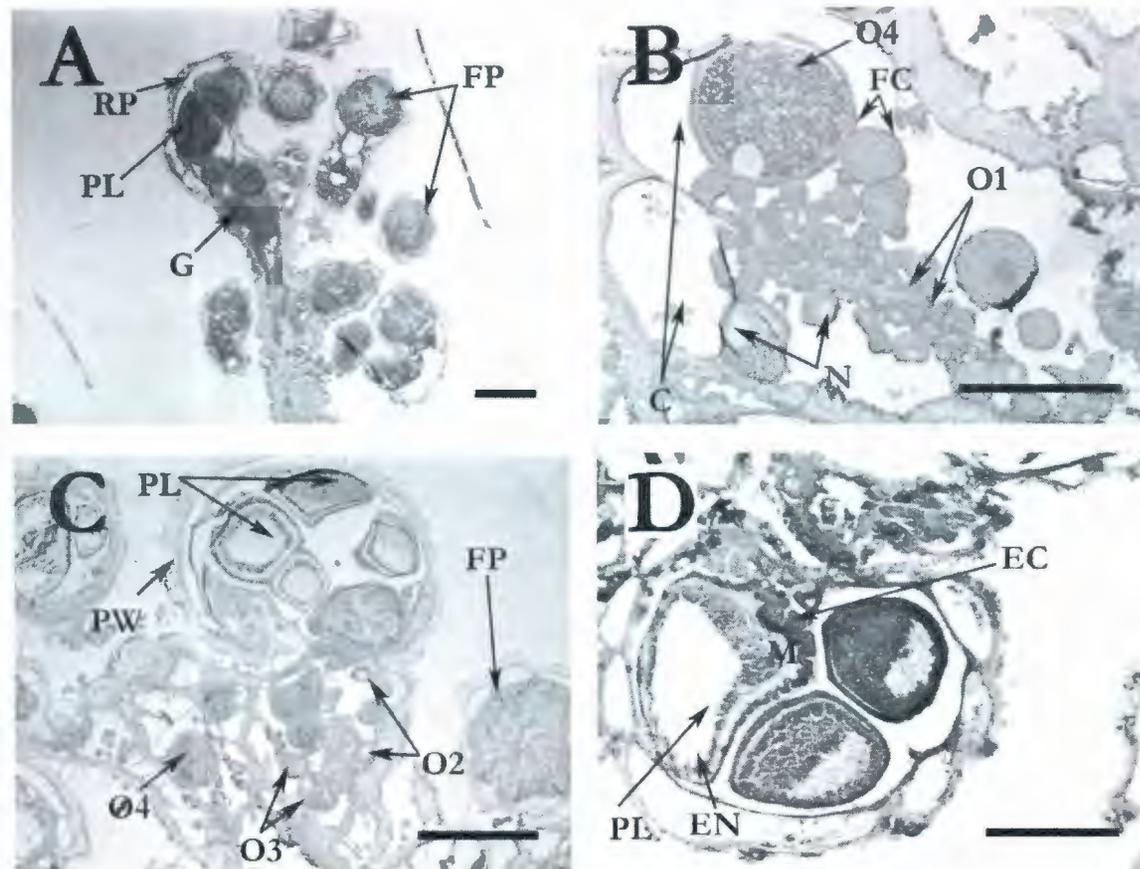


Figure 3-4. A) General longitudinal view of reproductive and feeding polyps. B) Micrograph showing stages of oocyte development. C) Micrograph showing gametes in one reproductive polyp. D) Well-developed planula within one reproductive polyp. RP: reproductive polyps, FP: feeding polyp, PL: planula, O1: stage I oocyte, O2: stage II oocyte, O3: stage III oocyte, O4: stage IV oocyte, C: coelenteron, FC: follicle cell layer, N: nucleus, PW: polyp wall, EC: ectoderm, M: mesoglea, EN: endoderm. The scale bars in A and C represent 0.5 mm; the scale bars in B and D represent 1 mm.

Moreover, data from live colonies showed that polyps containing planulae and/or gametes were present throughout the year under laboratory conditions. However, the spermaries were observed only twice in December 2007 in two different colonies. More precisely, a single spermary was observed in each colony.

In *Drifa* sp., mature planulae are reared in what appears to be specialized reproductive polyps that do not possess tentacles for feeding and are shaped like a trunk (Figure 3-3 E-G and Figure 3-3 A and C). When they contain planulae, these specialized polyps are visibly ridged and ca. 2~3 x larger than the feeding polyps, which exhibit a smoother surface (Figure 3-3 F-G).

Drifa glomerata is also an internal brooder. Female sexual cells and planulae are pinkish in colour. The early stages of female gametes are attached to or embedded in the mesenteries, develop into planulae and are retained in fertile polyps. In *D. glomerata*, no specialized reproductive polyps were observed; the fertile polyps that contained planulae were similar in shape to feeding polyps, despite becoming enlarged when containing planulae; however, the size differences between fertile polyps and feeding polyps are not as obvious as in *Drifa* sp.

Planula release and behaviour

***Drifa* sp.** The size at sexual maturity is fairly small: even the smallest colonies collected from 500 m (ca. 12 polyps, extended size ca. 3 x 2 x 2 cm) and 1200 m (ca. 25 polyps,

extended size ca. 3 x 1 x 1 cm) were fertile (i.e., contained planulae). The first evidence of planula release in *Drifa* sp. was observed in August 2007, several days after collection from the field. The planula was released through the opening of the specialized reproductive polyp. The process lasted about 15 minutes (Figure 3-5). During planulation, the adult colony was typically extended. If the adult colony was somehow disturbed and the polyps retracted, the planula release process was prolonged and could last up to ca. two weeks.

Large colonies (i.e., extended colony size during spawning season of ca. 11 x 7 x 7 cm, Figure 3-3 A) were observed to release planulae on a continuous basis from August 2007 to June 2008; however, planulation in several smaller colonies did not last as long, and one small colony (extended size ca. 3 x 2 x 2 cm) was observed to be totally spent after a few weeks of spawning in November 2007.

Overall, there was no obvious trend in the timing of planula release, which occurred at any time of the day or night. Adult colonies released more than one planula at a time, generally from different polyps. Furthermore, when more than one mature planula occurred in the same polyp and the planulae were released simultaneously, one could be released from the opening while the other was released through a tear in the polyp wall (Figure 3-5 D).

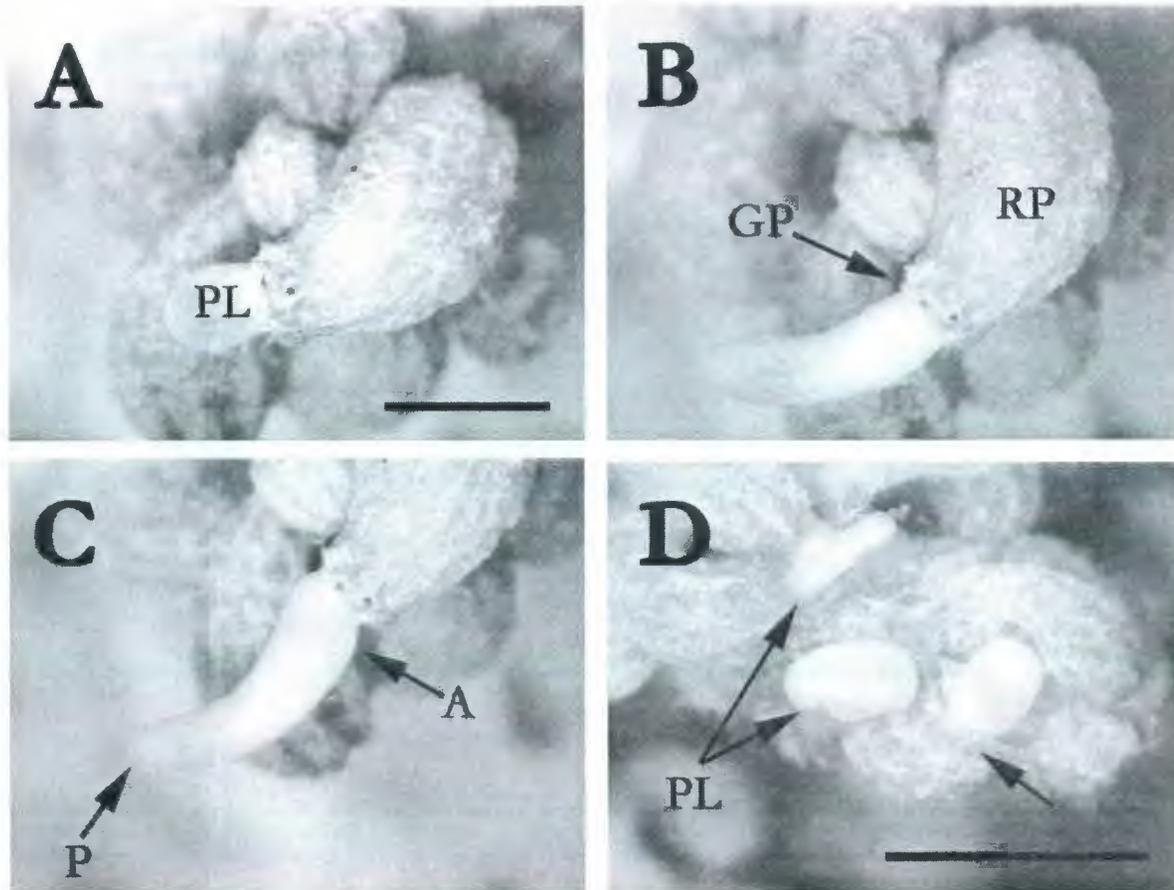


Figure 3-5. *Drifa* sp. A-C) Natural release of planula, completed in ca. 15 min. D) Planulae released from a tear in the reproductive polyp (arrow). PL: planula, GP: genital pore of reproductive polyp, RP: reproductive polyp, A: anterior end of planula, P: posterior end of planula. All scale bars represent 1 mm. The scale bar in A also applies to B and C.

Just after release, the mature planulae exhibited a very plastic shape. On average, fully extended mature planulae were 3-5 mm long and 1 mm wide: they were rod-shaped, and covered with cilia. In general, planulae sank to the bottom after their release. They did not actively swim, but instead exhibited cycles of contraction and expansion, that allowed them to alternately sink and float in the water column. Planulae actively probed the substratum with their aboral extremity; they used the oral extremity as an anchor to position the aboral extremity for attachment. A few smaller planulae (ca. 1-2 mm long) were observed to be released from reproductive polyps in colonies from 500 m (Figure 3-6), and were not as plastic as mature planulae. Some much smaller planulae (<1 mm) were also observed (Figure 3-6 E, F); but because they were rarely observed, they could not be studied in more detail.

Drifa glomerata. Planulation was observed in a single colony from January to June 2008 (for details on planulation periodicity, see Chapter 2). The average length of fully extended planulae was ca. 4-5 mm, and the maximum length recorded was ca. 5.5-6 mm. Planulae were released in the water column from the mouth of fertile polyps.

As was seen in *Drifa* sp. the adult colony of *D. glomerata* was extended during planulation. The colony released up to three planulae at a time from different polyps. Planulae were uniformly covered with ciliae and moved with their anterior end forward (Figure 3-7). Immediately after release, the demersal planulae exhibited complex crawling behaviours; they were able to climb onto a shell, and to circle vertically and

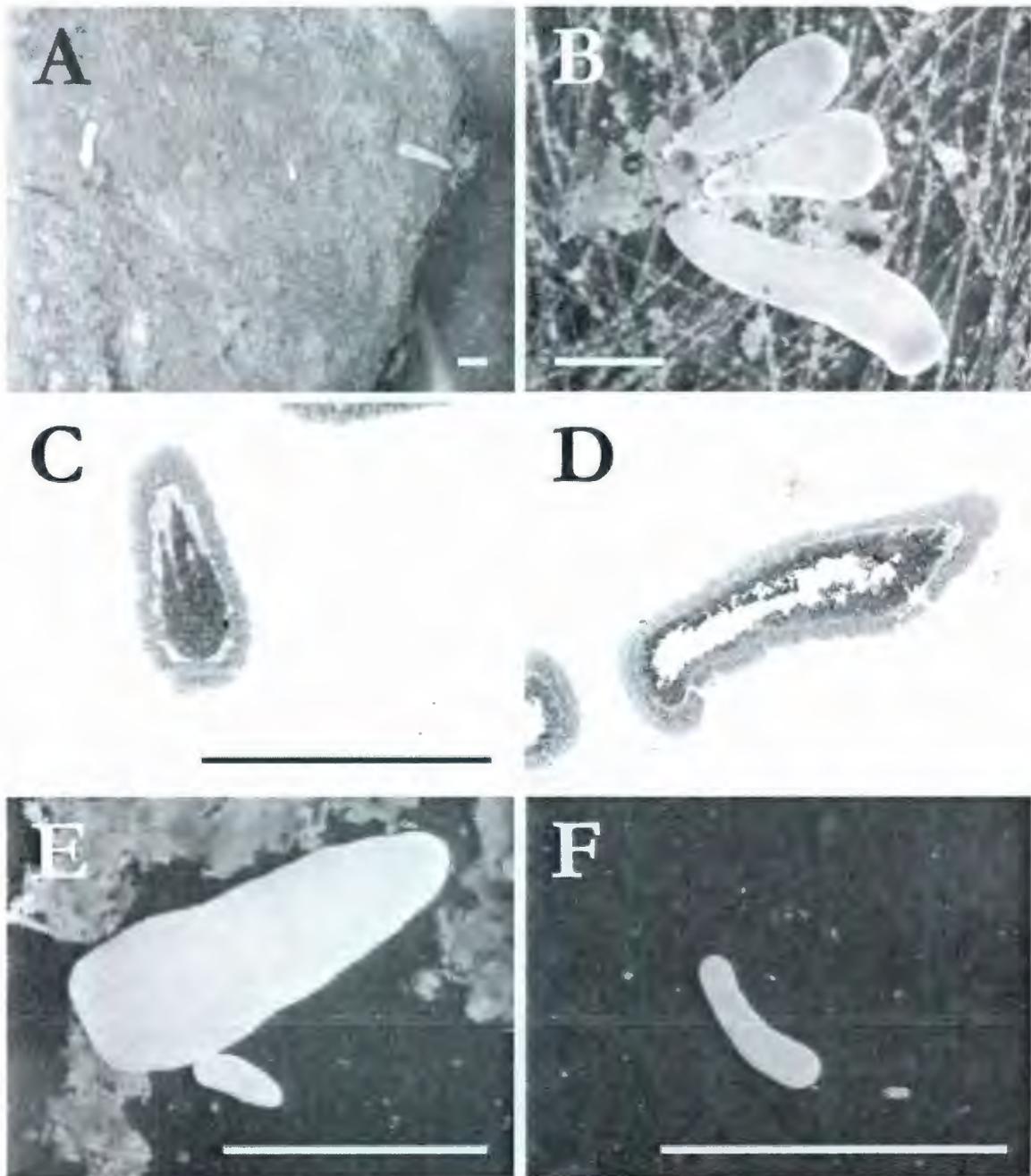


Figure 3-6. *Drifa* sp. A-B) Planulae of various sizes released during the same 24-h interval. C-D) TEM of planulae of various sizes. E) planulae of different sizes (0.05 vs 1.81 mm³), released simultaneously. F) Planula of small size. All scale bars represent 1 mm. The scale bar in C also applies to D.

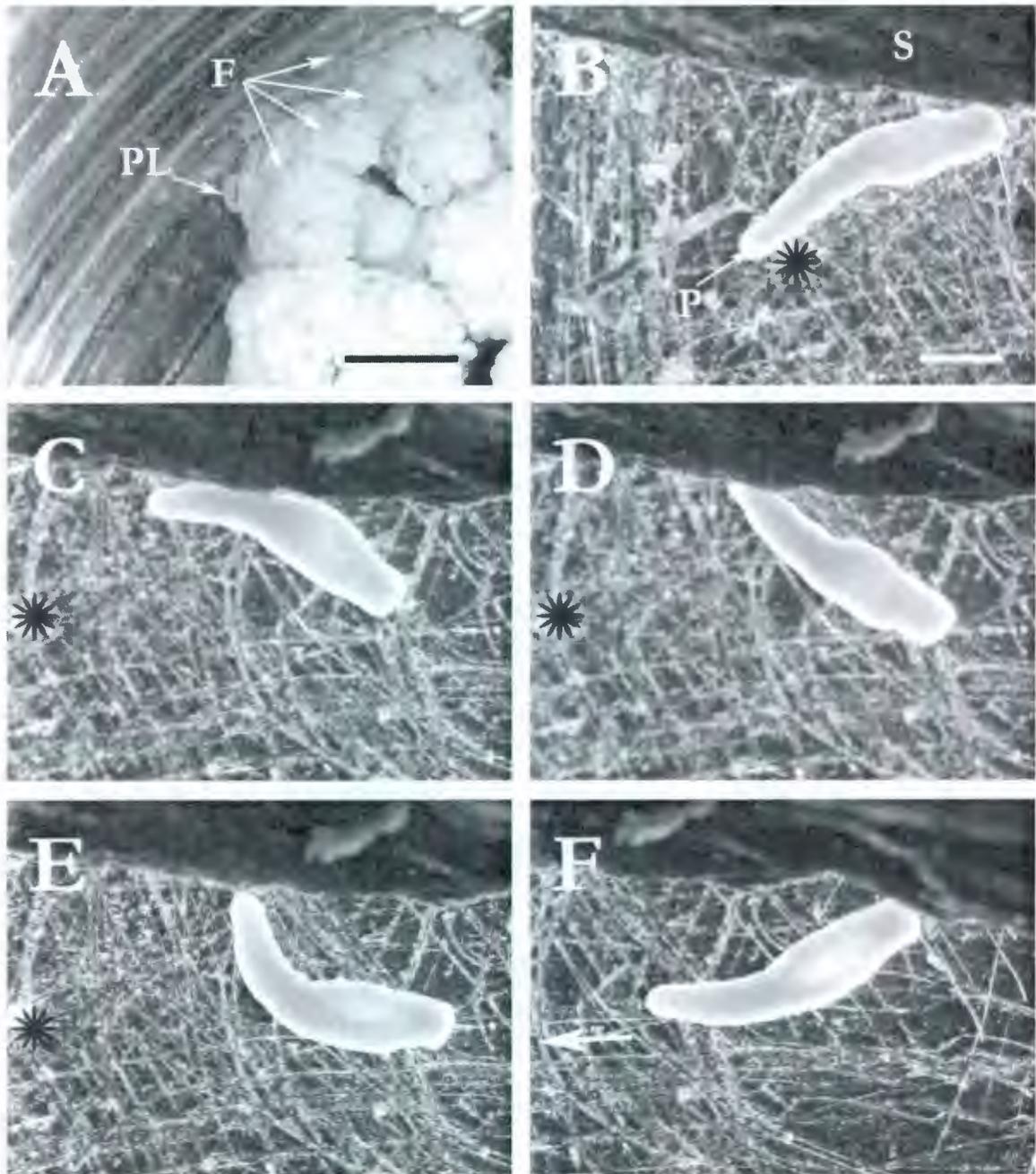


Figure 3-7. *Drifa glomerata*. A) Contracted live colony (3 x 3 x 2 cm) that spawned in the laboratory showing one newly released planula (PL) on its surface. B-F). Sequence showing planula behaviour monitored for two minutes post release. Note the reference point (asterisk). The arrow in F shows the direction of the reference point. PL: planula, F: fertile polyps containing reproductive cells, S: shell, A: anterior end of planula, P: posterior end of planula. The scale bar in A represents 5 mm. The scale bar in B also applies to C-F and represents 1 mm.

horizontally by contracting and expanding rapidly, i.e., changing from fully contracted to fully elongated within two minutes. Planulae typically settled one or two days post release, however, they were able to remain viable or to partially metamorphose in the water column and delay settlement for up to 50 days post release. Eventually, pre-metamorphosed juveniles were able to settle. One planula ejected a substance that was similar in appearance to fat-like granules through the oral pore ca. 40 days post release (Figure 3-8).

Numbers of planulae released

Drifa sp. From August 2007 to June 2008, 113 planulae were released by the eleven colonies from 1200 m (although two colonies did not possess any discernable reproductive polyp, and one had a single reproductive polyp filled with planulae). On the other hand, 289 planulae were released by seven colonies from 500 m (one colony contained fewer than 10 planulae-bearing reproductive polyps, and two died in February 2008). The number of planulae released during a single episode varied from 1 to 14 in the colonies from 500 m, and from 1 to 5 in the colonies from 1200 m.

Planulae were emitted more or less continuously by colonies from both depth ranges at irregular intervals (Figure 3-9). However, significant differences in planulation were detected between the two depth ranges and four lunar phases ($\chi^2_{3, 433} = 17.83, p < 0.001$).

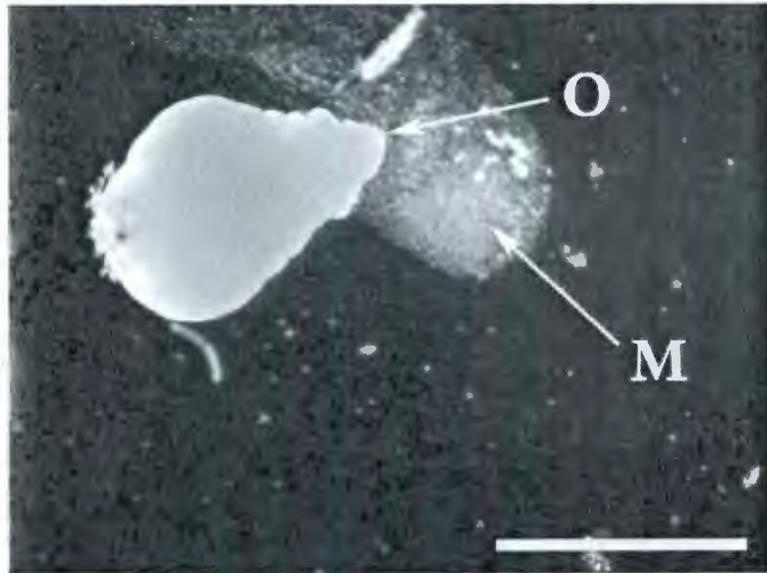


Figure 3-8. *Drifa glomerata*. Planula releasing a mucous or lipidic substance (M) through the oral pore (O). The scale bar represents 1 mm.

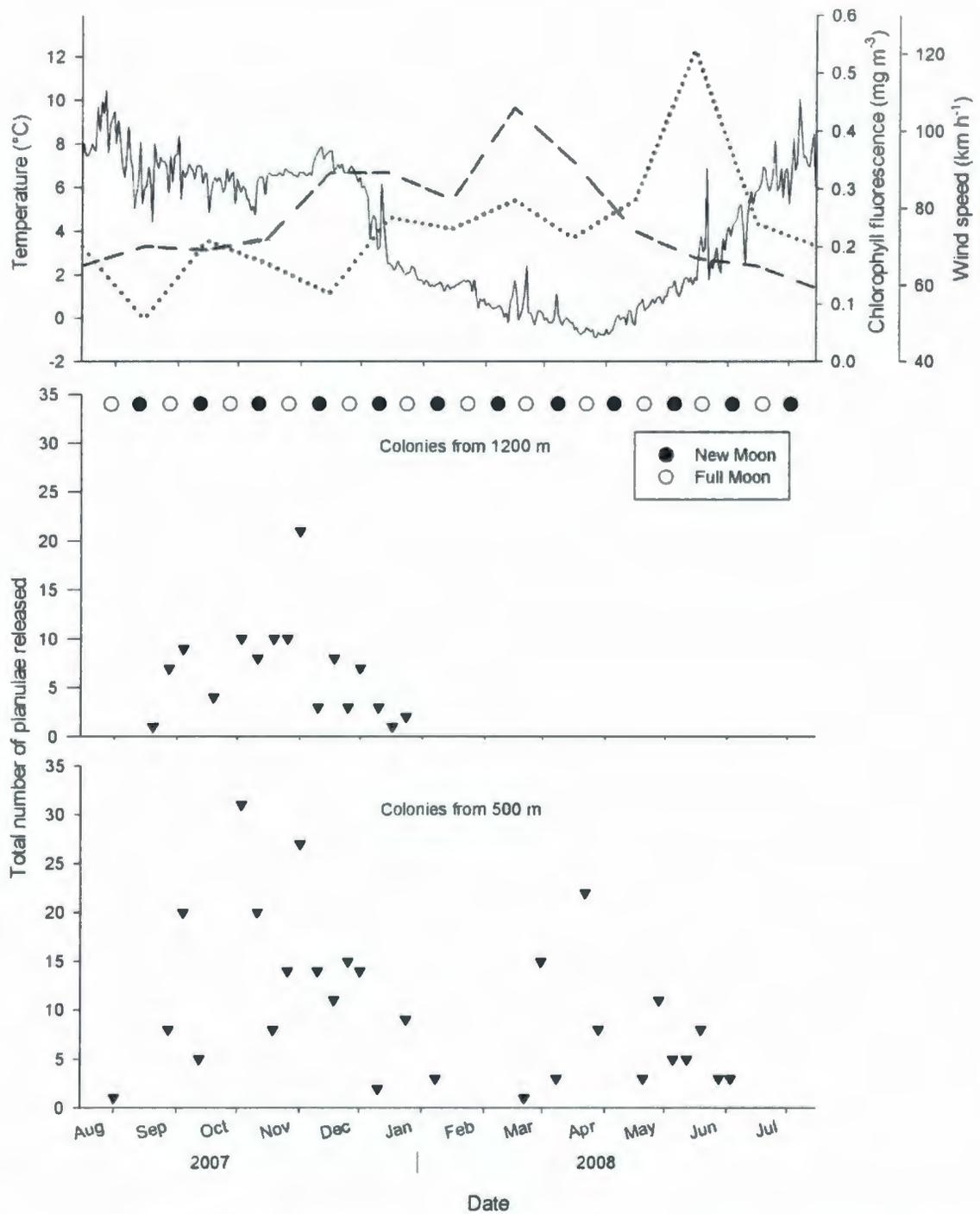


Figure 3-9. *Drifa* sp. Planulation of 500-m and 1200-m colonies from August 2007 to June 2008 corresponding to the fluctuation of temperature in the laboratory (solid line), chlorophyll fluorescence (an indicator of phytoplankton abundance, dotted line), wind speed (broken line), and the lunar phases. Planulae were plotted as number released per lunar-phase interval.

More precisely, colonies from 500 m released more planulae at the third quarter moon than at full moon ($\chi^2_{1, 144} = 36.07$, $p=0.026$, Figure 3-9) or new moon ($\chi^2_{1, 147} = 23.15$, $p<0.001$, Figure 3-9). In addition, more planulae were released at full moon than at the first quarter moon ($\chi^2_{1, 137} = 36.07$, $p<0.001$, Figure 3-9). Colonies from 1200 m also released more planulae at the third quarter moon than at full moon ($\chi^2_{1, 74} = 7.24$, $p=0.007$, Figure 3-9) or new moon ($\chi^2_{1, 73} = 12.21$, $p=0.005$). However, no significant differences were detected in the number of planulae released at full moon or first quarter moon ($\chi^2_{1, 73} = 0.00$, $p=0.987$, Figure 3-9).

Peak planulation occurred in October and November 2007 for colonies of both depth ranges, which coincided with the highest recorded seawater temperatures, a small peak of phytoplankton abundance and an increase in wind speed (Figures 3-9 and 3-10). Another peak season was observed in March 2008 but only in the colonies from 500 m; this timing coincided with the lowest seawater temperature and high phytoplankton abundance, and the period of strongest wind speed of the cycle (Figures 3-9 and 3-10).

Drifa glomerata. Planulation lasted through June 2008, with a total of 74 planulae released by the single colony (for details, see Chapter 2).

Metamorphosis and growth

Drifa sp. Metamorphosis generally coincided with settlement, however, some planulae exhibited partial metamorphosis or developed into polyps without settling on any

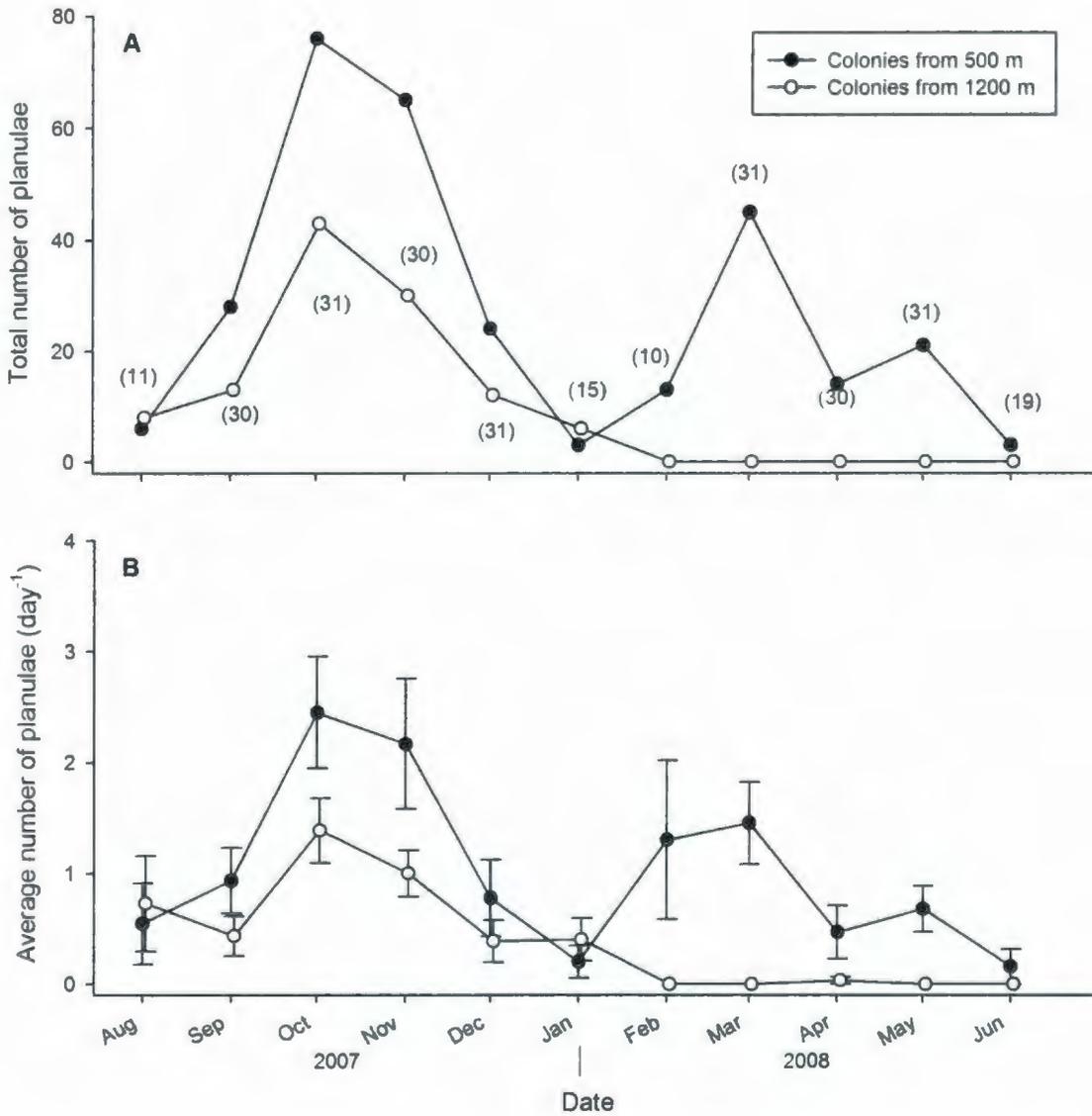


Figure 3-10. *Drifa* sp. Planulation of 500-m and 1200-m colonies from August 2007 to June 2008. A) Total number of planulae released every month, and B) Average daily number of planulae released in each month, expressed as Mean \pm SE. The values in parenthesis shown in A correspond to the number of days monitored every month.

substratum (see discussion in Chapter 4 regarding other species in which this feature is common). Most planulae settled and metamorphosed when they contacted with an appropriate substratum. When settling, the free-swimming planulae were elongated and probing the substratum. They then became cone-shaped or flattened and proceeded to metamorphose; they developed the eight primary mesenteries within 24 h post settlement. Primary polyps developed eight pinnulated tentacles within one week post settlement; however, this process required >50 days in some primary polyps (Figure 3-11 A-D). After pinnulated tentacles developed, polyps reached a maximum size of ca. 3-5 mm in length, with a stalk diameter of ca. 1 mm. No further growth or budding of primary polyps were observed in the tanks in over 21 months observation.

The only two-polyp colony observed after the settlement trials in the laboratory was formed by the fusion of two planulae. Planulae also settled on adult colonies (Figure 3-12). Polyps formed by fusion could be distinguished from naturally-budding polyps based on their different colouration (Figure 3-12 F-H): naturally-budding polyps were similar in colour to the adult colony (Figure 3-12 E). Normal two-polyp and four-polyp colonies (formed by budding) were observed in the holding tanks on the substrata that supported adult colonies. Though it was impossible to observe colonies constantly, daily observations allow me to assume with a high degree of certainty that these colonies were either the product of planulation in the laboratory or that they developed from tiny primary polyps present on the rocks at the time of collection.

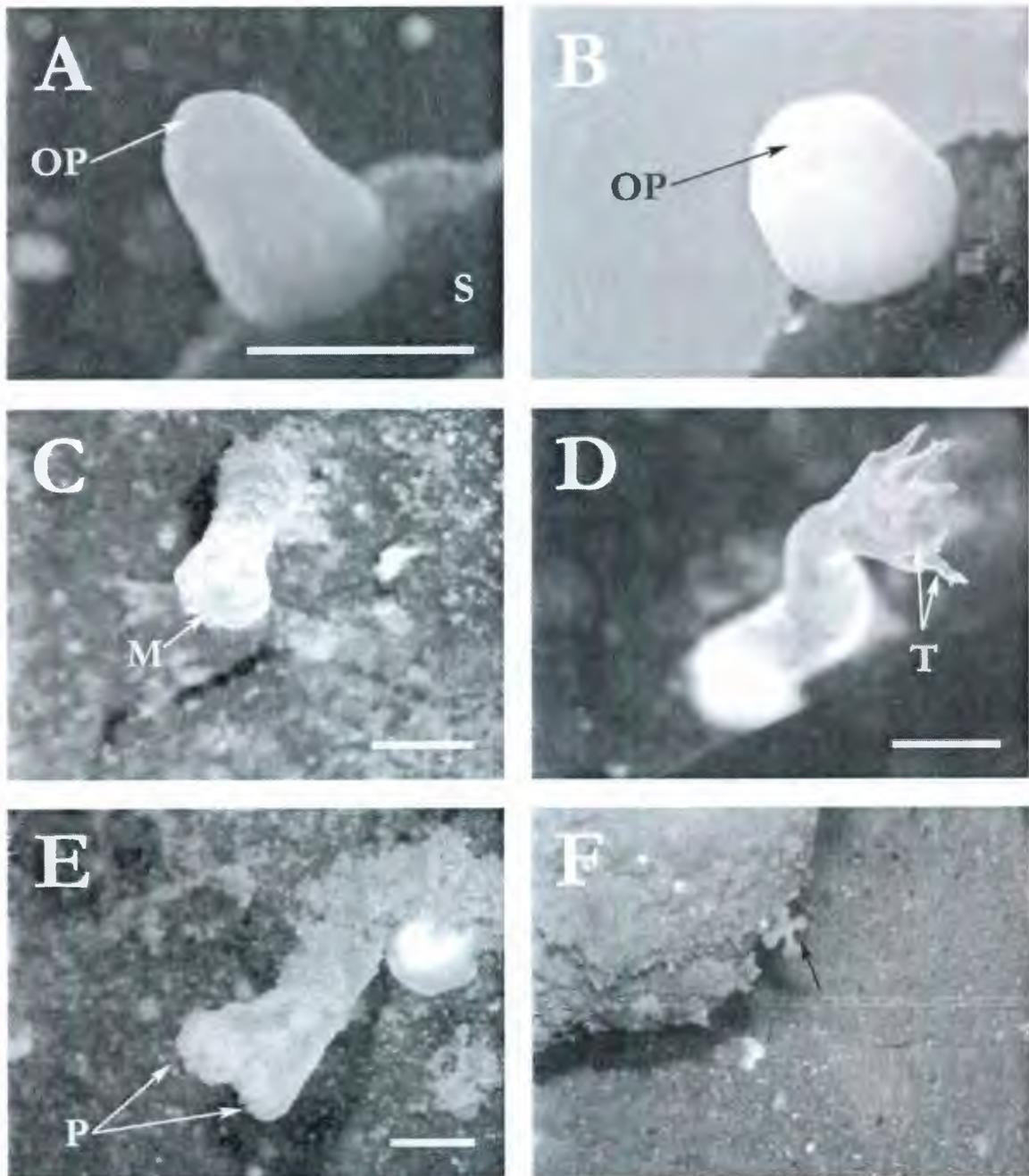
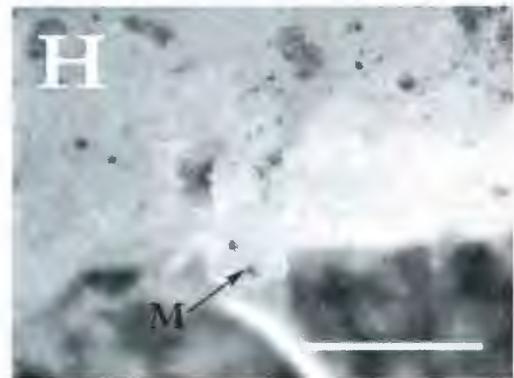
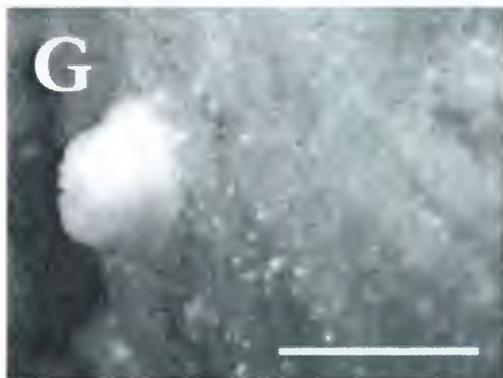
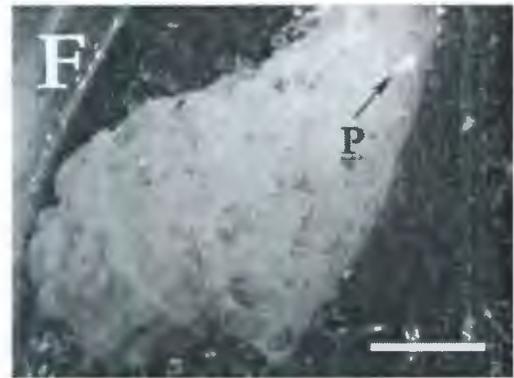
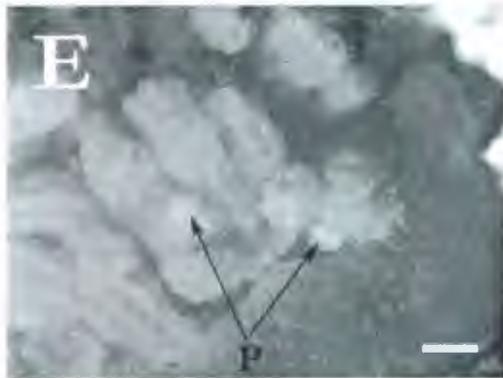
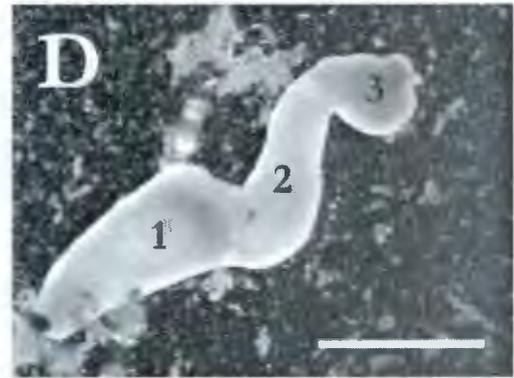


Figure 3-11. *Drifa* sp. A-D) Post-settlement growth of primary polyps from September to November 2007. E) Two-polyp colony and F) four-polyp colony (arrow) found in the holding tanks. OP: oral pore, S: shell, M: mouth, T: tentacle, P: polyp. All scale bars represent 1 mm. The scale bar in A also applies to B.

Figure 3-12. *Drifa* sp. A-C) Fused planulae that developed into a chimeric two-polyp colony, from August 16 to October 8, 2007. D) Fusion between three planulae. E) Polyps formed by natural branching. F-H) Polyp formed by fusion of a planula with adult colony (seven weeks post settlement). P: polyp, M: mouth. All scale bars represent 1 mm, except the scale bar in E which represents 1 cm. The scale bar in A applies to B and C.



Drifa glomerata. Planulae exhibited similar metamorphosis stages as *Drifa* sp. (Figure 3-13). The free-swimming elongated planulae probed the substratum with their aboral extremity. After settlement, planulae became cone-shaped or flattened. The eight primary mesenteries typically appeared within 24 h post settlement, and polyps developed small pinnules after two to three months of growth. The maximum size reached by polyps was ca.4-5 mm in length and 1-2 mm in stalk diameter after 6 months of growth. One single budding polyp was observed in the laboratory in March 2009 (Figure 13 F), ca. 9 months after release.

Settlement preferences

Drifa sp. Naturally-released planulae generally settled after 1 to 30 d, though a small portion of larvae took >3 mo to settle (Figure 3-14).

In *independent* treatments, planulae of colonies from 1200 m typically exhibited higher settlement rates ($\chi^2_{1, 88} = 28.17$, $p < 0.001$, Figure 3-14) and shorter time to settlement ($\chi^2_{1, 138} = 39.09$, $p < 0.001$, Figure 3-14) than those from 500 m. Furthermore, considering both depths studied, the type of substratum clearly influenced the settlement rates ($\chi^2_{4, 88} = 34.34$, $p < 0.001$, Figure 3-14) and the time to settlement ($\chi^2_{4, 138} = 36.45$, $p < 0.001$, Figure 3-14); however, significant interaction indicated that the trends were different ($\chi^2_{1, 138} = 39.09$, $p < 0.001$, Figure 3-14). At both depths, settlement occurred more frequently ($\chi^2_{1, 56} = 29.09$, $p < 0.001$, Figure 3-14) and faster ($\chi^2_{1, 85} = 7.59$, $p = 0.006$, Figure 3-14) on hard irregular substrata, such as conditioned shells (NS) than on smooth artificial surfaces

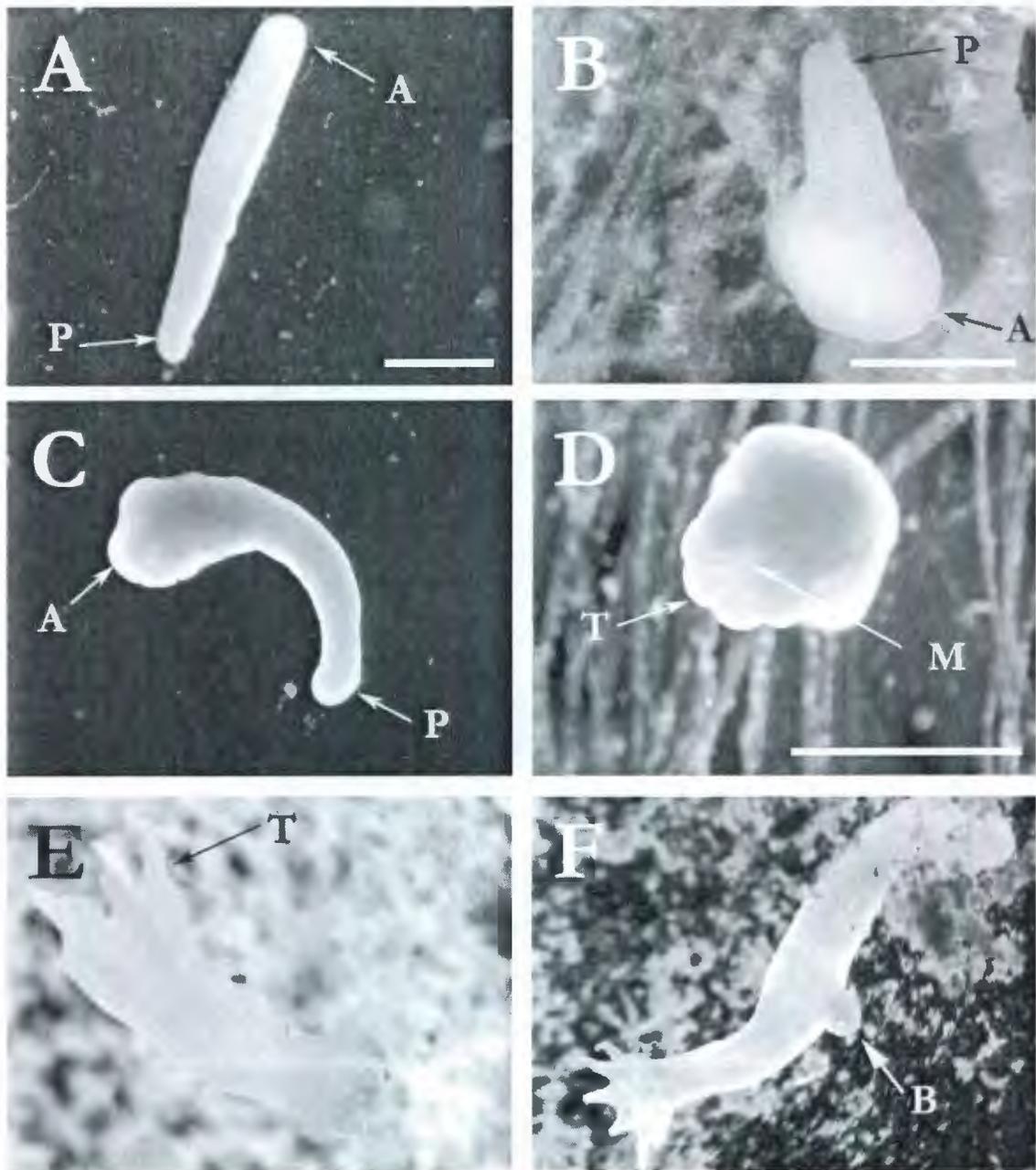


Figure 3-13. *Drifa glomerata*. A) Newly released planula, fully extended. B) Newly released planula exploring the substratum. C) Partial metamorphosis in the water column at 50 days post release. D) Newly settled primary polyp. E) Primary polyp after one month of growth in the laboratory. F) Budding polyp. A: anterior end of planula, P: posterior end of planula, T: tentacle, M: mouth, B, budding polyp. All scale bars represent 1 mm. The scale bar in A also applies to F; the scale bar in B also applies to C; the scale bar in D also applies to E.

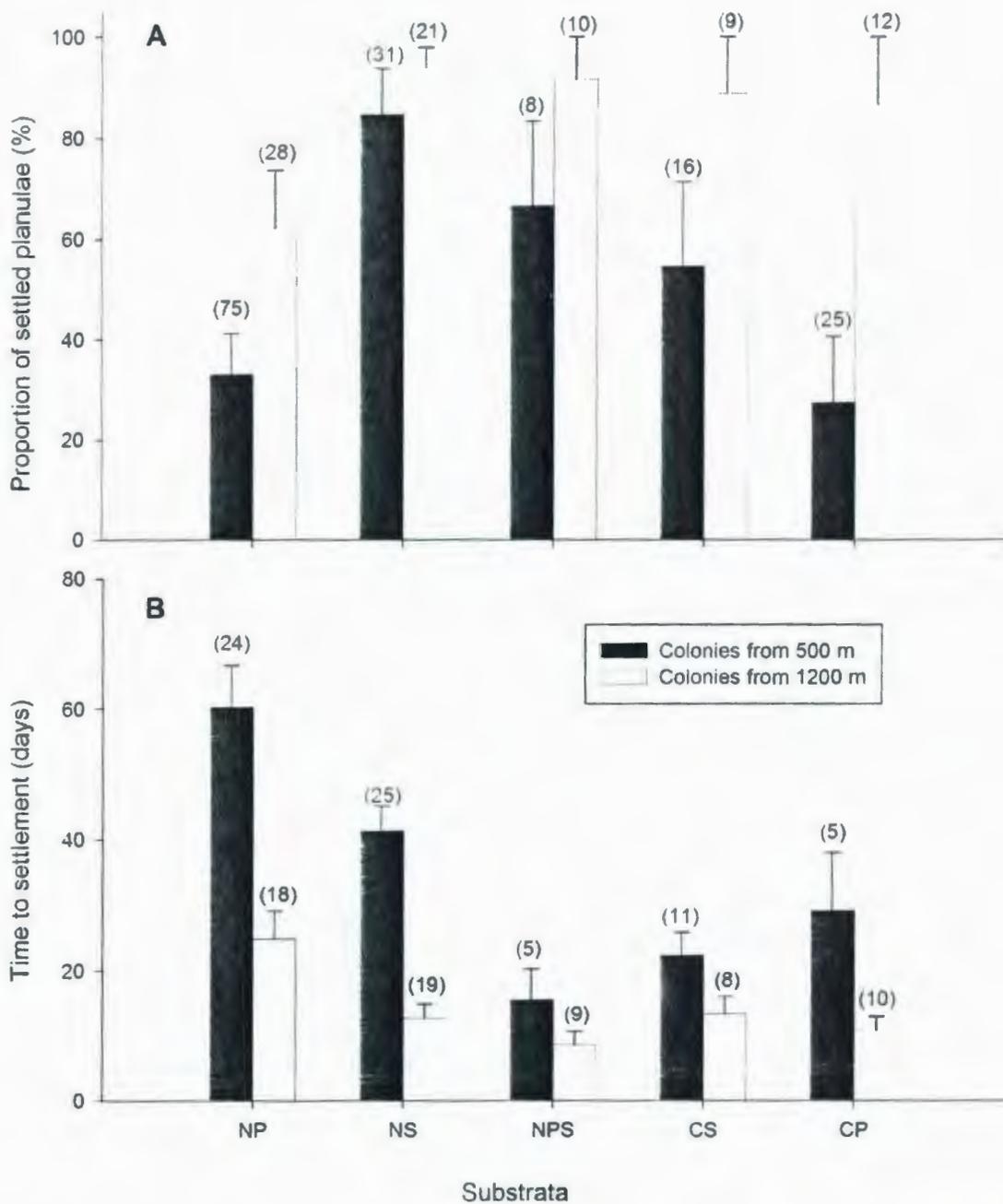


Figure 3-14. *Drifa* sp. A) Settlement rates and B) Time to settlement of planulae emitted by colonies from 500 m and 1200 m on different substrata. NP: conditioned culture plates; NS: conditioned shell fragments, NPS: rough and conditioned culture plate, CS: culture plates with cleaned shell fragments, CP: cleaned culture plates. Data are expressed as Mean \pm SE (total numbers of planulae tested for each treatment shown in parenthesis).

(NP) of conditioned culture plates. Planulae also settled more frequently ($\chi^2_{1,45} = 8.74$, $p=0.003$, Figure 3-14) and faster (at 500 m $\chi^2_{1,33} = 15.45$, $p<0.001$; at 1200 m $\chi^2_{1,26} = 6.34$, $p=0.012$, Figure 3-14) on rough (NPS) than smooth (NP) surfaces. Although planulae tended to settle more frequently on conditioned (NS) than clean (CS) shells at both depths, no significant differences in settlement rates were detected ($\chi^2_{1,27} = 2.7$, $p=0.100$, Figure 3-14). Furthermore, planulae from 500 m settled faster on clean shells (CS) than natural conditioned shells (NS) ($\chi^2_{1,35} = 8.78$, $p=0.003$, Figure 3-14), whereas no significant differences were detected in planulae from 1200 m ($\chi^2_{1,26} = 0.03$, $p=0.852$, Figure 3-14). No significant differences in settlement rates were detected between clean (CP) and conditioned (NP) culture plates ($\chi^2_{1,51} = 0.14$, $p=0.703$, Figure 3-14). However, planulae from both depths settled faster on clean (CP) than conditioned (NP) smooth surfaces ($\chi^2_{1,56} = 10.28$, $p=0.001$, Figure 3-14).

In pair-wise experiments, planulae tended to settle more frequently on natural conditioned shells (NS) than conditioned rough Petri dishes (NPS), although no statistical significance was detected ($\chi^2_{1,7} = 1.86$, $p=0.173$, Figure 3-15). Similarly, a larger proportion of planulae tended to settle on conditioned (N) than clean (C) surfaces, but no statistical significance was detected ($\chi^2_{1,15} = 3.24$, $p=0.072$, Figure 3-15). More planulae settled on rough (R) than smooth (S) surfaces ($\chi^2_{1,11} = 4.57$, $p=0.033$, Figure 3-15). Due to the small sample sizes, i.e., only one planula settled on clean (C) surfaces, no statistical analysis was performed on time to settlement on different substrata.

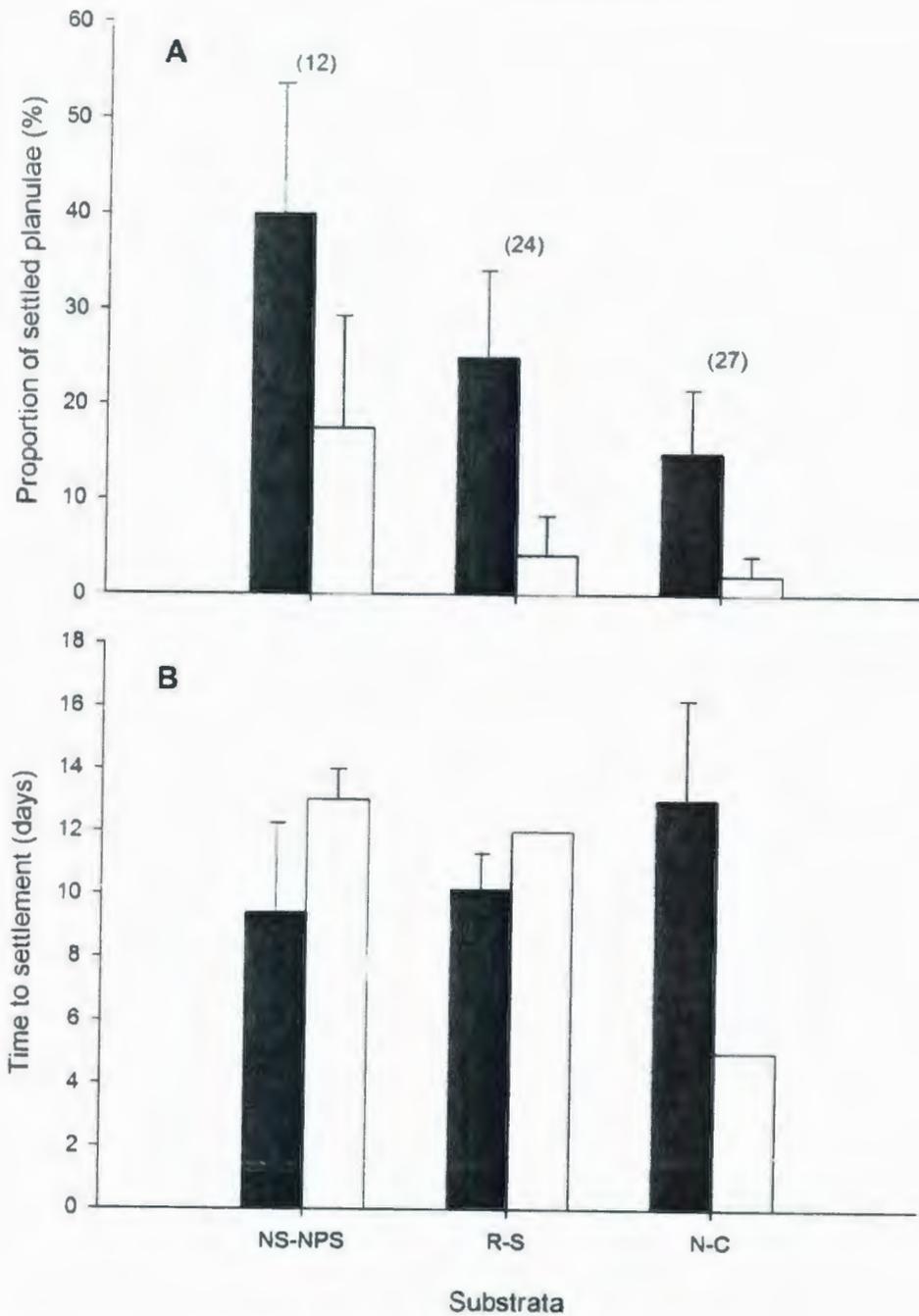


Figure 3-15. *Drifa* sp. A) Settlement rates and B) Time to settlement on different substrata. Data were collected in three pair-wise experiments: NS (conditioned shell fragments) versus NPS (rough and conditioned Petri dish); R (rough) versus S (smooth) surface; N (natural) versus C (clean) Petri dish. Data are expressed as Mean \pm SE (total numbers of planulae tested for each treatment shown in parenthesis).

Surgically-extracted planulae settled after 1 to 30 d (over a single month of monitoring). For each colony, the number of settled planulae was always lower than the number of elongated planulae available (Table 3-2). No clear differences in planula behaviour or juvenile growth rates were detected between surgically-extracted and naturally-released planulae.

Drifa glomerata. In the pair-wise trials, planulae tended to settle more frequently on conditioned shells (NS, natural substratum) than on conditioned culture plates (NPS, artificial substratum); however, no significant differences were detected ($\chi^2_{1,7} = 2.80$, $p = 0.094$, Figure 3-16). In addition, a larger proportion of planulae settled on conditioned rough (R) than conditioned smooth (S) surfaces ($\chi^2_{1,15} = 14.49$, $p < 0.001$, Figure 3-16), and on conditioned (N) surfaces than on clean (C) surfaces ($\chi^2_{1,13} = 5.96$, $p = 0.015$, Figure 3-16).

Larval settlement almost invariably occurred within one month; however, a small proportion of larvae partially metamorphosed in the water column and had not settled even two months post release. As explained for *Drifa* sp., no statistical analysis was performed on the time to settlement on different substrata due to small sample sizes, i.e., only one planula settled on smooth (S) and clean (C) surfaces.

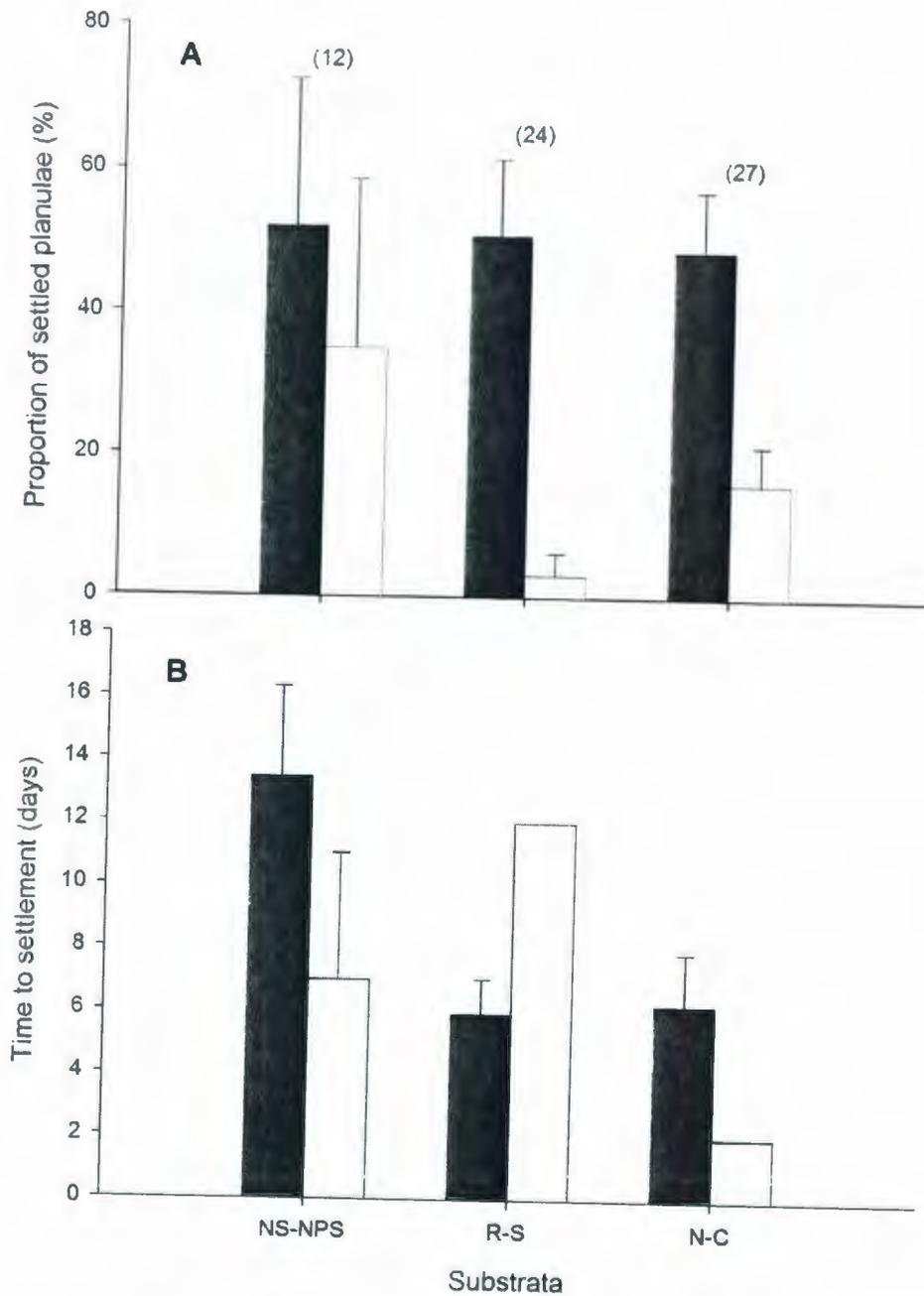


Figure 3-16. *Drifa glomerata*. A) Settlement rates and B) Time to settlement on different substrata. Data were collected in three pair-wise experiments: NS (natural shell fragments) versus NPS (rough and conditioned Petri dish); R (rough) versus S (smooth) surface; N (natural) versus C (clean) rough Petri dish. Data are expressed as Mean \pm SE (total numbers of planulae tested for each treatment shown in parenthesis).

3.5 Discussion

Reproductive features

Although the number of specimens examined was limited by the logistical constraints inherent to deep-sea research, both *Drifa* species appear to be female-dominated hermaphrodites with a short oogenesis, rapid embryogenesis and brief spermatogenesis. In *Drifa* sp. the oocytes and planulae were observed all year long, whereas spermaries were seen only twice during a short period in December 2007. Males of *D. glomerata* were never observed in the live colonies monitored here, nor were they present in serially preserved field samples (Chapter 2).

After embryogenesis (i.e., early cell cleavages, blastulation and gastrulation), embryos develop into a characteristic coral larva called the planula. The subsequent development of small planulae into large elongated planulae is termed the rearing period. Generally, internal brooders provide a safe environment for slow embryogenesis (Kruger et al. 1998); however in histological preparations of *Drifa* species examined here and in Chapter 2, all stages of oocytes were observed but no indication of embryonic development was ever recorded, suggesting rapid embryogenesis. Similar observations were reported in *Xenia macrospiculata* (Xeniidae), a shallow-water gonochoric brooder, for which internal fertilization within the polyp cavity and rapid embryogenesis was suggested (Achituv et al. 1992). To explain the coexistence of all stages of oocytes in three deep-sea scleractinian corals, Burgess and Babcock (2005) proposed an exponential growth of

oocytes: oocytes were produced at the start of the reproductive season and developed continuously based on the availability of food. In *Drifa* sp., the important intra-brood and intra-colony differences observed in the size of planulae are consistent with a prolonged rearing period before release. A few small planulae (1-2 mm) were released together with mature planulae (ca. 3-5 mm) in colonies of *Drifa* sp. from 500 m, and a few even smaller planulae (<1 mm) were observed on rare occasions. Overall, based on the coexistence of all four stages of oocyte development, the absence of embryogenesis and the size differences in planulae, I suggest that a rapid embryogenetic process and a slow maturation of planulae inside the reproductive polyps occur on a continuous or semi-continuous basis. This interpretation was strongly supported in my investigation of *Drifa* sp. but could not be verified unambiguously in *D. glomerata* because of the limited number of live specimens available that could not be sacrificed for histology. It should be noted that even the comparatively smaller naturally-released planulae in my study (ca. 1-2 mm) are of a size typical of planulae in other soft corals (Cordes et al. 2001), and that planulae of *D. glomerata* which can reach ca. 6 mm are possibly the largest ever recorded in alcyonaceans.

Planulation

Planulae released by colonies of *Drifa* sp. in the laboratory were observed throughout the year, with a peak in October and November. Based on these observations, I suggest an overlap between oogenesis and brooding. During the short appearance of spermaries and presumed sperm release in December 2007, a large number of oocytes may be fertilized

and the first cohort of planulae would correspond with the peak season for planulation. Subsequently, slower-maturing planulae would be released at irregular intervals. Extended and overlapping oogenic and brooding cycles were reported in the solitary coral, *Balanophyllia elegans*, off the Californian coast (Fadlallah and Pearse 1982). The evidence gathered from the single fertile colony of *D. glomerata* and the 26 colonies of *Drifa* sp. shows that even at the individual level, planula release can last several weeks or months. One particularly large colony of *Drifa* sp. intermittently released planulae throughout the 13 months of the study period and the single *D. glomerata* colony produced planulae for roughly six months before dying inadvertently. Nonetheless, I have seen that smaller colonies can become spent after a limited number of planulation episodes, suggesting a relationship between length of planulation period and size of colony.

Several factors have been proposed to affect planulation in shallow-water corals, including temperature, lunar cycle, wind speed and depth (Jokiel and Guinther 1978; Stimson 1978; Benayahu and Loya 1983; Benayahu and Loya 1984; Rinkevich and Loya 1987; Ben-David-Zaslow et al. 1999). However, the occurrence of annual or other patterns of planula release in the nephtheids studied here was difficult to define. Although planulation in *Drifa* sp. occurred year round, its intensity varied in that the maximum rate of planulation occurred in October and November. Similar patterns were observed in the study of a shallow-water soft coral species, *Heteroxenia fuscescens* in the Red Sea; *H. fuscescens* released planulae year round, however, more planulae were released in

summer and fall, in accordance with fluctuations in food and light levels (Ben-David-Zaslow et al. 1999). On the one hand, as a result of the logistical constraints already noted, this study was conducted over one year using a relatively small number of colonies and high inter-individual variability was observed. Thus, conclusions regarding the timing, intensity, and duration of planulation events over the annual scale should be drawn carefully. However, the colony of *D. glomerata* did not release any planulae until January 2008, which corresponded to the breeding period (between January and June/July) determined by using serial gonad samples of this species (for details see Chapter 2). This consistency lends strong support to my laboratory results.

The influence of seawater temperature and seasonal flux in food availability (i.e., phytodetritus) on reproduction has been documented for several benthic invertebrates (Tyler et al. 1982; Benayahu 1997; Ben-David-Zaslow et al. 1999; Wigham et al. 2003; Mercier and Hamel 2008, Chapters 2 and 4). In this study, planulation of *Drifa* sp. from both depths was first recorded in September, corresponding to a small fall phytoplankton bloom and an increased turbulence of the water due to stronger winds (generating more resuspended material in the water column). Planulation of colonies from 1200 m coincided precisely with the period of warmest water of the cycle in the laboratory. A similar planulation period was recorded in colonies from 500 m with intense activity in the fall; however, a second wave of planulation was observed starting in March, again coinciding with the beginning of the phytoplankton production and a slow increase of the water temperature.

No clear lunar patterns were found in *Drifa* sp. (see Chapter 2 for results with *Drifa glomerata*), although significant differences were detected in certain lunar phases at both depths. An even stronger correlation between planulation and the lunar phase was found in another deep-sea nephtheid, *Gersemia fruticosa* (Chapter 4). These results are unexpected for deep-sea species in that they suggest that the perception of lunar cycles may extend to deep ocean strata, possibly via modulation of tidal currents or hydrostatic pressure. On the other hand, as discussed more thoroughly in Chapter 2, the influence of light (including photoperiod and lunar cycles) cannot be completely discarded, at least for colonies living at depths above the limit of downwelling illumination (ca. 1000 m; Schiebel et al. 2007).

The depth at which the parent colonies of *Drifa* sp. were collected clearly had an impact on the observed breeding season, in that the planulation period of colonies from 1200 m was several months shorter than that of colonies from 500 m. In an investigation of the shallow-water scleractinian coral *Stylophora pistillata* (Rinkevich and Loya 1987), colonies from 5 m were more fecund and their reproductive season was two or three months longer than colonies from 24-45 m. The results obtained here must be interpreted with caution, however, because estimates of planulation duration might be biased by: (1) the small sample size (n= 7 from 500 m; n=11 from 1200 m); or (2) the difference in colony size at the two depths sampled (i.e., colonies from 1200 m had a maximum extended size of ca. 4 x 1 x 1cm and were smaller than those from 500 m, which had a maximum size of 11 x 7 x 7 cm). The influence of size on reproductive characteristics of

corals has been demonstrated in previous studies (McFadden 1991; Sakai 1998b; Kapela and Lasker 1999; Tsounis et al. 2006). For instance, the colony size of temperate soft coral species *Alcyonium* sp. was reported to influence the frequency of colony fission (asexual reproduction) and sexual reproduction, as well as fecundity (McFadden 1991). However, colony size alone cannot predict fecundity, because several large colonies of *Alcyonium* sp. produced only a few planulae (McFadden 1991). Incidentally, large non-fertile colonies were observed in the two *Drifa* species studied here.

Planula behaviour and metamorphosis

Planulae exhibited distinctive behaviours in the two *Drifa* species. The majority of planulae from *D. glomerata* were demersal, and actively crawled and probed the substratum immediately after their release. However, planulae of *Drifa* sp. frequently navigated between the water column and the bottom, shifting their position mainly via whole body expansion and contraction. The high proportion of planulae with complex crawling/searching behaviours will likely enhance local recruitment of new polyps in *D. glomerata* compared to *Drifa* sp. Furthermore, the small planulae (ca. 1-2 mm, observed only in colonies of *Drifa* sp. from 500 m), which presumably need more time before settlement, probably favour dispersal on a larger scale (Sebens 1983a). Larger planulae observed in colonies of *Drifa* sp. from both 500 and 1200 m appeared more competent to probe substrata and are presumably able to settle more quickly within the parental habitat. This is probably an adaptation to the deep-sea environment, especially considering that the main substratum available is mud at a depth of 1200 m. However, I did not notice the size

differences of planulae until late December 2007, and most of the 1200-m colonies had stopped planulating by that time. Thus, it is possible that I did not observe the smaller planulae in this group because of the shorter season in which planulation occurs. Similarly, planulae released in the shallow-water soft coral *Heteroxenia fuscescens* during summer were greater in length compared to the rest of the year (Ben-David-Zaslow et al. 1999). The ecological significance of the occurrence of different sizes of planulae is discussed below.

One planula of *D. glomerata* ejected fat-like granules from the oral pore. A similar phenomenon was also reported in brooding scleractinian corals *Stylophora pistilata* (Rinkevich and Loya 1979), *Agaricia* spp. (Van Moorsel 1983) and *Siderastrea stellata* (Neves and da Silveira 2003). Van Moorsel (1983) suggested that planulae decreased their buoyancy by releasing lipids in order to reach substrata. This type of release could be associated with the behaviour and buoyancy shifts observed in the planulae of *D. glomerata*. However, planulae of *D. glomerata* were able to rapidly change position in mid-water and sink to the bottom, and the release of "lipid" was observed only once during these movements, suggesting that the behaviour could be related to other factors, including stress. It is worth noting, however, that the spatial scales of movement in the laboratory observations were very small relative to those relevant to nature.

Demersal crawling planulae are believed to settle rapidly and aggregate within the parental habitat (Sebens 1983b; Dunstan and Johnson 1998). The planulae released by

both *Drifa* species were able to settle within two days or remain in the water column for several months, either prolonging the planula stage or metamorphosing partially before finally settling. Alternately, some individuals developed into polyps without ever settling, presumably to initiate feeding and thus enhance dispersal over long distances. This capacity to delay settlement is consistent with field observations of colonies distributed sparsely on mud, which may be the result of protracted pelagic movements of the planula before settlement. Generally, in benthic marine invertebrates, metamorphosis is triggered by a permanent attachment to the substratum (Rodriguez et al. 1993); however, metamorphosis before settlement has occasionally been reported (for a review Thorson 1950) including in the soft coral *Heteroxenia fuscescens* (Zaslow and Benayahu 1996). The ability of planulae to undergo partial metamorphosis is probably linked to an absence of an appropriate settlement cue, and is advantageous for increasing the probability of settlement on more suitable substrata (Zaslow and Benayahu 1996). Alternately, this behaviour may be a desperate survival measure on the part of the planula as it runs out of energy necessary for metamorphosis, in a manner consistent with the "desperate larva hypothesis" (Gibson 1995; Toonen and Pawlik 2001).

Prolonged competency periods and partial metamorphosis can make long-range dispersal possible, however they do not necessarily preclude short-range planulae settlement (Van Moorsel 1983). The dual ability to settle quickly and exhibit long competency periods enhances the possibility of both local recruitment and long-range dispersal. This capacity has been found in shallow-water Nephtheidae species, such as *Capnella gaboensis*

(Farrant 1986). Harii et al (2002) observed that planulae can remain competent to settle for up to 30 days post release in *Helipora coerulea* (Octocorallia) and for over 100 days in *Pocillopora damicornis* (Scleractinia). However, older planulae (i.e., longer time since release) had lower settlement rates in both species. Similar results were obtained in *Drifa* sp. (unpublished data).

Settlement preferences

Independent and pair-wise experiments showed that planulae generally settled faster and more frequently on hard irregular substrata covered with a natural biofilm. Hard substrata are important for the settlement of deep-sea soft coral planulae. In locations where mud is the dominating substratum, nephtheids were observed in clusters whenever firm substrata, such as sponges, shell debris, tube worms, etc. were found. This pattern has also been reported in deep-water scleractinians (Wilson 1979). Two non-exclusive explanations are suggested for the local aggregation observed in the field: (1) planulae are well developed and ready to settle immediately upon release; (2) conspecific adults induce or favour settlement. The influence of conspecific adults on settlement and juvenile growth has been documented for multiple benthic marine invertebrates (Rodriguez et al. 1993). One advantage of local aggregation is to increase fertilization success. Considering the rarity of male gonads in *Drifa* sp., which were observed only twice during the 12-month laboratory study, and the virtual absence of males in *D. glomerata* (see Chapter 2), aggregative distributions are not surprising.

Complementary field observations showed that colonies of soft corals occur on cliffs or rocky outcrops. This pattern likely results from: (1) a greater likelihood of settlement on vertical surfaces; and/or (2) the lower probability of sedimentation and suffocation on vertical substrata, which increases the survival rate of newly settled polyps (Rogers 1990). Shallow-water nephtheids favour outer and mid-shelf slopes where water clarity and near-bottom currents are high (Dinesen 1983). In addition, Fabricius (1997) found that shallow-water soft corals in the central Great Barrier Reef have high coverage on platforms exposed to relatively strong currents but low wave energy, and low settlement rates on shaded steep slopes.

There was weak evidence that planulae from 500 m were slightly more selective and typically took longer to settle than those from 1200 m. Two potential explanations can be offered: (1) planulae released by colonies from 1200 m require less precise/definite environmental inducers from the substrata (i.e., are more opportunistic); or (2) planulae released by colonies from 1200 m are more developed (i.e., brooded for a longer time inside the colonies until they are ready to settle). The former is consistent with the rarity of firm substrata at 1200 m, whereas the latter is supported by the fact that only colonies from 500 m released small planulae (ca. 1-2 mm long) in my study. Previous studies have demonstrated the importance of size differences in offspring with respect to their competency period and juvenile behaviour (Marshall et al. 2003; Marshall and Keough 2007). Though size variations were not as striking as those I observed in *Drifa* sp., Achituv et al (1992) found that a shallow-water xeniid species (*Xenia macrospiculata*)

released “young” planulae that were small and solid whereas its “mature” planulae were large and hollow. I noted the same solid/hollow distinction in *Drifa* sp. However, Isomura and Nishihira (2001) indicated that smaller planulae of three pocilloporid corals were able to settle and metamorphose with similar larval durations as larger individuals, and concluded that the small planulae were nonetheless sufficiently developed to settle and metamorphose. They found that size differences did not influence the settlement time but had an effect on the survival rates, and inferred that larger planulae, which had higher survivorship in the experiment, and presumably greater lipid reserves, had longer range dispersal. Both hypotheses are appealing; unfortunately, I did not have the chance to go beyond preliminary observations and experimentally test the influence of size on settlement and post-metamorphic survival.

Growth

Shallow-water soft corals species are commonly reported to exhibit fast growth and rapid colonization (Harrington et al. 2004). However, primary polyps of the species studied here exhibited very slow growth rates. Primary polyps virtually stopped growing when they reached a particular size (generally within two or three months post settlement). Furthermore, no natural budding of primary polyps of *Drifa* sp. was observed over the course of the monitoring (i.e., 13 months post settlement for the older polyps); and one natural budding polyp of *Drifa glomerata* was observed ca. 9 months after release. These observations lead to two main hypotheses. First, environmental factors in the laboratory might adversely influence growth rates. Although seawater temperatures were maintained

within a range that was appropriate for deep-sea corals, the pressure level and food availability were presumably very different from those found in the natural deep-water environment. Alternately, early growth rates of primary polyps are extremely slow, as inferred by the few other experimental growth studies that have been conducted on post-settled corals. For example, no budding of the shallow-water temperate soft coral *Dendronephthya gigantean* (Nephtheidae) was recorded during a one-month study (Hwang and Song 2007). In the alcyonacean coral *Anthomastus ritteri*, juvenile colonies developed a second autozoid after 1-2 months and displayed very slow growth in the first 8-9 months (Cordes et al. 2001). In addition, Coma et al. (1998) found a mean growth rate of 1.8 cm year^{-1} for the shallow-water Mediterranean gorgonian *Paramuricea clavata*; they inferred that the largest colony (55 cm) required 31 years to achieve its size. Even though growth rates were low in the two deep-sea *Drifa* species studied here and in two other deep-sea nephtheids (Chapter 4), the presence of planulae in small colonies (ca. 10-25 polyps) indicates that sexual maturity is reached in a relatively short time period, which might compensate for the slow growth.

Several multiple-polyp colonies obtained during my trials were formed when planulae settled on adult colonies and/or other planulae (i.e., chimerism). This behaviour can also be perceived as a mechanism to compensate for the slow growth rate, as suggested in the tropical scleractinian *Siderastrea stellata* (Neves and da Silveira 2003). Colony mortality was shown to be higher in colonies smaller than a critical size (Sakai 1998a); thus, fusion may enhance survival by increasing the growth rate to that minimum size. Another study

found no benefit to juvenile chimerism, suggesting that it resulted only from faulty ontogenetic allorecognition promoted by gregarious settlement (Barki et al. 2002). I cannot determine whether fusion is beneficial or detrimental to the deep-sea corals studied here. However, fusion is an important element to consider in studies of parentage and population genetic structure.

Conclusions

The year-round production of brooded lecithotrophic larvae and long-lived larval stage are mechanisms that can enhance conservation of local soft coral populations and promote long-range dispersal when local opportunities for settlement are limited. In addition, the finding that behaviour, settlement and post-metamorphic growth of surgically-extracted planulae were comparable to that of naturally-released larvae suggests that fertile colonies can produce offspring when damaged (e.g., by trawls). Though the production of recruits seems to occur on a nearly year-round basis, opportunities for settlement may be limited and the growth of settled polyps appears to be very slow compared to shallow-water species, further highlighting the vulnerability of deep-sea coral ecosystems.

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**CHAPTER 4 : PLANULA RELEASE, SETTLEMENT,
METAMORPHOSIS AND GROWTH IN TWO DEEP-SEA
SOFT CORALS (ALCYONACEA: NEPHTHEIDAE)**



Primary polyps of *Gersemia fruticosa*

4.1 Abstract

Live colonies of *Gersemia fruticosa* (< 300 m) and *Duva florida* (ca. 535 m) were collected from the continental slope southwest of the Grand Banks (eastern Canada). In the laboratory, planulation of *G. fruticosa* was monitored daily, and 79 planulae (1.5-2.5 mm long) were released from April to June 2007. Peak planulation in *G. fruticosa* was positively correlated with increases in seawater temperature and phytoplankton abundance, and planula release appeared to be more intense around the full moon. Metamorphosis and settlement occurred 3 to 70 d post release. The eight primary mesenteries typically appeared within 24 h. Primary polyps grew to a height of ca. 6-10 mm and a stalk diameter of ca. 1 mm within 2-3 months. Planulae of *Duva florida* (1.5-2.5 mm long) were extracted surgically from several colonies and were successfully reared in culture. Primary polyps (ca. 3-4 mm long) developed within 2-3 months. No budding of primary polyps was observed in either species over 11 months of monitoring, suggesting a very slow growth rate. The only two-polyp colony was formed by fusion of two planulae.

4.2 Introduction

Soft corals in the family Nephtheidae are widely distributed in temperate and cold waters around the world (Dinesen 1983; Gass and Willison 2005; Lumsden et al. 2007; Wareham and Edinger 2007). However, little research has been conducted on their reproduction and development (Farrant 1986; Benayahu et al. 1992; Dahan and Benayahu 1997; Hwang and Song 2007; Sun et al 2009 and Chapters 2 and 3) in part because of discrepancies in taxonomical identification (Watling and Auster 2005). Additional challenges for deep-water species include difficulties in maintaining adults and planulae under laboratory conditions.

Several factors have been proposed to affect planulation in shallow-water corals, including temperature, lunar cycle and depth. Reproduction coinciding with high water temperature in the summer have been observed in several soft corals (Benayahu 1997; Ben-David-Zaslow et al. 1999). For instance, the soft coral *Heteroxenia fuscescens* from the Red Sea has been shown to release planulae year round; however, maximum planula release was recorded in summer and fall, in association with fluctuations in food and light levels (Ben-David-Zaslow et al. 1999). Lunar reproductive cycles have been observed in several species (Benayahu and Loya 1983; Benayahu and Loya 1984; Coma et al. 1995; Kawahata et al. 2002), and Stimson (1978) suggested that low tides provide another mechanism to help synchronize and facilitate local recruitment. Rinkevich and Loya (1987) found that colonies of the scleractinian coral *Stylophora pistillata* from 5 m depth

were more fecund and had a reproductive season that was two or three months longer than colonies from 24-45 m, highlighting the influence of depth. Similarly, colonies of an unidentified deep-water *Drifa* sp. from Newfoundland (eastern Canada) from 500 m exhibited a longer planulation period than those collected from 1200 m (Chapter 3).

Generally, metamorphosis in benthic marine invertebrates is triggered by a permanent attachment to the substratum (Rodriguez et al. 1993). Based largely on studies of tropical corals, metamorphosis and settlement have been shown to be influenced by the larval type, larval competency period, and key environmental factors that include seawater temperature, physical texture and orientation of substrata, current, light quality, and presence of bacterial films (Atoda 1951; Jokiel and Guinther 1978; Hodgson 1985; Rogers 1990; Abelson 1997; Fabricius 1997; Heyward and Negri 1999; Baird et al. 2003; Harrington et al. 2004). Though it is not the norm in corals, metamorphosis prior to settlement has been reported in soft corals, including *Heteroxenia fuscescens* (Zaslow and Benayahu 1996).

The type of larvae and their competency period are important criteria in defining the recruitment and potential dispersal of corals (Richmond 1987). Shallow-water soft corals species commonly exhibit fast growth and rapid colonization (Fabricius et al. 1995); however, a few recent studies have shown that the primary polyps of temperate and deep-sea corals grow at very slow rates (Cordes et al. 2001; Hwang and Song 2007, Sun et al 2009 and Chapter 3).

The larval stage is a critical phase in the life history of benthic marine invertebrates, that increases the chance of finding appropriate substrata in both local or distant locations (Ostarello 1976; Müller and Leitz 2002). Although there has been considerable research on this issue in shallow-water coral species (Atoda 1951; Altieri 2003), few studies have focused on deep-sea corals as a result of the challenges of collecting deep-sea material and the constraints associated with *in situ* studies and captive breeding of live specimens.

Based on the limited availability of deep-sea specimens, my research was conducted on an opportunistic basis in an effort to gather key information on reproduction of deep-sea corals. This work focused on two deep-sea nephtheids, *Gersemia fruticosa* and *Duva florida*, collected in the NW Atlantic at depths of ca. 100-535 m. My goal was to conduct a comparative study of the two species to examine and characterize planula behaviour, settlement, metamorphosis and growth, and determine how these characteristics are influenced by environmental factors. These data could play a useful role in assessing the response of deep-water soft corals to manmade and natural perturbations.

4.3 Materials and Methods

Gersemia fruticosa colonies were collected during trawl surveys conducted by the Department of Fisheries and Oceans (DFO) aboard the CCGS *Teleost* in December 2006 at depths of 100 to 300 m. On the vessel, they were maintained in 5000 L tanks supplied

with running seawater and transferred to the Ocean Sciences Centre within one week. In the laboratory, all five colonies were kept in one 20-L tank, provided with unfiltered running seawater (ca. 1.5 L min⁻¹) in total darkness and with ambient temperatures that fluctuated between -1 and 9 °C. When necessary, running seawater was chilled to maintain it below 10 °C.

After newly-settled polyps were first observed on the side of the holding tanks on April 1, 2007, four of the five colonies were isolated in three 200-ml beakers until late June 2007 (20 days after the last observed natural release of planulae) in order to monitor daily planulation. The colonies were maintained in darkness at a temperature below 8 °C, and half of the seawater in the beakers was changed daily. Naturally-released planulae were routinely collected during the daily seawater change (within 24 h post release), generally on the bottom of the beaker. Planulae released on the same day were maintained together in 50-ml beakers to assess time to settlement. Once planulae had settled and metamorphosed into a primary polyp, they were reared in flow-through conditions and fed with a mixture of algae (*Isochrysis* sp., *Tetraselmis* sp., *Nannochloropsis* sp.) and rotifers on a continuous basis through a peristaltic pump (ca. 40 ml min⁻¹).

Duva florida colonies were collected from the continental slope southwest of the Grand Banks (eastern Canada) at a depth of ca. 535 m in November 2006 on board of the CCGS *Teleost* and in July 2007 on board of the CCGS *Hudson*. Colonies did not release larvae naturally; however, planulae were successfully extracted surgically and reared in the

laboratory. Extracted planulae and primary polyps were cultured under the same conditions as described for *G. fruticosa* above.

Environmental factors

Seawater temperatures in 2007 were monitored with a temperature-light logger HOBO Pendant (UA-002-XX) placed in the tank. Seasonal data on phytoplankton abundance (indicated by chlorophyll fluorescence) at a depth of ca 150 m in 2007 were obtained from the Department of Fisheries and Oceans Canada for station 27 (<http://www.meds-sdmm.dfo-mpo.gc.ca/isdm-gdsi/azmp-pmza/hydro/index-eng.html>, fixed zonal monitoring program; 47°32'04"N 52°35'06"W). Information on maximum wind speed (as an indicator of the amount of resuspended organic and inorganic materials) was gathered from Environment Canada, using data collected at St John's Airport (47°22'19"N 52°26'38"W) 140.5 m above sea level from April 2007 to June 2007 (http://www.climate.weatheroffice.ec.gc.ca/climateData/canada_e.html). Lunar cycle data were obtained from the StarDate Online website (<http://stardate.org/nightsky/moon/>).

4.4 Results

Environmental factors

Seawater temperature in the laboratory remained below 8 °C throughout most of the year in 2007. It decreased from 2-3 °C in January to 0 °C in March and increased from April to August to a maximum of 8-10 °C, remaining high until the end of October and early

November and then decreasing again (Figure 4-1). Phytoplankton abundance (indicated by chlorophyll fluorescence) was at a yearly minimum from January to April, and then increased rapidly during the spring bloom to reach a maximum around mid-May. It then decreased to its minimum value in August, with a low amplitude (compared to the spring bloom) in the early fall (Figure 4-1). Wind speed decreased from April to July in 2007, and no clear coincidence with planulation was observed (Figure 4-1).

Gersemia fruticosa

The release of planulae by colonies of *G. fruticosa* was monitored from April to early June 2007 in the laboratory (Figure 4-1). The number of planulae released during a single episode varied from 1 to 25; however, the release of larger numbers of planulae was observed in some colonies which were in poor shape and died soon after the spawning. Planulation began when the seawater temperature and phytoplankton abundance started to increase in April (Figure 4-1). Three major planulation events were identified: mid-April, early May, and late May, all of these events coincided with the days around the full moon (Figure 4-1).

From April to June 2007, 79 planulae were released by the five colonies, and 22 individuals settled successfully (Figure 4-2). Time to settlement varied from 3 to 70 days, with an average of 27.7 ± 4.6 days (Mean \pm SE). The proportion of settled planulae in each daily batch varied from 0 to 100 %, with an average of 48.2 ± 0.2 %. However, the

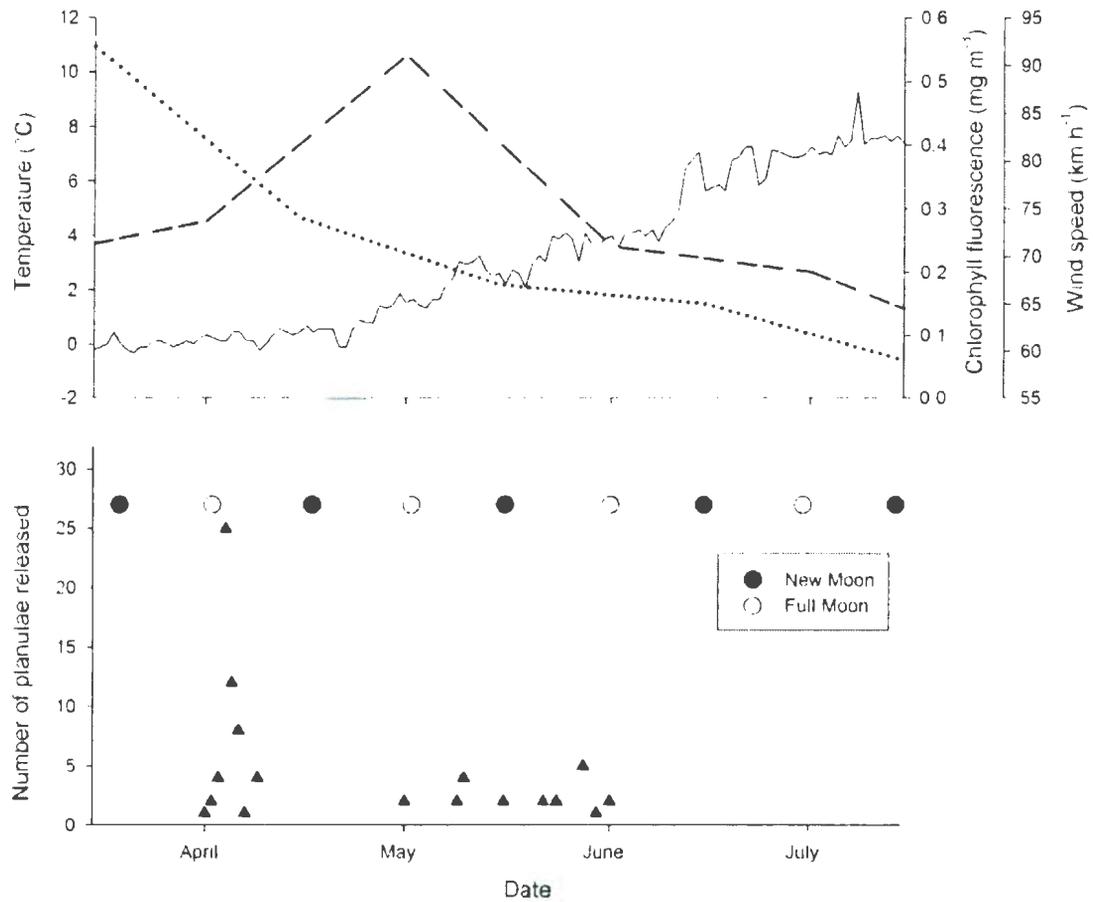


Figure 4-1. *Gersemia fruticosa*. Planulation events observed between April and June 2007 in correlation with temperature (solid line), chlorophyll fluorescence (an indicator of phytoplankton, broken line), wind speed (dotted line) and lunar cycle (open and closed circles).

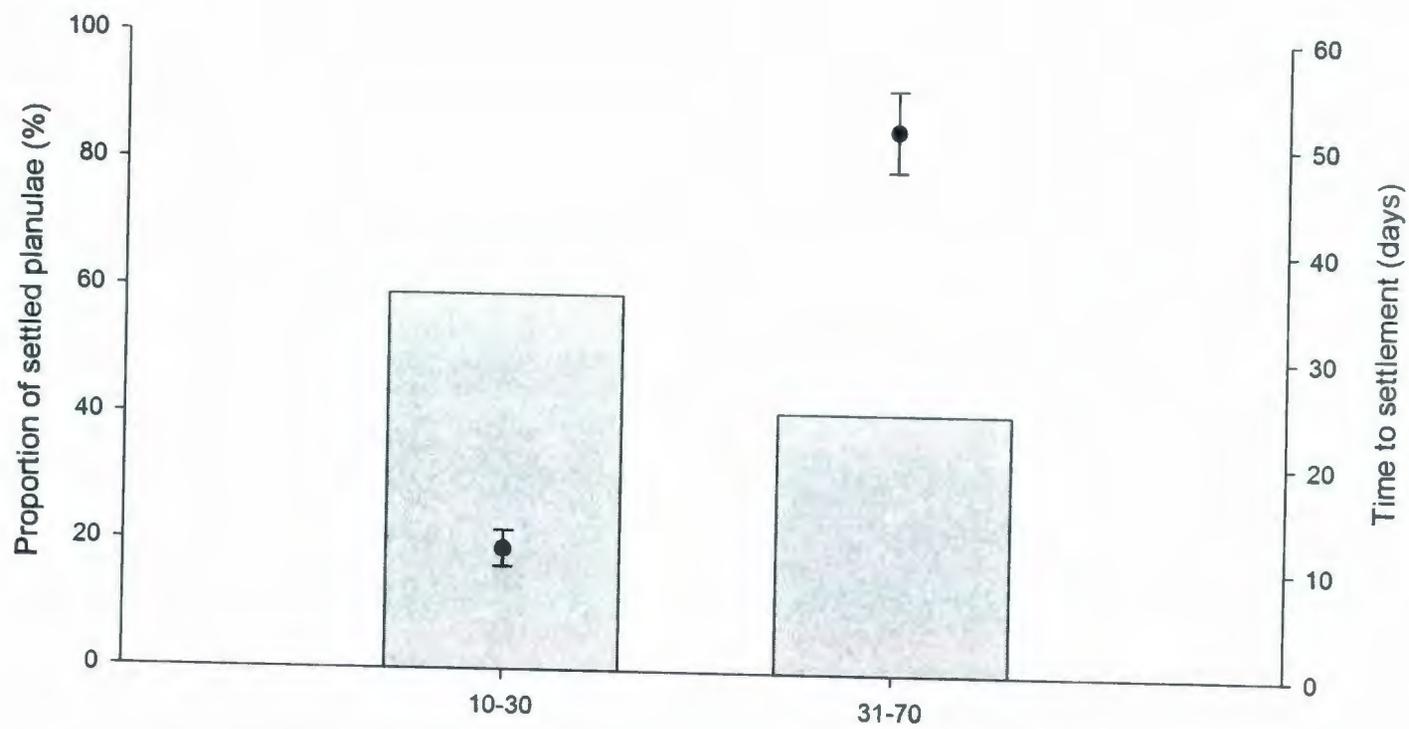


Figure 4-2. Settlement of *Gersemia fruticosa*. Bars indicate the proportion of planulae settled within 10-30 and 31-70 days, respectively. Dots indicate the average time to settlement in each group (Mean \pm SE, n=13 and 9, respectively).

laboratory settings did not offer optimal conditions for settlement (see Chapter 3) and time to settlement may be shorter in the field where natural rough substrata are available.

The size of fully extended planulae was ca. 1.5-2.5 mm long and 0.5 mm wide (Figure 4-3). Individuals were negatively buoyant, but were able to contract and expand their body to adjust their position in the water column (Figure 4-4). Planulae were also able to probe the substratum with their aboral extremity, and they used the oral extremity as an anchor to position the aboral extremity for attachment.

After the free-swimming elongated planulae settled on the substratum, they became cone-shaped or flattened. The eight primary mesenteries typically appeared within 24 h. Generally, primary polyps began to develop pinnules two or three weeks post settlement. Primary polyps developed long pinnules (ca. 3-4 mm long) and grew to a height of ca. 6-10 mm (tentacles included) with a stalk diameter of ca. 1 mm in 2 or 3 months of post-settlement growth. However, no further growth or budding of polyps was observed in the laboratory after 13 months of monitoring.

Duva florida

Surgically-extracted planulae when fully extended measured ca. 1-2.5 mm in length and ca. 0.5 mm in width (Figure 4-5). Planulae generally sank and remained on the bottom of the containers after their release, and they did not exhibit the complex post-release behaviours observed in *Gersemia fruticosa* planulae. Planulae became cone-shaped or

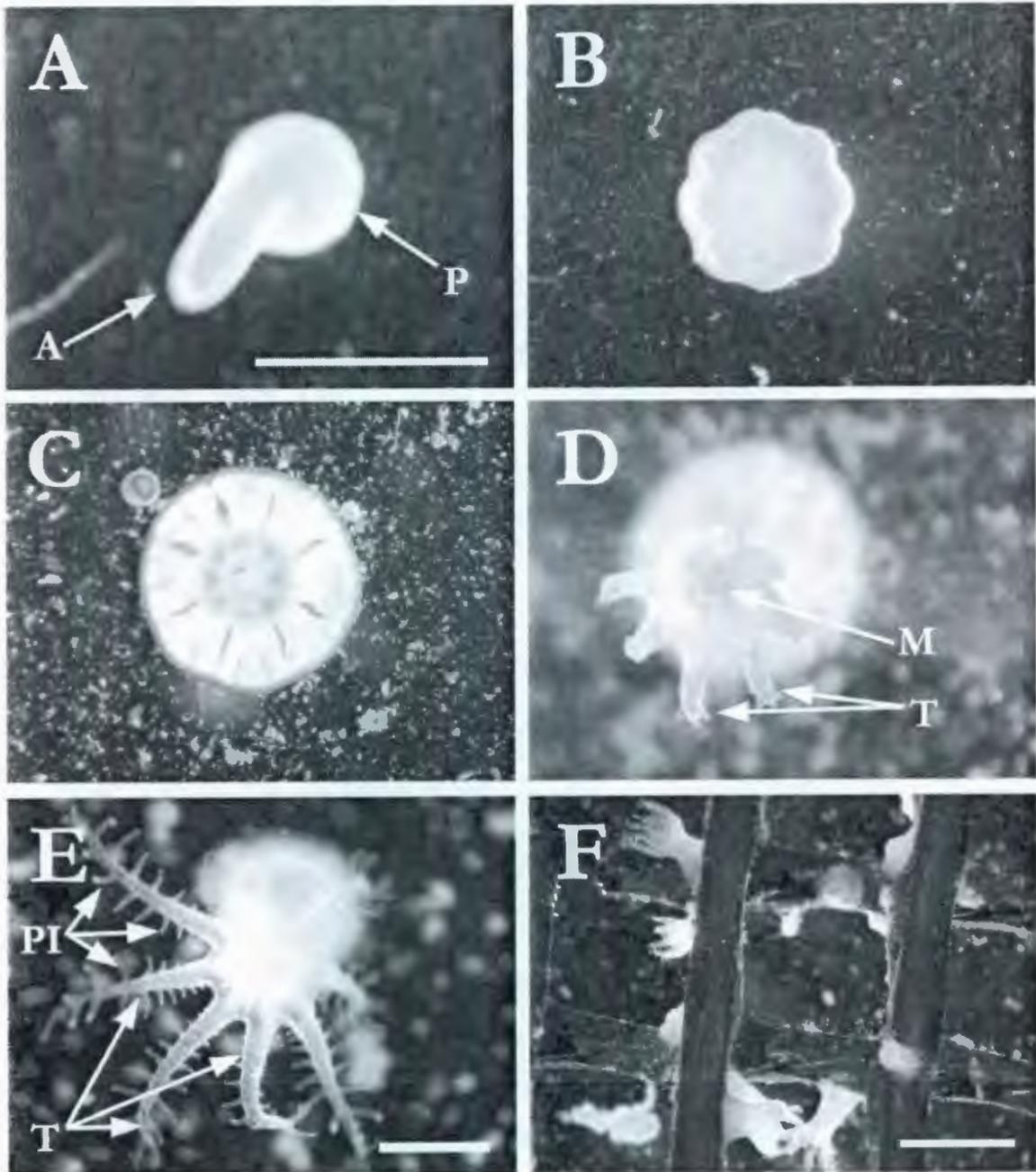


Figure 4-3. *Gersemia fruticosa*. A) Planula exploring the substratum. B) Newly settled planula. C) Early primary polyp at 3 days post settlement. D) Fully developed polyp at one month post settlement. E) Polyp at two month post settlement. F) General view of different stages of polyp development. A: anterior end of planula, P: posterior end of planula, T: tentacle, M: mouth. PI: pinnule. All scale bars represent 1 mm. The bar in A also applies to B, C and D.

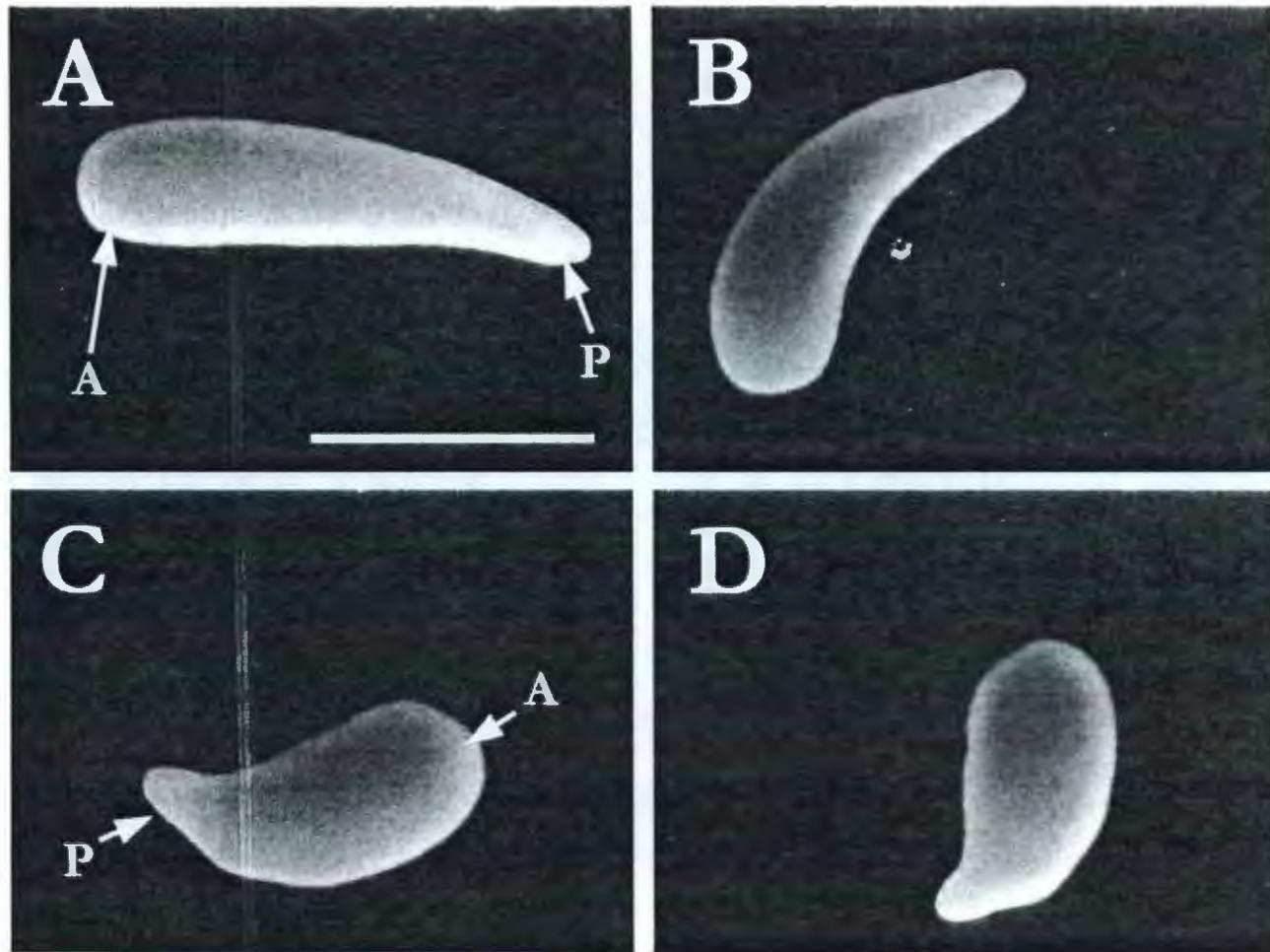


Figure 4-4. *Gersemia fruticosa*. A-D) Planula circling behaviour in close contact with the substratum monitored within two minutes. A: anterior end of planula, P: posterior end of planula. The scale bar in A also applies to B-D and represents 1 mm.

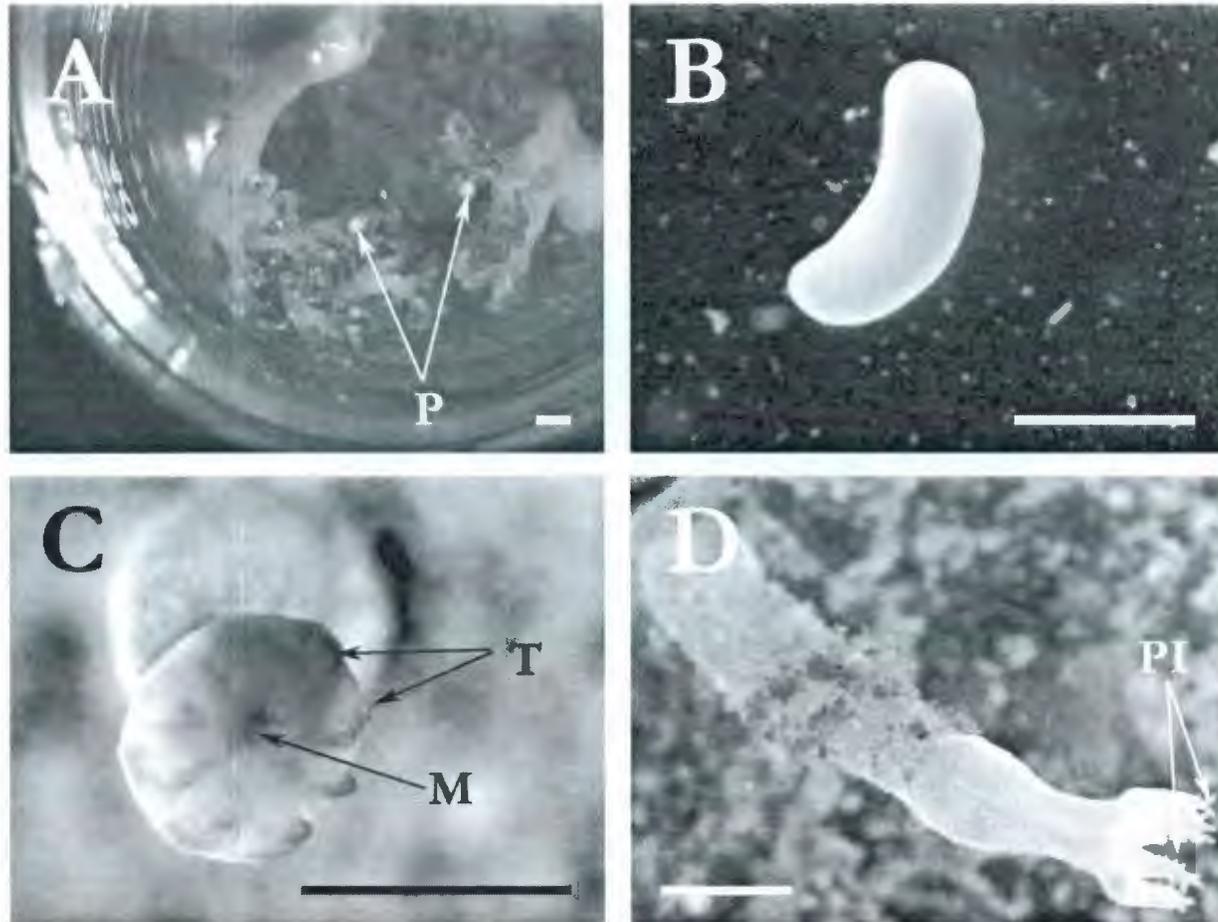


Figure 4-5. *Duva florida*. A) Branches containing planulae/oocytes. B) Planula. C) Early primary polyp at ca. one month post settlement. D) Fully developed polyp at ca. three months post settlement. T: tentacle (retracted), M: mouth. PI: pinnule. All scale bars represent 1 mm.

flattened and started to develop eight primary mesenteries within one week post settlement. Small pinnules were not observed until ca. two months post settlement. Primary polyps reached a maximum height of ca. 3-4 mm and a stalk diameter of ca. 1 mm, irrespective of whether they were obtained from extracted planulae in December 2006 or July 2007. Pinnules were less than ca. 1 mm long even when primary polyps attained their maximum size. Afterwards, no further growth or budding of polyps was observed over 11 months of monitoring.

Planulae typically stuck together when they came into contact, which did not prevent them from developing into polyps. Planulae also had the capacity to settle on another planula and develop into a two-polyp colony. The only two-polyp colony formed in the laboratory was formed by planula fusion (i.e., chimerism; Figure 4-6), and no further budding of the two-polyp colony was observed.

4.5 Discussion

Challenges of research on deep-sea corals

The reproductive features of deep-sea corals are a key aspect of their biology that contributes to their level of vulnerability or resilience to disturbances. However, the life histories of deep-sea octocorals are largely unresolved, because: 1) taxonomical identification is often uncertain; 2) opportunities for collection of deep-sea samples are

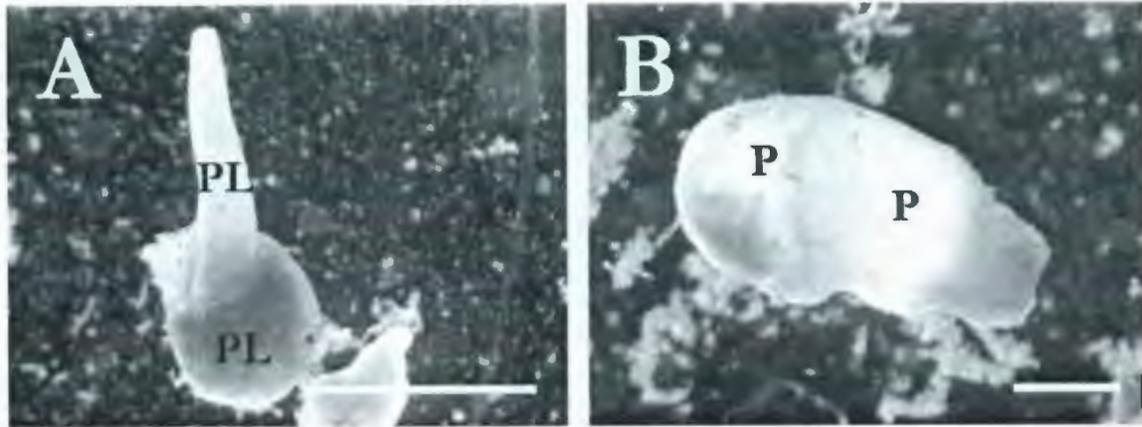


Figure 4-6. *Duva florida*. A) Fusion of two planulae. B) Two-polyp colony formed by fusion of two planulae. PL: planula, P: polyp. All scale bars represent 1 mm.

rare and costly; and 3) appropriate conditions for maintaining deep-sea animals alive for long periods in the laboratory are difficult to achieve.

The unique location and characteristics of the Ocean Sciences Centre facilities made it possible to overcome some of these challenges and successfully maintain and study aspects of reproduction in deep-sea coral species: 1) the research vessels used for sampling were equipped with a cold, running seawater supply; 2) the vessels were able to transport specimens to ports near the laboratory, thus reducing the stress associated with extended transport; and 3) high-quality, cold unfiltered running seawater was available for laboratory holding tanks.

The greatest spawning success was obtained with Nephtheidae species, which were in good physical condition and healthy enough to feed and spawn normally over several months of captivity. For the logistical reasons already outlined earlier, my research was performed in a laboratory setting on a small sample number of colonies. It is therefore difficult to compare these results directly to previous studies that have focussed on tropical and other shallow-water species of corals.

Planulation and planula behaviour

Planula release has rarely been described for deep-sea corals and this work is among the very few accounts of this activity, except for studies on *Anthomastus ritteri* (Cordes et al. 2001) and two *Drifa* species from the deep sea around Newfoundland (eastern Canada)

(Chapter 3) . The planulation I observed in *Gersemia fruticosa* extended from April to the end of May 2007. Although no natural planulation was observed in *Duva florida*, surgical extraction of viable planulae from fragments of colonies in December 2006 and July 2007 and their successful metamorphosis and subsequent growth as primary polyps suggests that this species may be able to reproduce at different times of the year and potentially year round. Similar year round patterns of planulation were observed in one yet unidentified deep-sea corals *Drifa* sp. (for details see Chapter 3).

Planulae of *G. fruticosa* (ca. 1.5-2.5 mm) and *D. florida* (ca. 1-2.5 mm) are the typical size of brooded soft coral larvae, and among the largest observed planulae in corals (Cordes et al. 2001). However, they are smaller than those of the two other deep-sea species I studied for which the longest planula was ca. 6 mm (Chapter 3). Larval size has been shown to have a significant influence on the performance of juvenile and adult colonial invertebrates. For instance, in the shallow-water colonial bryozoan *Bugula neritina*, colonies developed from larger larvae had higher survival rates, growth rates and reproductive output (Marshall et al. 2003).

Generally, brooded planulae are associated with limited dispersal scales (Sebens 1983a; 1983b; Harrison and Wallace 1990; Richmond and Hunter 1990). Nevertheless, the planulae of the two brooding species studied here displayed behaviours consistent with both short and long-range dispersal. Planulae of *Gersemia fruticosa* and *Duva florida* did not exhibit active searching activities as would be expected if they tended to increase

local recruitment of new polyps. However, some planulae postponed metamorphosis for weeks or partially metamorphosed before settlement, and some even developed to polyps (laying on the bottom without attaching) suggesting a dual ability to readily settle when possible or to prolong their pelagic phase to disperse and increase their chances of finding suitable substrata. The ability of planulae to undergo partial metamorphosis in the water column is probably related to the absence of appropriate inducers for settlement, and might also increase the probability of settlement on appropriate substrata as suggested by Zaslow and Benayahu (1996). According to the review by Thorson (1950), larvae of marine invertebrates might metamorphose and resort to growing in mid-water if they cannot find suitable substrata for a long period. The deep-sea corals *Duva florida* and *Drifa* sp. (Chapter 3) showed similar metamorphosis and development of primary polyps in the water column before settlement. The fact that polyps appear to feed whether or not they are permanently attached suggests that they metamorphose in the absence of appropriate substrata in order to extend their survival time in the plankton, and increase their chances of locating and colonizing suitable habitats. Whether this behaviour is a strategy or a desperate measure before running out of energy for metamorphosis remains to be clarified.

Planulation in correlation with environmental factors

The influence of seawater temperature and seasonal flux in food availability (i.e., phytodetritus) on reproduction has been documented for several benthic invertebrates (Tyler et al. 1982; Benayahu 1997; Ben-David-Zaslow et al. 1999; Wigham et al. 2003;

Mercier and Hamel 2008, Chapters 2 and 3). The onset of planulation in *Gersemia fruticosa* corresponded with an increase in seawater temperature and phytoplankton abundance in April, suggesting that these factors may trigger planula release. Furthermore, planulation started on the days around the full moon in April and continued more or less constantly through May. Lunar phases may be a cue to synchronize spawning, either directly or through tidal current and fluctuations in phytoplankton or zooplankton availability. Lunar cycles have been proposed to influence spawning and local recruitment in shallow-water octocorals (Benayahu and Loya 1983; Benayahu and Loya 1984; Coma et al. 1995).

Growth and fusion

Published studies on shallow-water soft corals indicate faster growth and more rapid colonization (Fabricius et al. 1995) than reported here for deep-sea species. New recruits of the two species studied here exhibited slow growth rates, and growth of primary polyps seemed to stop when they reached a specific size (generally within two or three months post settlement). Comparably slow growth rates of primary polyps were also observed in deep-sea *Drifa* species (Chapter 3). A few other studies on deep-sea or temperate corals have suggested that early growth rates of primary polyps might be extremely slow. In the deep-sea alcyonacean coral *Anthomastus ritteri*, juvenile colonies formed the second autozooid after 1-2 months and displayed very slow growth in the first 8-9 months (Cordes et al. 2001). In a shallow-water temperate soft coral,

Dendronephthya gigantean, (Nephtheidae), no branching of primary polyps was recorded during a one-month study (Hwang and Song 2007).

In the present study, no primary polyp budding was ever observed. The only two-polyp colonies obtained in the laboratory were formed by planula/polyp fusion. This behaviour, which is called chimerism, was observed in *Duva florida* (this study) and also in *Drifa* sp. (Chapter 3). Chimerism is presumably a common strategy to compensate for the slow juvenile growth rates in deep-sea corals and to increase recruitment rates. Similar fusion from aggregated settlement has been documented in various coral species (Barki et al. 2002). Several benefits of this phenomenon have been suggested, including increased genetic variability and body size, and improved survival, growth and reproductive output (Buss 1982). Research on *Stylophora pistillata*, a Red Sea stony coral, concluded that the benefits from increased of body size were the reason for the gregarious settlement (Amar et al. 2008). Research on *Pocillopora damicornis* in the Philippines also showed that fusion increased survival and growth rates, and thus benefited juvenile coral colonies (Raymundo and Maypa 2004). In addition, the latter authors found that fusion rate was influenced by colony age; colonies fused before 8 months were more stable than those fused after 8 months. However, research on four soft corals from the Red Sea found that genetically heterogeneous individuals formed by fusion in the laboratory had lower survival under field conditions, and the authors proposed that chimerism in juvenile cnidarians only occurred before the ontogenetic development of histocompatibility recognition when allorecognition failed (Barki et al. 2002). I cannot yet conclude whether

fusion is beneficial or detrimental in the deep-sea corals studied here. However, it is an important element to consider in studies of parentage and population genetic structure.

The resilience and vulnerability of deep-sea Nephtheidae corals

The deep-sea Nephtheidae corals studied here exhibited a degree of resilience to environmental variability, in that they survived and reproduced successfully under laboratory conditions (i.e., lower pressure and, potentially more importantly, temperature fluctuations and modified food supply). In addition, the fact that extracted planulae from colony fragments exhibited settlement rates and growth comparable to that of naturally-released larvae indicates that fertile colonies that are partially damaged by anthropogenic activities (e.g., bottom trawling) may still be able to produce offspring. Moreover, the small size at sexual maturity might help to compensate for slow growth rates; for example, the smallest fertile colony of *Gersemia fruticosa* in the laboratory was ca. 2 cm in length and 1 cm in width and the smallest fertile colony of *Drifa* sp. described in Chapter 3 had ca. 10 polyps (similar size as the smallest fertile colony size of *G. fruticosa*). Nonetheless, the slow growth rate of primary polyps highlights the vulnerability of deep-sea soft corals in recovering when ecosystems are damaged by natural or anthropogenic disturbances.

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CHAPTER 5 : GENERAL CONCLUSIONS



Deep-sea corals in NW Atlantic waters

5.1 Major findings

The study of four species of deep-sea nephtheids (Octocorallia: Alcyonacea) has allowed me to elucidate several aspects of their reproductive biology, including gamete development, mode and timing of reproduction, planulation, metamorphosis, settlement preferences and growth. The major findings and outcomes of this research are listed below:

- All four nephtheid species are internal brooders that release planulae in the water column.
- Planulation of the four nephtheids was investigated in the laboratory based on a small number of samples. *Drifa* sp. can release planulae year round. Successful culture of primary polyps of *Duva florida* collected at different periods of the year also suggests that mature planulae may be produced all year long at the population level. On the other hand, planulation of *Drifa glomerata* and *Gersemia fruticosa* appeared to be seasonal.
- Preliminary correlations with environmental factors suggest that temperature, photoperiod, phytoplankton abundance, wind speed and lunar phases may play a role in the reproductive cycle at different levels. Increases in temperature and phytoplankton abundance coincided with the initiation of planulation. In *Gersemia fruticosa*, lunar cycles may mediate planulation events within a given period of time.

- A specialized brooding polyp dubbed a “reproductive polyp” has been documented in *Drifa* sp, providing the first example of such a structure in nephtheid corals.
- In the four species studied, planulae exhibited distinctive behaviours. Specifically, the majority of planulae from *Drifa glomerata* actively crawled and probed the substratum after their release; however, the proportion of planulae exhibiting this behaviour was significantly lower in the other three species where individuals tended to lay relatively passively on the substratum.
- In the four species studied, planulae were extremely large, for example, the largest planula observed was ca. 6 mm long in *Drifa* sp. Intra-brood size differences in planulae were detected in *Drifa glomerata* and *Drifa* sp., suggesting two different degrees of dispersal potential.
- Both *Drifa* species settled at higher rates on hard substrata and rough surfaces, emphasizing the importance of such substrata in the successful recruitment of deep-sea soft corals. In *D. glomerata*, settlement occurred more rapidly on surfaces with a biofilm, whereas planulae of *Drifa* sp. from 500-m settled faster on clean surfaces. Physical and chemical factors likely play different roles in the metamorphosis and settlement of each species.
- Planulae of the four species were all able to metamorphose in the water column before settlement. This is probably related to the absence of suitable inducers for settlement and might increase the probability of eventually settling on appropriate substrata.

- New recruits of all species exhibit slow growth rates. Natural budding of a primary polyp was observed only once in *Drifa glomerata* ca. 9 months after settlement. No natural budding of polyp has been observed in the other three species studied.
- Two-polyp colonies in the laboratory were mainly formed by fusion, which is likely a common strategy in coral planulae of deep-sea corals that may help to compensate for low juvenile growth rates and thus increase recruitment rates.

In summary, though the production of recruits appears to occur in a long period of the year (either seasonal or on a continuous with some seasonal peaks), opportunities for settlement may be limited in habitats dominated by mud bottoms. The growth of settled polyps appears to be very slow, further highlighting the vulnerability of deep-sea coral ecosystems.

As a result of taxonomic uncertainties, there have been few investigations on the reproductive biology of even shallow-water species of the Nephtheidae, despite their widespread distribution. In the deep sea this is compounded by the common challenges associated with deep-sea research (i.e., limited number of samples, maintenance of deep-sea animals for long periods in the laboratory) further limiting our understanding of deep-sea nephtheids.

Most of the samples available for my study were frozen. Because frozen tissues are not always suitable for histological procedures, I decided to supplement the work with microsurgical investigations and studies of live specimens. Live specimens of Nephtheidae colonies were maintained under laboratory conditions for several months, allowing me to elucidate various aspects of the reproductive biology of deep-sea nephtheids and describe the processes of planula release, planula development, settlement preferences, and early growth of the juveniles for the first time. This work therefore provides one of the most complete investigations of deep-sea coral reproduction to date. It significantly enhances our understanding of the breeding and recruitment processes in deep-sea soft corals, and provides data that highlight the importance of limiting damage to and protecting deep-water ecosystems.

5.2 Future research

This study provided fundamental information on the reproduction, settlement and metamorphosis of four deep-sea nephtheids; however, the state of knowledge remains limited compared to the body of evidence gathered on shallow-water corals. More detailed studies would be helpful to obtain a better understanding of deep-sea corals.

- Proper identification of deep-sea soft corals should be one of the dominant foci of future investigations. Such studies should combine information from colony and sclerite morphology, reproductive features and molecular characteristics.

- The reproductive mode (sexual or asexual) is a significant factor that can influence variability in population genetics. Using histology, continuous and intense sampling would be needed to obtain a comprehensive view of deep-sea coral reproduction. However, genetic tools such as amplified fragment length polymorphism (AFLP) offer efficient alternative methods of distinguishing these two reproductive modes.
- Considering the influence of temperature, photoperiod, lunar cycles and other related factors on the reproduction of shallow-water species, and the preliminary evidence reported in this thesis, research on planulation/spawning of eurybathyal species at various depths would provide a better understanding of the influences of light cycles at different depths.
- The low occurrence or absence of male colonies in populations and the cycle of spermatogenesis are two other poorly understood topics in soft coral reproduction. The paternity of the brooded planulae in species with low male incidence such as those I studied, and the possibility of the parthenogenetic origin of planulae, should be investigated.
- Size differences of planulae are observed among and between species. However, their influence on settlement time and dispersal ability is largely unknown, especially where intra-brood variations are observed. Further research on the significance of planula size would help to provide a better understanding of the dispersal strategy of deep-sea corals. Furthermore, research on the influence of

different environmental factors (such as current speed and temperature) on settlement and survival rates would also be useful for conservation purposes.

- Studies on planula behaviour are generally based on observation. Research on lipid composition and planula fine structure would help clarify the reasons behind the distinct behaviours exhibited by planulae of different species or by conspecific planulae of different sizes.

Appendix. List of the presentations delivered.

- 1) Settlement preferences and planula behaviour in deep-sea soft corals (poster presentation). ASLO summer meeting, June 8-13 2008, St John's, NL, Canada.

- 2) Reproductive cycle of the deep-sea coral, *Drifa glomerata* (Octocorallia: Alcyonacea), in the NW Atlantic (oral presentation). 11th International Coral Reef Symposium (ICRS), July 7-11 2008, Fort Lauderdale, Florida, USA.

- 3) Planula release, settlement, metamorphosis and growth of deep-sea soft corals (poster presentation). 11th International Coral Reef Symposium (ICRS), July 7-11 2008, Fort Lauderdale, Florida, USA.

