THE EFFECT OF A SEASONAL PULSE OF SINKING PHYTODETRITUS ON THE REPRODUCTION OF TWO BENTHIC DEPOSIT-FEEDING SPECIES, YOLDIA HYPERBOREA AND CTENODISCUS CRISPATUS

J. ROBERTO JARAMILLO

INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps.

ProQuest Information and Learning
300 North Zeeb Road, Ann Arbor, MI 48106-1346 USA
800-521-0600

THE EFFECT OF A SEASONAL PULSE OF SINKING PHYTODETRITUS ON THE REPRODUCTION OF TWO BENTHIC DEPOSIT-FEEDING SPECIES, *YOLDIA HYPERBOREA* AND *CTENODISCUS CRISPATUS*

by

J. Roberto Jaramillo

A thesis submitted to the School of Graduate Studies in partial fulfilment
of the requirements for the degree of Doctor of Philosophy

Department of Biology
Memorial University of Newfoundland
2001

St. John's Newfoundland

ABSTRACT

Reproductive cycles of mollusc species have been extensively studied. Many of these studies demonstrated that fluctuating environmental factors affect the length of the spawning period and timing of reproduction. Since reproduction involves processes such as storage of energy and the production, accumulation and spawning of mature gametes, a close relationship has been proposed between the gametogenic cycle and food availability.

Species inhabiting unstable environments generally have episodic reproductive cycles whereas species living in more stable environments generally reproduce continuously. Recent studies suggest that many stable environments, such as the deep sea, are not as stable as previously thought, since many sublittoral and deep sea areas experience seasonal phytodetritus sinking which may affect the reproductive activity of some species.

In Conception Bay, Newfoundland, there is a deposition of phytodetritus which reaches the bottom at 240 m depth shortly after the spring and fall phytoplankton blooms. However, the reproductive response of the benthic community to this seasonal input is completely unknown.

In order to examine the reproductive response to a seasonal input of food in the benthic community in Conception Bay, two common deposit feeders were selected, *Yoldia hyperborea* and *Ctenodiscus crispatus*. This study provides new data on the role of phytodetritus deposition in the reproduction of these species and on the ecological significance of a seasonally-pulsed food supply on the deposit feeding community inhabiting Conception Bay.

Yoldia hyperborea had a mean egg diameter 120 μm, and a maximum apparent fecundity was of 8.5×10^4 eggs per individual. Larval development occurred through a lecithotrophic pericalymma larva that is restricted to protobranch species. Spawning occurred during the winter-spring period coincident or following phytodetrital deposition.

Laboratory feeding experiments showed that frequent addition of phytodetritus stimulates production of eggs, supporting field observations suggesting that gamete production

of *Y. hyperborea* is dependent on food availability. Laboratory experiments using ^{14}C labelled *Thalassiosira nordenskioldii* demonstrated incorporation of cell contents into the gonad.

Ctenodiscus crispatus eggs had a mean diameter of 450 μm. The maximum fecundity was 13.8×10^{6} eggs per individual. Eggs develop through a lecithotrophic larvae. Reproduction occurred continuously throughout the year, although fecundity was higher in spring due to seasonal phytodetritus sinking.

The reproductive cycle of both deposit feeders was affected by fluctuations in food availability. Individual fecundity was maximal coincident with or just following the seasonal peak in phytodetritus deposition. Energy provided by resuspension events appeared to be utilized for gametogenesis both in *Yoldia hyperborea* and *Ctenodiscus crispatus*.

Table of Contents

5. CHAPTER V LABORATORY EXPERIMENTS

Acknowledgments

I would like to express my sincere gratitude to my supervisor, Dr. R.J. Thompson, for his valuable advice, interest and support during the development of this research and the writing of this dissertation.

I would also like to thank the other members of my supervisory committee, Dr. D. Deibel and Dr. P. Snelgrove, for their critical evaluations of the dissertation. Dr. Deibel also provided the Wild Macroscope 420.

My thanks are due to the members of the Ocean Sciences Centre field services unit for assisting with collections of animals, to Elizabeth Hatfield for her valuable help with the laboratory work, and to Dr. R.A. Khan for his constant support and encouragement.

Financial support was provided by an NSERC grant to Dr. R.J. Thompson and an award from the MUN/CIDA fellowship programme in marine sciences.

List of Figures

List of Tables

List of Abbreviations

AF	Apparent Fecundity
ANOVA	Analysis of Variance
^{14}C	^{14}Carbon Radioisotope
CB	Conception Bay
EAF	Estimated Apparent Fecundity
ES	Extended Siphon
^{68}Ge	^{68}Germanium Radioisotope
GI	Gonad Index
GVF	Gametic Volume Fraction
GSI	Gonad Size Index
HE	Hematoxylin-Eosin
HS	Horizontally extended Siphon
IRV	Immediate Reproductive Value
MEAF	Mean Estimated Apparent Fecundity
POM	Particulate Organic Material
RRV	Residual Reproductive Value
SEM	Scanning Electron Microscopy
TAG	Triacylglycerol
V	Volume occupied by gonad

CHAPTER I

GENERAL INTRODUCTION

1.1. INTRODUCTION

1.1.1. Reproductive cycles in bivalves

A number of studies have examined the occurrence and timing of reproductive events in marine invertebrate species (Ropes and Stickney 1965; Heffernan et al. 1989a,b; Brousseau 1978; Newell et al. 1982; Dibacco et al. 1995; Hooker and Creese 1995; see reviews by Giese 1959; Sastry 1979; Dohmen 1983). As a result of these studies, authors have described reproductive activity as a cyclic phenomenon which is composed of a number of events including multiplication, growth and maturation of gametes, spawning and a resting period. This cyclical phenomenon can occur either on a seasonal (Sastry 1966; Brousseau 1978; Emmet et al. 1987; Heffernan and Walker 1989; Heffernan et al. 1989a, b; Paulet and Boucher 1991; Dibacco et al.1995) or a continuous basis (Malachowski 1988; Hooker and Creese 1995). Seasonal reproduction is typically annual (once a year) or semiannual (twice a year).

Reproductive cycles have been described as either synchronous, where many individuals spawn at the same time on a seasonal basis, or asynchronous, where individuals spawn at different times, typically with many spawning periods for the population spread throughout the year. Synchronous spawning is thought to be common for species living in unstable environments (Sastry 1966, 1968, 1970; Emmet et al.1987), whereas asynchronous spawning is more common in species living in more stable environments (Giese and Pearse 1974; Rokop 1974; Tyler and Young 1992).

1.1.2. Factors affecting the reproductive cycles of bivalves

Reproductive cycles of intertidal and shallow-water subtidal species have been studied extensively. Many of these studies suggest that the reproductive cycle of species inhabiting unstable environments is strongly affected by seasonal fluctuations in environmental factors. Consequently, these environmental factors may influence both the period and timing of the cycle. Because environmental factors may fluctuate on a variety of time scales, variables such as water temperature (Loosanoff 1937; Orton 1920; Thorson 1946; Ropes and Stickney 1965; Sastry 1966, 1970; Himmelman 1980; Newell et al.1982; Currie 1990), salinity (Loosanoff 1952), food availability (Sastry 1968; Bayne 1975, 1976), photoperiod (Paulet and Boucher 1991), and tides (Lammens 1967) may control the reproductive cycle. Differences in timing of reproduction between geographically separate populations of individual species may also be due to latitude (Sastry 1979).

1.1.2.1. Temperature

Temperature has long been considered to be one of the most important factors influencing the reproductive periodicity of shallow water species. Thus, authors have predicted that species inhabiting more stable environments such as the deep-sea may reproduce continuously because seasonal fluctuations of temperature are minimal or absent (Orton 1920). This hypothesis is supported by recent data on reproductive cycles of echinoderms and molluscs of northeast and western Atlantic deep-sea zones, suggesting that a continuous reproductive cycle appears to be the dominant pattern in most of these species (Rokop 1974; Tyler et al. 1992).

1.1.2.2. Food availability

In spite of evidence demonstrating the effect of temperature on reproduction, it is also well known that gametogenic cycles in marine invertebrates are related to the storage of energy and the production, accumulation and spawning of mature gametes. Thus, there may be a close relationship between gametogenic cycles and food availability (Ansell 1974; Kautsky 1982; Bayne 1985; MacDonald and Thompson 1986). Consequently, the onset of the gametogenic cycle may depend on a positive energy balance, stored energy reserves (Lubet 1986) and external factors that trigger gametogenesis.

Since Gabbott (1975) demonstrated that glycogen is the main energy reserve utilized for reproduction in marine molluscs, a number of studies have been carried out to establish the source of this energy. Depending on the species, the energy for gamete production is derived from "reserves of glycogen and protein stored in the adductor muscle", as reported for *Crassostrea virginica* (Thompson et al. 1996) and Pectinidae (Mathieu and Lubet 1993), or from protein and lipids stored in the adductor muscle e.g. *Argopecten irradians* (Epp et al. 1988), or from new ingested food as in *Chlamys septemradiata* and Baltic populations of *Mytilus edulis* (Ansell 1974; Kautsky 1982 respectively); or from both stored and ingested food as in *Placopecten magellanicus* (Thompson 1977).

It is likely that different energetic strategies represent adaptations of species to the environment in which they live. Thus, in any species, the available energy for growth and reproduction should reflect fluctuations in stored energy and food supply (Sibly and Calow 1989; Calow and Sibly 1990; Olive 1992). Consequently, because gametogenesis requires energy, species that have a steady input of energy (i.e. in stable environments) should exhibit a continuous reproductive cycle. On the other hand, species subject to seasonal pulses of food should exhibit a seasonal reproductive cycle, and those having a mixed strategy to obtain energy may exhibit a semiannual reproductive cycle.

1.1.2.3. Geographic variation

Some studies have reported differences in the timing of reproduction between populations of a species in different parts of its geographic range (Sastry 1970; McDonald and Thompson 1988; Barber et al. 1991). Authors have attributed these latitudinal differences primarily to local environmental factors, especially food supply, which determines the nutrient reserve and hence the capability to initiate gamete development (Sastry 1970; Crump 1971; Newell et al. 1982; Bricelj et al. 1987; MacDonald and Thompson 1988). However, genetic variations in the timing of gametogenesis have been reported for *Crassostrea virginica* along the east coast of the United States (Barber et al. 1991).

In general, it is accepted that at the lower latitudinal limit of its geographic range a temperate species tends to exhibit a more extended reproductive period (continuous reproductive cycle). In contrast, near the higher latitudinal limit reproduction tends to be seasonal as a result of seasonal fluctuations in food availability, temperature and light (Thorson 1950; Bricelj et al. 1987). However, this generalization does not apply to all species. *Placopecten magellanicus*, for example, exhibits a similar reproductive period through its distribution with no clearly identifiable latitudinal trends (MacDonald and Thompson 1988).

1.1.3. Reproductive cycles in echinoderms

As in other marine invertebrates, the reproductive cycles of echinoderms are seasonal in some species and continuous in others (Lessios 1984; Byrne 1991; Tyler and Gage 1984a,b; Young et al. 1992), and also appear to be affected by fluctuations of environmental factors such as temperature, photoperiod and food availability (Clarke 1988; Spirlet et al. 1998).

1.1.4. Factors affecting the reproductive cycle of echinoderms

1.1.4.1. Photoperiod

Photoperiodic control of gametogenesis in echinoids and other echinoderms was suggested by Giese (1959) and Boolootian (1966). Pearse et al. (1986) demonstrated that the reproductive cycle of *Strongylocentrotus purpuratus* is extremely sensitive to seasonal fluctuations in photoperiod. In this species, individuals are either stimulated by short days (< 12 hour light) or suppressed by long days. Similar results have been described for asteroid species (Pearse et al. 1986). Recent studies of holothurians suggest that although photoperiod can stimulate the onset of gametogenesis, a synergistic action with other factors, such as temperature and food availability, may be required to trigger this process (Hamel and Mercier 1996).

1.1.4.2. Food availability

In general, the available data suggest that for unstable environments (intertidal rocky shore and shallow waters, boreal and polar areas), fluctuations of some environmental factors drive the reproductive cycles of marine invertebrates by synchronizing both the gamete release and larval settlement with more favourable environmental conditions. There is some evidence that gametogenic activity in asteroid species depends directly on feeding. Comparative studies of *Odontaster validus* (Pearse 1965) and *Patiriella regularis* (Crump 1971) indicate that gamete production is related to food availability. Crump (1971) studied three geographically-separated populations of *P. regularis* and found that although the timing of reproduction was similar in all cases, marked differences occurred in the gonad index as a consequence of differences in food availability.

1.1.5. Reproduction in stable environments

It has been suggested that species inhabiting more stable environments (e.g. tropical and deep sea areas) should exhibit a continuous reproductive cycle (Tyler and Young 1992). In particular, continuous reproductive cycles may result from the suppression of seasonal fluctuations in environmental factors such as temperature, food availability and salinity. Conversely, it would be expected that any fluctuating environmental factor might convert the continuous pattern of reproduction in deep sea species into a semiannual or seasonal reproductive cycle, depending on variability in the environmental factor.

Several studies in coastal and oceanic environments (McCave 1975; Hinga et al. 1979; Deuser and Ross 1980; Billet et al. 1983; Lampitt 1985; Gage and Tyler 1991; Rice et al. 1986, 1991; Lampitt et al. 1990) have reported seasonal sinking of phytodetritus to the sea bed following the spring diatom increase in the photic zone. The seasonal input of phytodetritus is an important source of both energy and essential elements for benthic and cold water communities and may influence the onset of gametogenic cycles. Clarke (1988) reported that limitations of reproduction to the summer months in antarctic species are strictly related to the availability of food. Tyler and Young (1992) reported that whereas many deep-sea species in the northeast Atlantic reproduce on a continuous basis, a small number of them reproduce seasonally. Consequently, these authors proposed that this reproductive seasonality could be explained by a seasonal influx of phytodetritus, as suggested by other studies (George and Menzies 1967, 1968; Schoener 1967; Rokop 1974, 1977; Stockton and Delaca 1982; Tyler and Gage 1984a; Harrison 1988; Tyler 1988; Thiel et al. 1990; Gage and Tyler 1991, Tyler et al. 1992; Young et al. 1992, Sumido et al. 2000).

Thus, there is evidence that the seasonal sinking of phytodetritus can modulate the stable conditions of deeper waters (sublittoral, bathyal and abyssal zones) and cold water environments (Clarke 1988), and that this seasonal input of food may drive the reproductive activity of the small number of species that exhibit a seasonal reproductive cycle in the deep-sea, as well as explain seasonal reproduction in antarctic species (Clarke 1988; Tyler and Young 1992). However, only a few studies have been conducted on the reproductive response of

deep-sea communities to the input of sinking phytodetritus (Scheltema 1994), and evidence linking phytodetrital pulses to reproductive activity is still equivocal for most deep-sea, seasonally reproducing species (Eckelbarger and Watling 1995).

1.1.6. Studying reproduction

To determine the reproductive strategy of any species requires knowledge of three basic variables:

- the number of eggs released annually per individual and during the lifetime of the individual
- the mean size of the eggs produced, and
- the frequency of the reproductive cycle (how many times a given individual reproduces each year and at what times different individuals of a given species reproduce).

1.1.6.1. Reproductive Potential or Fecundity

Fecundity is defined as the potential number of gametes produced during the lifetime of an individual (Scheltema 1994). Fecundity in species reproducing only once during their lifetime (semelparous) is usually estimated by counting the total number of eggs within mature females (Scheltema 1994). In those species having more than one reproductive event during their lifetime (iteroparous), annual fecundity is estimated by counting the total number of gametes produced during a year. The lifetime fecundity for an individual may be obtained by summing the annual fecundity for each year over its lifetime (Scheltema 1994).

Fecundity is difficult to estimate in long-lived species. Consequently, most researchers assume that the number of gametes within a mature female reflects the actual lifetime fecundity (Scheltema 1994). This value is the so-called apparent fecundity (AF), because it represents only the number of eggs observed at one particular moment rather than an annual or lifetime cumulative gamete production.

An alternative method for estimating lifetime fecundity is reproductive value, which is based on average production of offspring expected from a female over her lifetime (Fischer 1930). This concept was modified by Williams (1966), who considered that reproductive value consisted of two components, the immediate reproductive value (IRV) or fecundity, and a second term called the residual reproductive value (RRV), which represents future reproductive potential (Bayne et al. 1982). This index appeals to life history theorists because it incorporates fecundity and mortality, both of which likely experience selective pressure. Thus, RRV is probably a better fitness correlate than other fecundity estimates. However, a realistic estimate of RRV requires meaningful mortality data which are difficult and tedious to obtain (Thompson and MacDonald 1991). Consequently, neither IRV nor RRV have been frequently used in fecundity estimates.

1.1.6.2. Periodicity of Reproduction

Several different techniques have been used to study the frequency of reproduction, including quantitative measurements of the gonad index, or GI (Sastry 1966, 1970; Beninger 1987; DiBacco et al. 1995), and gamete volume fraction, or GVF (MacDonald and Thompson 1985,1986; DiBacco et al. 1995). The most convincing data for reproductive periodicity comes from direct observations of oogenesis using histological preparations (Tyler et al. 1981b, Scheltema 1994). More recently, an accurate and sensitive method to determine reproductive activity has been developed based on analysis of histological sections using computerized image analysis. This method provides an oocyte size-frequency distribution (Morvan and Ansell 1988; Paulet and Boucher 1991; Die et al.1995; Pazos et al. 1996), which avoids the subjectivity often associated with microscopic examinations of gonadal tissue.

Periodicity of reproduction has also been inferred from size frequency distributions of individual animals in the population (Scheltema 1994). This method is based on observations of seasonal recruitment and population size structure throughout the year. However, it requires knowledge of the timing of spawning, which has to be obtained beforehand from gametogenic studies (Schoener 1967; Lightfoot et al. 1979; Gage and Tyler 1981b). Size frequency

distributions have been useful for estimating the periodicity of reproduction in many non-molluscan species but have not been useful for bivalve molluscs (Scheltema 1994). For example, the method has been successfully utilized to predict periodicity of reproduction in brittle star (ophiuroid) species, which exhibit discrete size-classes resulting from successive reproductive events in a single age-class (Scheltema 1994).

Bivalve molluscs (especially deep-sea bivalves) often exhibit a unimodal size frequency distribution as a result of heavy predation on spat and rapid growth rates of the survivors, differential temperature and differences in food concentrations. Thus, species exhibiting either seasonal or continuous modes of reproduction develop high growth rates to avoid predation (or as a result of differential effects of environmental factors), making it difficult to distinguish cohorts produced between two successive recruitment events. Consequently, size-frequency distribution analysis is not suitable for studying reproduction in these species.

1.1.6.3. Mode of Larval Development

Thorson (1950) classified benthic marine invertebrate larvae into several categories based largely on nutritional mode and length of the planktonic phase. Planktotrophic larvae are those that spend most of their development time swimming and feeding in the plankton, whereas lecithotrophic larvae are those that depend entirely on internal energy reserves. To distinguish between larval shells of planktotrophic or lecithotrophic gastropods Thorson (1950) stated that "As a general rule in gastropod species, a clumsy large apex points to a non-pelagic development while a narrowly twisted apex, often with delicate sculpture, points to a pelagic development." Within lecithotrophic larvae, Thorson (1950) distinguished forms that develop in the plankton, called pelagic, from brooded or encapsulated larvae lacking a planktonic stage, called non-pelagic or benthic larvae.

Direct field and laboratory observations of larval development are difficult, and larval development has therefore been described only for a small number of marine invertebrate species (Levin and Bridges 1995). Most evidence on the mode of larval development comes

from measurements of egg size (Levin and Bridges 1995) but the size of the early shell (prodissoconch) is a better indicator of development mode (Jablonski and Lutz 1980, 1983).

Ockelmann (1965) was one of the first authors to use this indirect method to describe the mode of larval development in bivalve mollusc species. He showed that certain quantitative and qualitative relations exist between egg size (defined as either a small yolk-poor egg, or a large yolky-egg), the size of the larval shell (prodissoconch I and II) and the type of development (planktotrophic or lecithotrophic) in marine bivalve species. Consequently, in the absence of field observations on larval development, Ockelmann's method seems to be the most reliable and most commonly used in studies of bivalve species (Knudsen 1979; Shein 1989; Allen and Sanders 1973; Sanders and Allen 1973).

1.1.7. Study area

Although seasonal deposition of phytodetritus to the deep-sea bed following blooms has been described in a number of coastal and oceanic environments at various latitudes (McCave 1975; Hinga et al. 1979; Deuser and Ross 1980; Gage and Tyler 1991; Lampitt 1985; Rice et al. 1986, 1991; Lampitt et al. 1990), there have been few investigations of the reproductive response of benthic communities to sinking spring blooms (Tyler and Young 1992). A number of studies have strongly suggested that a seasonal pulse of phytodetritus reaches the bottom of Conception Bay, an embayment in northeastern Newfoundland, after the spring bloom (Thompson et al. 1986, 1999; Deibel et al. 1992; Redden 1994; Redden et al. 1994). Consequently, Conception Bay is a good site to study the reproductive response of the benthic community to such seasonal input of energy, given that near-bottom and tidal currents are weak and because temperature is low and stable (-1.5 to 0.5 °C) throughout the year (De Young and Sanderson 1995). Thus, it is possible to evaluate the importance of seasonal phytodetrital flux in the absence of the potentially confounding temperature variation observed in many seasonal environments.

Studies in this area (Conception Bay) suggest that the spring diatom bloom generally starts in March-April as a response to increasing light and decreasing winds (Deibel et al. 1992).

The spring diatom bloom seems to be clearly utilized by water column bacteria and zooplankton (Thompson et al. 1992), so that much of this primary production reaches the bottom between 24 and 51 days after the start of the bloom (Thompson et al. 1999). Consequently, this phytodetritus deposition represents a potentially important source of energy and essential nutrients for benthic communities inhabiting the soft bottom. The purpose of this study is to establish how this seasonal pulse of energy affects reproduction in these benthic communities.

1.1.8. Study species

In order to evaluate the response of benthic species to the seasonal deposition of phytodetritus, the protobranch bivalve *Yoldia hyperborea* (Torell, 1859) and the asteroid *Ctenodiscus crispatus* (Retzius) were selected for study. Both are dominant deposit feeding species living in the soft bottom of the depositional zone at 200 to 270 m depth in Conception Bay.

1.1.8.1. Current knowledge of study species

Despite reports that protobranch bivalves are dominant in many sediments, they have been little studied. Some data on taxonomy, growth, population structure, size-dependent survivorship, zoogeography and lipid composition have been published to date (Drew 1899; Allen 1978; Warren 1989; Ockelmann 1965; Lewis et al. 1982; Hutchings and Haedrich 1984; Nakaoka 1996; Parrish et al. 1996), but data on protobranch reproduction are limited, even for shallow-water species (Tyler et al. 1992). Some data on reproduction of *Nuculana*, *Yoldiella*, *Ledella*, *Maletia* and a few *Yoldia* species have been reported (Scheltema 1972; Rokop 1974; Tyler et al. 1992; Nakaoka 1996). Within protobranch species, *Yoldia hyperborea* is one of the least studied and no data related to reproductive cycles or reproductive strategies have been reported except the lipid composition of individuals (Parrish et al. 1996).

The reproductive cycles of echinoderms are better known. Both continuously and seasonally reproducing species have been reported for this group (Tyler and Gage 1984a,b; Tyler et al. 1992; Tyler and Young 1992). Asteroid species have been extensively studied and all have a reproductive cycle strongly affected by fluctuations in food availability (Crump 1971; Jangoux and Van Impe 1977; Lowe 1978; Shick et al. 1981b, Falk-Petersen 1982a). However, information on the reproductive cycle of the mudstar *Ctenodiscus crispatus* is not consistent. The Damariscove Island (Maine) population reproduces continuously (Shick et al. 1981b), whereas the Ramfjorden (Norway) population exhibits a seasonal reproductive cycle (Falk-Petersen 1982a).

1.1.9. Objectives

Studying the reproductive response of *Yoldia hyperborea* and *Ctenodiscus crispatus* to the seasonal influx of phytodetritus by determining their respective reproductive cycles will provide insight into the relationship between food availability and gamete production in these species. However, other variables such as fecundity, egg size and larval shell size will provide data regarding the mode of development of these species under the environmental conditions in Conception Bay. This study will therefore examine the three basic aspects of reproduction, namely the reproductive potential, the periodicity of reproduction and the mode of larval development for each species. Specific goals will include:

1. To estimate apparent fecundity by determining the maximum number of eggs produced by individuals of different sizes.
2. To estimate the size at first maturity.
3. To establish the frequency of reproduction by establishing the gametogenic cycle.
4. To estimate mean oocyte diameter.
5. To infer the mode of reproduction from the estimated egg diameter.
6. To measure the length of the prodissoconch I for *Yoldia hyperborea*.

7. To determine the reproductive response of *Yoldia hyperborea* individuals under laboratory conditions when fed with decaying laboratory-grown microalgae.
8. To examine carbon incorporation into gonads using ^{14}C under controlled environmental conditions.

CHAPTER II

REPRODUCTIVE POTENTIAL

2.1. INTRODUCTION

2.1.1.Factors affecting fecundity

Many authors have related fecundity in marine invertebrate species with the mode of larval development, e.g. based on assumptions relating the size of the eggs to the mode of larval nutrition. Vance (1973a,b) proposed that marine invertebrate species exhibiting a planktotrophic mode of larval development should produce many small eggs, whereas lecithotrophic species should produce a small number of large eggs. Certainly this general rule applies for marine invertebrate species. Fecundity, however, like other reproductive characters, is also affected by individual phenotypic differences and fluctuations of environmental factors (Vance 1973a,b; George et al.1990).

2.1.1.1. Phenotypic differences

The number of eggs produced by a marine invertebrate depends on the size of the individual (Brousseau 1978,1987; Griffiths and King, 1979; Hughes and Roberts 1980; Brousseau 1981; Bayne and Newell 1983; Morvan and Ansell 1988; Scheltema 1994). Consequently, individuals from small species should produce fewer eggs than individuals from larger species. For example the protobranch *Nucula proxima* exhibited a fecundity of 4120 eggs for a 6.6 mm shell length individual, whereas *N. granulosa* exhibited a fecundity of 217 eggs for a 2.2 mm individual (Scheltema 1972). Many deep-sea bivalves produce only a few eggs per year, which is related to their small size relative to shallow-water species. Differences

in individual fecundity have also been described within populations, among individuals of the same size and between two successive spawnings by the same individual (Lawrence 1975; De Ridder and Lawrence 1982; Emlet et al.1987; Jangoux 1982; Morvan and Ansell 1988; George 1996).

Factors other than the size of the individual may also affect egg production. Morvan and Ansell (1988) reported that gonad volume in *Tapes rhomboides* was three times greater before summer spawning than before spring spawning. Furthermore, the gonad contained four times more oocytes during summer than spring.

2.1.2. Environmental factors

2.1.2.1. Food availability

A number of field studies have demonstrated that bivalves (Sastry 1968, 1970; Bayne et al. 1978; Newell et al 1982; MacDonald and Bourne 1987) and echinoderms (Crump 1971; Keats et al. 1984; Scheibling and Lawrence 1982; Thompson 1983, 1984; Andrew 1986; George 1994, 1996) respond to increasing food abundance by increasing their overall size and thereby increasing gonad size and fecundity. For example, George (1994) demonstrated that in the sea star, *Leptasterias epichlora*, somatic growth, egg production and egg size were greater in areas with an abundance and wide variety of prey. In contrast, a decrease in prey variety led to reduced growth and fewer and smaller eggs (George 1996). In bivalves, Thompson (1979) attributed annual differences in fecundity of the mussel *Mytilus edulis* to annual variations in the food supply in coastal inlets of Nova Scotia. Laboratory observations on echinoderms (Dehn 1980; Thompson 1983; Keats et al. 1984; George 1996) and bivalves (Sastry 1966, 1968, 1970; Bayne et al. 1982; Bayne 1975; 1976) have also demonstrated that fecundity is size-dependent, but also depends on food availability.

2.1.2.2. Temperature

According to Honkoop and van der Meer (1997, 1998) and Honkoop et al. (1998) the fecundity of the shallow water bivalve species *Macoma balthica* and *Cerastoderma edule* varies between years, and winter temperature is one of the main factors influencing variation in individual egg production. Honkoop et al. (1998) reported that water temperatures in winter influence individual egg production of *Macoma balthica* (fecundity was negatively correlated with temperature), suggesting that water temperatures explain a large percentage of the variation in egg production for this species.

2.1.2.3. Depth

The available information on factors affecting fecundity in marine invertebrates is derived primarily from studies on shallow-water species, although a few studies in deep sea asteroids (Falk-Petersen 1982b) and some protobranchs (Scheltema 1972; Tyler and Young 1992) have been conducted. Recently, Tyler and Young (1999) have comprehensively reviewed data for species living at vents and cold seeps. Considering the range of distribution only for lecithotrophic protobranch species, Scheltema (1972) proposed that species inhabiting shallower environments should exhibit higher fecundity than species living in deep-sea waters. For example *Nucula proxima*, a continental shelf species, exhibited a fecundity of 4120 eggs per individual, whereas *Malletia cuneata*, an abyssal species, exhibited a fecundity of 30 eggs per individual. According to these results, fecundity is reduced at greater depths.

Conception Bay, Newfoundland, is an ideal site for studying the effects of food availability on fecundity of benthic species at different sizes since water temperature at 265 m. depth remains around -0.5 °C throughout the year (DeYoung and Sanderson 1995), avoiding the confounding effect of temperature on reproduction.

With this objective the gamete volume fraction (meaning the total number of gametes present in the gonad) was obtained, then oocyte size frequency distribution determined in order to estimate the apparent fecundity for different sized individuals.

2.2. MATERIALS AND METHODS

2.2.1. Sampling

Specimens of *Yoldia hyperborea* and *Ctenodiscus crispatus* were dredged from the soft-bottom at a station situated at 47° 34' 02" N; 53° 08' 12" W, 265 m depth in Conception Bay, southeastern Newfoundland, Canada (Fig. 1). Thirty individuals of each species were collected (randomly selected) at ~ 4-6 week intervals during the winter (weather permitting), and on a monthly basis for the rest of the year from January 1997 to January 1999.

2.2.2. Histology

The shell length, total weight (including shell) and wet weight of soft tissues were recorded for individual *Y. hyperborea* (ranging between 25 mm and 35 mm length shell). Because the gonad envelops the digestive gland and spreads into the foot, only the part of the gonad-foot complex closest to the digestive gland was removed, weighed and fixed in Baker's fixative for histological purposes.

For *C. crispatus*, individuals were weighed and the length of the longest arm (R) recorded (between 24 mm to 32 mm arm length). For apparent fecundity estimations, the wet weight of the gonad was recorded for 10 individuals each month (randomly selected). For histological purposes, the portion of the gonad situated beneath the madreporite was removed, weighed and fixed.

After 48 to 96 hrs, histological samples were dehydrated, embedded, sectioned at 7μm and mounted on slides using standard procedures (Humason, 1979). Sections were dewaxed, rehydrated in a graded series of ethanol solutions, stained with hematoxylin-eosin (HE), dehydrated in an ethanol series, cleared in xylene and mounted in Permount (Merck).

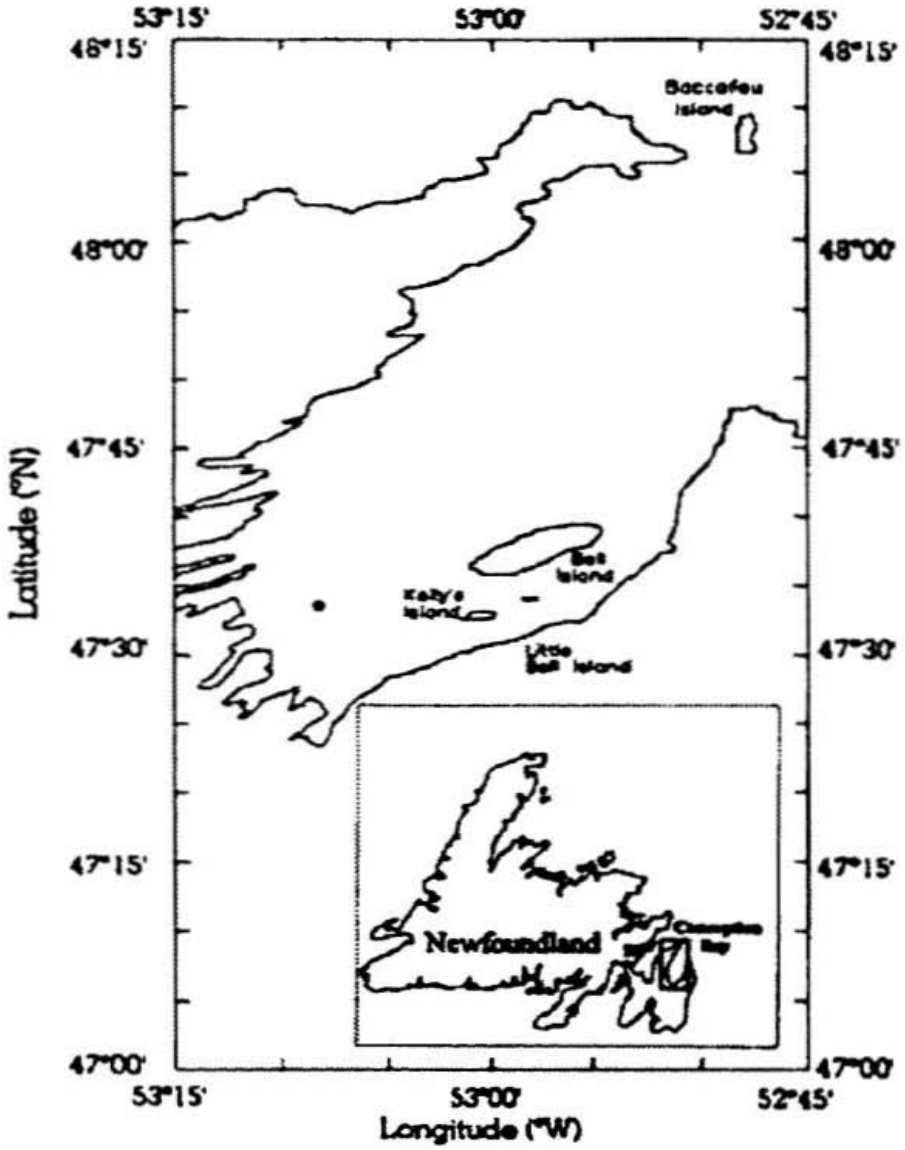

Fig.1. Map of Conception Bay (CB) showing the study area location (*). The insert shows the location of CB in Newfoundland, Canada.

2.2.3. Oocyte frequency distributions

For the analysis of oocyte frequency distributions, a sub-sample of 5 to 10 individuals of each species from each sampling period was examined by image analysis (MOCHA version 1.2., Jandel Scientific). For each individual, two strips of serial sections (8 to 10 sections per strip) were mounted on a slide, and a string of 5 serial sections was videotaped completely field by field. For *C. crispatus* specimens, one section (containing more than 60 oocytes) was selected from each slide and videotaped completely field by field.

2.2.4. Image Capture

Optical images of gonad sections of *Y. hyperborea* obtained from an inverted microscope (Axiovert 35, Karl Zeiss) were captured and converted into a video signal by means of a digital video camera (COHU model. 4815-5000). The video signal was recorded on Hi8 video tape (Hi8ME, SONY) with a Hi 8 VCR (model EV S2000, SONY). The video image was then digitized by a frame grabber (Targa+, Truevision Inc.) and stored as a BMP file. Images were captured at 10x magnification with a constant field area of 1576 mm^2.

For *C. crispatus*, images were obtained from a stereomicroscope (Makroskop 420, WILD) at 25x magnification with a constant field area of 3507 mm^2. Digitized images were obtained as described for *Y. hyperborea*.

2.2.5. Apparent fecundity

2.2.5.1. Indirect estimations

Apparent fecundity of *Y. hyperborea* and *C. crispatus* was estimated from oocyte frequency distributions in histological sections of the gonads of individuals collected throughout the sampling period. Because the gonad of *Y. hyperborea* is small and only those oocytes displaying a full nucleus were considered, the number of measured oocytes in each

section was small. Consequently, in order to estimate the oocyte number per unit volume of gonad (Nv), a mean oocyte number was obtained from the 5 videotaped serial sections and considered as the oocyte number for that individual. For *C. crispatus*, which has a larger gonad than *Y. hyperborea*, the oocyte number for each individual was estimated from all oocytes present in only one histological section. Later, Nv was estimated according to the following formula (Williams 1981):

$$Nv = 4.664 * \frac{(Na)^{3/2}}{D^4} * \left(\frac{\sum_{i=1}^{n} (Di)^3}{n} \right)^{1/3}$$

where Nv = oocyte number per unit volume of gonad
Na = oocyte number per unit area of gonad
D = mean diameter of the oocyte
Di = diameter of individual oocytes
n = number of measurements

The apparent fecundity was estimated as

$AF = Nv * v$

where v = the volume of the sampled gonad. Because measurements of volume for small samples of gonad (less than 1 g) may produce equivocal results, the volume of the gonad was obtained as determined by volume displacement, estimated by immersing weighed gonads in a 10 cc-graduated cylinder (0.1 cc) containing sea water at room temperature (16°C ±1). Results for this volumetric estimation gave approximately a 1:1 weight: volume ratio, so, 1 g of gonad tissue was assumed to correspond to 1 cc of gonad tissue.

The total volume occupied by the gonad (v) was obtained from the gamete volume fraction (GVF), as described below. The values for each cell type were summed and the total volume of the gonad was obtained by subtracting the empty space and values for other tissues.

v = Previtellogenic Oocytes + Vitellogenic Oocytes + Mature Oocytes - free spaces - other tissues

Oocytes were categorized as previtellogenic, vitellogenic, or mature cells depending on the size of the oocyte and its staining affinities for HE. For *Y. hyperborea*, oocytes less than 40 μm in diameter, recognized by their basophilic cytoplasm and large nucleus, were considered as previtellogenic oocytes, those between 40 μm and 80 μm in diameter having an eosinophilic cytoplasm and yolk platelets were termed vitellogenic oocytes, and those 80 μm or larger and pale blue in colour were termed mature oocytes.

A similar procedure was applied for *C. crispatus*. Oocytes up to 100 μm diameter were classified as previtellogenic oocytes, those between 100 μm and 200 μm diameter as vitellogenic oocytes, and those greater than 200 μm diameter as mature oocytes. Both the muscle tissue and the connective tissue between gonad septa were considered as "other tissues" (non-reproductive tissues), and only those free spaces present within gonad tissue were considered to be part of the gonad.

Apparent fecundity for both species was estimated for ripe individuals of various lengths. As described previously, for each body size the highest estimated apparent fecundity (EAF) value was considered as the AF representing that size. A ripe individual was considered to be one containing fully-grown oocytes.

Apparent fecundity values throughout the sampling period were obtained from monthly pooled data. A mean estimated apparent fecundity (MEAF) value was calculated for each month. To obtain this monthly MEAF, the highest EAF values for each size sampled were summed and the mean value obtained.

2.2.5.2. Direct estimations

Direct estimates of the AF of *Y. hyperborea* individuals were obtained by counting the number of eggs released by spawned individuals (induced). Spontaneous spawning occurred during February and March 1998, 12 to 24 hrs after individuals were transferred from the field into the laboratory.

Individuals brought from the field were washed in filtered sea-water and separately placed in beakers filled with filtered sea water and maintained at 5°C in a cold room. After spawning, the egg suspension was diluted to 1 litre with filtered sea-water. A number of 1 ml sub-samples was taken from this egg suspension, later videotaped as described for image analysis, and a mean number of eggs obtained. Finally, this mean value was corrected to obtain the total number of eggs spawned.

Individuals of *Y. hyperborea* maintained in the laboratory were induced to spawn by thermal shock. Each individual was washed with seawater and placed separately in a beaker filled with sea water at 10°C. Once spawned, eggs were treated as previously described for non-induced individuals. Attempts to induce spawning in *C. crispatus* were unsuccessful, and no spontaneous spawning was observed in the laboratory.

2.2.6. Gamete Volume Fraction

GVF was determined by analyzing 5 fields of one section per individual with a Weibel sampling matrix graticule. The maximum number of possible counts on 5 fields observed was 210. Counts were scored for each cell type, and the sum of counts per cell type was determined and expressed as a percentage of the total possible. The original Weibel graticule was enlarged 2.2 times and printed on a transparency taped onto the monitor screen. The GVF for each cell type was obtained from the following equation:

$$GVF = \frac{\text{Number of counts per cell type}}{42} * 100$$

2.3. RESULTS

2.3.1. *Yoldia hyperborea*

Estimated apparent fecundity (EAF) was calculated for 102 *Yoldia hyperborea* individuals, ranging from 25 mm to 35 mm shell length. EAF fluctuated between 2.6 $x10^3$ eggs for a 25.2 mm individual and 1.7 $x10^5$ eggs for a 34 mm individual (Fig. 2). A positive exponential relationship was observed between the EAF and size of *Y. hyperborea* ($y = a \cdot b^x$ $a = 3.4 \times 10^{10}$, $b = 2.06$; $r = 0.95$).

Fluctuations in MEAF were observed throughout the sampling period, but no clear pattern was observed (Fig. 3). In addition high fluctuation of EAF values resulted in a high SD in the MEAF. A mean value of 3 $x10^4$ eggs per spawn ($\pm$ 7.9 $x10^3$, n = 5) was obtained for AF in individuals of shell length 28.3 mm to 30.5 mm (pooled data for induced and non-induced spawnings), and no differences were observed between data obtained by induced spawning and non-induced.

2.3.2. *Ctenodiscus crispatus*

Fluctuations in the MEAF for *C. crispatus* did not follow a clear pattern (Fig. 4). The estimated apparent fecundity was obtained for a total of 65 *C. crispatus* individuals. Values varied from 6.6 $x10^4$ eggs for a 24.5 mm arm length individual to 11 $x10^6$ eggs for a 32.5 mm arm length individual. There was an exponential relationship between EAF and arm length (Fig. 5, $y = a^* b^x$; $a = 4.4 \times 10^5$, $b = 1.47$, $r = 0.91$).

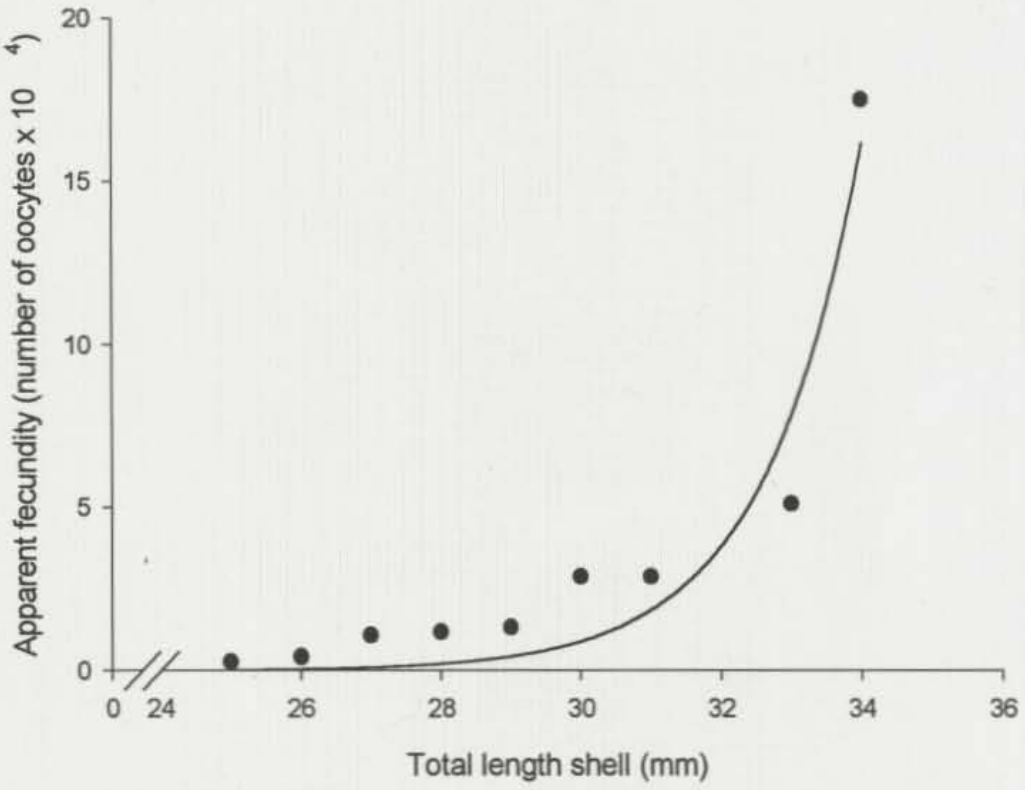

Fig. 2. Relationship between apparent fecundity and total shell length in the protobranch *Yoldia hyperborea*. Plotted dots represent the estimated apparent fecundity obtained for individuals potentially ready to spawn ($r = 0.95$)

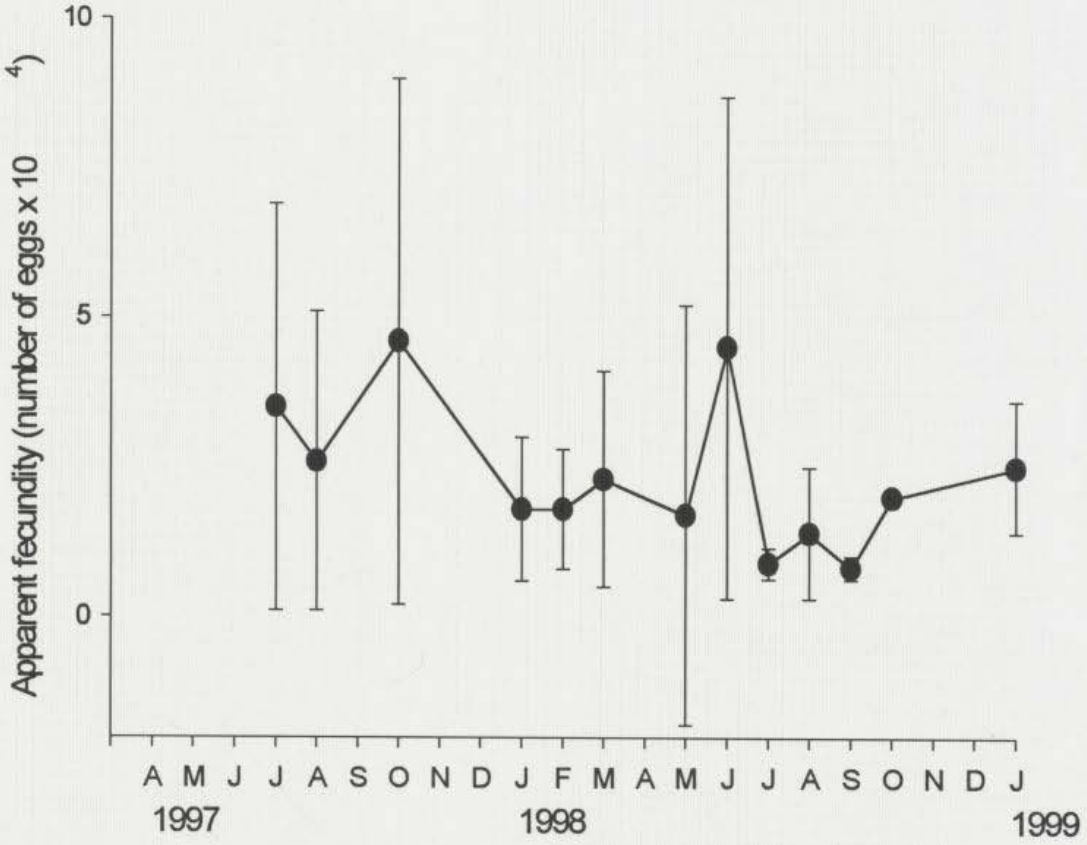

Fig. 3. Temporal mean apparent fecundity fluctuations for *Yoldia hyperborea,* data from individuals 25 to 35 mm shell length (mean ± SD, N = 1 ~ 4 each point).

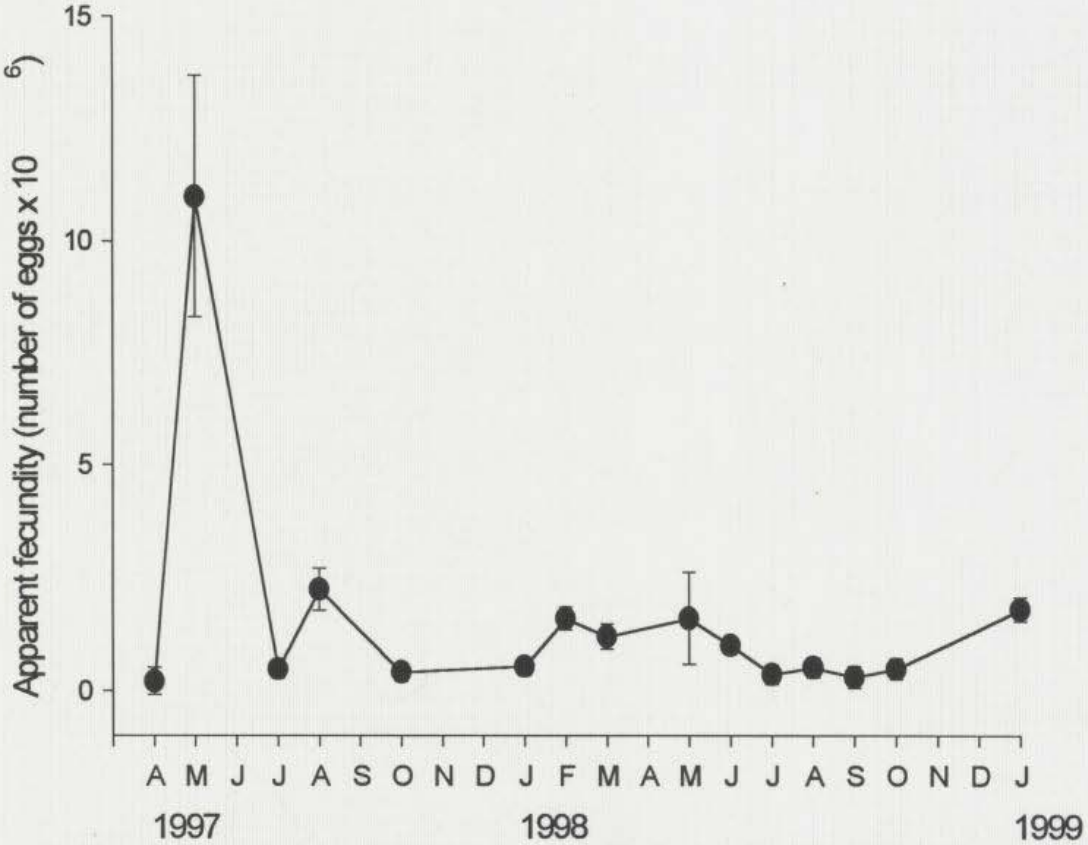

Fig. 4. Temporal mean apparent fecundity fluctuations for *Ctenodiscus crispatus*, data from individuals 24 to 32 mm arm length (mean ± SD, N = 1 ~ 6 each point)

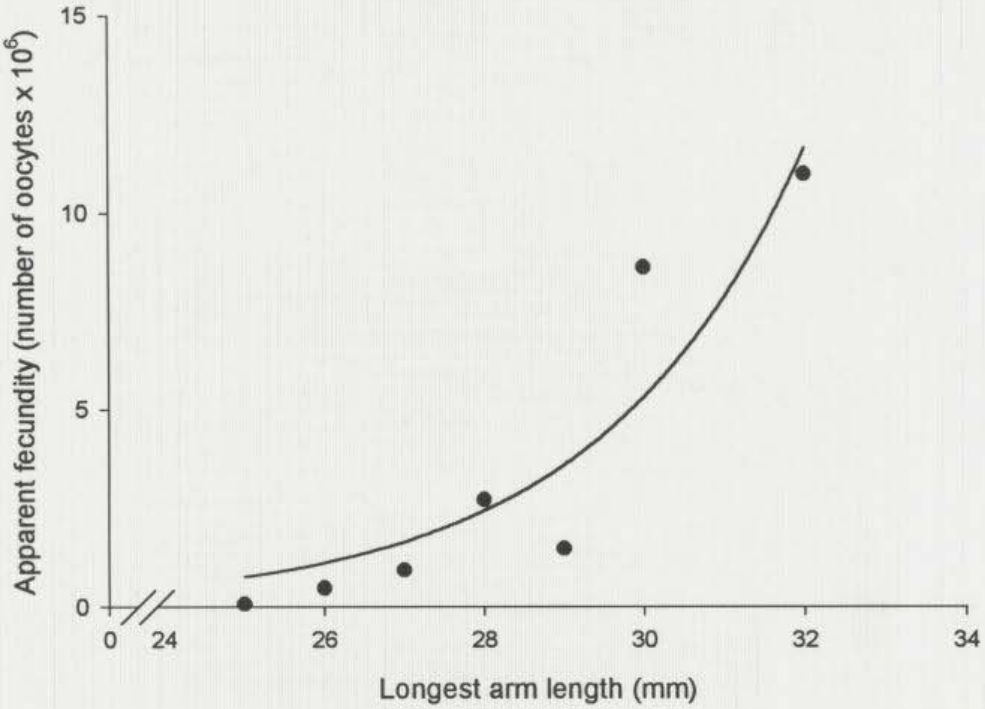

Fig. 5. Relationship between apparent fecundity and arm length (longest arm) in the asteroid *Ctenodicus crispatus.* Points represent the estimated apparent fecundity for individuals potentially ready to spawn (r = 0.91).

2.4. DISCUSSION

2.4.1. *Yoldia hyperborea*

It is known that gamete production is not only dependent on a positive energy balance favouring reproduction, but also on the size of the individual. Bayne and Newell (1983) suggested that fecundity is an increasing function of size, which has been confirmed for bivalves by a number of authors (MacDonald and Thompson 1986; Bricelj et al. 1987; MacDonald and Bourne 1987). Furthermore, larger size is attained in those individuals inhabiting areas with an abundant food supply. For example, MacDonald and Thompson (1986) reported that individuals of the sea scallop *Placopecten magellanicus* maintained in suspended culture exhibited greater shell growth rates and produced more gametes than those growing on the bottom nearby. Similar results have been reported for echinoderm species. Laboratory experiments (Thompson 1983) showed that all sizes of the sea urchin *Strongylocentrotus droebachiensis* grew in diameter at high ration levels with a corresponding increase in fecundity. Field observations by George (1996) demonstrated that sea urchins and sea stars from sites with an abundant supply of food were larger and produced large numbers of eggs than conspecifics from poorer sites. It is therefore to be expected that *Yoldia hyperborea* being larger than most other protobranchs studied, should exhibit the highest fecundity value reported for a protobranch species (8.7×10^4 eggs per individual EAF), since abyssal or bathyal species rarely produce more than 500 eggs per individual (Scheltema 1972; Allen and Hannah 1986; Tyler et al. 1992). For example the fecundity of *Nucula proxima*, a protobranch inhabiting the continental shelf, has been reported as 4120 eggs for a 6.6 mm shell length individual, whereas the fecundity of the continental slope species *N. granulosa* has been estimated as 217 eggs for a 2.2 mm individual (Scheltema 1972). Consequently, differences in the EAF among protobranch species may be mainly attributable to body size variation, although it is also known that in a number of species, fecundity varies significantly among individuals of the same size, depending on food availability and how efficiently individuals utilize energy (Brousseau 1978,1981, 1987; Griffiths and King 1979; Hughes and Roberts 1980; Bayne and Newell 1983; Morvan and Ansell 1988; Scheltema 1994).

The influence of food availability on fecundity has been examined in several marine invertebrate species. In bivalves, for example, MacDonald and Thompson (1985) and MacDonald (1986) reported that good food quality resulted in greater gamete production in both natural and cultured *Placopecten magellanicus* populations. Rodhouse et al. (1984) came to a similar conclusion for *Mytilus edulis* and suggested that food quantity as well as food quality may influence gamete production. Studies on echinoderms (Thompson 1983, George et al. 1990; George 1996; Guillou and Lumingas 1999) have shown that better nutritional conditions result in greater numbers of large, high quality eggs in sea urchins and sea stars.

Studies in Conception Bay have demonstrated that much of the seasonal primary production observed during the spring diatom bloom reaches the bottom one or two months after the start of the bloom (Deibel et al 1992; Thompson et al. 1992, 1999). Large variations in AF were observed for *Y. hyperborea* in the present study (e.g. two different individuals of 25.8 mm shell length during February 1998 exhibited fecundities of 1144 and 497 eggs respectively), which may have masked any seasonal cycle. However, it is reasonable to conclude that *Y. hyperborea* produces gametes continuously rather than seasonally, because resuspension of bottom sediments creates steady food availability. Bioresuspension is known to occur in near-bottom waters (Gardner et al. 1985; Graf and Rosenberg 1997) and is caused by feeding, locomotion and construction of mounds, pits and tubes by benthic organisms (Graf and Rosenberg 1997).

Resuspension of bottom sediments is a potentially important mechanism for recycling organic matter from the benthos to the water column (Wainright 1990). *Yoldia hyperborea* may have an important role in bioresuspension given that its feeding activities result in sediment resuspension through expulsion of faeces and pseudofaeces directly into the water column (Bender and Davis 1984). Resuspended particles are associated with high microbial activity which can convert suspended POM into material of greater nutritional value for deposit feeders like *Y. hyperborea* (Smith 1994). However, sporadic episodes of settlement of dead fishes, whales or wood can also provide fresh nutrients to the benthic community (Gage and Tyler 1991). Consequently, resuspension or other episodic events may account for the presence of gametes in the gonad of *Y. hyperborea* at all times of the year, although presumably

the labile fresh phytodetrital material arriving at the sea bottom seasonally may also contribute to gamete growth and maturation. Laboratory experiments with *Y. hyperborea* (see Chapter V) demonstrated that individuals periodically supplied with senescent algae produced more and larger oocytes than control individuals receiving no algal supplement, which supports the effect of the seasonal food deposition.

Methodological differences may also account for variation in fecundity values between studies. Samples of species inhabiting the slope, bathyal and abyssal zones are difficult to obtain, and most of the few reports on AF are based on data obtained from only one individual (Sanders and Allen 1973; Allen and Sanders 1973). In other cases, authors do not report the number of specimens studied (Scheltema 1972). Consequently, these data, although valuable, may represent biased values of fecundity in the deep sea given that variations in AF are known to occur within and among same size individuals. Variation between years may also partially explain differences in fecundity.

2.4.2 *Ctenodiscus crispatus*

The EAF of 11×10^6 eggs reported for *Ctenodiscus crispatus* in this study is much higher than the value of 10^3 eggs reported by Falk-Petersen (1982a) for this species from Balsfjorden (69°31' N; 19°01' E), northern Norway. These differences may be attributable to the size of the sampled individuals but it is also known that differences in fecundity among conspecific females inhabiting different geographic zones can be explained by variations in the amount of food available. An alternative explanation for such a difference in egg production is that individuals sampled in Balsfjorden were not *Ctenodiscus crispatus* specimens (Tyler, personal communication). Crump (1971) studied the effect of food availability on three geographically separated populations of the asteroid *Patiriella regularis* (Verrill), and suggested that both the quantity and quality of available food may be responsible for differences in potential fecundity among individuals from adjacent areas. Laboratory studies conducted by Crump (1971) supported these field observations. Scheibling and Lawrence (1982) and George (1994) demonstrated that females of *Echinus spp.* and *Leptasterias epichlora*, inhabiting

environments with high food quality and quantity, were larger and produced more and bigger eggs than conspecifics from poorer environments. For example, *Leptasterias epichlora* individuals were larger and able to produce more eggs at sites with a wide variety of large molluscs, and were smaller and produced few small eggs at sites with a variety of small molluscs (George 1994). Thus under favorable environmental conditions (food quantity and quality) sea stars are bigger and produce more eggs, which agrees with predictions that the relationship between fecundity and food availability varies according to the size of the individual. A number of studies have reported that echinoderms with a rich food supply respond by increasing body length and consequently body weight, size and weight of the gonad (Menge 1975; Scheibling and Lawrence 1982; Keats et al. 1984; Thompson 1984; Andrew 1986; Parker and Begon 1986; Mc Ginley 1989; 1994; Venable 1992; Bertram and Strathmann 1998).

The water temperature below the thermocline of Conception Bay is around -0.5°C, which reduces the rate of pelagic microbial decomposition during the spring bloom and allows a significant proportion of the primary production to reach the bottom (Pomeroy and Deibel 1986). This flux likely enhances the total production (gametic and somatic) of benthic organisms. The Balsfjorden area, in contrast, exhibits temperatures between 2 °C and 3 °C throughout the year (Eilertsen et al. 1981), which results in more rapid pelagic microbial growth and reduced flux in primary production reaching the bottom. This difference in the quality (primary production reaching the bottom) and the quantity (little grazing in the water column) of food observed in Conception Bay may explain differences in EAF from values obtained from Balsfjorden.

MEAF peaks for *C. crispatus* exhibited little variation throughout the year, suggesting that gametes were continuously present in the gonad. Data on the effect of food availability and reproduction of *C. crispatus* and asteroid species suggest that the reproductive cycle of this species is strongly affected by food availability (Shick et al. 1981b; Falk-Petersen 1982a; Chia and Walker 1991). Consequently a constant food supply would facilitate a continuous transfer of energy from the gut to the ovary (Shick et al. 1981b) and also could explain the constant presence of gametes in *Ctenodiscus crispatus* as proposed by Falk-Petersen (1982a).

Deposit feeder species in general contribute to bioresuspension of sediments (Davis

1993). Thus, the deposit feeder community (including *Ctenodiscus crispatus*) inhabiting the soft-bottom may be responsible for biosuspension of sediments and subsequently facilitate increases in the microbial mass associated with the POM. The year-round availability of resuspended POM may allow the continuous gamete production found in *Ctenodiscus crispatus*. However, echinoderms inhabiting polar regions take advantage of the seasonal vertical flux of primary production occurring during the summer by transferring part of the energy directly to the gonad and some to the pyloric caeca for storage. Consequently, during winter months reserves in the pyloric caeca can be used to sustain gamete production (Clarke 1988).

Meanwhile, other deposit feeding communities do exhibit strong seasonality in reproduction because they are capable of responding to seasonal nutrient pulses (Eckelbarger and Watling 1995), e.g. in the northeast Atlantic the echinoderms *Ophiura ljungmani*, *Plutonaster bifrons*, *Dytaster grandis* and *Echinus affinis* have seasonal reproductive cycles (Tyler 1988, Tyler et al., 1992), and the apparent driving force for these seasonal patterns of reproduction is the seasonal influx derived from surface primary production sinking rapidly to the deep-sea bed (Tyler 1988). However, not all species subjected to seasonal pulses of food should exhibit a seasonal reproductive cycle, since species having a mixed strategy to obtain energy may also exhibit a semiannual or continuous reproductive cycle.

The results presented here for *Ctenodiscus crispatus* reveal a strong correlation between AF and body size ($r = 0.91$). This type of relationship between fecundity and body size has not been previously described for asteroid species, and consequently it represents a new approach in understanding fluctuations in AF values.

Resuspension is an alternative means by which deposit feeders such as *Y. hyperborea* may obtain organic material from the surface sediments. There is evidence that *Yoldia ensifera* (Stacek, 1965) and some nuculanid species (Caspers 1940; Owen 1956) have the capacity to ingest particles suspended in the water. Similar observations have been made on *Y. hyperborea* (See Chapter V; Stead 2001, PhD thesis).

CHAPTER III

REPRODUCTIVE CYCLES

3.1. INTRODUCTION

3.1.1. Classification of reproductive cycles

Since marine invertebrate species are subjected to a number of environmental factors such as temperature, salinity, light and food availability, the occurrence of daily, monthly or seasonal fluctuations of any of these factors may affect reproductive activity (Loosanoff 1937; Sastry 1966, 1970; Newell et al. 1982; Currie 1990; Bayne 1976). Reproductive activity in marine invertebrates may be continuous or seasonal (annual or semiannual) and is synchronous if all individuals of a population spawn at the same time (generally for species reproducing seasonally) or asynchronous if individuals spawn at different times (Sastry 1979). In general, reproduction is seasonal or cyclical in species inhabiting predictable environments, but continuous in species inhabiting more stable environments (Giese and Kanatani 1987). However, exceptions to this general rule have been observed in species inhabiting the shallow-non intertidal zone e.g. *Placopecten magellanicus* exhibits a seasonal reproductive cycle (MacDonald and Thompson 1988; Parsons et al. 1992, Dibacco et al. 1995).

3.1.2. Reproductive cycles and the environment

In general, it is accepted that near the lower latitudinal limit in the geographic distribution (closer to tropical or subtropical areas) a marine invertebrate species tends to exhibit a continuous reproductive cycle as a result of more stable environmental conditions (little or no fluctuation in temperature, photoperiod or salinity). In contrast, near the higher

latitudinal limit (closer to boreal or polar areas) reproduction tends to be more seasonal as a result of fluctuations in food availability, temperature, photoperiod, etc. (Thorson 1950; Sastry 1979; Giese and Kanatani 1987). According to this concept, invertebrate species living in shallow waters should exhibit a seasonal reproductive pattern, whilst deeper water species (species living in the continental slope, bathyal and abyssal zones), submitted to little or no seasonal fluctuations of environmental factors, should spawn continuously rather than seasonally (Scheltema 1972). However, recent reports suggest that a seasonal input of sinking material (Billet et al. 1983; Lampitt 1985; Rice et al. 1986; Lampitt et al 1990) can reach the bottom of the sea bed and may affect reproduction of benthic invertebrate species (Tyler and Young 1992). Most tropical species reproduce continuously (although some massive spawns have been observed in corals), whilst polar species tend to reproduce seasonally (Sastry 1970; Giese and Kanatani 1987), although there are exceptions e.g. some tropical echinoderm species reproducing seasonally (Lessios 1984).

Differences in the timing of reproduction have been reported for bivalve and echinoderm species inhabiting the same geographic area. Differences between geographically separated populations of the same species have also been reported (Ropes 1968; Chia and Walker 1991) as a result of adaptation to local variations in environmental factors such as food availability (Newell et al. 1982; Bricelj et al.1987; MacDonald and Thompson 1988). However, differences in the reproductive cycle of *Crassostrea virginica* have been attributed to genetic variation (Barber and Blake 1991).

Some deep-sea bivalve species exhibit seasonal reproductive cycles (e.g. *Ledella pustulosa* and *Yoldiella jeffreysi* (Lightfoot et al.1979; Tyler et al. 1993), and some shallow water bivalve species such as *Modiolus modiolus* (Seed and Brown, 1977), *Mercenaria mercenaria*, (Heffernan et al. 1989a), and *Hinnites giganteus* (Malachowski 1988) reproduce continuously. Similar exceptions have been reported for echinoderm species, e.g. the northeast Atlantic species *Poriocidaris purpurata* exhibits continuous reproduction whilst *Echinus affinis*, *E. alexandri* and *E .acutus* reproduce seasonally (Tyler and Gage 1984a, b; Gage et al. 1986). Data on the periodicity of reproductive cycles for deeper water bivalve and echinoderm species provide evidence for both seasonal and continuous reproductive cycles.

3.1.3. Factors affecting reproductive cycles

Since a number of studies (Loosanoff 1937; Ropes and Stickney 1965; Sastry 1966, 1970; Machell and DeMartini 1971; Newell et al. 1982; Malachowski 1988; Arsenault and Himmelman 1998; Currie, 1990) suggest that fluctuations in environmental factors drive the periodicity of either seasonal or continuous reproductive cycles in shallow water species, it is reasonable to assume that similar environmental fluctuations should also influence the frequency of reproduction in bathyal and abyssal species.

For many years, areas beyond the continental shelf were considered to be very stable environments, without temperature, salinity, light or food availability fluctuations. However, more recent studies (McCave 1975; Hinga et al. 1979; Deuser and Ross 1980; Lampitt 1985; Rice et al. 1986, 1991; Lampitt et al. 1990; Gage and Tyler 1991) have reported seasonal sinking of phytodetritus to the sea bed following the spring diatom bloom in the photic zone. Many of these studies suggest that these phytodetrital particles are a rich food source for deposit feeding species and that phytodetritus input may be responsible for the seasonal reproductive pattern described for some deep-sea species inhabiting the northeast Atlantic (Tyler and Young 1992). This seasonal phytodetrital pulse has been well documented for the continental slope (Lampitt 1986; Billett et al. 1983) in the northeast Atlantic on the northwest Atlantic slope (Aller and Aller 1986; Hecker 1990) and in the central equatorial Pacific (Smith et al. 1996, 1997).

3.1.4. Study area

Available evidence (Thompson et al. 1986, 1992; Deibel et al. 1992; Redden 1994; Redden et al. 1994) strongly suggests that a seasonal pulse of phytodetritus reaches the bottom of Conception Bay (where a depositional area exists between 200 and 300 m depth), following the spring diatom increase. However, to date no research has been conducted to determine whether this food pulse influences the reproduction of the benthic community inhabiting the soft-bottom in this area. The benthic macrofauna that inhabits the soft bottom of Conception Bay is dominated by polychaetes, molluscs, sipunculids and echinoderms (Scheibe 1991). *Yoldia hyperborea* and *Ctenodiscus crispatus* are prevailing bivalve and echinoderm species respectively. Both are deposit feeders.

Information on the reproduction of benthic species inhabiting the depositional zone of Conception Bay is scarce and no data exist on reproduction of *Y. hyperborea* and *C. crispatus* of this region. Because both species share a similar environment and have a similar feeding strategy, it is of considerable importance to determine how the seasonal input of energy may affect their reproductive activity. Although studies on the reproduction of *C. crispatus* have been carried out in two different geographic areas, Maine, USA and Balsfjorden, Norway, (Shick et al. 1981a, b and Falk-Petersen 1982a respectively), it would be informative to study the reproductive strategy for a population inhabiting cold waters, in the sublittoral zone and which can be easily reached for a long term sampling period.

3.5.1. Study species

Studies of protobranch bivalve species are scarce and many of them have emphasized taxonomy and shell morphology (Drew 1899; Allen 1978; Knudsen 1979; Warren 1989), although Allen and Sanders (1973, 1982), Sanders and Allen (1973, 1977), Rokop (1979) and Tyler and Young (1992) have provided details of reproduction for several families. However, most of these reports refer to deep-sea species (Scheltema 1972; Rokop 1979; Tyler and Young 1992), and there are few reports on reproduction in shallow-water protobranchs (Drew

1899; Ockelmann 1965; Lewis et al. 1982; Davis and Wilson 1983). Current knowledge for *Yoldia* species is limited to reports on taxonomy (Allen 1978; Cowan 1968; Warren 1989), growth and population structure (Lewis et al. 1982; Hutchings and Haedrich 1984; Nolan and Clarke 1993; Peck et al 2000), feeding behavior (Stacek 1965; Davenport 1988), size-dependent survivorship (Nakaoka 1996, 1998) lipid composition (Parrish et al. 1996), degradation of algal lipids (Sun et al. 1999).

Studies on asteroid species are more numerous (Crump 1971; Jangoux and Van Impe 1977; Nauen and Böhm 1979; Dehn 1980; Shick et al. 1981a; Falk-Petersen 1982a; Chia and Walker 1991) and some of these studies refer to *Ctenodiscus crispatus* (Grossert et al. 1973; Walker 1974; Shick et al. 1981a; Falk-Petersen 1982a). However, data for *C. crispatus* reproduction are inconsistent. Shick et al. (1981a) reported that the reproductive cycles for the Damariscove Island and the Cuckolds (Maine, USA) populations are continuous, with asynchronous oocyte development, but on the other hand Falk-Petersen (1982a) found that the reproductive cycle for individuals living in Balsfjorden (northern Norway) exhibited a seasonal reproductive pattern. In addition, differences have also been observed in egg size; Shick et al. (1981a) reported 400 μm as the maximum egg diameter whilst Falk-Petersen (1982b) reported 650 μm.

This chapter will examine the effect of food availability on the multiplication, growth and maturation of gametes in the gonad of *Yoldia hyperborea* and *Ctenodiscus crispatus*. An attempt will then be made to determine the frequency of reproduction in these species. Because there are no data on the effect of a seasonal pulse of phytodetritus on the reproduction of *Yoldia hyperborea*, and data on *Ctenodiscus crispatus* are contradictory, the goal of the present study is to describe the frequency of reproduction in *Y. hyperborea* and attempts to explain the inconsistencies in published data for *C. crispatus*. Conception Bay is an ideal location to conduct this type of study, because temperature is constant but food availability varies.

3.2 MATERIALS AND METHODS

3.2.1. Sex Ratio

Sex ratios for both species were estimated for the total number of individuals sampled. The frequency distribution of males and females was compared with a 1:1 ratio with the Goodness of fit test (G test) for a sample with n > 200 with one degree of freedom (Sokal and Rohlf 1995)

3.2.2. Gonad Index

As an indicator of the reproductive state of *C. crispatus* individuals, the total of the gonad tissue present for each individual was isolated to estimate the Gonad Index as the following equation:

$$GI = \frac{\text{Gonad wet weight}}{\text{Total body wet weight}} \text{ X } 100$$

Because protobranchs have a divided gonad, with part being embedded in the digestive gland and part in the foot, the GI of *Y. hyperborea* is not easy to determine. An alternative index, the Gonad Size Index (GSI), was calculated based on quantitative measurements of the number of gametes present in the ovary (corresponding to GVF values). This GSI (which corresponds to the GI) was obtained by summing the values for all stages of gametes observed in the gonad expressed as the percentage of the field occupied by gonad tissue (GVF results are already expressed as a percentage). This analysis was performed for both species.

3.2.3. Gamete volume fraction (GVF)

Procedures for determining GVF for both species have already been described in Chapter II.

3.2.4. Size at first maturity

During sampling of individuals for analysis of oocyte frequency distributions, a number of smaller individuals was obtained (<up to 23 mm in arm length for *C. crispatus* and up to <24 mm shell length for *Y. hyperborea*). The arm length/shell lengths of these individuals were measured and the gonads fixed separately for histological purposes.

Gonad tissue was processed as described in chapter II, and examined to determine the gametogenic stage. Those males having mature spermatozoa and those females exhibiting mature oocytes were considered sexually mature. Size at first maturity was defined as the size at which 50 % of the individuals sampled in that size group (either male or female) were mature.

3.2.5. Environmental Factors

Salinity, temperature and in situ fluorescence (to estimate chlorophyll concentrations) were determined at the sampling site on a regular basis with a SEABIRD 25 CTD equipped with a SeaTech Fluorometer.

3.2.6. Statistical analysis

3.2.6.1. Anova

A one-way analysis of variance for frequencies (ANOVA type I) was performed to establish whether monthly mean values during the sampling period were significantly different

(Grant and Tyler 1983a). Assuming that sampled individuals exhibit significant differences between oocyte size classes, some oocyte size classes should be represented more than others.

3.2.6.2. Contingency analysis

Size-frequency distributions for oocytes were examined by contingency analysis (Grant and Tyler 1983b). Oocyte size classes and oocyte frequencies were arranged in an (r x c) contingency table, where r is the number of sampled months and c is the number of oocyte size classes. If each oocyte size class is represented in the same ratio as the other classes, the expected frequency for each e_{ij} cell is given by

$$e_{ij} = (Ri \times Ci) / \sum Cj$$

Where R_i is the total number of oocytes in the i^{th} class summed over all months, Ci is the total number of oocytes measured in the J^{th} month, and $\sum Cj$ is the total number of oocytes measured during the study period.

The statistic G was computed as :

$$G = \sum \left((o_{ij} - e_{ij})^2 / e_{ij} \right)$$

Where o_{ij} is the number of observed oocytes for each i^{th} class and e_{ij} is the number of expected oocytes, assuming the same ratio (1) for each oocyte size class estimated

If a similar stage of development is assumed to be attained each month, the G value will be distributed as χ^2 with $(r - 1)(c - 1)$ degrees of freedom. If the computed value is greater than the value of χ^2 for the significance level $P = 0.05$ we conclude that gonad development is different between individuals. Examining standardized residuals makes it possible to determine which oocyte size classes and which months contribute most to the χ^2 value, and plotting the positive residual values indicates what type of reproductive pattern is occurring in the species (Grant and Tyler 1983b).

The residual value is calculated as:

$$r_{ij} = (o_{ij} - e_{ij}) / \sqrt{e_{ij}}$$

The residual values are then standardized by dividing by the expected variance V_{ij} calculated as:

$$V_{ij} = [1 - R_i / n] [1 - (c_j / n)]$$

where n is the total number of oocytes measured.

Results of these calculations are reported in a contingency table. Positive residual values in the table signify that the frequency of oocytes in that particular size class is greater than the expected frequency (represented by positive and boldfaced values in the table).

Displacement of positive residuals towards greater oocyte size classes represents a maturation phase, whereas a spawning is indicated by displacement towards smaller oocyte size classes (Grant and Tyler 1983b).

3.3. RESULTS

3.3.1. *Yoldia hyperborea*

3.3.1.1. Sex ratio

The sex ratio was estimated for 269 males and 219 females (470 in total individuals). There was no significant deviation from a 1:1 ratio of males to females $P < 0.05$ (Table 1A).

3.2.1.2. Gonad size index (GSI)

The gonad size index (Fig. 6) increased slightly from January-April 1997, followed by a decrease in May, a short recovery in July, and a further decrease in August. GSI then increased from September to January when over 50% of each microscope field was occupied by gonad tissue. GSI decreased slightly in February 1998 and then decreased steadily until May 1998. In June 1998, the GSI exhibited a sharp increase, remaining constant from July to October followed by a sharp decrease with the lowest GSI value recorded in January 1999. GSI values observed in July 1997 and June 1998 may be attributable to phytodetrital deposition beginning one month before, although increases in GSI also occurred during fall-winter at a time that was presumably unrelated to the seasonal input of phytodetritus. A one-way ANOVA test showed that GSI values for April 1997 and January 1998 ($P \leq 0.025$ and 0.021 respectively) were significantly different from other months, suggesting that each one represents the beginning of a spawning period.

Data for oocyte diameter (Fig. 7) suggested that a main spawning of *Y. hyperborea* occurred from early spring to early summer (April-July 1997). However, it is possible that gamete release may have occurred by January 1997. Another spawning occurred in winter (February-March 1998), which was corroborated when non-induced individuals spawned spontaneously after being transferred from field to laboratory in February-March (non-induced spawnings were observed only during February and March 1998).

Table 1. Goodness of fit tests for sex ratio estimations of *Yoldia hyperborea* and *Ctenodiscus crispatus*.

A) ***Yoldia hyperborea***

Individual	o Observed	e Expected	o - e Deviation from expected	$(o - e)^2$ Deviation squared	$(o - e)^2 / e$
Male	261	pn (0.5*470) = 235	21	441	1.876
Female	219	pn (0.5*470) = 235	-21	441	1.876
Total	480		0		χ^2 = **3.752**

Goodness of Fit for n > 200

$\chi^2 = (o - pn)^2 / pq\ n)$
$= (266 - 235)^2 / 0.5 * 0.5 * 470$
$= (961/117.5)$
$\chi^2 = 8.1787234$
$\chi^2 < \chi^2\ 0.05_{[1]} = 3.841$

B) ***Ctenodiscus crispatus***

Individual	o Observed	e Expected	o - e Deviation from expected	$(o - e)^2$ Deviation squared	$(o - e)^2 / e$
Male	235	pn (0.5*480) = 240	- 5	25	0.106
Female	245	pn (0.5*480) = 240	5	25	0.102
Total	480		0		χ^2 = **0.208**

Goodness of Fit for n > 200

$\chi^2 = (o - pn)^2 / pq\ n)$
$= (235 - 240)^2 / 0.5 * 0.5 * 480$
$= (25/120)$
$\chi^2 = 0.2083333$
$\chi^2 < \chi^2\ 0.05_{[1]} = 3.841$

where p = probability of male occurrence
q = probability of female occurrence
n = total number of individuals

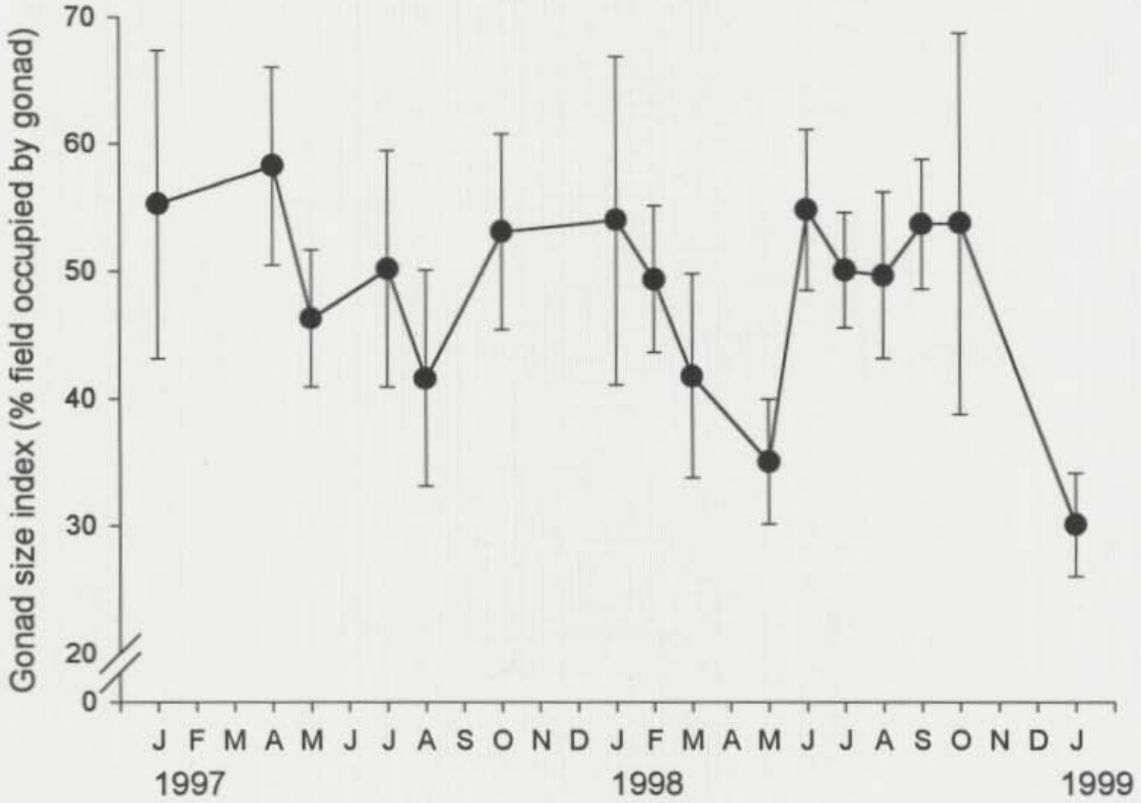

Fig. 6. *Yoldia hyperborea*. Gonad size index (mean ± SD).

Fig. 7. *Yoldia hyperborea*. Oocyte diameter (mean ± SD) measured in histological sections. Where no bars are shown, SD is smaller than the symbol.

3.3.1.3. Gamete Volume Fraction (GVF)

The ovaries of *Yoldia hyperborea* were dominated by vitellogenic and previtellogenic oocytes, while mature oocytes were less frequently observed throughout the sampling period.

3.3.1.3.1. Previtellogenic oocytes

According to volume fraction estimations, the percentage of previtellogenic oocytes dropped steadily from January 1997 to October 1997 (Fig. 8). Frequencies then increased by January-February 1998 followed by a drop from March to June 1998. A sharp increase occurred by July-August 1998, ending with a drop in late August to October. Another increase was recorded in January 1999. This pattern suggests that multiplication of gametes was initiated shortly after October and this process was extended until January of the next year (when higher values were reached).

3.3.1.3.2. Vitellogenic oocytes

Vitellogenic oocytes were the most frequent category in every field examined. Peaks (representing more than 30 % of the field occupied by vitellogenic oocytes) were observed during April and October 1997 and June and September 1998 (Fig. 8), and were coincident with higher GSI, but only the June 1998 peak appeared closely related to phytodetritus sinking, which occurred in June 1998 (see Fig. 9). The effect of food availability on this peak was expressed as higher percentages of vitellogenic oocytes recorded shortly after the actual phytodetritus sinking.

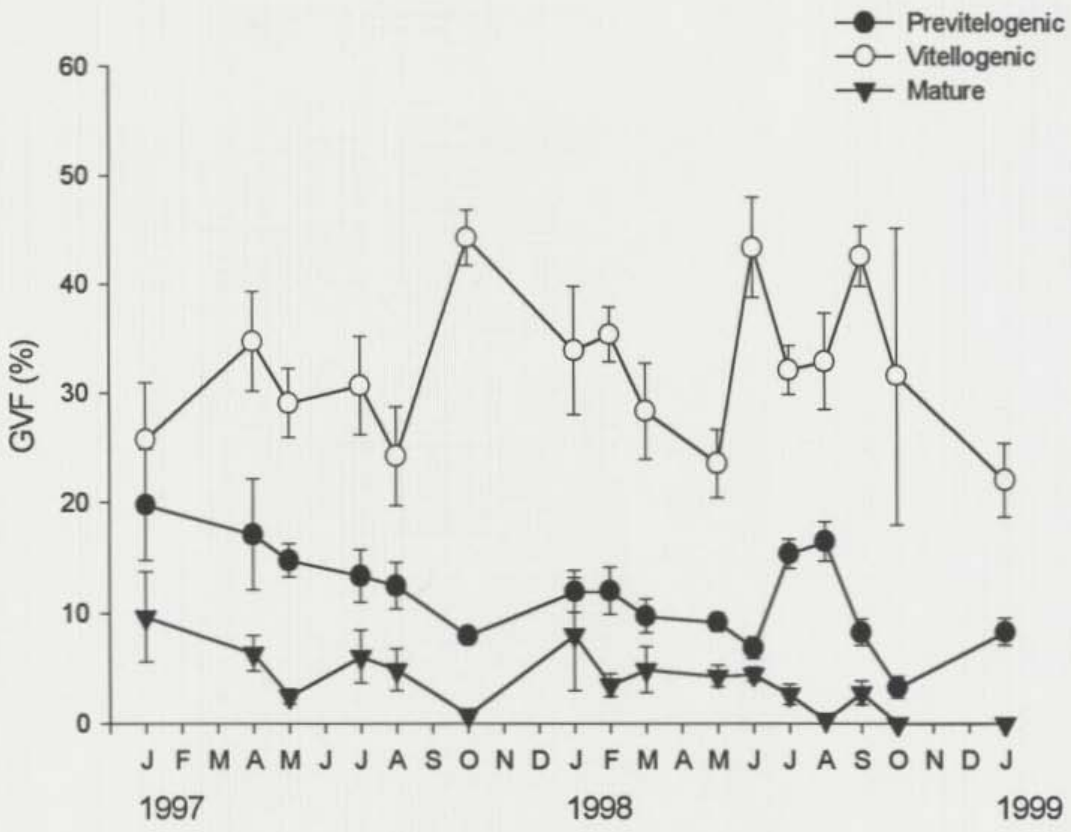

Fig. 8. *Yoldia hyperborea*. Gamete volume fraction (GVF) for previtellogenic, vitellogenic and mature oocytes. Data from individuals 25 - 35 mm shell length (mean ± SD).

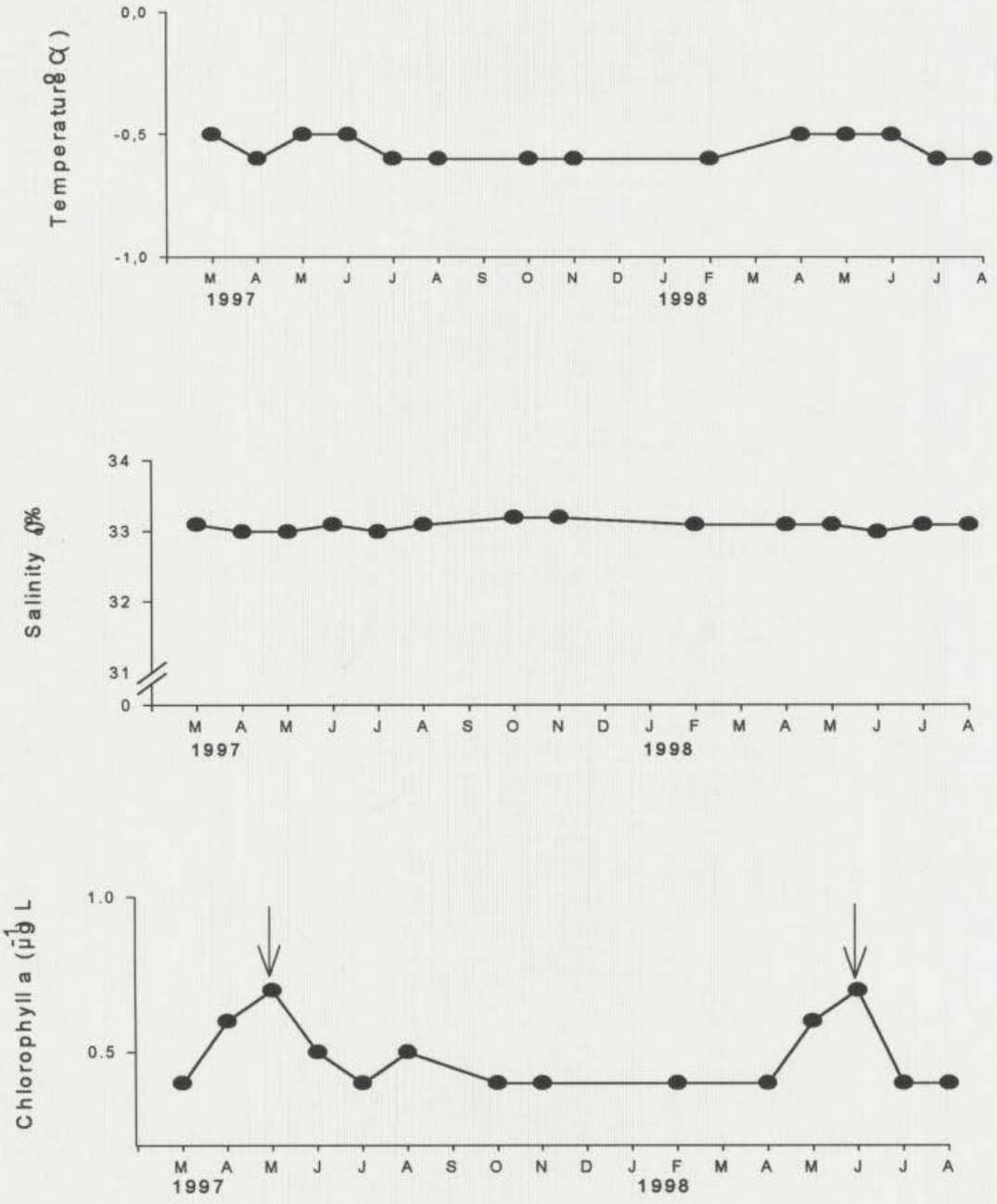

Fig. 9. Temperature, salinity and chlorophyll a at the sampling site at 240 m depth in Conception Bay, March 1997-August 1998. Arrows representing phytodetritus sinking.

3.3.1.3.3. Mature oocytes

Mature oocyte percentages decreased from January to May 1997 followed by a slight increase in July 1997 and another decrease from August to October 1997 (Fig. 8). For January 1998 the GVF value for mature oocytes was high, but fell steadily in February 1998, then remained stable to June 1998 followed by a drop in during July-August 1998. These results suggest that the main spawning period may have occurred during winter-spring 1997 (January to May) and perhaps during winter 1998 (January-February). This finding is consistent with oocyte mean size fluctuations (Fig. 7), higher mean oocyte diameters being observed in the middle of, or by the end of, these spawning periods (April 1997 and February 1998 respectively). Contingency table analysis (Table 2) showed that oocyte size classes 80 μm and larger prevailed in April 1997 and January-February 1998 .

3.3.1.3.4. Free spaces

Free space percentages fluctuated between 18.3 % in January 1997 and 39.7 % in January 1999, the highest frequencies occurring during February 1998 and January 1999 (Fig. 10). However, there were also high values in July 1997 and July 1998. These free spaces indicate that a spawning had already occurred and that the gonad was entering the post-spawning phase.

3.3.1.3.5. Other tissues

Proliferation of other tissues (primarily connective tissue) fluctuated from January to August 1997 followed by a sharp drop from August 1997 to January 1998. A sharp increase occurred from February to May 1998, followed by another drop in September 1998 (Fig. 10). The percentages for this category fluctuated with variations in gonad maturity, decreasing when the gonad was ripe and individuals were ready to spawn.

Table 2. Standardized residuals values for oocyte size classes in *Yoldia hyperborea*. The positive residuals are in bold type.

Sampling	Oocyte size classes													
Date	30 µm	40 µm	50 µm	60 µm	70 µm	80 µm	90 µm	100 µm	110 µm	120 µm	130 µm	140 µm	150 µm	>160 µm
Jan 97	**11.4281**	**7.6770**	**2.6831**	-1.9698	**0.9516**	-3.8080	-4.4267	-3.7827	-2.3987	-2.3572	-1.4843	-0.7404	-0.0894	-0.1236
Apr 97	-3.5052	-4.7966	-4.7504	-2.6629	-1.1083	**2.2276**	**1.8579**	**2.0857**	**5.3915**	**3.8381**	**3.2168**	**2.1493**	**0.9471**	**3.9477**
May 97	**2.3609**	-1.6742	**0.6597**	**1.8291**	**2.0549**	-1.8345	-1.0534	-1.3787	**0.4712**	-0.7056	**0.3764**	-1.4016	-0.6941	-0.6997
Jul 97	-1.7746	-1.6126	-0.8281	**1.7607**	**4.3279**	-0.5055	**0.3133**	-0.1626	-2.2620	-1.7513	-0.9776	-0.2937	**0.3475**	**0.2907**
Aug 97	-1.5085	-1.8187	-1.7709	**0.1112**	**0.6388**	**0.9924**	**0.8227**	-0.0301	**0.7566**	**0.0111**	**0.1671**	**3.0411**	**0.1882**	**0.1398**
Oct 97	-2.1571	-1.0451	-2.8219	-0.5046	**1.9267**	**1.5967**	**2.7396**	**0.4602**	-0.4619	-0.7388	-0.6767	-0.5815	**0.0631**	**0.0212**
Jan 98	-3.0737	-3.7942	-1.7931	-1.6335	-1.6052	-0.5273	**1.4636**	**3.9603**	**3.0440**	**4.9320**	**2.4158**	**1.1194**	-0.1304	**0.1786**
Feb 98	-2.9690	-5.4180	-4.7175	-3.7312	-3.9346	-1.1004	**1.8489**	**5.5407**	**4.6397**	**9.1046**	**7.3446**	**3.3844**	**4.7743**	**1.1459**
Mar 98	-0.2641	**2.1542**	**1.9863**	**0.8929**	**0.0536**	-1.0553	**0.3010**	-0.4529	-1.4101	-2.6235	-0.3523	**1.5406**	-0.8162	-0.8162
May 98	**0.7096**	**0.8985**	**1.1747**	-0.2097	-1.7193	**1.3285**	-1.4920	-0.2566	**1.9729**	-0.7086	-0.7878	-0.0354	-1.1656	**1.1506**
Jun 98	-0.8876	-1.8976	-0.0910	-1.4100	**0.6274**	-0.7676	**4.1686**	**1.6846**	-0.3288	-0.0641	-1.4932	-1.1586	-0.4769	-0.4924
Jul 98	-2.7049	-1.1843	**4.0310**	**6.0686**	**3.1969**	**0.8709**	-3.3818	-4.3024	-3.1039	-2.6624	-2.2623	-0.8031	-0.6837	-0.6897
Aug 98	-0.0685	**0.7149**	**2.1029**	**2.1776**	**0.6463**	**0.3267**	-0.0020	-0.4482	-3.2901	-3.0970	-2.0817	-1.2421	-0.5522	-0.5642
Sep 98	**0.4652**	**5.0346**	**0.1046**	**1.3151**	-1.5946	**0.4407**	-1.2316	-1.5901	-0.3463	-1.3559	-2.2719	-0.2279	-0.6907	-0.6964
Oct 98	**4.7597**	**2.5959**	**0.4467**	-1.8414	-1.2834	-0.4473	-0.1307	**0.6228**	-2.3455	-1.4658	**0.0693**	-0.0672	**0.5791**	**0.5097**
Jan 99	**2.6040**	**5.9353**	**3.0459**	-0.6838	-0.7000	**1.0442**	-1.3307	-2.5071	-4.0999	-2.5683	-2.1986	-1.3379	-0.6378	-0.6458

G = 1018.52, r = 195

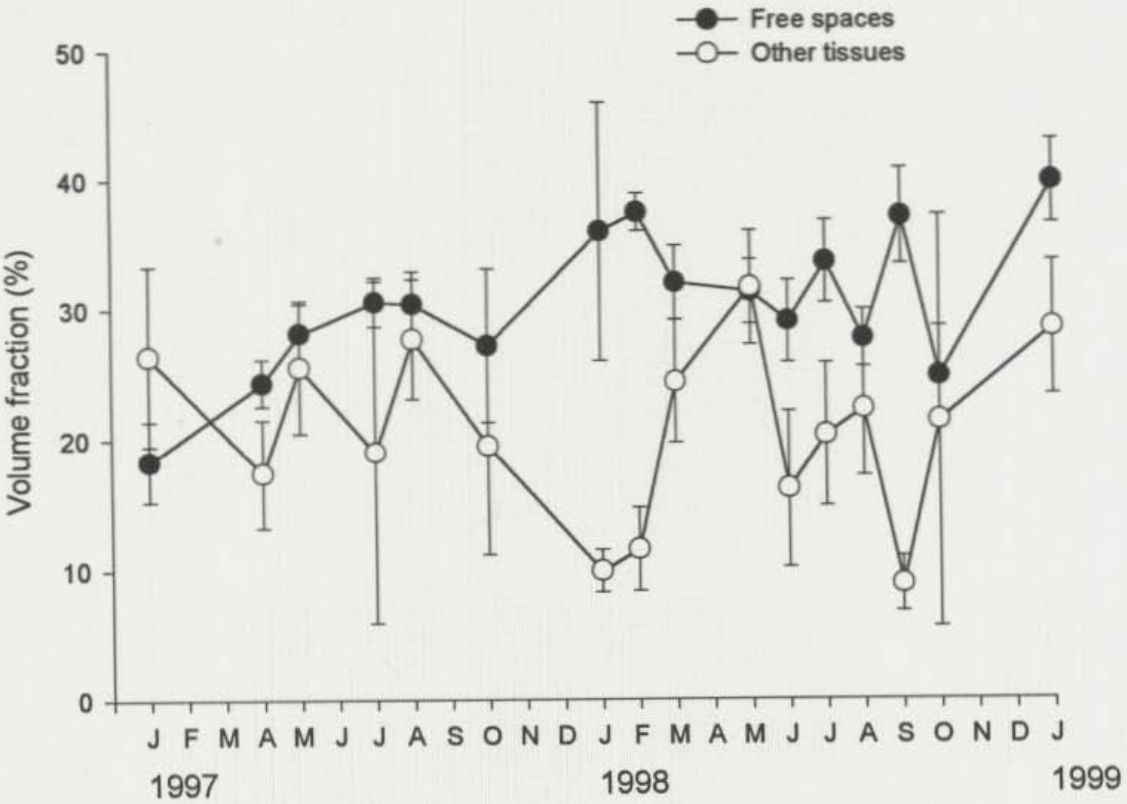

Fig. 10. *Yoldia hyperborea*. Volume fraction for other tissues and "free spaces". Data from individuals 25 - 35 mm shell length (mean ± SD).

3.3.1.4. Size at first maturity

A total of 99 *Y. hyperborea* individuals of shell length ≤ 23 mm was collected during the sampling period. There were 19, 18, 33 and 29 specimens of ≤ 20 ≤21 ≤22 and ≤ 23 mm shell length respectively and the percentage of mature individuals for each shell range was 15%, 37%, 57% and 63% respectively (Fig. 11). Individuals smaller than 20 mm in shell length exhibited little or no gonad development. The size at first maturity was estimated at around 21 mm for females and 22 mm for males.

3.3.1.5. Environmental factors

Temperature and salinity at the sampling site at 240 m depth in Conception Bay varied little during the sampling period, temperature remaining around -0.5 °C, and salinity 33 ‰. Fluorescence (representing chlorophyll a values) was the only factor seasonally variable, peaks values being observed in May 1997 and June 1998 (Fig. 9).

3.3.1.6. Contingency analysis

Positive residual values (Table 2, Figs. 12, 13) were observed for oocyte size classes 30 μm to 70 μm in January 1997, while oocyte size classes of 90 μm to 160 μm were present in April 1997, August 1997 (Fig. 12), January - February 1998 and June 1998 (Fig. 13). These results suggest that a main spawning event occurred in April 1997 (January - May 1997), and another in January - February 1998. This finding was consistent with observations of induced and non-induced spawning of *Y. hyperborea* specimens. A minor spawning event took place in August 1997 which can be explained as a secondary spawning of gametes not released during the main spawning.

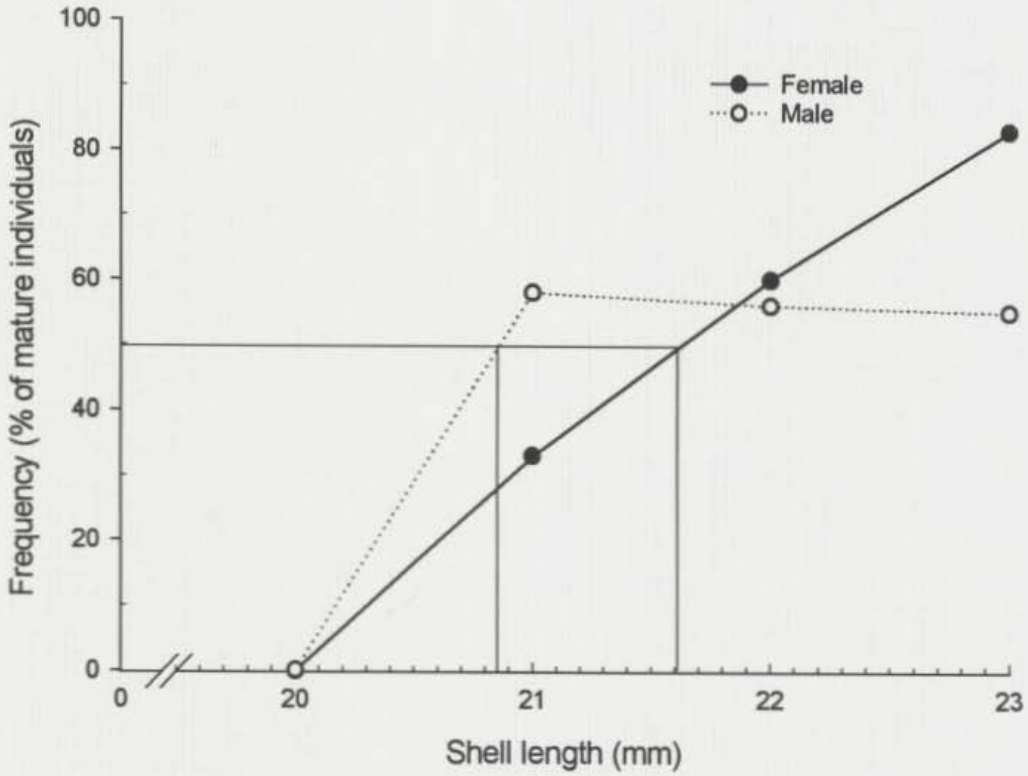

Fig. 11. Estimation of size at first maturity for *Yoldia hyperborea* in Conception Bay (Newfoundland). Size at first maturity defined as the size 50% of the individuals sampled in each size class (either male or female) exhibited any mature gametes.

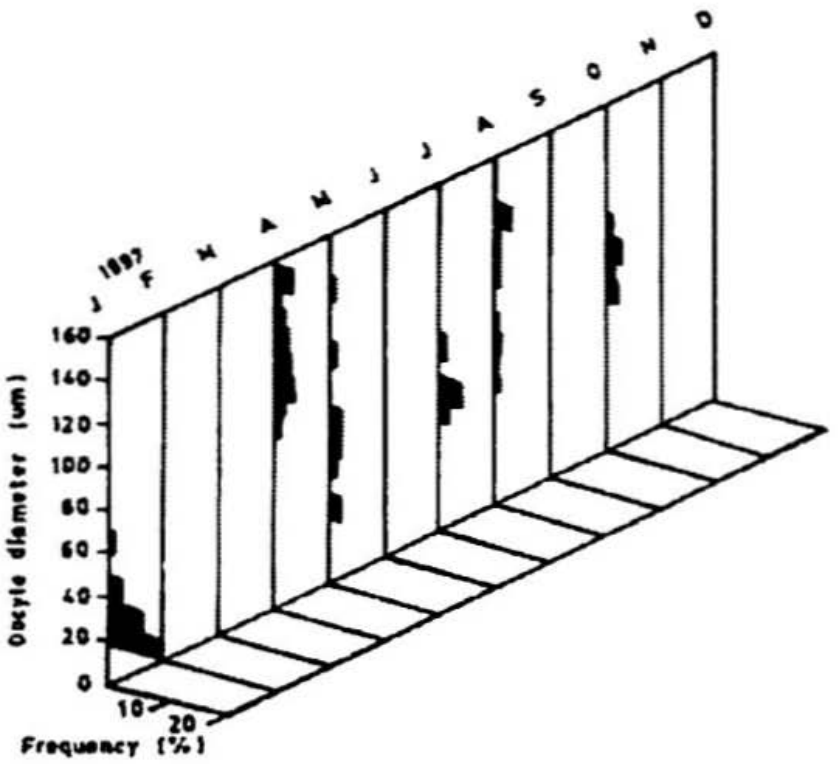

Fig. 12. Positive residual values plotted against oocyte size class frequency data in *Yoldia hyperborea* (1997). months without histograms signify no data

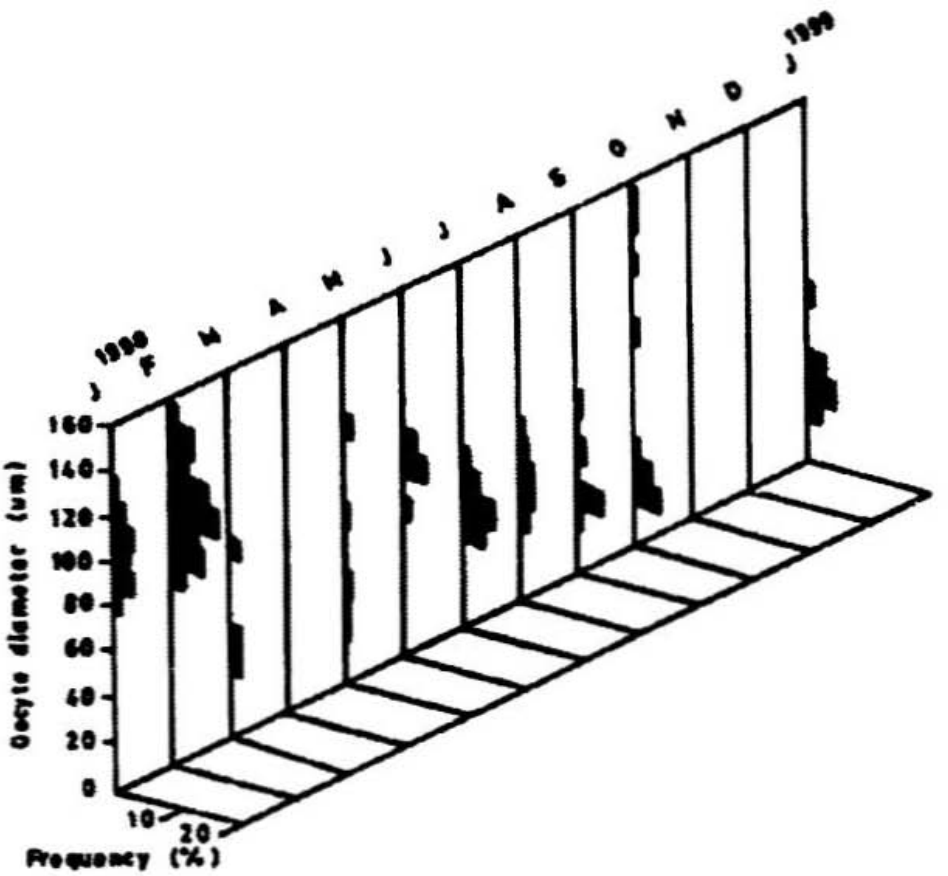

Fig. 13. Positive residual values plotted against oocyte size class in *Yoldia hyperborea* (1998-1999).months without histograms signify no data

3.3.2. *Ctenodiscus crispatus*

3.3.2.1. Sex ratio

The sex ratio was estimated for a total of 480 *C. crispatus* individuals (235 males and 235 females). Males and females were present in a 1 : 1 ratio over the study period according to results of a goodness of fit test (Table 1b) for a sample with $n > 200$ with one degree of freedom ($P < 0.05$).

3.3.2.2. Gonad Index

The mean gonad index was relatively constant during April-September 1997, followed by a decrease from October 1997 to January 1998 (Fig. 14). Two increases were recorded in February and June 1998 followed by a sharp drop in March and July 1998 respectively. Another increase begun in August 1998, reaching a maximum value in October 1998 before decreasing. High GI values measured recorded in June 1998 were coincident with phytodetritus sinking to the bottom of Conception Bay (see Fig. 9), but there is no clear explanation for the peak observed in August 1997.

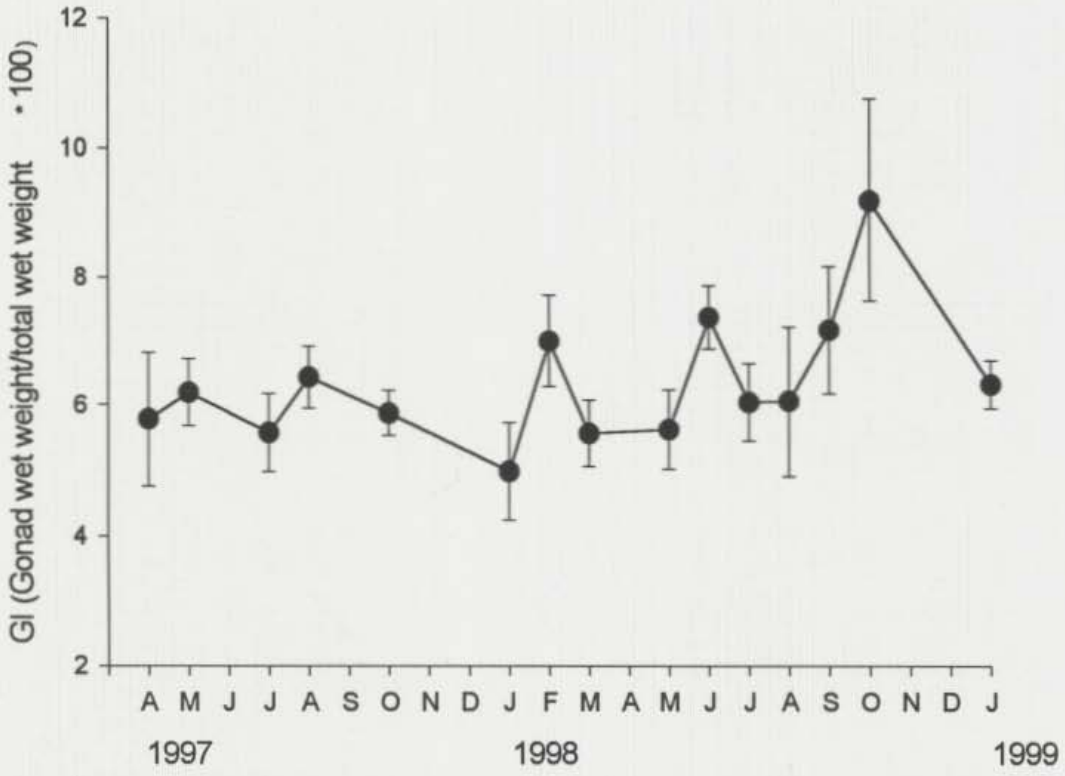

Fig. 14. *Ctenodiscus crispatus.* Gonad index (mean ± SD).

3.3.2.3. Gonad size index

Figure 15 shows that the gonad size index for *C crispatus* varied very little (between 63 % and 75 %) during the sampling period .

3.3.2.4. Gamete Volume Fraction

A full range of oocytes ranging from 50 μm to 550 μm in diameter was observed throughout the sampling period. Oocytes ranging between 100 μm and 150 μm were the most frequently observed, oocytes measuring from 200 μm and 500 μm were less frequent, and finally oocytes smaller than 100 μm or larger than 500 μm were scarce.

3.3.2.4.1. Previtellogenic oocytes

The volume fraction for previtellogenic oocytes was relatively constant from January to August 1997 (Fig. 16), then decreased steadily from August 1997 to January 1998. A further increase then occurred with a peak value in March 1998. There was little change from August 1998 to January 1999.

3.3.2.4.2. Vitellogenic oocytes

The percentage of vitellogenic oocytes dropped steadily from January to July 1997 (Fig. 16), remaining unchanged from August 1997 to March 1998. A sharp increase occurred in May 1998 followed by a sudden drop in June 1998. Again, this category increased from July to September 1998, but dropped by October 1998 and remained constant until January 1999.

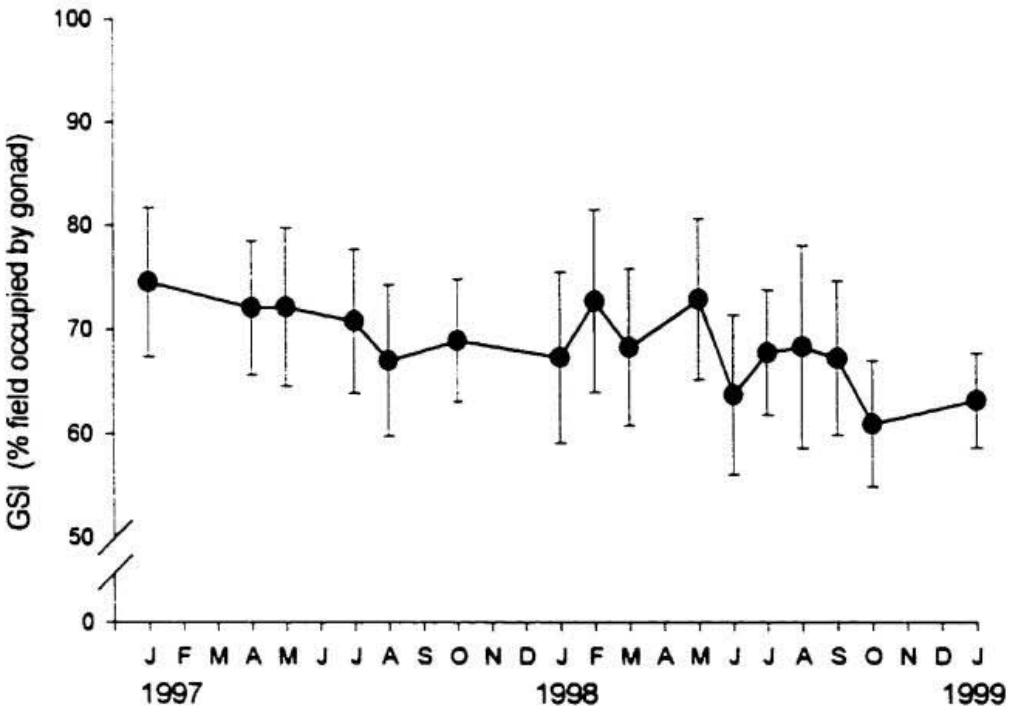

Fig. 15. *Ctenodiscus crispatus*. Gonad size index (mean ± SD).

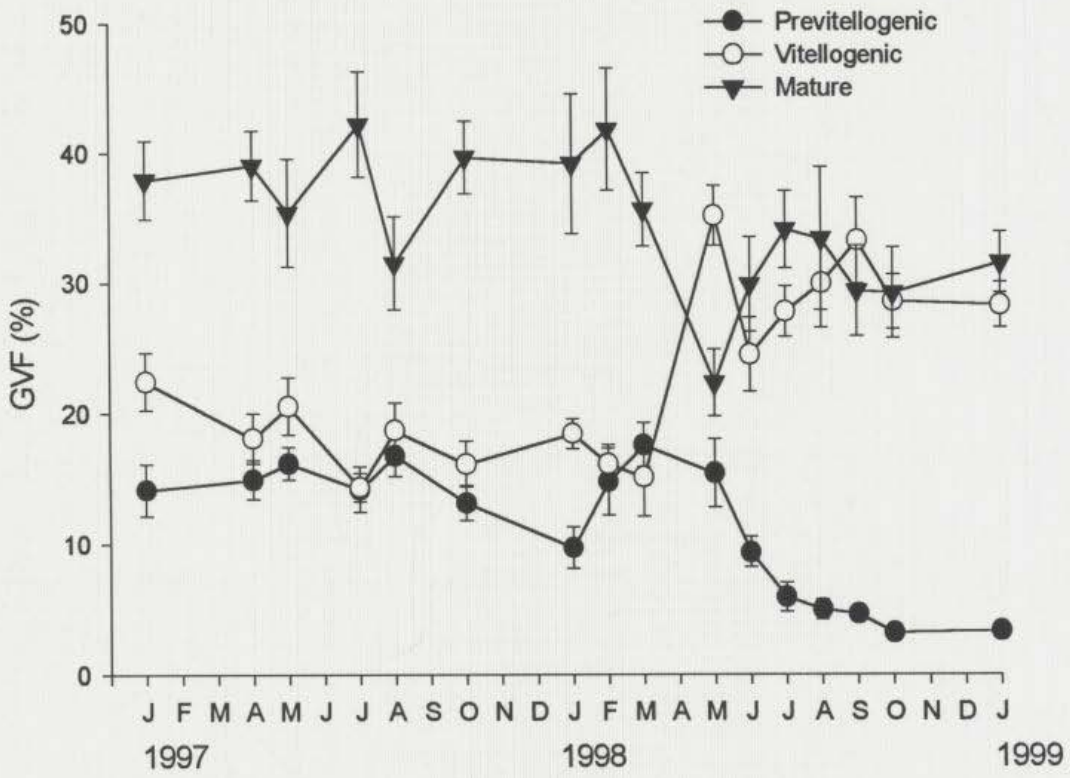

Fig. 16. *Ctenodiscus crispatus.* Gamete volume fraction (GVF) for previtelogenic, vitellogenic and mature oocytes. Data from individuals 28 - 31 mm arm length (mean ± SD).

3.3.2.4.3. Mature Oocytes

Mature oocytes were the dominant stage throughout the sampling period (Fig.16). The percentage of mature gametes remained constant from January to May 1997, but increased slowly to July 1997, followed by a sharp drop in August 1997. By October 1997 the percentage had increased before remaining constant until February 1998. A steady decrease occurred between March and May 1998. During June-July 1998 the percentage again increased, but then decreased until September 1998. Subsequent GVF values remained constant.

3.3.2.4.4. Free spaces

Fluctuations in the percentage of free spaces increased slowly from January 1997 to August 1997 followed by a steady decreases between November 1997 to February 1998 (Fig. 17) Following this period, values fluctuated from month to month, with increases starting in March 1998 and ending by November 1998. A decrease followed in January 1999. Free spaces were recorded throughout the sampling period, suggesting that the release of gametes may have occurred continuously and that no resting period occurred in this *C. crispatus* population .

3.3.2.4.5. Other tissues

The percentage of other tissues present in the gonad of *C. crispatus* specimens was zero at almost all times because only gonad tissue was sampled, in contrast to the percentages observed in *Y. hyperborea*, where the gonad is embedded in muscle tissue (Fig. 17). A few sampling months (January and June 1998) exhibited higher values than zero as a consequence of increases in the amount of gonad connective tissue.

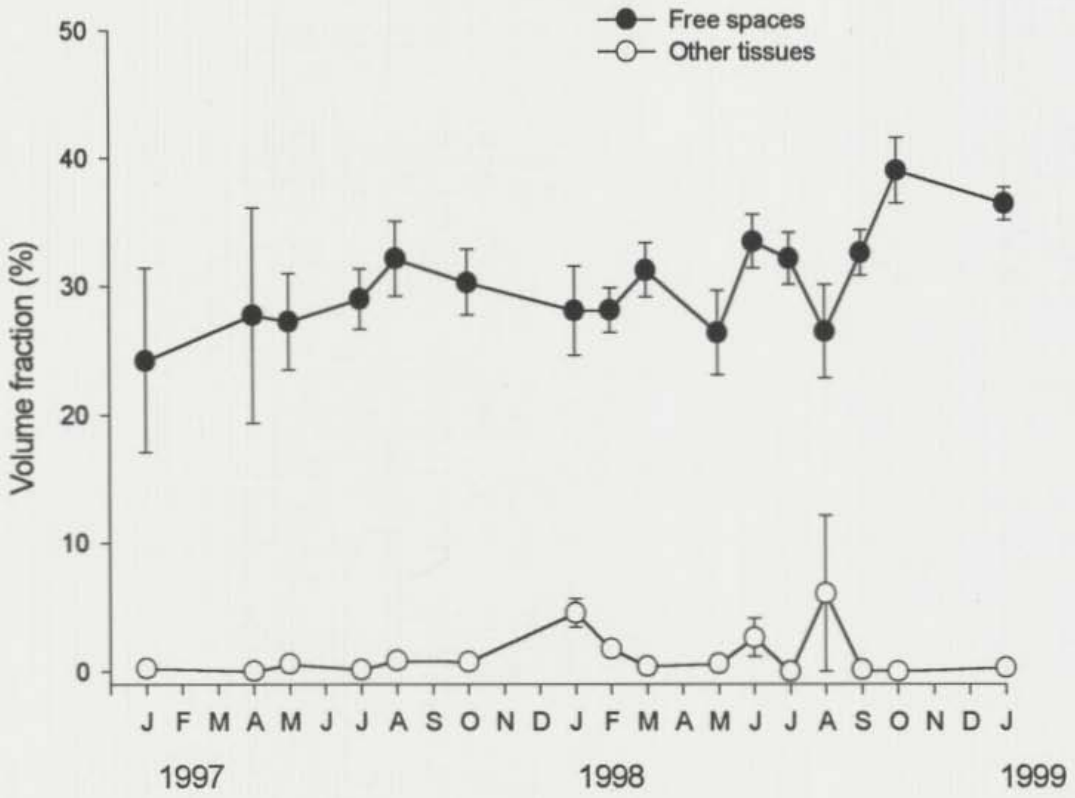

Fig. 17. *Ctenodiscus crispatus*. Volume fraction for other tissues and "free spaces". Data from individuals 28 - 31 mm arm length (mean ± SD).

3.3.2.5. Size at first maturity

Thirty individuals of *Ctenodiscus crispatus* (≤ 24 mm longest arm size) were collected. Frequencies of 2, 3, 6, and 19 individuals were registered for ≤ 21, ≤ 22, ≤ 23, and ≤ 24 mm longest arm size, respectively. The percentage of mature individuals in these length classes was 0%, 33 %, 66% and 94%, respectively (Fig. 18). Individuals smaller than 20 mm with longest arm size <20 mm showed no gonad development. The size at first maturity for female *C. crispatus* specimens was estimated to be 22 mm, and for males 23 mm longest arm size.

3.3.2.6. Contingency analysis

A contingency table for oocyte class data showed positive residuals values for oocyte size classes smaller than 500 μm during almost all the year (Table 3, Figs. 19, 20), while oocytes in the class 500 μm or larger were observed during January to April 1997, July 1997 to March 1998 and July 1998 to January 1999 . These results suggest that mature gametes were available for spawning during January to April 1997, July 1997 to February 1998, and July to September 1998 coinciding with high GVF values for mature oocytes as reported above.

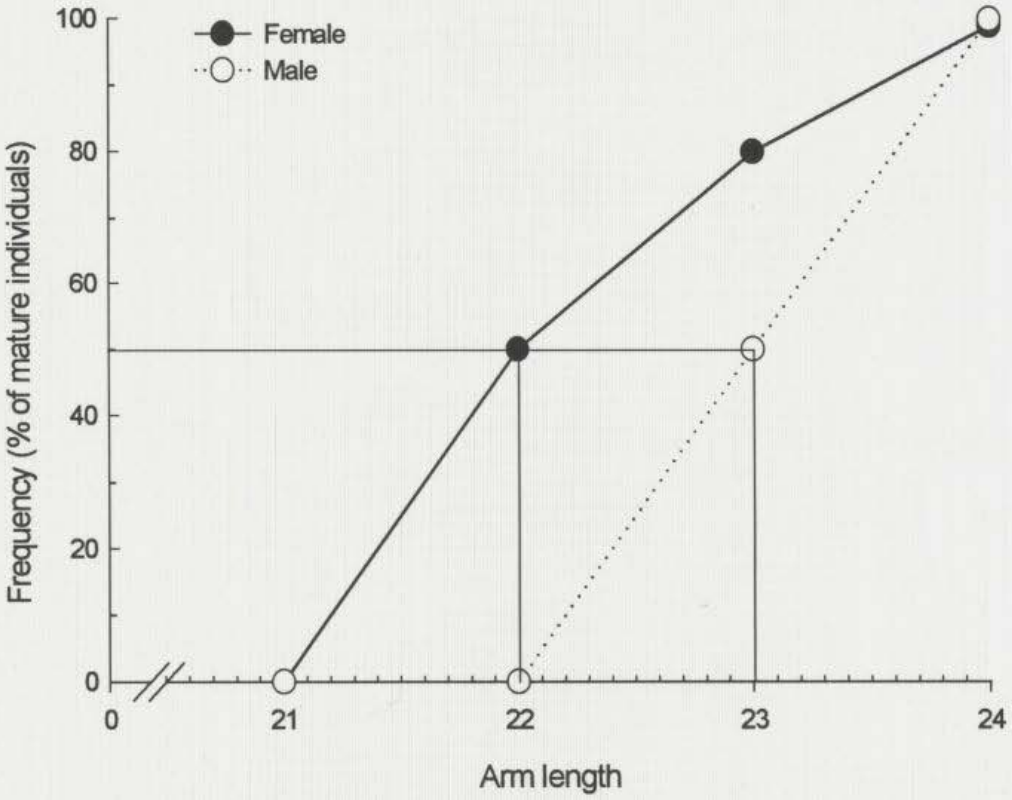

Fig. 18. Estimation of size at first maturity for *Ctenodiscus crispatus* in Conception Bay (Newfoundland). Size at first maturity defined as the size when 50% of the individuals sampled in each size class (either male or female) exhibited any mature gametes.

Table 3. Standardized residual values for oocyte size classes in *Ctenodiscus crispatus*. The positive residual values are in bold type.

Sampling	Oocyte size class											
Date	50 μm	100 μm	150 μm	200 μm	250 μm	300 μm	350 μm	400 μm	450 μm	500 μm	550 μm	600 μm
Jan97	-3.0768	-16.961	**7.7221**	**7.047**	**1.1542**	-1.1331	-1.4135	-1.4961	**0.5936**	**0.7519**	**1.4788**	-1.1559
Apr97	-2.2315	-9.5507	-1.4961	**1.0748**	**1.7678**	**2.4197**	**4.4648**	**4.2659**	**1.6727**	**1.3387**	-2.2741	-0.1829
May97	-17.193	-7.7151	-0.9841	**4.8598**	**4.2206**	**0.2373**	**2.3328**	**2.0411**	-1.4981	-1.1678	-0.2766	-0.7265
Jul97	-1.5696	-0.2629	**5.1438**	-0.3764	-2.7085	-3.8517	-4.0243	-1.1097	-0.65	**1.3611**	-0.7064	**0.3938**
Aug97	**0.9739**	-3.4367	-1.7734	**2.3774**	**1.4519**	-1.0285	**0.69**	**0.3896**	**2.3996**	**1.4518**	**0.5475**	-0.1201
Oct97	-0.0966	-4.5544	-1.721	**2.4758**	**0.7787**	**0.9333**	**1.8668**	**0.5362**	**2.8126**	-0.8594	**0.2694**	**0.7685**
Jan98	-6.7318	-1.8995	**4.9768**	-0.4287	-2.9216	-3.5345	-2.5951	**0.682**	**2.0794**	**1.3262**	**1.7168**	**0.909**
Feb98	-1.3671	**1.3697**	**1.0231**	-1.9749	-2.6479	-4.2632	**1.3633**	**1.0308**	**1.9368**	**2.8227**	**0.7471**	-0.3005
Mar98	-1.3828	**2.8291**	**3.311**	-4.5959	-3.2517	-0.8718	-2.468	-0.2289	**1.7661**	**0.5716**	**0.5722**	-0.4444
May98	-2.1302	-1.488	**0.3254**	**0.3606**	**2.1608**	**1.8305**	**0.0542**	-0.4863	-4.91	-2.0476	-0.6676	-0.0585
Jun98	-0.6213	-0.9661	-2.0979	**0.4264**	**2.8136**	**2.2869**	**0.4811**	**0.2203**	-3.0344	-5.5994	-1.9699	-0.071
Jul98	**4.1006**	**7.0135**	-9.7383	-3.6413	**2.0285**	**1.5931**	-0.8769	-3.4014	-8.6293	-3.5534	-1.0386	**0.2716**
Aug98	**3.8505**	**7.4605**	-6.2474	-4.7017	-0.264	**1.1452**	-5.5017	-3.4012	-5.8453	-2.2302	-0.4363	**0.4831**
Sep98	**1.3335**	**7.3477**	-4.4027	-2.9403	-0.4152	**1.9942**	-3.1715	-4.5321	-10.3658	-4.3786	-1.4142	**0.1334**
Oct98	**3.735**	**8.001**	-1.8282	-5.043	-3.2585	-2.134	-0.7924	-2.0114	-6.2159	-4.1427	-1.0671	-0.2937
Jan99	**2.12**	**10.6905**	-2.894	-8.6411	-9.8954	**0.3096**	-0.7336	-1.4398	-0.3473	-1.8278	-2.5473	-0.2834

G = 1148.7 v = 165

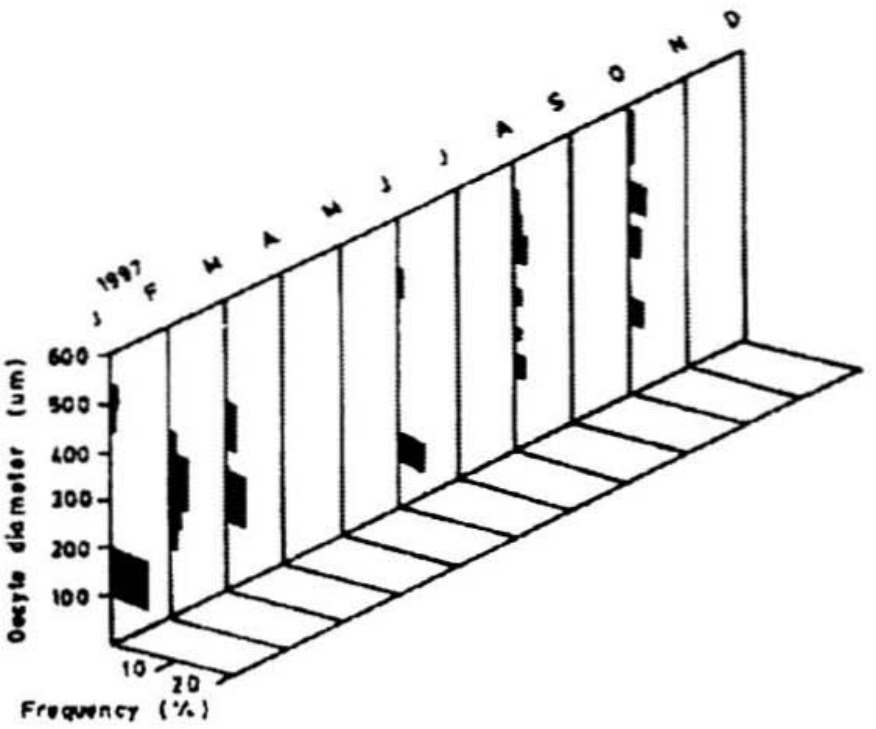

Fig. 19. Positive residual values plotted against oocyte size class frequency in *Ctenodiscus crispatus* (1997). months without histograms signify no data.

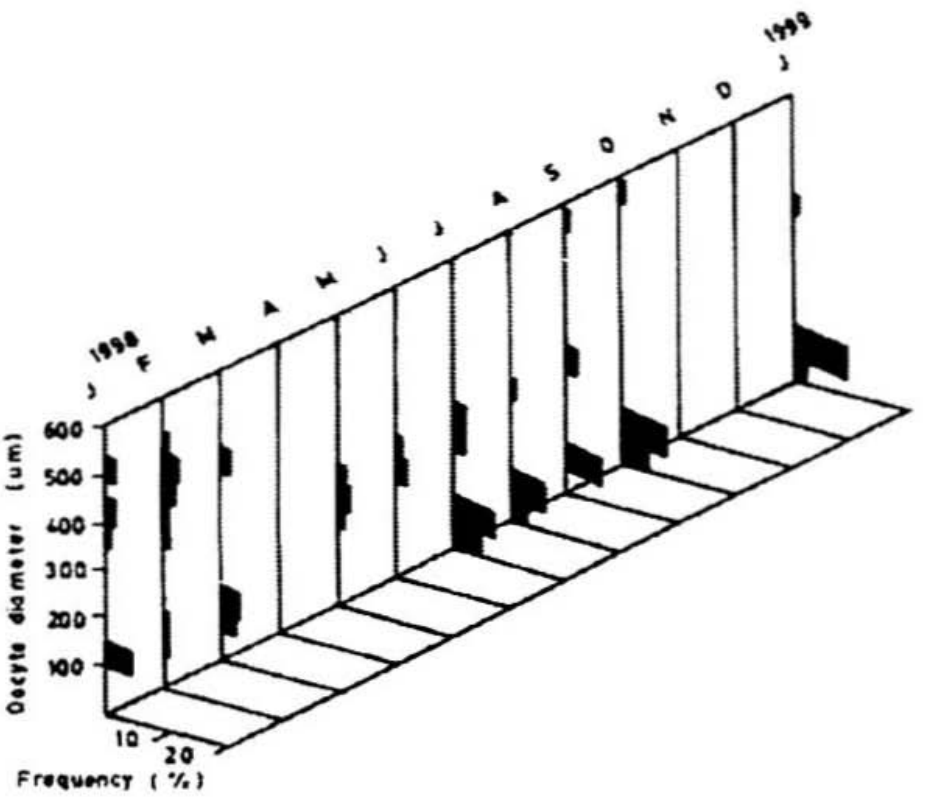

Fig. 20. Positive residual values plotted against oocyte size class frequency in *Ctenodiscus crispatus* (1998- 1999) months without histograms signify no data

3.4. DISCUSSION

3.4. 1. *Yoldia hyperborea*

Gametogenesis involves a maturation process in which germinal cells develop into mature gametes either oocytes or sperm. During oogenesis, this process starts with the multiplication of germinal cells to form oogonia which become previtellogenic oocytes, then grow into vitellogenic oocytes, and finally become mature oocytes. This morphological definition of different gamete stages can be quantified by summarizing standardized residual values obtained for each morphological stage with the aid of a contingency table. Thus displacement of standardized positive residuals values towards greater oocyte size classes (mature oocytes) indicates a maturation process, whereas displacement of standardized positive residual values towards smaller oocyte sizes (previtellogenic or vitellogenic oocytes) indicates a spawning event (Grant and Tyler 1983b; Pazos et al.1996).

Standardized positive residuals values for *Y. hyperborea* indicated that oocyte size classes 100 μm or larger were frequently observed during the sampling period, whereas displacement of standardized positive residuals towards smaller oocytes occurred from January to May 1997 and March to May 1998, suggesting spawnings during these periods. Similar results were observed by GVF, mean oocyte size diameter analysis and oocyte size frequency distributions and supported by spontaneous, non-induced spawnings occurring during February and March 1998. These results suggest that a main spawning period occurred during winter-early spring (January 1997-May 1997 and January - March 1998 in *Y. hyperborea* individuals from Conception Bay. However, decreases in the percentage of mature gametes also occurred during July to October 1997 and July to August 1998 which may be interpreted as a minor spawning.

Seasonal patterns of reproduction have been frequently reported for shallow water species but less frequently for deep sea species. Seasonal reproduction in shallow water species has been associated with seasonal fluctuations of environmental factors such as temperature,

salinity, tides, waves, food availability, etc. (Sastry 1966, 1970; Machell and DeMartini 1971; Newell et al.1982; Currie 1990; Arsenault and Himmelman 1998; Loosanoff 1937; Ropes and Stickney 1965; Malachowski 1988). By contrast, for a number of years, sublittoral, bathyal and abyssal zones were thought to be very stable environments, and consequently many biologists suggested that species inhabiting these areas should exhibit a continuous reproductive cycle because it was assumed that they were not affected by such environmental fluctuations occurring near the ocean's surface. However, seasonal reproduction has been reported for some deep-sea bivalve and echinoderm species (Lightfoot et al 1979, reviewed by Tyler 1986, Tyler et al. 1992), and attributed to seasonality in the sinking of phytodetritus reported by George and Menzies (1967, 1968), Schoener (1967), Rokop (1974, 1977), Stockton and Delaca (1982), Tyler and Gage (1984), Harrison (1988), Tyler (1988), Thiel et al. (1990), Gage and Tyler (1991), Tyler et al. (1992), Young et al. (1992) and Smith (1994).

Seasonal sinking of phytodetritus has also been observed in Conception Bay in a yearly basis (April, May and June; Redden 1994). The observed increase in the gonad development in *Yoldia hyperborea* (increased percentages of vitellogenic and/or mature gametes) which occurred during May - July 1997 and May - June 1998, followed by a late spring-summer spawning (July - October 1997, June - August 1998) may be a direct result of this seasonal food pulse which provides deposit feeders (including *Yoldia hyperborea*) with a fresh and direct source of nutritive organic carbon, proteins and lipids that can be richer and more labile than occurs in the typical deep-sea sediments (Billett et al. 1983; Rice et al. 1986; Smith 1994). Some of this material can be directly assimilated from the water column (Kristensen et al. 1992). Then the seasonal food pulse can explain minor spawning events that occurred shortly after phytodetritus sinking, but does not explain spawning occurring during winter-early spring.

A possible explanation for the presence of vitellogenic and mature gametes during most of the year (including the spawning period) is that *Y. hyperborea* has a steady supply of material able to sustain this continuous gamete growing phase. The phytodetritus therefore may be one of several food sources available to deposit feeding species, which may also ingest bacteria, protozoans etc (Phillips 1984; Jumars et al. 1990). However, deposition of animal

carcasses and remains of macrophytes may also contribute to enrichment of benthic sediments (Gage and Tyler 1991).

Turbulence (close to the sea floor) and/or bioresuspension (reworking) may cause resuspension of organic material as a result of feeding activities or construction of mounds, pits, or tubes by the benthic community modifying the bottom microtopography and providing a source of particulate organic matter (POM). POM can result in increased microbial activity (Wainright 1990), which represents a constant primary food source ((Newell 1965; Phillips 1984; Jumars et al. 1990). Important evidence supporting the effect of bioresuspension was provided by Bender and Davis (1984), who reported that the feeding activity of *Yoldia limatula* results in sediment bioresuspension through expulsion of loose pseudofaeces directly into the water column. Consequently, during most of the year *Y. hyperborea* may obtain energy to develop gametes either from resuspension or bioresuspension, of bacteria and/or bacterial degradation of resuspended nutritive material which facilitates gamete maturation. However, during or shortly after phytodetrital deposition, individuals probably feed directly from a suite of phytodetritus, compounds of nutritive value obtained directly from primary production (Phillips 1984; Jumars et al. 1990).

Laboratory feeding experiments (see Chapter V) demonstrated that laboratory-grown, senescent algae added to an experimental aquarium remained suspended in the water column for a few days, stimulating siphon activity in *Y. hyperborea*. Once phytodetrital particles settled onto the bottom of the aquarium, however siphon activity was reduced and most individuals kept their siphons buried in the sediment, expelling loose faeces and/or pseudofaeces directly into the water column (bioresuspension). These experimental observations support suggestions that *Y. hyperborea* obtains energy from both newly settled phytodetritus and material which has been incorporated into the sediments for long periods.

3.4.2. *Ctenodiscus crispatus*

Results from analysis of oocyte frequency distributions, contingency analysis and histological sections of the ovaries of *Ctenodicus crispatus* individuals indicated the presence

of a full range of oocytes throughout the sampling period. Moreover, the percentages of previtellogenic vitellogenic and mature frequencies changed little throughout the sampling period. These results suggest that the gametogenic cycle for *C. crispatus* in Conception Bay is non-seasonal. Oocyte maturation was also asynchronous, as previously described for *C. crispatus* from Damariscove Island, Gulf of Maine (Shick et al. 1981b). The continuous presence of gametes, including mature oocytes, reported in this study has also been previously described for Damariscove Island specimens (Shick et al. 1981b). However the presence of large oocytes does not necessarily mean that spawning is imminent, although intermittent spawning may be explained by a year round food availability, allowing a constant transfer of nutrient stored in somatic tissues to the ovary (Shick et al. 1981b; Eckelbarger and Watling 1995). However, as in polar echinoderm species, *C. crispatus* may transfer some of the energy available during the seasonal deposition of phytodetritus directly to the gonad, and some to the pyloric caeca for storage. Consequently, during periods of low food availability, stored reserves are available for transfer to the ovary for gamete production.

A continuous transfer of energy from gut to ovary is the most likely mechanism to produce asynchronous gamete maturation in *C. crispatus*. Studies on the biochemical composition of gonads of *C. crispatus* individuals at Damariscove Island have reported no variations throughout the year (Shick et al. 1981 b), suggesting that there is a steady flow of nutrients moving from the gut to the ovary via the pyloric caeca as a result of a year round food supply obtained from organically-rich sediments (Falk-Petersen and Sargent 1982). This may be explained by the fact that *C. crispatus* is a non-selective deposit feeder which feeds on bulk sediment, although amphipods, polychaete tube fragments and bivalve shells have also been found in the sediment-filled stomachs of many specimens (Shick et al. 1981a). However, these authors reported that most of the organic detritus at Damariscove Island was refractory and not directly available to deposit feeders, suggesting that bacteria associated with the POM were the primary source of energy for deposit feeders (Newell 1965), although stored reserves may also have provided the energy required for gamete production. The availability of POM through bioresuspension has previously been discussed as a constant source of organic matter and bacteria associated with POM which could play an important role in providing *C. crispatus* with

a primary source of energy (Wainright 1990).

Because a full range of oocytes was observed in female *C. crispatus*, it is possible that throughout the year both resuspension and bioresuspension activities and storage reserves may provide enough energy to support the non seasonal maturation of a small number of gametes (Eckelbarger and Watling 1995). If maturation starts coincidently with, or shortly before, the arrival of phytodetritus, a greater number of gametes can undergo vitellogenesis, producing a greater number of mature oocytes for release during spawning. In contrast, during autumn or winter resuspension regularly provides a steady supply of energy which is sufficient to promote the maturation of a small number of gametes. Consequently, it is possible that the energy required to produce a small number of mature gametes may be lower than required to produce a large number of gametes once a year (Eckelbarger and Watling 1995). This low energy requirement may be met by the resuspension or continuous bioresuspension of sediment by the soft-bottom benthic community in Conception Bay (Redden 1994) or from stored energy in the pyloric caeca .

Not only does *Y. hyperborea* play an important role in bioresuspending sediments in Conception Bay, but deposit feeding species in general tend to resuspend sediments (Davis 1993). Thus, other deposit feeding species (e.g. *C. crispatus*, protobranchs, and some polychaetes) inhabiting the soft bottom of Conception Bay, probably contribute to bioresuspension of sediment and subsequently facilitate increases in the microbial biomass associated with POM.

Decreases in GVF values for mature oocytes occurred in July-August 1997, February - May 1998 and July - September 1998, shortly after or coincidently with phytodetritus sinking, which can be interpreted as spawning of mature oocytes. However, the presence of mature gametes continuously throughout the year suggests that several minor spawnings may also have taken place throughout the year. The absence of seasonal trends for all size classes of oocyte may suggest that oocyte maturation in the population is continuous. Because biochemical composition and the content of the gonad of *C crispatus* showed little seasonal variation at Damariscove Island, reproduction was interpreted as continuous rather than non-seasonal, and related more to changes in phytoplankton production than to temperature (Shick et al., 1981b).

However In Conception Bay, temperature and salinity remained stable during the sampling period and food was the only fluctuating factor (phytodetritus sinking). The evidence suggests that reproduction in *C. crispatus* is non-seasonal and is more related to changes in food availability than to any other environmental factor.

Results from the contingency analysis (positive residuals) show a clear trimodal oocyte size-frequency distribution during the last year of sampling, suggesting that maturation of gametes may take longer than one year. Long oocyte development times have been observed in the echinoid *Sterechinus neumayeri* and the asteroid *Odontaster validus* (18-24 months for both species) (reviewed by Clarke 1988).

Both Conception Bay (present study) and Damariscove Island (Shick et al. 1981b) populations exhibited similar non seasonal reproductive cycles which were more intense during late spring and/or summer months. Spawnings occurred during or shortly after phytodetritus sinking in Conception Bay, suggesting that late spring-summer spawnings (July-September 1997 and July-September 1998) were influenced by the fresh phytodetritus reaching the bottom. Billett et al. (1983) and Rice et al. (1986) proposed that during phytodetrital deposition (May-June in Conception Bay), high concentrations of rich and labile POM are available to deposit feeders and can be immediately absorbed and utilized to increase gamete production. Similar observations have been reported for other asteroid, echinoid and ophiuroid species inhabiting the northeast Atlantic, which show elevated vitellogenic activity from June to November following phytodetrital deposition in late May through August (Tyler 1988; Gage and Tyler 1985).

The Conception Bay and Damariscove Island *C. crispatus* populations (this work, Shick et al. 1981b) exhibited a slightly different reproductive cycle than Balfsjorden populations, which possess an aseasonal reproductive cycle, with spawning during mid-winter coincident with the highest water temperature in that area (Falk-Petersen and Sargent 1982). This suggests that spawning in this population can be stimulated by increases in water temperature, but the timing of gametogenesis may be affected by fluctuations in food availability.

CHAPTER IV

MODE OF DEVELOPMENT

4.1. INTRODUCTION

The survival of any species depends on successful spawning, fertilization and settlement of recruits. Every species has developed a specific reproductive strategy that ensures the survival of a number of recruits until they reach reproductive maturity. These strategies include adopting either a seasonal or continuous gametogenic cycle, releasing mature gametes with enough energy reserves to ensure complete larval development (lecithotrophy), or releasing smaller eggs that develop into planktotrophic larvae. Other species exhibit a direct mode of development that permits recruitment of an individual that is effectively a small sized adult, by eliminating the larval stage completely.

According to Thorson (1950), the mode of larval development depends upon the environment in which the species lives. Thus species inhabiting higher latitudes (polar or subpolar areas) should reproduce mainly by a non-pelagic mode of larval development (lecithotrophy, either with a short pelagic life or through direct development), whereas tropical and subtropical species should primarily exhibit a pelagic mode of larval development (planktotrophy). Thorson (1936) demonstrated that a number of crustacean, echinoderm, bivalve, and gastropod species inhabiting higher latitudinal zones have larger eggs than those observed at lower latitudes, and these eggs eventually pass through either a lecithotrophic or direct mode of development. This theory has been questioned, since it has been validated only for gastropod species, and some polar species known to produce planktotrophic larvae (Menge 1975; Bosch, 1989). Production of a small adult (direct development) or a lecithotrophic larva, either with a short pelagic stage or a non-pelagic stage, requires more energy reserves in the egg than production of a planktotrophic larva, and the mode of larval development is closely related to the size of the egg (Thorson 1950; Clarke 1992).

It is well-known that marine invertebrates exhibiting a lecithotrophic mode of larval development produce a small number of large eggs (between 10 and 10^3 eggs per individual, (Thorson 1946), which generally develop into either big larvae with a short pelagic stage or non-pelagic larvae. Neither type of larva requires phytoplankton to survive because it primarily feeds on yolk stored in the egg itself. In contrast, species that produce planktotrophic larvae release a large number (between 10^2 and 10^9 eggs per individual, Thorson 1946) of smaller eggs that develop into larvae that generally feed on phytoplankton and possibly on organic detritus (Thorson 1950; Mileikowsy 1971; Jablonski and Lutz 1983).

Published studies for protobranch species report that egg diameter varies between 100 μm (*Nucula proxima*, Scheltema 1972) and 240 μm (*Malletia cuneata*, Tyler et al. 1992). Larval development in protobranchs appears to be restricted to a lecithotrophic pericalymma larva with a short pelagic life in some species (Drew 1899; Gustaffson and Reid 1986), or direct development in others (Scheltema 1994). Shelf and abyssal protobranch species exhibit either a lecithotrophic or direct mode of development (Drew 1899; Lebour 1938; Zardus and Morse 1996), but no data are available for slope protobranchs.

Egg diameter in asteroid species varies from 105 μm (*Luidia cidaris*, Emlet et al.1987) to 3500 μm (*Diplasterias octoradiata* and *Rhophiella koehleri*, Emlet et al.1987). There is a bimodal distribution of egg diameter among asteroids, with the first group containing almost exclusively species that develop through a planktotrophic larva (eggs ranging from 100 μm to 230 μm diameter), and the second group mostly containing species that develop through a lecithotrophic larval stage (eggs ranging between 300 μm and 3500 μm). In this latter group, however, there are also some brooding species (Emlet et al.1987; Sewell and Young 1997).

Direct field and laboratory observations of larval development are difficult to obtain, and conclusions about the mode of larval development of a number of species have therefore been drawn indirectly from measurements of egg diameter (Levin and Bridges 1995) or from measurements of the size of the early shells (protoconch I and II for gastropods and prodissoconch I and II for bivalves) as suggested by Jablonski and Lutz (1980, 1983). However, the method developed by Ockelmann (1965) demonstrated that a relationship exists between egg size (small yolk-poor eggs or large yolky-eggs), larval shell size (prodissoconch

I or II), and mode of larval development (planktotrophic or lecithotrophic). This method, although helpful, should be applied carefully when inferring the mode of larval development, because the criteria used are based solely on observations of taxonomically related shallow-water species (Gustafson and Lutz 1992). The most reliable method for determining mode of larval development is to include comparisons between closely related species whose developmental biology is already known (Jablonski and Lutz 1983).

The main objective of this chapter is to determine by indirect methods the mode of larval development for *Y. hyperborea* and *C. crispatus* inhabiting CB. Measurements of both the egg diameter and prodissoconch I length allow prediction of the mode of larval development for *Y. hyperborea*, and egg diameter measurements are appropriate for *C. crispatus* because asteroids develop through either bipinnaria or brachiolaria larvae.

4.2. MATERIAL AND METHODS

4.2.1. Obtaining eggs

4.2.1.1. *Yoldia hyperborea* eggs

Fresh *Y. hyperborea* oocytes were obtained by stimulating spawning of mature individuals (October 1997, February 1998 and March 1998) with temperature increases. Individuals brought from the field were maintained for two or three days in running sea water at 0 °C, then switched to tanks with sea water at 5°C. Spawning took place after two hours.

Released oocytes were washed in filtered sea water, then observed under a stereomicroscope (Makroskop 420, Wild). Images of spawned oocytes were obtained by videotaping, then digitized and analyzed as described in Chapter II for analysis of oocyte frequency distributions. A total of 526 oocytes was measured, and values for egg diameters obtained by image analysis were plotted as size-frequency histograms.

4.2.1.2. *Ctenodiscus crispatus* eggs

In order to estimate the size of newly spawned eggs, a number of attempts were made to induce spawning in *C. crispatus* individuals by injecting 0.5 m NaCl into the body cavity, but they were unsuccessful. Consequently, fresh eggs were obtained by gonad stripping. Each month, gonads from two or three individuals, sampled for histological purposes, were stripped and the eggs pooled and washed with filtered sea water. Later, eggs were observed, videotaped, digitized and analyzed as previously described for *Yoldia* specimens.

4.2.2. Prodissoconch measurements

4.2.2.1 *Yoldia hyperborea*

Small *Y. hyperborea* ranging between 500 μm and 800 μm were collected from the muddy soft bottom of CB using a hyperbenthic sledge fitted with a 500 μm mesh net, sorted by size and then fixed in 4% formaldehyde to await processing for SEM (Scanning Electron Microscopy).

Individuals stored in 4% formaldehyde were post-fixed in 1% osmium tetroxide in 0.2 M cacodylate buffer for 30 minutes, then washed twice with 0.1 M cacodylate buffer (10 minutes per wash). Postfixed, rinsed samples were then dehydrated in a graded ethanol series, ending with two washes in absolute ethanol. The samples were then dried to the critical point (Polaron Critical Point Dryer), mounted, sputter coated with gold and observed by SEM (S 570 Hitachi).

4.3. RESULTS

4.3.1. *Yoldia hyperborea*

4.3.1.1. Egg size

Freshly spawned *Y. hyperborea* eggs were light brown in color and spherical in shape. After a few seconds they all settled to the bottom of the beaker. Pooled data for both months showed that the size of fresh eggs varied between 26 μm and 170 μm diameter (Fig. 21). The monthly mean egg diameter was 116 μm (±15 μm SD) for October 1997, 123 μm (± 20 μm SD) for February 1998, and for March 1998, with a overall mean of 118 μm (± 7 μm SD, N= 831).

4.3.1.2. Prodissoconch shell

SEM images of shells of young *Yoldia hyperborea* individuals revealed that, like other protobranchs, they lack a prodissoconch II stage. A large oval prodissoconch I, extending to the border of the dissoconch, was observed (Fig. 22). The length of the prodissoconch was measured as the straight-line distance from the prodissoconch-dissoconch boundary to the opposite side of the same boundary. The mean length was estimated as 210 μm (±10 SD).

4.3.2. *Ctenodiscus crispatus*

4.3.2.1. Egg size

Freshly stripped *C. crispatus* eggs were bright orange in color and spherical in shape. Pooled data for all months sampled showed that the stripped eggs varied between 260

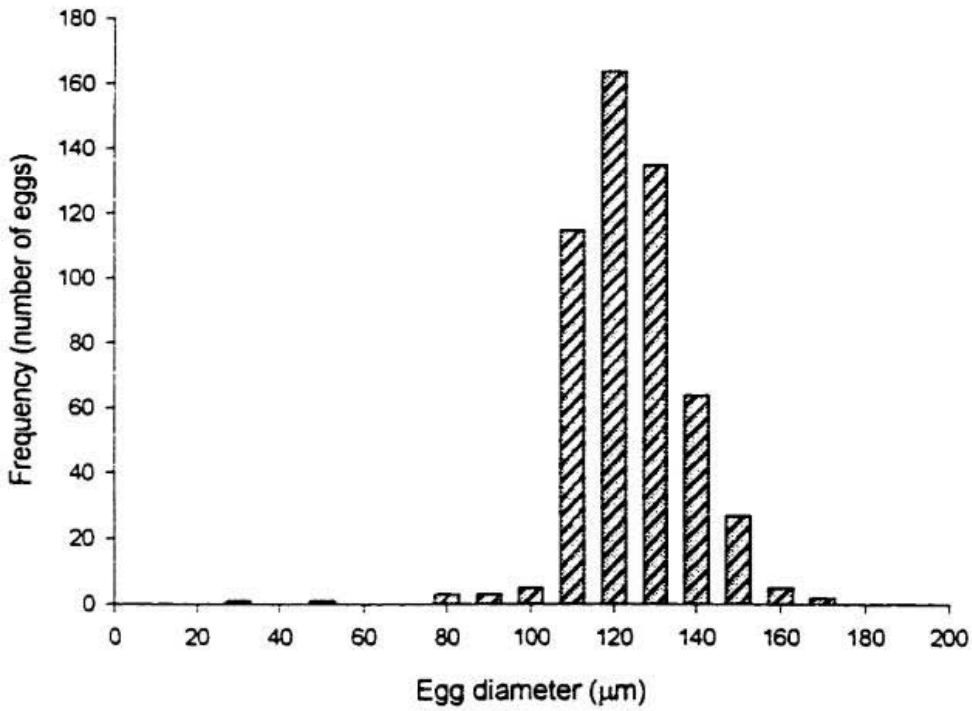

Fig. 21. Distribution of egg size frequencies for *Yoldia hyperborea* Data pooled from all sampling periods (N = 526).

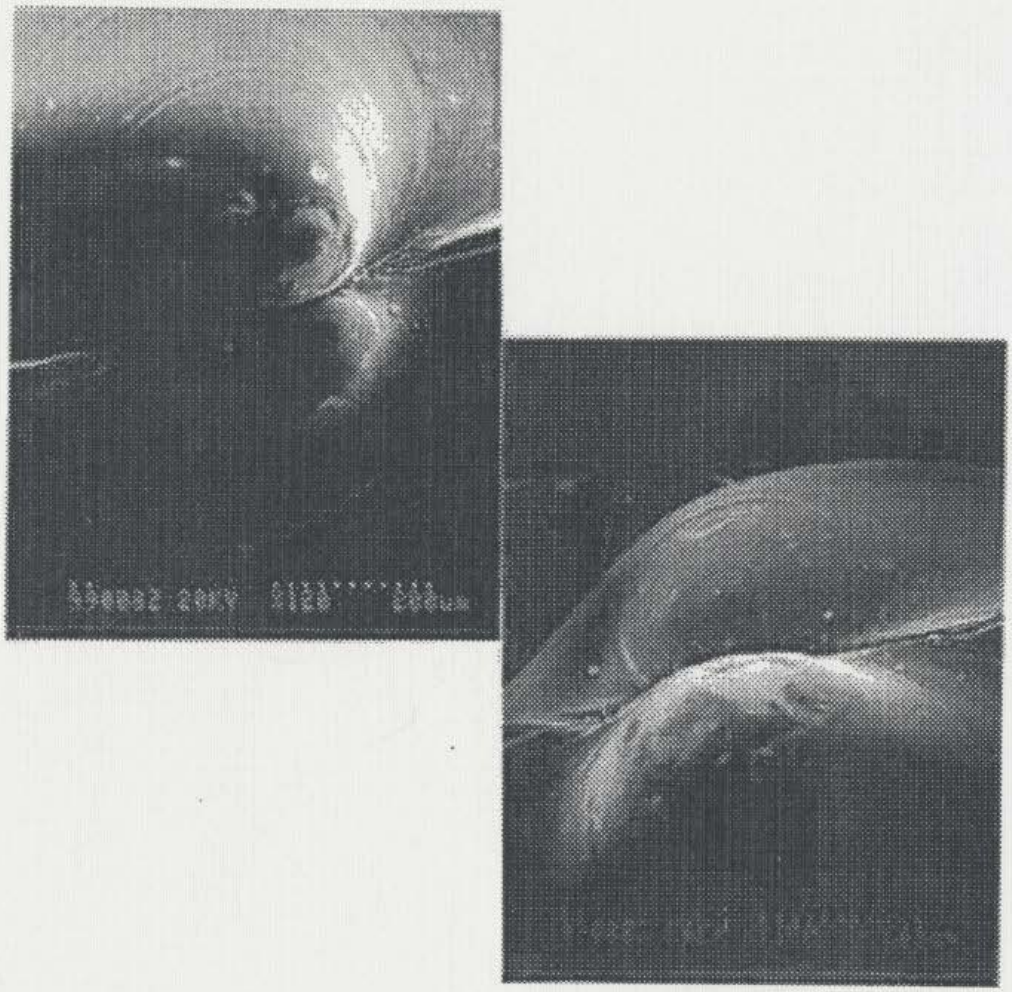

Fig.22. Photomicrograph at top left: general view of *Yoldia hyperborea* young individual shell. Bottom right: prodissoconch at higher magnification.

μm and 982 μm in diameter (Fig. 23). The mean oocyte diameter varied from 388 μm (± 40 μm SD) in March 1998 to 490 μm (± 69 μm SD) in July 1998 with an overall mean of 450 μm (± 56 μm SD, N = 1413).

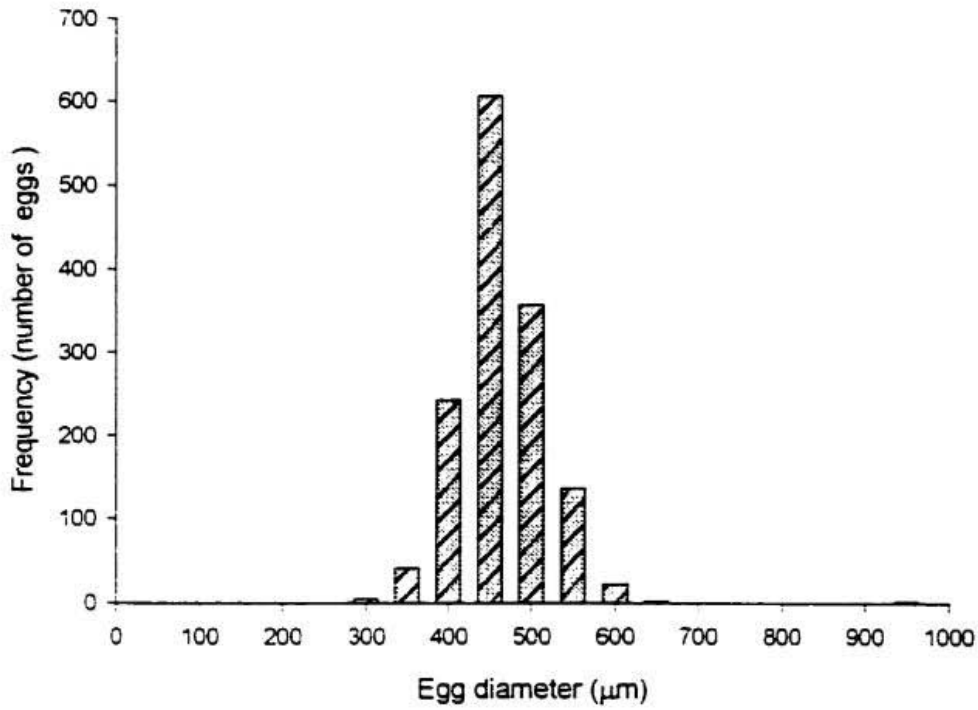

Fig. 23. Distribution of egg size frequencies for *Ctenodiscus crispatus*. Data pooled from all sampling periods (N = 1413).

4.4. DISCUSSION

4.4.1. *Yoldia hyperborea*

Early attempts to predict the mode of larval development were based on the premise that planktotrophic larvae (with a long pelagic life) originate from very small eggs, whereas lecithotrophic larvae develop from large eggs (Thorson 1950). This basic tenet has been considered by many authors to determine the mode of larval development when field or laboratory observations are difficult to make (Levin and Bridges 1995). Consequently, much of the present data on the mode of larval development of marine invertebrates may be equivocal, since they have been based on this indirect method (Levin and Bridges, 1995).

Ockelmann (1965) demonstrated that a positive relationship exists between egg size, larval shell size and the type of larval development of gastropods, and this relationship can also be applied to bivalve species. According to the above criteria a bivalve species with a mean egg size 40 μm to 85μm diameter, a prodissoconch I of 70 μm to 150 μm length, and a prodissoconch II of 200 μm to 600 μm in length will exhibit a planktotrophic mode of development, whereas a bivalve species with mean egg diameter 90 μm to 140 μm and a prodissoconch I 135 μm to 230 μm in long will be lecithotrophic (Gustafson and Reid 1986). Considering that the mean oocyte diameter of protobranch species varies from 90 μm diameter in *Nucula proxima* (Drew 1899) and *Nucula turgida* (Lebour 1938) to 271 μm in *Solemya reidi* (Gustafson and Reid 1986), the mode of larval development for these species should be either lecithotrophic or direct, and this has indeed been reported for these protobranchs, including some brooding examples (e.g., *Solemya reidi*, Gustafson and Reid 1986). According to the egg size criteria previously cited, protobranch species should not have a planktotrophic larvae (Drew 1899; Gustafson and Reid 1986). However, one larval characteristic seems to be important in limiting the non-planktotrophic mode of larval development in these species. Protobranch species developing through a pericalymma larva lack a feeding organ (Gustafson

and Lutz 1992), preventing the larva from feeding on plankton or any other seston. Consequently, larvae must exhibit either lecithotrophic or direct development, as already described.

The second criterion considered in Ockelmann's theory (prodissoconch I and II sizes) is not applicable to protobranch species, since pericalymma larvae do not exhibit a prodissoconch II stage (no planktotrophic development). Consequently, the total length of the prodissoconch has been substituted for prodissoconch I length in order to predict the mode of larval development in these species.

The data reported in this study show that *Yoldia hyperborea* in individuals from CB possesses a mean oocyte diameter of 120 μm, lacks a prodissoconch II stage, and has a prodissoconch 210 μm in long, suggesting that the species exhibits a lecithotrophic mode of larval development.

4.4.2. *Ctenodiscus crispatus*

The mode of larval development in asteroid species has been classified into four types: planktotrophy, pelagic-lecithotrophy, demersal development (meaning nonfeeding, free-swimming larvae which remain on or close to the bottom) and brooding (McEdward and Jenies 1993; Sewell and Young 1997). This classification is based on the habitat where the larva develops and the source of nutrition of the larva. Because Ockelmann's method is not suitable for echinoderm species, egg size is the only available predictor of the mode of larval development.

The eggs of asteroid species vary between 105 μm and 3500 μm diameter, and all four development patterns cited are represented in this oocyte size range. A suggested classification proposes that all species with egg sizes around 100 μm to 250 μm diameter should exhibit a planktotrophic mode of development, species with a pelagic lecithotrophic mode of development should possess eggs from 300 μm to 1460 μm diameter, and brooding species (including those with demersal development) should produce eggs ranging from 300 μm to 3500 μm diameter (Emlet et al. 1987). According to this egg size classification, it should be

easy to identify a planktotrophic mode of development because it is restricted to species with eggs measuring 250 μm diameter or less. However, because egg size ranges for lecithotrophy and direct development overlap, they are difficult to separate. Sewell and Young (1997) contributed to the clarification of this egg size overlapping problem by pointing out that many brooding species have eggs larger than 650 μm. Consequently, species having an egg diameter between 300 μm and 650 μm diameter are closer to a pelagic-lecithotrophic mode of larval development than to brooding. However, additional field observations on brooding behavior would be helpful in order to clarify the mode of development in species having egg sizes in the overlapping range (Emlet et al. 1987).

According to results reported here (450 μm mean egg diameter), and following the egg size-based classification discussed above, the CB *Ctenodiscus crispatus* population should exhibit a pelagic-lecithotrophic mode of larval development, as suggested for the Damariscove Island and Balsfjorden populations (Shick et al. 1981a; Falk-Petersen 1982a respectively). All three of these *Ctenodiscus* populations compared (CB, Damariscove Island and Balsfjorden) exhibit a similar mode of larval development but different egg diameter. Damariscove Island and CB populations have a mean egg diameter of 400 μm and 450 μm egg diameter respectively, whereas, the Balsfjorden population has a mean egg diameter of 650 μm egg diameter. These egg size differences might be explained by the close relationship between egg size and the geographic location demonstrated by Emlet et al. (1987). Thus species inhabiting high latitudinal zones (polar or subpolar areas) have bigger eggs than similar species that inhabit temperate areas.

A positive correlation between egg size and latitudinal location among populations of the same species has not often been reported, but a few examples are given by West et al. (1984), Levin and Bridges (1995) and Qian and Chia (1991, 1992), who indicated that geographically separated populations of an opisthobranch, a polychaete, and a gastropod species respectively exhibit different egg sizes. In addition, different modes of larval development were observed between these same species populations. Similar relationships have also been reported for some echinoderm species, e.g. the echinoid species *Strongylocentrotus droebachiensis* and *Strongylocentrotus pallidus* show increases in egg size

with increases in latitude along the coast of Norway (Hagström and Lonning 1961). Results from the present study suggest that such a positive correlation between egg size and latitude may exist among *Ctenodiscus crispatus* populations, a pattern which has not previously been shown for any asteroid.

Variations in egg size of echinoderm species between sites have also been attributed to differences in food quantity; echinoderms from sites with a wide variety of food produce larger eggs than those from sites with a poor variety of food (George et al. 1990). Variations between two successive reproductive periods at the same site may also be explained by fluctuations in both the food availability and the food quality available (Scheibling and Lawrence 1982; George et al. 1990; George 1994, 1996). Thus observed differences among *Ctenodiscus crispatus* populations may also be explained by differences in both food quantity and quality between sites. Since there are insufficient data to distinguish between any of the given explanations, further research is needed to determine whether differences in egg size between *C. crispatus* populations are a result of either geographical location or food availability.

CHAPTER V

LABORATORY EXPERIMENTS

5.1. INTRODUCTION

5.1.1. General overview

As previously described, a gametogenic cycle involves a number of events which depend on the energy available for reproduction, and the main energy reserve used for reproduction in adult bivalves is glycogen (Gabbott 1975). The gametogenic cycle will depend on the energy reserve stored by individuals (Lubet 1986), the amount of energy obtained directly from the environment and finally on the proportion of energy allocated to gametogenesis. Although individuals may have the required energy for reproduction, the influence of some environmental factors may also be required for triggering the onset of gametogenesis.

5.1.2. Factors influencing gametogenesis

5.1.2.1 Shallow water species

A number of studies in shallow water mollusc species suggest that differentiation of gametes begins at certain critical threshold temperatures, but the onset of both growth and maturation of gametes requires higher temperatures. Sastry (1966, 1968) demonstrated that individuals of the scallop *Aequipecten irradians* developed oogonia at 15°C, but the oogonia failed to develop into oocytes even when the animals were fed. When food was available and temperature increased, however, oocytes started growing immediately. Consequently, once initiated, gametogenesis can be accelerated by increasing the temperature, since increases in

temperature facilitate nutrient mobilization and the subsequent development of gametes (Sastry 1968; Sastry and Blake 1971). Nutrient mobilization occurs only if the basic metabolic needs of the individual have been satisfied (Sastry 1979). Consequently, mobilization of energy compounds, either from stored reserves or directly from ingested food, is required to start gamete development. Shallow water species inhabit very unstable environments and are exposed to fluctuating environmental factors such as temperature, salinity, light and food availability (Sastry 1979; Eckelbarger and Watling 1995). Variation in these factors can contribute to seasonally predictable reproductive cycles in some species (Pearse et al. 1986; MacDonald and Thompson 1986, 1988).

5.1.2.2. Deeper waters

Because deeper water species live in an environment less variable, they were once thought to be unaffected by variability in surface conditions (Tyler and Young 1992; Smith 1994), and most species were assumed to reproduce continuously. More recently, however, evidence has been found for a seasonal phytodetrital pulses reaching the deep-sea floor in some areas following the spring phytoplankton bloom in surface waters. This phenomenon may account in part for the seasonal reproductive cycle observed in some deeper water species (George and Menzies 1967, 1968; Schoener 1967; Rokop 1974, 1977; Stockton and Delaca 1982; Tyler and Gage 1984a; Harrison 1988; Tyler 1988; Thiel et al. 1990; Gage and Tyler 1991, Tyler et al. 1992; Young et al. 1992). Seasonal reproduction is well documented among echinoderm and bivalve species,. Several deep-sea studies (Tyler 1988, Tyler and Gage 1984 b, Tyler and Young 1992) reported that a few northeast Atlantic species clearly exhibited a seasonal pattern of reproduction, which is probably associated with seasonal phytodetritus deposition (Tyler 1988, Tyler and Gage 1984 b, Tyler and Young 1992; Sun 1999). To date, evidence suggests that the phytodetrital pulse stimulates the rapid growth of bacteria, flagellates, foraminiferans and meiofauna (Gooday and Turley 1990), but there is no direct evidence to support the hypothesis that seasonal deposition of phytodetritus can stimulate an immediate reproductive response in benthic macrofauna, despite the existence of seasonal reproductive cycles among some benthic invertebrates (Eckelbarger and Watling 1995).

To date, only northeast Atlantic echinoderm species (asteroids, echinoids and ophiuroids) have been shown to exhibit active vitellogenesis following phytodetrital deposition (Tyler 1988; Gage and Tyler 1991). For example, *Echinus affinis* exhibited maximal vitellogenesis activity during the later phases of phytodetrital deposition and immediately afterwards (Tyler and Gage 1984a). These results suggest that although the reproductive response is not immediate, vitellogenesis may be initiated by an influx of phytodetritus (Eckelbarger and Watling, 1995). Consequently, a study of the effects of the seasonal influx of phytodetritus on the reproductive activity of deeper- water species other than echinoderms could contribute to a better understanding of the relationship between reproduction and food availability in these species.

5.1.2.3. Conception Bay as an example

Results reported in previous chapters for *Yoldia hyperborea* inhabiting Conception Bay support the hypothesis that reproduction of some bivalve species living in deeper waters are affected by phytodetritus pulses. Phytodetrital deposition was reflected in an increase in the percentage of developing oocytes in *Y. hyperborea* and then subsequently maturation, which takes place two or three months after phytoplankton deposition. However, these reproductive events may occur as a consequence of either a seasonal input of energy after the spring phytoplankton bloom, (Deuser and Ross 1980; Gage and Tyler 1991; Hinga et al. 1979; Lampitt 1985; Lampitt et al. 1990; McCave 1975; Rice et al. 1986, 1991), or after periodic resuspension of surface sediments (Redden et al. 1994, Graf and Rosenberg 1997). In *Y. hyperborea* from Conception Bay, an influence of the seasonal pulse of phytodetritus was observed during or shortly after the spring months, although gamete maturation during the fall and winter may be induced by resuspension of surface sediment (see chapter III).

Field observations suggest that at 240 m in Conception Bay factors such as temperature and salinity do not change seasonally and therefore are unlikely to have a significant effect on the reproductive cycle of *Y. hyperborea*. Food availability, however, varies seasonally, and experiments were carried out in the laboratory using a simulated pulse of algae, to determine whether the reproductive responses of *Yoldia* observed in the field can be

reproduced under controlled conditions, and whether organic nutrients in the algae are incorporated into the gonad. The main purpose of these experiments was to establish whether phytodetritus is utilized for gamete production. No experiments were undertaken with *C. crispatus* since this species is much more difficult to keep in the laboratory than *Y. hyperborea*.

5.2 MATERIAL AND METHODS

5.2.1. Feeding experiments

5.2.1.1. Experiment one

5.2.1.1.1. Sediment

Sediment collected in epibenthic sled samples with *Yoldia hyperborea* individuals during the sampling period was brought into the laboratory, separated from macrofauna, and then kept frozen for at least one week. A few days before starting the experiment, the sediment was thawed at room temperature and allowed to settle to the bottom of an experimental and a control aquarium to form a bottom layer 4-cm. thick. Aquaria were maintained inside a refrigerated tank with running sea water (0.8 l/hour) at 0°C . Each aquarium was provided with both outflow and inflow pipes in order to ensure a constant water flow. A low airflow into the aquarium provided oxygenation, but flows were kept low enough to prevent sediment resuspension.

5.2.1.1.2. Animals

Yoldia hyperborea individuals collected in Conception Bay were maintained for four months under the conditions described above (running sea water at 0°C) before starting the experiments. Twelve individuals ranging between 28.0 mm and 33.0 mm shell length were selected and allowed to burrow in the sediment of each of the experimental and the control aquaria (30 L capacity each, 12 individuals per aquarium).

After 65 days animals were opened and the gonad tissue removed and processed for histology as described in Chapter II. Oocyte size and oocyte frequency distributions were obtained as described in Chapter II.

5.2.1.1.3. Senescent algae

A concentrate of a very dense slurry of senescent algae (a mixture of *Isochrysis galbana*, *Tetraselmis suecica* and *Chaetoceros affinis*), obtained from the bottom of algal culture vessels, was carefully layered onto the sediment surface of the experimental aquarium with a pipette at 0, 10, 37 and 51 days. Animals in the control aquarium did not receive any food other than that available through the running water system (not quantified).

5.2.1.1.4. Carbon/Nitrogen quantification

Carbon and nitrogen in both the algal concentrate and the sediments were estimated with a Perkin-Elmer CHN analyzer model 240A calibrated with acetanilide. For this purpose, three samples (1 cc per sample) were taken from the senescent algae concentrate shortly before each addition to the tank. For sediment C/N estimate, nine samples (1 cc sediment each sample) were obtained from the sediment surface at 0, 16, 37 and 65 days. Samples were taken from the edges and the center of each aquarium.

5.2.1.2. Experiment two

Preliminary observations suggested that siphon activity is related to the presence of particulate material suspended in the water column of the aquarium. Therefore, an experiment was also carried out in which particular attention was paid to the activity of the inhalant siphon. Animals, food and sediment utilized in this experiment were obtained as previously described.

5.2.1.2.1. Suspended particles

Shortly after the addition of senescent algae, replicate water samples were taken 0.5 cm and 2.5 cm above the sediment. The numbers of suspended particles present in the experimental and control aquaria were determined with a Coulter Multisizer II.

5.2.1.2.2. Siphon activity

Daily observations of the activity of the exhalant siphon were made from the first addition of algae until completion of the experiment 50 days later. The presence or absence of the exhalant siphon and either the vertical (ES) and/or horizontal position of this siphon (HS) were also recorded for each animal. During the ES condition the siphon was oriented vertically in the water column, whereas during the HS behavior the siphon was observed either in a horizontal position or swirling around. Procedures for gonad sampling, histological processing and estimations of oocyte size were as previously described.

5.2.1.2.3. Oocyte size and number

Procedures for gonad sampling, histological processing and estimations of oocyte size were previously describe on Chapter II. The gonad for each experimental individual was sampled and processed separately. After estimating the number of eggs per individual, data were pooled and represented in an oocyte frequency distribution histogram.

5.2.1.2.4. Statistical analysis

A one way ANOVA was performed in order to compare the mean size of oocytes between experimental and control individuals. The data were normally distributed according to a normal distribution test.

5.2.2. Radioactive tracer experiment

Given that the results from the feeding experiments suggested that addition of laboratory-grown senescent algae affected gamete development, an experiment was carried out to determine whether the cell contents of senescent algae are incorporated into the gonad tissue. For this purpose, a dual marker experiment using ^{14}C and ^{68}Ge radiolabelled algae was carried

out. This technique has been successfully used to determine the absorption efficiency of the cold water appendicularian *Oikopleura vanhoeffeni* feeding on the laboratory-grown diatom *Thalassiosira nordenskioldii* (Bochdansky et. al. 1999). The method is based on the fact that some bivalves (e.g. *Mercenaria mercenaria*) have separate pathways for processing cell and cell wall (meaning the outer rigid structure present in diatoms which helps the cell keep its shape and gives the cell strength) contents of microalgae. The cell contents (^{14}C labelled) are incorporated into the digestive gland and they remain there for intracellular digestion. Thus ^{14}C is retained for a longer period of time than cell wall constituents, resulting in greater exposure to ^{68}Ge attached to the cell surface. A dual labelling technique (using either ^{68}Ge or ^{51}Cr as a marker) is more sensitive and more useful for small samples and has already been applied by several investigators (Calow and Fletcher 1972, Bricelj et. al. 1984; Bochdansky et. al.1999).

5.2.2.1. Animals

For each experiment (3 in total) 12 to 18 *Yoldia hyperborea* individuals ranging between 28.0 and 33.0 mm total length were individually placed on a small plastic stand immersed in a beaker containing 200 mL filtered (GF/C) seawater and senescent radiolabelled algae at concentrations of 10 cell*μl^{-1}. Beakers were placed on a cold stirrer plate to provide a constant suspension of particles in the water. Experimental individuals were kept at 0°C for 60 to 80 hours.

5.2.2.2. Radiolabelled food sources

Thalassiosira nordenskioldii was grown at 5°C under continuous light in f/2 medium containing 100μCi liter^{-1} ^{14}C ($NaH\ ^{14}CO_3$) and 80μCi liter^{-1} ^{68}Ge ($^{68}Ge\ (OH)_4$, New England Nuclear Corp.). After 10 to 12 days of culture the unincorporated label was removed from the radiolabelled algae by reverse flow filtration using a 7 μm Nitex mesh. The concentrate of senescent radiolabelled algae was diluted in (1μm) GF/C-filtered seawater and added to the culture flasks until a concentration of approximately 10 cell*μl^{-1} was reached.

5.2.2.3. Radionuclide counting

After incubation for periods varying between 66 to 83 hours, experimental *Y. hyperborea* individuals were opened and part of the gonad tissue, adjacent to the digestive gland, was excised and placed on a 25 mm GF/C filter paper disk. Free ^{14}C was removed from the filter by washing with 0.25 mL of 0.2 N perchloric acid and placed individually in a 7-mL glass scintillation vials that were left loosely capped. Twelve hours later, 5 mL of BioLume (ICN Biomedical), a liquid scintillation cocktail, was added to the vials. Radioactivity of samples was measured with a Packard TriCarb Liquid Scintillation Analyzer (model TR 2500) 48 forty eight hours later. Remaining tissues for experimental individuals were taken for phisiological analysis.

The maximum β energy for ^{68}Ge is 1.9 MeV and 156 KeV for ^{14}C. The ratio of these maximum energies (12.2) is high enough for complete separation of the two isotopes. For each isotope, standard quench curves were constructed using internal standards, with chloroform as the quenching agent. In addition, radioactive counts were corrected for background radiation (background radiation fluctuated between 10 and 20 dpm for ^{14}C and zero for ^{68}Ge).

5.3. RESULTS

5.3.1. Feeding experiments

5.3.1.1. Experiment one

5.3.1.1.1. Carbon and Nitrogen levels in sediment

Carbon concentrations increased after the addition of senescent algae. Whereas in the experimental aquarium, carbon increased from 87 $\mu g^{-1} * mg^{-1}$ (± 1.86 SD) at the beginning of the experiment to 95 $\mu g^{-1} * mg^{-1}$ sediment (± 6.05 SD) by the end of the experiment (Fig 25a), in the control aquarium carbon concentrations decreased from 82 $\mu g^{-1} * mg^{-1}$ (± 1.89 SD) to 76 $\mu g^{-1} * mg^{-1}$ (± 2.13 SD) during the same period.

Nitrogen concentrations in the control aquarium decreased from 10.6 $\mu g^{-1} * mg^{-1}$ (± 0.31 SD) at the beginning of the experiment to 7.9 $\mu g^{-1} * mg^{-1}$ sediment (± 1.14 SD) by the end of the experiment whereas nitrogen concentrations in the experimental aquarium increased from 11.3 $\mu g^{-1} * mg^{-1}$ (± 0.15 SD) to 11.9 (± 0.77 SD) during the same period (Fig 25b).

5.3.1.1.2. Oocyte diameter

Differences in both the mean oocyte size and the frequency of distributions produced per individual were observed between experimental and control individuals. A total of 77 oocytes with a mean size of 61 μm (± 23.1 SD) in diameter was obtained from experimental individuals, whereas control individuals exhibited a mean oocyte size of 57 μm (± 27 SD) diameter and a total of 62 oocytes were counted. A frequency polygon (Fig. 24) showed that the most frequently observed oocyte size class among control individuals was 40 μm diameter, whereas the oocyte size class 60 μm was the most frequently observed among experimental individuals.

A one-way ANOVA demonstrated that differences in the mean oocyte size between control and experimental individuals were significant ($P < 0.004$ for experiment one and P<

0.001 for experiment two, Table 4). A heterogeneity G-test demonstrated that the frequency of oocyte size classes was dependent on the treatment ($P < 0.01$ for experiment one and $P < 0.001$ for experiment two).

5.3.1.2. Experiment two

5.3.1.2.1. Siphon behaviour

Increases in both ES and HS activity were observed among experimental individuals immediately after addition of laboratory-grown senescent algae to the aquarium (Fig 27b). A few days later, when the suspended particles had been cleared by *Yoldia hyperborea* and/or had settled to the bottom, the water column became clear and ES activity decreased with, around ~20% of the individuals remaining in this state. HS activity remained for a longer period than ES then decreased, with around ~20% of the individuals exhibiting this activity (Fig 27 b). Control individuals exhibited only ES activity (Fig. 28b). Concentrations higher than about 200 x 10^3 particles ml^{-1} resulted in ES activity by *Yoldia hyperborea* in the control aquarium. Thus, ES and HS activities appeared closely related to higher amounts of suspended particles in the water column. Most of the experimental individuals developed a thin layer of gonad tissue adjacent to the digestive gland, which was removed for counting radiolabels

5.3.1.2.2. Oocyte size and number

Differences between experimental and control individuals in both the mean oocyte size and the total number of oocytes produced were also observed in this experiment. A total of 48 control animal oocytes was compared with 171 experimental animal oocytes. The mean oocyte diameter was 29 μm (± 7.4 SD) and 54 μm (± 21.8 SD) diameter for control and

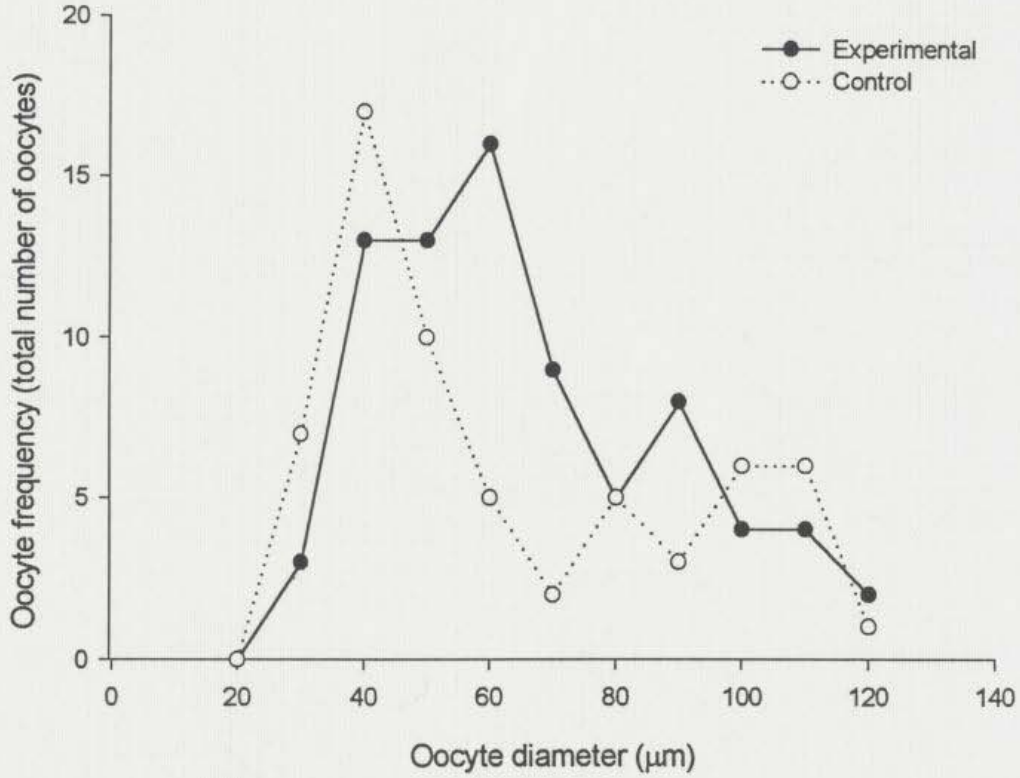

Fig. 24. Distribution of oocyte size frequencies observed in *Yoldia hyperborea* after experiment 1.

Table 4. Comparative one-way ANOVA to test for differences in oocyte diameter between experimental and control *Yoldia hyperborea* individuals

Analysis of Variance:
One Way

Experiment 1	***N***	***Sum***	***Mean diameter***	***Variance***
Control	62.000	3582.811	57.787	757.849
Experimental	77.000	4707.987	61.143	534.523

Analysis of Variance

Source of Variation

	SS	***df***	***MS***	***F***	***P-value***
Control vs Experimental	9099.499	1.000	9099.499	8.733	0.004**
Within groups	162547.929	156.000	1041.974		
Total	171647.427	157.000			

Experiment 2	***N***	***Sum***	***Mean diameter***	***Variance***
Control	48.000	1393.621	29.034	55.147
Experimental	171.000	9269.876	54.210	461.860

Analysis of Variance

Source of Variation

	SS	***df***	***MS***	***F***	***P-value***
Control vs Experimental	181390.054	1.000	181390.054	559.580	0.000***
Within groups	110212.370	340.000	324.154		
Total	291602.424	341.000			

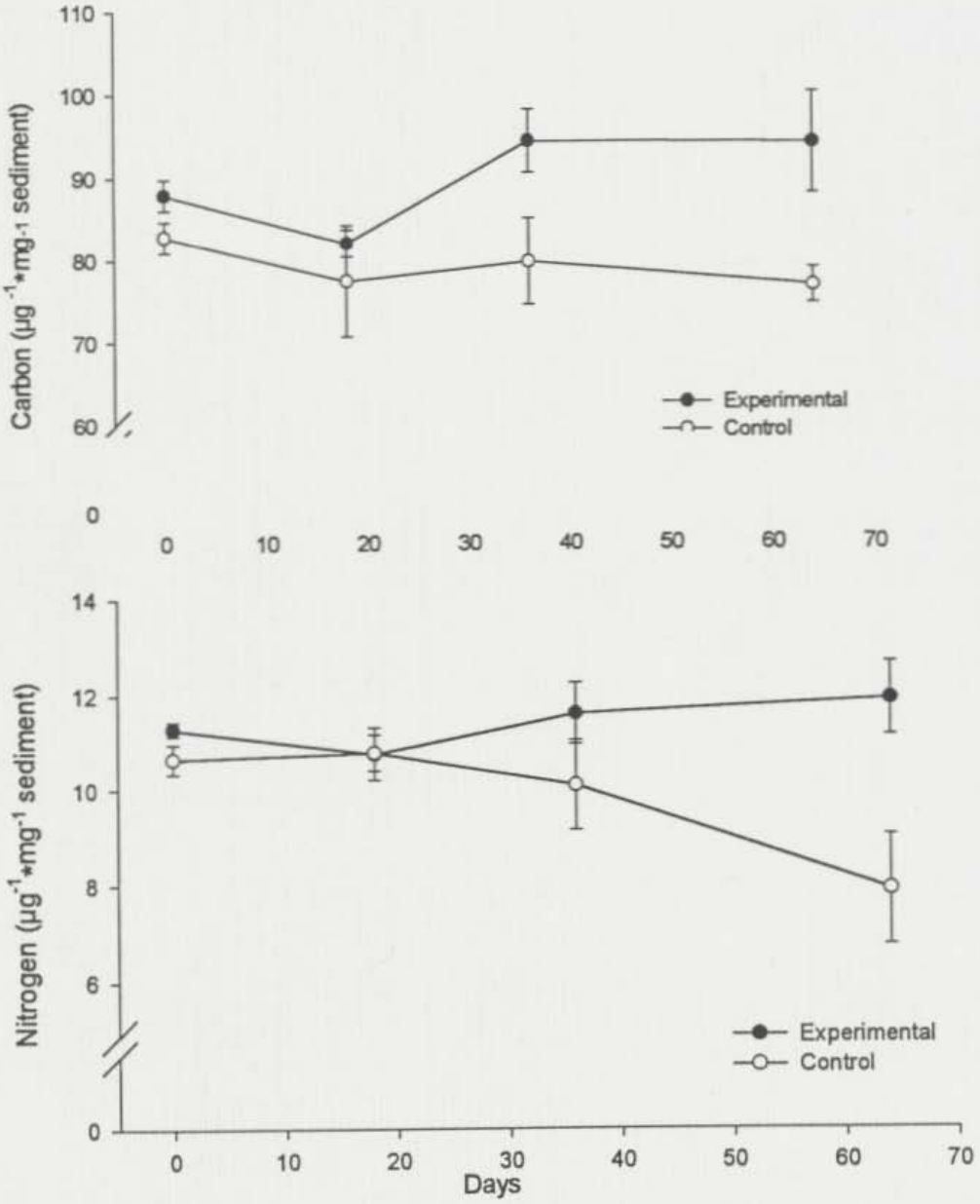

Fig. 25. Temporal fluctuationsin sediment Carbon (A) and Nitrogen (B) concentrations for the experimental aquarium and control aquarium (mean ± SD)

experimental individuals respectively (Fig 26). Control individuals produced oocytes with a narrower range of classes (between 20 and 50μm in diameter) than did experimental individuals oocytes (between 20 μm and 120 μm in diameter). The size class 30 μm size class diameter was the most frequent among control individuals whereas 40 μm was the most frequent class in the experimental group (Fig 26).

Once placed on the sediment surface, *Y. hyperborea* started burrowing into the sediment, and after a few hours the individuals were completely buried with their siphons extended above the sediment surface. After the addition of phytodetritus, the water column became cloudy as a result of the high concentrations of suspended particles (Fig 27a), and experimental individuals began to hold their siphons straight up in the water column (ES activity).

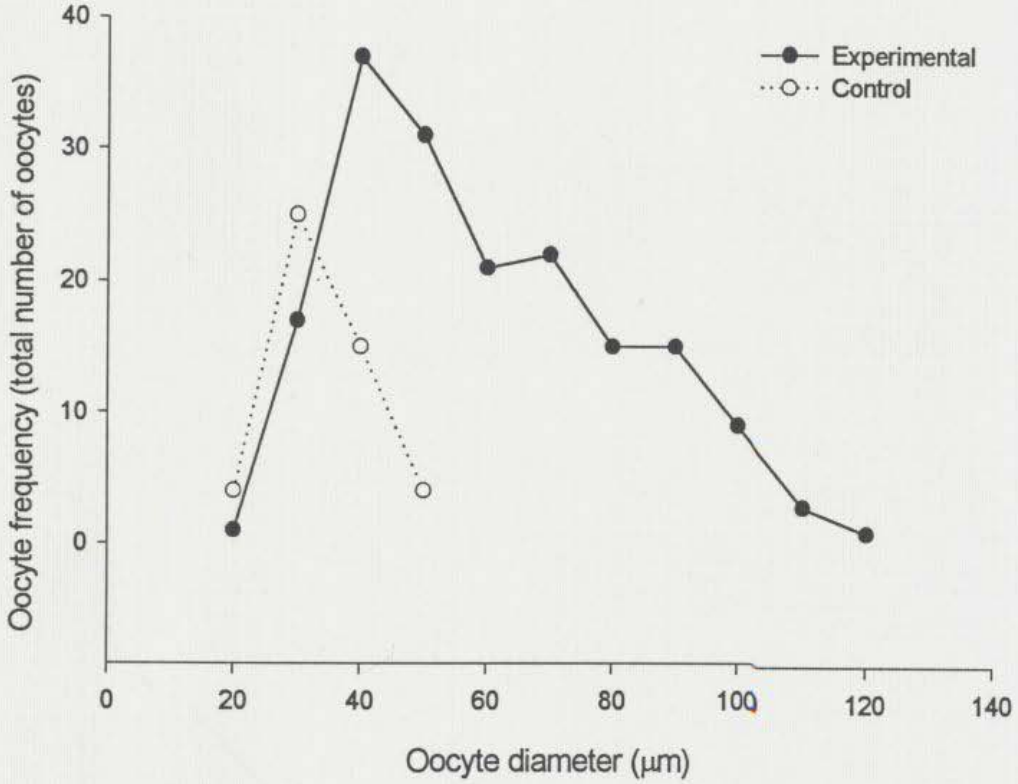

Fig. 26. Distribution of oocyte size frequencies observed in *Yoldia hyperborea* after experiment 2.

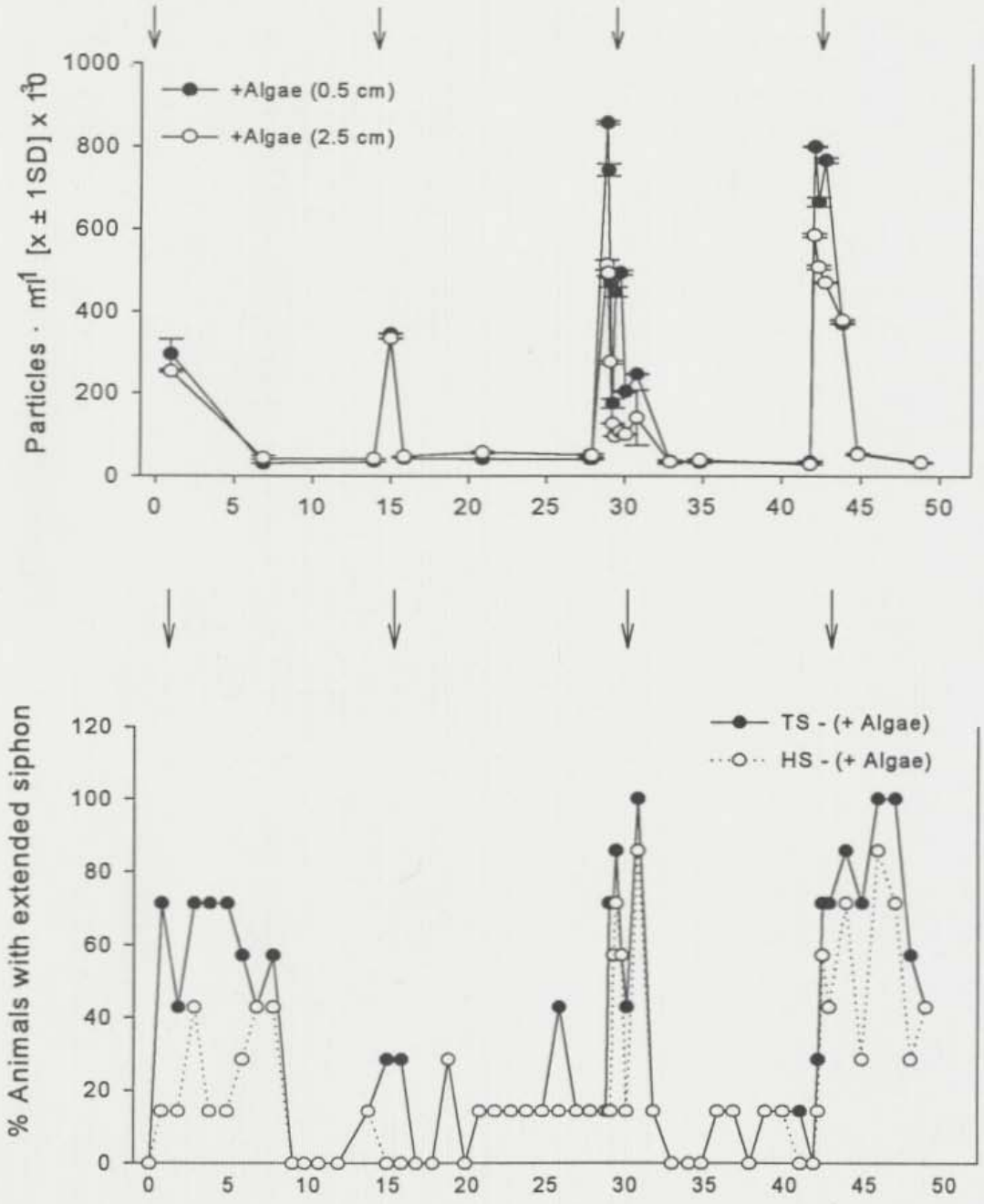

Fig. 27. (A) Temporal variations in particle concentrations at 0.5 cm and 2.5 cm above the sediment surface in the experimental. aquarium. (B) Variations of the percentage of all individuals exhibiting HS ES and activity in the experimental aquarium. Arrows: addition of senescent alga.

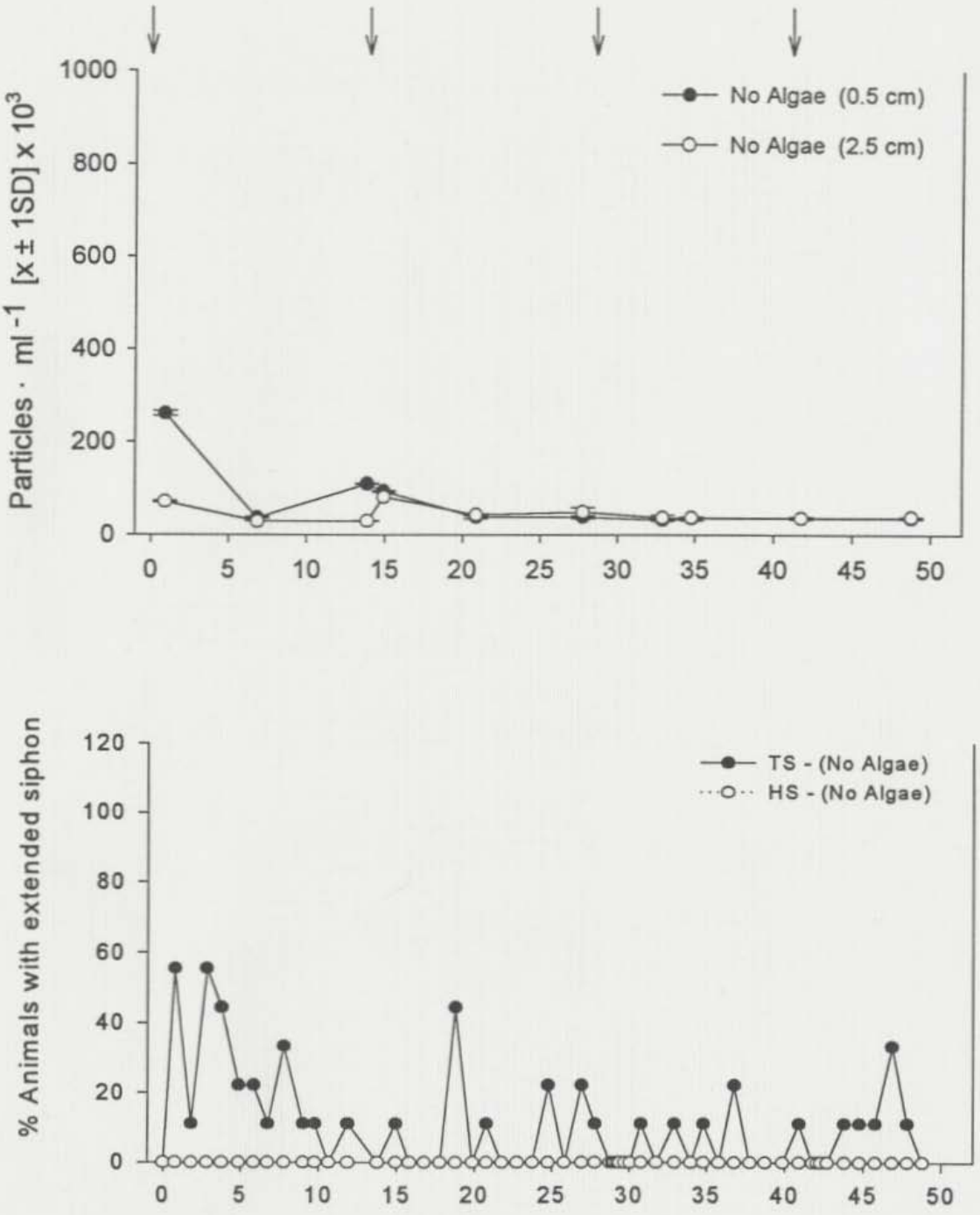

Fig. 28.(A) Temporal variations in particle concentrations at 0.5 cm and 2.5 cm above the sediment surface in the control aquarium. (B) Variations of the percentage of all individuals exhibiting ES and HS activity in the experimental aquarium. Arrows: addition of senescent algae.

5.3.2. Radiotracer experiments

Results from absorption efficiency studies (Stead, Thesis submitted) revealed that *Yoldia hyperborea* fed on radiolabelled *Thalassiosira nordenskioldii* during the incubation period. After 66.5 hours of incubation, a mean of 141 dpm ^{14}C per gonad was detected. This value increased to 279 dpm ^{14}C after 82.5 hours of incubation (Table 5). No evidence of incorporation of ^{68}Ge was detected.

Table 5. Incorporation of ^{14}C into the gonad of *Yoldia hyperborea* individuals. Animals were fed with the radiolabelled laboratory-grown diatom *Thalasiossira nordenskioldii*.

Date	Ambient temperature °C	Duration of experiment (h)	Experimental animals	Density (cells·µl^{-1}) [x, (SD, n)]	^{14}C (totals dpm/gonad) [mean, SD,n]
18.05.99	0	82.5	16	8.69 (1.29, 20)	278.9 (110.1, 5)
15.06.99	0	74.0	19	9.94 (2.48, 20)	147.43 (124.0,13)
07.07.99	0	66.5	19	7.54 (2.43, 24)	141.0 (91.35, 4)

5.4. DISCUSSION

Higher C and N concentrations in the experimental aquaria provided a better diet for *Y. hyperborea*, which may represent an increase in the total amount of energy available for reproduction (Libes 1992). The data reported here demonstrate significant increases in both the number of oocytes produced and the frequency of oocyte size class 60 μm (experiment 1) and size class 40 μm and above (experiment 2) in experimental individuals. In contrast, the control aquarium exhibited decreases in C and N concentrations by the end of the experiment, possibly as a consequence of the feeding activity of animals. In this case, degradation of organic compounds without the addition of phytodetrital particles resulted in a decrease in the amount of POM.

Results in the mean oocyte size and total number of oocytes produced per individual in experiment one can be explained by the fact that both gonad development and timing of reproduction are energy-driven processes (Sastry 1979). Thus the amount of energy available in the aquaria at the beginning of the experiment was sufficient to sustain gametogenesis in both the experimental and the control group. This statement is supported by field observations for *Y. hyperborea* in Conception Bay (present study) which show that phytodetrital deposition promoted increases in the percentage of developing oocytes as well as their maturation. As the experiment proceeded, the energy available in the sediment of the control aquarium decreased, reducing the transfer of nutrients to the gonad, so that gametogenesis ceased and only a few oocytes in a narrow size range were produced; few or no gametes reached maturity in these individuals.

For experiment two, experimental individuals periodically supplied with senescent algae concentrate also produced more and larger oocytes than control individuals, perhaps as a result of a continuous energy mobilization. Since no temperature fluctuations occurred during experiments one and two, it can be concluded that gamete maturation and production depend mainly on food availability. Many field and laboratory studies have considered food availability to be an important factor driving reproduction in mollusc species (Sastry 1966, 1970; Newell

et al. 1982; MacDonald et al. 1987; George et al. 1990; George 1996). Unlike the present work however, the studies could not distinguish unequivocally between the effect of food and temperature. These authors suggest that increases in food availability stimulates production of a large number of larger eggs. These observations on the effect of food supply on gamete production under experimental conditions are consistent with field observations reporting that shallow-water deposit feeding species respond to seasonal fluctuations of organic matter (Ólaffson 1986; López and Levinton 1978). Gamete production is sustained by energy- rich particles obtained from phytodetritus (Smith 1994).

Yoldia hyperborea, like other protobranchs, has two long fused siphons (exhalant and inhalant), which actively participate in the acquisition and rejection of particles. Regardless of the source of the sediment, only a fraction is ingested, and the remainder is expelled as pseudofaeces (Bender and Davis 1984). This expelling activity, which they described for *Yoldia limatula*, allows pseudofeces to be ejected through the exhalant siphon as a cloud of loose sediment and cylindrical faecal pellets (Bender and Davis 1984, Davis 1993).

Field observations on feeding activity of *Macoma balthica* (Ólaffson 1986; López and Levinton 1987; Kamermans 1994) demonstrated that during spring blooms the stomach of this shallow water facultative deposit feeder contains large amounts of algal particles collected mostly from the water column by suspension feeding. Suspension feeding individuals hold their siphons straight up in the water column (ES) or swirling them around (HS) in the water column in a circular fashion (Ólaffson 1986). During this activity, *Macoma* acquires suspended particles from the water column by pumping large amounts of water through the inhalant siphons (Kamermans 1994). The ES activity observed in both control and experimental *Y. hyperborea* appeared to correspond to the suspension feeding activity previously described for *Macoma* individuals; consequently, this ES activity in *Y. hyperborea* may correspond to a suspension feeding mode of nutrition. Suspension feeding behavior has also been reported in other protobranch species such as *Nucula* sp (Caspers 1940; Owen 1956) and *Yoldia ensifera* (Stasek 1965)

Experimental *Y. hyperborea* individuals, compared with control individuals, exhibited increased HS and ES activities throughout the experimental period. Assuming that both HS and ES activities normally occur during suspension feeding, the additions of senescent algae

stimulated these activities as a result of greater amounts of suspended particles in the aquarium water column. Thus the inhalant siphon collected and transported suspended particles to the labial palps, and later the remaining particles were expelled as faeces or pseudofaeces through the exhalant siphon (Bender and Davis 1984). After settlement of the phytodetrital particles added to the aquarium, ES and HS activities were reduced and individuals started deposit feeding by extending the appendages of the labial palps and subsequently transporting sediment to the labial palps (Bender and Davis 1984). Since *Yoldia* also feeds on subsurface deposits, no records of labial palp activity were obtained.

ES activity was observed in both control and experimental individuals, suggesting that this activity is related to the water inhalation process (including inhaling particles suspended in the water column), but it is also related to the ejection of faeces and pseudofaeces by means of the exhalant siphon. This water inhalation process may explain ES activity in control individuals which were not fed but resuspension of sediment particles occurred Thus, in a facultative deposit feeder, suspension feeding is a response to increased amounts of suspended particles present in the water column, whereas deposit feeding is a response to decreases in the concentration of these suspended particles (Ólaffson 1986).

The data suggest that *Yoldia hyperborea* is a deposit feeder species like the tellinid clams *Macoma balthica* and *Scrobicularia plana* (Bradfield and Newell 1961; Hughes 1969; Thompson and Nichols 1988) and as other protobranchs such as e. g. *Nucula* sp. (Caspers 1940; Owen 1956) and *Yoldia ensifera* (Stasek 1965), which also may behave as a facultative suspension feeder. Consequently, *Yoldia hyperborea* may switch from deposit feeding to suspension feeding as a response to increased amounts of suspended organic particles in the water column. This suggestion is supported by differences recorded between fluorescence and transmittance measurements during field observations in Conception Bay. Occasionally when fluorescence was nearly zero at 240 m depth, higher values of transmittance were recorded, indicating that a resuspension of sediment but not chlorophyll was occurring. Such resuspension events may supply *Y. hyperborea* with suspended organic particles to stimulate suspension feeding. Sun et al. (1999) reported that *Y. limatula* uses a selective ingestion strategy, and one way to ingest sediment particles is to sort them on the labial palps, selectively ingesting and retaining certain particles (Lopez and Levinton 1987). However, although *Y.*

hyperborea exhibits suspension feeding, the small size of the gills suggests that this mode of energy acquisition may not be important. The remaining question is whether suspension feeding may significantly influence the reproductive output of this species.

Yoldia hyperborea in the control aquarium exhibited ES activity, suggesting that sediment resuspension occurred. Consequently, they could feed on bacteria associated with POM, as they almost certainly do in the field, and they produce a limited number of gametes. Since individuals under environmental stress (decrease in food ration) produce gametes only when sufficient resources are available beyond the maintenance requirements of the adult (Thompson 1977; Newell et al. 1982; Thompson 1983), conditions in the control aquarium apparently did not represent starvation stress. Consequently, differences in the number of gametes produced between control and experimental individuals can be attributed to the food supplement.

Individuals in the control aquarium developed gametes by obtaining energy from the fresh sediment present in the bottom of the aquarium. Later, resuspension of particles caused by *Yoldia hyperborea* individuals expelling faeces and pseudofaeces provided an additional source of energy for these individuals. However, it is probable that a shortage of energy eventually occurred, inhibiting subsequent gametogenesis. Similar results were observed in field observations conducted in this study. Since *Y. hyperborea* exhibited early and developing oocytes for most of the year the seasonal food pulse can explain the presence of mature gametes and the subsequent minor spawning occurred shortly after phytodetrital deposition

This effect of food supply on gamete production may be explained by the fact that some species develop gametes from energy either absorbed directly from the environment (Ansell 1974; Kautsky 1982) or from both stored and ingested food (Thompson 1977), as appears to occur in *Yoldia hyperborea*. Miller et al. (2000) reported that deposit-feeding species feed selectively on particles recently settled on the sea floor, as suggested by Smith et al. (1993). Parrish et al. (1996) found that *Y. hyperborea* individuals from Conception Bay exhibited high levels of triacylglycerols (TAG), usually utilized for long-term energy storage. These TAG are stored in the digestive gland and may serve as an energy source during conditions of food shortage and during gametogenesis (Parrish et al. 1996). TAG stored in the digestive glands of *Y. hyperborea* in the control aquarium, together with a reduced amount of

energy available in the sediment, may account for the weak reproductive response observed in these individuals. In contrast, experimental individuals provided with a supplementary food source (senescent algae) exhibited a stronger response.

Experimental results in this study showed that *Y. hyperborea* individuals respond to seasonal fluctuations of phytodetritus by increasing gametogenic production, and field results suggest that a similar response occurs in this species. However, direct evidence of the incorporation of phytodetrital particles into the gonad tissue has not yet been reported.

Evidence of nutrient transfer to gonads at the initiation of gametogenesis was obtained by Sastry (1979) in *Argopecten irradians* by injecting [^{14}C] leucine into the digestive gland of scallops with inactive gonads. Radiotracer experiments with *Mytilus edulis* have also demonstrated that the digestive gland controls the distribution of assimilated food (Bayne 1975, 1976; Gabbott 1975). Incorporation of ^{14}C isotope into the gonad and digestive gland of *Y. hyperborea* individuals indicates that algal food was ingested, assimilated and utilized to produce gametes. Similar results were reported for an *in situ* radiolabelled experiment conducted by Cahet and Sibuet (1986) showing that the benthic communities (bacteria and meiofauna) responded in less than three hours when ^{14}C labelled glucose solution and particulate organic substances (carbohydrate mixed with ^{14}C labelled algae) were injected into the sediment-water interface. A large amount of the ^{14}C radioactivity was incorporated into the benthic biota after 24 hours incubation. According to Eckelbarger and Watling (1995), nutrients must be mobilized from the gut to the storage tissue (the digestive gland) before being transferred to the gonad. However, incorporation of nutrients directly from gut to gonad is also possible (Ansell 1974; Kautsky 1982).

The experimental results of the current study demonstrated that some of the phytodetrital particles are ingested and incorporated into the gonadal tissue. Since both field and experimental results report that *Y. hyperborea* individuals responded to fluctuations of phytodetritus by increasing gametogenic production it is suggested that the rain of phytodetritus represents a fresh and direct source of nutrition which is directly assimilated and transformed into gonad tissue.

CHAPTER VI

6.1. General conclusions

Three aspects of the reproductive strategy of *Yoldia hyperborea* and *Ctenodiscus crispatus*, namely the reproductive potential, the periodicity of reproduction and the mode of larval development were studied. The estimated apparent fecundity (reproductive potential) for *Yoldia hyperborea* and *Ctenodiscus crispatus* inhabiting Conception Bay, Newfoundland were estimated as 8.5×10^4 and 11×10^6 eggs per individual respectively. These estimated fecundity values were higher than values reported for other protobranch species and other *Ctenodiscus* populations respectively. While in protobranchs the variation in the number of produced gametes differences can be explained by differences the body size of compared species, variations in the size of sampled individuals account for differences in fecundity between *Ctenodiscus* populations. However, it is known that gamete production is not only dependent on the size of the individual but also on a positive energy balance favoring reproduction. Consequently, larger size (more eggs) is attained in those individuals inhabiting areas with an abundant food supply.

Yoldia hyperborea releases light brown, spherical eggs of 120 μm diameter. As in other protobranchs, there is a pericalymma larvae lacking a feeding organ, limiting these species a to non-planktotrophic mode of development. Pericalymma larvae do not exhibit a prodissoconch II stage, therefore the length of prodissoconch I has been utilized to predict the mode of development in these species. The prodissoconch I of *Yoldia hyperborea* reaches a maximum length of 210 μm and, as in other protobranchs, the larva is lecithotrophic.

Freshly stripped eggs from *Ctenodiscus crispatus* are bright orange and spherical with a mean diameter 450 μm. Estimation of the mode of development for asteroid species is normally based on the egg sizes, and according this method the CB *Ctenodiscus crispatus* population should exhibit a pelagic-lecithotrophic mode of development.

Reproduction in *Yoldia hyperborea* occurred on a seasonal basis with a main spawning period occurring during winter-early spring (January-May 1997 and January-March 1998) shortly before the sinking of phytodetritus. However, other minor spawnings occurred throughout the year (July-October 1997 and July-August 1998).

Ctenodiscus crispatus individuals exhibited a full range of oocyte sizes throughout the year, suggesting that the gametogenic cycle in this population was continuous, aseasonal and asynchronic with several minor spawnings throughout the year.

In general, the reproductive cycle of both deposit feeders appeared to be affected by food availability. Since *Y. hyperborea* and *C. crispatus* exhibited mature gamete most of the year it is possible that a steady food supply is able to sustain continuous gametogenesis. A constant primary food source may be obtained by a increased microbial activity stimulated by POM from turbulence and bioresuspension (reworking) of organic material caused by the deposit feeding community Consequently, throughout the year both turbulence and bioresuspension may provide enough energy to support the continuous maturation of a small number of gametes. This energy requirement may be lower than that required to produce a large number of gametes once a year. The occurrence of minor spawnings shortly after phytodetritus sinking, may be a direct result of the seasonal food pulse which occurs in CB. This seasonal phytodetrital deposition contributes with a fresh and rich food source which can be directly assimilated from suspended particles in the water column.

Experimental evidence revealed that *Y. hyperborea* responded to an increased number of phytodetrital particles suspended in the water column as a suspension feeder (individuals kept their siphons straight up into the water column). After settlement of particles individuals began to feed on deposits. Evidence obtained from a study using radiolabelled (^{14}C) laboratory-grown senescent algae *(Thalassiosira nordenskioldii)* demonstrated that the sinking algal material was incorporated into the gonad tissue of *Y. hyperborea* ,after a few hours. Additional evidence was provided by experimental individuals fed with a sludge of laboratory-grown, senescent algae producing more and bigger eggs (mean size 61 μm) than control individuals (mean size 57 μm).

The results suggest that *Y. hyperborea*, like some other protobranch species studied, may behave as a facultative deposit feeder. This ability allows these species to switch from

deposit feeding to suspension feeding depending on the availability of food. Such behaviour would have an important ecological impact, since during the absence of fresh phytodetritus *Y. hyperborea* may feed on bacteria associated with POM, whereas when the seasonal phytodetritus pulse reaches the bottom the species may compete with bacteria and other benthic biota such as meiofauna and infaunal macrofauna for the fresh organic material.

LITERATURE CITED

Allen, J. A. and H. L. Sanders. 1973. Studies on Deep-sea protobranchs (Bivalvia): The families Siliculidae and Lametilidae. Bull. Mus. Comp. Zool., Harvard. 145: 263-309.

Allen, J. A. 1978. Evolution of the deep sea protobranch bivalves. Phil. Trans. Roy. Soc. London B. 284: 387-401.

Allen, J. A. and H. L. Sanders. 1982. Studies on the deep-sea Protobranchia (Bivalvia). The subfamily Spinulinae (family Nuculanidae). Bull. Mus. Comp. Zool. Harvard. 150: 1-30.

Allen, J. A. and F. J. Hannah 1986. A reclassification of the recent genera of the subclass Protobranchia (Mollusca:Bivalvia). J. Conchol. 32. 225-249.

Aller, J. Y. and R. C. Aller. 1986. Evidence for localized enhancement of biological activity associated with tube and burrow structures in deep-sea sediments at the HEBBLE site, western North Atlantic. Deep Sea Research. 33: 755-790.

Andrew, N. L. 1986. The interaction between diet and density in influencing reproductive output in the echinoid *Evechinus chloroticus* (Val). J. Exp. Mar. Biol. Ecol. 97: 63-79.

Ansell, A. D. 1974. Seasonal changes in biochemical composition of the bivalve *Nucula sulcata* from the Clyde Sea area. Mar. Biol. 25: 101-108.

Arsenault, D. J. and J. H. Himelmann. 1998. Spawning of the Iceland scallop (*Chlamys islandica* Muller, 1776) in the Northern Gulf of St. Lawrence and its relationship to temperature and phytoplankton abundance. The Veliger 41(2): 180-185.

Barber, B. J. and N. J. Blake. 1991. Reproductive Physiology. pp 377-428. In: Scallops: Biology Ecology and Aquaculture. Ed. By S. E. Shumway. Elsevier Science Pub. The Netherlands.

Barber, B. J. S. E. Ford. and R. N. Wargo. 1991. Genetic variation in the timing of gonadal maturation and spawning of the eastern oyster (Gmelin). Biol. Bull. 181: 216-221.

Bayne, B. L. 1975. Reproduction in bivalve molluscs under environmental stress. In:Vernberg, F.J. (Ed) Physiological ecology of estuarine organisms. pp. 259-277. University South Caroline Press, Columbia.

Bayne, B. L. 1976. Aspects of reproduction in bivalve molluscs. In: Wiley, M. (Ed) Estuarine processes, Vol I. pp. 432–448. Academic Press, New York,

Bayne, B. L., D. L. Holland, M. N. Moore, D. M. Lowe and J. Widdows. 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*. J. Mar. Biol. Assoc. U.K. 58: 825-841.

Bayne, B. L., J. Widdows, M. N. Moore, P. Salkeld, C. M. Worral and P. Donkin.1982. Some ecological consequences of the physiological and biochemical effects of petroleum compounds on marine molluscs. Phil. Trans. R. Soc. Lond. B. 297: 219-239.

Bayne, B. L. and Newell, R. C. 1983. Physiological Energetic of Marine Invertebrates In "The Mollusca" Vol. 4. Physiology Part 1. pp 407- 515. A. S. M. Saleuddin and K. L. Wilbur Eds. Acad. Press.

Bayne, B. L. 1985. Responses to environmental stress, tolerance, resistance and adaptation Proceedings 18th European Marine Biology symposium. pp. 331-349. J. S. Gray and M.E. Christiansen Eds., Chichester, Wiley Interscience.

Bender, K and W. R. Davis. 1984. The effect of feeding by *Yoldia limatula* on bioturbation. Ophelia. 23: 91-100.

Beninger, P. G. 1987. A qualitative and quantitative study of the reproductive cycle of the giant scallop, *Placopecten magellanicus*, in the bay of Fundy (New Brunswick, Canada). Can. J. Zool. 65: 495–498.

Bertram, D. F. and R. R. Strathmann. 1998. Effects of maternal and larval nutrition on growth and form of planktotrophic larvae. Ecology. 79(1): 315-327.

Billett, D. S. M., R. S. Lampitt, R. S. Rice and R. F. C. Mantoura. 1983. Seasonal sedimentation of phytoplankton to the deep sea benthos. Nature. 302: 520-522.

Bochdansky , A. B. D. Deibel and R. B. Rivkin, 1999. Absorption efficiencies and biochemical fractionation of assimilated compounds in the cold water appendicularian *Oikopleura vanhoeffeni*. Limnol. Oceanogr. 44(2): 415–424.

Boolootian, R. A. 1966. Reproductive physiology. In: Physiology of Echinodermata, pp 561-613. Ed. By R.A. Boolotian. New York: Wiley - Interscience 1966.

Bosch. I. M. 1989. Contrasting modes of reproduction in two antarctic asteroids in the genus *Porania*, with a description of unusual feeding and nonfeeding larval types. Biol. Bull. 177: 77-82.

Bradfield, A. E. and G. E. Newell. 1961. The behaviour of *Macoma balthica* (L). J. Mar. Biol. Assoc. U.K. 41: 81-87.

Bricelj, V. M. Epp, J. and R. E. Malouf. 1987. Intraspecific variation in reproductive cycles of the bay scallop *Argopecten irradians*. Mar. Ecol. Prog. Ser. 36: 123-137.

Bricelj, V. M. A. E. Bass and G. R. Lopez. 1984. Absorption and gut passage time of microalgae in a suspension feeder: An evaluation of the ^{51}Cr: ^{14}C twin tracer technique. Mar. Ecol. Prog. Ser. 17: 57-63.

Brousseau, D. J. 1978. Population dynamics of the Soft-shell clam *Mya arenaria*. Mar. Biol. 50: 63-71.

Brousseau, D. J. 1981. Spawning cycle and fecundity in a population of *Petricola pholadiformes*. (Pelecypoda:Petricolidae) from Milford, Connecticut. The Veliger 24: 56-61.

Brousseau, D. J. 1987. A comparative study of the reproductive cycle of the soft-shell clam *Mya arenaria* in long island sound. J. Shellfish Res. 6(1): 7 -15.

Burke M. V. and K. H .Mann. 1974. Productivity and production: biomass ratios of bivalve and gastropod populations in an eastern Canadian estuary. J. Fish. Res. Bd. Can. 31: 167-177.

Byrne, M. 1991. Reproduction, development and population biology of the Caribbean ophiuroid *Ophionereis olivacea*, a protandric hermaphrodite that broods its young. Mar. Biol. 111: 387-399.

Cahet, G. and M. Sibuet. 1986. Activité biologique en domaine profond: transformations biochimiques *in situ* de composés organiques marqués au carbono-14 a l'interface eau-sediment par 2000 m de profondeur dans le Golfe de Gascogne. Mar. Biol. 90: 307-315.

Calow P.and C. R. Fletcher 1972. A new radiotracer technique involving ^{14}C and ^{51}Cr, for estimating the assimilation efficiencies of aquatic, primary consumers. Oecologia. 9: 155-170.

Calow, P. and R. M. Sibly. 1990. A physiological basis of population processes: ecotoxicological implications. Functional Ecology. 4: 283-289.

Caspers, H., 1940. Über Nahrungserwerb und Darmverlauf bei *Nuculla*. Zool. Anz. 129: 48-55.

Chia, F. S. and Walker, C. W. 1991. Echinodermata: Asteroidea. In Reproduction of Marine Invertebrates. Vol VI. pp.301 - 353. Giese, A.C. , Pearse, J. S. and V. B. Pearse. (Ed) Acad. Press. N.Y.

Clarke, A. 1988. Seasonality in the antarctic marine environment. Comp. Biochem. Physiol. 90B(3): 461-473.

Clarke, A. 1992. Reproduction in the cold: Thorson revisited. Invert. Reprod. Dev. 22 (1-2): 175-184.

Crump, R. G. 1971. Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill) a common New Zealand asteroid. J. Exp. Mar. Biol. Ecol. 72:137-162.

Cowan, I. McT I. 1968. The interrelationship of certain boreal and arctic species of *Yoldia* Möller 1842. The Veliger. 11(1): 51-58.

Currie, D. R. 1990 Reproductive periodicity of three species of chitons at a site in New South Wales, Australia (Mollusca:Polyplacophora). Invert. Reprod. Dev. 17(1): 25-32.

Davenport, J. 1988. The feeding mechanism of *Yoldia (= Aequiyoldia). eightsi* (Couthouy). Proc. R. Soc. (Ser B)282: 431-442

Davis, W. R. 1993. The role of bioturbation in sediment resuspension and its interaction with physical shearing. J. Exp. Mar. Biol. Ecol. 171: 187-200.

Davis, K. K. 1985. DNA synthesis and the annual spermatogenetic cycle in individuals of the sea star *Patiria miniata*. Biol. Bull. 169: 313-327.

Dehn, P.F. 1980. The annual reproductive cycle of two populations of *Luidia clathrata* (Asteroidea) I. Organ indices and occurrence of larvae. In: Echinoderms: present and past. pp 361-367. Ed. by M. Jangoux. Rotterdam: A.A. Balkema.

Deibel, D., R. J. Thompson, C.H. Mckenzie and A.M. Redden. 1992. Initiation and limitation of a north-temperate spring diatom bloom at sub-zero water temperatures. American Society of Limnology and Oceanography, Santa Fe.

De Ridder, C. and J. M. Lawrence. 1982. Food and feeding mechanisms: Echinoidea. pp.57-115. In Echinoderm nutrition. Jangoux M. and Lawrence, J. M. Eds. Rotterdam, Netherlands: A.A. Balkema

Deuser, W.G. and E. H. Ross. 1980. Seasonal change in the flux of organic carbon to the deep sea. Nature 283: 364-365.

DeYoung, B. and B. Sanderson. 1995. The circulation and Oceanography of Conception Bay, Newfoundland. Atmospheric Ocean. 33(1): 1335-1362.

Dibacco, C., G. Robert, and J. Grant. 1995. Reproductive cycle of the sea scallop, *Placopecten magellanicus* (Gmelin, 1791), on northeastern Georges Bank. J. Shellfish. Res. 14(1): 59-69.

Die, D. J. , J. G. Mc Gilbray, A. J. Courtney and M. G. Cosgrove. 1995. A quantitative method for staging penaeid prawn ovaries using image analysis. Invert. Reprod. Dev. 28(3): 177-183.

Dohmen, M. R. 1983. Gametogenesis.. In: N.H. Verdonk, J. A. M. van den Biggelaar and S. Tompa. (Eds) The Mollusca. Vol. 3. pp. 1-48. Development. Academic Press, New York.

Drew, G. A. 1899. *Yoldia limatula*. Memoirs from the Biological laboratory of the Johns Hopkins University. IV. 3: 1-37.

Eckelbarger, K. L. and L. Watling. 1995. Role of the phylogenetic constrains in determining reproductive patterns in deep-sea invertebrates. Invert. Biol. 114(3): 156-269.

Eilertsen, H. Chr., S. Falk-Petersen, C. C. Hopkins and K. Tande. 1981. Ecological investigations on the plankton community of Balfsjorden northern Norway. Program for the project, study area, topography and physical environment. Sarsia. 66: 25-34.

Emlet, R. B., L. R. McEdward and R. R. Strathmann. 1987. Echinoderm larval ecology viewed from the egg. In: Echinoderm Studies Vol 2. pp. 55-136. M. Jangoux and J. M. Lawrence (Eds). A. A. Balkema Publishers. Netherlands.

Emmet, B., K. Thompson and J. D. Popham. 1987. The reproductive cycle and energy storage cycles of two populations of *Mytilus edulis* (Linne) from British Columbia. J. Shellfish Res. 6(1). 29-36.

Epp, J., V. M. Bricelj, and R. E. Malouf. 1988. Seasonal partitioning and utilization of energy reserves in two age classes of the bay scallop *Argopecten irradians irradians* (Lamarck). J. Exp. Biol. Ecol. 121: 113-136.

Falk-Petersen, I. B. 1982a. Reproductive and biochemical studies of the asteroid *Ctenodiscus crispatus*. Sarcia. 67(2): 123-130.

Falk-Petersen, I. B. 1982b. Breeding season and egg morphology of echinoderms in Balsfjorden, Northern Norway. Sarsia. 67(4): 215-221

Falk-Petersen, I. B. and J. R. Sargent 1982. Reproduction of asteroids from Balfsjorden,Northern Norway: Analyses of lipids in the gonads of *Ctenodiscus crispatus*, *Asterias lincki* and *Pisaster militaris*. Mar. Biol. 69: 291-298.

Fisher, R. A. 1930. The genetical theory of natural selection. Oxford University Press, London

Gabbott, P. A. 1975. Storage cycles in marine invertebrates molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In. Ninth European Marine Biology, Symp. Pp. 191-211.(H. Barnes, Ed.) Aberdeen University Press. Aberdeen.

Gage, J. D. and P.A. Tyler. 1981a. Non-viable seasonal settlement of larvae of the upper bathyal brittlestar *Ophiocten gracilis* in the Rockall Trough abyssal. Mar. Biol. 64: 153-161.

Gage, J. D. and P. A. Tyler.1981b. Reappraisal of age composition, growth, and survivorship of the deep-sea brittle star *Ophiura ljungmani* from size structure in a time series from the Rockall Trough. Mar. Biol. 64: 163-174.

Gage, J. D. and P. A. Tyler.1985. Growth and recruitment of the deep-sea urchin Echinus affinis Mar. Biol. 90 (1): 41-53.

Gage, J. D. and P. A Tyler. 1991. Deep sea Biology: a Natural History of Organisms at the Deep Sea Floor. 504 pp. Cambridge: Cambridge University Press.

Gage, J. D. and P. A. Tyler, G. Davies and R. Harvey. 1986. Life history studies on deep sea protobranch bivalves from the Rockall Trough (NE Atlantic). In: D. Heppell (Ed) Ninth International Malacological Congress, Edinburgh, Scotland, 31 August - 6 September 1986. National Museums of Scotland, Edinburgh. pp. 27 (abstract).

Gardner, W.D., J. B. Southard and C.D. Hollister.1985. Sedimentation, resuspension and chemistry of particles in the Northwest Atlantic. Mar. Geol. 65: 199-242.

George R. Y .and R. J. Menzies. 1967. Indication of cyclic reproductive activity in abyssal organisms. Nature 215: 878.

George R. Y. and R. J. Menzies. 1968. Further evidence for seasonal breeding cycles in deep sea. Nature 220: 80-81.

George, R. S., C. Cellario, and L. Fenaux. 1990. Population differences in egg quality of *Arbacia lixula* (Echinodermata:Echinoidea): Proximate composition of eggs and larval development. J. Exp. Mar. Biol. Ecol. 141: 107-118.

George, R. S. 1994. Population differences in maternal size and offspring quality for *Leptasterias epichlora* (Brandt) (Echinodermata:Asteroidea). J. Exp. Mar. Biol. Ecol. 175: 121-131.

George, R. S. 1996. Echinoderm egg and larval quality as a function of adult nutritional state. Oceanologica Acta. 19 (3-4): 297-308.

Giese, A. C. 1959. Comparative physiology: Annual reproductive cycles of marine invertebrates. Ann. Rev. Physiol. 21: 547-576.

Giese. A. C. 1965. Reproductive periodicities in several constrains populations of *Odontaster validus* Koehler, a common antarctic asteroid. Antarctic Research Series 5: 39-85.

Giese. A. C. and J. S. Pearse. 1974. Introduction: General principles. In Reproduction of Marine Invertebrates. Vol.1, pp 1- 49. A.C. Giese and J.S. Pearse, Eds. Acad. Press, New York.

Giese. A. C. and H. Kanatani. 1987. Maturation and spawning. In: Reproduction of Marine Invertebrates, vol. 8: General Aspects: Seeking Unity from Diversity. pp. 251-329. A. C. Giese, J. S. Pearse, and V. B. Pearse, Eds. Palo Alto and Pacific Grove, Calif. Blackwell/Boxwood.

Gooday, A. J. and C. M. Turley. 1990. Responses by benthic organisms to inputs of organic material to the ocean floor: a review. Phil. Trans. R. Soc. Lond. A. 331: 119-138.

Graf. G., and R. Rosenberg. 1997 Bioresuspension and biodeposition: a review. J. Mar. Systems. 11: 269-278.

Grant, A. and P. A. Tyler. 1983a. The analysis of data in studies of invertebrate reproduction. I. Introduction and statistical analysis of gonad indices and maturity indices. Int. J. Invert. Reprod. 6: 259-269.

Grant, A. and P. A Tyler. 1983b. The analysis of data in studies of invertebrate reproduction. II. The analysis of oocyte size/frequency data, and comparison of different types of data. Int. J. Invert. Reprod. 6: 271-283.

Griffiths, C. L. and J. A. King. 1979. Energy expended on growth and gonadal output in the ribbed mussel *Aulacomya ater*. Mar. Biol. 53: 217-222.

Grossert, J. S., P. Mathiaparanam, G. D. Hebb, P. Price, I. M. Campbell. 1973. The sterols of the echinoderm *Ctenodiscus crispatus* (Retzius). Experientia 29: 258-259.

Guillou, M. and L. J. L. Lumingas. 1999. Variation in the reproductive strategy of the sea urchin *Sphaerechinus granularis* (Echinodermata:Echinoidea) related to food availability. J. Mar. Biol. Assoc. U.K. 79:131-136.

Gustafson, R. G. and R. A. Lutz. 1992. Larval and early post-larval development of the protobranch bivalve *Solemnya velum* (Mollusca:Bivalvia). J. Mar. Biol. Assoc. U. K. 72: 383-402.

Gustafson, R. G. and R. G. B. Reid. 1986. Development of the pericalymma larva of *Solemnya reidi* (Bivalvia: Cryptodonta: Solemnyidea) as revealed by light and electron microscopy. Mar. Biol. 93: 411-427.

Hagström , B. E. and S. Lonning 1961. Experimental studies of *Strongylocentrotus droebachiensis* and *S. pallidus* . Sarcia 29:165-176.

Hamel, J. F. and A. Mercier. 1996. Evidence of chemical communication during the gametogenesisi of holothurids. Ecology. 77(5): 1600-1616.

Harrison, K. 1988. Seasonal reproduction in deep sea crustacea (Isopoda:Asellota). J. Nat. Hist. 22: 175-197.

Hecker, B. 1990. Photographic evidence for rapid flux of particles to the sea floor and their transport down the continental slope. Deep Sea Research. 37: 1773-1782.

Heffernan, P. B. and R.L. Walker. 1989. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: III. *Geukensia dimissa* (Dillwyn, 1817). J. Shellfish Res. 8 (2): 327-334.

Heffernan, P. B., R. L. Walker and J. L. Carr. 1989a. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: I. *Mercenaria mercenaria* (Linneus, 1758). J. Shellfish Res. 8 (1): 51-60.

Heffernan, P. B., R. L. Walker and J. L. Carr. 1989b. Gametogenic cycles of three bivalves in Wassaw Sound, Georgia: II. *Crassostrea virginica* (Gmelin,1791). J. Shellfish Res.8 (1): 61-70.

Hinga, K. R. , J. Mc N. Sieburth, and G. Ross Heart. 1979. The supply and use of organic material at the deep sea floor. J. Mar. Res. 37: 557-557.

Himmelman, J. H. 1980. Reproductive cycle patterns in the chiton genus mopalia (polyplacophora). The Nautilus. 94(2): 39-49.

Hooker, S. H. and R. Creese. 1995. The reproductive biology of pipi, *Paphies australis* (Gmelin, 1790) (Bivalvia:Mesodesmatidae). I. Temporal patterns of the reproductive cycle. J. Shellfish Res. 14(1): 7- 15.

Honkoop, P. J. C. and J. Van der Meer. 1997. Reproductive output of *Macoma balthica* populations in relation to winter-temperature and intertidal-height mediated changes of body mass. Mar. Ecol. Prog. Ser. 149: 155-162.

Honkoop, P. J. C. and J. Van der Meer. 1998. Experimentally induced effects of water temperature and immersion time on reproductive output of bivalves in the Waden sea. J. Exp. Mar. Biol. Ecol. 220: 227-246.

Honkoop, P. J. C., J. van der Meer, J. J. Beukema, and D. Kwast. 1998. Does temperature-influenced egg production predict the recruitment in the bivalve *Macoma balthica*?. Mar. Ecol. Prog. Ser. 164: 229-235.

Hughes R. N.1969. A study of feeding in *Scrobicularia plana*. Jour. Mar. Biol. Ass. U.K. 49: 805-823.

Hughes, R. N. and D. J. Roberts. 1980. Growth and reproductive rates of *Littorina neritoides* (L) in North Wales. J. Mar. Biol. Assoc. U.K. 60: 591-595.

Humason, G. L. 1979. Animal Tissue Techniques. 4th ed. W. H. Freeman, San Francisco.

Hutchings J. A. and R. L. Haedrich. 1984. Growth and populations structure of two species of bivalves (Nuculanidae) from deep sea. Mar. Ecol. Prog. Ser. 17: 135-142.

Jablonski, D. and R.A. Lutz. 1980. Molluscan larval shell morphology: Ecological and paleobiological applications. pp. 323-377. In: Skeletal growth of aquatic organisms. D.C. Rhoads and R.A. Lutz. Eds. New York. Plenum Press.

Jablonski, D. and R.A. Lutz 1983. Larval ecology of marine invertebrates: paleobiological implications. Biol. Rev. 58: 21-89.

Jangoux, M. and E. Van Impe. 1977. The annual pyloric cycle of *Asterias rubens* L. (Echinodermata:Asteroidea). J. Exp. Mar. Biol. Ecol. 30: 165-184.

Jangoux, M. 1982. Food and feeding mechanisms: Asteroidea: pp 117-159. In: Echinoderm nutrition. Jangoux M. and Lawrence,J.M. Eds. Rotterdam, Netherlands: A. A. Balkema.

Jumars, P.A., L. M. Mayer, J. W. Deming, J. A. Baross, and R. A. Wheatcroft. 1990. Deep-sea deposit - feeding strategies suggested by environmental and feeding constrains. Phil. Trans. R. Soc. Lond. A. 331: 85-101.

Kamermans, P. 1994. Similarity in food source and timing of feeding in deposit-and suspension-feeding bivalves. Mar. Ecol. Prog. Ser. 104: 63-75

Kautsky, N. 1982. Quantitative studies on the gonadal cycle, fecundity, reproductive output and recruitment in a Baltic *Mytilus edulis* population. Mar. Biol. 68: 117-133.

Keats, D. W., D. H. Steele, and G. R. South. 1984. Depth dependent reproductive output of the green sea urchin *Strongylocentrotus droebachiensis* (O. F. Müller) in relation to the nature and availability of food. J. Exp. Mar. Biol. Ecol. 80: 77-91.

Knudsen, J. 1979 Deep Sea Bivalves. pp. 195-224. In S. V.P. Spoel, A.C. V. Bruggen, and J. Lever, Eds., Pathways in Malacology, Ultrecht : Scheltema, Holkema

Kristensen, E., F. O .Anderson, and T. H. Blackburn. 1992. Effects of benthic macrofauna and temperature on degradation of macroalgal detritus: The fate of organic carbon. Limn. and Oceanogr. 39: 1404-1419.

Lammens, J. J. 1967. Growth and reproduction in a tidal flat population of *Macoma balthica* (L). Neth. J. Sea Res. 3: 315-382.

Lampitt, R. S. 1985. Evidence for the seasonal deposition of detritus to the deep sea floor and its subsequent resuspension. Deep Sea Research. 32: 885-897.

Lampitt, R. S., T. Moji, and B. Von Bodungen. 1990. What happens to zooplankton fecal pellets? Implications for material flux. Mar. Biol. 104: 15-23.

Lawrence, J. M. 1975. On the relationship between marine plants and sea urchins. Oceanogr. Mar. Biol. Ann. Rev. 13: 213-286.

Lebour, M., 1938. Notes on the breeding of some lamellibranchs from Plymouth and their larvae. J. Mar. Biol. Assoc. U.K. 23: 119-144.

Lessios, H.A. 1984. Annual reproductive periodicity in eight echinoid species on the Caribbean coast of Panama. Proc. of the Fifth Internat. Echinoderm Conference, Galway. 1984 pp. 303-311.

Levin, L. A. and T. S. Bridges. 1995. Pattern and diversity in reproduction and development In: Ecology of Invertebrate Larvae. pp. 1-47. L. Mac Edward, Ed., CRC Press. New York.

Lewis, J. B., S .Saleh, H. M. Reiswig and C. M. Lalli. 1982. Growth, production and biomass of the burrowing protobranch mollusc *Yoldia limatula* in the Bideford river, Prince Edward Island, Canada. Mar. Biol. 70: 173-179.

Libes, S. M. 1992. An Introduction to Marine Biogeochemistry. Chapter 23. pp 394-422. John Wiley and Sons, Inc. New York

Lightfoot, D. H., P. A. Tyler and J.D. Gage. 1979. Seasonal reproduction in deep-sea bivalves and brittlestars. Deep Sea Research, 26A: 967-973.

Loosanoff, V. L. 1937. Seasonal gonadal changes in adult clams, *Venus mercenaria* (L). Biol. Bull. 72: 406-416.

Loosanoff, V. L. 1952. Behavior of oysters in eater of low salinities. Proc. Natl. Shellfish. Assoc. 43: 135-151

López G. R. and J. S. Levinton 1987. Ecology of deposit feeding animals in marine sediments. The Quarterly Review of Biology. 62(3): 235-259.

Lowe, G. F 1978. Relationship between biochemical and caloric composition and reproductive cycle in *Asterias vulgaris* (Echinodermata-Asteroidea) from the Gulf of Maine, Orono. pp 95.

Lubet, P. 1986. The reproductive strategies of marine bivalve molluscs. In: Advances in Invertebrate Reproduction 4. pp 401-408. M. Porchet, J. C. Andries and A. Dhainaut Eds. Elsevier Science Publishers

Machell, J. R. and D. DeMartini. 1971. Annual reproductive cycle of gaper clam *Trexus capax* in South Humboldt Bay, California. Calif. Fish. Gam. 57(4): 274-282

McCave, I. N. 1975. Vertical flux of particles in the ocean. Deep Sea Research. 22: 491-502.

McEdward, L. R. and D.A. Janies. 1993. Live cycle evolution in asteroids: what is a larva. Biol. Bull. 184: 255-268.

MacDonald B. A. and R. J. Thompson. 1985. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus* II. Reproductive output and total production. Mar. Ecol. Prog. Ser.25: 295-303.

MacDonald B. A., R. J. Thompson. 1986. Production dynamics and energy partitioning in two populations of the giant scallop *Placopecten magellanicus* (Gmelin). J. Exp. Mar. Biol. Ecol. 101: 285-299.

MacDonald B. A., N. F. Bourne. 1987. Growth rate and reproductive output, and energy partitioning in wheatervane scallops, *Patinopeten caurinus*, from British Columbia . Can. J. Fish. Aquat. Sci.44:152-160.

MacDonald, B A., R. J. Thompson and B.L. Bayne. 1987. Influence of temperature and food availability on the ecological energetics of the giant scallop *Placopecten magellanicus*. IV. Reproductive effort , value and cost. Oecologia. 72: 550-556.

MacDonald, B. A. and R. J. Thompson. 1988. Intraspecific variation in growth and reproduction in latitudinally differentiated populations of the giant scallop *Placopecten magellanicus* (Gmelin). Biol. Bull. 175: 361-371.

MacDonald B. A. and R. J. Thompson. 1988. Intraspecific variation in growth and reproduction in latitudinally differentiated populations of the giant scallop *Placopecten magellanicus*. Biol.Bull. 175: 361-371.

McGinley, M.A. 1989. The influence of a positive correlation between clutch size and offspring fitness on the optimal offspring size. Evol. Ecol. 3: 150-156.

Malachowski, M. 1988 The reproductive cycle of the rock scallop *Hinnites giganteus.*(Grey) in Humboldt Bay, California. J. Shellfish. Res. 7(3): 341- 348.

Mathieu, M. and Lubet, P. 1993. Storage tissue metabolism and reproduction in marine bivalves- a brief review. Invert. Reprod. Dev. 23(2): 123-129.

Menge, B. A. 1975. Brood or Broadcast?. The adaptative significance of different reproductive strategies in two intertidal seastars *Leptasterias exactis* and *Pisaster Ochraceus.* Mar. Biol. 31: 87-100.

Mileikowsky, S. A.1971. Types of larval development in marine bottom invertebrates their distribution and ecological significance: A reevaluation. Mar. Biol. 10: 193-213.

Miller, R. J., C.R. Smith, D.J. DeMaster and W.L. Fornes. 2000. Feeding selectivity and rapid particle processing by deep-sea megafaunal deposit feeders: A ^{234}Th approach. Jour. Mar. Res.58:653-673.

Morvan, C. and A. D. Ansell. 1988. Stereological methods applied to reproductive cycle of *Tapes rhomboides*. Mar. Biol. 97: 355-364.

Nakaoka, M. 1996. Size dependent survivorship of the bivalve *Yoldia notabilis* (Yokohama 1920): The effect of crab predation. J. Shellfish. Res. 15(2): 355-362.

Nakaoka, M. 1998. Optimal resource allocation of the marine bivalve *Yoldia notabilis*- the effects of size-limited reproductive capacity and size dependent mortality. Evol. 12 (3): 347-361.

Nauen, C.E. and L. Böhm.1979. Skeletal growth in the echinoderm *Asterias rubens* L. (Asteroidea: Echinodermata) estimated by ^{45}Ca labeling. J. Exp. Mar. Biol. Ecol. 38: 261-279.

Newell, R. I. E., T. J., Hilbish, R. K., Koehn, and C. J. Newell. 1982. Temporal variations in the reproductive cycle of *Mytilus edulis* (Bivalvia:Mytilidae) from localities on the east coast of North America. Biol. Bull. 162: 299-310.

Newell, R. C. 1965. The role of detritus in the nutrition of the marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macoma balthica*: Proc. Zool. Soc. of London.144: 25-45.

Nolan, C. P. and A. Clarke. 1993. Growth in the bivalve *Yoldia eightsi* at Signy Island, Antarctica, determined from internal shell increments and calcium-45 incorporation. Mar. Biol. 117: 243-250.

Olive, P. J. 1992. The adaptative significance of seasonal reproduction in marine invertebrates, the importance of distinguishing between models. Inv. Reprod. Dev. 22(1-3): 165-174

Ockelmann, K. W. 1965. Developmental types in marine bivalves and their distribution along the Atlantic coast of Europe. pp. 25-35. In: L. R. Cox and J. F. Peake, Eds., Proc. 1st Europ. Malac. Congr. London 1962.

Ólaffson, E. B. 1986. Density dependence in suspension-feeding and deposit-feeding populations of the bivalve *Macoma balthica*: a field experiment. J. Anim. Ecol. 55: 517-526.

Orton, J. H. 1920. Sea temperature, breeding and distribution in marine animals. J. Mar. Biol. Assoc. UK. 12: 339 - 366.

Owen, G. 1956. Observations on the stomach and digestive diverticula of the Lamellibranchia.II. The Nuculidae. Quart. J. Micr. Sci. 97: 541-567.

Parker, G. A. and M. Begon. 1986. Optimal egg size and clutch size: Effects of environment and maternal phenotype. Amer. Nat. 128: 573-592.

Parrish, C. C., Z. Yang, A. Lau and R. J. Thompson. 1996. Lipid composition of *Yoldia hyperborea* (Protobranchia), *Nephtys ciliata* (Nephthyidae) and *Artacama proboscidea* (Terebellidae) living at sub-zero temperatures. Comp. Biochem. Physiol. 114B (1): 59-67.

Parsons, G. J., S. M.C. Robinson, R.A. Chandler, L.A. Davison, M. Lanteigne and M. J. Dadswell 1992. Intra-annual and long term patterns in the reproductive cycle of giant scallop *Placopecten magellanicus* (Bivalvia:Pectinidae) from Passamaquody Bay, New Brunswick, Canada. Mar. Ecol. Prog. Ser. 80: 203-214.

Paulet, I. M. and J. Boucher. 1991. Is reproduction mainly regulated by temperature or photoperiod in *Pecten maximus* Invert. Reprod. Dev. 19(1): 61-70.

Pazos, A. J., G. Roman, C.P. Acosta, M. Abad and J. L. Sanchez. 1996. Stereological studies on the gametogenic cycle of the scallop *Pecten maximus* in suspended culture In Ria de Arousa (Galicia NW Spain). Aquaculture 142: 119-135.

Pearse, J. S., 1965 Reproductive periodicities in several constrasting populations of *Odontaster validus* Koehler, a commomn antarctic asteroid. Antarctic Res. Ser.5: 39-85

Pearse, J. S., D. J. Fernisse, V. B. Pearse, and K. A. Beauchamp. 1986. Photoperiodic regulation of gametogenesis in sea stars with evidence for an annual calendar independent of fixed daylength. Amer. Zool. 26: 417–431.

Peck, L.S., J.G. Colman, and A.W.A. Murray. 2000. Growth and tissue mass cycles in the infaunal bivalve *Yoldia eightsi* at Signy Island, Antarctica. Polar Biology 23: 420–428.

Phillips, N. W. 1984. Role of different microbes and substrates as potential suppliers of specific, essential, nutrients to marine detritivores. Bull. Mar. Sci. 3513: 283-298.

Pomeroy, L. R. and D. Deibel. 1986. Temperature regulation of bacterial activity during the spring bloom in Newfoundland coastal waters. Science. 233:359-361.

Qian, P. Y. and F. S. Chia. 1991. Fecundity and egg size are mediated by food quality in the polychaete worm *Capitella* sp. J. Exp. Mar. Biol. Ecol. 148: 11-25.

Qian, P. Y. and F. S. Chia.1992. Effects of diet type on the demographics of *Capitella* sp. (Annelida:Polychaeta): lecithotrophic development vs planktotrophic development. J. Exp. Mar. Biol. Ecol. 157: 159-179.

Redden, A. M., R. J. Thompson, and D. Deibel. 1994. Vertical flux of spring bloom production at subzero temperatures in a Newfoundland fjord. American Society of Limnology and Oceanography, San Diego.

Redden, A. M. 1994. Grazer mediated chloropigment degradation and the vertical flux of spring bloom production in Conception Bay, Newfoundland. Ph. D. Thesis. Biology Department. Memorial University of Newfoundland.

Rice, A. L., D. S., Billet, J. Fry, A. W. G. John, R. S. Lampit, R. F.C. Mantoura and R. J. Morris. 1986. Seasonal deposition of phytodetritus to the deep sea floor. Proceedings of the Royal Society of Edinburgh 88B: 265-279.

Rice, A. L., D. S. Billett, M. H. Thurston and R. S. Lampit. 1991. The Institute of Oceanographic Science Biology Program in the Porcupine Seabight: Background and General Introduction. J. Mar. Biol. Assoc. U. K. 71: 281-310.

Rodhouse, P. G., C. M. Roden, M. P. Hensey, T. McMahon, B. Ottway and T. H. Ryan.1994. Food resource, gametogenesis and growth of *Mytilus chilensis* on the shore and in suspended culture: Killary Harbour, Ireland. J. Mar. Biol. Asocc. U. K. 64: 513-529.

Rokop, F. J. 1974. Reproductive patterns in deep sea benthos. Science 186: 743-745.

Rokop, F. J. 1977. Seasonal reproduction of the brachiopod *Frieleia halli* and the scaphopod *Cadulus californicus* at bathyal depths in the Deep Sea. Mar. Biol. 43: 237-246.

Rokop, F. J. 1979. Year round reproduction in the deep sea bivalve molluscs. In: Reproductive ecology of marine invertebrates, pp. 189-198. S. Stancyk, Ed. University of North Carolina Press. Columbia.

Ropes, J. W. and A. P. Stickney. 1965. Reproductive cycle of *Mya arenaria* in New England. Biol Bull. 128: 315-327.

Ropes, J. W. 1968. Reproductive cycle of the surf clam *Spisula solidissima*, in offshore New Jersey. Biol. Bull. 135: 349-365.

Sanders, H. L. and J. A. Allen. 1973. Studies on Deep-sea protobranch (bivalvia); prologue and pristiglomidae. Bull. Mus. Comp. Zool., Harvard. 145:237-262.

Sanders, H. L. and J. A. Allen. 1977. Studies on Deep-sea protobranch (bivalvia); The family Tindariidae and the genus *Pseudotindaria*. Bull. Mus. Comp. Zool., Harvard. 148:23-59.

Sastry, A.N. 1966. Temperature effects in reproduction of the bay scallop *Aequipecten irradians* Lamarck. Biol. Bull. (Woods Hole Mass.)130:118-134.

Sastry, A. N. 1968. Relationships among food, temperature and gonadal development of the bay scallop *Aequipecten irradians*. Lamarck. Physiol. Zool. 6:374-375.

Sastry, A. N. 1970. Reproductive physiological variation in the latitudinally separated populations of the bay scallop *Aequipecten irradians*. Biol. Bull. 138: 56-65

Sastry, A. N. 1979. Pelecypoda (Excluding Ostreidae) In Reproduction of Marine Invertebrates. Vol V. pp. 113-292. Giese, A. C. and Pearse, J. S. Ed. Acad. Press. 1979.

Sastry, A. N. and N. J. Blake. 1971. Regulation of the gonadal development in the bay scallop *Aequipecten irradians* Lamarck. Biol Bull. (Woods Hole Mass.)140: 274-283.

Seed, R. and R. A. Brown, 1977. Growth as a strategy for survival in two marine bivalves *Cerastoderma edule* and *Modiolus modiolus*. Jour. Of Anim. Ecol. 47: 283-292.

Sewell, M. A. and C. M. Young. 1997. Are echinoderm egg size distributions bimodal? Biol. Bull. 193: 297-305.

Scheltema R. S. 1972. Reproduction and dispersal of bottom dwelling deep sea invertebrates: a speculative summary. In R.W. Bauer, ed. Barobiology and Experimental Biology of the Deep Sea. pp. 58-68. Chapel Hill: University of North Carolina Press.

Scheltema R. S. 1994. Adaptations for Reproduction Among Deep Sea Benthic Molluscs: An Appraisal of the Existing Evidence. In Reproduction, Larval Biology and Recruitment of the Deep Sea Benthos. pp 44-75. C.M. Young, K. J. Eckelbarger Eds., Columbia University Press. New York.

Scheibe, S. 1991. The benthic macrofauna of the deep sublittoral in Conception Bay (Newfoundland, Canada). Master Thesis. Christian-Albrechts-University, Kiel.

Scheibling, R. E., and J. M. Lawrence. 1982. Differences in reproductive strategies of morphs of the genus *Echinaster* (Echinodermata:Asteroidea) from the eastern gulf of Mexico. Mar. Biol. 70: 51-62.

Schoener, A. 1967. Evidence for reproductive periodicity in the deep sea. Ecology 49: 81- 87.

Shein, E. 1989. Pectinidae (mollusca,bivalvia) bathyaux et abyssaux des carmpagnes BIO-GAS (Golf de Gascone). Systematique et Biogeographique. Ann. Ins. Oceanogr. (Paris) 65: 59-129.

Shick J. M., K. C. Edwards and J. H. Dearborn. 1981a. Physiological ecology of the deposit-feeding sea star *Ctenodiscus crispatus*: ciliated surfaces and animal-sediment interactions. Mar. Ecol. Prog. Ser. 5: 165-184.

Shick J. M., W.F. Taylor and A.N. Lamb. 1981b. Reproduction and genetic variation in the deposit feeder sea star *Ctenodiscus crispatus*. Mar. Biol. 63: 51-66.

Sibly, C.M. and P. Calow. 1989. A life cycle theory of response to stress. Biol. J. Linn. Soc. 37: 101-116.

Smith, C.R., R. H. Pope, D. J. Demaster, and L. Magaard. 1993. Age-dependent mixing of the deep-sea sediment. Geochim. Cosmochim. Acta.57: 1473-1488.

Smith, C.R. 1994. Tempo and mode in deep-sea benthic ecology: punctuated equilibrium revisited. Palaios. 1994 (9): 3-13.

Smith, C. R., D. J. Hoover, S. E. Doan, R. H. Pope, D. J. Demaster, F. C. Dobss, and M. A. Altabet. 1966. Phytodetritus at the abyssal seafloor across 10° of latitude in the central equatorial Pacific. Deep Sea Res.II. 43(4-6):1309-1338.

Smith, C. R., W. Berelson,D. J. Demaster, F. C. Dobss, D. Hammond, D. J. Hoover, R. H. Pope, and M. Stephens. 1997. Latitudinal variations in benthic processes in the abyssal equatorial Pacific. Control by biogenic particles flux. Deep Sea Res. II. 44(9-10): 2295-2317.

Sokall, R. R. and F. J. Rohlf. 1995. Biometry. The principles and practice of statistics in biological research, 3rd ed. W.H. Freeman and Co. San Francisco.

Spirlet, C., P. Grosjean and M. Jangoux. 1998. Reproductive cycle of the echinoid *Paracentrotus lividus*: analysis by means of the maturity index. Invert. Reprod. Dev. 34(1): 69-81.

Stacek, C. R. 1965. Feeding and particle sorting in *Yoldia ensifera* (Bivalvia:Protobranchia), with notes on other nuculanids. Malacologia. 2(3): 349-366.

Stockton, W. L and T. E. Delaca. 1982. Food falls in the deep sea: occurrence, quality and significance. Deep Sea Research. 29(2A): 157-169.

Sumida, P. Y. G., P. A. Tyler, R. S. Lampitt and J. G. Gage. 2000. Reproduction, dispersal and settlement of the bathyal ophiuroid *Ophiocten gracilis* in the NE Atlantic Ocean. Mar. Biol. 137: 623-630.

Sun, M. Y., R. C. Aller, C. Lee and S. G. Wakeham. 1999. Enhanced degradation of algal lipids by benthic macrofaunal activity: Effect of *Yoldia limatula*. Jour. Mar. Res.57: 775-804.

Thiel, H., O. Pfannkucke, G. Shriever, K. Lochte. A. J. Gooday, C. Hemleben, R.F. G Mantoura, C.M. Turley, J.W. Patching, and F. Riemann. 1990. Phytodetritus on the deep sea floor in the central oceanic region of the northeast Atlantic. Biol. Oceanography 6: 203-239.

Thompson, J.K. and F.H. Nichols. 1988. Food availability controls seasonal cycle of growth in *Macoma balthica* (L.) in San Francisco Bay, California. J. Exp. Mar. Biol. Ecol. 116: 43-61.

Thompson, R. J. 1977. Blood chemistry, biochemical composition of tissues and the annual reproductive cycle in the giant scallop *Placopecten magellanicus* (Gmelin) from Southeast Newfoundland. J. Fish. Res. Board Can. 34: 2104-2116.

Thompson, R. J. 1979. Fecundity and reproductive effort in the blue mussel *Mytilus edulis* , the sea urchin *Strongylocentrotus droebachiensis*, and the snow crab *Chionoecotes opilio* from populations of Nova Scotia and Newfoundland. J. Fish. Res. Board. Can. 36: 955-964.

Thompson, R. J. 1983. The relationship between food ration and reproductive effort in the green sea urchin *Strongylocentrotus droebachiensis*. Oecologia (Berl). 56: 50-57.

Thompson, R. J. 1984. Partitioning of energy between growth and reproduction in three populations of the sea urchin *Strongylocentrotus droebachiensis*. Advances in Invertebrate Reproduction 3: 425-432.

Thompson, R. J., D. Deibel, R. Lawrence, R. Pomeroy, D.J. Douglas and P.C. Griffith. 1986. Cold ocean productivity experiment (COPE 86): particle flux and seston characteristics. ASLO meeting Abstracts.

Thompson, R. J. and B. MacDonald. 1991. Physiological Integrations and Energy Partitioning. pp. 347-375. In Scallops: Biology, Ecology and Aquaculture. Ed. By S. E. Shumway. Elsevier Science Pub. The Netherlands.

Thompson, R. J., D. Deibel, A. M Redden and C.H. McKenzie. 1992. Sinking of the spring diatom bloom in a cold ocean. American Society of Limnology and Oceanography, Santa Fe.

Thompson R. J., R. I. E. Newell, V.S. Kennedy and R. Mann. 1996. Reproductive processes and early development. In: The eastern oyster *Crassostrea virginica*. pp.335-370. V. S.Kennedy, R. I. E. Newell and A. Eblet. Edits. Maryland Sea Grant Book. College Park, Maryland

Thompson, R. J., C. C. Parrish, D. Deibel, and N. Choe. 1999. Effect of a sinking spring bloom on lipids in suprabenthic and benthic invertebrates in a cold ocean coastal environment American Society of Limnology and Oceanography, Santa Fe.

Thorson, G. 1936. The larval development, growth and metabolism of arctic marine bottom invertebrates. Medd. Grønland. 100: 1-155.

Thorson, G. 1946. Reproduction and larval development of Danish marine bottom invertebrates. Medd. Komm. Damn. Fisk.-og Havunders. Ser. Plankton: 4. 523 pp.

Thorson, G. 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25; 1- 45.

Tyler, P.A. 1986. Studies of a benthic time-series: reproductive biology of benthic invertebrates in the Rockall Trough. Proc. R. Soc. Edinb. 88b: 175-190.

Tyler, P.A. 1988. Seasonality in the deep sea. Ocean Mar. Biol. Ann. Rev. 26: 227-258.

Tyler, P. A and J.D. Gage. 1984a. Seasonal reproduction of *Echinus affinis* (Echinodermata; Echinoidea) in the Rockall Trough, northeast Atlantic Ocean. Deep Sea Research. 31(4): 387-402.

Tyler, P.A and J.D. Gage. 1984b. The reproductive biology of echinothurid and cidarid sea urchins from the deep-sea (Rockall Trough, North-East Atlantic Ocean). Mar. Biol. 80: 63-74.

Tyler, P.A. and C.M. Young. 1992. Reproduction in marine invertebrates in "stable"environments: the deep sea model. Inv. Reprod. Dev. 22: 185-192.

Tyler, P.A. and C.M. Young. 1999. Reproduction and dispersal at vents and cold seeps. J. Mar. Biol. Ass. U.K. 79: 193-208.

Tyler, P.A., R. Harvey., L. A. Giles, and J. D. Gage. 1992. Reproductive strategies and diet in deep-sea nuculanid protobranchs (Bivalvia : Nuculoidea) from the Rockall Trough. Mar. Biol. 114: 571 - 580

Vance, R. 1973a. On reproductive strategies in marine benthic invertebrates. Amer. Nat 107 (955): 339-352.

Vance, R. 1973b. More on reproductive strategies in marine benthic invertebrates. Amer Nat 107 (955): 353-361.

Venable, D. L. 1992. Size-number trade-offs and the variation of seed size with plant resource status Amer. Nat. 140: 287-304.

Wainright, A. C. 1990. Sediment-to-water fluxes of particulate material and microbes by resuspension and their contribution to the planktonic food web. Mar. Ecol. Prog. Ser. 62: 271-281.

Walker, C. E. 1974. Studies on the reproductive systems of sea-stars. I. The morphology and histology of the gonad of the gonad of *Asterias vulgaris*. Biol. Bull. 146: 661-677.

Walker, W. W. 1974. Histology and ultrastructure of the gonad of *Ctenodiscus crispatus* (Asteroidea). Amer. Zool. 14(4): 1264.

Warren, A. 1989. Taxonomic comments on some protobranch bivalves from the northeast Atlantic Sarcia. 74: 223-259.

West, H.H. J. F. Harrigan, and S. K. Pierce. 1984. Hybridization of two populations of a marine opisthobranch with different developmental patterns. The Veliger 26: 199-206.

Williams, G.C. 1966. Natural selection, the cost of reproduction and a refinement of Lack's principle. Amer. Natur. 100: 687-690.

Williams, M.A. 1981. Quantitative Methods in Biology. 2. Stereological Techniques. Vol. 6. In: Glauert, A. M. (Ed). North Holland Publishing Company, Amsterdam: pp 5-84.

Young, J. S. Tyler, P.A. Cameron, J. L. and S.G. Rumrill. 1992. Seasonal breeding aggregations in low density populations of the bathyal echinoid *Stylocidaris lineata*. Mar. Biol. 113: 603-612.

Zardus, J.D. and. P. Morse.1998. Embryogenesis, morphology and ultrastructure of the pericalymma larvae of *Acila castrensis* (Bivalvia:Protobranchia:Nuculoidea). Invert. Biol. 117(3): 221-244.