THE RELATIONSHIP BETWEEN THE FEEDING ECOLOGY OF THE PROTOBRANCH BIVALVE YOLDIA HYPERBOREA AND THE SEASONAL CHANGES IN THE DEEP-DEPOSITIONAL ZONE IN CONCEPTION BAY, NEWFOUNDLAND

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by

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Abstract

The primary purpose of this study was to further the understanding of the deposit-feeding strategies through an overall comprehension of the environmental and physiological constraints and the feeding opportunities encountered by the obligate deposit feeding protobranch *Yoldia hyperborea*.

Seasonal variation in the feeding response by *Yoldia hyperborea* from the deep-depositional zone of Conception Bay (~250 m) was monitored through periodic quantification of morphological (digestive cell height) and biochemical changes (protein content, activity of acid protease and α-amylase) in the digestive gland. Results showed an increase of digestive cell height and protein content as soon as sinking organic material from the spring bloom fallout reached the benthic zone, suggesting storage of metabolic energy during spring and summer, this was followed by a decline of both variables in late summer, coinciding with gamete development. Sharp increases of digestive enzyme activity occurred only in early spring of each year, coinciding with the timing of the primary bloom fallout, and suggests activation of the formerly depressed lysosomal system after a prolonged period of low food availability (*i.e.* late summer to autumn). Individuals exposed to laboratory simulated events of sedimenting algae showed an increase of digestive cell height to similar levels observed in the field, whereas digestive cell height in animals exposed to impoverished sediment remained at the low levels shown by animals within the inter-bloom period.

Yoldia hyperborea is primarily a sub-surface deposit feeder, but switches to surface deposit-feeding when surface sediment is enriched with algae. Behavioural changes to the downward flux of sedimenting microalgae was displayed as it extended its siphons into the

water column when suspended algae were in high concentration. This behaviour was followed by partial reemergence of individuals and extension of the palp proboscides over the sediment surface once suspended algal concentration decreased and algae accumulated on the sediment. Concurrently, orientation of the siphon changed from vertical to horizontal, thereby always keeping closer contact with the area of highest algal concentration. In contrast, activity of animals not exposed to algae was primarily restricted to strata below the sediment surface.

Siphon extension into the water column and active ventilation during periods of high suspended algal concentration suggest active suspension-feeding during algal sedimentation events, although deposit-feeding would be resumed once suspended algae decreased. Despite the fact that feeding experiments demonstrated the feasibility of suspension-feeding behaviour in *Yoldia hyperborea*, ingestion rates were extremely low and individuals were not capable of meeting their metabolic energy demands, although absorption efficiency of organic carbon was high (50-72%). In contrast, high ingestion rates (4.358 mg sed. · ind^{-1} · h^{-1}) were observed during deposit-feeding on algae-enriched sediments, which together with high assimilation efficiency of algae-derived organic carbon (87.7-95%), supplied sufficient energy to meet metabolic demand and provide a positive scope for growth.

The results of both field and laboratory studies suggest that the suite of behavioural changes displayed by *Yoldia hyperborea* during algal sedimentation events are linked to its nutritional dependence on this food source.

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List of Abbreviations

AE	absorption efficiency
AFDW	ash-free dry weight
CCD	charge coupled device
CTD	conductivity, temperature and depth probes
DG	digestive gland
DGI	digestive gland index
DGW	digestive gland weight
DW	dry weight
GF C	glass fibre filter, type C
GF F	glass fibre filter, type F
GPT	gut passage time
HI	hydrolysis index
HPLC	high performance liquid chromatography
Hb	haemoglobin
Hc	haemocyanin
ind	individual
IR	ingestion rate
LI	lypolisis index
MD	macrophytic detritus
NE	north-east
NW	north-west
O:N	oxygen to nitrogen ratio
PAR	photosynthetically active radiation

PCB	polychlorinated biphenyls
PM	particulate matter
POC	particulate organic carbon
POM	particulate organic matter
PON	particulate organic nitrogen
PUFA	polyunsaturated fatty acid
RFU	relative fluorescence units
SFG	scope for growth
SW	south west
TAG	triacyl-glycerid
TPM	total particulate matter
TTW	total tissue weight
VNH_4-N	ammonia excretion rate
VO_2	oxygen uptake rate
W	west
WW	wet weight

CHAPTER 1

GENERAL INTRODUCTION

1.1. Deposit-feeding

The seabed of the world's oceans is mostly covered with fine-grained sediment, the organic content of which constitutes the food source for a myriad of organisms we call deposit-feeders. It has long been recognised that the population dynamics of deposit-feeders depends on the nutritional characteristics of the sedimentary environment and in particular of detritus (Levinton & Stewart 1988). Detritus is a heterogenous pool derived from plant material of varying nutritional quality (*e.g.* seagrass, seaweeds and microalgae), but also includes animal remains, faecal pellets and their association to bacteria, fungi and Protozoa. The importance of these different sources of food varies according to their relative contents of particulate organic carbon (POC) and nitrogen (PON) and of typically limiting substrates such as protein, essential amino acids and fatty acids (Levinton *et al.* 1984, Phillips 1984, Rice & Rhoads 1989, Dauwe *et al.* 1999). However, the rate at which these nutrients are supplied and consumed (*e.g.* sedimentation and ingestion rates, competition) as well as their rate of decay, has a considerable impact on the physiology, growth and production of the detritivore community.

Food supply is one of a variety of controlling factors that determine a species' distribution, abundance, and food chain parameters. Other control mechanisms are bound to its physiological limitations, strategies for food exploitation and interaction with other species (see Fig. 1.1). Tenore (1989) suggested that these factors act as an "interactive hierarchy" of potential regulatory mechanisms, with levels that control the final expression

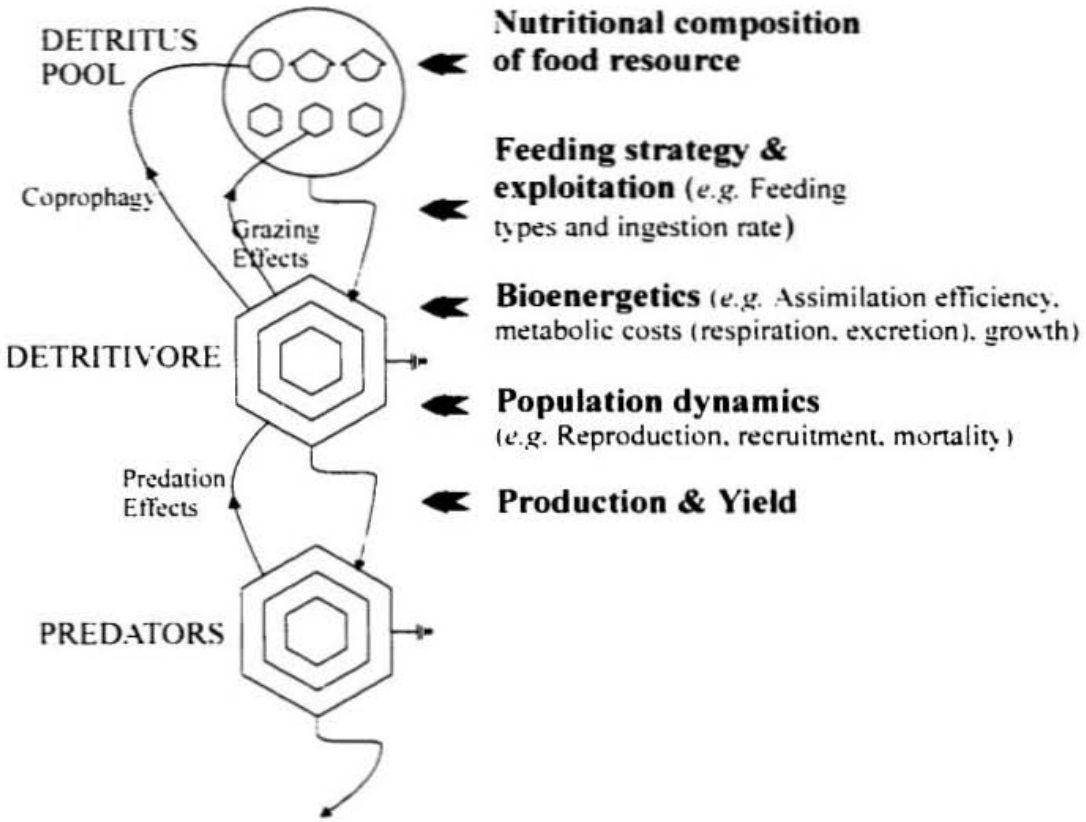

Fig. 1.1: Hierarchy of regulatory mechanisms controlling the detritus food chain (redrawn from Tenore, 1989). Symbols represent different components of the detrital food chain where, ○ represents an outside source of inflow, ⬠ is storage, ⬡ indicates a consumer, —||► symbolises non-utilisable energy or heat sink, is the material source (detritus pool), and —► indicates the direction of energy flow.

of production and trophic transfer within the detrital food chain. Thus in order to understand population dynamics in deposit-feeders it is necessary to focus on some of the regulatory aspects within the detrital food chain. Much of the work done so far on deposit-feeding has focussed on the nutritional role of sediment, but information on other regulatory mechanisms is comparatively less (*cf.* Bender & Davis 1984, Brock *et al.* 1986, Lopez & Levinton 1987, Bayne & Thurberg 1988, Cammen 1989, Cheng & Lopez 1991, Bock & Miller 1996).

Although deposit-feeders can inhabit a wide range of sediments, most are found in deposits with a high proportion of fine particles with a relatively high organic content (see Snelgrove & Butman 1994, for discussion of mechanisms). To date it has been established that many deposit-feeders efficiently digest bacteria and most microalgae, as well as macroalgae-derived and other non-living detritus (Newell 1965), but growth rate is more likely to be limited by nitrogen rather than carbon (Rice & Rhoads 1989), as well as by some essential nutrients such as fatty acids, vitamins and amino acids provided by bacteria and, especially, by algae (Phillips 1984). In addition, nutrient concentration varies with sediment depth, so that the highest detritivore biomass is usually found nearest the surface where food value is highest (Rhoads 1974). Episodic sinking of plankton detritus replenishes and enriches the sediment surface with macro- and micronutrients (Clarke 1988). However, the concentration of labile and fresh detritus, and hence its nutritional value, decreases with increasing depth of sediment, whereas the proportion of inorganic and refractory organic material increases (Graf 1992).

A knowledge of the depth within the sediment at which a detritivore obtains its nutrients is important for understanding its feeding strategy and physiological capabilities. Animals feeding on different strata will possess different suites of physiological and behavioural adaptations that will enable them to maximise their exploitation of the available resources. These feeding strategies will be reflected in the population dynamics of the species (*sensu* Tenore 1989). Thus, secondary production of surface deposit-feeders from a range

of water depths increases following episodic pulses of fresh plankton detritus, although populations of subsurface deposit-feeders, normally provided with a steady supply of reworked POM, tend to show their relative independence from sediment surface fluctuations by remaining remarkably stable (Rice & Rhoads 1989).

Although there may be a wide variety of potential food resources in a given habitat, the physiological, morphological and behavioural characteristics of a deposit-feeder determine what, and how much of, these food resources can be potentially utilised (Tenore 1989). In addition, the capacity of a potential food resource to meet the energy demands of deposit-feeders is poorly understood, mainly because there have been few studies of energy budgets in these organisms (*e.g.* Worrall *et al.* 1983, Hummel 1985a).

Since deposit-feeders are normally presented with particles of low organic content, it is important to distinguish species that ingest whole sediment (*i.e.* bulk or non-selective deposit-feeders) from those that selectively ingest some particles over others (selective deposit-feeders). A better understanding of how they perceive or sense the food value of particles is required both for the formulation of models and for the appropriate experimental testing of model predictions. Food selection and ingestion are sensitive to physical and chemical cues in a number of species, although the role of cues as feeding stimulants or deterrents needs further investigation (Cammen 1989, Taghon 1989, Watling 1989).

The role of temporal variation in the foraging behaviour of deposit-feeders has also received little attention, although this deficiency was identified more than a decade ago (Taghon 1989). A knowledge of variations in feeding physiology and food uptake and storage would help to identify the nutritional constraints to which a specific natural population is subjected, especially in species exposed to strong fluctuations in food supply.

1.2. Deposit-feeding in protobranch bivalves

Deposit-feeding in bivalves is restricted to most of the sub-class Protobranchia (=Paleotaxodonta) and the Tellinacea of the sub-class Lamellibranchia. Most studies of deposit-feeding in bivalves have been done on tellinaceans, despite the fact that the degree of adaptation to deposit-feeding in this group varies among the different families, although none of them are obligate deposit-feeders (Pohlo 1969). For example, members of the Donacidae possess no deposit-feeding adaptations and thus are obligate suspension-feeders, whereas the Tellinidae and Semelidae are facultative deposit-feeders (Pohlo 1969).

In contrast, current knowledge of the ecology and biology of the deposit-feeding Protobranchia is relatively scarce and based primarily on monographs published in the late 19th and the first half the 20th century (e.g. Mitsukuri 1881, Drew 1899, 1901, Kellogg 1915, Atkins 1936, Yonge 1939) together with a few more recent publications (e.g. Allen 1992, Schaefer 2000). This early work established that protobranchs have some important morphological and functional differences from the Lamellibranchia.

1.2.1. The Ctenidia

The most striking of these differences is that the Protobranchia possesses a pair of small ctenidia lacking reflected demibranchs (Fig. 1.2), and which are believed to be used primarily for gas exchange (Yonge 1939, Levinton *et al.* 1996), although some authors suggest that they may also play a role in feeding (Atkins 1936, Stasek 1963, Davenport 1988a, b, Nakaoka 1992). However, members of the order Solemyoida have gills with endosymbiotic chemoautotrophic bacteria which contribute more than 98% of the carbon requirements of *Solenomya velum* (Conway *et al.* 1989). Due to the unique character of this latter taxon, which in addition lacks a digestive gut, I will only consider extant members of

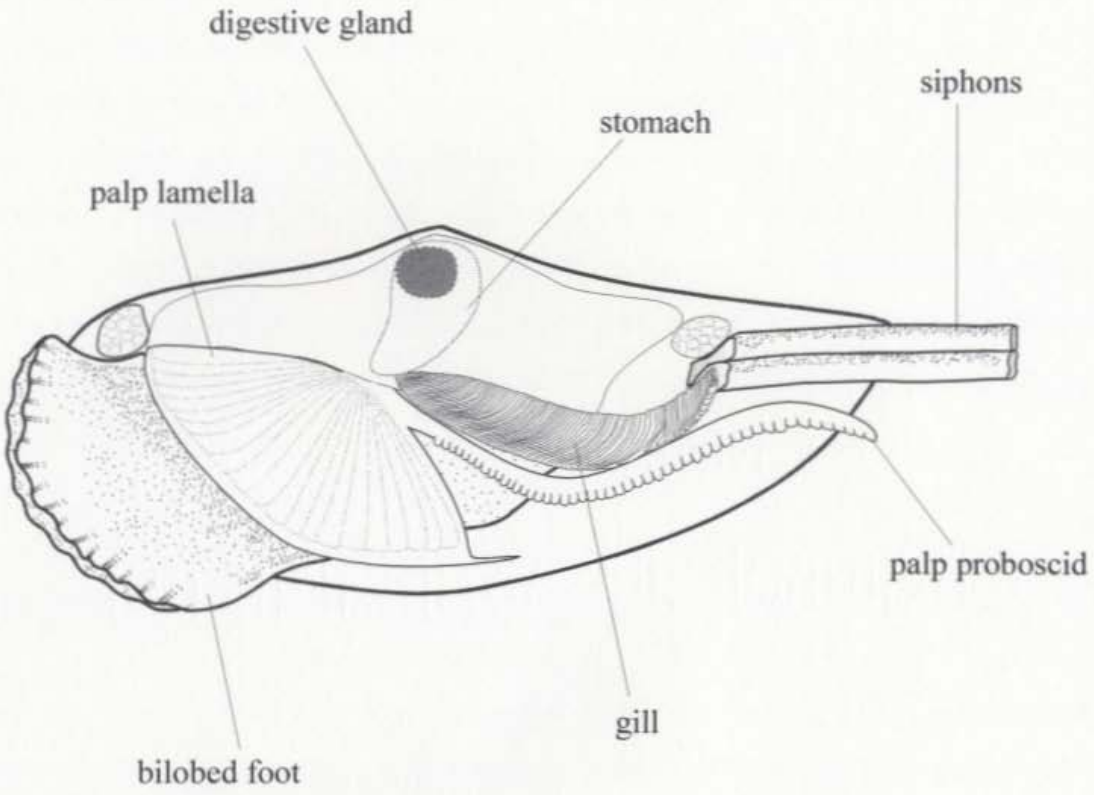

Fig. 1.2.: Schematic representation of the anatomy of *Yoldia hyperborea*.

the order Nuculoida (Superfamilies Nuculacea and Nuculanacea) when referring to the Protobranchia.

The filaments of the protobranch gill alternate on either side of the chitinous gill axis (Morse & Zardus 1997). Laterofrontal cilia lie adjacent to frontal cilia on the exposed surfaces of the filaments, whereas lateral cilia (found between the filaments), are responsible for water movement through the gill. The relatively smooth abfrontal surface shows scattered groups of cilia, their numbers varying between species and individuals (see Stasek 1963). Protobranch gills are known to contain high concentrations of cytoplasmic haemoglobin (Angelini *et al.* 1998), whereas the haemolymph contains the blood pigment haemocyanin as in gastropods and cephalopods, but unlike lamellibranchs, some of which possess only haemoglobin (Houlihan *et al.* 1982, Herskovits & Hamilton 1987, Mangum *et al.* 1987).

1.2.2. Feeding and digestion

The main food gathering structure within the mantle cavity of the protobranch is a pair of large labial palps with a differentiated pair of palp proboscides which gather food and transfer it to the labial palp lamellae on the way to the mouth. These palp proboscides are unique to protobranchs, and are specialised for gathering sediment particles. Unlike the Tellinacea, which use their siphons to suck particles from the sediment surface (Hughes 1969, Gilbert 1977), protobranchs may use these structures to obtain sediment from either the surface or subsurface (see Bender & Davis 1984) or to clear sediment from within the mantle cavity (Stasek 1965). Stasek (1961, 1963) described the anatomy, function and ciliation of the labial palps of *Acila castrensis* and other protobranchs, and showed that particles captured by the distal part of the palp proboscides are transferred along their ciliated troughs to the posterior edges of the lamellae. The inner ridges of the lamellae are heavily

ciliated, whereas the outer surfaces are relatively smooth with patches of rejectory cilia (Morse & Zardus 1997).

In protobranchs food is conveyed via the labial palps into the mouth, then down the short tubular oesophagus into the complex stomach. A mass of mucus-laden detritus, the protostyle, projects from the style sac of the midgut into the stomach and rotates to draw in the food from the oesophagus. The protostyle is not a discrete structure like the lamellibranch crystalline style, but a cohesive mass formed by secretions of the style-sac and digestive diverticula mixed with particulate material (Owen 1966, Purchon 1977, Morton 1983). Sorted food particles in the cuticle-lined stomach are squeezed through primary unciliated ducts which branch into multiple heavily ciliated secondary ducts ending in masses of blind tubules forming the left and right diverticula of the digestive gland. The cells lining these sacs participate in moving the food, secreting materials into the lumina, and phagocytosing and endocytosing particulates for further intracellular digestion in the lysosomal systems of the digestive cells (Owen 1972, Morse & Zardus 1997).

In addition to its digestive role, the digestive gland of bivalves acts as a site for the storage of metabolic reserves, later used for gametogenesis or during periods of physiological stress (Thompson *et al.* 1974). Cells of the digestive diverticula also show cyclical changes associated with intracellular digestion, which may be indicative of the feeding cycle (Langton 1975, Lowe *et al.* 1981, Lowe & Moore 1985, Moore & Lowe 1985). Changes at cellular and tubular levels are linked to the influx of food (Owen 1974, Thompson *et al.* 1974, Langton 1975) or stressors such as oil pollution (Lowe *et al.* 1981, Widdows *et al.* 1982), spawning (Bayne *et al.* 1978) and temperature (Thompson *et al.* 1978) and therefore can be used as an indicator of animal response to environmental changes, particularly seasonal food supply. However, there has been little work on the digestive processes of protobranch bivalves (Morton 1983).

1.2.3. Ecology and physiology

There are very few studies on the physiology of protobranchs. For example, oxygen uptake rates are known for only a handful of species (*e.g.* Wilson & Davis 1984, Mangum *et al.* 1987, Davenport 1988a, Bernard & Noakes 1990, Taylor *et al.* 1995), and there is only one study of ammonia excretion rates (Gray & Follum 1987). The relatively recent discovery of haemocyanin in the haemolymph of protobranchs has led to renewed interest in this group (Morse *et al.* 1986, Mangum *et al.* 1987, Terwilliger *et al.* 1988, Angelini *et al.* 1998), but mainly for the elucidation of phylogenetic relationships.

Although the deposit-feeding mechanism of protobranchs is unique amongst bivalves, studies of their feeding physiology, *e.g.* ingestion selectivity, ingestion rate, assimilation efficiency, are few (Lopez & Cheng 1982, 1983, Cheng & Lopez 1991), although there are detailed qualitative descriptions of their feeding habits (Drew 1899, 1901, Yonge 1939, Rhoads 1963, Bender & Davis 1984, Davenport 1988b). However, in spite of a lack of knowledge of their feeding rates and assimilation efficiency (AE), the importance of protobranchs has been recognised in the monitoring of sediment contamination with heavy metals and PCB's (*e.g.* Brand *et al.* 1984, Means & McElroy 1997, Burgess & McKinney 1999).

Aspects of the population biology of a few protobranch species have been recently considered, notably *Yoldia notabilis* (Nakaoka 1992, 1994, 1996, 1998, Nakaoka & Matsui 1994), *Yoldia eightsii* (Nolan & Clarke 1993, Peck *et al.* 2000), *Nuculana* spp. and *Nucula* spp. (Ansell 1974, Davis & Wilson 1983, Hutchings & Haedrich 1984, Craig 1994). One possible reason for this paucity of information is the relatively low abundance of protobranch species in the coastal region, with the exception of the genus *Yoldia* which can dominate in some areas (Wildish *et al.* 1980, Scheibe 1991, Lee *et al.* 1992). Although some protobranch bivalves are found in soft sediments in shelf waters, their abundance increases considerably

with water depth and they dominate in the deep sea (Taylor *et al.* 1995). At the shelf/slope break they account for 30% of the bivalves present, but at the slope bottom this increases to 50% and then to between 80% and 90% on the abyssal plain (Manly 1993).

1.3. Objectives

The protobranch bivalve *Yoldia hyperborea* (Lovén) Torrell 1859 is a benthic species with circumpolar distribution. Its range in eastern Canada extends from the high Arctic (~75°N) to inshore bays in Newfoundland (47°N) (Lubinsky 1980). Despite its wide distribution and dominance within soft sediments (Scheibe 1991), little is known about its physiology (see Parrish *et al.* 1996, for lipid composition) or ecology.

The present thesis complements the work undertaken simultaneously by Jaramillo (2001) on the reproductive strategy of *Yoldia hyperborea* from Conception Bay, Newfoundland.

The general objective of this thesis is to contribute towards the knowledge of deposit-feeding strategies through the understanding of feeding in the protobranch *Yoldia hyperborea*. Specific objectives are separated into each chapter, as follows:

- Chapter 2: Relationships between some important environmental variables and the feeding response of *Yoldia hyperborea* from Conception Bay, Newfoundland throughout an annual cycle:
    - Quantification of the temporal variability of physiological changes at the enzymatic (digestive) and cellular level, in order to detect changes in digestive capability and nutrient storage and consumption and their relation to changes in environmental conditions.

- Chapter 3: Determination of basic physiological variables (oxygen uptake, ammonia excretion) to expand current knowledge of protobranch physiology and energetics, and of deposit-feeders in general.

- Chapter 4: Examination of the feeding physiology of *Y. hyperborea* through the measurement of standard feeding variables (ingestion rate, gut passage time, absorption efficiency).
    - Determine the relative contributions of suspension-feeding and deposit-feeding.
    - Derive the energy balance for individuals from data presented in chapters 3 and 4.

- Chapter 5: Determination of suspension- and deposit-feeding behaviour in relation to commonly observed environmental changes (resuspension and bloom-fallout events).

CHAPTER 2

THE IMPACT OF BENTHIC-PELAGIC COUPLING ON A *YOLDIA HYPERBOREA* POPULATION FROM CONCEPTION BAY, NEWFOUNDLAND

2.1. INTRODUCTION

Heterotrophic life in the aphotic benthic zone of the sea is largely based on primary production that takes place in the euphotic pelagic or benthic zones (Graf 1992). Carbon supply to the benthos results from the settlement of phytoplankton and deposition of faecal material from zooplankton and from the carcasses of vertebrates and invertebrates, but coastal areas may have additional carbon input from local freshwater runoff carrying plant material and topsoil. Thus the supply of particulate organic matter depends primarily on the timing of climatic events (*e.g.* snow and ice meltdown or rain season), tidal exchange, and most importantly, the occurrence of phytoplankton blooms (Wassman 1991).

The organic fraction of the particle input to the sediment is a potential energy source for benthic organisms. It is well established that in shallow water the input of fresh organic matter of high nutritional value from primary production creates a burst of activity amongst the benthic population (Tyler 1988, Boon *et al.* 1998, Sun & Wakeham 1999). Graf *et al.* (1982) showed that the response to organic matter input is more rapid in those species that utilise the organic matter directly rather than those species which feed on other smaller meiofauna and sediment.

However, availability of food particles decreases with increasing depth and becomes more episodic at higher latitudes, imposing specific constraints on the organisms, which must be able to withstand periods of reduced food availability. Thus energy demanding metabolic processes such as growth and reproduction are often restricted to periods of high food abundance. For example, Antarctic suspension feeders show a marked seasonal variation in feeding rates, with highest activity occurring in the short but very productive summer, whereas during two or three winter months most animals cease feeding in order to reduce metabolic costs when food is scarce (Barnes & Clarke 1995). In Antarctic crustaceans that maintain high metabolic costs during winter, energy is provided from large lipid stores, whereas metabolic costs in other species, such as bivalves, can be met from normal lipid, glycogen and protein pools (Vassallo 1973, Clarke 1983, Emmett *et al.* 1987, Henry *et al.* 1991). Thus the seasonal pattern of growth in many species, together with the frequent limitation of reproduction to the summer months, indicates that many processes are regulated strictly by the availability of food. However, the degree of seasonality in the biology of polar marine organisms varies with their position in the food web (Clarke 1988).

Deposit feeders, may either utilise organic matter as it arrives at the sediment surface or use the fraction that has been buried through bioturbation (Middelburg *et al.* 2000). Although the quality of organic matter in the underlying sediment layers may differ from that of recently deposited material (Graf 1992, Mayer 1994), deposit feeders should be able to maintain a relatively constant feeding activity throughout the year, resulting in a continuous storage and utilisation of energy reserves and a reproductive pattern marked by non-seasonal or continuous spawning in the population. As a result, metabolic processes should reflect a weak seasonal pattern.

Monitoring reproduction or feeding activity has become a useful tool for evaluating the response of a population to the quantitative and qualitative variability of food resources (*e.g.* Hummel 1985b, Johnson & Wiederholm 1992, Karrh & Miller 1994, Boon *et al.* 1998).

For example, bivalves respond to variations in food availability by exhibiting changes at cellular and tubular level (Owen 1974, Thompson *et al.* 1974, Langton 1975) but may also exhibit cellular changes resulting from stressors such as oil pollution (Lowe *et al.* 1981, Widdows *et al.* 1982), spawning (Bayne *et al.* 1978) or temperature (Thompson *et al.* 1978) and therefore can be used as an indicator of whole organism response to environmental changes, particularly in seasonal food supply.

The digestive gland of bivalves, in addition to its digestive role, acts as a site for the storage of metabolic reserves that are later used for gametogenesis or during periods of physiological stress (Thompson *et al.* 1974, Johnson *et al.* 1996). Cells of the digestive diverticula also show cyclic changes associated with intracellular digestion which have been characterised as (1) absorption, (2) digestion, (3) fragmentation and excretion stages. In many mollusc groups these cellular changes are often synchronised so that each tubule is generally uniform in appearance (Langton 1975, Moore & Lowe 1985). In *Mytilus edulis* individuals exposed to different feeding conditions, Langton (1975) identified four tubule phases, (I) normal or holding, (II) absorptive, (III) disintegrating and (IV) reconstituting. However, Langton (1975), Lowe *et al.* (1981) and Lowe and Moore (1985) recognised that this qualitative categorisation is often ambiguous, and preferred to relate these changes to the variability of epithelial cell height.

Enzymes produced by the digestive gland undergo fluctuations in activity which have been related not only to changes in food supply (Ibarrola *et al.* 1998a,b, 2000b), but also to temperature (Seiderer *et al.* 1982, 1984, Brock *et al.* 1986), anoxia (Greenway & Storey 1999), nutritional regime (Reid & Rauchert 1976), pollutants (Widdows *et al.* 1982) and requirements for specific biochemical components (Cancio *et al.* 1999). However, all these studies were conducted on suspension-feeding lamellibranch species, whereas in deposit-feeding bivalves, such as protobranchs, such aspects have not yet been approached.

The benthic community of the deep-depositional zone of Conception Bay (Newfoundland, Canada) is dominated by deposit-feeders (50-60%), whereas suspension-feeders make up only 8 to 25% of total species in the area (Scheibe 1991). As phytoplankton biomass at this latitude is highly seasonal (Navarro & Thompson 1995), benthic animals should experience a strong seasonal input of organic matter that accumulates over time since bacterial turnover is not significant in the area as a result of low temperatures (Pomeroy & Deibel 1986). Hence, deposit-feeders should be able to utilize the potential food source over an extended period before depleting it.

In this study, the feeding response of the protobranch *Yoldia hyperborea* from Conception Bay was studied indirectly by quantifying enzymatic and cellular changes in the digestive gland in relation to seasonal changes in the transfer of organic matter from the water column the water column to the benthos.

2.2. MATERIAL AND METHODS

2.2.1. Study site

All sampling was carried out in the depositional zone of Conception Bay, Newfoundland, Canada (240-270 m) (Fig. 2.1). This bay is 60 km long and 23 km wide and is exposed to the Atlantic Ocean (orientation NE-SW). Its mouth has a sill at 150 m depth which closes off isobaths in the bay and restricts water access of the inshore Labrador (-1.7 to 2.0°C, 32.6‰) (de Young & Sanderson 1995). Pack ice often covers the bay from mid-March to late April, but little of it is formed locally (Cote, 1989). Local freshwater is relatively unimportant compared to the influence of ice-melt upstream of the bay, which

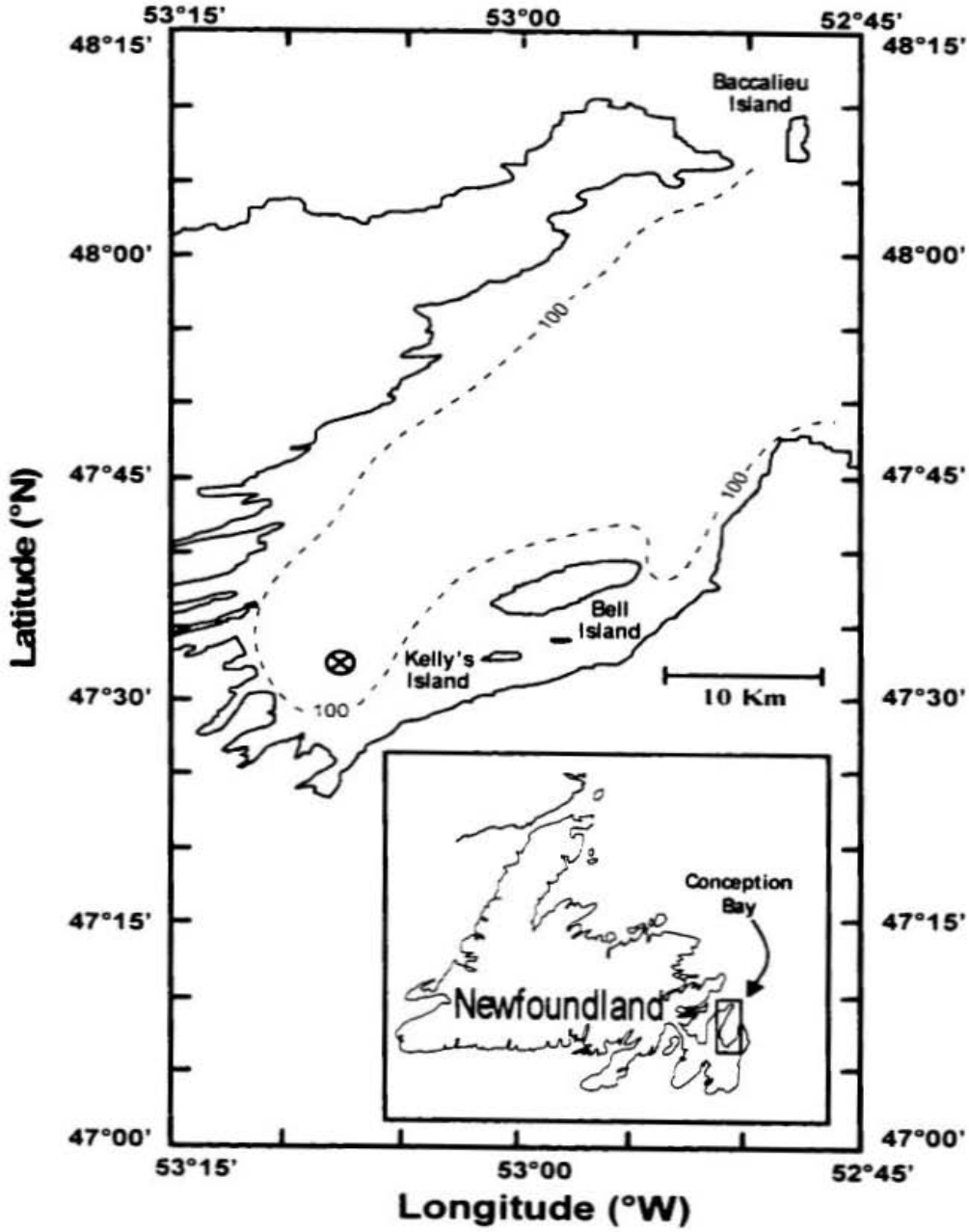

Fig. 2.1: Map of Conception Bay on the east coast of the Island of Newfoundland, showing the location of the sampling site (⊗).

accounts for most of variability in salinity (de Young & Sanderson 1995).

The depositional zone of Conception Bay corresponds closely to station "B" described by Scheibe (1991) and is characterized by muddy sediment (92% <63µm, 8% >63µm) with 60-70% water content and 20% of organic matter in the upper 10 cm layer. The benthic fauna is dominated by the polychaetes *Prionospio steenstrupi*, *Chaetozone setosa*, *Eteone longa* and *Nephtys cilliata*, the bivalve *Yoldia hyperborea* and the echinoderm *Ctenodiscus crispatus*. *Yoldia hyperborea* at this station attained a dominance of 6.1%, a mean abundance of 12 ind·m^{-2} and a mean biomass of 1.38 g AFDW·m^{-2} (Scheibe 1991).

2.2.2. Water column

The water column was sampled with a Seabird SBE25 CTD equipped with a SeaTech fluorometer. Temperature [°C], salinity [‰], *in situ* fluorescence [RFU], light transmission [%] and photosynthetically active radiation (PAR) [µmol · m^{-2} · s^{-1}] were measured. Relative fluorescence units [RFU] were converted to chlorophyll *a* concentration [µg chl*a* · l^{-1}] through the equation:

$$Y = 0.3973 \cdot X - 0.3015 \quad (r^2 = 0.6482,\ n = 253)$$

which was calculated from several years of CTD casts in Conception Bay (Ru Cheng Tian, personal communication).

Measurements were made 61 times between March 1996 and December 1999, with more casts during the spring and summer months than in autumn and winter of each year. Data were reduced to 2 metre bin averages. Only the downcast sampling was used to plot the results after smoothing the data with the inverse distance method and applying a weight

of 5 (see Appendix I for formula).

2.2.3. Sampling of *Yoldia hyperborea*

A 1.2 m diameter dredge fitted with a 2.54 cm mesh was towed from the R/V Karl & Jackie (Memorial University of Newfoundland) for 20 minutes from 47°34.0' N, 53°08.1' W to 47°32.5' N, 53°07.8' W. Material trapped in the dredge was brought to the surface and washed with ambient seawater over a 1 cm mesh steel screen. Adult *Y. hyperborea* (> 23 mm shell length) were removed, held in seawater at approximately 0°C, and placed later in a refrigerated holding tank (0.0 ± 1.0 °C) at the Ocean Sciences Centre in Logy Bay (Newfoundland, Canada).

2.2.4. Protein content and enzyme assays

Protein content and the activity of acid protease and α-amylase were determined in the digestive gland tissue (DG) after each sampling trip. The complete digestive gland (without stomach) was excised from 4 to 8 large animals (size range 23.49 to 42.03 mm, $\bar{x}$=32.71, S.D.= 3.54) and weighed (± 0.001 g WW). Since the size of the gland fluctuated throughout the year it was necessary at times to pool up to 2 individuals per replicate to obtain a minimum of 450 µl of homogenised tissue, thus producing 2 to 5 replicate samples.

A fraction of each tissue homogenate was used to determine its protein content according to Lowry *et al.* (1951) as modified by Hartree (1972) using bovine serum albumin (BSA) as a standard (Sigma B-6917).

Acid protease activity was determined as described by Anson (1938) using 2%

azocasein (Sigma A2765) as a substrate. The method for determining α-amylase was based on Rinderknecht *et al.* (1967) with amylopectin azure (2%) (Sigma A6808) as a substrate. At the end of the incubation period, the reaction of the acid protease and α-amylase assays was stopped and the remaining substrate precipitated with 5% TCA and absolute ethanol, respectively. The concentration in solution of the products of digestion (amino acids and glucose, respectively) were determined with a spectrophotometer (Beckmann DU-65®) at 366 nm and 595 nm for the acid protease and α-amylase assays respectively.

The pH optimum of each enzyme assay was determined within the range pH 2.0 and 8.5 (pH 0.5 intervals) (see Appendix II). The enzyme studies showed acid protease activity within the range of pH 2.0 and 7.5, but peaking at pH 4.5 (Fig. AII.1). Amylase activity occurred within the range of pH 4.5 and 7.0, although the highest activity was observed at pH 6.0 to 6.5 (Fig. AII.2). pH 4.5 was chosen for assaying acid protease and pH 6.0 for α-amylase.

All assays were carried out in duplicate and incubated at 25°C. Reactions were stopped after 0.5 and 2.5 hours in the case of acid protease and 5 and 20 hours in the case of α-amylase assays after initial trials indicated that reaction velocity did not change within the respective time frame (see Appendix III, Figs. AIII.1 & AIII.2.).

Specific activity, $U \cdot mg^{-1}$, was related to protein content of the tissue homogenate supernatant and calculated using the equation:

$$U \cdot p^{-1} = U \cdot mg^{-1} = \frac{\Delta A(t_2) - \Delta A(t_1)}{\alpha \cdot p \cdot \Delta t}$$

where,

U = µmol of product equivalents (amino acids, maltose) obtained per minute

$\Delta A(t_x)$ = difference between absorbance values of the sample mean and the blank mean after

the reaction was stopped at times t_1 and t_2, respectively

α = slope of the reaction velocity between times t_1 and t_2

p = protein content in the extract (mg)

Δt = incubation time (min). Difference between times t_1 and t_2

2.2.5. Histology

Histological examination of the digestive gland *Yoldia hyperborea* was carried out on 13 occasions from April 1997 to the end of October 1998. Animals (n = 30) were selected from within the 24 to 42 mm shell length range and processed within 48 hours of collection. The wet weight of each individual was obtained with and without the shell. The digestive gland was carefully removed, weighed, fixed in 6 ml Baker's formol calcium with 2.5% sodium chloride, and refrigerated at 4 °C (Lowe & Moore 1985).

Total animal tissue and whole digestive gland wet weights were used to calculate the digestive gland index (DGI), with the formula:

$$DGI = \frac{DGW}{TTW} \times 100$$

where,

DGI = digestive gland index (%)

DGW = digestive gland wet weight (g)

TTW = total tissue weight (g)

Tissue samples were fixed at 4 °C for at least a week before transferring to a gum sucrose solution for storing prior to dehydration, clearing and embedding in paraffin wax (Paraplast®). Tissue sections (7 µm) were left to dry for a minimum of 5 days at 20°C before

staining with hematoxylin and eosin.

Sections from 6 to 10 randomly selected individuals from each sample were examined at 250x magnification on a Zeiss Axiovert* inverted microscope. A Sanyo* colour CCD high resolution camera (model VDC-2972) connected to a computer equipped with image processing software (Image-Pro Plus* v. 4.1.0.0 for Windows*) was used to capture 5 to 7 different portions of the tissue to complete a minimum of 50 tubule measurements. Measurements were made by placing a sampling matrix (lines 76.84µm from each other) over the image and measuring the height of intercepted cells as described by Lowe *et al.* (1981) and Lowe and Moore (1985). No measurements were made on tubules that had intersected obliquely and which appeared to be several cell layers thick. From these data the mean cell height and standard deviation were calculated for each animal and each sampling.

2.3. RESULTS

2.3.1. Water column

2.3.1.1. Temperature

Water temperature in Conception Bay showed a seasonal pattern with surface values that fluctuated between 15.9 °C in summer (19 August 1997, 0 to 8 metres) to -1.5 °C in late winter (21 March 1997, 22 to 40 m) (Fig. 2.2). Although this pattern was similar for most years, a maximum surface temperature of only 11.6°C was observed in the summer of 1998.

The thermocline started to develop within the top 25 to 40 metres of the water column, in mid-May in 1996 and 1997, at the beginning of May 1998 and in early June

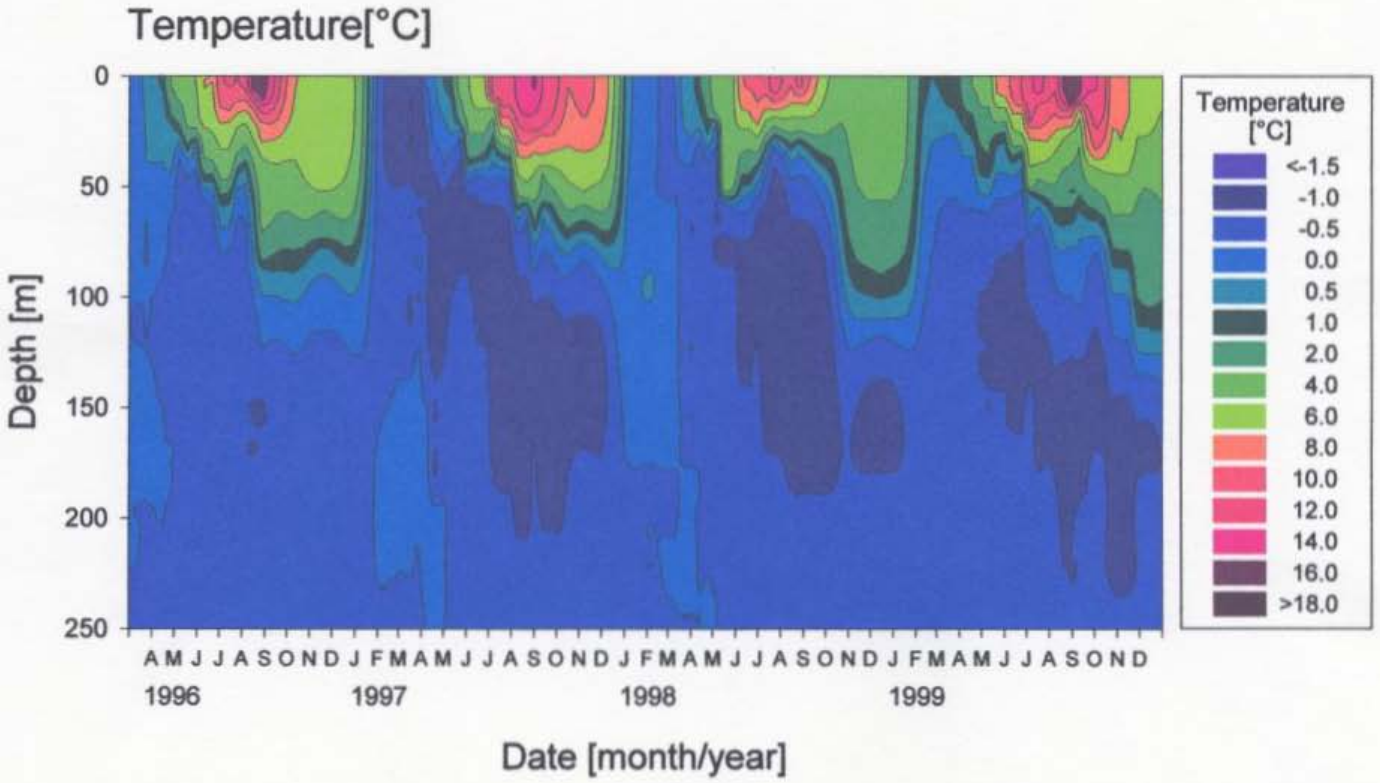

Figure 2.2: Temporal variation of temperature [°C] from 1996 to 1999 in Conception Bay, Newfoundland, Canada.

1999. The thermocline persisted throughout the late summer, autumn and early winter to mid-January (1997, 1998) or mid-February (1999), as autumn and winter mixing occurred to depths of 90 to 125 metres (*cf.* 0°C isotherm, Fig. 2.2).

Bottom temperature remained fairly stable throughout the year, averaging -0.63 °C (S.D. = 0.15, n= 732, min= -1.12°C, max = -0.36°C) below 200 metres depth.

A cold water mass with temperatures below -1°C develops every year below the thermocline, although it reached the surface in February and March 1997. This cold water mass was usually found between 60 and 200 metres and coincided with the thermocline, except for 1996 when only a small water mass appeared briefly for approximately two weeks at around 150 metres.

2.3.1.2. Salinity

Salinity fluctuated between 24.97 and 33.27 ‰ between March 1996 and December 1999 (Fig. 2.3). However, much of this fluctuation corresponded to one measured event (24 February 1998) in which salinity ranged from 24.97‰ at the surface to 32.09‰ at 58 metres. Throughout the rest of the study period the salinity of the top 15 metres remained between 30.91 and 32.15‰. Surface salinity values above 31‰ were observed only during late winter and early spring. Highest salinity values (31.78 - 33.23‰) were always observed below the 150 m depth mark. Lowest values at this depth were recorded on one sampling occasion (June 1998), and otherwise ranged from 32.89 to 33.23‰.

2.3.1.3. Light transmission

Light transmission at different depths is related to the presence of particulate matter in the water column, decreasing values representing an increase in suspended particulate material (Siegel *et al.* 1989, Gardner *et al.* 1993). From March 1996 to December 1999, light transmission within the water column ranged from 0.08 to 91.18% but averaged 75.92%

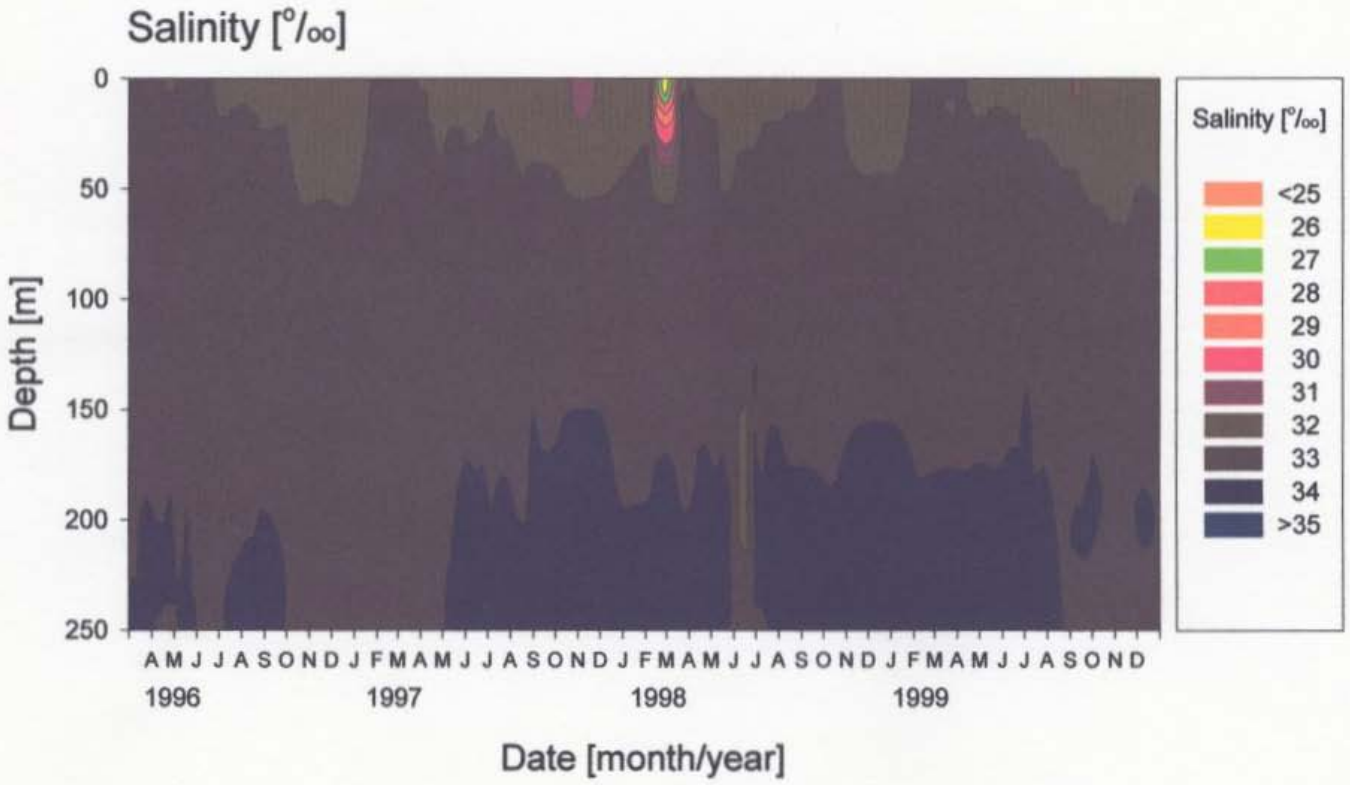

Figure 2.3: Temporal variation of salinity [°/oo] in Conception Bay, Newfoundland from 1996 to 1999.

(S.D. = 16.57). Thus most of the water column always showed light transmission values between 70 and 90%, and only occasionally were lower values recorded (Fig. 2.4). Values below 20%, when observed, were usually found near the surface, as in March 1997, February, June to August and November 1997, July and August 1998 and June to July and October to December 1999. In February 1998, the 20% isopleth could be seen extending from the surface to around 120 metres, whereas in August 1997 and July 1999 it extended from 150 m to the bottom of the bay.

By contrast, isopleths between 20% and 70% were observed at the surface at the same times as the isopleths below 20% but also developed from the bottom of the bay to around 100 metres from July to mid-October 1996, March 1997 to March 1998, July to the end of November 1998 and again from mid-June to the end of October 1999.

2.3.1.4. Photosynthetically Active Radiation

Photosynthetically active radiation (PAR) showed a seasonal pattern in Conception Bay. PAR values in the first 10 metres of the water column ranged from 23.02 to 949.43 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Fig. 2.5). Highest radiation values appeared at different times of the year, although a trend of radiation increase was apparent from early spring to mid-summer. Thus values above 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were observed up to 10 metres depth from mid-April to mid-May in 1996, sporadically between July and August 1997, mid-February to March and May 1997, and mid-April, June to mid-July and mid-September to mid-October 1999. Furthermore, the 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ isopleth was observed always in the first 25 metres and showed a seasonal pattern starting in February or March of each year, but disappeared between August and December (October 1996, September 1997, August 1998, December 1999). A similar trend was observed for the 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ isopleth which reached about 35 m depth. Low values of 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ were observed throughout the year within the 30 to 100 metre depth band but were also recorded at 145 metres in May 1997.

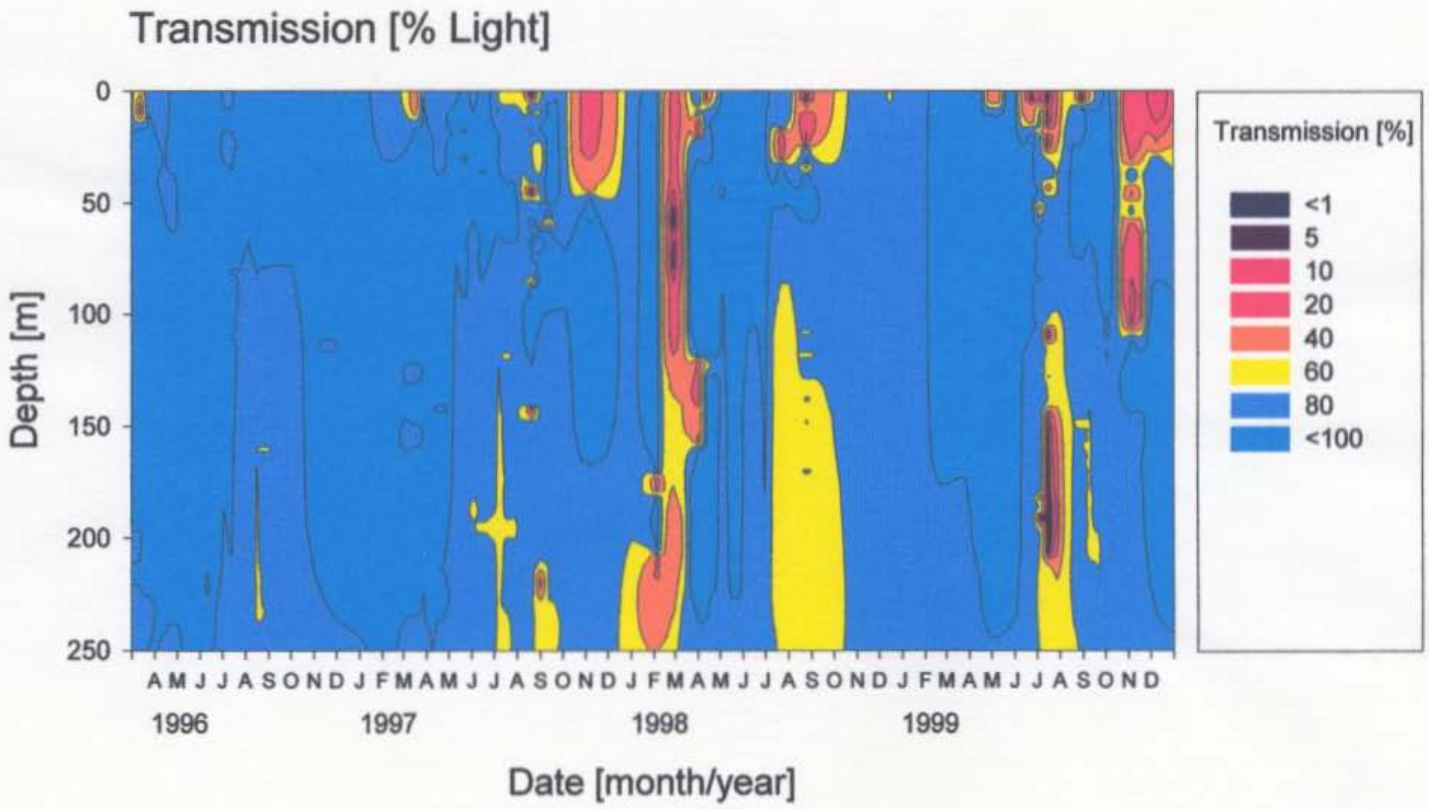

Figure 2.4: Temporal variation of light transmission [%] fom 1996 to 1999 in Conception Bay, Newfoundland, Canada

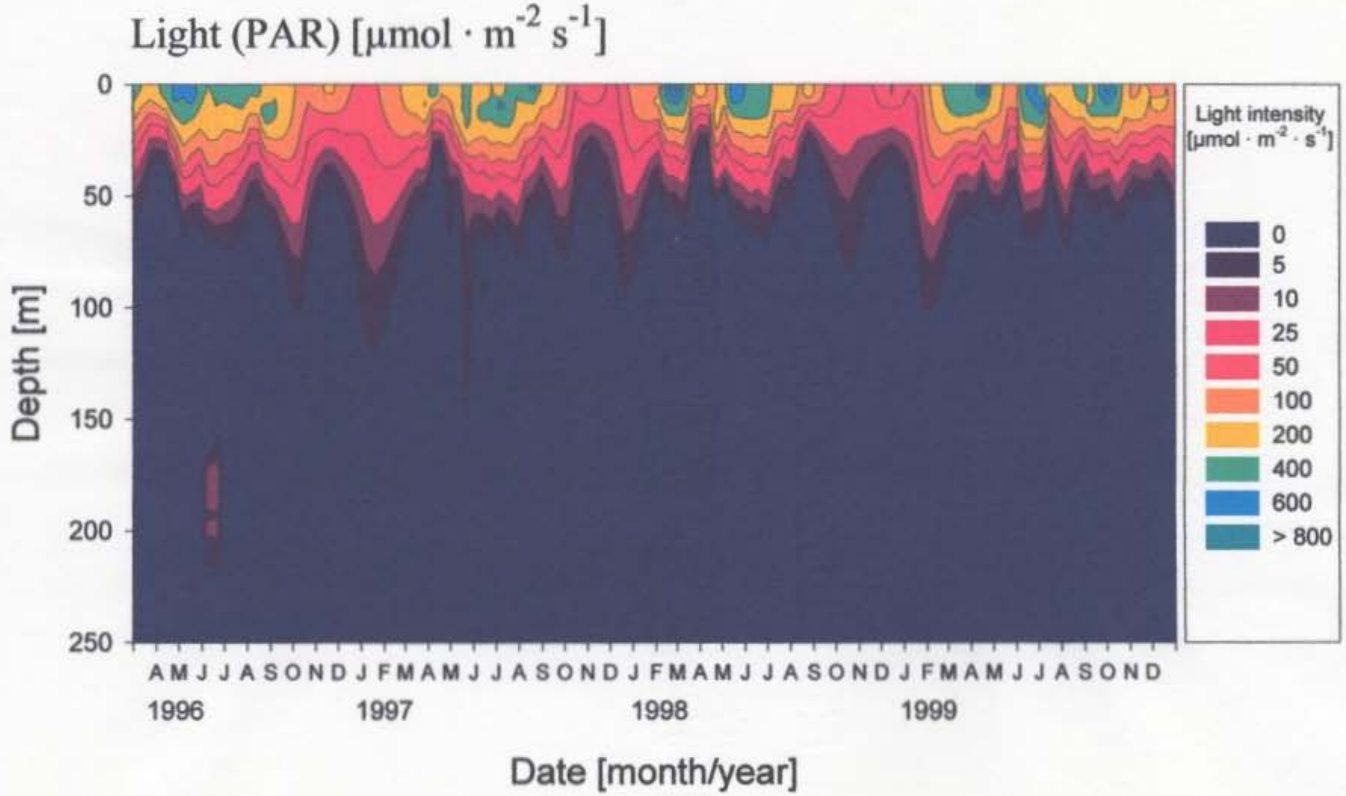

Figure 2.5: Temporal variation in light penetration in Conception Bay, Newfoundland, Canada. Corresponding values span the years 1996 to 1999.

2.3.1.5. Chlorophyll *a*

Chlorophyll *a* concentration in Conception Bay from 1996 to 1999 ranged from 0.138 to 5.090 $\mu g \cdot l^{-1}$ and averaged 0.414 $\mu g \cdot l^{-1}$ (S.D. = 0.399) (Fig. 2.6). Maximum values were always observed between 24 and 50 metres during the third week of April of each year, and reached 3.370, 4.260, 5.090 and 2.036 $\mu g \cdot l^{-1}$ in 1996 to 1999 respectively. Lowest values were invariably observed near the surface during the summer months and below 60 metres from June- July to late December.

The development of the spring bloom was usually abrupt, starting in mid-March or early April and extended to mid-April or May of each year. An exception to this pattern was observed in 1997, when the spring bloom showed a gradual development starting as early as mid-January, with values chlorophyll *a* above 0.6 $\mu g \cdot l^{-1}$ from the surface to around 40 metres depth.

A smaller, secondary bloom can be observed by following the 0.6 $\mu g \cdot l^{-1}$ isopleth, although this bloom is more patchy and variable from year to year. In 1996, the secondary bloom was observed from mid-August to mid-September. This pattern was repeated in 1997, and included a third bloom event from mid-October to November. However, in 1998 the secondary bloom started in early July and extended to late September, peaking in late August with chlorophyll *a* concentrations near 1 $\mu g \cdot l^{-1}$. In December of the same year a less prolonged increase was observed but chlorophyll *a* again reached concentrations up to 1 $\mu g \cdot l^{-1}$. Standing stock was in 1998, which contrasts with the lower values and shorter events seen in 1999. The secondary bloom in 1999 started developing in July and extended throughout August with a peak above 1 $\mu g \cdot l^{-1}$ chlorophyll *a* in mid-July.

Phytoplankton bloom fallout events can be clearly observed by following the 0.3 $\mu g \cdot l^{-1}$ isopleth which appears from around 50 metres to the bottom shortly after the full development of the primary bloom in early April to around June of each year. In addition,

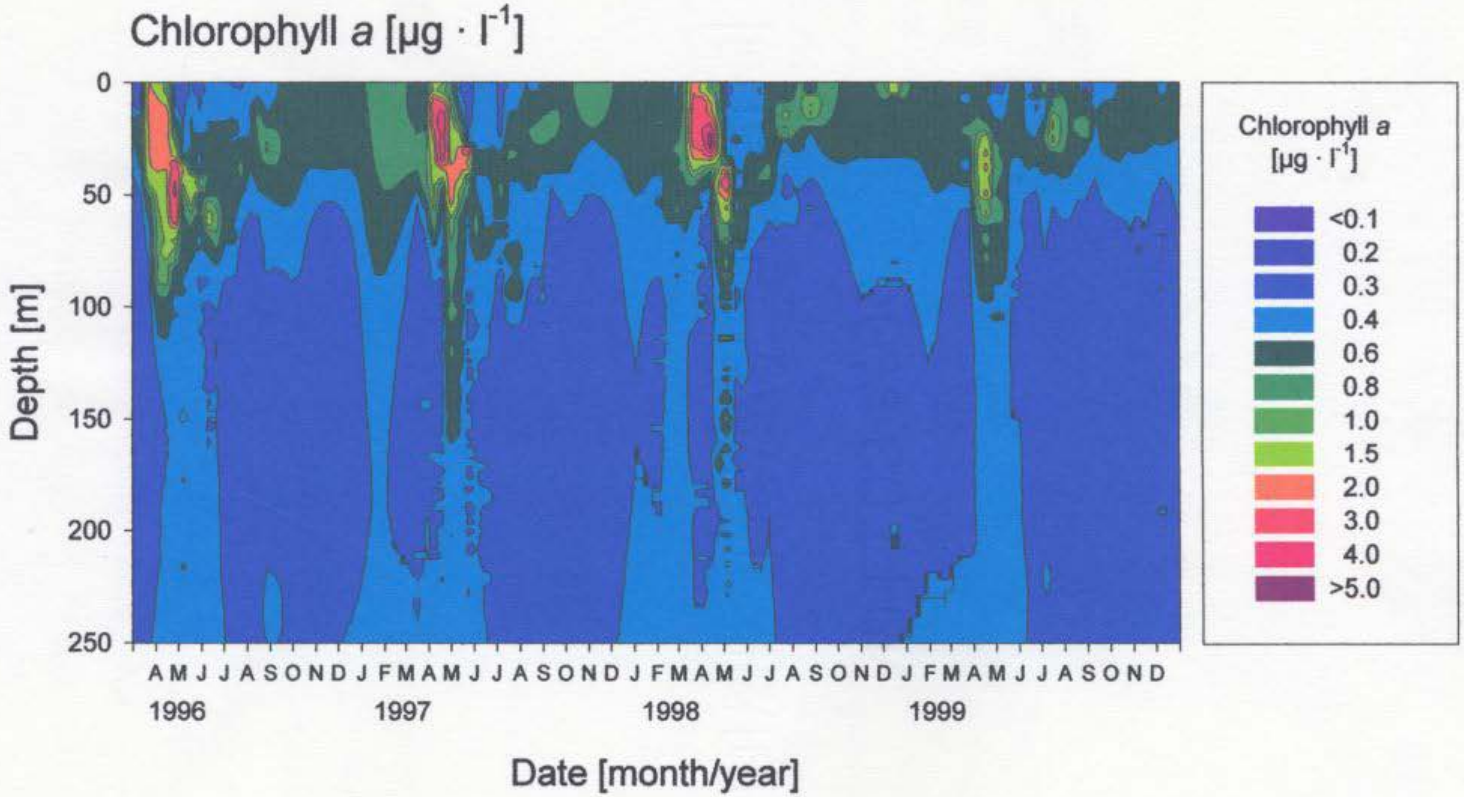

Figure 2.6: Temporal variation in water column chlorophyll *a* concentration for Conception Bay, Newfoundland for the years 1996 to 1999.

an earlier, secondary sinking event in 1997 and 1998 probably occurred from January to early March of both years. However, an increase of chlorophyll *a* concentrations above 0.3 $\mu g \cdot l^{-1}$ were observed below 200 metres before the sinking events from around early January to early June of each year.

2.3.2. Protein content and enzyme assays

The mean protein concentration in the digestive gland of *Yoldia hyperborea* was 0.0610 $mg \cdot mg^{-1}$ DG (S.D. = 0.0264) (Fig. 2.7). Lowest monthly means were observed in April 1997 (0.0118 $mg \cdot mg^{-1}$ DG), whereas maximum means occurred in May and October of that year (0.0957 and 0.1061 ± 0.0121 $mg \cdot mg^{-1}$ DG). High activities of acid protease and α-amylase were recorded from April to May 1997, sharply decreasing in late May 1997 (Figs. 2.8 & 2.9). From late August 1997 to early February 1998 activity levels remained around 2 $U \cdot mg^{-1}$ and more than doubled in late February, only to decline steadily until early July. An increase was observed in late August 1998 and values progressively declined thereafter.

Both α-amylase and acid protease activity followed a similar pattern throughout the study period (*cf.* Figs. 2.8 and 2.9). Mean activity of α-amylase was 3.90 $U \cdot mg^{-1}$ (S.D. = 2.86), whereas maximum activity was observed at the end of April 1997 (12.81 $U \cdot mg^{-1}$). Lowest α-amylase activity was observed in May 1997 (0.93 $U \cdot mg^{-1}$). In 1998 activity of this enzyme showed very little fluctuation, ranging between 1.97 in June and 4.10 in July.

Acid protease activity ranged between 0.71 (May 1997) and 12.25 $U \cdot mg^{-1}$ (April 1997) but averaged 3.80 $U \cdot mg^{-1}$ (S.D.= 2.79) throughout the period. As with α-amylase, acid protease activity did not fluctuate as strongly in 1998, showing values in the range of 1.96 (July) and 5.19 (February).

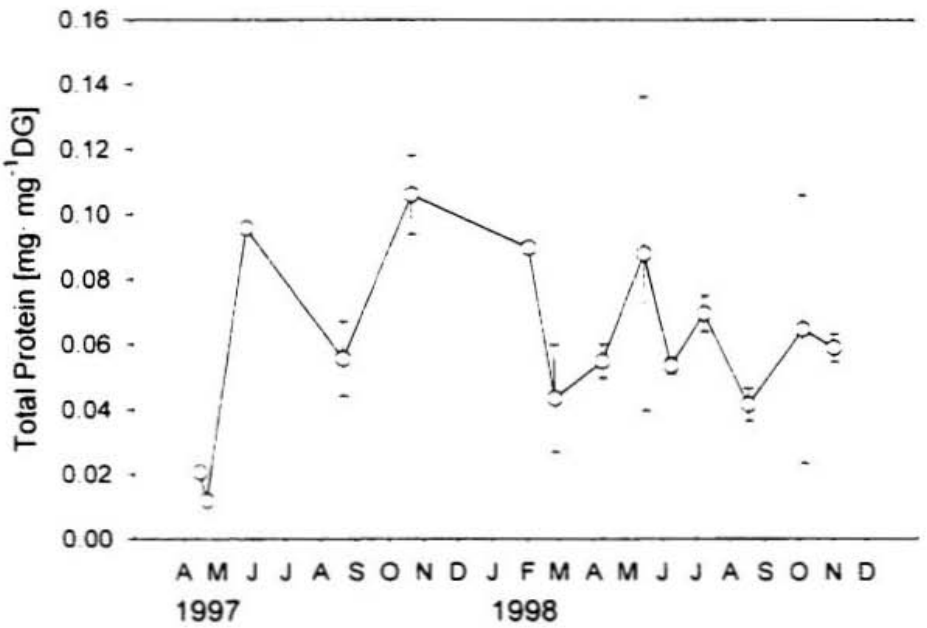

Fig. 2.7: *Yoldia hyperborea*. Temporal variation of mean digestive gland total protein content [mg·mg^{-1} DG] (± S.D.) from individuals sampled in Conception Bay, Newfoundland. Values from April 1997 are single values from pooled digestive glands (n=5), whereas . Standard deviations in May 1997 and early February 1998 are smaller than the plotted points.

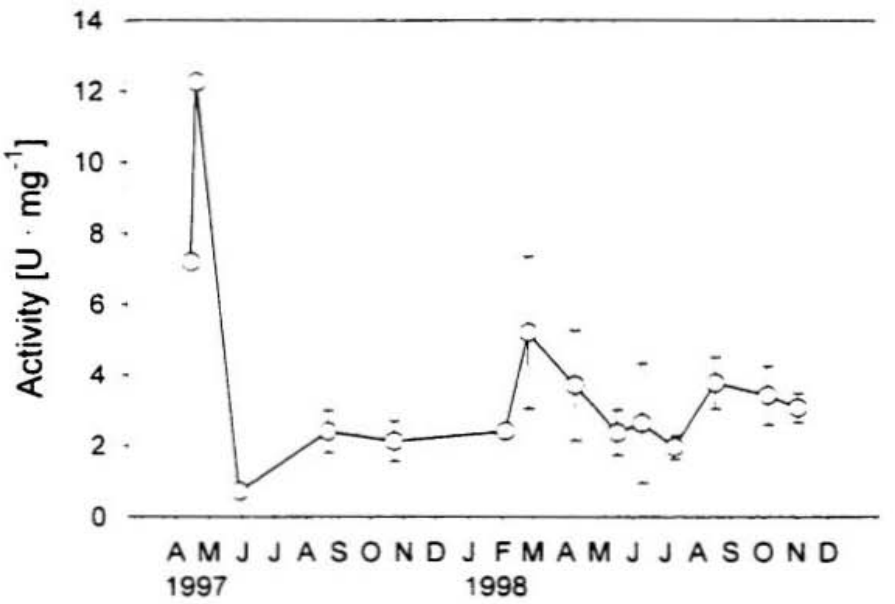

Fig. 2.8: Relative activity of acid protease (U · mg^{-1} protein in extract) ($\bar{x}$ = S.D.) from the digestive gland of *Yoldia hyperborea* individuals from Conception Bay, Newfoundland. Values from April 1997 are single values from pooled digestive glands (n=5). Standard deviations in May 1997 and early February 1998 are smaller than plotted points.

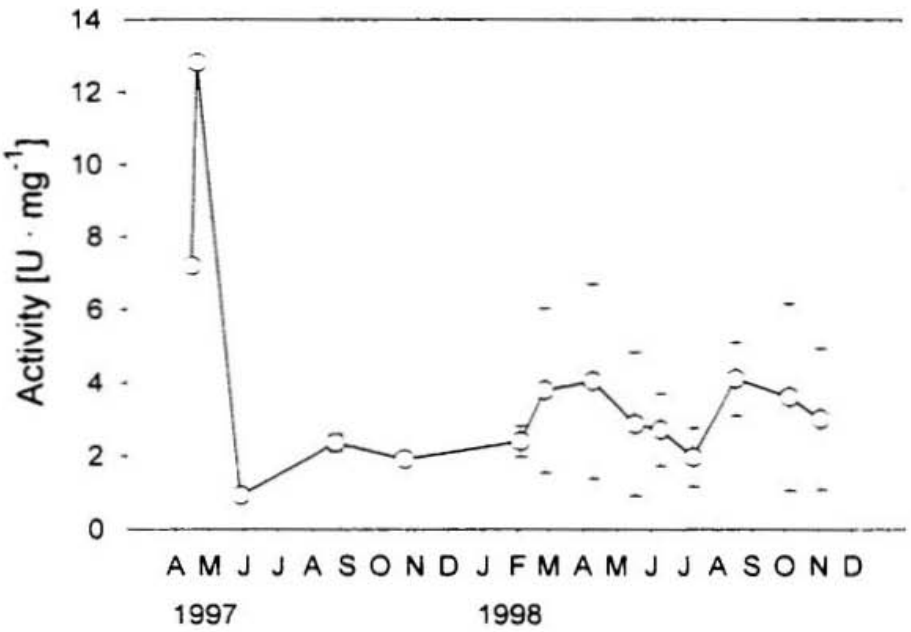

Fig. 2.9: Relative activity of α-amylase (U · mg^{-1} protein in extract) ($\bar{x}$ = S.D.) from the digestive gland of *Yoldia hyperborea* individuals from Conception Bay, Newfoundland. Values from April 1997 are single values from pooled digestive glands (n=5). Standard deviations in May and October 1997 are smaller than plotted points.

2.3.3. Digestive gland index

Although no data were obtained for April and May 1997, the digestive gland was green in colour, presumably because *Yoldia hyperborea* had been feeding on algal cells. However, in August the tissue had become light brown, suggesting a change in diet. In July 1997 the digestive gland index was 14.42%, decreasing to a low of 10% from October to January 1998 (Fig. 2.10). In 1998 the DGI increased steadily from February to early April, when it reached 15.24%. An abrupt loss to 9.91% was observed in May, but was regained in early June (17.77%). In July 1998 the DGI dropped to 9.14% and remained at this level until the end of the study period in January 1999.

2.3.4. Histology

Although qualitative observations were not the purpose of this study, qualitative descriptions have been included for comparison with studies done on other species.

Digestive tubules from *Yoldia hyperborea* were well organised, with the prominent digestive cells being easily distinguishable from the basiphil cells (Fig. 2.11). A conspicuous feature was the large number of darkly staining inclusions in the basal region of the digestive cells. The principal feeding and digestive phases in the digestive cells appeared to be synchronised and did not vary significantly between individuals sampled at the same time.

Although the digestive phases described by Owen (1966) and Langton (1975) correspond to short-term changes occurring within hours of food intake, in *Yoldia hyperborea* each phase seemed to progress in an annual cycle as food energy was ingested, stored and later used as food quality decreased. In tissue sections the phases of the digestive

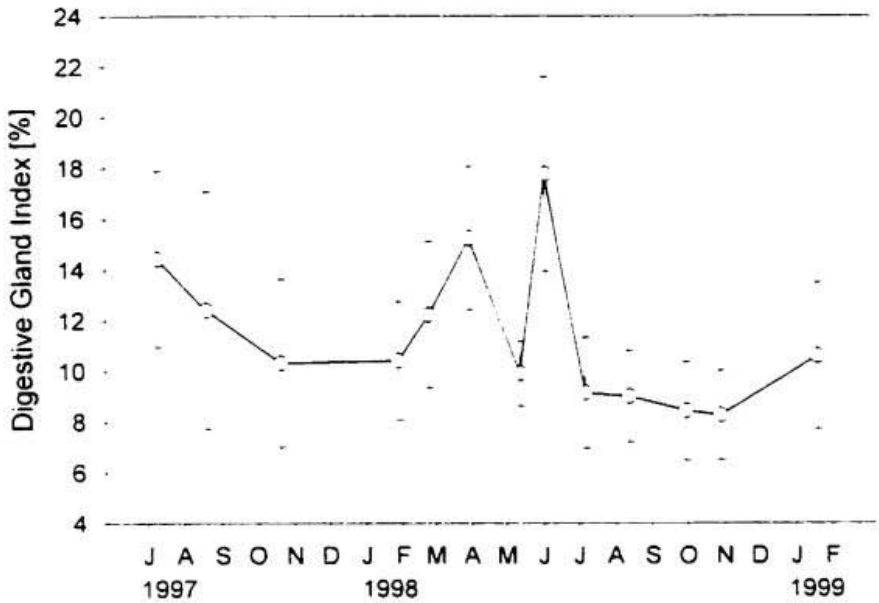

Fig. 2.10: *Yoldia hyperborea*. Fluctuation of the Digestive Gland Index (DGI) ($\bar{x}$ ± S.D, n= 30) from individuals obtained between July 1997 and January 1999 from Conception Bay, Newfoundland.

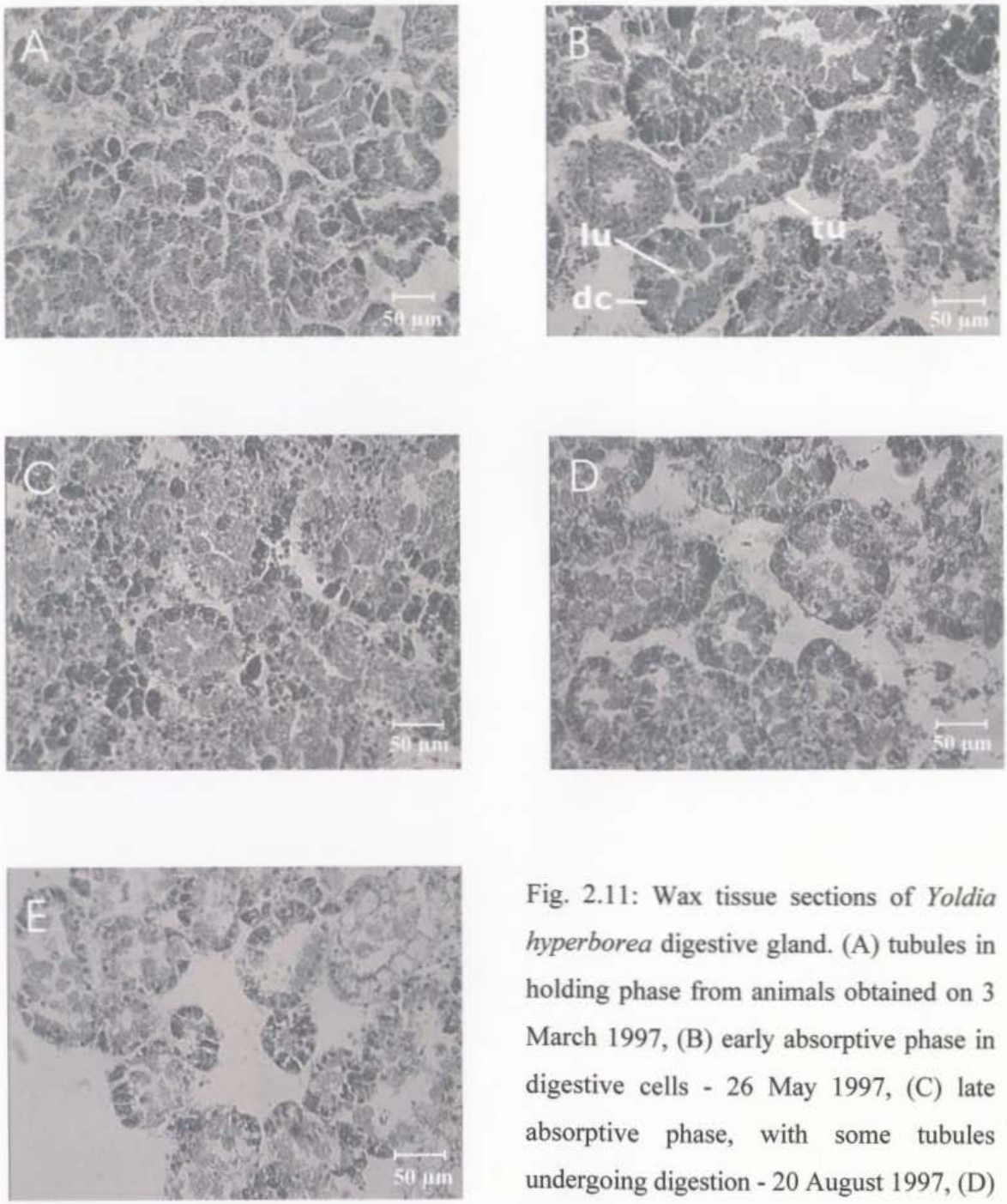

Fig. 2.11: Wax tissue sections of *Yoldia hyperborea* digestive gland. (A) tubules in holding phase from animals obtained on 3 March 1997, (B) early absorptive phase in digestive cells - 26 May 1997, (C) late absorptive phase, with some tubules undergoing digestion - 20 August 1997, (D) disintegrating/digestive phase - 21 October 1997, (E) reconstitution of tubules from animals obtained on 31 March 1998. dc = digestive cell, tu = tubule, lu = lumen

tubules (holding, absorption, disintegrating and reconstituting, *sensu* Langton 1975) were strongly related to the digestive cell height (see Fig. 2.12). A holding phase was initially seen in digestive tubule cells from April 1997 which rapidly increased in size as food was stored in the cells, giving way to the absorptive phase. Cells continued the absorptive phase until late August when they were so large that the lumina of the diverticuli were hardly visible. However, this late absorptive phase also contained some tubules undergoing disintegration. The disintegrating phase became more apparent shortly afterwards as cell height decreased and the tubule lumen became visible again. As stored nutrients in the cell became depleted the tubules entered the reconstituting phase, reaching the holding state in late March after which a new cycle began. However, in 1998 absorption in the cell was not as marked, leading to a more ambiguous interpretation of the different phases.

Digestive gland cell height showed a seasonal pattern with smaller cells observed in winter and early spring and larger cells occurring in summer (Fig. 2.12). Mean cell height throughout the period was 23.67 µm (S.D.= 4.96) with highest values in July and August 1997 (33.72 µm and 34.31 µm, respectively) and again in July and August 1998 (26.47 µm and 25.77 µm, respectively). Cell height was at its minimum in early April 1997 (18.15 µm) and in late March 1998 (19.03 µm). Because qualitative observations also indicated that tubule diameter was directly proportional to cell height (see stages in Fig. 2.11), it is suggested that this measurement may be used as an alternative or an addition to cell height.

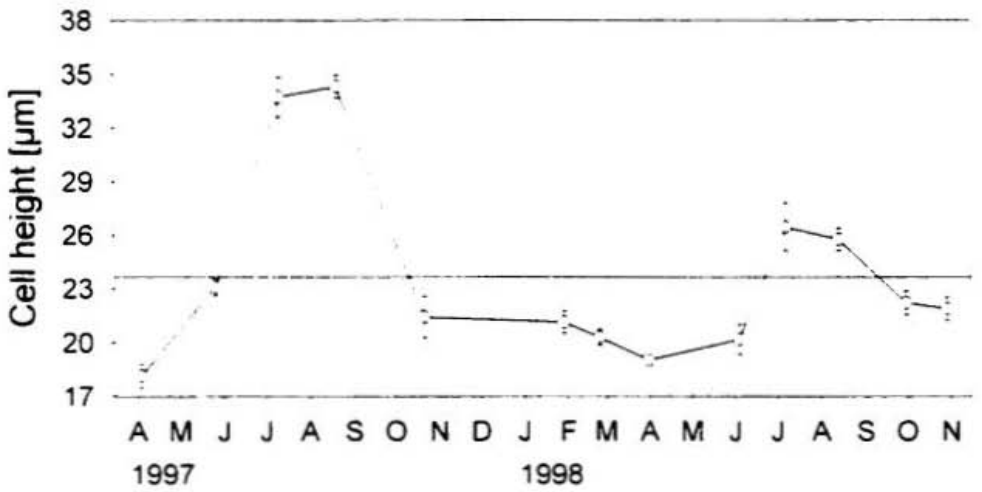

Fig. 2.12: *Yoldia hyperborea.* Temporal variability of mean digestive gland cell height ($\bar{x}$ ± S.D.) from a population in Conception Bay, Newfoundland sampled between April 1997 and November 1998. Standard deviation in late March 1998 are smaller than plotted points. Horizontal line indicates the mean cell height for the entire study period (23.67 µm).

2.4. DISCUSSION

The primary phytoplankton bloom in Conception Bay started to develop between mid-March and early April, when the water temperature was below 0°C and photosynthetically active radiation was at its highest. The bloom continued until it was disrupted by an increase in water temperature followed by water column stratification in mid-April or May of each year. This pattern is recurrent in this area, as de Young and Sanderson (1995) showed that the head of the bay is normally sheltered from the winds in spring, leading to reduced mixing and increased thermal stratification.

The timing of the spring bloom coincided with observations by Deibel *et al.* (1992), who sampled the same area from March to June 1986, 1988 and 1990. Thus, conditions for the development of the primary bloom in Conception Bay may include the disappearance of the thermocline in early winter, water temperature near 0°C and an increase of photosynthetically active radiation to around 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (*cf.* Figs. 2.2, 2.5 & 2.6). Photosynthetically active radiation above 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ was observed in February of each year, but Deibel *et al.* (1992) indicated that light intensity is sufficient in January and February to initiate the bloom, but this does not happen until the winter wind velocity decreases in intensity in late March to mid-April.

In this study, primary bloom maxima always occurred in the third week of April when water temperature was below 0.5°C. However, chlorophyll *a* concentrations were considerably lower than in 1986, 1988 and 1990 when values of 24, 8 and 12 $\mu g \cdot l^{-1}$ respectively, were recorded (Deibel *et al.* 1992). Lowest chlorophyll *a* values were observed in 1999, probably as a result of surface temperature above 0.5 °C from late February onwards. Thus Conception Bay is a cold water system, and although the bay has a mid-latitude light regime, the spring bloom occurs at high-arctic temperatures, a characteristic

only shared in the northern hemisphere with parts of the Sea of Okhotsk (Shiomoto 1997).

The development of a secondary bloom in late summer or autumn was usually associated with water temperatures between 4 and 6°C in 1996, 1997 and 1999. However, in 1998 a bloom that peaked in August occurred when water temperature was between 6 and 10°C, whereas the December bloom occurred at around 2°C. Deibel *et al.* (1992) indicated that the early bloom proceeded through a succession of diatoms beginning with *Skeletonema* spp., followed by *Thalassiosira* spp. and dominated by 17 species of the genus *Chaetoceros*, whereas the prymnesiophyte *Phaeocystis* sp., *Skeletonema* sp. and *Chaetoceros cf. dicepens* (E. Hatfield, pers. comm.) were common in the middle and late bloom.

The phytoplankton bloom fallout to the benthos in early April of each year occurred less than two weeks after its initial development. Chlorophyll *a* concentrations in the bottom 50 metres at this time were 5.89 to 14.73% (1998 and 1999, respectively) of surface concentration maxima. However, an earlier phytoplankton bloom fallout starting in January 1997 and February 1998 was observed in which approximately 50% of surface chlorophyll *a* concentration reached the benthos, resulting in a similar degree of chlorophyll *a* input. The difference in the magnitude of these fallout events is attributable to the higher zooplankton abundance at the time of the primary spring bloom (Davis 1982). Thus grazing pressure in the water column in January and February is relatively low so that most of the algae settles on the bottom floor. According to Pomeroy and Deibel (1986), the bacterial population of Conception Bay utilise particulate material at very low rates when temperature is as low as -0.2°C, which means that most of the primary production is available to zooplanktonic and benthic animals.

A decrease of light transmission from spring to early summer can be explained by the increased phytoplankton concentration followed by a rapid proliferation of microzooplankton and microaggregates (McKenzie *et al.* 1997), as well as the resulting increase in zooplankton

faecal pellet production. Deibel *et al.* (1992) suggest that copepods are not significant grazers in the water column of Conception Bay until June and July because of their temperature-dependent hatching rates. This pattern would lead to increased faecal pellet production after the bloom has subsided, consistent with the timing of increased particle concentration observed in Conception Bay (see Fig. 2.4).

Renewal of bottom water was evident by a water mass of higher salinity observed intermittently below 150 metres. De Young and Sanderson (1985) indicate that high density water enters the bay at sill level (150 m) from late autumn to early winter, and sinks to greater depths, causing renewal. However, this seasonal pattern was not as clearly observed here as the higher salinity water mass persisted throughout most of the period between May 1997 and late August 1999, with a short interruption in the summer months.

Tidal currents are weak in the area (1-2 $cm \cdot s^{-1}$, de Young & Sanderson 1995), but occasional bottom water renewal periods may provide strong convection currents that promote resuspension of fine particulate material, as suggested by the particle increase near the bottom during periods of salinity increase (*cf.* Figs. 2.3 & 2.4). Strong currents may produce a slight but constant bedload transport or resuspension of particulate material and microalgae or may create sufficient turbulence to impede settlement of this matter on the bottom floor. This would also explain why chlorophyll *a* concentration in the bottom 50 metres of the bay (see 0.3 $\mu g \cdot l^{-1}$ contour, Fig. 2.6) is slightly higher than in mid-water throughout most of the year. However, Thomsen and van Weering (1998) indicate critical shear velocities of 0.5 to 1.7 cm s^{-1} for shelf slope sediments from the NW European continental margin. Hence low-speed currents commonly found in Conception Bay would be potentially capable at least of initiating bedload transport of fine particles (see Butman 1987 and Snelgrove & Butman 1994 for discussion of mechanisms). In addition, macrofaunal organisms such as *Yoldia* spp. increases the destabilisation of sediment through movement and reworking during feeding (Rhoads 1963, Bender & Davis 1984, Davis 1993).

thus further decreasing the sediment critical shear velocity (Graf 1992, Snelgrove & Butman 1994). Rhoads (1963), and Bender and Davis (1984) illustrated the overwhelming role of benthic infauna as bioturbators after estimating that a 14 mm (shell length) *Yoldia limatula* individual resuspended approximately 440 g or 23-51 litres of sediment a year.

Resuspension of sediment organic matter also provides higher concentrations of POM for hyperbenthic filter-feeding invertebrates, thus helping to sustain the high biomass typically found in this layer (Mees & Jones 1997). Choe and Deibel (2000) found high concentrations of the chaetognath *Parasagitta elegans* one metre off the bottom of Conception Bay. High zooplankton concentrations have also been observed in the first 10 metres of the hyperbenthic layer, including species such as *Calanus finmarchicus*, *C. glacialis* and *C. hyperboreus*, which attain densities ten times higher one metre above the bottom (~250 metres depth) than at 50 to 175 metres depth in the water column (Choe 1999).

An unusual event was observed on 24 February 1998. At this time salinity had reached its lowest point in the top 50 metres, whereas temperature (0°C) was completely uniform throughout the water column to 180 metres and was also homogenous below that depth between March and early May of that year (see 0°C isopleth, Fig. 2.2). A high particle load was also observed throughout the water column until early March, whereas dredge samples and sediment cores from May showed that the top 25 cm of sediment had been completely mixed as evidenced by its high water content and low compactness. Such conditions were also found in the top 7 cm and 3 cm of sediment cores from June and July 1998, respectively (Elizabeth Hatfield, pers. comm.) but not in February, which suggests that the anomaly observed in late February was related to movement in the benthic boundary layer around a month later, probably as a result of convective forces (see Fig. 2.2). Animals obtained in May 1998 were sluggish and showed a strong tendency to gape, which resulted in 100% mortality within a week of collection. Sediment disturbance of this magnitude was

not observed throughout the rest of the study period and has not been reported in previous years. No unusual or prolonged periods of high wind were observed for February to May 1998, suggesting the presence of an unusually strong intrusion from the Labrador Current into the bay, a view also supported by bottom salinity data (Fig. 2.3). However, an alternative explanation is that the anomaly observed in the water column as from February 1998 triggered bottom turbidity currents, leading to strong sediment disturbance and particle resuspension. Particulate material, collected shortly after the bloom peak of April 1996 and April 1998 from sediment traps deployed between 50 and 220 metres in the same area as this study, indicated 3 times more material in April 1998 than in to April 1996 (C. C. Parrish, unpublished data). Lipid analysis of particles captured in April 1996 showed a high content of total PUFAs (>32.8%) from 80 and 220 metres depth. In addition to low bacterial fatty acids (2.40 and 2.41%, respectively), low lipolysis (LI = 14.72 and 19.25%, respectively) and hydrolysis (HI = 19.79 and 33.79%, respectively) indexes were also observed (see Weeks *et al.* 1993, Parrish *et al.* 1995), indicating a high concentration of fresh and relatively undegraded material throughout the water column. However, trap material obtained in April 1998 from 50 and 100 m contained a lower concentration of total PUFAs (23.18 and 16.14%, respectively) and also showed higher lipolysis (LI = 20.55 and 32.90, respectively) and hydrolysis (36.22 and 51.91%, respectively) indexes with increasing depth, although bacterial fatty acids were approximately the same as in 1996 (2.88 and 2.44%, respectively). These results indicate that in April 1998 sedimenting material at 100 metres was of poorer quality than surface particles (50 m), although this difference cannot could not be ascribed to increased bacterial degradation (see also Pomeroy & Deibel 1986) and may be attributable to massive resuspension of bottom sediments.

The digestive gland of *Yoldia hyperborea* from Conception Bay shows marked seasonal variation, particularly in digestive cell height, which is interpreted as a cycle of uptake, storage and use of energy reserves. Changes in the biochemical composition of the digestive gland in *Mytilus edulis* led Thompson *et al.* (1974) to suggest it stores metabolic

energy reserves which are subsequently transferred to the gonad during gametogenesis. Carbohydrates accumulated in the digestive gland are transferred to the gonad during vitellogenesis or in response to stress. Furthermore, when stock of carbohydrates reserves are low, lipids are synthesized and stored for subsequent distribution to other tissues (Vassallo 1973, Thompson *et al.* 1974, Sastry 1979). In addition, similarities between fatty acid composition of the triglyceride fraction (TAG) of the gonad and the digestive gland in the scallop *Argopecten purpuratus* demonstrate the transfer of lipids from the lipid-rich digestive gland to the gonad for gametogenesis (Caers *et al.* 1999). However, the digestive gland also plays a major role in the storage of lipids originating from phytoplankton (Soudant *et al.* 1999).

An increase in digestive cell volume was observed after April 1997 and 1998, as soon as the phytoplankton cells from the spring bloom fallout reached the benthos, and values increased dramatically thereafter, suggesting a storage of metabolic reserves until late August 1997 and July 1998. Ibarrola *et al.* (2000b) demonstrated that the presence of food affected significantly the characteristics of the lysosomal system of *Cardium edule* in less than three days, and that the lysosomal volume density was significantly lower in starved than in fed cockles. Furthermore, the lysosomal volume density appeared to increase with improved feeding condition.

Yoldia hyperborea gonad tissue analysed from the same samples by Jaramillo (2001) show an increase in developing gametes in August and early October 1997 and May and August to September 1998, coinciding with the period when digestive cell volume declines sharply in each year.

The digestive gland index (DGI), however, showed a different pattern, as evidenced by its decline as early as July 1997 and June 1998. An increase in DGI was observed after February 1998 and in February 1999, probably as a result of the early phytoplankton fallout

observed in those months. However, this increase did not result in the accumulation of energy reserves, as digestive cell height was at its minimum, and the observed increase in DGI therefore must be a result of an increase in the number of digestive cells and proliferation of tubules; qualitative observations during dissection of individuals and examination of tissue sections indicated a proliferation of digestive gland tissue at this time. According to Moore and Lowe (1985), the digestive gland may undergo autolytic changes during stress or starvation (*e.g.* Thompson 1974, Bayne *et al.* 1978) but may be reconstituted once conditions improve.

Results obtained here strongly suggest that *Yoldia hyperborea* depends on the annual cycle of phytoplankton production for storage of metabolic reserves, growth and reproduction. The less productive months of the year (*i.e.* autumn and winter) were marked by utilization of what was left of the metabolic energy reserves in the digestive gland and a decrease in size of the digestive tissue. Thompson *et al.* (1974) also noted a decrease in size and number of the digestive cells of laboratory fed *Mytilus edulis* and also found that the structural integrity of digestive tubules was lost in individuals starved for 5 months.

Although the pattern of phytoplankton production in both years was somewhat similar, the magnitude of metabolic reserve accumulation was very different in 1997 and 1998. Smaller cell height in 1998 could have been the result of differences in phytoplankton composition and thus of different qualitative characteristics. However, this seems unlikely as no marked differences were observed in the algal composition of blooms occurring in both years (Elizabeth Hatfield, personal communication). Cumulative standing stock could also have been less in 1998 as compared to 1997, and although this possibility cannot be ruled out, integration of snapshot sampling throughout these years suggests otherwise (*cf.* Figs 2.4 & 2.6). A more probable explanation can be found in stress induced by the strong sediment disturbance that occurred in early May, which also explains the sharp decline in digestive gland index observed during that month. Animals that survived such a disturbance may have

been unable to feed until most of the particle load had settled. Observations during subsequent feeding experiments with *Yoldia hyperborea* (see Chapter 4) indicated no faecal production and increasing high mortalities when suspended particle load was higher than 84.5 $mg \cdot l^{-1}$ for prolonged periods (>96 hours). Because the disturbance likely resuspended at least the top 25 cm layer of sediment, it would also have released entrapped H_2S (see Graf 1992), further affecting *Y. hyperborea*.

The stages of the digestive process described by Langton (1975) in mussels were not observed occurring at the same time in *Yoldia hyperborea* tubules, indicating synchrony of the cells (see Fig. 2.11). Langton (1975) related changes in the condition of the digestive tubules to the periods of exposure and submersion of mussels. Individuals affected by increasing exposure showed more tubules in the holding phase, whereas during submersion the absorptive phase was more common. However, Taïeb (2001) suggested that during *ad libitum* feeding part of the cycle cannot be observed because digestive processes occur in a short and well defined period of the digestive cycle. Thus during feeding periods the digestive cells will be mainly in the absorptive phase whereas the disintegrating and reconstituting phases are dominant when food availability is reduced and stored energy is made available.

As *Yoldia hyperborea* is a subtidal species, phasic activity in the digestive cells are not likely to occur during the tidal cycle, but rather over a longer period. Only as food is depleted or its quality decreases will the digestive cells enter a predominantly disintegrating phase. Ibarrola *et al.* (2000b) showed that *Cerastoderma edule* fed high quality diets for three days had significantly higher proportions of tubules in the absorptive phase and lower proportions in the disintegrating phase than those fed low quality diets. Food quality is therefore a significant factor in determining digestive tubule stage.

Low protein content of the digestive gland in April 1997, together with qualitative

and quantitative histological examination, indicate that animals were at the end of a period of low food availability, coinciding with a reduction in digestive cell size. The sharp increase in protein content (~ 9 fold) in late May of that year shows that growth of the digestive gland coincides with the arrival of the spring bloom fallout. Ibarrola *et al.* (2000a) found that protein is absorbed with very high efficiency by the bivalve *Cerastoderma edule*, especially when food quality is low. In addition, digestive gland protein levels rise with enhanced food quality (Ibarrola *et al.* 1998b). Protein content of the digestive gland from *Y. hyperborea* decreased shortly after an increase in developing gametes was observed in late August of each year (Jaramillo 2001), a pattern observed in other bivalve species and which may be linked to the high protein demands of gametogenesis (reviewed by Newell and Bayne, 1980). During this time the digestive cells were still large but some tubules had started to disintegrate (*cf.* Fig. 2.11.C).

The next important drop in digestive gland protein content was observed towards the end of February 1998 as digestive cells were reaching their minimum size and the early bloom fallout was beginning to reach the benthos. During the early- and spring bloom fallout of 1998 protein content doubled but varied strongly between individuals, probably as a result of a differential effect on individuals of the sediment disturbances observed in May.

Attempts to establish a correlation between feeding mode and digestive capacity in benthic invertebrates have been contradictory. Whilst Pal *et al.* (1980) and Teo and Sabapathy (1990) reported high carbohydrase and very low protease activity in mussels, Ibarrola *et al.* (2000a) indicated high protease activities for the suspension-feeding bivalve *Cardium edule*. On the other hand Brock and Kennedy (1992) found no differences in enzymatic activity between suspension and deposit- feeding bivalves. Owen (1974) noted that extracts of a number of bivalve species show relatively weak proteolytic activities. Although in this study α-amylase activities were similar or lower than acid protease activity, α-amylase activity its activity may have been underestimated due to degradation by proteases

in the extract.

It appears that in the majority of molluscs the secretion of enzymes is a continuous and frequently rhythmic process accelerated by feeding (Morton 1983, Bayne *et al.* 1989, Brock 1989). In addition, enzyme composition and activity levels tend to reflect the biochemical nature of the digestible food substrate, regardless of animal feeding mode (Mayer *et al.* 1997). For example, Stuart *et al.* (1985) demonstrated that in the amphipod *Corophium volutator* enzyme production was regulated by external factors such as food availability and composition.

The ability to regulate the rate of digestive enzyme production has frequently been proposed as a possible mechanism that tends to maximise absorption (Bayne *et* 1987,1989, Navarro *et al.* 1994). Moreover, advantages derived from increasing digestive investments in response to enhanced food quality have been analysed in terms of an optimal feeding behaviour (Willows 1992). Changes in the rate of digestive enzyme production have been considered to operate only as a long-term response in bivalves (Newell *et al.* 1980). However, a few studies have attempted to establish a relationship between digestive enzyme activity and nutritional status in bivalves. Brock (1989) observed that digestive glands of *Crassostrea gigas* individuals acclimatised for three weeks to a diet of *Tetraselmis suecica* showed a significantly higher capacity to hydrolyse phytoplanktonic cells than did glands of starved oysters. By contrast, Ibarrola *et al.* (1996) described variations in total cellulase activity in the digestive gland of the cockle *Cerastoderma edule* (L.) during a short-term response to simultaneous changes in food organic content and particle concentration, and showed that the acute response to increasing food organic content included an increase in digestive gland weight as well as an adjustment in digestive enzyme activities. This digestive response may be considered a maximising strategy, as digestive investments tend to increase with increasing quality of available food.

In April 1997 activities of digestive gland enzymes were at their highest in *Yoldia hyperborea*. It is likely that the animals at this time had undergone a prolonged period of low quality food as the digestive cells attained the minimum size and tubules were in the holding phase. Experiments in which the bivalve *Cerastoderma edule* was starved for 20 days and then fed showed that a long starvation period before feeding induced a rapid increase in protease and a gradual but significant increase in amylase activity (Ibarrola *et al.* 1999). However, upon resumption of feeding, protease activity quickly returned to the pre-starvation levels (Ibarrola *et al.* 1999). A similar pattern was also observed in *Yoldia hyperborea*: in May 1997 activities of acid protease and α-amylase reached the lowest values recorded, although chlorophyll *a* concentration and digestive cell size had started to increase. Ibarrola *et al.* (1999) suggested that since most protein digestion in bivalves occurs through the action of lysosomal proteases (Reid & Rauchert 1972, 1976), the recorded fast reaction of proteases may reflect activation of the formerly depressed lysosomal system.

Acid protease and α-amylase activity remained relatively constant from August 1997 to the beginning of February 1998, then doubled in the third week of February. This pattern suggests an increase of food quality in late February 1998, consistent with the earlier conclusion that algae had settled in the early months of 1998. Although stronger activities were observed at the end of winter of each year, minor increases were observed between June and late August 1997 and between July and late August of 1998, coinciding with the times that the secondary summer bloom fallout reached the benthos. Ibarrola *et al.* (1999) also found that enzyme activity in the digestive gland of *C. edule* was greater in winter (*i.e.* after prolonged period of starvation), especially in the case of protease.

Thus activities of acid protease and α-amylase are potential indicators of the physiological state of *Yoldia hyperborea*, high activities occurring after periods of starvation or decreased food quality. The magnitude of the difference between low- and subsequent high activities may be a good indicator of the degree of reduced food quality in the preceding

months. As *Yoldia hyperborea* had accumulated a lot of energy reserves during the spring and early summer of 1997, low food quality in subsequent months did not have a large impact on the physiological state of the animals, which explains why enzymatic activity in late February of 1998 did not increase as sharply as in April of 1997.

CHAPTER 3

PHYSIOLOGICAL ENERGETICS OF *YOLDIA HYPERBOREA*

3.1. INTRODUCTION

The physiological ecology of marine organisms inhabiting extreme conditions is important for understanding the adaptations of these animals to limiting habitat conditions. Most physiological studies of bivalve molluscs have been done on lamellibranch species inhabiting temperate and tropical areas, and only a few studies have centred on cold water species. In addition, few studies have focussed on protobranch bivalves, and have only addressed one aspect of physiological energetics such as oxygen consumption or nitrogen excretion (Gray & Follum 1987).

Protobranch bivalves show some morphological and physiological differences from lamellibranch bivalves. For example, it is possible that their respiratory adaptations have enabled them to dominate lamellibranchs in extreme habitats such as deep-sea sediments and the muds of the continental shelf in which they deposit feed. Since gills of all protobranchs have a smaller surface area than those of lamellibranchs, it could be assumed that they would be restricted to well oxygenated habitats. Nevertheless, Moore (1931) demonstrated that the protobranch *Nuculoma tenuis* survived anoxic conditions for up to 17 days, in contrast to the veneroid lamellibranch *Abra abra*, which survived for no more than 3.5 days. *Nuculana sulcata* can survive for two weeks in an oxygen free environment at 10°C (Taylor *et al.* 1995). However, basic aspects of protobranch physiology are still poorly understood and little studied. For example, oxygen uptake experiments have been conducted in

approximately five species (*e.g.* Wilson & Davis 1984, Mangum *et al.* 1987, Davenport 1988a, Bernard & Noakes 1990, Taylor *et al.* 1995), whereas ammonia excretion rates are known for only one species (Gray & Follum 1987), though these parameters are necessary for estimating energy budgets.

Yoldia hyperborea is an infaunal bivalve (Protobranchia: Nuculanidae) inhabiting soft sediments of the circumboreal region and the east coast of Newfoundland, Canada (Cowan 1968, Scheibe 1996). Newfoundland ambient temperature in coastal embayments below the thermocline remains between -1.5 and 0.5°C throughout the year, whereas surface temperature fluctuates between -1.6 and 17°C (de Young & Sanderson 1995). This and other species of the genus *Yoldia* can inhabit reduced muddy sediments with low oxygen tension in the pore water which it can avoid by extending its siphons above the sediment surface or emerging from it altogether (see Bender & Davis 1984, Davenport 1988b).

The characteristic gills of the genus *Yoldia* are dark red in colour as a result of a high concentration of cytoplasmic (intracellular) haemoglobin. Haemolymph however, contains no Hb but a very low concentration of haemocyanin (Angelini *et al.* 1998). These characteristics further differentiate the respiratory capacity of the taxon when compared to lamellibranchs, as the haemolymph of the latter may only possess Hb.

The purpose of this chapter was to investigate the physiological energetics of *Yoldia hyperborea* and thus contribute towards the understanding of protobranch physiology. This knowledge was also necessary in order to design feeding and behavioural experiments requiring maintenance of animals in the laboratory.

3.2. MATERIAL AND METHODS

Specimens of *Yoldia hyperborea* (Lovén) Torrell 1859 were collected at a depth of 240-265 m from the muddy sediments of the deep-depositional zone of Conception Bay, Newfoundland (47°34.0' N, 53°08.1'W to 47°32.5'N, 53°07.8'W) (for area description see de Young & Sanderson 1995) by means of a 1.20 m wide dredge. Collected animals were transported on ice to the Ocean Sciences Centre in Logy Bay and transferred to sediment-containing aquaria placed in a holding tank with recirculating seawater kept at 0.0 °C (± 1.0°C). A constant supply of suspended particles was provided by running a steady flow of ambient seawater into the holding tank. Animals were acclimated for a minimum of 4 weeks before being used for oxygen uptake and excretion experiments.

3.2.1 Oxygen uptake

Oxygen uptake was determined for individual animals (shell length 12.4- 45.1 mm) between May 1997 and May 1998. The experimental setup consisted of sealed acrylic chambers (63.5, 131.5, 216.0 or 367.0 ml) filled with oxygen-saturated, Whatman GF C-filtered seawater at 31 to 35.5 ‰ salinity ($\bar{x}$ = 33.56, S.D.= 1.81). Animals were transferred to a cup overlying a stirbar within the acrylic container which in turn was attached to a submersible magnetic stirrer in order to keep the seawater continuously mixed (Fig. 3.1).

To test the effect of lack of sediment on *Y. hyperborea* O_2 uptake, a few experiments (n = 12) were carried out in which animals were allowed to bury in pre-combusted ambient sediment (450°C, 6 hours) added to the cup. However, subsequent experiments were carried out without sediment, after no difference in O_2 uptake was detected between buried and non-buried animals (see Results).

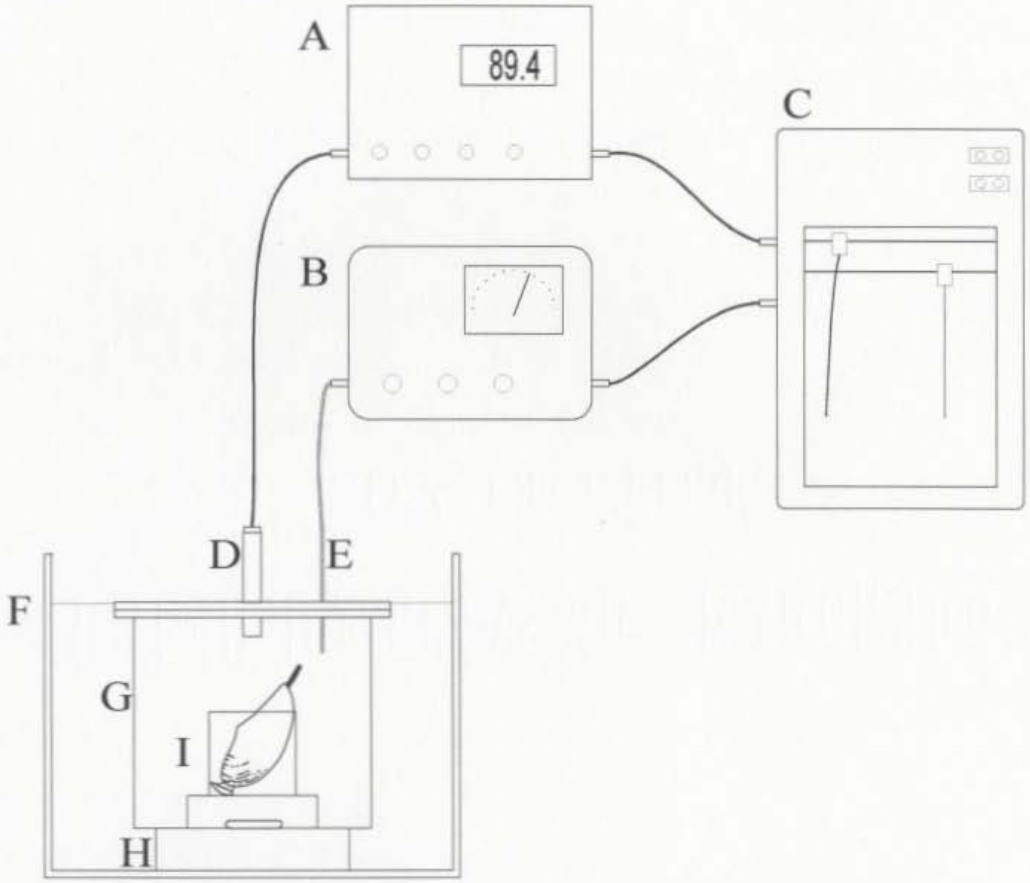

Fig. 3.1: Experimental setup for the oxygen consumption experiments. (A) oxygen meter, (B) temperature monitor, (C) chart recorder, (D) pO_2 electrode, (E) temperature probe, (F) temperature controlled bath, (G) experimental chamber, (H) submersible magnetic stirrer, (I) perforated cup overlying a stirbar and holding the experimental animal. Size of the experimental chamber varied according to animal size. In the experiments that included sediment, a cup without perforations was used to contain the sediment and was large enough to allow animals to bury completely.

Water temperature was permanently kept at -1.0 °C (= 0.1) by submersing the chamber in a temperature controlled bath. A temperature probe connected to a YSI model 42SF telethermometer was inserted into the chamber and values were recorded on a chart throughout the experiment. Oxygen consumption of individual animals was measured, for a period of up to 28 hours, with a polarographic electrode (Radiometer Copenhagen model E5046-O) inserted through a hole in the chamber lid and connected to a Strathkelvin oxygen-meter (Model 781b) which fed the amplified signal to a chart recorder. Dissolved oxygen was generally maintained above 70% of air saturation level to avoid any compensation in O_2 uptake (Bayne 1971). However, in some instances animals were allowed to consume oxygen below 5% pO_2 for 28 to 96 hours in order to determine the response of the animal to declining oxygen tension. Animals were returned to the holding tank after each experiment and only used in a subsequent trial after 72 hours had elapsed. However, no animal was used more than three times and always within 15 days of the first experiment. Oxygen uptake was calculated as:

$$VO_2 = \frac{([\%O_2]_{t_0} - [\%O_2]_{t_1}) \times C \times 0.01 \times (V_{chamber} - V_{animal})}{(t_1 - t_0)}$$

where,

VO_2 = oxygen uptake (ml $O_2 \cdot h^{-1}$)

t_0 = time at start of linear decline (h)

t_1 = time at end of linear decline (h)

$V_{chamber}$ = Volume of chamber in litres (subtracting sediment volume of 65.9 ml when required)

V_{animal} = Volume of animal (litres)

C = solubility of O_2 in seawater at a given temperature and salinity ($ml \cdot l^{-1}$), calculated after Weiss (1970).

Animals were dissected for total dry weight (with shell, TDW), tissue dry weight

(DW), and tissue ash-free dry weight (AFDW) determinations as described for length-weight relationships.

3.2.2 Ammonia Excretion

Ammonia excretion (µg NH_4-N) was determined by the phenol-hypochlorite method of Solórzano (1969), using ammonium sulphate as a standard (Widdows 1985b). Precautions were taken before and during the experiment to avoid contamination by washing all materials with 0.25 N HCl and rinsing three times with deionised distilled water (Milli-Q), which was also used in the preparation of all reagents.

Experimental chambers consisted of plexiglass sealed containers (216 ml) with 100 ml filtered seawater (Whatman GF/F) and a minimum of 100 ml air. These were submerged in a temperature controlled bath at -1.0°C (± 0.1°C). A total of ten chambers was used in every experiment: each of the first seven contained three bivalves of similar size whereas three chambers without bivalves were used as replicate controls. As excretion rates were low, three adult individuals of similar size were placed in each chamber. The experimental animals ranged between 27.93 and 36.51 mm shell length, weighed between 0.221 and 0.308 g DW, and were left without food for a minimum of 48 hours in filtered seawater (0.2 µm) before the measurement period.

Replicate water samples (0.25 ml) were drawn from all containers at 1, 3, 4.5, 7.5, 10.5, 19.5, 26.5, 50.5, 74.5, 95.5, 116.5 and 140.5 hours. After addition of reagents, samples and standards were allowed to stand in darkness for 2 to 24 hours at room temperature before reading absorbance at 640 nm with a spectrophotometer (Beckman DU®-65). Absorbance values were converted to ammonia concentrations (µg NH_4-N) from a standard curve prepared with ammonium sulphate. All animals were dissected after each experiment in

order to record total dry weight (TDW), tissue dry weight (DW) and ash-free dry weight (AFDW). Ammonia excretion rate per unit DW of animals ($\mu g\ NH_4\text{-}N \cdot h^{-1} \cdot g^{-1}$) was calculated according to the following formula:

$$VNH_4\text{-}N = \frac{([E_c] - [C]) \times 28 \times (V_{chamber})}{(\delta t) \times (DW)}$$

where,

VNH_4-N = excretion rate of animals per gram DW ($\mu g\ NH_4\text{-}N \cdot h^{-1} \cdot g^{-1}$)

E_c = Ammonia concentration in the experimental chamber (μM)

C = Ammonia concentration in the control chamber (μM)

28 = 1 μM $(NH4)_2SO_4$ contains 28 $\mu g\ NH_4\text{-}N \cdot l^{-1}$

$V_{chamber}$ = Volume of seawater in the chamber (l)

δt = time between consecutive samplings (h)

DW = total tissue dry weight of animals within each chamber (g)

3.2.3 Length-weight relationship

At the end of each physiological measurement, the soft tissue and shell of each individual were dried separately to constant weight (72 hours) at 60°C and weighed to the nearest 0.0001 g. In addition, ash-free dry weight (AFDW) was obtained separately for shells and soft tissues of each individual after combustion at 450 °C for 12 hours in a muffle furnace.

3.2.4 Statistical Analysis

The relationships between physiological rates and body weight were described by an

allometric equation of the form:

$$Y = aX^b$$

where,

Y = physiological rate ($\mu g\ NH_4\text{-}N \cdot h^{-1}$) or ($ml\ O_2 \cdot h^{-1}$)

X = body dry weight (g)

a = intercept of the regression

b = slope of the regression

Both variables Y and X were transformed to logarithms to reduce the dependance of the sample variance on the mean, and in addition achieved normalisation of data distribution.

Differences in the oxygen consumption (VO_2) of animals with and without sediment were determined by comparison of regression slopes by ANCOVA. SPSS* version 10.0 for Windows* (SPSS* Inc.) was used for all statistical analyses.

3.3. RESULTS

3.3.1. Length-weight relationships

The shell length of animals used for these measurements ranged from 12.4 to 40.3 mm with a mean of 25.67 mm (S.D.= 7.10, n= 75), whereas their tissue dry weights ranged from 0.011 to 0.409 g ($\bar{x}$ = 0.134, S.D.= 0.103, n=75). The relationship between shell length and tissue dry weight of *Y. hyperborea* showed that DW increased exponentially with shell

length (a= $3.07 \cdot 10^{-5}$, b= 2.548, n= 75) (Fig. 3.2 and Table 3.1). Ash-free dry weight (AFDW) of tissue from the same animals ranged between 0.005 and 0.362 g ($\bar{x}$ = 0.098 g, S.D.= 0.099, n=75). The shell represented 63.16 to 80.92 % of total body dry weight ($\bar{x}$ = 71.83%, S.D. = 5.76, n = 75). Tissue AFDW increased almost proportionally with tissue dry weight (a= 1.170, b= 1.280, n= 75) (Fig. 3.3, Table 3.2).

3.3.2. Oxygen consumption

As oxygen uptake by *Yoldia hyperborea* showed no differences for measurements taken between animals buried in sediment and those without sediment (Table 3.3), all data were pooled in one regression equation (Figure 3.4, Table 3.4).

Oxygen consumption increased with animal size (tissue DW) (Fig. 3.4, Table 3.4). A highly significant linear regression of log VO_2 versus log dry tissue weight was obtained ($P < 0.0001$, $r^2 = 0.887$, n= 55) and defined by the equations:

$$\log_{10} VO_2 = -0.294 - 0.735 \cdot \log_{10} DW$$

or $$VO_2 = 0.051 \cdot DW^{0.735}$$

where,

VO_2 = oxygen uptake (ml $O_2 \cdot ml^{-1}$)

DW = individual dry weight (g)

Yoldia hyperborea showed a high level of respiratory independence under hypoxic conditions, since VO_2 was independent of pO_2 from 5 to 100% saturation (Fig. 3.5). Mean oxygen consumption for a 1 g (DW) individual under these conditions was 0.051 ml O_2 (± S.D. 0.002) (Fig. 3.5).

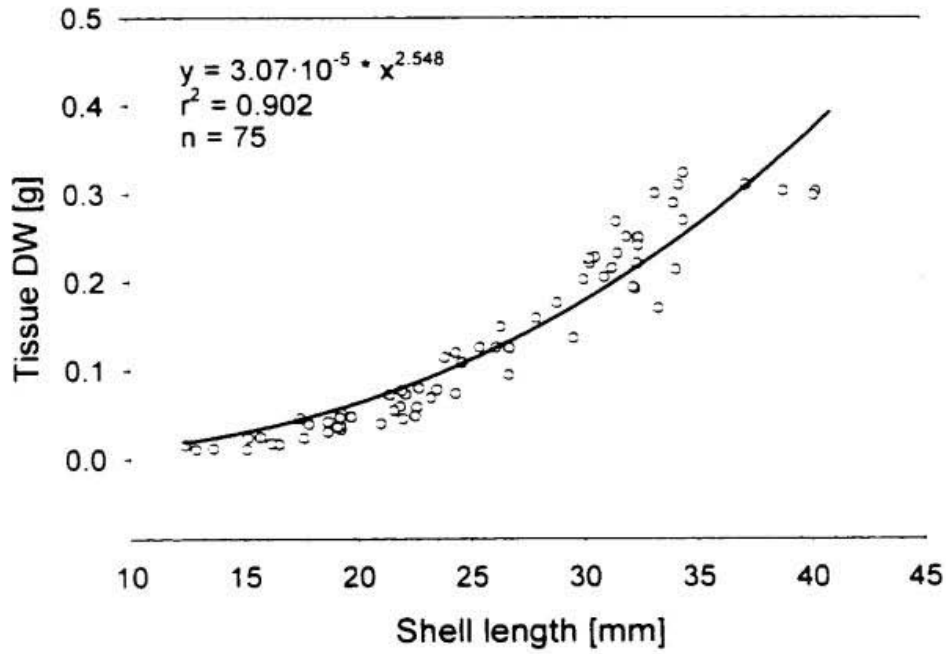

Fig. 3.2: Relationship between tissue dry weight [g] and shell length [mm] in *Yoldia hyperborea* from Conception Bay.

Table 3.1: Regression parameters and analysis of variance of *Yoldia hyperborea* tissue dry weight [g] against length [mm] for animals of 12.4 to 40.3 mm shell length. The regression equation is in the form $Y = a \cdot X^b$

Parameter		Value	Std Error	t	P
a		$3.07 \cdot 10^{-5}$	0.0000	2.13	0.0369
b		2.5476	0.1346	18.92	<0.0001
r^2		0.9020	0.0314		
ANOVA	**df**	**SS**	**MS**	**F**	**P**
Regression	1	0.5979	0.5979	607.62	<0.0001
Residual	66	0.0649	0.0010		
Total	67	0.6629	0.0099		

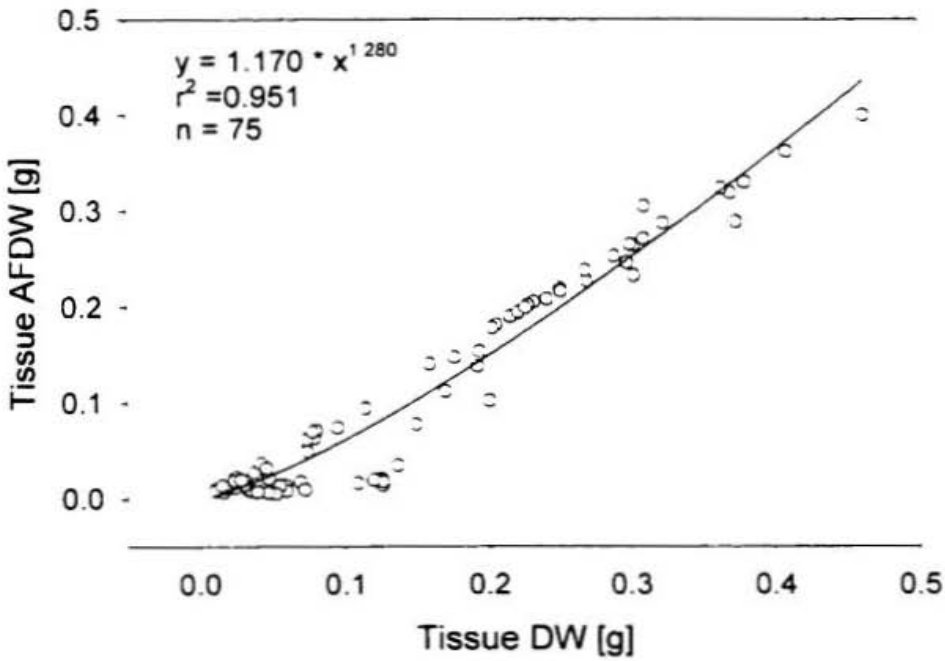

Fig. 3.3: Relationship between tissue ash-free dry weight [g] and dry weight [g] in *Yoldia hyperborea* animals from Conception Bay, Newfoundland.

Table 3.2: Regression parameters and analysis of variance of *Yoldia hyperborea* tissue dry weight [g] against tissue ash-free dry weight [g] for animals of shell length 12.4 to 40.3 mm. The regression equation is in the form $Y = a \cdot X^b$

Parameter		**Value**	**Std Error**	**t**	**P**
a		1.1696	0.0778	15.03	<0.0001
b		1.2797	0.0529	24.17	<0.0001
r^2		0.9514	0.0251		
ANOVA	**df**	**SS**	**MS**	**F**	**P**
Regression	1	0.9129	0.9129	1448.22	<0.0001
Residual	74	0.0466	0.0006		
Total	75	0.9595	0.0128		

Table 3.3: One way analysis of variance for *Yoldia hyperborea* oxygen consumption rate (VO_2)[$\log_{10}$ ml $O_2 \cdot h^{-1}$] against tissue dry weight [$\log_{10}$ g] for animals (A) with and (B) without sediment. (C) Comparison of regressions of oxygen consumption (VO_2) [$\log_{10}$ ml $O_2 \cdot h^{-1}$] with and without sediment.

(A) In sediment

Parameter	Value	Std Error	t	P
a	0.0647	0.0224	2.89	0.0162
b	0.7985	0.2226	3.59	0.0050
r^2	0.5264			

ANOVA	df	SS	MS	F	P
Regression	1	0.0005	0.0005	11.11	0.0076
Residual	10	0.0005	0.0000		
Total	11	0.0010	0.0001		

(B) No sediment

Parameter	Value	Std Error	t	P
a	0.0449	0.0042	10.73	<0.0001
b	0.6563	0.0683	9.60	<0.0001
r^2	0.8522			

ANOVA	df	SS	MS	F	P
Regression	1	0.0030	0.0030	236.42	<0.0001
Residual	41	0.0005	0.0000		
Total	42	0.0036	0.0001		

(C) sediment effect on VO_2

Source	df	SS	MS	F	P
corrected model	11	$1.66 \cdot 10^{-3}$	$0.15 \cdot 10^{-3}$	6.79	0.000
intercept	1	$8.31 \cdot 10^{-3}$	$8.31 \cdot 10^{-3}$	374.38	0.000
(1) sediment	1	$0.013 \cdot 10^{-3}$	$0.013 \cdot 10^{-3}$	0.60	0.445
(2) dry weight	8	$1.48 \cdot 10^{-3}$	$0.185 \cdot 10^{-3}$	8.31	0.000
(1) x (2)	2	$0.082 \cdot 10^{-3}$	$0.041 \cdot 10^{-3}$	1.84	0.179
Error	26	$0.58 \cdot 10^{-3}$	$0.022 \cdot 10^{-3}$		
Total	38	$13.15 \cdot 10^{-3}$			
Corrected total	37	$2.24 \cdot 10^{-3}$			

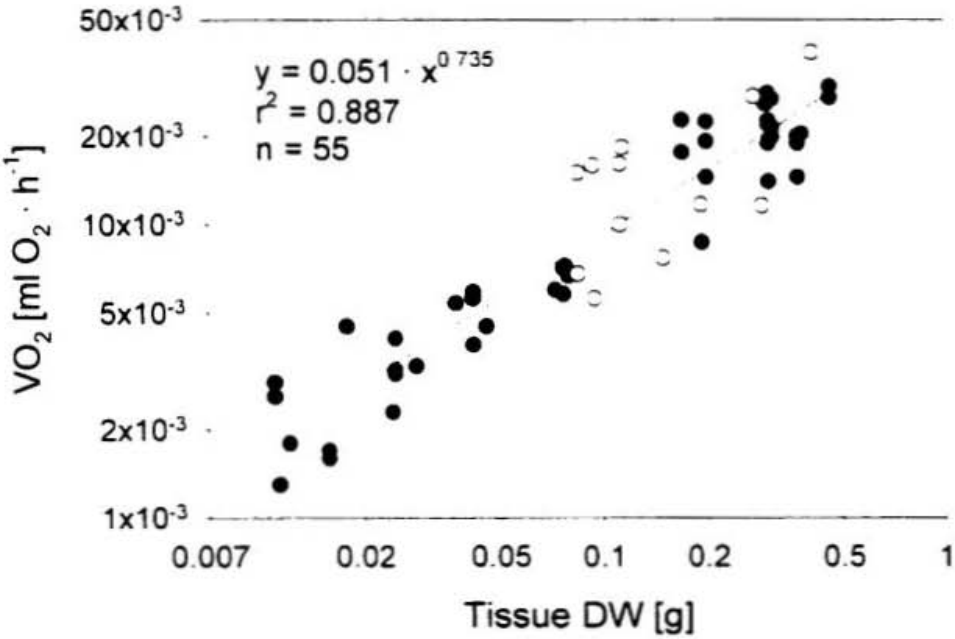

Fig. 3.4: Relationship between $\log_{10}$ oxygen consumption (VO_2) [ml $O2 \cdot h^{-1}$] and $\log_{10}$ dry tissue weight [g] at -1.0°C in *Yoldia hyperborea*. Black circles indicate consumption rate of individuals not provided with sediment (n = 43). White circles indicate consumption rates of individuals placed in mud within the chamber (n = 12). Regression line is based on all values.

Table 3.4: Regression parameters and analysis of variance for *Yoldia hyperborea* oxygen consumption rate ($\log_{10}$ VO_2)[ml $O_2 \cdot h^{-1}$] against tissue dry weight [$\log_{10}$ g] for animals in the range 12.4 to 40.3 mm shell length. The regression equation is in the form $\log_{10}Y = a - b \cdot \log_{10} X$.

Parameter		**Value**	**Std Error**	**t**	**P**
a		-1.2943	0.0396	-32.64	<0.0001
b		0.7349	0.0357	20.57	<0.0001
r^2		0.8868	0.1333		
ANOVA	**df**	**SS**	**MS**	**F**	**P**
Regression	1	7.5214	7.5214	423.20	<0.0001
Residual	54	0.9597	0.0178		
Total	55	8.7811	0.1542		

Note: This regression is equivalent to the form $Y = a \cdot X^b$ (a=0.051, b = 0.735)

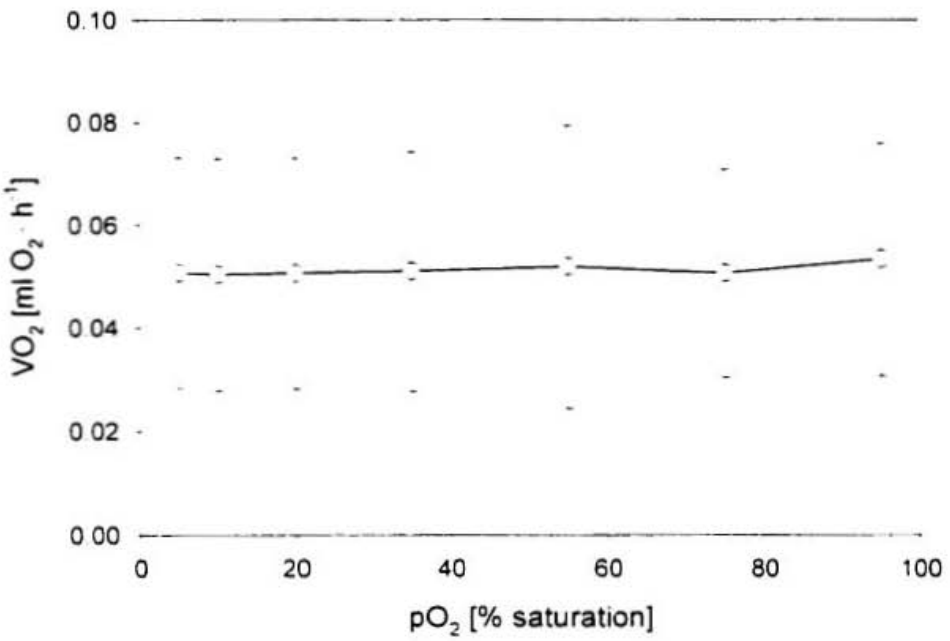

Fig. 3.5: Relationship between oxygen consumption rate (VO_2)[ml $O_2 \cdot h^{-1}$] and relative oxygen content (pO_2)[% saturation] of the water in the experimental respiration chambers. Values are means from 10 animals (±S.D.) standardized to 1 g DW.

3.3.3. Ammonia excretion

Ammonia excretion for a 1g (DW) *Yoldia hyperborea* was relatively low ($\bar{x}$ = 3.545, S.D.= 0.232 µg NH_4-N · h^{-1} · g^{-1}) throughout 141 hours (Fig. 3.6). The excretion rates for the first 4.5 hours were variable, probably as a result of acclimation or stress. However, after this initial period the excretion rate became constant. In addition, ammonia excretion rate was independent of ammonia concentration (Fig. 3.7).

The relationship between excretion rate (VNH_4-N) and individual dry weight ($P < 0.0001$, n = 12, r^2= 0.872) (Fig. 3.8, Table 3.5) is defined by the equation:

$$VNH_4\text{-}N = 4.224 \cdot DW^{1.261}$$

where,

VNH_4-N = excretion rate of animals (µg NH_4-N · h^{-1})

DW = individual dry weight (g)

The ratio between VO_2 and VNH_4-N (O:N), calculated in atomic equivalents after Widdows (1985b), indicates the proportion of lipid and carbohydrate relative to protein that is metabolised for energy metabolism. A low O:N ratio close to 10 is indicative of exclusive protein metabolism and hence of a stressed condition (Bayne & Newell 1983, Widdows 1985a). The O:N ratio for *Yoldia hyperborea* in this study was 36.21.

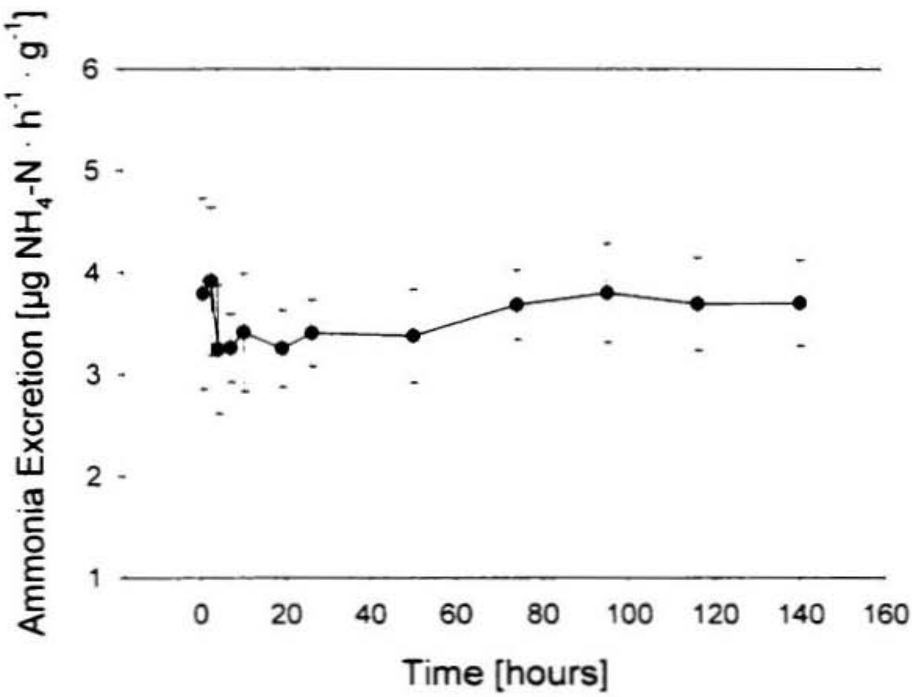

Fig. 3.6: Mean ammonia excretion rate (= S.D.) of *Yoldia hyperborea* during a 141 hour period. Mean VNH_4-N for 1 g DW of animal tissue throughout the experiment was 3.545 µg NH4-N · h^{-1} · g^{-1} (S.D. = 0.232, n = 21).

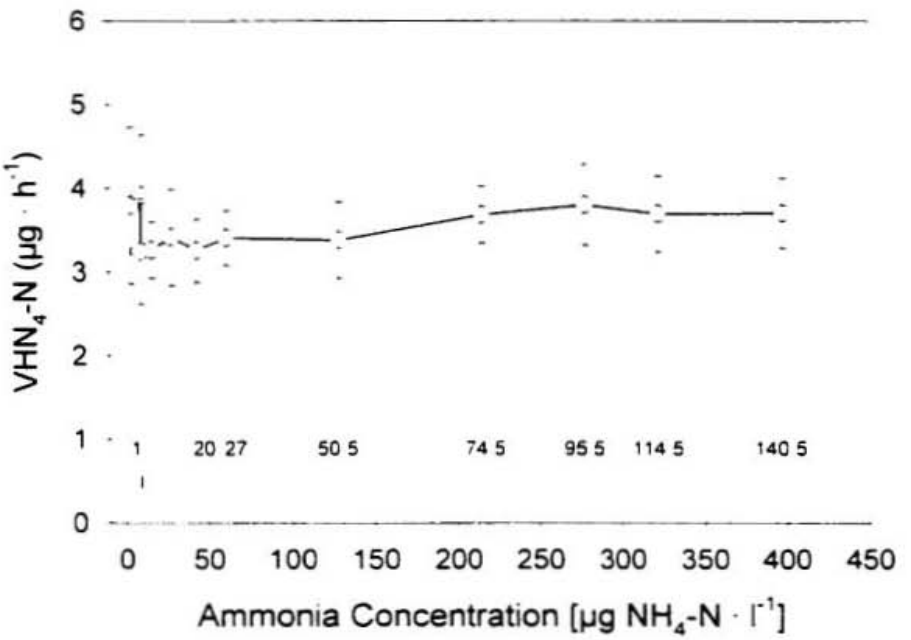

Fig. 3.7: Relationship between ammonia excretion rate (µg NH_4-N · h^{-1}) (1 g DW) and ammonia concentration (µg NH_4-N · l^{-1}), illustrating the independence of excretion rate from the ammonia concentration within the experimental chambers. Values are means (± S.D., n=21). Numbers and vertical lines within the figure show the time in hours when water samples were taken.

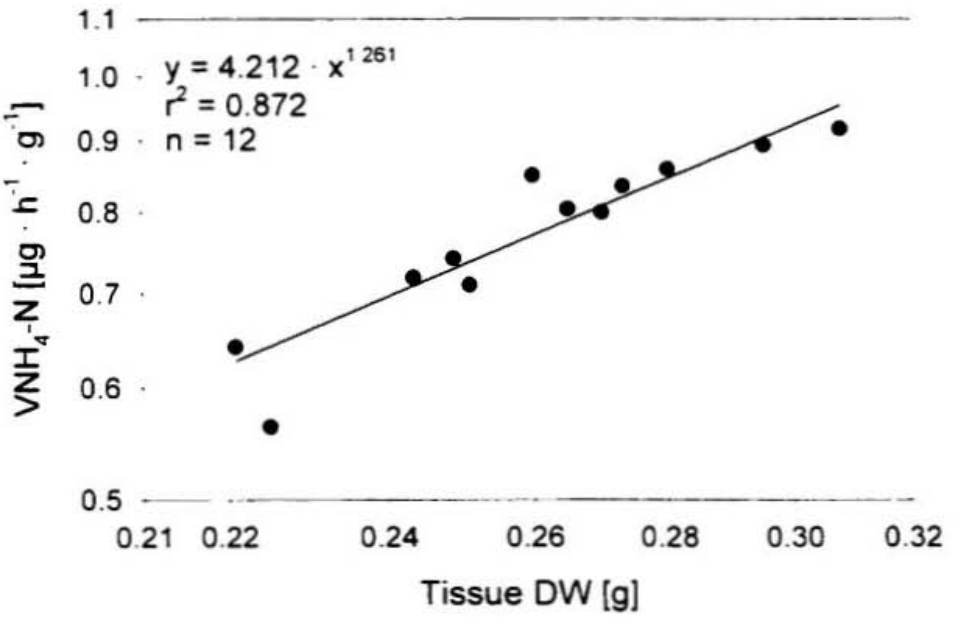

Fig. 3.8: Relationship between ammonia excretion rate (VNH_4-N) [ml NH_4-N · h^{-1}] and dry tissue weight [g] at -1.0°C in *Yoldia hyperborea*.

Table 3.5: Regression parameters and analysis of variance for *Yoldia hyperborea* excretion rate (VNH_4-N) [$\mu g \cdot h^{-1} \cdot g^{-1}$ DW] against tissue dry weight [g] for animals in the range 27.93 to 36.51 mm shell length (0.1589 to 0.4093 g DW). The regression equation is in the form $Y = a \cdot X^b$

Parameter		**Value**	**Std Error**	**t**	**P**
a		4.2122	0.8744	4.82	0.0007
b		1.2610	0.1566	8.05	<0.0001
r^2		0.8868	0.0398		
ANOVA	**df**	**SS**	**MS**	**F**	**P**
Regression	1	0.1075	0.1075	67.87	<0.0001
Residual	11	0.0158	0.0016		
Total	12	0.1233	0.0112		

3.4. DISCUSSION

3.4.1. Length-Weight relationships

Length-weight relationships reported here are, to my knowledge, the first for *Yoldia hyperborea*. Lewis *et al.* (1982) found that similar sized (10-50 mm) *Y. limatula* from Malpeque Bay, Prince Edward Island, showed a regression equation ($a= 7.206 \cdot 10^{-7}$ g, b=3.4227) which indicated that individuals between 15 and 35 mm shell length contained from 2 to 4 times less dry tissue than *Y. hyperborea* animals of the same length. These trends correspond to shell morphology, because *Y. limatula* has a more slender body with less shell height than *Y. hyperborea*.

3.4.2. Oxygen uptake

Estimation of oxygen uptake rates for infaunal animals living in natural sediment may be confounded by the presence of micro- and meio-organisms that also consume oxygen. The removal of animals from the sediment, on the other hand, may result in stress and/or abnormal behaviour. However, in the case of deposit-feeders such as protobranchs, placing them in sediment may overestimate their standard metabolic rate as they may be stimulated to ingest and process it. Newell (1977) determined that there was no significant difference in oxygen consumption and filtration rate of the infaunal *Cardium* (=*Cerastoderma*) *edule* maintained in sediment and without sediment. In the present set of experiments the absence of sediment did not affect the rate of oxygen uptake nor the survival of the individuals and I decided therefore to exclude sediment in the remaining trials.

Comparisons of oxygen uptake rates between *Yoldia hyperborea* and other

protobranch bivalves is possible for only a handful of studies (Table 3.6) of which only one is for a species from a cold water habitat (Davenport 1988a). However, the VO_2 values obtained here lie within the range observed for protobranchs and a few lamellibranch species, but are lower than those found in most lamellibranch bivalves listed in Table 3.6. Low metabolic rates have been found in a number of cold water species (Table 3.6) and may be an important mechanism for energy conservation in areas (*e.g.* Antarctica) where food supply is strongly seasonal (Ahn 1998). The highest oxygen consumption rates for protobranchs have been observed in *Solemya reidii* and *Yoldia thraciaeformis*, where measurements were made of active metabolic rates in animals that had undergone short acclimation periods (Bernard & Noakes 1990), and in *Nucula turgida* (Wilson & Davis 1984), where the procedure of stepwise temperature increase may have placed the animals under stress. The importance of adequate acclimation periods has been clearly demonstrated for the cockle *Cerastoderma edule*, in which oxygen consumption consistently decreased with increased time held in the laboratory, although the ambient seston concentration was constant (Navarro *et al.* 1992). In addition, Widdows and Bayne (1971) found that complete acclimation of oxygen consumption and filtration rate in *Mytilus edulis* occurred within 14 days of the mussels being exposed to a sudden increase of 5°C, although individuals held at ambient temperature (10°C) and those exposed to a 5°C decrease showed complete acclimation of oxygen uptake rate.

The comparison of oxygen consumption data between studies is usually complicated by the use of different techniques and the close relationship between metabolism and food supply or temperature (Griffiths & Griffiths 1987, Jorgensen 1990). Metabolic rates in bivalves vary from active metabolism, when the animal is actively feeding or exercising other metabolic demanding processes (*e.g.* moving from one place to another, burrowing), through maintenance metabolism, which is adequate to sustain life but cannot support other functions (*e.g.* feeding, digestion, growth, movement), to standard metabolism, which is considered to be a resting state. Standard metabolic rate, as used in this study, eliminates

variation as a result of short-term changes in quality and quantity of food ingested and provides a meaningful comparison with metabolic rates of other species (see Table 3.6).

The slope value obtained here (0.73) compares well with values from the literature. Newell (1979) discussed the influence of size on oxygen uptake and showed that the data from various studies approach a common regression line with a slope around 0.75, whereas Winter (1978) argued that values lay between 0.66 and 0.82. This value signifies that metabolic rate becomes slower as body size increases and shows that energy flow per unit body mass is much greater in smaller individuals.

One of the most striking findings in this study was the ability of *Y. hyperborea* to regulate its oxygen consumption rate at very low pO_2 levels, suggesting that this species did not switch to anaerobic metabolism, although invocation of anaerobiosis cannot be ruled out under more prolonged hypoxic conditions. Invertebrates may be classified as oxygen conformers or oxygen regulators, according to their response to declining oxygen tensions. Shumway (1983) suggested that maintenance of aerobic respiration at normal rates is advantageous for animals faced with continuously changing environmental conditions. *Yoldia hyperborea* is always found in muds with a well developed oxygenated layer which the animal helps to maintain as it actively moves both horizontally and vertically within the sediment (R. Stead, pers. obs.; see also Rhoads 1963, Bender & Davis 1984, Davenport 1988b). In contrast, Wilson and Davis (1984) demonstrated the inability of *Nucula turgida* to regulate oxygen consumption at lowered oxygen tensions and suggested that this shortcoming may be attributable to the inability of the small protobranch gill to compensate for a reduction in oxygen availability. On the other hand, other protobranchs can survive anoxic conditions, *e.g.* the nuculid *Nucula sulcata*, which can survive two weeks in an oxygen-free environment, exceeding the capability of any known lamellibranch bivalve (Taylor *et al.* 1995). However, this capacity may be an adaptation of the non-siphonate and less active *Nucula*, which respires mainly via the interstitial water, as opposed to the

Table 3.6. Comparison of oxygen consumption rate (VO_2) and tissue dry weight (DW) relationships in various protobranch and lamellibranch bivalves and cold water organisms at their ambient temperatures. Data were made to fit the allometric equation $VO_2 = a \cdot DW^b$ when described otherwise in the original work. n/a – data not available

Species	Feeding type	Mean ± S.D / range VO_2 [ml $O_2 \cdot g^{-1} DW \cdot h^{-1}$]	slope (b)	Temp. [°C]	Source
Bivalvia -Protobranchia					
Solemya reidi	autotrophic	0.18	n/a	n/a	Bernard & Noakes 1990
Yoldia thraciaeformis	deposit	0.25	n/a	n/a	Bernard & Noakes 1990
Yoldia eightsi	deposit	0.0997, 0.0955	0.794, 0.824	0.2, 2.5	Davenport 1988
Yoldia hyperborea	deposit	0.0508	0.7349	-1.0	This study
Nucula turgida	deposit	0.2569 ± 0.0660	0.737 ± 0.097	5.0 - 15.0	Wilson & Davis 1984
Nucula sulcata	deposit	0.0354	n/a	10.0	Taylor *et al.* 1995
Acila castrensis	deposit	0.0020 ± 0.0009	n/a	11.5	Mangum *et al.* 1987
Bivalvia - Lamellibranchia					
Chlamys hastata	suspension	0.38	n/a	n/a	Bernard & Noakes 1990
Mytilus edulis	suspension	0.4856 ± 0.1449	0.445	9.0 - 16.6	Bayne & Widdows 1978
Mytilus edulis	suspension	0.2311	0.7	-0.5 - 1.5	Loo 1992
Mytilus edulis	suspension	0.10 - 0.42	0.280 - 1.044	0 - 15	Thompson 1984
Clinocardium nuttalli	suspension	0.55	n/a	n/a	Bernard & Noakes 1990
Saxidomus giganteus	suspension	0.41	n/a	n/a	Bernard & Noakes 1990
Mya truncata	suspension	0.63	n/a	n/a	Bernard & Noakes 1990
Modiolus modiolus	suspension	0.143 ± 0.093	0.743 ± 0.142	-1.0 - 14.0	Navarro & Thompson 1996
Venericardia ventricosa	suspension	0.2695 ± 0.0016	n/a	11.5	Mangum *et al.* 1987
Noetia ponderosa	suspension	0.0148	n/a	10.0	Mangum *et al.* 1987

Table 3.6: Continued.

Species	Feeding type	Mean ± 1 S.D - range VO_2 [ml $O_2 g^{-1} DW \cdot h^{-1}$]	slope (b)	Temp. [°C]	Source
Pisidium personatum	suspension	0.0194 ± 0.0035	n/a	5.0	Bleck & Heitkamp 1980
Pisidium obtusale	suspension	0.0296 ± 0.0059	n/a	5.0	Bleck & Heitkamp 1980
Scrobicularia plana	deposit	0.1411 ± 0.0537	n/a	10.0	Oeschger & Pedersen 1994
Mya arenaria	suspension	0.11 - 0.33	0.76	12.0	MacDonald *et al.* 1998
Placopecten magellanicus	suspension	0.22 - 0.33	0.76	12.0	MacDonald *et al.* 1998
Limopsis narionensis	suspension	0.1808 ± 0.0235	n/a	-1.5, 0.0	Portner *et al.* 1999
Placopecten magellanicus	suspension	0.066 - 0.447	0.70 - 0.93	0 - 12	MacDonald & Thompson 1986
Cold water organisms					
Amphipoda: *Orchomene plebs*	scavenger	1.78	0.44	-1.8	Rakusa-Suszczewski 1982
Amphipoda: *Eusirus perdentatus, Cyphocaris richardi, Eurythenes gryllus**	herbivores, scavenger*	0.2437 ± 0.1628	n/a	-1.4, -1.0	Opalinski & Jazdzewski 1978
Copepoda: *Calanus finmarchicus, C. hyperboreus, C. glacialis*	herbivores	0.300 - 0.454	n/a	-0.8 -0.0	Hirche 1987
Plankton community	n/a	0.0233 - 0.0127	n/a	0.2 - 1.8	Robinson & Williams 1993
Euphausida: *Euphausia superba*	herbivore	0.36 ± 0.11	n/a	0.2	Hernandez-Leon *et al.* 1999
Gastropoda: *Nacella concinna*	herbivore	0.1112	0.834	-1.5	Houlihan & Allan 1982
Gastropoda: *Trophon* sp. A	omnivore	0.0559	0.822	-1.7	Houlihan & Allan 1982
Gastropoda: *Pellilitorina setosa*	herbivore	0.1328	0.931	-1.7	Houlihan & Allan 1982
Gastropoda: *Margarella antarctica*	n/a	0.0264	0.623	-1.7	Houlihan & Allan 1982
Chephalopoda: *Pareledone charcoti*	carnivore	0.1075 ± 0.0109	0.75	0.0	Daly & Peck 2000
Brachiopoda: *Liothyrella uva*	suspension	0.017	0.717	0.0	Peck *et al.* 1986
Pisces: *Boreogadus saida*	carnivore	0.0511 ± 0.0022	n/a	0.4	Hop & Graham 1995

siphonate *Yoldia* which may obtain oxygen from several cm above the sediment surface.

Protobranch bivalves are adapted to environments characterised by short-term fluctuating oxygen tensions, as is commonly observed in low-energy physical environments. Although the genus *Yoldia* has a bathymetric distribution which ranges from intertidal sandflats and shallow subtidal areas (*Yoldia eightsii*, pers. obs., Davenport 1988b) to deep waters (824 m, *Y. eightsii* in Villarroel & Stuardo 1998), shelf waters are dominated by Lamellibranchia whereas Protobranchia predominate in the deep sea (Allen & Sanders 1997). At high latitudes and in the deep sea, water temperature at the bottom is usually low and may come close to freezing (*e.g.* Conception Bay, Newfoundland). Dissolved oxygen levels in seawater at such temperatures are approximately 1.6 times greater than at 20°C. In addition, the affinity of oxygen carrier proteins for ligands increases at low temperatures but the increased viscosity of body fluids makes their circulation more difficult (Clarke, 1983). The reason for protobranch dominance over lamellibranchs in these habitats may partly lie in the functional morphology of their respiratory system as well as their deposit-feeding mode.

The respiratory pigments haemocyanin (in the haemolymph) and haemoglobin (within gill cells) are both found in protobranchs. Angelini *et al.* (1998) recently reported low concentrations of haemocyanin in the haemolymph and very high concentrations of haemoglobin in gill cells of *Yoldia eightsii*, an antarctic species. Most bivalves with gill Hb (which gives the deep red colour to the gills of *Y. hyperborea*) inhabit reduced sediments rich in H_2S or deep sea hydrothermal vents and all of them, except for the members of the genus *Yoldia*, contain symbiotic intracellular chemoautotrophic sulphur oxidising bacteria (Doeller *et al.* 1990). A possible function of cytoplasmic Hb is to support aerobic metabolism when oxygen is lacking by storing it, allowing animals to burrow through anaerobic mud for prolonged periods. According to Weber (1980) gill haemoglobin occurs most frequently in invertebrate species living under hypoxic stress.

3.4.3. Ammonia excretion

Although excretion rates have been thoroughly investigated in pelagic and epibenthic animals, little work has been done with infaunal animals, including only one other protobranch species (Gray & Follum 1987). Determinations of nitrogen excretion in cold water invertebrates are also sparse. Results obtained in this study are on the lower end of the range of values reported for bivalves (Griffiths & Griffiths 1987). White (1975, in Peck *et al.* 1986) showed excretion rates in the antarctic scavenging isopod *Glyptonotus antarcticus* of 5.027 μg NH_4-N·h^{-1}·g^{-1}WW (WW=wet weight), whereas Peck *et al.* (1986) recorded rates of 0.333 μg NH_4-N· h^{-1}·g^{-1}WW for the antarctic brachiopod *Liothyrella uva*. Gray and Follum (1987) reported VNH_4-N values of 5.755 and 4.199 μg NH_4-N·h^{-1}·g^{-1}DW for the protobranch *Nucula tenuis* with and without sediment, respectively. Bayne & Widdows (1978) recorded comparable values of VNH_4-N for *Mytilus edulis* from the Lynher and Cattewater estuaries during winter to those values reported here for *Y. hyperborea*, but much higher values were recorded at other times of the year, reaching maximum rates of 40 μg NH_4-N· h^{-1}·g^{-1}DW. Low excretion rates reported here compare well with values reported for intertidal species from Newfoundland such as *Modiolus modiolus* (6.27 ± 4.15 μg NH_4-N·h^{-1}·g^{-1}DW), and *Mytilus edulis* (6.15 ± 3.22 μg NH_4-N· h^{-1}·g^{-1} DW), although these populations are generally exposed to temperatures from -1 to 15°C (Thompson 1984, Navarro & Thompson 1996).

Relationships between rates of ammonia excretion and body dry weight in various bivalves show that the exponent in the allometric equation with body size lies in the range 0.417 to 1.480 (Griffiths & Griffiths 1987) and a similar range has been found in the mussel *Mytilus edulis* (Bayne & Scullard 1977), including a population from the east coast of Newfoundland (Thompson 1984). Although the value (b=1.261) reported here is at the upper end of the range, it is similar to that found by Gray and Follum (1987) for *Nucula tenuis* (b=1.192).

As ammonia is the major excretory product of protein catabolism in aquatic animals, temporal variability in the rate of excretion of nitrogenous waste products can show strong seasonal fluctuations which relate to environmental variables (*e.g.* water temperature, food concentration) and the reproductive and nutritional state of the animal. High ammonium excretion rates have been observed in mussels in late winter when particle concentration is low and individuals are initiating gametogenesis with energy obtained from short-term reserves (Bayne & Widdows 1978, Navarro & Thompson 1996, Hatcher *et al.* 1997). However, if animals have enough lipid and carbohydrate reserves, protein catabolism should be minimal and excretion rates relatively low, even at high POM concentration. Navarro *et al.* (2000) observed that oxygen uptake and ammonia excretion in *Argopecten purpuratus* were not affected by temperature and food ration. This pattern has also been reported for many other bivalves (*e.g.* Widdows *et al.* 1979, Bayne & Newell 1983, MacDonald *et al.* 1998). Gray and Follum (1987) observed that excretion rate fluctuated with the spawning cycle of the nuculid *Nucula tenuis*, peaking during the spawning season and decreasing to a low level immediately afterwards. On the other hand, Navarro and Thompson (1997) recorded a strong correlation of ammonia excretion with gonad weight in *Modiolus modiolus* from Newfoundland, similar to results obtained from other lamellibranch bivalves (*e.g.* Widdows *et al.* 1979, Worrall *et al.* 1983).

Although no seasonal study was conducted in this case, an O:N value of 36.21 suggested that reasonable amounts of carbohydrate and lipid reserves were present and thus protein was not a major source of energy in experimental animals. Thompson (1984) did not observe a clear seasonal trend in the ratio of protein lost to protein absorbed in mussels from Newfoundland. However, the lowest O:N ratios for mussels were observed in March, just before the occurrence of the spring bloom, suggesting that when glycogen is low at the end of winter, protein is used as an energy substrate. As described in chapter 2, the energy reserves in *Yoldia hyperborea* followed a seasonal cycle, with the lowest reserves occurring in winter. As these excretion experiments were conducted at that time of year, results

obtained here should reflect the lowest O:N ratios. Thus, it may be expected that excretion rates would not increase much further and could even drop as more energy is accumulated in the form of lipid and carbohydrates after the spring bloom. However, these conclusions must be treated with caution until a seasonal study is conducted.

CHAPTER 4

FEEDING PHYSIOLOGY OF *YOLDIA HYPERBOREA*

4.1. INTRODUCTION

Deposit-feeding animals ingest a complex assortment of mineral grains and organically dilute detritus, some of which has low nutritional value (*e.g.* refractory material) (Lopez & Levinton 1987, Jumars 1993). However, deposit-feeders show a considerable diversity of feeding mechanisms and degrees of specialisation, which enables them to survive in a seemingly nutritionally deficient environment.

Supply of food to a deposit-feeding community occurs mainly through lateral and vertical advection (Taghon & Jumars 1984, Snelgrove & Butman 1994) or microbial regeneration within the sediment (*e.g.* Newell 1979). Benthic communities dominated by deposit-feeders, such as the deep sea, are considered to be food-limited and depend on the amount of food that reaches the sediment surface, primarily through processes occurring in the photic zone of the water column (Gooday & Turley 1990). In te mperate and high latitude areas, where input of particulate food matter is highly seasonal (Clarke 1988, Wassman 1991, Boon *et al.* 1998), animals tend to accumulate energy reserves when food is available for use in maintenance, growth and reproduction during periods of low food availability (*e.g.* Nakaoka 1992, Brockington & Clarke 2001). Thus benthic animals must utilise food material efficiently when particulate material is especially nutritious, *e.g.* during phytoplankton fallout events. Strategies may include physiological adaptations such as high absorption efficiencies and long gut passage times (or vice versa), particle selection before

ingestion, and behavioural plasticity (Lopez & Levinton 1987 Levinton 1989, Taghon & Greene 1992).

There are many examples of animals that show behavioural plasticity when acquiring food. Dauer *et al.* (1981) used the term "interface-feeders" to refer to species that are not obligatory suspension- or deposit-feeders. A combination of suspension-feeding and deposit-feeding has been observed in many invertebrate taxa (*e.g.* Lopez & Levinton 1987, Okamura 1990, Taghon & Greene 1992, Bock & Miller 1996) including several lamellibranch bivalves, all of which belong to the tellinid family (Pohlo 1969, Olafsson 1986,1989, Levinton 1991). Olafsson (1986) found that switching from deposit- to suspension-feeding in *Macoma balthica* occurred with changes in flow velocity. He further suggested that suspension-feeding was a response to higher amounts of suspended particles present in high flows, whereas deposit-feeding was a response to a decrease in the concentration of suspended material, a view later substantiated by Lin and Hines (1994). However, Hummel (1985b) indicated that although this species behaves as a deposit-feeder most of the time, it depends largely on food present in the water column.

Although protobranch bivalves have usually been considered deposit-feeders (Rhoads & Young 1970, Lopez 1988), some authors have suggested *Yoldia* spp. may be capable of suspension-feeding, based on anatomical features of the gills and palps (Kellogg 1915, Stasek 1965). However, Levinton *et al.* (1996) suggested that these features only have a role in the removal of particles from the gill surface and mantle cavity. Furthermore, qualitative observations on *Yoldia eightsii* by Davenport (1988b) suggest that this species is capable of passively trapping phytoplankton when ventilating, whereas Nakaoka (unpubl., in Nakaoka 1992) indicated that *Yoldia notabilis* was capable of suspension-feeding, but did not explain how he reached this conclusion.

Although no study has yet described an accurate account of suspension-feeding behaviour in any protobranch bivalve, personal observations on the nuculanid *Yoldia hyperborea* suggest that it can produce faeces when deprived of sediment and in the presence of suspended particles.

Although protobranchs are one of the dominant infaunal taxa in muddy sediments, little is known about their feeding physiology, particularly aspects such as ingestion and assimilation of organic carbon (Lopez & Cheng 1982, 1983, Cheng & Lopez 1991). However, these variables are used for estimating the energy budget of a species in conjunction with other physiological variables such as oxygen uptake and nitrogen excretion (Widdows 1985b). The estimation of a species' energy budget can contribute towards an overall comprehension of its feeding strategies and behaviour, as well as its strategies for energy storage in a nutrient-limited environment.

Thus the purpose of the study in this chapter is to establish the importance of suspension-feeding in *Yoldia hyperborea* at different particle concentrations, and to compare this strategy with deposit-feeding through the quantification of ingestion rate, assimilation efficiency and gut passage time. In addition, an energy budget is also derived for inferring the potential impact of feeding strategy and variation of food supply on the feeding activity, energy gain and expenditure of *Yoldia hyperborea*.

4.2. MATERIAL AND METHODS

4.2.1. Feeding Experiments

Most feeding studies on deposit-feeding animals have used various techniques involving radiolabelled food material to determine ingestion rate, gut passage time and absorption efficiency in order to understand the feeding strategy of a species (*e.g.* Forbes & Lopez 1989a, 1989b, Kofoed *et al.* 1989, Decho & Luoma 1991, Charles *et al.* 1995) The advantage of this approach over gravimetric and indicator methods (*sensu* Lopez *et al.* 1989) is its increased sensitivity for measuring small changes (Kofoed 1975a, b) Dual labelled food is particularly useful as it can be partitioned into organic and inorganic fractions One of these techniques is that developed for diatoms by Rivkin (1986) and Bochdansky *et al.* (1999), using ^{14}C to label the organic matter fraction, whereas ^{68}Ge is incorporated into the structure of the frustule and thus cannot be absorbed by the animal (Bochdansky *et al.* 1999)

In the present study, I used the ^{68}Ge:^{14}C dual-labelled *Thalassiosira nordenskioldii* to understand the relative importance of deposit- and suspension-feeding in *Yoldia hyperborea*.

4.2.1.1 Collection and handling of animals

Animals were obtained by dredge from the deep-depositional zone of Conception Bay, Newfoundland, and placed in sediment within a refrigerated holding tank (0.0 ± 1.0 °C) at the Ocean Sciences Centre in Logy Bay Since most physiological rates vary with body size, only large animals of similar size were used for feeding experiments (mean shell length = 28.96 mm, S.D. = 2.14, n=96).

4.2.1.2. Preparation of experimental food source

Laboratory experiments were carried out with the cold water diatom *Thalassiosira nordenskioldii* as a food source. This species was chosen because of its size (equivalent spherical diameter = 15 µm) and its frequent presence in Newfoundland waters. Cultures were grown under continuous light in f/2+ medium (33‰ salinity) at 5-8°C. Cell counts were routinely done on a haemocytometer with a Neubauer grid. Once the culture entered the exponential growth phase it was inoculated with $^{68}Ge(OH)_4$ (Brookhaven National Laboratories) and $NaH^{14}CO_3$ (ICN Radiochemicals) to a final concentration of 80 to 100 $\mu Ci \cdot l^{-1}$ for ^{14}C (Nielsen & Olsen 1989) and 40 to 60 $\mu Ci \cdot l^{-1}$ for ^{68}Ge (Penry & Frost 1991). No detectable effects of these isotope concentrations on the rate of algal growth have been found in previous studies (Rivkin 1986, Nielsen & Olsen 1989, Penry & Frost 1991, Bochdansky *et al.* 1999). ^{68}Ge acts as an unabsorbed marker wich passes through the gut of the animal and is recovered in the faeces. Dissolved label was removed after 8 to 13 days from inoculation by a series of reversed flow filtrations, using a 5µm Nitex mesh, until the label was not more than 3% higher than background levels.

4.2.1.3. Suspension-feeding experiments

Experimental animals were transferred, 7 days before the start of each experiment, to a cup overlying a stirbar within individual glass jars (200 ml) containing filtered seawater (2 µm) and unlabelled *T. nordenskioldii* cells. Containers were kept in an incubator (0°C ±1°C) in the dark and placed on a magnetic stirring plate that kept the algae in suspension. This setup allowed the animals to maintain the orientation they most frequently adopt when in sediment, but prevented them from reaching any deposited algae with their palp proboscides. Filtered seawater with algae was renewed daily.

The experiment began after filtered seawater (Whatman GF/F) had been renewed and different concentrations of dual-labelled and unlabelled algae introduced into the jars. A total of 11 experiments was done on 5 different occasions (Table 4.1). Experiments carried

Table 4.1. Main characteristics of the food fed to *T. hyperborea* in different sets of suspension- and deposit feeding experiments. M_a – dual labelled *T. nordenskioldii*, U_a – unlabelled *T. nordenskioldii*, POM – particulate organic matter.

Date (D/M/Y)	Exp. #	*T. nordenskioldii* density [cells·ml-1] × (S.D., n)	M_a:U_a (1:U_a)	*T. nordenskioldii* ^{68}Ge:^{14}C × (S.D., n)	Particle concentration [mg·ml^{-1}] (%POM, n)	Particle activity [dpm·mg^{-1}]	Food Value [J·mg^{-1}]
suspension-feeding							
18/05/1999	1	8.69 (1.29, 20)	0	0.123 (0.005, 6)	0.016 (36.54, 3)	114984	7.593
15/06/1999	2	9.94 (2.48, 20)	0	0.312 (0.035, 41)	0.020 (37.48, 3)	76637	7.788
07/07/1999	3	7.54 (2.43, 24)	0	0.317 (0.034, 23)	0.022 (35.32, 3)	67591	7.339
07/07/1999	4	89.21 (11.33, 24)	10.83	0.317 (0.034, 23)	0.257 (35.32, 3)	67357	7.339
07/07/1999	5	170.87 (22.66, 24)	21.66	0.317 (0.034, 23)	0.493 (35.32, 3)	67357	7.339
27/07/1999	6	9.52 (2.61, 20)	0	0.666 (0.114, 34)	0.020 (40.59, 3)	43350	8.435
27/07/1999	7	19.51 (2.82, 20)	1.05	0.666 (0.114, 34)	0.042 (40.59, 3)	43349	8.435
27/07/1999	8	39.51 (3.23, 20)	3.15	0.666 (0.114, 34)	0.085 (40.59, 3)	43349	8.435
25/08/1999	9	9.8 (1.1, 20)	0	0.526 (0.045, 19)	0.021 (36.54, 3)	106267	7.593
25/08/1999	10	29.55 (3.94, 20)	1.21	0.526 (0.045, 19)	0.063 (36.54, 3)	106265	7.593
25/08/1999	11	49.89 (6.87, 20)	2.46	0.526 (0.045, 19)	0.107 (36.54, 3)	106265	7.593
deposit-feeding							
20/08/2000	12		0	5.544 (3.905, 9)	4910.0 (11.88, 8)	85.9	2.468
27/08/2000	13		0	7.191 (1.071, 28)	491.0 (11.88, 8)	84.9	2.468
07/09/2000	14		0	9.14 (0.661, 46)	491.0 (11.88, 8)	84.9	2.468

out on each date included a treatment with a fixed algal density (7.54 - 9.94 cells·ml^{-1}), whereas an additional 6 treatments with 19.51 to 170.87 cells·ml^{-1} were included to test the effect of varying algal concentration on the feeding physiology of *Yoldia hyperborea* (Table 4.1). Four to 17 replicates of each algal concentration were used on each occasion. Replicate water samples (n = 3) were taken at the beginning of the experiment for cell counts, whereas suspended particulate matter was estimated by filtering 50 to 100 ml of unlabelled algae on a GF/F filter, followed by drying (60°C, 72 hours) and combustion at 450°C (6 hours) for dry and ash-free dry weight calculations.

Faeces were collected every hour for the first five hours and then every three hours until the end of the experiment (66.5 to 82.5 hours). All faecal pellets produced were removed with Pasteur pipettes from the jar bottom and washed three times with 20 ml GF/F-filtered seawater to remove attached algae before transferring onto a 25 mm GF/F filter. A 2 ml water sample was also removed from the jar and filtered through a glass fibre filter (GF/F) at the beginning of the experiment and also when faeces were present. Residual $NaH^{14}CO_3$ was removed by adding 0.25 ml of 0.2 N perchloric acid to the sample. Vials were left loosely capped for twelve hours and 5 ml of scintillation cocktail (Ecolume) was then added. Samples were stored for 48 hours to ensure that ^{68}Ge attained a transient equilibrium with ^{68}Ga (Rivkin 1986). Counting was performed with a Packard Tri-Carb liquid scintillation spectrometer (model TR 2500); all sample counts were corrected for both quench (external standard method) and background activity.

4.2.1.4. Deposit-feeding experiments

Ambient sediment, which had been previously frozen (-20°C) to eliminate all fauna, was used to fill the bottom of the experimental jars (~ 8 cm layer). All jars were kept in an incubator at 0°C (±1°C) and total darkness. After sediment had settled in the jars, the overlying water was removed and replaced with filtered seawater (2µm). An equal amount of labelled *T. nordenskioldii* (see Table 4.1) was introduced into each jar and allowed to

settle for three days before the overlying seawater was replaced. After ensuring that the seawater contained no traces of radiolabel, animals were carefully placed on the sediment surface and allowed to bury. A total of five replicate jars with animals was used on each occasion. Jars were checked for faeces every hour for the first five hours and every three hours thereafter until the end of the experiment (46 to 99.5 h). Sediment samples were taken with a glass tube (3 mm diameter) from the top 7 mm layer at the beginning of the experiment and each time faeces were detected on the sediment surface. Sediment samples and faeces were processed as in the suspension-feeding experiments.

4.2.1.5 Handling of animals after the experiment

At the end of each experiment (*i.e.* after 68.5 to 82 hours), animals were removed from the experimental jars, placed in filtered seawater (2 µm) and checked for mortality. In order to confirm ingestion of algae by *Yoldia hyperborea*, a small portion of the digestive gland was removed from each individual after this period and separately processed for counting as described above for labelled algae, faeces and sediment.

4.2.1.6 Dual-labelling protocol

The maximum β energies for ^{85}Ge and ^{14}C are 1900 and 156 keV, respectively. The ratio of these maximum energies (*i.e.* 12.2) is high enough for complete separation of the two isotopes (Simmonet 1990). Standard quench curves were constructed for each isotope using internal standards and chloroform as quenching agent. The procedure outlined in the Packard Tri-Carb® liquid scintillation counter manual was followed for the dual labelling counting protocol. Accuracy of the technique was tested by counting known concentrations of ^{14}C and ^{85}Ge with the dual-label scintillation counting protocol.

For logistic reasons (hardware upgrade of scintillation counter), two different protocols of similar characteristics had to be used for counting label activity in suspension- and deposit-feeding experiments. After testing a wide range of known ^{85}Ge to ^{14}C ratios

with each protocol, it was determined that both isotopes could be accurately and predictably separated (Appendix IV, Figs. A.IV.1 & A.IV.2). To adjust the obtained ratios to the ideal 1:1 (^{68}Ge:^{14}C) - measured (X) to actual values (Y), all ratios were transformed in both suspension- ($Y = 0.829 \cdot X^{1.0247}$, $n = 6$, $r^2 = 0.999$) and deposit-feeding ($Y = 0.3052 \cdot X^{1.1298}$, $n = 9$, $r^2 = 0.999$) experiments.

4.2.2 Physiological measurements

4.2.2.1 Absorption Efficiency

Absorption efficiency (AE) of labelled microalgae was calculated from the formula devised by Conover (1966) as modified by Calow and Fletcher (1972)

$$AE = \left(1 - \frac{(^{68}Ge_a \; ^{14}C_a)}{(^{68}Ge_f \; ^{14}C_f)}\right) \cdot 100$$

where,

AE = absorption efficiency of organic carbon (%)

$^{68}Ge_a$ $^{14}C_a$ = ratio of ^{68}Ge to ^{14}C in dual-labelled algae

$^{68}Ge_f$ $^{14}C_f$ = ratio of ^{68}Ge to ^{14}C in faeces

Mean absorption efficiency (AE) was calculated from values obtained each time faeces were collected.

4.2.2.2. Gut passage time

Minimum gut passage time (GPT) was defined by the time elapsed between ingestion of radiolabelled algae and the first appearance of ^{68}Ge in the faeces

4.2.2.3. Ingestion rate

Ingestion rate was calculated from the total amount of ^{65}Ge (dpm) defecated during the experiment, after converting to reflect seston concentration (see formula). Gut passage time provided a correction for material still in the digestive tract as indicated in the formula.

$$IR = \frac{[(\Sigma^{65}Ge) \cdot (1 - U_a)]}{(\Delta t - GPT)} \cdot \frac{1}{PM}$$

where,

IR = ingestion rate ($mg \cdot h^{-1}$)

Σ^{65}Ge = sum of ^{65}Ge activity in all collected faeces (dpm)

U_a = number of unlabelled cells per labelled *T. nordenskioldii* cell

Δt = duration of experiment (h)

GPT = Gut passage time (h)

PM = concentration of particulate matter ($dpm \cdot mg^{-1}$)

No unlabelled *T. nordenskioldii* cells were added to the sediment in deposit-feeding experiments (*i.e.* $U_a = 0$).

4.2.2.4. Scope for Growth

The physiological index of energy balance given by scope for growth (SFG) represents the energy available for growth and reproduction of an individual after all the physiological demands of respiration and excretion have been met (Widdows 1985a). Rates of oxygen consumption (VO_2) and excretion (VNH_4-N) were calculated for each individual from the equations obtained in chapter 3 and converted to energy equivalents ($J \cdot h^{-1}$) using the conversion factors suggested by Widdows (1985b).

$$C\text{-}F = A = R + U + P_u + P_g$$

and $$SFG = A - (R + U)$$

where:

C = ingestion rate ($mg \cdot d^{-1}$) x energy content of food ($J \cdot mg^{-1}$)

F = energy lost as faeces ($J \cdot d^{-1}$)

A = energy absorbed (C x AE) ($J \cdot d^{-1}$)

R = oxygen uptake ($ml\ O_2 \cdot d^{-1}$) x 20.33 J

U = ammonia nitrogen excretion rate ($\mu g\ NH_4\text{-}N \cdot d^{-1}$) x $24.87 \cdot 10^{-4}$ J

P_s = production of somatic tissue ($J \cdot d^{-1}$)

P_g = production of gametes ($J \cdot d^{-1}$)

SFG = scope for growth ($J \cdot d^{-1}$)

Particulate organic matter (POM) was converted to energy equivalents by considering 1mg of POM as 20.78 J (Crisp 1984). This conversion factor was appropriate, as similar values were obtained after converting carbon values with the equation given by Platt and Irwin (1973).

4.2.3. Statistical Analysis

The effect of food conditions (*i.e.* food source and particle concentration) (=experiments) on absorption efficiency (AE)[%], gut passage time (GPT) [h] and ingestion rate (IR) [$mg\ PM \cdot h^{-1}$] was examined using a multivariate analysis of variance (MANOVA) after normalisation with arcsine square-root or $\log_{10}$ transformations when appropriate (Sokal & Rohlf 1995). In cases where ANOVA revealed significant differences, *a posteriori* pairwise comparisons of each independent variable (AE, GPT, IR) were performed between experiments (= food conditions) (see Appendix V, Table A.V.1), by employing the Tukey-Kramer HSD test (Sokal & Rohlf 1995). As food conditions and all other experimental conditions were similar in all three deposit-feeding experiments, the experiments were considered as replicates. All data transformations and analyses were conducted using the

SPSS® v. 10 statistical analysis package (SPSS Inc.)

4.3. RESULTS

4.3.1. Absorption efficiency

Mean absorption efficiency (AE)[%] of *T. nordenskioldii* carbon was 50.06 to 71.70 % in suspension-feeding and 87.68 to 94.76% in deposit-feeding experiments (Table 4.2). Since animals fed the two highest seston concentrations in suspension-feeding experiments (exp. 4 & 5) did not ingest algae, an AE of 0% was assumed in their case, so these treatments were significantly different from all other feeding conditions (Table 4.3). Significantly different absorption efficiencies were also observed between experiments 1 and 3, although both had similar feeding conditions, and the difference in AE may have resulted from the high variability in individual response to suspended particulate matter. Absorption efficiencies for *T. nordenskioldii* carbon were significantly higher in deposit-feeding than suspension-feeding animals, except for experiments 3 and 10 (0.022 and 0.063 mgPM · ml^{-1}) (*cf.* Tables 4.2 & 4.3).

4.3.2. Gut passage time

Gut passage time ranged from 8.15 to 57.31 hours in suspension-feeding experiments and 9.81 to 17.31 hours for deposit-feeding experiments, although mean values were similar ($\bar{x}$= 19.35 h, S.D. = 13.82 and $\bar{x}$= 13.80 h, S.D. = 3.31, for suspension- and deposit-feeding groups, respectively) (Table 4.2). Statistical comparison between

Table 4.2: Suspension- and deposit-feeding experiments with *Yoldia hyperborea* using suspended or sediment-mixed $^{68}Ge:^{14}C$ dual labelled *Y. nordenskioldii* as food. Experiments were conducted in the dark at 0°C. ^{68}Ge ^{14}C is the ratio of the isotopes in the faeces. AE – absorption efficiency of carbon contained in *Y. nordenskioldii*. n i – ingestion not observed, total time – experimental period. t_2 – time after removal from experimental setup

Date (D/M/Y)	Exp. #	Total Time [h]	*Y. hyperborea* shell length [mm] × (SD, n)	AE [%] × (SD, n)	Gut passage time [h] × (SD, n)	Ingestion rate [µg · h^{-1}] × (SD, n)	Mortality rate [%]	t_2 [h]
suspension-feeding								
18/05/1999	1	82.5	32.98 (1.68, 11)	50.06 (7.68, 11)	20.11 (11.73, 11)	1.91 (0.90, 8)	0.00	152.0
15/06/1999	2	74.0	30.35 (1.30, 17)	59.55 (12.33, 17)	18.50 (12.18, 11)	3.25 (2.47, 11)	0.00	147.5
07/07/1999	3	66.5	27.93 (0.56, 6)	67.77 (15.56, 6)	15.20 (14.78, 5)	-0.01 (0.00, 4)	0.00	148.5
07/07/1999	4	66.5	28.12 (0.55, 6)	n i (0.00, 4)	n i (0.00, 4)	0.00 (0.00, 6)	66.66	148.5
07/07/1999	5	66.5	27.77 (0.49, 6)	n i (0.00, 6)	n i (0.00, 6)	0.00 (0.00, 6)	50.00	148.5
27/07/1999	6	77.3	25.78 (0.53, 5)	55.30 (14.14, 5)	14.63 (13.51, 4)	0.18 (0.09, 5)	0.00	151.3
27/07/1999	7	77.3	27.44 (0.59, 6)	70.20 (6.41, 6)	12.75 (11.30, 5)	5.77 (4.99, 5)	0.00	151.3
27/07/1999	8	77.3	28.76 (1.42, 5)	71.70 (14.42, 5)	8.15 (7.90, 5)	93.66 (95.42, 5)	16.66	151.3
25/08/1999	9	75.8	28.20 (0.87, 5)	58.40 (20.28, 4)	12.01 (6.81, 3)	0.19 (0.18, 5)	0.00	149.5
25/08/1999	10	75.8	28.70 (1.43, 4)	61.96 (13.27, 4)	15.50 (20.93, 4)	-0.01 (0.00, 3)	0.00	149.5
25/08/1999	11	75.8	27.74 (0.93, 5)	69.76 (8.96, 5)	57.31 (31.93, 4)	-0.01 (0.00, 5)	0.00	149.5
deposit-feeding								
20/08/2000	12	99.0	26.58 (0.93, 3)	87.68 (5.96, 3)	13.67 (2.49, 3)	4135.0 (1734.8, 3)	0.00	99.0
27/08/2000	13	46.8	29.80 (1.99, 4)	98.52 (0.04, 3)	17.92 (3.77, 3)	2067.4 (2670.3, 3)	0.00	46.8
07/09/2000	14	99.5	30.98 (1.28, 4)	94.76 (3.39, 4)	9.81 (4.59, 4)	6872.3 (6050.8, 4)	0.00	168.0

Table 4.3. Pairwise comparison of absorption efficiency (AE)[%] between each of the suspension- (1-11) and deposit-feeding (12-14) experiments. Data from three deposit-feeding experiments were considered as replicates. Values represent P values (significant at P< 0.05*, P< 0.01**, P< 0.001***).

Experiment	2	3	4	5	6	7	8	9	10	11	12-14
1	1.000	0.018*	0.000***	0.000***	1.000	0.268	0.133	1.000	1.000	0.636	0.000***
2		1.000	0.000***	0.000***	1.000	1.000	1.000	1.000	1.000	1.000	0.000***
3			0.000***	0.000***	0.316	1.000	1.000	1.000	1.000	1.000	1.000
4				1.000	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
5					0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
6						1.000	1.000	1.000	1.000	1.000	0.000***
7							1.000	1.000	1.000	1.000	0.027*
8								1.000	1.000	1.000	0.058*
9									1.000	1.000	0.002**
10										1.000	0.108
11											0.044*
12-14											

experimental feeding conditions showed that as a result of their inability to feed, gut passage time in animals from experiments 4 and 5 (0.257 and 0.493 mg · ml^{-1}) was significantly different from all other experiments, after assuming total GPT of 66.5 h (*i.e.* duration of experimental period) (Table 4.4). The highest gut passage time ($\bar{x}$ = 57.31 h, S.D. = 31.93) was observed in animals from experiment 11 fed with 0.107 mg · ml^{-1} of suspended particles. The high variability in GPT observed in animals from this experiment may have resulted from the fact that these animals were exposed to the third highest particle concentration (0.107 mg · ml^{-1}). GPT from experiment 11 was significantly different than that in all other except experiments 1, 3 and 10, possibly as a result of their high variability and low n.

4.3.3 Ingestion rate

Faecal pellets collected from both suspension- and deposit-feeding individuals varied in size from 0.5 to 1.5 mm, although most were around 2 mm in length. Pellets of similar size were also observed in *Y. hyperborea* deposit-feeding in unlabelled mud (R. Stead, unpublished observations). Faecal pellets were easily obtained from the sediment surface in deposit-feeding experiments as the sediment surface was not greatly disturbed. In addition, faeces were not buried by particles from the pseudofaecal plume, as previous observations on *Yoldia hyperborea* feeding on un-enriched sediment showed, indicating that no significant amount of pseudofaeces was produced during the experiment.

Ingestion rate (IR) varied considerably between individuals in all suspension-feeding experiments (Table 4.2, Fig. 4.1). Animals fed low seston concentrations (0.016 to 0.022 mg · ml^{-1}) ingested ~0.01 to 3.25 µg · h^{-1}, whereas intermediate seston concentrations of 0.042 and 0.085 mg · ml^{-1} increased ingestion rates to 5.77 and 93.66 µg · h^{-1}, respectively. However, this increase was not apparent at 0.063 mg · ml^{-1} when animals showed ingestion

Table 4.4. Pairwise comparison of gut passage time (GPT) [h] between each of the suspension- (1-11) and deposit-feeding (12-14) experiments. Data from three deposit-feeding experiments were considered as replicates. Values represent P values (significant at $P<0.05^{*}$, $P<0.01^{**}$, $P<0.001^{***}$)

Experiment	2	3	4	5	6	7	8	9	10	11	12-14
1	1.000	1.000	0.000***	0.000***	1.000	1.000	1.000	1.000	1.000	0.352	1.000
2		1.000	0.000***	0.000***	1.000	1.000	1.000	1.000	1.000	0.020*	1.000
3			0.000***	0.000***	1.000	1.000	1.000	1.000	1.000	0.028	1.000
4				1.000	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
5					0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***
6						1.000	1.000	1.000	1.000	0.030*	1.000
7							1.000	1.000	1.000	0.009**	1.000
8								1.000	1.000	0.002**	1.000
9									1.000	0.037*	1.000
10										0.274	1.000
11											0.002**
12-14											

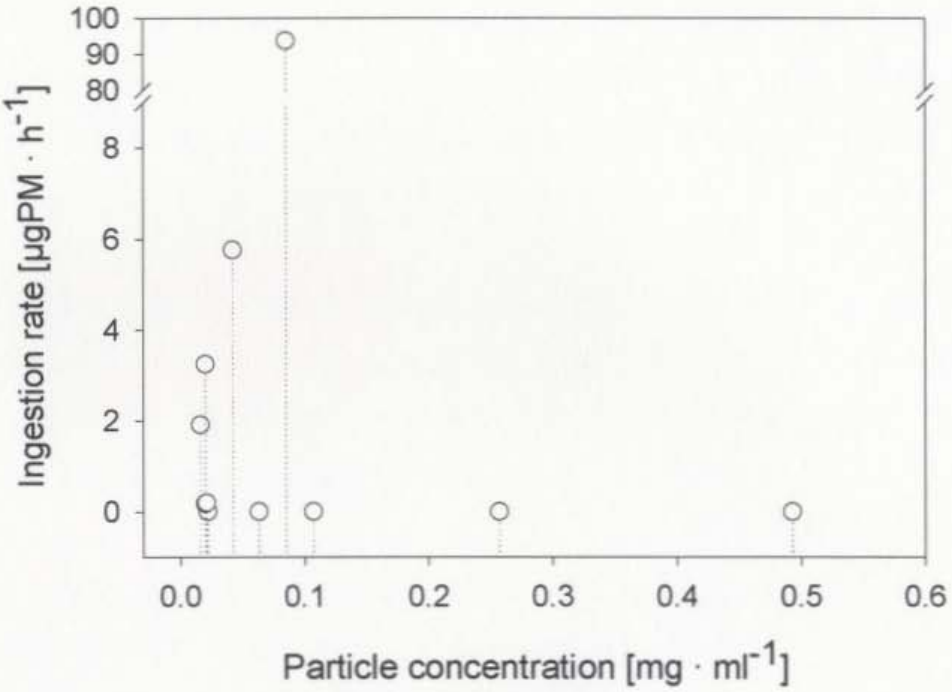

Fig. 4.1: *Yoldia hyperborea*. Suspension-feeding experiments. Mean ingestion rates at different concentrations of suspended particulate matter.

rates of $7 \cdot 10^{-4}$ mg · h^{-1} (Fig. 4.1). A low value of IR ($8 \cdot 10^{-4}$ mg · h^{-1}) was observed at a seston concentration of 0.107 mg · ml^{-1}, and animals ceased to ingest suspended particles at higher concentrations (Fig. 4.1).

In contrast, animals from all deposit-feeding experiments showed high mean ingestion rates, although the food value was only 31.86% (S.D. 1.69) (see Table 4.1) of particles offered to suspension-feeders. However, mean values also varied strongly between individuals (Table 4.2) and ranged from 2.07 to 6.87 mg·h^{-1}.

However, feeding rates were not significantly different between suspension-feeding experiments as a result of the high variability of individual response to suspended particulate matter. Significant differences were, however, observed between suspension- and deposit-feeding experiments (Table 4.5).

4.3.4 Mortality rates

No mortality was observed in animals from deposit-feeding and suspension-feeding experiments with low seston concentrations (Table 4.2). One individual died at 0.85 mg·ml^{-1} (16.66%) but no mortality was observed at the slightly higher concentration of 0.107 mg·ml^{-1}. However, 50.00 to 66.66 % of experimental animals exposed to the highest seston concentration (0.493 and 0.257 mg·ml^{-1}, respectively) died during, or after 150 hours from the start of the experiment. Death of an individual at high suspended particle concentration was usually preceded by behavioural changes, such as increased valve gaping and a strong loss of orientation. In the latter case, individuals were usually found inverted within the holding cup, with their anterior end towards the bottom of the jar and their foot slightly outside the valves pointing towards the top. Individual orientation was corrected each time an animal was found in this position, but without effect, as the animal always reverted to the

Table 4.5: Pairwise comparison of ingestion rate (IR) [mgPM · h^{-1}] between each of the suspension- (1-11) and deposit-feeding (12-14) experiments. Data from three deposit-feeding experiments were considered as replicates. Values represent P values (significant at P< 0.10', P< 0.05*, P< 0.01**, P< 0.001***). PM = particulate matter.

Experiment	2	3	4	5	6	7	8	9	10	11	12-14
1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.002**
2		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.002**
3			1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.032*
4				1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.032*
5					1.000	1.000	1.000	1.000	1.000	1.000	0.032*
6						1.000	1.000	1.000	1.000	1.000	0.032*
7							1.000	1.000	1.000	1.000	0.012*
8								1.000	1.000	1.000	0.013*
9									1.000	1.000	0.100'
10										1.000	0.100'
11											0.032*
12-14											

inverted position.

4.3.5. Scope for Growth

Scope for growth for *Y. hyperborea* under different feeding conditions is presented in Table 4.6. The metabolic cost of excretion and oxygen uptake corresponds to standard metabolic rates and therefore does not include the cost of handling and digestion of particulate matter. Particulate organic matter in the sediment was 32% of that contained in suspended particles (Table 4.1).

Mean oxygen uptake varied between 5.25 and 8.06 $J \cdot d^{-1}$, whereas mean ammonia excretion rates lay in the range between 0.018 and 0.037 $J \cdot d^{-1}$ (Table 4.6).

Ingestion rate of organic matter varied between 0.04 and 0.61 $J \cdot d^{-1}$ at low suspended particle concentrations (0.016 to 0.022 $mg \cdot ml^{-1}$). Suspension-feeding animals showed the highest ingestion rates (18.97 and 1.17 $J \cdot d^{-1}$) when exposed to seston concentrations of 0.085 and 0.042 $mg \cdot ml^{-1}$, respectively. However, at all other intermediate seston concentrations IR was always below 0.15 $J \cdot d^{-1}$. No ingestion was observed at seston values above 0.257 $mg \cdot ml^{-1}$. In contrast, deposit-feeding *Y. hyperborea* ingested 122.48 to 407.14 $J \cdot d^{-1}$. A similar trend was observed for absorption rate of organic carbon in all experiments.

Deposit-fed *Yoldia hyperborea* have a higher SFG than suspension-feeding individuals. Scope for growth for deposit-feeders ranged between 113.79 and 372.38 $J \cdot d^{-1}$, although their food ration remained constant in all three experiments (9.57 $J \cdot d^{-1}$). Scope for growth in suspension-feeding animals was negative except at a particle concentration of 0.85 $mg \cdot ml^{-1}$ ($\bar{x} = 9.57\ J \cdot d^{-1}$, S.D. = 16.14).

Table 4.6: *Yoldia hyperborea* Scope for growth calculated for *Y. hyperborea* in suspension- and deposit-feeding experiments. Mean shell length of all experimental animals was 28.50 mm (S.D. = 1.93)

Date (D/M/Y)	Exp. #	VO_2 [J·d^{-1}] ×	(SD, n)	VNH_4-N [J·d^{-1}] ×	(SD, n)	IR [J·d^{-1}] ×	(SD, n)	Absorption rate [J·d^{-1}] ×	(SD, n)	SFG [J·d^{-1}] ×	(SD, n)
suspension-feeding											
18/05/1999	1	7.86	(0.58, 8)	0.035	(0.005, 8)	0.35	(0.17, 8)	0.18	(0.11, 8)	-7.71	(0.66, 8)
15/06/1999	2	7.08	(0.61, 11)	0.029	(0.004, 11)	0.61	(0.46, 11)	0.41	(0.38, 11)	-6.69	(0.49, 11)
07/07/1999	3	6.05	(0.26, 4)	0.022	(0.002, 4)	0.54	(0.52, 4)	0.46	(0.46, 4)	-5.62	(0.31, 4)
07/07/1999	4	6.18	(0.07, 6)	0.023	(0.000, 6)	0.00	(0.00, 6)	0.00	(0.00, 6)	-6.20	(0.07, 6)
07/07/1999	5	6.03	(0.22, 6)	0.022	(0.001, 6)	0.00	(0.00, 6)	0.00	(0.00, 6)	-6.06	(0.22, 6)
27/07/1999	6	5.25	(0.19, 5)	0.018	(0.001, 5)	0.04	(0.02, 5)	0.02	(0.01, 5)	-5.25	(0.19, 5)
27/07/1999	7	5.87	(0.23, 5)	0.021	(0.001, 5)	1.17	(1.01, 5)	0.80	(0.69, 5)	-5.09	(0.77, 5)
27/07/1999	8	6.51	(0.28, 5)	0.025	(0.002, 5)	18.97	(19.32, 5)	16.10	(16.40, 5)	9.57	(16.14, 5)
25/08/1999	9	6.22	(0.59, 5)	0.024	(0.004, 5)	0.036	(0.03, 5)	0.02	(0.02, 5)	-6.23	(0.60, 5)
25/08/1999	10	6.49	(0.36, 3)	0.025	(0.012, 3)	0.13	(0.09, 3)	0.09	(0.05, 3)	-6.43	(0.33, 3)
25/08/1999	11	6.03	(0.59, 5)	0.022	(0.004, 5)	0.15	(0.11, 5)	0.12	(0.10, 5)	-5.94	(0.51, 5)
deposit-feeding											
20/08/2000	12	5.61	(1.08, 3)	0.020	(0.006, 3)	244.97	(102.78, 3)	216.67	(94.60, 3)	211.04	(95.51, 3)
27/08/2000	13	6.91	(0.87, 3)	0.028	(0.006, 3)	122.48	(155.95, 3)	120.73	(155.95, 3)	113.79	(156.05, 3)
07/09/2000	14	8.06	(1.25, 4)	0.037	(0.010, 4)	407.14	(358.47, 4)	380.48	(337.38, 4)	372.38	(337.98, 4)

4.3.6. Carbon assimilation

A comparison of $^{15}N:^{14}C$ ratios in the food source, faeces and digestive gland (Table 4.7) confirms the assimilation of carbon from labelled *T. nordenskioldii* into the tissues of *Y. hyperborea*. Faeces in suspension-feeding animals (excluding experiments 4 & 5) exhibited ratios of 4.16 (S.D. = 1.60), higher than in the food source, whereas in deposit-feeders this difference was 11.31 (S.D. = 1.54) times. This consistent difference could be attributable to the higher AE estimated for deposit-feeders.

Lower $^{15}N:^{14}C$ ratios were generally observed in the digestive gland than in the food source, meaning that carbon had been accumulated in the digestive gland. However, considerable differences were observed between suspension- and deposit-feeding individuals, since deposit-feeders contained 52.31 times (S.D. = 3.69) more carbon than their food source, whereas in suspension-feeders the mean difference was only 1.04 (S.D. = 0.42).

4.4. DISCUSSION

4.4.1. Labelling of microalgae as a food source

This study was based on the uniform labelling of *T. nordenskioldii* as a food source for *Y. hyperborea* under deposit- and suspension-feeding conditions. Several different approaches have been followed in deposit-feeding studies such as the widely used ash-ratio method developed for quantifying AE in herbivorous zooplankton (Conover 1966). However, this method is not always suitable for studies of deposit-feeders (see Lopez and Cheng 1983 and review by Lopez *et al.* 1989), as it requires large amount of faeces for a

Table 4.7: Comparison of mean ^{68}Ge to ^{14}C ratios (± S.D., n) in the food source (*T. nordenskioldii* in suspension and within sediment), and in the faeces, digestive gland and gonad of *T. hyperborea*. (n.d. - no available data)

Date (D/M/Y)	Exp. #	food source (^{68}Ge : ^{14}C) × (SD, n)	faeces (^{68}Ge : ^{14}C) × (SD, n)	digestive gland (^{68}Ge : ^{14}C) × (SD, n)
suspension-feeding				
18/05/99	1	0.123 (0.005, 6)	0.304 (0.073, 11)	0.060 (0.025, 9)
15/06/99	2	0.312 (0.035, 41)	1.148 (0.506, 17)	0.275 (0.050, 10)
07/07/99	3	0.317 (0.034, 23)	1.500 (0.983, 6)	0.713 (0.270, 6)
07/07/99	4	0.317 (0.034, 23)	0.000 (0.000, 4)	0.000 (0.000, 4)
07/07/99	5	0.317 (0.034, 23)	0.000 (0.000, 6)	0.000 (0.000, 4)
27/07/99	6	0.666 (0.114, 34)	1.571 (0.396, 5)	0.775 (0.255, 5)
27/07/99	7	0.666 (0.114, 34)	2.669 (1.080, 6)	0.491 (0.303, 6)
27/07/99	8	0.666 (0.114, 34)	3.841 (2.309, 5)	0.582 (0.083, 5)
25/08/99	9	0.526 (0.045, 19)	1.405 (0.758, 4)	0.525 (0.104, 4)
25/08/99	10	0.526 (0.045, 19)	2.201 (1.155, 4)	0.564 (0.227, 4)
25/08/99	11	0.526 (0.045, 19)	3.970 (2.598, 5)	0.776 (0.127, 5)
deposit-feeding				
20/08/2000	12	5.544 (3.905, 9)	72.057 (13.164, 3)	0.099 (0.028, 3)
27/08/2000	13	7.191 (1.071, 28)	83.930 (13.121, 3)	n.d.
07/09/2000	14	9.140 (0.661, 46)	84.774 (4.818, 4)	0.188 (0.081, 3)

reasonable estimation, a problem encountered when testing this method on *Yoldia hyperborea* under suspension-feeding conditions.

Although some authors have developed novel methods for quantifying feeding parameters (*e.g.* protein coated beads, Taghon & Jumars 1984), most have used various forms of radiolabelled sediment detritus. These methods, collectively called "slurry-methods", include labelling freeze-dried detritus or algal material with ^{14}C-formaldehyde or ^{14}C-dimethyl sulphate (Lopez & Cheng 1982, Lopez & Crenshaw 1982, Charles 1993, Charles *et al.* 1995) and mixing it with sediment. However, Amouroux *et al.* (1991) and Charles *et al.* (1996) demonstrated a high instability in the radioactivity contained in natural sediment radiolabelled with these methods. Lopez & Cheng (1983) considered these slurry methods as suitable for estimating ingestion rate and ingestion selectivity of organic matter, but not AE.

Absorption efficiency has been successfully measured by labelling microalgae with ^{14}C-bicarbonate and comparing the ratio of this label with a conservative tracer such as biogenic silica (Tande & Slagstad 1985) or another radiolabel such as ^{51}Cr (Lopez & Cheng 1983, Forbes & Lopez 1989a) and ^{75}Ge (Bochdansky *et al.* 1999). The ^{75}Ge method was chosen for this study because it requires a smaller amount of faeces and involves fewer analytical steps than the Si method. On the other hand, ^{51}Cr is not specific enough as it is adsorbed to various surfaces, and hence may be lost to large body surfaces like ctenidia and palps (*e.g.* Stuart *et al.* 1982), thus increasing the possibility of error (see Bochdansky *et al.* 1999 for a detailed discussion on problems). In addition, Decho and Luoma (1996) also indicated that a moderate presence of ^{51}Cr in bacteria and food reduced the feeding rate in the suspension-feeding bivalve *Potamocorbula amurensis*.

Nevertheless, in the present study the assumptions for the use of radiotracers in assessing ingestion of organic matter according to Calow and Fletcher (1972), Tande and

Slagstad (1985) and Lopez *et al.* (1989) have been met according to the criteria discussed by Bochdansky *et al.* (1999)

4.4.2. Absorption efficiency

Absorption efficiency has only been studied for three other protobranch bivalves (Bubnova 1972, Lopez & Cheng 1983, Cheng & Lopez 1991) and hence this study should contribute to further the knowledge of this bivalve group

Mean absorption efficiency of organic carbon was 62.74 % (S.D. = 7.12) for suspension-feeding *Yoldia hyperborea* and 93.65 % (S.D. = 4.49) when deposit-feeding on *T. nordenskioldii*. In contrast, only 50% of sedimentary organic matter and 51% of sedimentary protein is absorbed by the protobranch *Portlandia arctica* (Bubnova 1972). Furthermore, Lopez and Cheng (1983) found a mean AE of 72.1 % (range: 66.0 to 78.2) for sediment-associated bacteria in the nuculid *Nucula annulata*, whereas *Nucula proxima* (Cheng & Lopez 1991) absorbed bacteria with 65.25 % (S.D. = 15.93) efficiency. These results obtained from the literature are comparable to those obtained from *Y. hyperborea* suspension-feeding on the diatom *T. nordenskioldii* in this study. However, under natural conditions animals ingest sediment containing a variety of organic compounds of potentially different, mostly lower, digestibility and thus should exhibit lower AE values when feeding on bulk sediment. Cheng and Lopez (1991) recorded a mean AE of only 18.85 % (S.D. = 15.24) in natural sediment detritus containing 10.5% POM, but their values showed high seasonal variation, ranging between 1.42 and 42 %.

In order to meet metabolic demands animals should attain a minimum absorption efficiency of carbon, which may range between 4 and 13 % of ingested food (Cammen 1989). Cammen (1989) indicated that because non-living detritus is poorly absorbed in

comparison microbes (primarily bacteria and microalgae), it may be assumed that the latter form the major food source for deposit-feeders. Although bacteria may contribute some essential nutrients, such as B-complex vitamins, they lack polyunsaturated fatty acids (PUFA) which are essential to most metazoans, but which are abundant in diatoms (Phillips 1984). The sediment offered here to *Y. hyperborea* was highly refractory (R. Stead, unpublished) and thus it may be assumed that most of the digestible components obtained by this species in these experiments was provided by *Y. nordenskioldii* in the sediment.

Food quality has also been seen to affect AE in deposit-feeding lamellibranchs as shown by Charles *et al.* (1996) in *Abra ovata* fed with detritus from 11 macrophytes and labelled with ^{14}C-formaldehyde. Whereas detrital food from five of the macroalgae resulted in an AE below 3.9‰, four other diets showed AEs between 7.0 and 12.1‰ and the remaining two diets were hardly ingested (Charles 1993). On the other hand, when the same species was fed with detritus derived from the microalga *Pavlova lutheri* the authors observed AEs that ranged between 13 and 24‰ (Charles *et al.* 1995). However, their results may have been confounded by methodological problems (Amouroux *et al.* 1991, Charles *et al.* 1996) and by an indirect method for calculating AE (see Kofoed *et al.* 1989 for method).

Low AE was also observed in the mussel *Mytilus edulis* (18.4 - 28.2 ‰) after suspension-feeding dual-labelled (^{14}C and ^{51}Cr) *Isochrysis* sp. (T-Iso), although Thompson (1984) indicated AE values between 23 and 56‰ for this species from Newfoundland feeding on natural seston. AE values between 30 and 60‰ are typically found in bivalves feeding on natural seston but are usually high (~80‰) for suspension-feeders feeding on fresh algal diets, except at high concentrations (Bayne & Newell 1983). Thus *Mytilus edulis* showed AEs between *ca.* 81 and 87‰ and *ca.* 65 to 74‰ when fed with 1500 and 5500 cells·ml^{-1}, respectively, of the algae *Tetraselmis suecica* (Widdows & Bayne 1971). However, high AE can also be found under natural conditions, as demonstrated by Navarro

and Thompson (1996) for *Modiolus modiolus* from Newfoundland, which exhibited a seasonal variation in AE between 50.3 and 93.3%. The authors suggested that these high values could have been the result of low particle loads.

Although AE normally declines with higher particle concentrations in lamellibranch bivalves (Widdows & Bayne 1971, Bayne & Newell 1983), this was not observed for *Yoldia hyperborea*. Similar results were obtained by Cheng and Lopez (1991), where bacterial abundance did not relate to AE in the protobranch *Nucula proxima*.

The partitioning of food into a rapidly processed fraction in the intestine and a slowly digested fraction in the digestive gland was not immediately observable in these experiments, as faeces egested at the beginning of the experiment did not show lower absorption values than faeces obtained subsequently. Intestinal and glandular digestion (*sensu* Widdows *et al.* 1979) has been demonstrated for a number of suspension- and deposit-feeding lamellibranch bivalves (*e.g.* Decho & Luoma 1991, see also Bayne & Newell 1983), suggesting that particles are selectively routed into the digestive gland. This mechanism allows the animal to increase digestion rates without compromising effective absorption of nutrients.

However, the relative ^{15}N ^{13}C ratios in digestive gland and faeces indicated that glandular digestion was occurring in *Yoldia hyperborea*. If the digestive gland were to function solely as a nutrient storage area, no ^{15}N should have been observed in this tissue. Thus, it is possible that under deposit- and suspension-feeding conditions, most of the ingested algae was digested in the digestive gland, resulting in high AE values.

4.4.3. Gut passage time

Gut passage time (GPT) was approximately the same in deposit- and suspension-feeding *Yoldia hyperborea* individuals. Mean GPT from all experiments (except exp. 11) was 14.39 hours (S.D. = 3.47). These data are the first for a protobranch bivalve, and compare with 9.6 hours (S.D. = 1.8) for the facultative de posit-feeding *Macoma balthica* and contrast with 0.86 h (S.D. = 0.8) for the suspension-feeding *Potamocorbula amurensis* (Decho & Luoma 1991). However, Hummel (1985b) recorded a GPT of only 1.6 hours for *Macoma balthica*, although this occurred under suspension-feeding conditions. Navarro *et al.* (1992) reported a GPT for the suspension-feeder *Cerastoderma edule* between 2.28 and 4.85 hours, with higher values observed at lower concentrations of suspended particles. Levinton *et al.* (1996) calculated gut residence times for *Macoma nasuta* of 8.92 hours, whereas Hughes (1969) recorded times of 8 to 48 hours in the deposit-feeding *Scrobicularia plana*. On the other hand, Charles (1993) indicated that the GPT in the deposit-feeding *Abra ovata* is inversely correlated with ingestion rate, and is normally between 2 and 14 hours.

Bayne *et al.* (1989) suggested that bivalves balance variations in GPT and filtration rate to achieve relatively constant AEs under different feeding conditions, although Decho and Luoma (1991) indicate that such a balance can also be achieved by partitioning food into digestive processes with different absorption capabilities.

Jumars (1993) suggested that deposit-feeders in general have short gut residence times in order to process large numbers of particles. However, this does not apply to *Y. hyperborea* and other deposit-feeding bivalves, as they exhibit longer GPTs than suspension-feeding bivalves and bulk deposit-feeders (see Bayne & Newell 1983). Allen (1992) argued that the maximisation of extracellular digestion of refractive food material or of that attached to silt particles requires increased residence time within the gut, which in

protobranchs is facilitated by an elongated hindgut for more complete digestion and absorption. Lopez and Levinton (1987) stated that gut morphology reflects the adaptation of an animal to availability of labile organic matter. Thus animals exposed to high amounts of digestible food are equipped with short and wide digestive tracts and are adapted to high feeding rates and low gut passage times, whereas the contrary is observed in species with narrow and long intestines such as *Yoldia hyperborea*.

Although GPT was highly variable between individuals, no overall difference was observed amongst suspension- and deposit-feeding groups or between animals exposed to different particle concentrations. Kofoed *et al.* (1989) argued that rapid saturation of the absorption mechanism is likely to occur with easily absorbed food types such as diatoms and bacteria, and thus AE should not change considerably over a wide range of GPTs.

4.4.4 Ingestion rate

The data suggest that ingestion of suspended particulate matter (SPM) by *Yoldia hyperborea* is possible, although the rates observed were mostly very low. Nevertheless, in one case where *Y. hyperborea* was offered seston concentrations of 0.085 mg·ml^{-1} mean ingestion rates were 93.66 µg·h^{-1}. Estimated ingestion rates per tissue dry weight (0.118 mg·mg^{-1} DW·d^{-1}) during suspension-feeding, are much lower than values obtained for the cockle (*Cerastoderma edule* (4.986 mg·mg^{-1} DW·d^{-1}) (Navarro *et al.* 1994) and the horse mussel *Modiolus modiolus* (0.242 mg·mg^{-1} DW·d^{-1}) (Navarro & Thompson 1996), but higher than in *Mytilus galloprovincialis* (Labarta *et al.* 1997) and *Placopecten magellanicus* (Cranford & Hargrave 1994) (0.054 and 0.026 mg·mg^{-1} DW·d^{-1}, respectively) (Table 4.8). However, the observed rates occurred at suspended particle concentrations (SPM) ~50 times higher than those normally encountered by suspension-feeders, which indicates a low efficiency of suspended particle capture. Although these results reinforce the idea that

Table 4.8: Comparison of ingestion rates (IR) in different bivalves species when suspension-feeding (susp.) or deposit-feeding (dep.). Values of ingestion rate as a function of dry body mass (1 mg tissue DW) are shown as indicated by each source or estimated (e) with the equation $IR_e = IR_o \cdot (DW_e/DW_o)^{0.579}$, where IR_e and IR_o are the estimated and observed ingestion rates, respectively, and DW_e and Dw_o are the estimated and observed tissue dry weights, respectively. The exponent 0.579 was obtained from Bayne and Newell (1983). MD– Macrophytic detritus (source algae indicated), TPM – total particulate matter in suspension, POM– organic content of particulate matter (%), SPM– suspended particulate matter; n.d. – no available data. Parenthesis following species name indicates Superfamily: T– Tellinacea, M– Mytilacea, C– Carditacea, P– Pectinacea, Nc– Nuculida, Ncn– Nuculanacea. (*) values calculated by Lopez & Levinton (1987) from indicated source.

Species	Feeding mode dep./susp.	Temp. [°C]	Animal DW [mg]	Food source	TPM [mg·l^{-1}]	POM [%]	IR [mg·ind^{-1}·h^{-1}]	IR [mg·mg^{-1}·d^{-1}]	Source
Abra ovata (T)	dep.	n.d.	1	MD: *Ulva rigida*	n.d.	68.5	0.0085	0.204	Charles *et al.* (1996)
Abra ovata (T)	dep.	n.d.	1	MD: *Cystoseira mediterranea* & *Dilophys spiralis*	n.d.	70.3	0.0002	0.004	Charles *et al.* (1996)
Macoma balthica (T)	dep.	6	1	n.d.	n.d.	n.d.	9	216	Kofoed, unpub., in Lopez & Levinton (1987)
Macoma balthica (T)	dep.	6	5.1	Ambient sediment	n.d.	20	0.187	1.748 e	Bubnova (1972)
Macoma nasuta (T)	dep.	n.d.	1	Ambient sediment	n.d.	n.d.	1-2	24-48	Hylleberg & Gallucci (1975) (*)
Scrobicularia plana (T)	dep.	15	380	Ambient sediment	n.d.	3.4	1.792	1.380 e	Hughes (1969)
Cerastoderma edule (C)	susp.	17	200	Suspended silt + microalgae	2.191	56.14	4.465	4.986 e	Navarro et al. (1994)

Table 4.8: continued

Species	Feeding mode dep./susp.	Temp. [°C]	Animal DW [mg]	Food source	TPM [mg·l^{-1}]	POM [%]	IR [mg·ind^{-1}·h^{-1}]	IR [mg·mg^{-1}·d^{-1}]	Source
Modiolus modiolus (M)	susp	0-15	2000	Ambient SPM	1 203	64 5*	0 821	0 242 e	Navarro & Thompson (1996)
Mytilus galloprovincialis (M)	susp	14 5	1000	sediment + microalgae	1 035	56 21	2 265	0 054	Labarta *et al.* (1997)
Placopecten magellanicus (P)	susp	9 7	42000	Ambient SPM	1 5	58	47 900	0 026	Cranford & Hargrave (1994)
Nucula proxima (Nc)	dep	12-25	1	Ambient sediment + bacteria	n d	9 -13	0 32 - 0 45	7 68-10 8	Cheng & Lopez (1991)
Nucula annulata (Nc)	dep	15	1	Ambient sediment + bacteria	n d	19 3	0 3	7 201	Lopez & Cheng (1983)
Portlandia arctica (Ncn)	dep	6	19 9	Ambient sediment	n d	8 8	0 148	0 627 e	Bubnova (1972)
Yoldia limatula (Ncn)	dep	8-24	1	Ambient sediment	n d	n d	10-20	240-480	Bender & Davis (1984) (*)
Yoldia hyperborea (Ncn)	susp	0	161 8	suspended diatoms	85	40 59	0 094	0 118 e	this study
Yoldia hyperborea (Ncn)	dep	0	162 1	Ambient sediment + diatoms	n d	11 88	4 358	5 497 e	this study

suspended particles are only passively taken into the mantle cavity during ventilation as suggested by Levinton *et al.* (1996). *Y. hyperborea* was observed extending its siphons into the water column as if actively capturing these particles. This active behaviour could have been the result of stimuli other than particle concentration associated with the type of food offered to *Y. hyperborea*. Jumars (1993) indicated that at least four sets of stimuli seem to affect ingestion rate: smell, taste, distention of the gut and internal detection of the levels of absorbed products in body fluids. A good example of this is given by Charles *et al.* (1996), who offered sediment mixed with detritus derived from different macrophytes to the deposit-feeding *Abra ovata*: whereas maximum IR values were observed when individuals were feeding on detritus derived from *Ulva rigida* (0.204 mg·mg^{-1} DW·d^{-1}), very low IRs were observed when sediment was mixed with either *Cystoseira mediterranea* or *Dylophis spiralis* (0.004 mg·mg^{-1} DW·d^{-1}) (Table 4.8).

Cammen (1980, 1989) pointed out that the importance of a particular diet to an individual is given by the resulting IR and AE. However, IR and AE are in turn affected by digestive system morphology, gut residence time and digestive processing, which are unique and complicated in bivalves (Owen 1974, Bayne & Newell 1983, Decho & Luoma 1996). Bivalves are also selective deposit-feeders, as food is pre-sorted on the basis of size and organic content at one of the following stages: (1) before ingestion on the gills and labial palps, and additionally on the palp proboscides in the case of protobranchs, and (2) after ingestion in the stomach and digestive gland (glandular and intestinal digestion). However, because of this feeding strategy, bivalves also exhibit the lowest ingestion rates observed within the deposit-feeding guild (see Cammen 1980 and Bayne & Newell 1983, for review). In contrast, bulk deposit-feeders such as some polychaetes, usually have high ingestion rates to compensate for high inorganic content of their food (Linton & Taghon 2000a). However, feeding rates in polychaetes can also increase progressively with rising protein concentration until a plateau is reached (Gremare *et al.* 1991, Linton & Taghon 2000b).

Mean ingestion rate per body mass of *Yoldia hyperborea* during deposit-feeding was 5.497 mg·mg^{-1} DW · d^{-1}, which is similar to values recorded for other deposit-feeding protobranchs such as *Nucula proxima* (7.68-10.8 mg·mg^{-1} DW · d^{-1}) and *Nucula annulata* (7.201 mg·mg^{-1} DW · d^{-1}), but higher than in other deposit-feeding bivalves (0.004 to 1.748 mg·mg^{-1} DW · d^{-1}, see Table 4.8). Higher ingestion rates were reported by Lopez and Levinton (1987) and Cammen (1980) for *Yoldia limatula*, *Macoma balthica* and *Macoma nasuta* (240-480, 216 and 24-48 mg·mg^{-1} DW · d^{-1}, respectively). However, those values were not provided by the original authors (Hylleberg & Galluci 1975, Bender & Davis 1984) but calculated from information contained in their published work after making various assumptions in order to accommodate the data. Hence, the aforementioned ingestion rates are likely to have been overestimated and should be accepted with some caution. Nevertheless, descriptions of deposit-feeding in *Yoldia limatula* indicate that it is a more active species than *Y. hyperborea* (Drew 1899, Rhoads 1963, Bender & Davis 1984, Davis 1993) thus the former is likely to exhibit higher ingestion rates than the latter.

During deposit-feeding experiments, *Yoldia hyperborea* consumed 20 to 70 times more particles than it did at the highest ingestion rate observed during suspension-feeding. However, deposit-feeding is carried out primarily by the palp proboscides on a physically stable food source, as opposed to the involvement of the gill during suspension-feeding (see Levinton *et al.* 1996). In addition, intake of food by the palp proboscides only occurs on demand from the labial palps, so that particle capture does not occur at a constant rate, being limited by the handling capabilities of the labial palps as well as GPT.

4.4.5. Mortality

Although deposit-feeding animals are adapted to handling high particle loads, the high mortalities recorded at SPM levels above 0.085 mg·ml^{-1} indicate a negative impact of

these conditions on *Y. hyperborea* which may result from structural limitations.

The protobranch families Nuculidae and the Nuculanidae are known to have a ctenidial-palp association similar to that found in lamellibranch bivalves (Stasek 1965). Furthermore, endoscopic observations of *Yoldia limatula* indicated that during deposit-feeding, the mantle cavity is relatively free of suspended material (Levinton *et al.* 1996). However, suspended particles increased in numbers after digging action by the foot, or during particle ejection via the siphon. These suspended particles were rapidly captured and sorted by size on the ctenidia and later transported to around the 15th plate where they formed a mucous-bound ball before being transferred to the labial palps for ingestion (Stasek 1965, Levinton *et al.* 1996). Thus an increase in particle concentrations within the mantle cavity is a common occurrence, albeit of short duration. However, in experiments where animals were exposed to particle concentrations exceeding 0.107 $mg \cdot l^{-1}$, it is possible that the ctenidial-palp association system could have become overloaded, causing it to shut down altogether. It is likely that due to the lack of sediment animals were not capable of isolating themselves from their external environment by sealing off the mantle cavity and maintaining ventilation at a minimum rate. At high particle concentrations *Y. hyperborea* increased the valve gape, suggesting a lack of oxygen, probably as a result of increased numbers of particles on the gill surface which could have reduced gas exchange significantly.

Differences in individual survival may be explained by variations in ciliation of the ctenidia. After observing several individuals of *Yoldia scissurata*, Stasek (1965) indicated that the ctenidia of one individual contained complete ciliation, whereas cilia in another was restricted to some platelets near the 15th plate. If these differences also occur in *Y. hyperborea*, then it could be argued that some individuals are more capable of coping with higher suspended particles than others. A closer look to ctenidial structure morphology should clarify this point.

4.4.6. Scope for growth

This is the first report of scope for growth (SFG) for a protobranch bivalve. However, these results must be taken with some caution as measurements that led to this estimation (VO_2, VNH_4-N, AE) were carried out independently on different sets of animals.

Ammonia excretion has a low impact on the energy budget, contributing a maximum of less than 0.5% in non-feeding animals. Because of its low impact, the energy lost in excretion has not been considered in SFG calculations by many authors (Bayne & Newell 1983), but was included in the present study.

Mucus production may be a significant source of energy loss and is usually ignored in energy balance studies (Bayne & Newell 1983). Although much of the mucus produced by bivalves during feeding may be reingested, a considerable amount is lost during the production of pseudofaeces. However, energy loss due to mucus production is likely to be negligible in *Yoldia hyperborea*, because sediment particles in pseudofaeces are not bound together when expelled as a cloud from the exhalent siphon.

The fraction of energy expenditure resulting from oxygen consumption depended on the amount of POM ingested. Thus, in suspension-feeding *Y. hyperborea* it amounted to 68% of the total energy budget at 0.085 mg·ml^{-1} and over 99% in all other experiments. During deposit-feeding animals ingested more POM, and oxygen consumption accounted for less than 5% of the total energy budget. However, these values do not include the cost of feeding and processing of food. Griffiths and Griffiths (1987) found that standard respiration values increased two to four times in *Mytilus californianus* after initiation of feeding, and particle concentration also affected oxygen uptake in *Cardium edule* (Navarro *et al.* 1992). In contrast, other studies have found that oxygen consumption of some bivalve species, such as *Aulacomya ater* (Stuart *et al.* 1982), *Mytilus edulis* (Widdows *et al.* 1979)

and *Spisula subtruncata* (Møhlenberg & Kiorboe 1981) are independent of the concentrations of suspended microalgae and sediment. The scallop *Placopecten magellanicus* also shows O_2 uptake rates that are independent of seston concentration and quality, in contrast to values obtained at the same time in the clam *Mya arenaria*, which increased VO_2 twofold when exposed to increased seston concentration and improved food quality (MacDonald *et al.* 1998).

Hummel (1985b) studied a population of *Macoma balthica* that alternated between suspension- and deposit-feeding and concluded that standard O_2 uptake (*i.e.* no feeding) in this species was increased 3 to 5 times during suspension-feeding and 4 to 9 times during deposit-feeding. If the cost of metabolism in *Y. hyperborea* were arbitrarily increased to 9 times the standard value obtained here, it would still account for 19.5 to 43.8% of the energy budget during deposit-feeding.

However, SFG also depends on the organic content of food and the estimations obtained here are for sediments containing around 12% of POM. Seasonal values for organic matter from Conception Bay sediments range between 8 and 13% DW (R. Thompson, unpublished), with highest POM values typically found in early spring after bloom fallout events have occurred and lowest values between the end of summer and late winter. Thus, SFG of *Y. hyperborea* from Conception Bay would should fluctuate with changes in organic matter content of sediment.

Y. hyperborea needs to ingest a minimal amount of particulate organic matter in order to sustain its metabolic cost. If absorption rates were to decrease 20 times to account for a decrease in sediment quality, individuals would still maintain positive SFG. The experimental conditions included enriched sediment or suspended particles that simulated specific conditions that would be observed only during the phytoplankton bloom fallout, so that a reduction in energy gain would be expected under ambient conditions.

In addition, SFG calculations assume that all the POM is metabolisable. However, POM in sediment includes refractory organic matter which was not quantified but which could account for a significant proportion of the organic matter ingested by *Y. hyperborea* in its natural habitat. Rice and Rhoads (1989) assumed that 33% of deposited POC was metabolisable, whereas Mayer (1989) suggested that the fraction of sediment POM utilisable by deposit-feeders was only 5 to 30%. Thus, if five-fold standard respiration rates and only 20% of ingested POM were considered to be metabolisable (see Table 4.3), animals would still be able to meet their minimum metabolic demands.

As 60 to 80% of suspended particulate organic matter is usually metabolisable (*e.g.* Navarro & Thompson 1996), suspension-feeding could become a beneficial strategy during the first bloom fallout, when sediment quality is at its lowest, but not once the sediment has been enriched. Although deposit-feeding is the main strategy followed by *Yoldia hyperborea* and related species (personal obs., Bender & Davis 1984, Davenport 1988b, Davis 1993), results obtained here suggest that suspension-feeding can be advantageous under certain conditions, possibly as a response to water-borne chemical cues released by settling microalgae after a prolonged period of low food availability (Ward & Targett 1989). However, these conditions are likely to be sporadic and of short duration (see chapter 2). In addition, suspension-feeding may be beneficial in order to capture high quality food particles before they are ingested by competitors (*e.g.* benthic and hyperbenthic species). However, because capture of suspended particles by *Yoldia hyperborea* is not an efficient mechanism, only very low ingestion rates would be observed, thus reinforcing the view that protobranch bivalves are primarily deposit-feeders.

CHAPTER 5

BEHAVIOURAL RESPONSES OF *YOLDIA HYPERBOREA* TO SETTLING MICROALGAE AND RESUSPENSION EVENTS

5.1. INTRODUCTION

Deposit-feeding bivalves graze upon food present in the surrounding sediment. Local production and deposition of food particles renew their food supply, but fresh food will often be limiting for a deposit-feeder. Furthermore, in areas with strong seasonal primary production, the annual energy cycle (*e.g.* energy storage, reproduction and growth) of deposit-feeders is regulated by the input of settling organic matter from bloom fallout events (Jumars *et al.* 1990, Allen 1992), emphasising the fact that deposit-feeder nutrition is limited by algae-derived food, particularly in populations inhabiting the subphotic zone. Although local bacterial production may contribute in part to deposit-feeder nutrition, its nutritional role does not seem as critical as that of the essential nutrients contained in algae (Phillips 1984, Cammen 1989, Charles *et al.* 1999).

Many marine species are known to vary their feeding behaviour in response to changes in their surrounding environment (*e.g.* food, flow speed), but it is of interest to know what cues trigger the behavioural shift. Some infaunal species are known to modify their behaviour for reproductive purposes (Ansell *et al.* 1998), in response to the presence of predators (Vadas *et al.* 1994, Smith & Jennings 2000) or prey (Weissburg & Zimmer-Faust 1994) or in order to avoid stress associated with unfavourable oceanographic conditions, parasitism or contaminants (Tyurin 1991, Roper *et al.* 1995). On the other hand,

suspension-feeding organisms are known to respond to changes in suspended particle concentration or organic content (Okamura 1990). In addition, ingestion of food with higher value has led some species to increase switch from deposit- to suspension-feeding and *vice-versa*, thus potentially increasing their fitness (Levinton 1991, Taghon & Greene 1992, Bock & Miller 1997).

Protobranch bivalves have long been recognised as deposit-feeders (Drew 1899, Yonge 1939), but some authors have noted the capacity of members of the genus *Yoldia* to capture particles in suspension (Stasek 1965, Davenport 1988b, Nakaoka 1992), although the importance of this mechanism has been questioned (Yonge 1939, Levinton *et al.* 1996). Quantitative feeding experiments with *Yoldia hyperborea* indicated that this species is capable of ingesting suspended particles, but ingestion rates were low and implied more energy expenditure than gain (see Chapter 4). However, qualitative observations showed that *Y. hyperborea* actively extended its siphons into the water column when offered suspended algae, indicating an active behavioural response despite the energetic limitations.

Although there have been many studies on the feeding behaviour of suspension- and deposit-feeding bivalves, the great majority have dealt with physiological responses (*e.g.* feeding and ingestion rate, gut passage time, AE, VO_2, VNH_4). Of the few studies reporting direct observations of behaviour in bivalves, most were done more than 80 years ago (*e.g.* Drew 1899,1901, Kellogg 1915), and these as well as more recent reports (Davenport 1988b) were limited to qualitative descriptions. Although qualitative accounts of behaviour are useful in understanding ecological patterns and physiological responses, they do not provide information on their significance to the species or population under consideration. Underwood *et al.* (2000) stress the necessity of more quantitative observations as the basis for providing the context for studying mechanisms and processes in ecology. In addition, the growing recognition that behaviour can be quite flexible within and between populations emphasises the importance in quantifying its variation (Chapman 2000).

Yoldia hyperborea is a protobranch bivalve inhabiting soft sediments in Conception Bay, Newfoundland. Periodic monitoring of environmental conditions showed that this population is exposed to strong seasonal input of organic matter during one principal and one secondary phytoplankton bloom fallout event, in addition to resuspension of local or nearby bottom sediments (see Chapter 2). Although both resuspension and bloom fallout events lead to an increase of suspended particles, food quality should be greater in the latter.

The purpose of the study in this chapter was to quantify the behavioural response of *Yoldia hyperborea* to pulses of settling algae and sediment resuspension events, in order to understand the relative importance of previously reported behavioural patterns and the role of suspended particle concentration as a food source for this species.

5.2. MATERIAL AND METHODS

5.2.1. Experimental Setup

The experimental setup consisted of 2 identical refrigerated holding tanks (444 litre capacity). Each tank was aerated and supplied with flowing filtered ambient seawater (2 µm) maintained at 0°C (± 1 °C). The bottom of each tank was fitted with a perforated tray over which four glass aquaria of similar dimensions were placed (L=50.2 cm, W= 25.3 cm, H= 32.0 cm). Before introducing animals, each aquarium was filled with a n 8 cm-thick layer of muddy sediment obtained by dredge or corer from the deep-depositional zone of Conception Bay.

Since the seawater level in the tank was usually 2 cm above the top of the aquarium,

a 1 mm black plastic mesh was placed over the aquarium in order to allow water exchange while avoiding disturbance of the sediment by the turbulence caused by constant air bubbling. Light was also minimised by this mesh and by a black cover placed over each tank.

5.2.2. Treatments

Each of the four aquaria in one tank represented one of four treatments which were replicated in the second tank. Three of these treatments (Treatment 1: *algae addition*, Treatment 2: *no addition*, Treatment 3: *resuspension*) contained 11 adult *Yoldia hyperborea* of 33.04 mm mean shell length (range: 29.07 to 44.08 mm), whereas one treatment (Treatment 4: *Control*) contained no animals and served as control for sediment and suspended particle concentration.

Monocultures of the diatom *Thalassiosira nordenskioldii* were harvested in the senescent phase, washed with filtered seawater to eliminate traces of nutrients, and added to one of the animal-containing aquaria (Treatment 1: *algae addition*). The purpose of this treatment was to observe the response of *Yoldia hyperborea* to settling algae, as would be expected under natural conditions during phytoplankton bloom fallout events. Algae were added at days 20.2, 34.0, 44.6 and 55.3 after the start of observations. The water level was lowered below the top of the aquaria for 24 hours during algal addition in order to avoid accidental contamination of Treatments 2, 3 and 4 with algae from Treatment 1.

The effect of sediment resuspension events (*i.e.* particle increase) on *Yoldia hyperborea* behaviour was simulated by resuspending the sediment of a third aquarium that also contained animals (Treatment 3: *resuspension*). At the same time that algae were added to the *algae addition* treatment, short resuspension events were simulated by carefully

blowing over the sediment surface for a few seconds, with a pipe (0.7 cm diameter), away from areas where animals were present.

A fourth aquarium with animals acted as control for animal behaviour. No changes or additions were performed in this group (Treatment 2, *no addition*).

5.2.3 Handling of specimens

Adult specimens of *Yoldia hyperborea* (Loven) were collected by dredge from muddy sediments of the deep-depositional zone (240-265 m) of Conception Bay, Newfoundland (47°34.0' N, 53°08.1' W to 47°32.5' N, 53°07.8' W), transported on ice to the Ocean Sciences Centre in Logy Bay and placed in a refrigerated tank (0 ± 1 °C) containing aquaria with ambient sediment and recirculating seawater (~33‰ salinity). After two weeks these individuals were briefly removed from the aquaria, measured with a vernier caliper (shell length) and numbered with a permanent felt pen (Staedler® Lumocolor 318) for identification.

After all animals had been held in the aquaria with sediment for an additional month they were randomly separated into six groups of 11 animals each (one group per aquarium). To minimise handling at the start of observations, each group was placed in individual beakers without sediment five days earlier.

5.2.4 Quantification of behaviour

A preliminary experiment, carried out between August and October 1998 (56 days), with similar characteristics to the one described here, indicated the potential variables to be

measured in this experiment as well as improvements to the original methods.

The experiment reported here began on 22nd December 1999 (Day Time 0), when animals were placed on the sediment surface of each aquarium, and ended on 20th February 2000 (Day 60). To allow for adequate acclimation, only days 20 to 60 were considered for comparative purposes. All observations were made by the same investigator daily around midday, although additional observations were carried out during the first day after addition of animals and during the first 3 days after addition of algae and sediment resuspension.

The positions of the individuals were recorded immediately after placing them on the sediment surface, using a metal grid with 1.2 cm^2 squares placed a few mm above the surface. The grid also served as a ruler (± 0.2 cm) within the aquarium.

Daily observations were carried out for 20 minutes on each aquarium, in which the following properties of each animal were recorded: (1) Position - as shown by the siphon openings, valves or palp proboscides, unless the animal had altered its position since the previous recording, in which case the route followed was also estimated from marks left on the sediment surface by using the grid. (2) Burial [% of shell length] - estimated proportion of the shell length below the sediment surface (0, 25, 50, 75, 90, 100 %)(Fig. 5.1). (3) Estimated angle - if part of the shell was visible above the surface the angle of the individual with respect to the horizontal plane was noted (see Fig. 5.1 B). (4) Presence of palp proboscides - the presence of one or both palp proboscides on the sediment surface was recorded in addition to whether they had left fresh striations on the sediment surface since the previous observation (see Fig. 5.1 B). (5) Length of the palp proboscides [cm] - the portion of the palp proboscides present on the sediment surface was measured with the grid (± 0.2 cm). (6) Siphon position - position of the portion of the siphon visible above the sediment surface was rated "horizontal" when lying on the sediment surface or close to it, "vertical" when the siphon was extended into the water column, or "absent" when no siphon

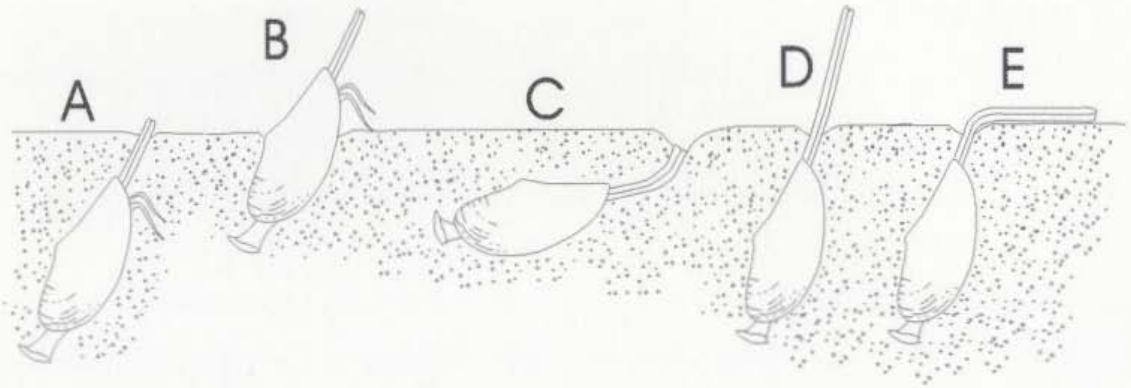

Fig. 5.1: *Yoldia hyperborea*. Commonly adopted positions within the sediment: (A) subsurface deposit-feeding with extended palp proboscides; (B) surface deposit-feeding with extended palp proboscides during reemergence of an individual (approximate angle = 50°); (C) subsurface position of an animal only showing the tip of its siphon; (D) vertical extension of the siphon into the water column; (E) horizontal positioning of the siphon over the sediment surface.

was visible (*cf.* Fig. 5.1 C, D and E); (7) Siphon length [cm] - length of the visible portion of the siphon as measured with the grid (*cf.* Fig. 5.1 A to E).

5.2.5 Faeces expulsion and ventilation frequency

During the course of the experiment, differences in the frequency of faeces production became apparent between animals exposed to algae addition and those from other treatments, the former producing more faeces and less pseudofaeces, although the ventilation frequency appeared to be the same. In order to determine the significance of this pattern, observations were carried out on 6 occasions (days 33.06, 36.21, 42.18, 47.07, 51.17 and 58.18) starting before the second algae addition/ resuspension event.

All observations were carried out for 10 minutes per animal, during which the ventilation frequency (*i.e.* expulsion of water from the mantle cavity) was noted. A record was also made if ventilation was accompanied by the expulsion of faeces and/or pseudofaeces.

5.2.6 Water column and sediment

Suspended particle concentration was measured by taking 2 replicate samples of 20 ml of seawater from 0.5 and 2.5 cm above the sediment surface with syringes attached to a rod placed just above the sediment surface. Samples were obtained from each aquarium on 26 occasions, between day 16 and day 58. Sampling intervals varied between 0.23 and 6.10 days, with shorter intervals just after algae addition or resuspension events. Water samples were allowed to reach ambient temperature (~20°C) before particles between 1.6 and 25.6 µm were counted with a Coulter® Multisizer II® fitted with a 70µm orifice tube.

Sediment from each aquarium was sampled 7 times between day 19 and day 60, before and after the addition of algae and resuspension of sediment. Triplicate sediment samples were randomly taken with a 1.1 cm corer (internal diameter) from the top 1.5 cm layer, and stored at -70°C. Pigments were extracted from lyophilised sediment at -20°C for 20 hours in the dark with 90% acetone (HPLC grade). Chlorophyll *a* and phaeopigments were quantified with a Turner Designs® Model 10 fluorometer.

5.2.7 Histology

At the end of day 60, 4 animals from each aquarium were dissected and their digestive glands removed for histological examination in order to quantify the overall physiological feeding response to the various treatments. The complete digestive gland of each individual was then fixed in Baker's formol calcium with 2.5% sodium chloride and refrigerated at 4 °C (Lowe & Moore 1985). Tissue samples were maintained in fixative for at least a week before transferring to gum sucrose solution for storing prior to dehydration, clearing and embedding in paraffin wax (Paraplast®). Tissue sections (7 µm) were left to dry for a minimum of 5 days at 20°C before staining with haematoxylin and eosin.

Inspection of sections was carried out at 250x magnification on a Zeiss Axiovert® inverted microscope. A Sanyo® colour CCD high resolution camera (model VDC-2972) connected to a computer equipped with image processing software (Image-Pro Plus® v 4.1.0.0 for Windows®) and frame grabber board was used to capture 5 to 7 different fields to complete a minimum of 50 tubule measurements. Measurements were made by placing a sampling matrix (lines 76.84µm apart) over the image and measuring the height of intercepted cells as indicated by Lowe *et al.* (1981) and Lowe and Moore (1985). No measurements were made on tubules that had intersected obliquely or which appeared to be several cell layers thick. From these data the mean cell height and standard deviation was

calculated for each animal

5.2.8. Statistical analyses

Sediment pigments, particle concentration and behavioural measurements between treatments were compared separately using a multivariate repeated measures ANOVA after adjusting individual treatment values to averages (Sokal & Rohlf 1995). Data in the form of percentages was transformed to arcsine $\sqrt{x}$, whereas categorised observations were treated as nominal data. The data were also analysed to test for both within-subject treatment effects and between groups on either sediment pigments, particle concentration or behavioural measurements. Greenhouse-Geisser adjustments to the P-Values were done to protect against possible violations of the sphericity assumptions. Tukey-HSD comparisons were used as a post-hoc test of specific differences between the treatments. In addition, pairwise comparisons of treatments between tanks were performed when the analysis of variance indicated significant differences between tanks. All analyses were performed with SPSS* v. 10 statistical analysis package (SPSS Inc.).

5.3. RESULTS

5.3.1. Particle concentration

Mean particle concentration (1.6 to 25.6 µm particle size range) from all treatments fluctuated between $13.6 \cdot 10^3$ and $390.5 \cdot 10^3$ particles·ml^{-1} (Fig. 5.2). Highest values were usually observed in the *algae addition* and *resuspension* treatments after algae addition and

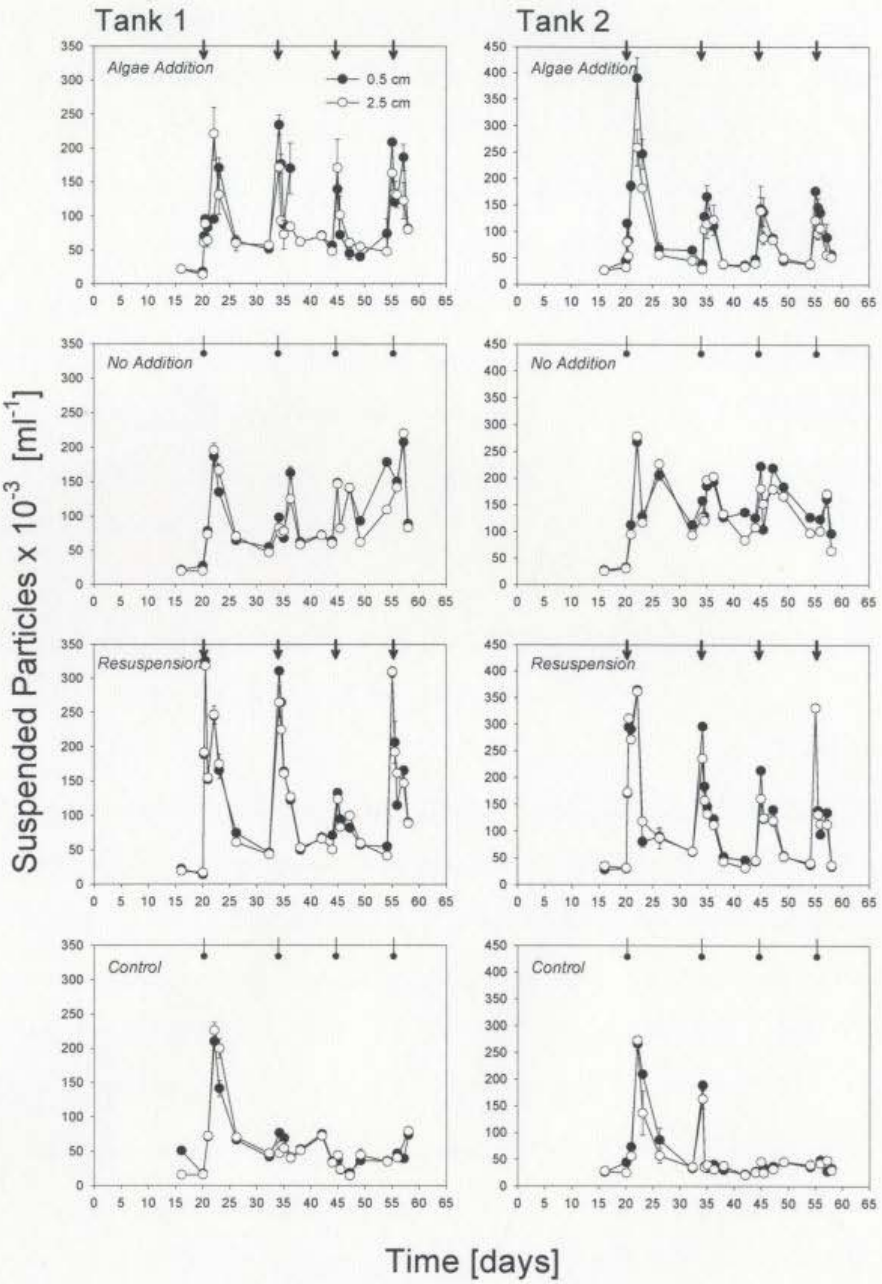

Fig. 5.2: Particle concentration [ml^{-1}] at 0.5 and 2.5 cm above the sediment surface in all four treatments from both tanks throughout the experimental period. Particles measured were within the range 0.5 to 25 μm in diameter. Times of algae addition and sediment resuspension are represented by arrows (algae addition and resuspension treatments) or lines (no addition and control treatments).

resuspension events, although high particle concentrations, up to $278.2 \cdot 10^3$ particles·ml^{-1}, were also observed on occasion in the *no addition* treatment. The *control* aquaria showed the lowest particle concentration except around day 22 (both tanks) and day 34 (tank 2) where mean values were between $188.5 \cdot 10^3$ and $266.5 \cdot 10^3$ particles · ml^{-1}. Particle increase in treatment 2 (*no addition*) and treatment 4 (*control*) occasionally increased 24 hours after the algae addition/resuspension events, coinciding with the restoration of the water level above the top of the aquaria, suggesting that some resuspension may have occurred after the first event. However, particle increase in treatment 2 (*no addition*) at other times was more erratic and was possibly the result of higher frequency of pseudofaeces expulsion in animals not exposed to enriched food.

Although suspended particle concentration was high in all treatments, mean particle diameter from all samples was only 2.58 µm (S.D. = 0.50).

Statistical comparison between treatments (Table 5.1, Fig. 5.3) indicated significant differences in particle abundance between treatment 1 (*algae addition*) and treatments 2, 3 and 4 (*no addition*, *resuspension* and *control*, respectively), particularly after times of algae addition to the aquaria. As time progressed this difference was not as strong or not significant (see P value categories in Fig. 5.3). However, for treatments *algae addition* and *resuspension*, differences were generally only significant within 2 or 3 days of algae addition and resuspension events. A similar trend was observed when comparing *resuspension* and *control* treatments. However, when comparing *no addition* to *resuspension* and *control* treatments, differences were found to be highly significant throughout most of the experimental period, but these differences showed no particular trend.

Mean sediment chlorophyll *a* from treatments 2, 3 and 4 was 10.2 ng·mg^{-1} sediment (S.D. =1.5) throughout the entire experimental period, whereas it increased steadily from

Table 5.1: Two way analysis of variance for testing between-subject effects for particle concentration [No. x ml^{-1}] against time [days] in 4 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	5.039×10^{12}	1	5.039×10^{12}	3376.517	0.000
Treatment	1.713×10^{11}	3	5.711×10^{10}	38.268	0.000
Same treatment between tanks	4.879×10^{10}	4	1.22×10^{10}	8.173	0.006
Error	1.194×10^{10}	8	1.492×10^{9}		

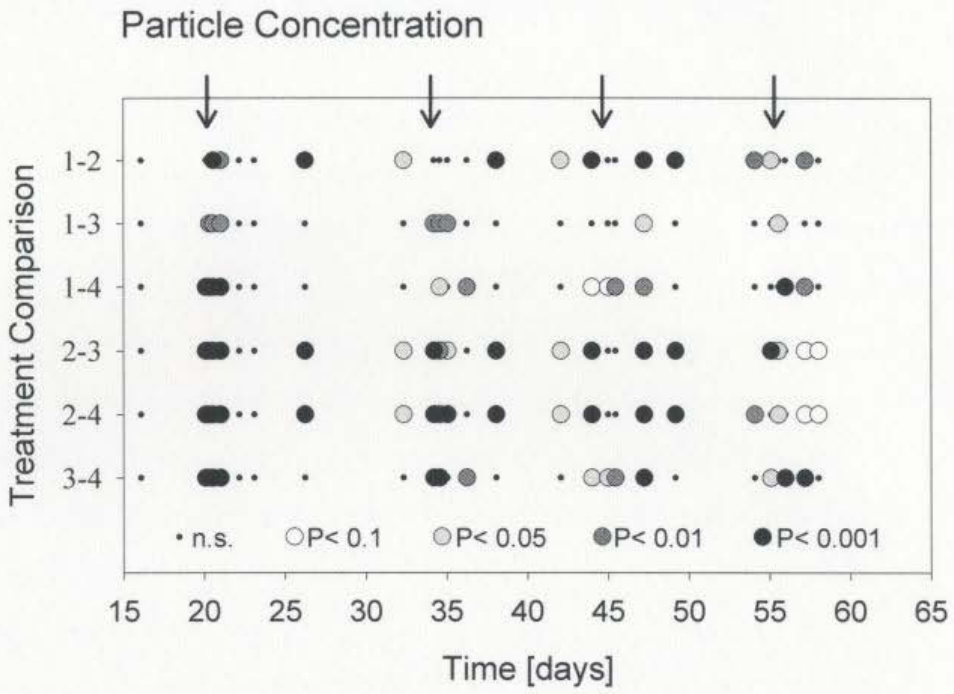

Fig. 5.3: Comparison of particle concentration between treatments from both tanks during the experimental period. Significance of the interaction is represented by circles of varying shade. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension, 4= Control. Times of algae addition and resuspension events are indicated by arrows.

10.4 to 41.6 ng·mg^{-1}sediment ($\bar{x}$ = 23.6, S.D. = 9.0) as algae were added to sediments in treatment 1 (*algae addition*) (Fig. 5.4). Phaeopigments, however, showed mean concentrations of 32.6 ng·mg^{-1} sediment (S.D. = 3.5) in sediments from treatments 2, 3 and 4, similar to values found in treatment 1 sediments ($\bar{x}$ = 34.302, S.D. = 1.7) (Fig. 5.4).

Statistical comparison of treatments showed that sediment chlorophyll *a* content was significantly different between treatment 1 (*algae addition*) and treatments 2, 3 and 4 (*no addition*, *resuspension* and *control*, respectively) primarily after the first addition of algae (Table 5.2, Fig. 5.5). In contrast, significant differences between treatments 2, 3 and 4 were observed in samples of days 22 and 32, but not during the rest of the experimental period. On the other hand, phaeopigment *a* concentration was not significantly different between sediments of all treatments except on the last sampling date (day 60)(especially in treatment 1), possibly as a result of breakdown of algae added to these aquaria throughout the study period (see Table 5.3, Fig. 5.6).

5.3.2. *Yoldia hyperborea* behaviour

After being added to the aquaria, some animals buried immediately whereas most took two or three days to bury completely, the last doing so on day 16 (Fig. 5.7). During the experimental period animals in treatments 2 and 3 (*no addition* and *resuspension*, respectively) remained buried most of the time with only a few cases of re-emergence being observed, although usually no clear temporal pattern was evident in these cases. However, a few cases of slight reemergence in the *no addition* treatment from tank 1 coincided with the third and fourth algal addition/resuspension, suggesting that some algae may have entered in that aquarium as a result of tank aeration. In contrast, animals from aquaria where *Y. nordenskioldii* was added consistently reemerged from the sediment 3 hours to a day after each algal addition (Fig. 5.7). Reemergence of *Y. hyperborea* individuals had a duration of

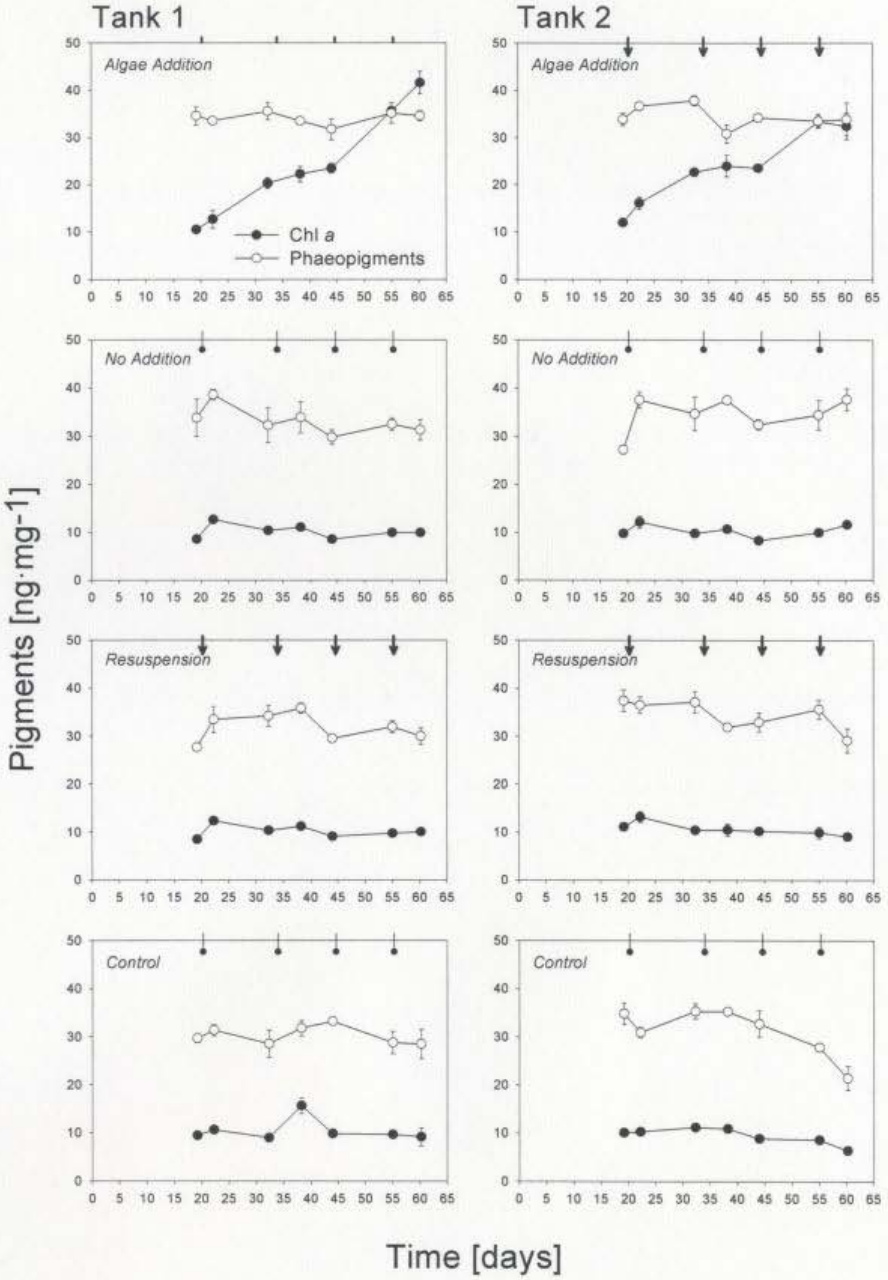

Fig. 5.4: Temporal variability of sediment chlorophyll *a* and phaeopigment concentration [ng·mg^{-1} sediment] from each treatment within each tank. Times of algae addition and resuspension are indicated by arrows (algae addition and resuspension treatments) or lines (no addition and control treatments).

Table 5.2: Two-way analysis of variance for testing between-subject effects for sediment chlorophyll *a* concentration [ng · mg sediment^{-1}] against time [days] in 4 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	30367.42	1	30367.42	13957.94	0.000
Treatment	5644.15	3	1881.39	864.75	0.000
Same treatment between tanks	29.62	4	7.41	3.40	0.034
Error	34.81	16	2.18		

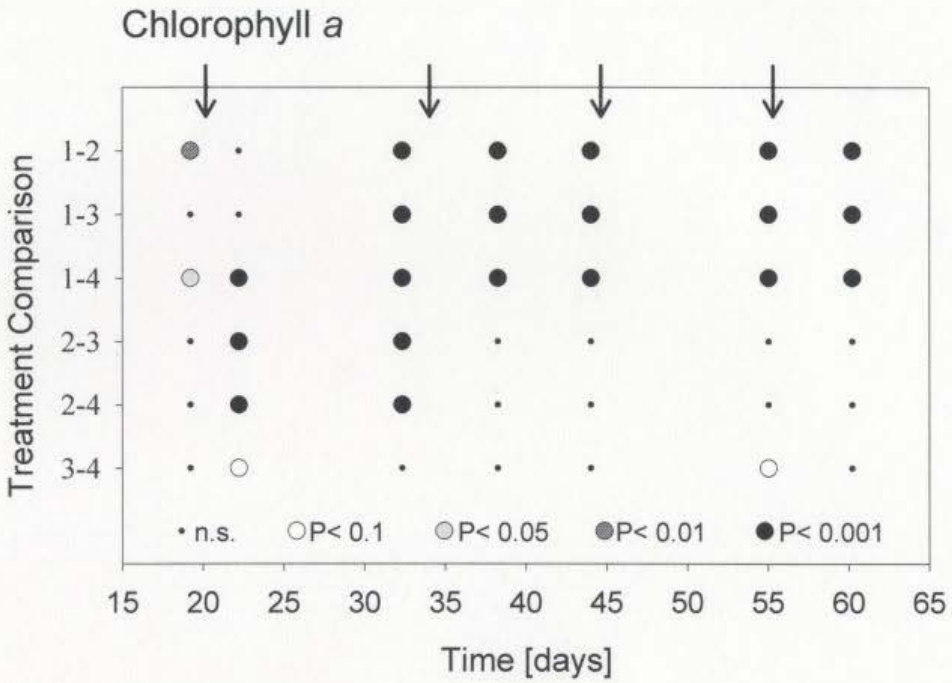

Fig. 5.5: Comparison of sediment chlorophyll *a* concentration between treatments from both tanks during the experimental period. Significance of the interaction is represented by circles of varying shade. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension, 4= Control. Times of algae addition and resuspension events are indicated by arrows.

Table 5.3: Two-way analysis of variance for testing between-subject effects for sediment phaeopigment concentration [ng · mg sediment^{-1}] against time [days] in 4 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	180036.61	1	180036.61	12303.19	0.000
Treatment	558.81	3	186.27	12.73	0.000
Same treatment between tanks	79.30	4	19.87	1.36	0.293
Error	234.13	16	14.63		

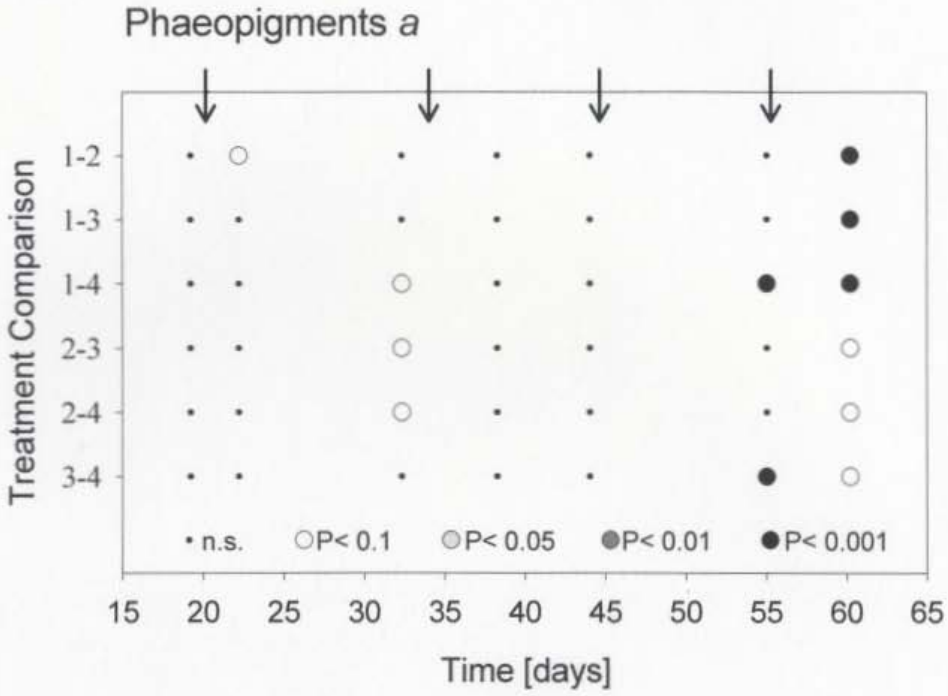

Fig. 5.6: Comparison of sediment phaeopigment concentration between treatments from both tanks during the experimental period. Significance of the interaction is represented by circles of varying shade. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension, 4= Control. Times of algae addition and resuspension events are indicated by arrows.

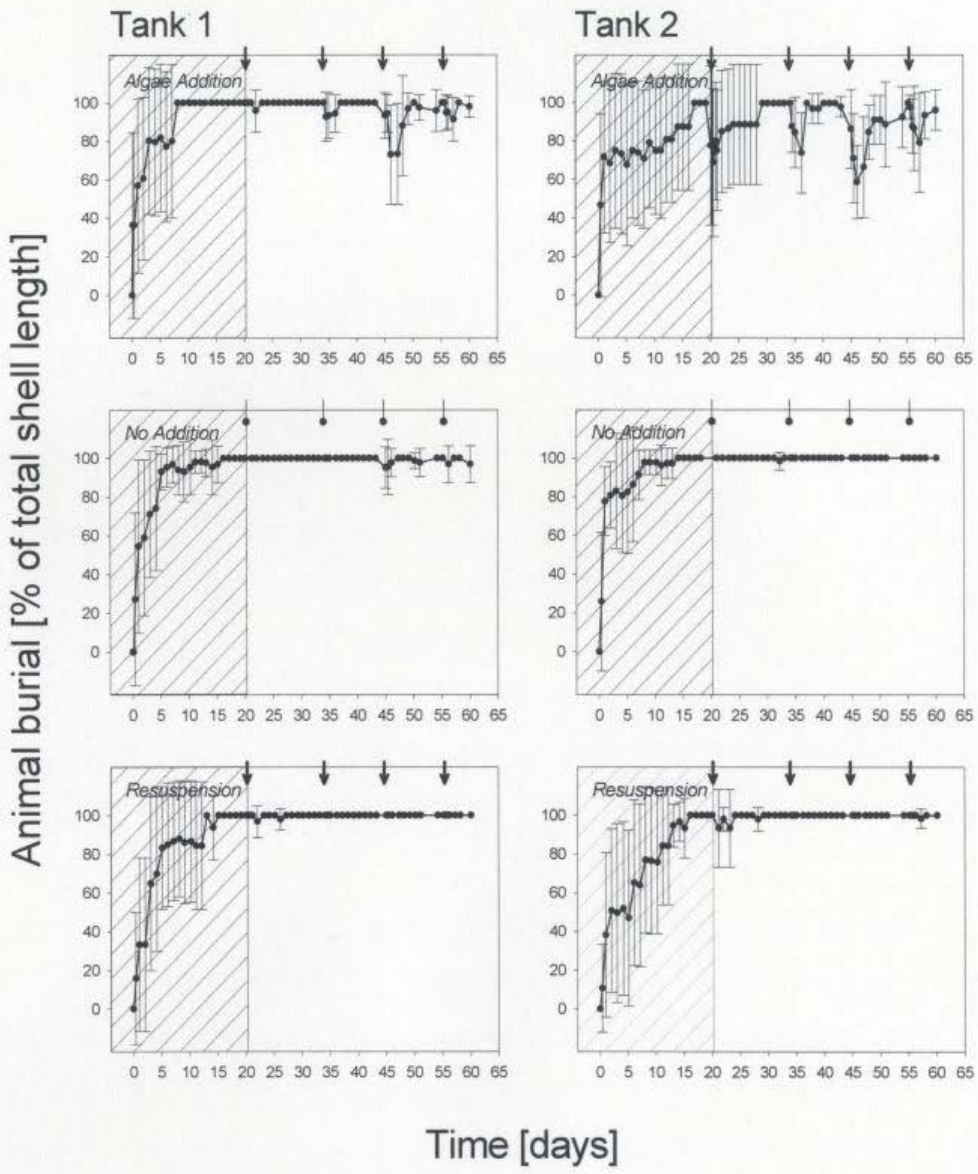

Fig. 5.7: Temporal variability of *Yoldia hyperborea* burial throughout the acclimation (crosshatched area, days 0 to 20) and experimental period (days 20 to 60) in each treatment and tank. Values are mean proportions (± S.D.) of shell length buried below the sediment surface. Times of algae addition and resuspension events are indicated by arrows (algae addition and resuspension treatments) and lines (no addition treatment).

2 days after the first and second addition, but was long-lasting after the third and fourth addition of algae. This pattern of *Y. hyperborea* burial behaviour showed that differences between treatment 1 and treatments 2 and 3 were significant at these times (Table 5.4, Fig. 5.8).

As animals reemerged from the sediment, they exposed 10% to 75% of their whole shell length (see Fig. 5.1). In addition, their longest axis (antero-posterior axis) was between approximately 10° and 90° with respect to the sediment plane, although more than 57% of the observed cases showed shells at an approximately 50° angle (*cf.* Fig. 5.1).

Reemergence of individuals was usually accompanied by the display of one or both palp proboscides on the sediment surface which could often be observed carrying surface sediment into the mantle cavity. However, the palp proboscides could also appear on the sediment surface without prior reemergence and their activity in drawing sediment into the mantle cavity was evident by marks left around the surface in a fan-like pattern with the siphon opening at its centre.

Palp proboscid activity on the sediment surface occurred in all treatments but with a higher frequency in treatment 1 (*algae addition*), especially after the addition of algae (Fig. 5.9). Differences in palp proboscid activity were statistically significant between treatment 1 and treatments 2 and 3 (*no addition* and *resuspension*, respectively) but not between *no addition* and *resuspension* (Table 5.5, Fig. 5.10). Palp proboscid activity was evident for 2 days after the first and second addition in treatment 1 but ceased thereafter. However, after the third addition, palp proboscides remained on the sediment surface for 4 days, whereas after the fourth addition they did so until the end of the experimental period (5 days). A similar trend was observed for palp proboscides size, as a significant increase in their length was observed immediately after each algae addition in treatment 1, only to decrease thereafter (see Table 5.6 and Fig. 5.11).

Table 5.4: Two-way analysis of variance for testing between-subject effects for *Yoldia hyperborea* burial [% of total shell length] against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	5690.477	1	5690.48	41242.71	0.000
Treatment	7.275	2	3.64	26.36	0.000
Same treatment between tanks	3.378	3	1.13	8.16	0.000
Error	6.761	49	.14		

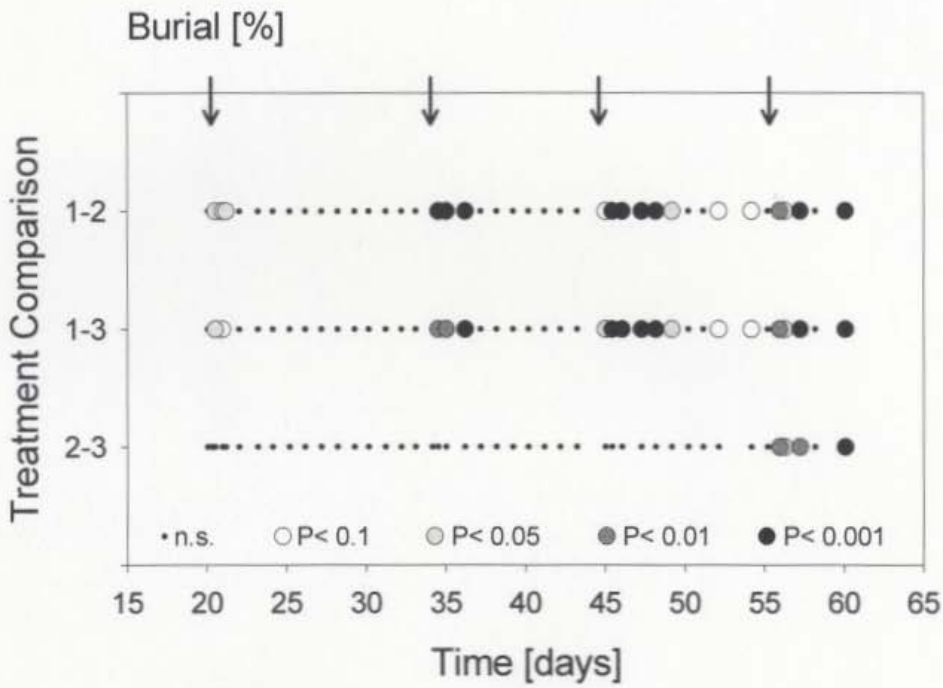

Fig. 5.8: Comparison of *Yoldia hyperborea* burial between treatments combined from both tanks during the experimental period. Significance of the interaction is represented by circles of varying shade. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows.

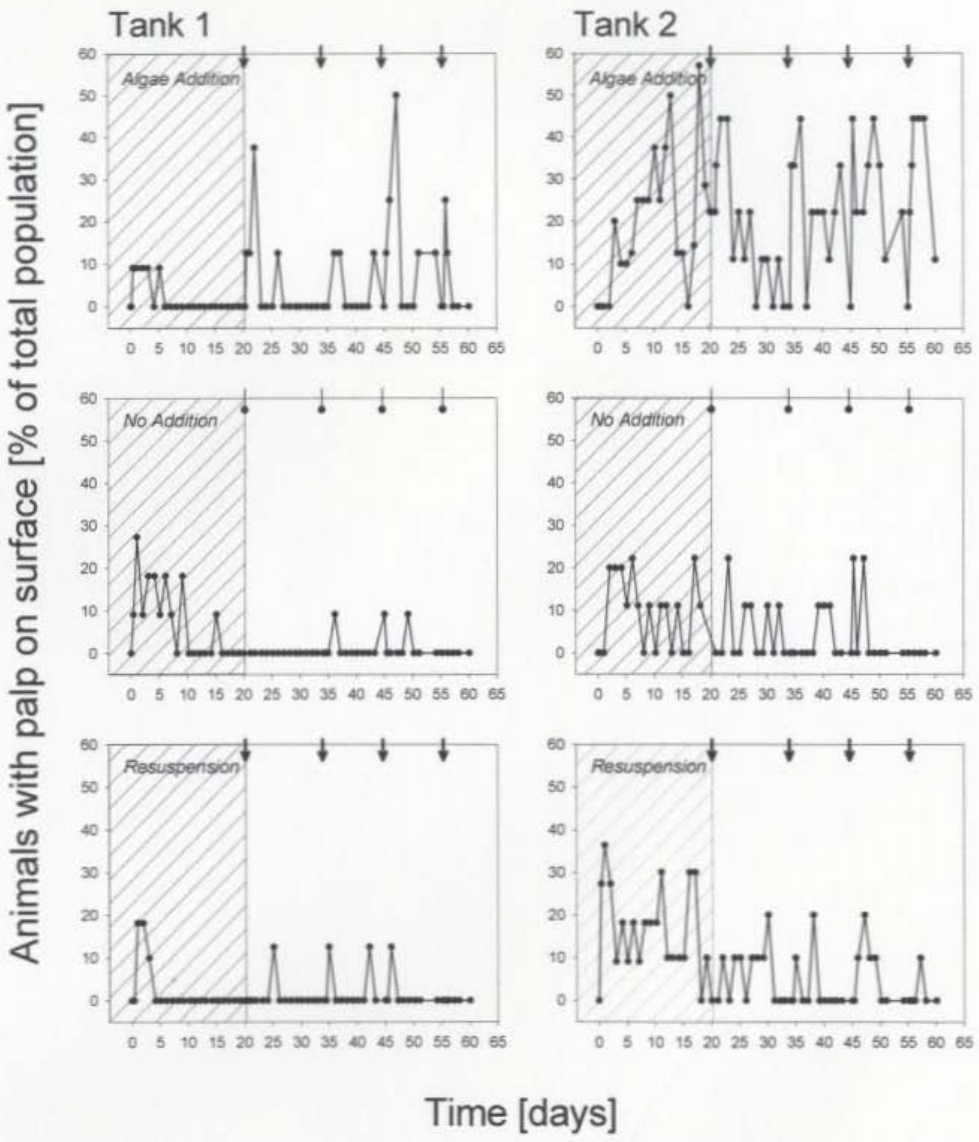

Fig. 5.9: Temporal variability in the percentage of *Yoldia hyperborea,* from each treatment and tank, with one or both palp proboscides present on the sediment surface throughout the acclimation (crosshatched area, days 0 to 20) and experimental periods (days 20 to 60). Times of algae addition and resuspension events are indicated by arrows (algae addition and resuspension treatments) and lines (no addition treatment).

Table 5.5: Two-way analysis of variance for testing between-subject effects for *Yoldia hyperborea* palp presence on sediment surface [% of total population] against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	540	1	5.40	45.52	0.000
Treatment	5.44	2	2.72	22.93	0.000
Same treatment between tanks	3.83	3	1.28	10.78	0.000
Error	5.81	49	0.12		

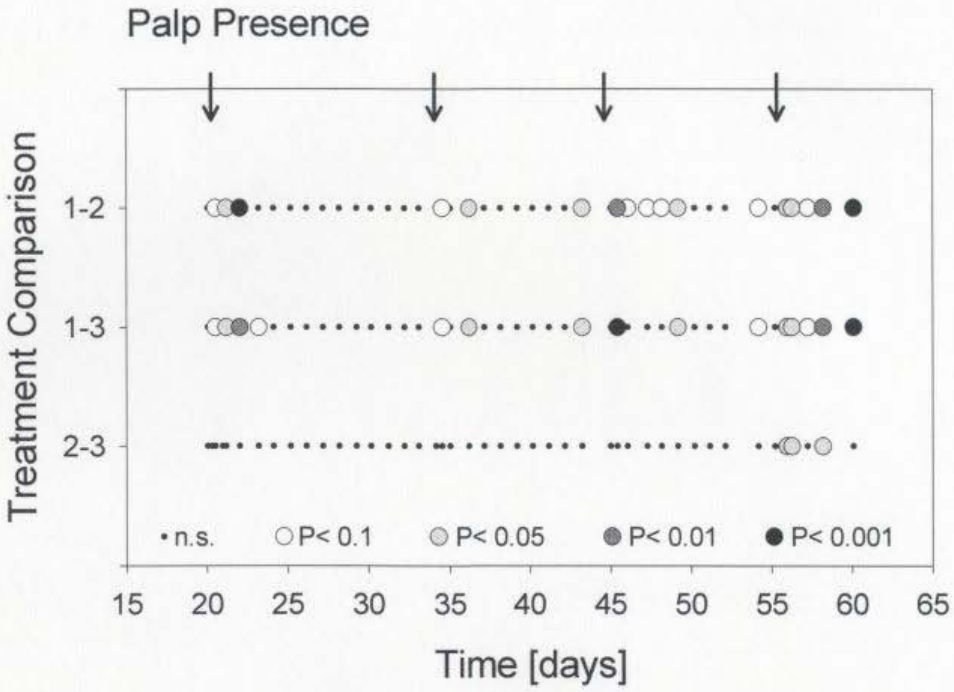

Fig. 5.10: Comparison of *Yoldia hyperborea* palp proboscides present on the sediment surface of each treatment. Significance of the interaction is represented for combined data from both tanks, by circles of varying shade. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows.

Table 5.6: Two-way analysis of variance for testing between-subject effects for *Yoldia hyperborea* palp length [cm] against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	9.43	1	9.43	41.22	0.000
Treatment	7.26	2	3.63	15.86	0.000
Same treatment between tanks	5.15	3	1.72	7.50	0.000
Error	11.22	49	0.23		

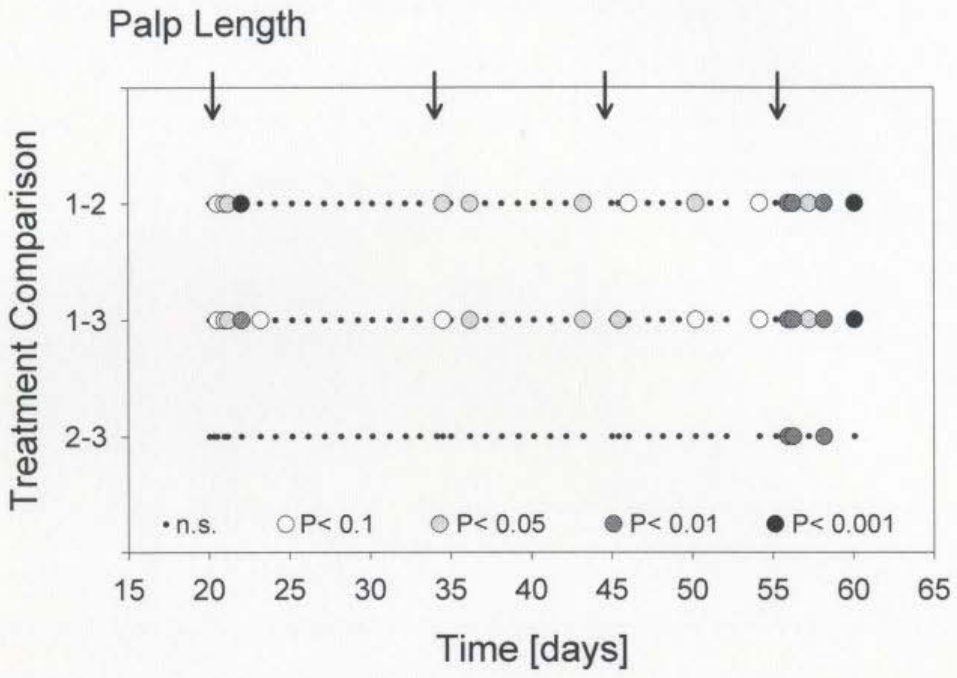

Fig. 5.11: Comparison of the length of *Yoldia hyperborea* palp proboscides extended on the sediment surface of each treatment. Significance of the interaction is represented by circles of varying shade for combined data from both tanks. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows.

Increased activity of *Yoldia hyperborea* in treatment 1 compared with the other two treatments was also evident from the differences in siphon length displayed throughout the experimental period (Fig. 5.12). Mean siphon length in treatment 1 (*algae addition*) was 0.96 cm (S.D. = 0.79), whereas in treatments 2 and 3 it was only 0.28 and 0.31 cm respectively (S.D. = 0.27 and 0.27, respectively). Although siphon length did change throughout the experimental period in treatments 2 and 3, in treatment 1 an increase of siphon length occurred after each addition, whereas the shortest siphons were always observed in the days preceding the addition (Fig. 5.12). Statistical analyses showed that siphon length was significantly greater in treatment 1 than in treatments 2 and 3 throughout most of the experimental period, although greater differences were always observed within 3 or 4 days of each addition (Table 5.7, Fig. 5.13).

Siphon orientation was also different between treatments (Table 5.8, Fig. 5.14). Whereas on many occasions siphons were lying horizontally on the surface, or lay below the sediment surface, on occasion they were extended vertically into the water column. Comparison of siphon position in *Yoldia hyperborea* from all three treatments showed differences in orientation in treatment 1 (*algae addition*) as a higher frequency of vertical siphons was observed, especially within 3 or 4 days after algae addition, compared with the other treatments. However, significant differences were also detected at times between treatments 2 and 3 (*no addition* and *resuspension*, respectively), especially towards the end of the study period (Fig. 5.15).

Although the position of animals was recorded for each sampling, after a few days it was no longer possible to follow the movements of individuals, as the movement of one animal was confounded with the positions and/or movements of others. Furthermore, although some animals left clear movement patterns on the sediment surface as they moved on or near the surface (sometimes more than 30 cm in a day), others moved well below the surface so that the individual and its route could not be determined. However, surface and

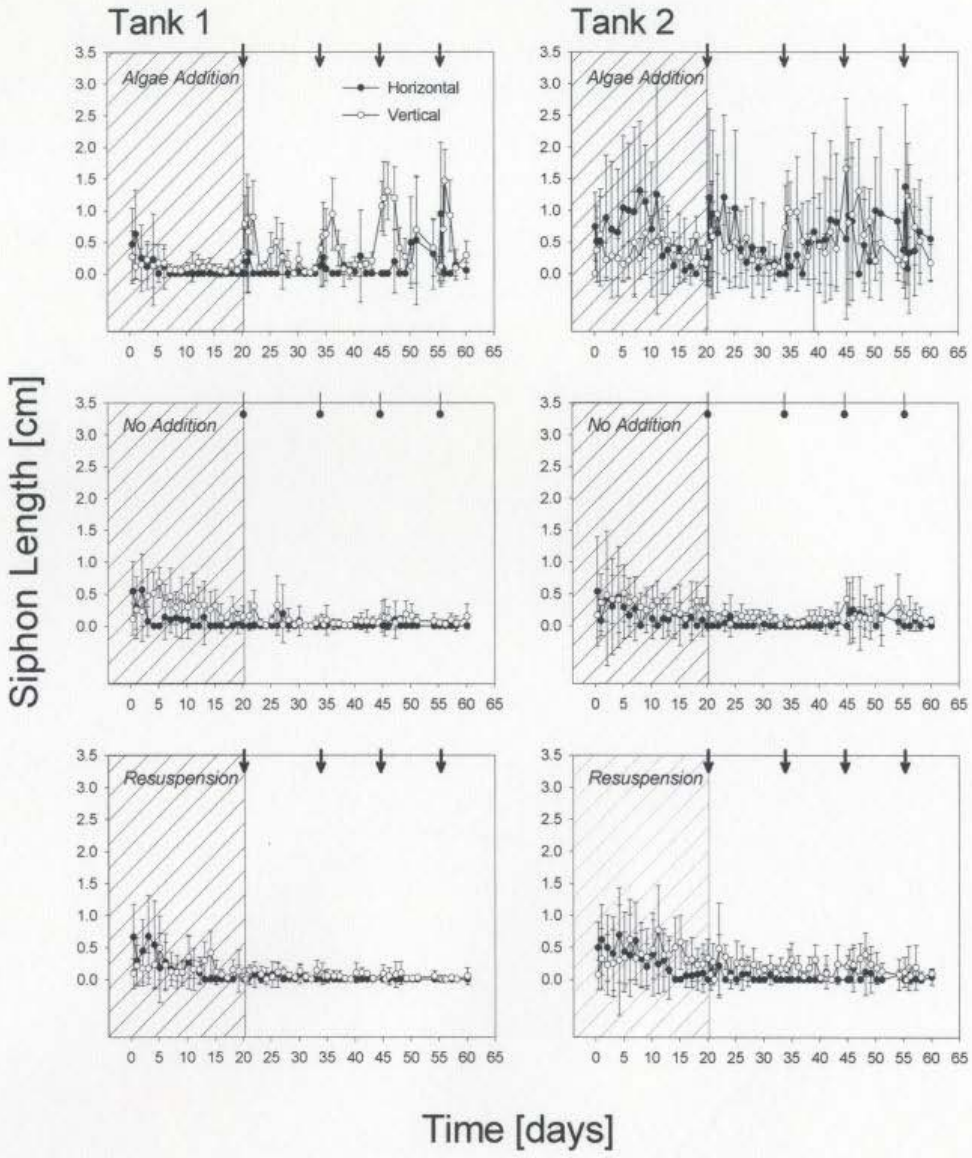

Fig. 5.12: *Yoldia hyperborea*. Temporal variability in mean siphon length [cm] (± S.D.) oriented in both horizontal or vertical position within each treatment and tank throughout the acclimation (crosshatched area, days 0 to 20) and experimental periods (days 20 to 60). Times of algae addition and resuspension events are indicated by arrows (algae addition and resuspension treatments) and lines (no addition treatment).

Table 5.7: Two-way analysis of variance for testing between-subject effects for *Yoldia hyperborea* siphon length [cm] against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	140.77	1	140.77	176.72	0.000
Treatment	146.88	2	73.44	92.20	0.000
Same treatment between tanks	3.40	3	1.13	1.42	0.248
Error	39.03	49	0.80		

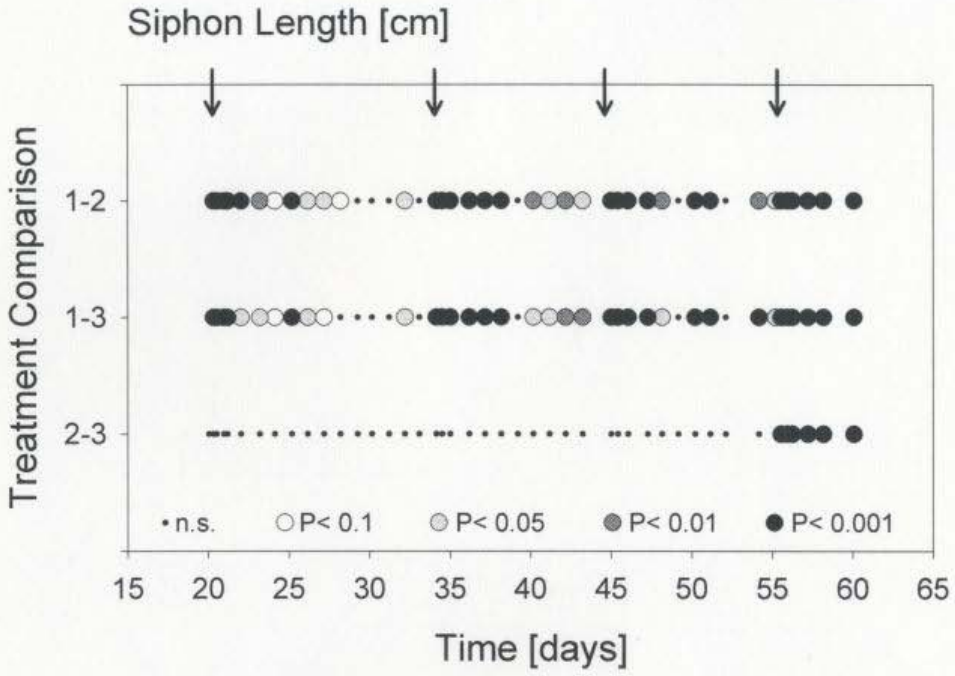

Fig. 5.13: Between-treatment comparisons of *Yoldia hyperborea* total siphon length, regardless of orientation. Significance of the interaction is represented by circles of varying shade for combined data from both tanks. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows

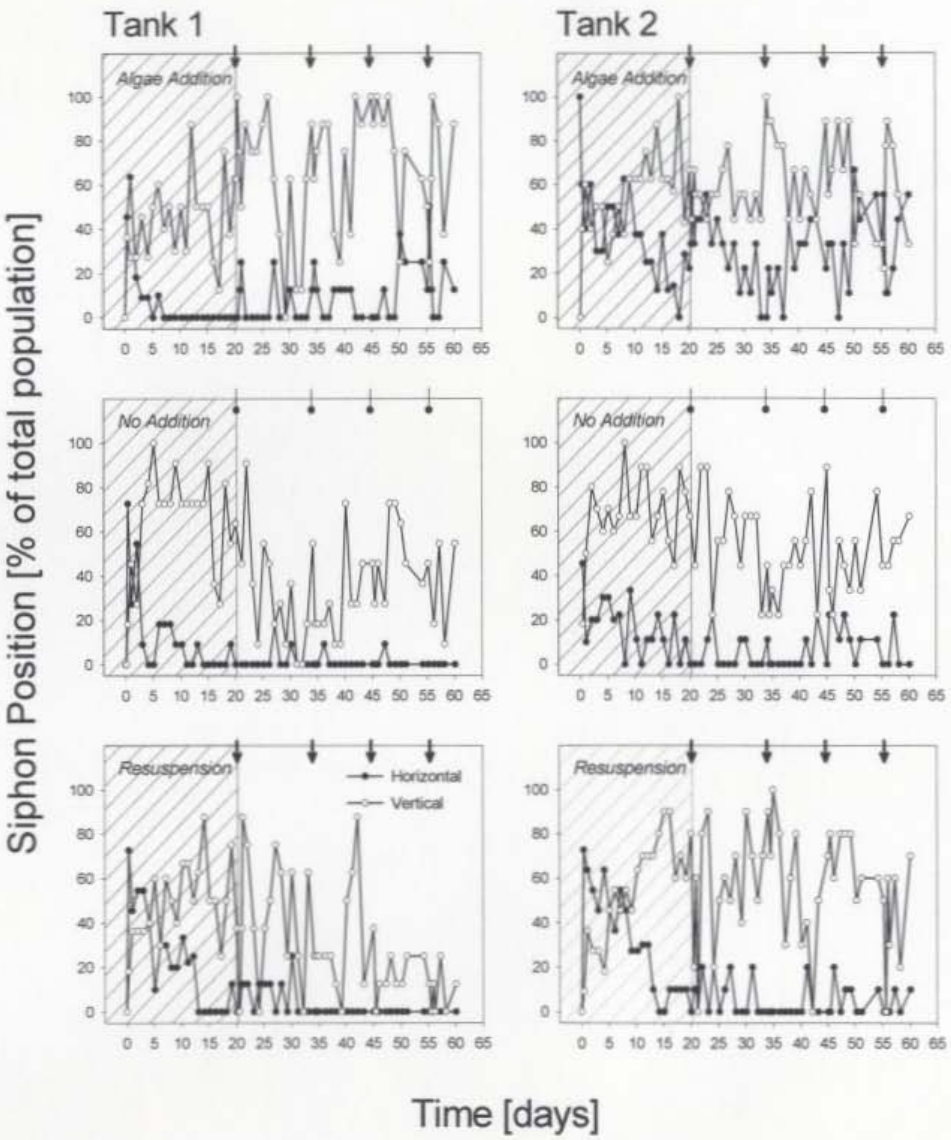

Fig. 5.14: *Yoldia hyperborea*. Temporal variability in the proportion of individuals with their siphons in either the horizontal or the vertical position within each treatment and tank throughout the acclimation (crosshatched area, days 0 to 20) and experimental periods (days 20 to 60). The difference between both positions accounts for absent siphons. Times of algae addition and resuspension events are indicated by arrows (algae addition and resuspension treatments) and lines (no addition treatment).

Table 5.8: Two-way analysis of variance for testing between-subject effects for *Yoldia hyperborea* siphon position (absent, horizontal or vertical) against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	2567.49	1	2567.49	197.29	0.000
Treatment	198.43	2	99.22	7.62	0.001
Same treatment between tanks	83.07	3	27.69	2.13	0.109
Error	637.69	49	13.01		

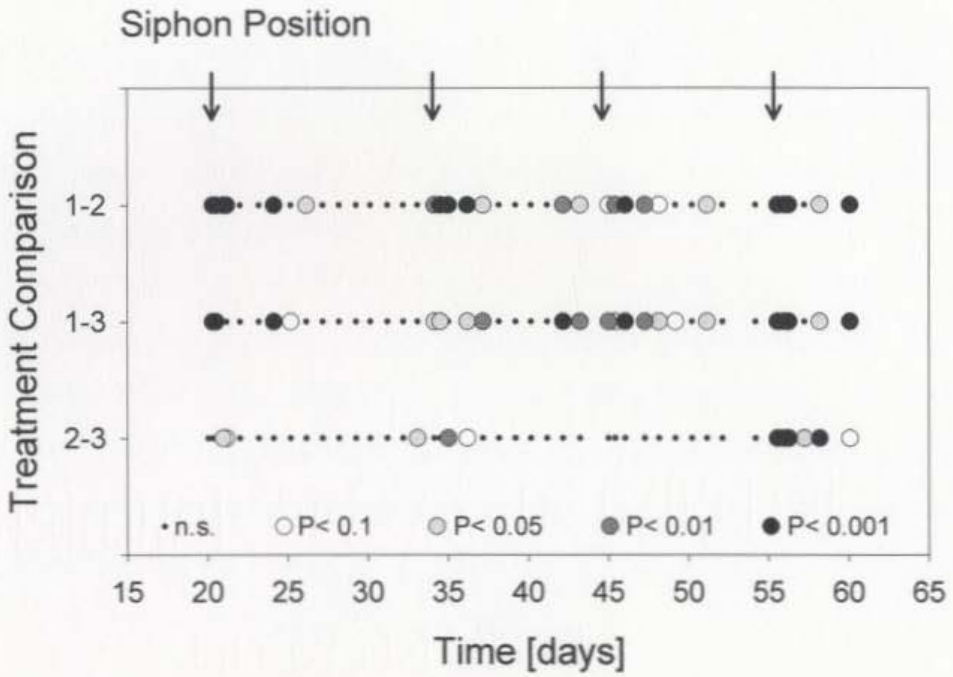

Fig. 5.15: Comparison of siphon position between treatments in *Yoldia hyperborea*. Significance of the interaction is represented by circles of varying shade for combined data from both tanks. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows

near surface migration was more commonly observed in animals from treatment 1, whereas animals from treatments 2 and 3 moved less and usually did so well below the surface.

5.3.3 Faeces expulsion and ventilation frequency

Ventilation frequency varied between zero and six events in 10 minutes, with a mean occurrence near 1 (Fig. 5.16). Statistical comparison showed no significant difference in ventilation frequency between treatments, except for one occasion between treatments 1 and 3 (*algae addition* and *resuspension*, respectively) as a result of high ventilation frequency in treatment 1 (Table 5.9, Fig. 5.17). However, on 3 of 5 occasions significantly more faeces were expelled per ventilation by animals exposed to algae addition (treatment 1) compared with treatments 2 and 3 (*no addition* and *resuspension*, respectively) (see Figs. 5.16 & 5.18, Table 5.10). In addition, animals in treatments 2 and 3 produced few faeces in the 10 minute observation period (mean near zero), with no significant difference between treatments. Although faeces were not weighed, there was no appreciable difference in the size of the pellets, most of them being around 2 mm long.

5.3.4 Histology

Histological examination of digestive gland cells showed that individuals from treatment 1 had taller digestive cells ($\bar{x}$ = 28.44µm, S.D. = 2.23, n= 8) than individuals from treatments 2 ($\bar{x}$ = 20.48µm, S.D. =0.89, n = 8) and 3 ($\bar{x}$ = 20.20µm, S.D. = 1.67, n = 6), whereas treatments 2 and 3 were similar to each other (Fig. 5.19).

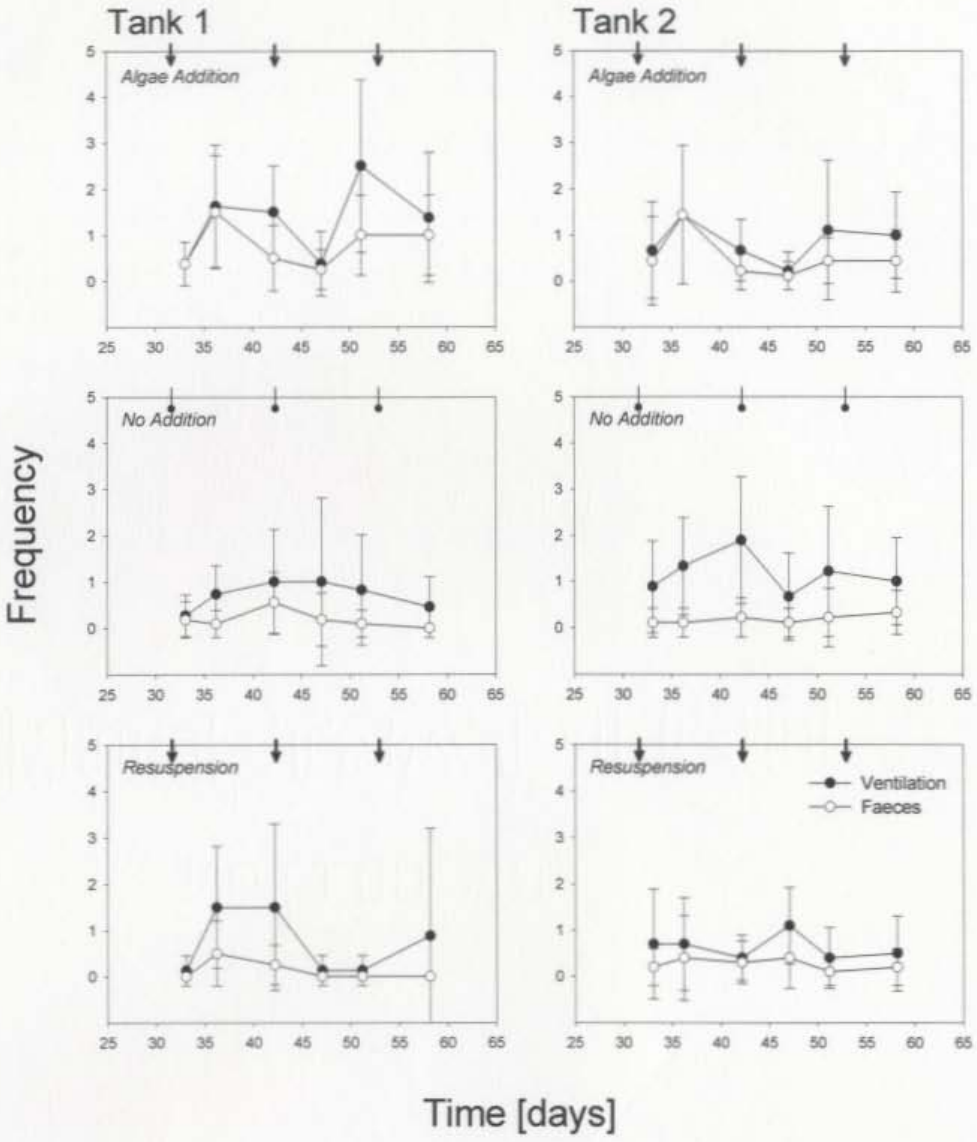

Fig. 5.16: *Yoldia hyperborea*. Temporal variability in mean (± S.D.) ventilation and faeces expulsion frequencies (events · 10 min^{-1}), quantified at different times during a 10 minute observation period. Times of algae addition and resuspension events are indicated by arrows (algae addition and resuspension treatments) and lines (no addition treatment).

Table 5.9: Two-way analysis of variance for testing between-subject effects for ventilation frequency in *Yoldia hyperborea* against time [days] in three treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	260.33	1	260.33	250.391	0.00
Treatment	8.769	2	4.384	4.217	0.02
Same treatment between tanks	11.202	3	3.734	3.591	0.02
Error	50.945	49	1.04		

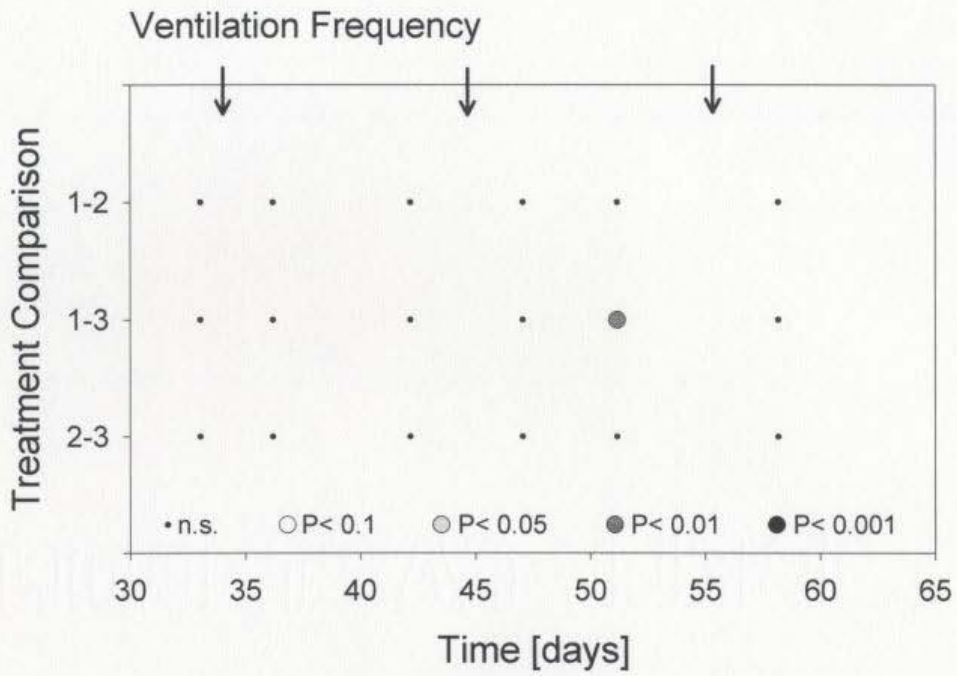

Fig. 5.17: Comparison of ventilation frequency differences between treatments in *Yoldia hyperborea*.. Significance of the interaction is represented by circles of varying shade for combined data from both tanks. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows

Table 5.10: Two-way analysis of variance for testing between-subject effects for faeces production frequency in *Yoldia hyperborea* against time [days] in 3 treatments and two tanks.

Source	SSQ (III)	df	MS	F	P
Intercept	37.951	1	37.951	108.895	0.000
Treatment	14.535	2	7.267	20.853	0.000
Same treatment between tanks	2.153	3	0.718	2.06	0.118
Error	17.077	49	0.349		

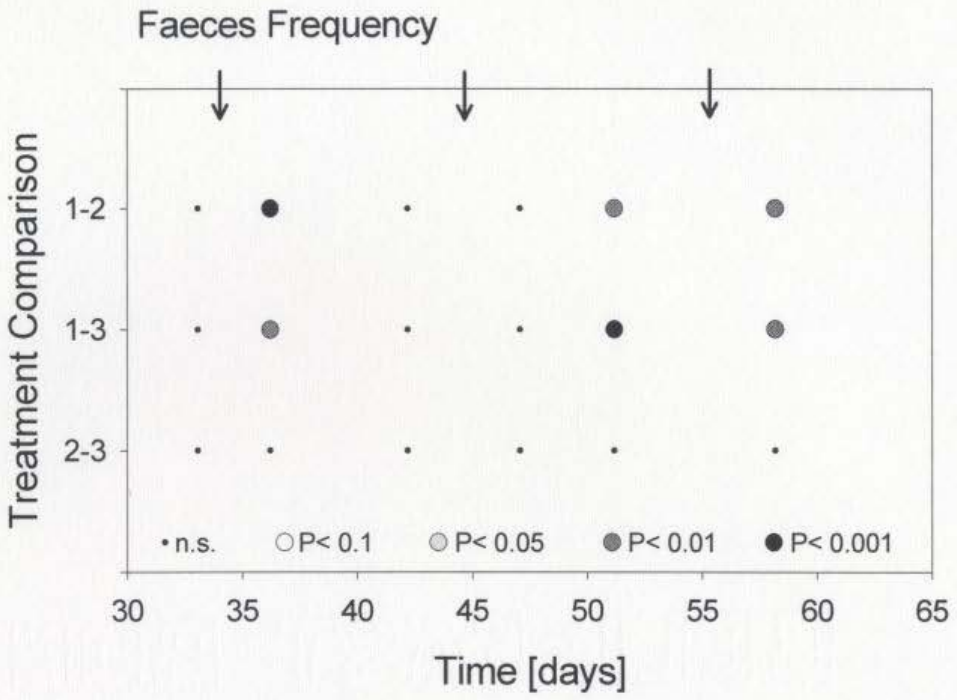

Fig. 5.18: Comparison of *Yoldia hyperborea* faeces expulsion frequency between treatments. Significance of the interaction is represented by circles of varying shade for combined data from both tanks. Treatments are represented by numbers, where 1= Algae addition, 2= No algae addition, 3= Resuspension. Times of algae addition and resuspension events are indicated by arrows

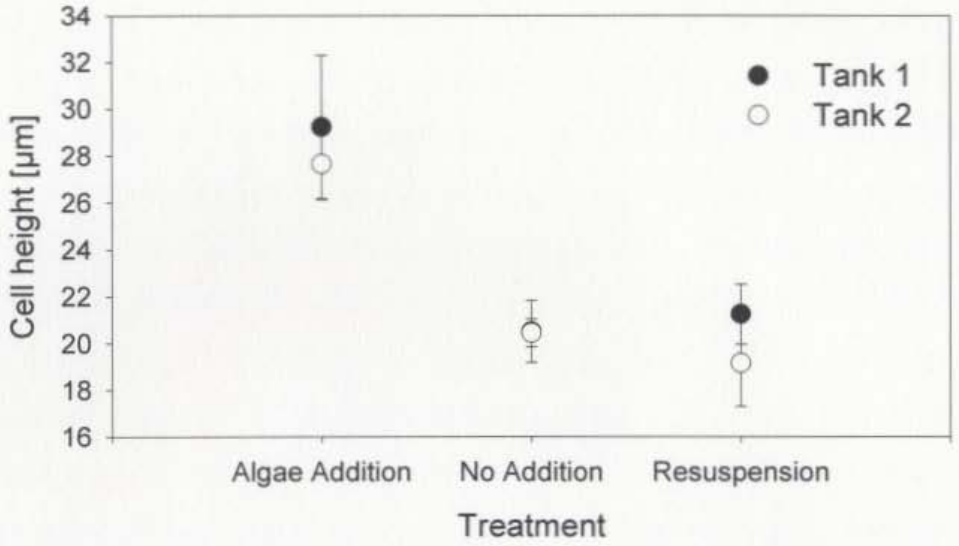

Fig. 5.19: *Yoldia hyperborea*. Mean digestive cell height [µm] (± S.D.) in individuals from all three treatments and both tanks at the end of the experimental period (day 60, 20 February 2000).

5.4. DISCUSSION

Suspended particle concentration increased after each algae addition and resuspension event. Furthermore, sediment in treatment 1 was enriched after algae settled on its surface. Thus *Yoldia hyperborea* increases its activity with each episodic elevation of suspended and sediment algae concentration. In contrast, when ambient sediment with low organic content was resuspended, animals did not react differently than individuals exposed to either resuspension or algae addition events. Increased activity of *Yoldia hyperborea* exposed to algae addition was marked by patterns of reemergence, changes in siphon position and increased siphon length as well as the presence of palp proboscides on the sediment surface. Furthermore, animals exposed to algal additions responded by increasing the height of the digestive gland cells, and is consistent with field observations in which digestive cell height in *Yoldia hyperborea* from Conception Bay, increased in response to the bloom fallout (see Chapter 2). Although occasional significant differences of the various variables within the same treatment were detected between the tanks, these differences were the result of individual variation in the magnitude of the response rather than to the overall behavioural trend.

The siphons of *Yoldia hyperborea* were always the first structures to respond to the increase in suspended algae by being extended into the water column (*i.e.* increased length of vertical siphons). As suspended algae decreased and algal material accumulated on the sediment surface, there was a tendency to withdraw the siphon from the water column to a position nearer the sediment surface (either flush or close to horizontal), thus maintaining a closer contact with the area of highest algal content. Although changes in position and length of siphons were also observed in treatments 2 and 3 (*no addition* and *resuspension*, respectively), these were not as frequent and no clear pattern could be observed. Thus extension of the siphons into the water column may be for the purpose of testing the

overlying water column for water-borne cues.

Although *Yoldia hyperborea* is primarily a deposit-feeding bivalve, it is also capable of engaging in suspension-feeding behaviour by ingesting suspended particles (see chapter 4, and also Davenport 1988b, Levinton *et al.* 1996). At high suspended algae concentrations, individuals were seen extending their siphons into the water column and dilating the inhalent siphon, indicating that water was being actively pumped into the mantle cavity. Although suspension-feeding in *Yoldia hyperborea* is not in itself an efficient strategy (see chapter 4), it may prove beneficial in combination with sediment intake by the palp proboscides by enriching nutrient-poor sediment with fresh material. The frequency of pseudofaeces production may therefore be greatly reduced, and thus the task of particle selection minimised. Individuals exposed to sediment containing algae produced more faeces, suggesting higher ingestion rates. Similarly, Davenport (1988b) observed that *Yoldia eightsii* never produced pseudofaecal plumes when feeding on algae-enriched surface sediments with the palp proboscides, although the opposite was observed during sub-surface feeding. In a previous experiment (see Chapter 4), individuals exposed to sediment with homogeneously mixed algae (12% organic matter) showed low or no production of pseudofaeces, suggesting that *Yoldia hyperborea* stops feeding selectively once critical levels of food enrichment are reached.

On the other hand, the observed suspension-feeding behaviour may be a mechanism to obtain specific essential nutrients (*e.g.* amino acids, sterols, fatty acids) that the protobranch cannot synthesise *de novo*, are not available from detritus and only found in fresh algae (Phillips 1984), *e.g.* essential PUFAs detected in *Yoldia hyperborea* shortly after the spring bloom sinks in Conception Bay (Parrish *et al.* 1996). In *Yoldia hyperborea*, high level of triacylglycerides (TAG) may serve as an energy source during food shortages and reproductive periods (Vassallo 1973, Parrish *et al.* 1996). However, results from chapter 2 indicate that in April *Yoldia hyperborea* is just beginning to store nutrients after a prolonged

period of food shortage, suggesting that sedimenting algae are ingested and their nutrients stored as soon as they are made available. Phillips (1984) argues that the procurement of essential nutrients may drive the feeding strategy of marine detritivores. Changes in feeding strategy would be advantageous in capturing food of higher quality before it is ingested by competitors, even if this means lower efficiency of food acquisition.

Furthermore, comparisons of the behavioural changes observed in *Yoldia hyperborea* exposed to increased concentrations of algae and resuspended sediment show that the individual does not respond to the increase in particle concentration *per se*, but rather in response to specific cues contained in the algae.

A few studies suggest that bivalves are affected by, and respond to, the physical properties of particles or to substances excreted by microalgae that are collectively known as ectocrines, which include sugars, amino acids, fatty acids, vitamins, steroids and numerous other secondary metabolites (see Ward & Targett 1989). For example, substances in culture filtrates of the flagellate *Tetraselmis suecica* stimulate oxygen uptake in *Mytilus edulis*, but have no effect on its clearance rate (Thompson & Bayne 1972). However, some species of microalgae reduce the clearance rate of various bivalves, and some bivalves are capable of selectively regulating the intake of particulate matter according to its nature and composition. This selection can occur, for example, between two microalgal species (Shumway *et al.* 1985), between microalgae and bacteria (Charles *et al.* 1999), or between microalgal cells and inorganic particles (Kiorboe *et al.* 1980, Bacon *et al.* 1998, Wong & Cheung 1999), although responses vary with different algal species and their concentrations (Ward & Targett 1989). To my knowledge this is the first study to show that a microalga can actually stimulate feeding responses in a bivalve.

Many benthic invertebrates, including bivalves, increase their chances of survival by responding to water-borne cues from the early larval stages onwards, *e.g.* by inducing

or delaying metamorphosis (Woodin 1991, Cohen & Pechenik 1999, and see Pawlik 1992, for review). Some of these cues originate from conspecific individuals, predators, the substrate or from potential food sources. For example, *Mytilus edulis* produces significantly thicker shells in the presence of the dogwhelk *Nucella lapillus* or the crab *Carcinus maenas*, both of them potential predators (Smith & Jennings 2000). Dogwhelks themselves can also sense the presence of predators through water-borne cues, and avoid taking risks when in their presence, although starved animals are more likely to take risks during foraging than satiated ones (Vadas *et al.* 1994). In addition, the crab *Callinectes sapidus* is capable of finding its bivalve prey by odours released from their siphons (Weissburg & Zimmer-Faust 1994).

Bivalve sensory structures include cephalic eyes, statocysts, osphradia and Stempell's sense organ (Morse & Zardus 1997). In the case of protobranchs, the siphonal tentacle may also serve as a sensory organ, although there is no unequivocal evidence. No information regarding this structure could be obtained in the present study, as the tentacle was only rarely seen lying on the sediment surface. However, the principal sensory structures in bivalves consist primarily of ciliated receptors associated with siphons and the mantle edge (Morse & Zardus 1997).

A comparison of the digestive cells from individuals exposed to the *algae addition* treatment indicated that their mean cell height (28.44 ±2.23 µm) was comparable to cells of animals obtained after the main bloom fallout events in early July 1997, and greater than the mean cell height in animals obtained throughout 1998 (max. ± = 26.47 ± 1.36 µm, July 1998). On the other hand, mean cell height in animals from treatments 2 (20.48 ± 0.89) and 3 (20.20 ± 1.67) (*no addition* and *resuspension*, respectively) was similar to the minimum cell height observed in individuals from the period between bloom fallout events, *i.e.* between October 1997 and June 1998. The data suggests that under ideal conditions, *Yoldia hyperborea* is capable of storing large amounts of energy in less than 40 days. In addition,

nutrient storage is primarily an annual event which depends on the bloom fallout. Assuming that sediments become nutrient-impoverished in autumn, the animals may subsist with the remaining sedimentary organic matter and nutrients stored in tissues such as the digestive gland. However, temporal variability of the nutritional quality of ambient sediment must first be determined before further extrapolation of these results.

Siphons were never observed taking in sediment by suctioning it from the sediment surface, as reported for other deposit-feeding bivalves, particularly tellinaceans *e.g. Macoma balthica* (Yonge 1949, Hughes 1969, Gilbert 1977, Lin & Hines 1994). Deposit-feeding behaviour in *Yoldia hyperborea* varied from one animal to another (see Fig. 5.1), but could basically be separated into surface- and subsurface deposit-feeding.

Subsurface deposit-feeding was not observed directly, but its occurrence was inferred during expulsion of clouds of pseudofaeces whilst animals remained below the surface. In this case sediment was taken into the mantle cavity by the action of the foot or the palp proboscides (see Drew 1899, Rhoads & Young 1970 and Bender & Davis 1984, for description). On the other hand, surface deposit-feeding was evidenced by the action of the palp proboscides of the animal, which left small striations on the sediment surface which radiated from the postero-ventral margin (see Fig. 2 in Bender & Davis 1984). Furthermore, when palp proboscides were exposed above the sediment surface, particles were often observed moving along the palp proboscides groove towards the mantle cavity.

When Drew (1899) and Kellogg (1915) first described the biology of *Yoldia limatula*, they indicated that it was a surface deposit feeder. However, later studies indicated that both *Y. limatula* and *Y. eightsii* feed on subsurface as well as surface sediment, although the relative importance of each strategy could not be determined (Rhoads 1963, Davenport 1988b).

In this study, subsurface deposit-feeding predominated in animals not exposed to algae addition (*no addition* and *resuspension*) as palp proboscides were observed in no more than 20% of individuals. However, in animals exposed to pulses of sedimenting algae the palp proboscides were more frequently observed on the surface, particularly in the first days after each event, suggesting a predominance of surface deposit-feeding. In addition, as surface sediment became increasingly enriched by algae, surface deposit-feeding became more prolonged (*cf.* additions 1 & 2 with additions 2 & 3).

The presence of palp proboscides on the sediment surface was often accompanied by reemergence of the animal. The early descriptions of *Yoldia limatula* by Drew (1899, 1901) indicated that the animal frequently exposed about one third of its posterior shell above the mud surface, and this description of feeding and figures has subsequently often been used to describe "typical" feeding behaviour of protobranch bivalves in both research papers and textbooks alike (see Kellogg 1915, Yonge 1939 and recently Pechenik 2000). However, results obtained here indicate that *Y. hyperborea* is most commonly found below the sediment surface, as described by Bender and Davis (1984) for *Y. limatula* and by Davenport (1988b) for *Y. eightsii*. In the present study this behaviour was only occasionally disrupted by reemergence of individuals, coinciding with the deposition of settling algae on the sediment.

Reemergence of infaunal bivalves has been observed, mainly during nighttime, in juvenile *Macoma* spp., *Ensis directus* and *Cerastoderma edule*, which then drift with the currents for migratory purposes (Armonies 1992, Beukema 1993, Cummings *et al.* 1993, Garrison & Morgan 1999). In addition, juvenile *Macomona liliana* have been observed to emerge and then escape from sediment contaminants (Roper *et al.* 1995). In the case of the surf clam *Donax vitatus*, reemergence occurs as a response to reduced incident light intensity during night hours, although upward movement is also observed prior to spawning (Ansell *et al.* 1998).

The behavioural mechanism of reemergence described above maximises the survival of the individual by allowing it to escape from unfavourable conditions, or by facilitating the dispersal of gametes, although the risk of predation is increased for the individual. This may also be the case for *Yoldia* spp., and therefore the observed pattern of repeated reemergence should result in direct benefit for the individual at risk, possibly by allowing a more efficient intake of surface sediment by the palp proboscides. On the other hand, as individuals re-bury, surface sediment is mixed and moved below the surface and out of reach of many potential competitors from the hyperbenthos or from redistribution by bottom currents, thus enabling the individual to ingest it at a later time.

The rapid storage of algae-derived nutrients, together with the immediate behavioural response of *Yoldia hyperborea* to an increase in suspended and sediment algae concentrations, emphasise the important role of seasonal episodic events of settling algae on the life cycle of this species. Although resuspension events do not stimulate feeding, a different response may be obtained if *Yoldia hyperborea* were exposed to resuspended algae-enriched sediment. The question remains as to which specific cues are responsible for triggering a change of behaviour, and whether the same responses would be obtained in animals of different physiological status.

CHAPTER 6

GENERAL CONCLUSIONS

The primary purpose of this study was to further the understanding of deposit-feeding strategies through an overall comprehension of the environmental and physiological constraints and the feeding opportunities encountered by the obligate deposit-feeding protobranch *Yoldia hyperborea*.

Yoldia hyperborea is a protobranch bivalve that inhabits extensive areas of the arctic and subarctic region which is primarily characterised by low but stable temperatures and strong seasonal patterns of ice coverage, light regime and primary production (Tranter 1982, Clarke 1988, Smith & Sakshaug 1990). Some of these environmental characteristics (*e.g.* temperature, seasonality in particle flux) are shared with areas of the open ocean (*cf.* Clarke 1988 and Tyler 1988) and some coastal areas at temperate latitudes, such as the cold-water system of Conception Bay, Newfoundland (de Young & Sanderson 1995).

Most of the information regarding energy flow to benthic marine invertebrates have been obtained for species living in shallow waters of the littoral and sub-littoral areas of near-shore regions, whereas there is little information on benthic species inhabiting the continental shelves and deep-sea, which make up most of the earth's oceans (Valiela 1995). Furthermore, benthic research from polar and deep-sea areas is also dominated by snapshot studies and studies of composition and distribution of fauna, but rarely includes temporal variability of the processes that regulate them (but see Barnes & Clarke 1995 and Brockington & Clarke 2001). The reason for this bias is that these areas are often remote and inaccessible (Gage 1991). Boreal inshore systems such as Conception Bay provide the

possibility of year-round study of populations, and some of the variables that control their dynamics, with good temporal resolution.

Periodical sampling of the *Yoldia hyperborea* population in Conception Bay between April 1997 and November 1998 indicated that this species was exposed to stable temperatures that averaged -0.63°C (-1.12 to -0.36°C). However, the standing stock of phytoplankton in the photic zone showed strong seasonal fluctuations with primary peaks always occurring during the third week of April (4.260 and 5.090 $\mu g \cdot l^{-1}$ chlorophyll *a* in 1997 and 1998, respectively), although its development started between mid-March and early April, when water temperature was below 0.5°C. In addition, a secondary bloom developed during the summer of each year (0.8 -1.5 $\mu g \cdot l^{-1}$). Arrival of the phytoplankton bloom fallout at the benthos started within 2 weeks of its initial development in early May and lasted until late June of each year. Lowest chlorophyll *a* concentrations (< 0.4 $\mu g \cdot l^{-1}$) in the benthic boundary layer nearest to the bottom were always observed between mid-July and late December and were followed by an increase in chlorophyll *a* which persisted until approximately the end of February, suggesting a significant input of fresh algal material in the first two months of the year.

Seasonal variation in the feeding response of *Yoldia hyperborea* was monitored through periodic quantification of morphological (digestive cell height) and biochemical changes (protein content, enzymes: acid protease and α-amylase) in the digestive gland. There was an increase in digestive gland protein content and digestive cell height after April 1997 and 1998, as soon as the sinking organic material from the spring bloom fallout reached the benthic zone. A marked increase of both variables was observed, suggesting storage of metabolic energy in the summer (August 1997, July 1998). A sharp decline in both digestive cell height and digestive gland protein content was observed in the late summer, coinciding with gamete development (Jaramillo, 2001). Furthermore, enzymatic activity in digestive gland extracts remained relatively stable throughout most of the study period. However, activity of both enzymes (α-amylase and acid protease) doubled at the beginning of February

1998, suggesting a sudden increase of food quality after a prolonged period of food limitation, thus coinciding with the earlier conclusion that algae had settled in the early months of 1998. Minor enzymatic activity increases were also observed after the secondary bloom fallout events of the summers of 1997 and 1998.

An important sediment disturbance event occurred between March and May of 1998. This conclusion is supported by observations of high water content and low compactness in sediment cores obtained from May through July 1998, and also from the low food value of sediment obtained from traps deployed in the area during April. The condition of *Yoldia hyperborea* obtained at this time was poor as evidenced by the high incidence of gaping and subsequent high mortalities, as well as low or zero feeding activity during the period of maximum organic flux to the benthos. A sharp decline in digestive gland index and a cessation of nutrient storage (*cf.* digestive cell height), as well as a decrease in digestive enzyme activity, was observed at this time. Furthermore, digestive cell height did not reach the values observed in 1997, although chlorophyll *a* concentrations during that year were slightly greater.

Digestive cell height also increased in animals exposed to laboratory simulated events of algae sedimentation. This increase occurred over a period of 40 days and reached similar levels to those observed in animals from the spring and summer of 1997. However, the digestive cell height in animals not exposed to sedimenting algae, and to resuspension of impoverished sediments remained at similar levels shown by animals within the inter-bloom period (*i.e.* October 1997 to June 1998). These results from experimental simulations of the sinking event substantiate the conclusion that the increase in digestive gland size observed in the field is attributable to food input, and emphasise the nutritional dependence of *Yoldia hyperborea* on the annual cycle of sedimenting microalgae in Conception Bay.

Yoldia hyperborea responded rapidly to the downward flux of sedimenting microalgae by extending its siphons into the water column. This behaviour was followed by

partial reemergence of individuals and extension of the palp proboscides over the sediment surface, once suspended algal concentration decreased and algal material accumulated on the sediment. Concurrently, orientation of the siphon changed from vertical to horizontal, thereby always keeping closer contact with the area of highest algal concentration. In contrast, activity of animals not exposed to algae was primarily restricted to strata below the sediment surface. Thus the predominant subsurface feeding strategy of *Yoldia hyperborea* is modified to surface deposit-feeding as a response to cues, which in this case was provided by the diatom *Thalassiosira nordenskioldii*. Whether these cues are of a physical or chemical nature should be a subject of future research, in addition to the determination of mechanisms involved in cue detection. However, given the immediate response of siphons to sedimenting algae, it is suggested that these structures play a significant role in the detection process Observation of the siphonal ultrastructure in various bivalve species show the presence of primary ciliated receptors associated to different regions of the siphons (Morse & Zardus 1997), but no specific information is known for *Yoldia* spp.

Early detection of sedimenting algae constitutes an advantage in a nutrient-limited environment and may constitute an adaptation for fitness maximisation by increasing the possibilities of ingesting high quality food material (*i.e.* essential nutrients) before it is depleted by competitors. This strategy includes the switching from deposit- to suspension-feeding or vice-versa, as has often been demonstrated in facultative deposit-feeding polychaetes and bivalves (Levinton 1991, Taghon & Greene 1992, Shimeta 1996, Bock & Miller 1997). Siphon extension into the water column by *Yoldia hyperborea*, together with concurrent observations of active ventilations also suggest active suspension-feeding during algal sedimentation events, although deposit-feeding would be resumed once suspended algae concentration decreased. Despite the fact that feeding experiments demonstrated the feasibility of suspension-feeding behaviour in *Yoldia hyperborea*, ingestion rates were extremely low and individuals were not capable of meeting their metabolic energy demands, although absorption efficiency of organic carbon was high (50- 72%). In contrast, high ingestion rates ($\bar{x}$ = 4.358 mg sed · ind^{-1} · h^{-1}) were observed during deposit-feeding on algae-

enriched sediments, which, together with high assimilation efficiency of algae-derived organic carbon (87.7 - 98.5%), supplied sufficient energy to meet metabolic demand and provide a positive scope for growth.

The estimated standard metabolic energy demand indicated that ammonia excretion was not an important component, in contrast to oxygen consumption. As oxygen uptake would be expected to increase during feeding, the cost of handling food should be an aspect taken into account in future research.

Yoldia hyperborea is a selective deposit-feeder which ingests particles after sorting them on the labial palps. Uningested particles are eliminated from the mantle cavity, through the exhalent siphon, as a pseudofaecal plume. When sediment was homogenously mixed with algae, pseudofaeces were rarely expelled from the mantle cavity, indicating a cessation of particle selection. In contrast, animals exposed to surface and near-surface enriched sediment showed a reduced frequency of pseudofaeces production and to higher faeces expulsion rates compared with individuals exposed to un-enriched sediment, meaning that although surface sediments had been enriched, part of the sedimentary diet was obtained from the subsurface. Future research should address the variability of pseudofaeces production in relation to changes in nutritional value of sediment in more detail, especially as pseudofaeces are known to be the principal cause of bioturbation and sediment resuspension by protobranch bivalves (Rhoads 1963, Bender & Davis 1984, Davis 1993).

It is now recognised that the behaviour of marine invertebrates responds to environmental and physiological cues and is therefore likely to vary spatially and temporally (Chapman 2000). However, for most species the precise cues that cause variation in behaviour have not yet been identified. Chapman (2000) indicates that many behavioural experiments would not have been done, nor the particular cues investigated if close attention had not been paid to quantifying the patterns of distribution, abundance and field conditions under which the particular behaviours were shown. Coastal marine ecology has increasingly

benefited from its focus on experimental testing of hypothesis explaining processes, which are done to explain observations and patterns (Underwood 2000). In this study, long-term monitoring of the environmental variability to which the *Yoldia hyperborea* population from Conception Bay was exposed, provided the framework for understanding the physiological constraints encountered. Periodic sampling of the population also provided important information regarding the nutritional status of individual *Yoldia hyperborea*, although without laboratory measurements of its general- and feeding physiology as well as its behavioural flexibility, interpretation of patterns in aspects of the population dynamics of this species would not have been as meaningful.

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APPENDIX I

A.I. Inverse Distance Method

The inverse distance method generates Z values for an evenly spaced XY grid from XYZ triplet data. Given n (X,Y,Z) triplets (x1,y1,z1), (x2,y2,z2),...(xn,yn,zn), and a mesh of X_{int} uniformly spaced X intervals from x_{min} to x_{max} and Y_{int} uniformly spaced Y intervals from y_{min} to y_{max}, interpolated Z values are computed, corresponding to the X and Y values at the intersection of the X_{int} Y_{int} grid.

$$z = \begin{cases} \dfrac{\sum_{i=1}^{n} w_i z_i}{\sum_{i=1}^{n} w_i} & x_i \neq x,\ y_i \neq y \\ zi & x_i = x,\ y_i = y \end{cases}$$

where

$$w_i = [\,(x_i - x)^2 - (y_i - y)^2\,]^{-p/2}$$

p is the distance weight value that can be specified in the Interpolate 3D Mesh dialogue.

APPENDIX II

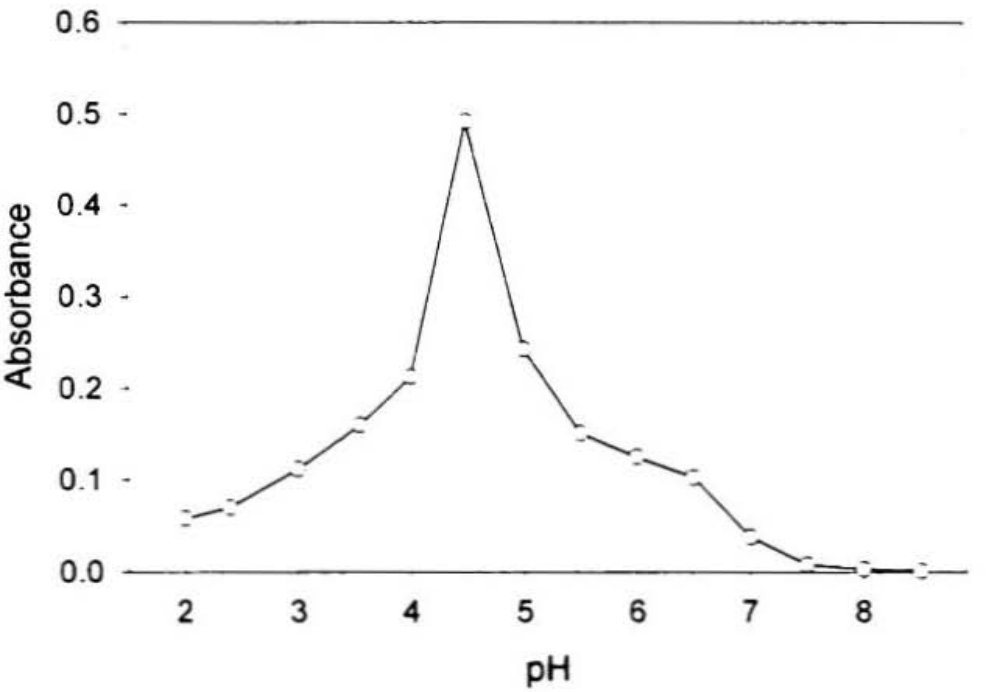

Fig A.II.1: *Yoldia hyperborea*. pH dependency of digestive gland acid protease activity. Values are expressed in absorbance [%] (λ=366 nm).

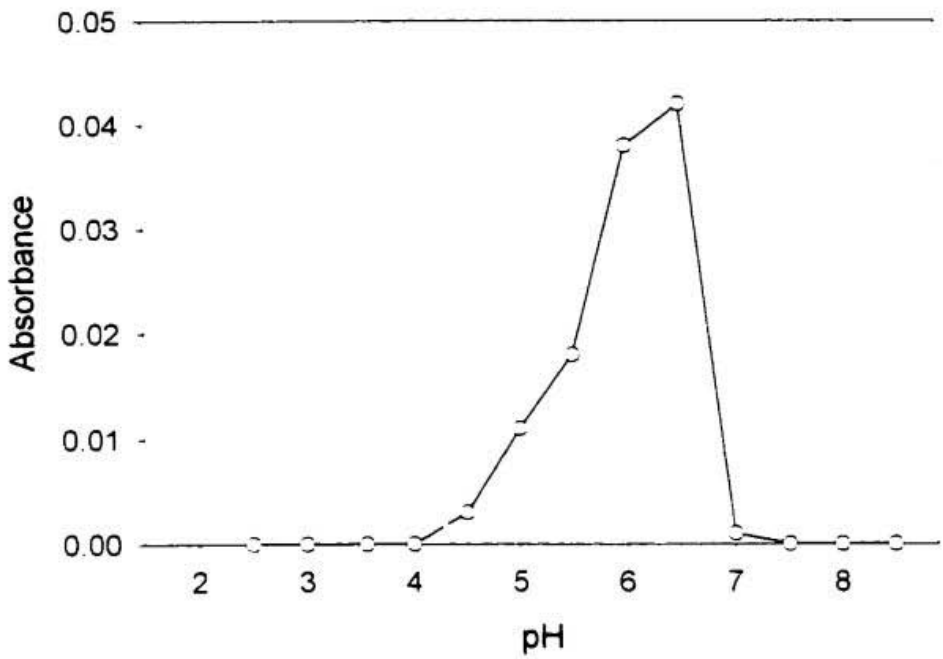

Fig. A.II.2: *Yoldia hyperborea*. pH dependency of digestive gland α-amylase activity. Values are expressed in absorbance [%] (λ= 595 nm).

APPENDIX III

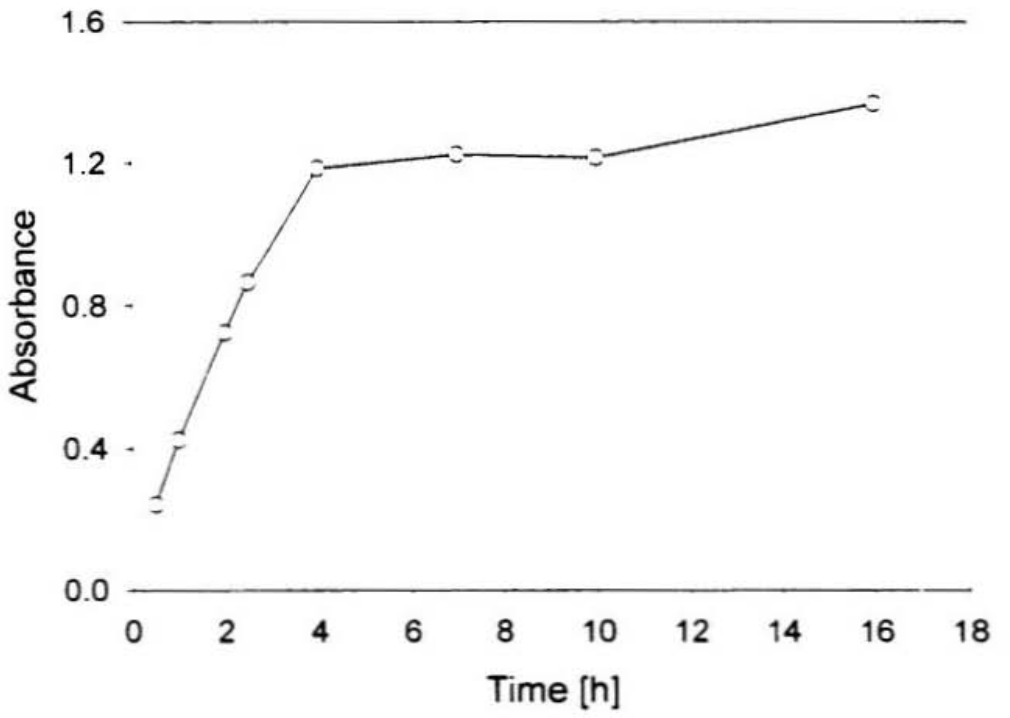

Fig. A.III.1: Time course of amino acid release by acid protease from *Yoldia hyperborea* digestive gland extract incubated at 25°C. Results are indicated as a function of absorbance [%] at 366 nm.

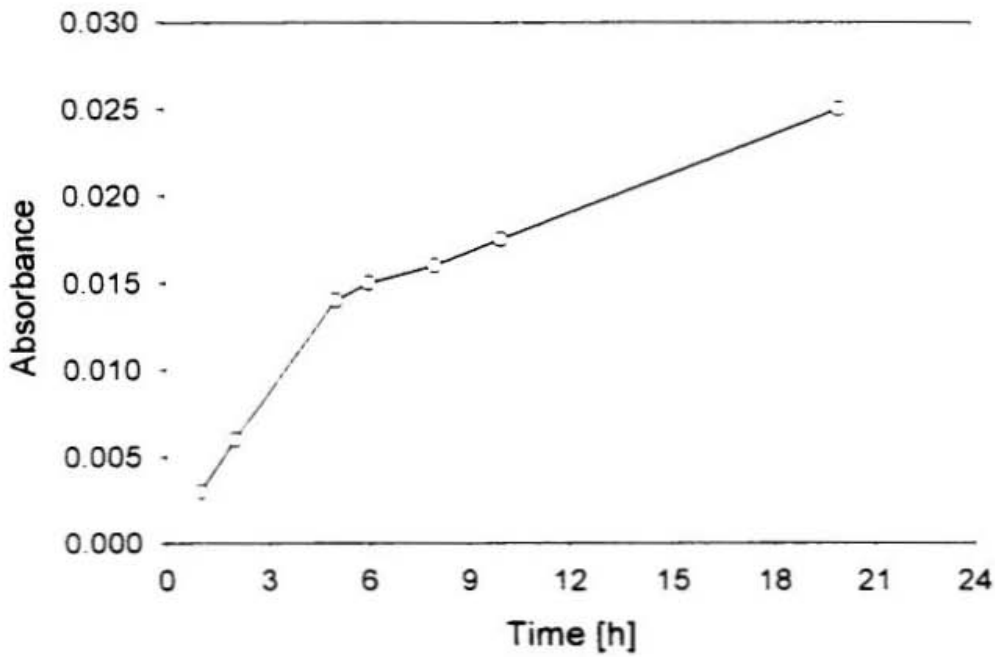

Fig. A.III.2. Time course of glucose release during an α-amylase assay of digestive gland extract of *Yoldia hyperborea*. Values are expressed as a function of absorbance.

APPENDIX IV

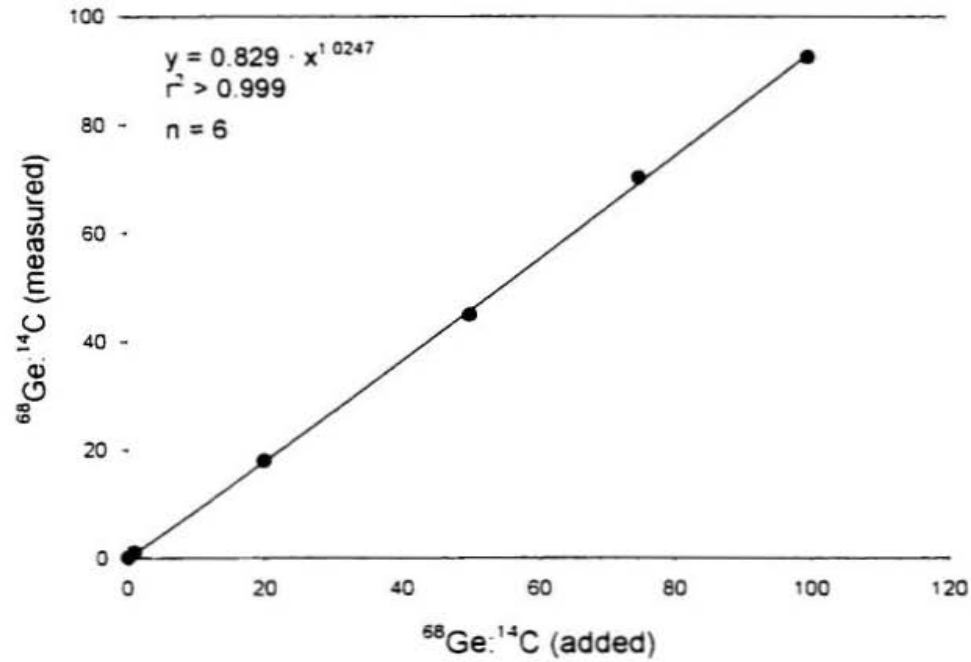

Fig. A.IV.1. Comparison of added and measured ^{68}Ge to ^{14}C ratios to test the effective separation of both isotopes by the dual-labelling protocol used in suspension-feeding experiments. All experimental ratios were within the 0 to 30 range.

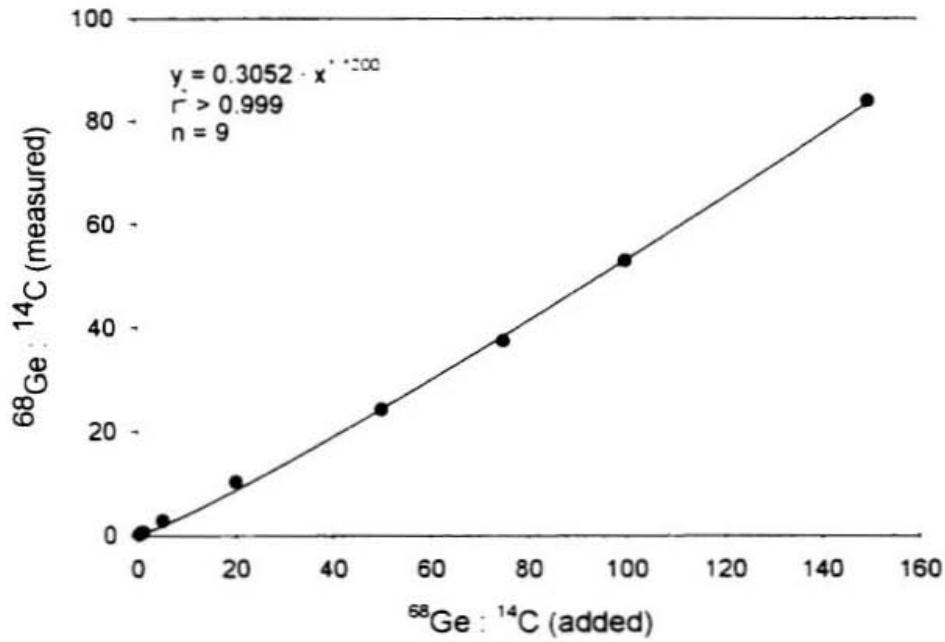

Fig. A.IV 2: Comparison of added and measured ^{68}Ge to ^{14}C ratios to test the effective separation of both isotopes by the dual-labelling protocol used in deposit-feeding experiments. All experimental ratios were within the 0 to 90 range.

APPENDIX V

Table A.V.1. Multiple regression analysis of absorption efficiency (AE)[%], gut passage time (GPT) [h] and ingestion rate (IR) [mg · h^{-1}] in each experiment. All three deposit feeding experiments were treated as replicates.

Source	Dependent Variable	df	SS	MS	F	P
Corrected	AE	11	44112.8	4010.26	30.90	0.000
Model	GPT	11	123616.0	11237.82	43.47	0.000
	IR	11	177.9	16.18	3.72	0.001
Intercept	AE	1	171474.4	171474.39	1321.25	0.000
	GPT	1	92922.8	92922.77	359.42	0.000
	IR	1	8.1	8.09	1.86	0.018
Experiment	AE	11	44112.8	4010.26	30.90	0.000
	GPT	11	123616.0	11237.82	43.47	0.000
	IR	11	177.9	16.18	3.72	0.001
Error	AE	50	6489.1	129.78		
	GPT	50	12926.7	258.54		
	IR	50	217.4	4.35		
Total	AE	62	274718.5			
	GPT	62	218270.8			
	IR	62	429.9			
Corrected	AE	61	50601.9			
Total	GPT	61	136542.8			
	IR	61	395.4			