THE EFFECT OF CHANGES IN QUANTITY AND QUALITY OF FOOD ON THE FEEDING BEHAVIOUR OF THE SOFT-SHELLED CLAM, Mya Arenaria, USING A NEW TECHNIQUE TO DETERMINE GUT RETENTION TIME

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by

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A thesis submitted to the
School of Graduate Studies
in partial fulfilment of the
requirements for the degree of
Master of Science

Department of Biology
Memorial University of Newfoundland

March, 1994

St. John's Newfoundland

ISBN 0-315-91621-4

Dedicated to my family

with all my love.

Abstract

This study investigates the effect of changes in the quantity and quality of food on the feeding behaviour of *Mya arenaria*, the soft-shelled clam. *M. arenaria* is a suspension-feeding bivalve, ingesting particles suspended in the water column. The laboratory component of this study presented *M. arenaria* with an artificial diet, consisting of silicon dioxide (SiO_2) particles and the single-celled diatom *Chaetoceros muelleri*, for inorganic and organic components respectively. These feeding experiments were performed using a flow-through apparatus. This included measurements of clearance and ingestion rates, absorption efficiencies, and gut retention times of both organic and inorganic fractions.

In response to a 3-fold increase in food concentration, *M. arenaria* decreased clearance rate by almost 9-fold, and therefore ingested less material, even though more food was available. These clams with lower ingestion rate also had a shorter gut retention time, and thus a smaller amount of material in the gut. It as possible that clams feeding on the low quantity diet were expending more energy in obtaining particles (higher ingestion rate despite lower food concentrations) but perhaps less energy in digesting them (clams feeding on the low quantity diet had an absorption efficiency equal to those feeding on the 10 mg diet, but over a longer gut retention time. If the net energy gained in the digestive process (i.e. lower amounts of enzymes working over a longer time) was greater than that expended in filtering, this would be an effective feeding strategy for clams to adopt when experiencing low quantities of food.

In response to an increase in the quality of the food (proportion of organic material increases), clams decreased clearance rate and ingestion rate, to maintain a constant ingestion rate of organic material. Although gut retention time lengthened, absorption efficiency remained unchanged. Therefore, clams regulated both their intake of food and gut retention time to keep their ingestion rate of organic material constant, and to maintain absorption efficiency levels.

This study also measured the clearance rate, ingestion rate, gut retention time and absorption efficiency of *M. arenaria* feeding on natural particle assemblages at a clam flat in Platter's Cove, Terra Nova National Park, NF. Clearance rates were significantly higher than any measured in the laboratory study. Field measurements of gut retention time were

comparable to those measured in the laboratory study, however, field measurements of absorption efficiency were strongly negative, indicating possible metabolic faecal loss or periodicity of digestion in the intertidal population.

This study also describes the development and use of a new technique to assess the gut retention time of suspension-feeding bivalves. The green alga *Tetraselmis succica* was used as an organic marker, detectable in faecal pellets by high performance liquid chromatography because of its characteristic chlorophyll b signature. The inorganic marker used was silicon carbide particles which can be detected in faecal pellets by particle size analysis using a Coulter Multisizer. Results obtained by this method are comparable to those of other studies, and the technique is sensitive enough to detect post-ingestive selection of particles within the gut of individual clams.

Acknowledgements

The following people were invaluable in their support and assistance throughout the course of this project: Greg Bacon, Betty Hatfield, Margaret Miller, Evan Ward, Anna Redden, Jonathon Gardner, Donna Gardner, Pat Dabinett, Alexander Bochdansky, Ed Downton, Steve Sooley, and Roy Bartlett. Special thanks go to Dr. D. Innes, my supervisory committee member.

I thank the Natural Sciences and Engineering Council of Canada, Memorial University of Newfoundland, R.J. Thompson and B.A. MacDonald for project funding.

Completion of this work would not have been possible without the tremendous advice and encouragement from my two supervisors, Drs. R.J. Thompson and B.A. MacDonald.

Finally, I would like to thank my friends and family for all their support and kindness.

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List of Abbreviations

AE - Absorption efficiency

CR - Clearance rate

GRT - Gut retention time

HPLC - High performance liquid chromatography

IR - Ingestion rate

IR_o - Ingestion rate of organic material

SiC - Silicon carbide

SiO_2 - Silicon dioxide

CHAPTER I

Introduction

1.1. Feeding Ecology of Suspension-Feeding Bivalves

Suspension-feeding bivalves form a significant component of the benthic populations of marine ecosystems, dominating many estuarine and coastal habitats world-wide, and are therefore the subjects of many physiological and ecological research projects. Because of their dominance in many marine ecosystems, it is necessary to investigate the ecological role of bivalves in order to understand productivity and energy dynamics of benthic populations. Many bivalve species are also important economically for both the harvesting of natural populations and in aquaculture, so much research is being directed at maximizing growth efficiencies in these species (Griffiths and Griffiths 1987).

Despite this great volume of research (see Bayne 1976, Bayne and Newell 1983, Griffiths and Griffiths 1987, Jørgensen 1990, Gosling 1992 for reviews), there remain many aspects of bivalve physiology which are not well understood. Recently, attention has turned towards bivalve feeding ecology, particularly in response to changes in the available food source (e.g. Bayne et al. 1984, Hawkins and Bayne 1984, see reviews by Bayne 1976, Bayne and Newell 1983, Griffiths and Griffiths 1987, Bayne et al. 1988, 1989). Although basic models of feeding behaviour have been developed (to be discussed more fully in a later section of this chapter), bivalve responses do not always conform to expected results. This is further complicated by the fact that responses to environmental stimuli, such as a change in the composition or concentration of a food source, fall into essentially two categories: there are responses for survival at environmental extremes, and there are responses for the maintenance of rate functions at optimal levels over a normal environmental range. Furthermore, the adaptational responses can be expressed as either decreased energy expenditure (energy-conserving) or increased energy input (energy-supplementing) (Gillmor 1982, Green et al. 1985).

There is usually a hierarchical response to environmental stimuli (Slobodkin 1968). The initial response level to environmental change is usually a change in the behaviour or physiology of the animal. This type of compensation by many individuals can, in turn,

affect such population parameters as mortality rates, fecundity and dispersal of pelagic larvae, which could in turn alter the genetic composition of future generations. Finally, changes in the structure of a population can alter the structure of the community as a whole. Bivalves have been identified as a group which can show adaptation to environmental stimuli at all hierarchical levels of response, again making them good subjects for experimental research (Green et al. 1985). The research of this thesis deals with responses of bivalves at behavioural and physiological levels. Inferences will be made into the resulting physiological energetics of the animals, in terms of gains and losses of energy and energy transformation (Hibbert 1977, Bayne and Newell 1983).

1.2. The Bivalve Digestive Tract and Measurements of Feeding

In order to understand the range of adaptations available to a bivalve in terms of its feeding behaviour, it is first necessary to understand the anatomical structures (and their functions) involved. The feeding and digestive structures of a bivalve has been illustrated schematically in Fig. 1.1 (modified from Widdows et al. 1979, Bayne and Newell 1983, Bricelj and Malouf 1984).

1.2.1. The Gills

Suspension-feeding (= filter-feeding) bivalves ingest micro- and macroscopic particulates suspended in the water column above the bivalve population. Ciliary currents pump water through the bivalve mantle cavity and across the animal's gills. Particles are captured from suspension by the laterofrontal cilia of the gills (Jørgensen 1966, Dral 1967). Pumping rates probably correlate with the beat frequency of the cilia, although the relation between the action of the cilia and the transport of water is still disputed (Jørgensen and Riisgård 1988, Jørgensen 1990, Jørgensen et al. 1990, Ward et al. 1993a). Rates of water transport are also controlled by regulation of the diameter of the exhalant siphon (Foster-Smith 1976b), and in the degree of gape of the two valves (Bayne and Newell 1983).

The rate of feeding by a bivalve is dependent not only on the rate of water transport (= pumping rate) but also on the functional state of the gill. Jørgensen (1976) describes three functional states of the gills of suspension-feeders: 1) non-retentive, indicative of

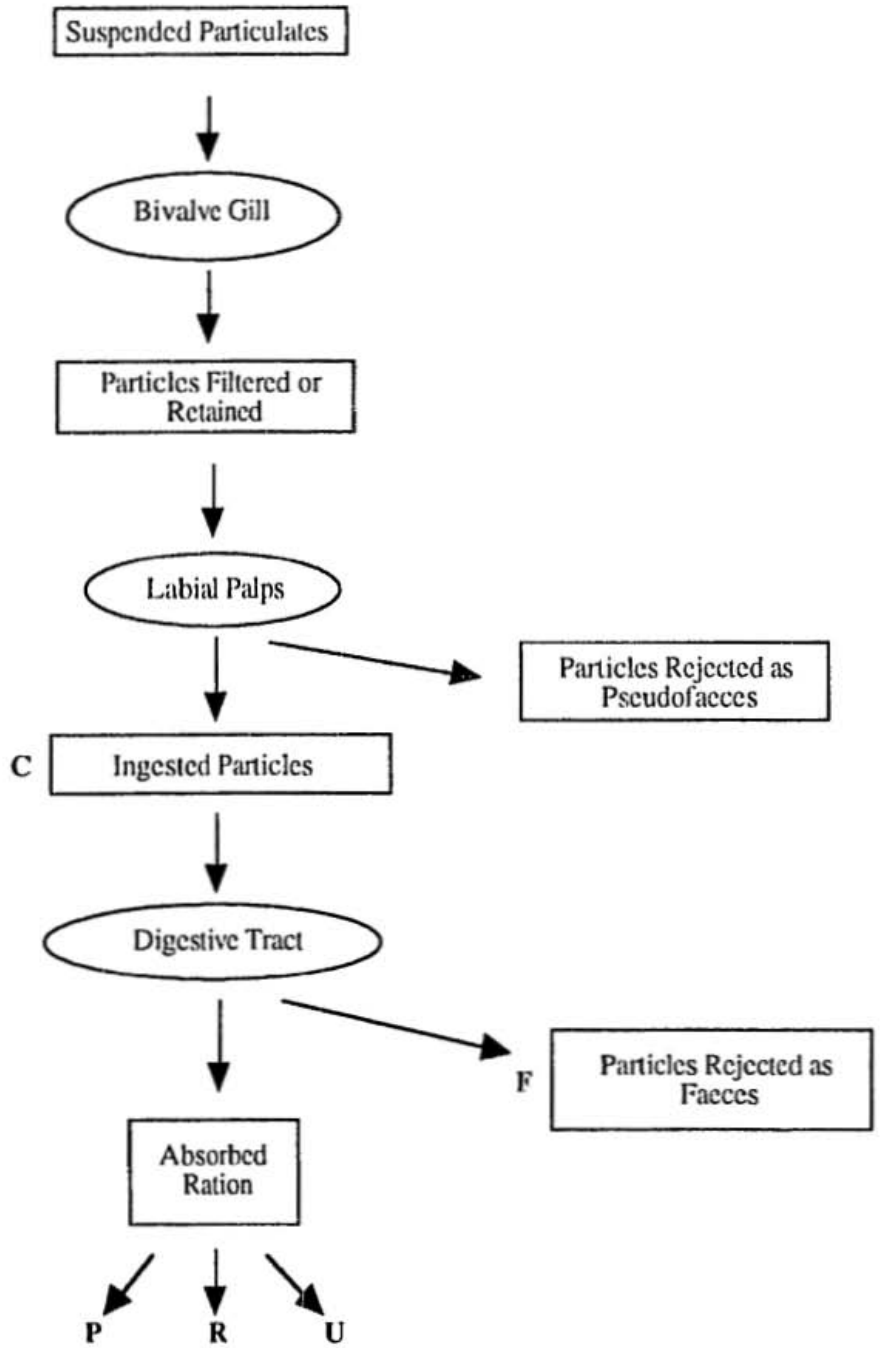

Fig. 1.1: Schematic diagram of the components of feeding and digestion in a suspension-feeding bivalve. Letters in bold type refer to the Equation 1.1. Circled areas indicate points along the digestive tract where particle selection may occur.

stressed or disturbed animals, 2) a cleaning state, characterized by copious mucus production, which may be favourable in conditions of high particle concentration and increased frequency of clogging of the gill, and 3) a "normal" state, with high pumping rates and high particle retention efficiency. Research is still needed to understand the morphological basis and the physiological roles of these three functional states (Jørgensen 1990). However, it is known that some bivalves can change their efficiency of particle retention in response to changes in the seston by changing the porosity of the gills (Dral 1967, Hummel 1985).

Because of the combined effects of pumping rate and the functional state of the gill on rates of feeding, most studies of bivalves have used the "clearance rate" as an indication of feeding behaviour. Clearance rate (CR) can be defined as the volume of water cleared of particles above a certain minimum size in a given period of time.

The gills are known to be the first site of particle selection within the bivalves. Selection by the gills is based primarily on particle size, which in turn depends on the gill ostia size: particles smaller than the ostia generally are not retained. Most bivalves do not retain particles below about 1.5 µm in diameter (Griffiths and Griffiths 1987).

1.2.2. The Labial Palps

Once particles have been retained by the gills, they are bound in mucus (Ward et al. 1993a, but see comments by Jørgensen 1993 and Ward et al. 1993b) and travel along a food groove on the gills to structures in front of the mouth called the labial palps (Fig. 1.1). The palps are the second site of particle selection, where rejected particles are expelled from the mantle cavity by muscular contraction of the valves, reversing the direction of water flow and forcing particles back through the inhalent siphon. These particles rejected by the labial palps, as well as particles arising from the mantle surface after dropping off the gills, are collectively termed "pseudofaeces". Unlike the gills, the palps are known to select or reject particles on the basis of their chemical or nutritional properties (Kiørboe and Möhlenberg 1981). Many studies have demonstrated that certain bivalve species are capable of preferentially retaining the organic fraction of a food suspension, with inorganic particles being rejected as pseudofaeces (Kiørboe and Möhlenberg 1981, Newell and Jordan 1983, Shumway et al. 1985). The ability to retain selectively more nutritious particles is of great

ecological advantage, since energy would otherwise be wasted on trying to digest poor quality food particles.

Although particle selection by the palps has been demonstrated (e.g. Kiørboe and Möhlenberg 1981), the mechanism behind it is still under dispute (Jørgensen 1990). Selection likely occurs in response to surface properties of the particle (such as charge, biochemical characteristics, shape, etc.), but this is complicated by the fact that particles reaching the palps are bound in mucus, which probably confounds detection of chemical properties (Kiørboe and Möhlenberg 1981) and size (Widdows et al. 1979). It is possible that different particles have a different likelihood of being caught in mucus (based on their surface chemical properties) and hence have different probabilities of being expelled as pseudofaeces (Kiørboe and Möhlenberg 1981). Regardless of the composition of the seston, pseudofaeces production occurs above a certain threshold concentration which varies considerably between species (Foster-Smith 1975b, Bayne and Newell 1983, Griffiths and Griffiths 1987). What determines that threshold concentration is not known.

Pseudofaeces production is important in the regulation of food intake by suspension-feeding bivalves (Widdows et al. 1979, Iglesias et al. 1992). For this reason many studies refer to "ingestion rate" (IR), which is the amount of material ingested by an animal of a certain size in a given period of time. When no pseudofaeces are produced, the amount of material cleared (= CR) is equal to the amount of material ingested. However, when pseudofaeces are produced, IR is less than CR. Ingestion rate can therefore be controlled 1) by changing the pumping rate and / or the CR (Winter 1978, Bayne et al. 1989), and 2) through the production of pseudofaeces (Widdows et al. 1979, Iglesias et al. 1992).

1.2.3. The Gut

After material has been accepted by the labial palps, it passes through the mouth and enters the digestive tract, which consists of an esophagus leading to the stomach and style sac with associated digestive gland, followed by a long intestine, and ending at the anus which is located at the bottom of the exhalent siphon.

The stomach is the third location at which selection of particles may occur. The mechanisms for selection in the gut are not clearly understood, although they are probably based on biochemical sensing (Bricelj et al. 1984). Selection can take two different forms. First, the retention time of food within the gut can be manipulated in response to the chemical properties of the food items. Food items of low dietary quality may be passed through the gut more rapidly than those of higher dietary quality (Self and Jumars 1978, Bricelj et al. 1984). This, coupled with the fact that items retained longer in the gut are expected to be digested and / or absorbed with greater efficiency (Taghon 1981), results in an efficient strategy to minimize energy wastage from digesting nutritionally poor material. Bivalves capable of this type of digestive selection may be at an ecological advantage when compared with those which are not. Secondly, bivalves can use two forms of digestion (Van Weel 1961, Widdows et al. 1979, Bayne and Newell, 1983): 1) extracellular "intestinal" digestion occurring in the stomach and resulting in low digestion and absorption efficiencies, and 2) more prolonged intracellular "glandular" digestion taking place in the digestive gland with much higher digestion and absorption efficiencies. It is possible that some bivalves are capable of shunting poorer quality particles directly into the intestine to be eliminated quickly, while higher quality particles are directed into the digestive gland for more thorough digestion. Bivalves capable of directing particles into the different digestive paths may be at an ecological advantage. These two types of selection in the gut (manipulating gut passage times and paths of digestion) are likely to be closely linked, and in some cases the former may be a direct result of the latter.

It is important to note that the sequence of digestion is as yet unknown. It was traditionally believed that intestinal and glandular digestion occurred simultaneously (Owen 1966, Purchon 1968). However, this view has been challenged, and a new theory has been proposed that feeding and intestinal digestion are completed before glandular digestion is initiated (i.e. there is a temporal as well as a spatial separation between the two forms of digestion; McQuiston 1969, Purchon 1971, Morton 1973, Langton 1975, 1977). However, a study by Robinson et al. (1981) does not support this latter theory: these researchers found high intra- and inter-animal variance in the digestive stages of tubules within the digestive gland of intertidal bivalves, meaning that different parts of the digestive gland were at different stages of the digestive cycle. These researchers hypothesize that despite cycles of food availability imposed by environmental factors (e.g. tidal rhythms),

bivalves are essentially opportunistic feeders, and utilize digestive strategies to exploit an erratic food supply.

To evaluate the treatment of food within the gut, researchers generally measure the length of time that food is retained within the gut (= gut retention time (GRT), gut passage time, -residence time, -throughput time or -clearance rate) and the absorption efficiency (AE) or digestive efficiency of the food. The methods for measuring these variables will be described in detail in later portions of this thesis. Previous studies have also looked at factors such as compartmentalization (= gut architecture) and the total amount of material within the gut, when studying the feeding ecology of different bivalve species.

1.2.4. An Energy Equation for Bivalve Feeding

Material within the gut can either be absorbed by the animal for use in growth, metabolism or excretion (substances other than faeces such as mucus and urine) or it can be eliminated as faeces (Fig. 1.1). This completes the digestive pathway of suspension-feeding bivalves.

This entire pathway can be summarized by the energy equation

$$C = P + R + U + F \qquad \text{Equation 1.1}$$

where C is the amount of energy consumed (CR x food concentration - food rejected as pseudofaeces), P is the energy incorporated as production (growth and reproduction), R is the energy allocated to basic metabolic functions, U is the energy content of excreta, and F is the energy voided as faeces (Bayne and Newell 1983). These symbols have been included in the appropriate places in Fig. 1.1.

By altering their feeding behaviour, bivalves can effectively change energy allocations within this equation, thereby altering their physiological and metabolic state. An animal must be able to balance its metabolic losses against energy gains from the environment in order to maintain positive somatic and reproductive growth. This "balancing" of the energy equation is carried out by a number of physiological adaptations, and is brought about, in part, by behavioural adaptations. In general, bivalves can

compensate for increased metabolic demands by increasing consumption (C, Equation 1.1), usually by increasing CR. However, an increase in CR also means that food may be processed through the digestive tract more quickly (decreased GRT) and could therefore be digested with lower efficiency (Taghon 1981). The interactions between CR, AE, and GRT are further compounded by things such as changes in gut volume, enzyme capacity, selection of food particles at various points in the digestive tract, and genetic effects (e.g. pre-disposition towards certain food compositions) among others. Therefore, feeding response to environmental changes can be extremely complex.

1.3. Study Objectives

The objective of this thesis was to investigate pre- and post- ingestive feeding activity (measured as CR, IR, GRT of organic and inorganic fractions, and AE) in the soft-shelled clam, *Mya arenaria* L., particularly in response to changes in food concentration and composition. Since most studies of feeding physiology have used epifaunal animals, this study specifically chose an infaunal species, since less is generally known about their feeding physiology.

The soft-shelled clam, *Mya arenaria* (K. Animalia, P. Mollusca, C. Bivalvia, O. Eulamellibranchia (Sub-order Heterodonta), F. Myidae, was selected for this study because, although much is known about its natural history, its physiology and feeding behaviour have not been extensively studied. In general, measurements of feeding behaviour or physiology of *M. arenaria* have been made as points of comparison with other species (e.g. Möhlenberg and Riisgård 1979, Kiørboe and Möhlenberg 1981, Shumway et al. 1985, Krueger et al. 1992). Studies centering on *M. arenaria* have included morphological work (MacDonald and Thomas 1980, Zwarts and Wanink 1989), biodeposit analyses (Allen 1962, Brown 1986), and feeding mechanics (Foster-Smith 1976a, Jørgensen and Riisgård 1988, Ward et al. 1991, 1993). Other studies have investigated various aspects of the behaviour and physiology of *M. arenaria*, but with respect to factors other than feeding strategies, including general culture and ecology (Hidu and Newell 1989), metabolic state (Lowe and Trueman 1972), substrate type (Swan 1952), role in the benthic community (Emerson et al. 1988), sediment disturbance (Emerson 1990), benthic-pelagic coupling (Loo and Rosenberg 1989), rates of recruitment (Andre and Rosenberg 1991), and oil spillage (Gilfillan et al. 1976, MacDonald and Thomas 1982).

Mya arenaria was also selected since it is an important species in many benthic marine communities throughout the northern hemisphere (Hidu and Newell 1989). There has been increased interest in studying *M. arenaria* as an aquaculture species throughout the Atlantic coast of New England and the Maritimes. *Mya arenaria* also occurs abundantly in many Newfoundland tidal flats, making it an important species ecologically if not economically.

Measurements of CR, IR, GRT, and AE were made to assess the feeding physiology of *M. arenaria* in this study. Methods for measuring CR, IR and AE are well established. However, before these behavioural studies could be undertaken, it was necessary to develop an appropriate method for determining the GRT of suspension-feeding bivalves. Chapter II explains the methods currently available, why they were considered inappropriate for this study, and the technique developed in response to this problem.

Chapter III uses this new GRT technique in a laboratory study to investigate the effect of changes in the concentration and composition of a food suspension on the feeding behaviour of *Mya arenaria*. The results from this study are then compared in Chapter IV to field measurements of feeding behaviour of a natural population of *M. arenaria*. The final chapter of this thesis is a critical evaluation of the new GRT technique.

CHAPTER II

Development of a New Technique to Measure Gut Retention Time in Suspension-feeding Bivalves

2.1. Introduction

There is substantial evidence that bivalves regulate feeding behaviour under different environmental conditions in order to optimize net energy yields (see reviews by Winter 1978, Bayne and Newell 1983, Bayne et al. 1988). This may include the regulation of clearance and ingestion rates, absorption efficiencies, and the preferential selection of particles both before and after ingestion. Considerable work has investigated how these physiological variables are interrelated, particularly in response to quantitative and qualitative changes in the food ration available to the animals. In research of this type, it is often useful to determine the length of time that food is retained within the gut, or the gut retention time, since it often bears a close relation to clearance rate (Bayne et al. 1984, 1989) and absorption efficiency (Bayne et al 1984, Sibly and Calow 1986, Bayne et al. 1987), and if GRT is compared for different types of particles, can be used to identify post-ingestive selection of particles within the gut (Bricelj et al. 1984).

In previous studies of feeding behavior in suspension-feeding bivalves, a variety of techniques to assess GRT have been used. All make use of marker particles which are either traced through the digestive system, or quantified in faecal material. Gut retention time is estimated by determining the amount of marker particles present in samples collected at known time intervals from bivalves fed a "pulse" of marker material. In most experiments, complete elimination of the marker requires a long sampling period, so previous studies have arbitrarily defined GRT according to a specified criterion, the most common being the time at which 90% of the recovered marker has accumulated in the faeces (e.g. Bayne et al. 1987, Hawkins et al. 1990).

Several types of marker particles have been used in previous studies of GRT, including radiolabelled particles, bovine red blood cells, and fluorescent or strongly

coloured particles or beads. The most common gut marker is perhaps radiolabelled particles (e.g. Bricelj et al. 1984, Hawkins and Bayne 1984, Bayne et al. 1987, Hawkins et al. 1990, Decho and Luoma 1991) which can be detected in faecal pellets by standard scintillation counting techniques. This method has the advantage that the labelled particles can be a natural component of the bivalve's diet, such as algal cells, and the labelled particles are easily quantified. Radiolabelling can also very sensitive: the high specific activity of certain labels, such as ^{14}C-bicarbonate, enables use of a very short exposure time to the markers, thereby narrowing the response peak and giving better temporal resolution. However, there are practical limitations to the use of radioisotopes, especially when dealing with non-recirculating, flow-through systems, or field- and ship-based research projects.

Bovine red blood cells, which are readily ingested by suspension-feeding bivalves, have also been employed as markers (Bayne et al. 1984) by using histochemical techniques to detect peroxidase activity within these cells as they pass through the bivalve digestive gland. Although bovine red blood cells are ingested by bivalves, it is not known whether their subsequent treatment in the gut is similar to that of typical diet components. Bivalves are capable of sorting different algal species in the gut, eliminating the more indigestible species more rapidly (Bricelj et al. 1984, Shumway et al. 1985). Thus, it is likely that foreign particles such as bovine red blood cells are also subjected to sorting within the bivalve gut, giving inaccurate estimates of the GRT of more standard dietary components.

Coloured particles such as latex particles or beads are a third type of marker in use (e.g. Bricelj et al. 1984, Hummel 1985). These can be detected visually, but are generally not quantified in faecal pellets, thereby giving only approximate estimates of GRT. Furthermore, these particles may also be subjected to post-ingestive sorting within the gut. It would be preferable to use more natural particles, such as algal cells, which may also be subjected to sorting, but are a natural dietary component.

There is some evidence that the inorganic fraction of the diet is voided from the gut more rapidly than the organic fraction (Bricelj et al. 1984). Therefore, it may be desirable in some studies of GRT to utilize two markers, one organic and one inorganic, to detect any differential selection within the gut. The use of two different marker particles, (organic and inorganic), administered simultaneously to bivalves in a flow-through experimental system,

is the basis for the technique described in this chapter. Both marker particles can be detected in each faecal pellet. The green flagellate *Tetraselmis suecica* was used as the organic marker, because its characteristic chlorophyll *b* signature is not found in most other types of algae including diatoms, dinoflagellates, and cryptomonads. The amount of chlorophyll *b* in the faeces can be determined by high performance liquid chromatography (HPLC), and used as an indicator of the number of *T. suecica* cells present. Silicon carbide particles (SiC, #600 grit, used for polishing geological sections) were chosen as the inorganic marker. These can be obtained in the same size range as *T. suecica* cells (approx. 8 - 12 μm diameter) which eliminates the problem of particle selection or retention based solely on size. In addition, they will pass through the gut undigested and their presence in the faeces can be detected using standard particle size analysis.

2.2. Technique Development

This section describes the major steps taken in the development of the double-marker technique. Additional information is included in Appendix D.

For all experiments in this chapter, clams of similar size (approx. 45 - 55 mm shell length) were collected from the mid- and lower littoral zones at Riverhead, St. Mary's Bay, Newfoundland. Collections were made approximately 1-2 weeks prior to experimentation. Clams were transported immediately on ice (trip duration = approx. 2h) to holding facilities at the Ocean Sciences Centre, Memorial University of Newfoundland. There were few transport-related mortalities. Clams were held in a 30 l tank supplied with unfiltered, flowing seawater at ambient temperature. All seawater used in the laboratory experiments was obtained from the Ocean Sciences Centre main seawater line. The water is not filtered, heated or recirculated, and is pumped directly from Logy Bay. Natural seston was supplemented daily with *Chaetoceros muelleri* and/or *Isochrysis galbana* at an approximate concentration of 66 x 10^3 cells ml^{-1} for 90 min (= approximately 9.5 mg dry weight $food^{-1}$ $clam^{-1}$ day^{-1}), during which time the seawater flow was shut off.

2.2.1. Ingestion and Digestion of *Tetraselmis suecica* by *M. arenaria*, and Detection of Chlorophyll *b* using HPLC

The purpose of this study was to ascertain whether *M. arenaria* would ingest *T. suecica*, and whether the chlorophyll *b* present in *T. suecica* could be detected from pigments extracted from faecal pellets.

Methods

Five clams were placed in individual fingerbowls with siphons oriented in the same direction, and labelled A to E. The fingerbowls were submerged in a plastic tray, with a constant 1 l min^{-1} flow of unfiltered seawater at ambient temperature. Clams were left undisturbed for 24 h to adjust to these conditions. After this adjustment period, faecal pellets (samples 0

h) were collected from all but two clams, which had not produced any faeces, and stored for pigment analysis as described below.

After the initial faeces samples had been collected, the bottom of each fingerbowl was siphoned clean of settled debris, and the seawater inflow line was shut off. Approximately one-half the volume of seawater was siphoned from each fingerbowl and replaced with 70 ml of *T. suecica* culture, to a final concentration of 50,000 cells ml^{-1}. Clams were allowed to feed on the *T. suecica* for 45 min, before the seawater inflow line was reopened, thereby flushing the *T. suecica* marker from the finger bowls. Faeces samples were collected at 1, 2 and 4 h after introduction of the *T. suecica* cells, and again from the fifth clam (E) after 24 h. One clam produced a bright green piece of pseudofaeces one hour after delivery of the marker, which was also collected for analysis (sample PF). All samples were prepared and analyzed on the HPLC as described below.

A seston sample was taken 24 h after delivery of the marker cells by filtering 700 ml of seawater onto a Whatman GF/C filter, from which pigments were extracted overnight at -20°C in 90% acetone and analyzed by HPLC as described below.

Pigment analysis of faecal pellets:

Pigment samples were stored in 1.5 ml Eppendorf tubes (with all seawater removed) in darkness at -20°C until analysis. Samples were then thawed, suspended in 1.5

ml 90% acetone, sonicated in an ice bath for 10 - 15 min, and held in darkness at -20°C for 24 h for extraction of pigments. Samples were then centrifuged at 16,000 rcf for 3 min. The supernatant was collected, transferred into another 1.5 ml Eppendorf disposable tube, and stored in darkness at -20°C for subsequent pigment analysis by HPLC. Pigment extraction is 95 -100% complete by this method.

Before analysis by HPLC, pigment samples were diluted with 90% acetone to a total pigment concentration of approximately 0.75 - 1.00 mg l^{-1} as determined with a Turner Designs (model TD10) fluorometer. Five-hundred µl of the diluted sample was mixed with 150 µl ion pairing solution (7.7g ammonium acetate in 100 ml milli-Q water) and 250 µl of this mixture was injected into the 100 µl loop of a Beckman reverse-phase HPLC. Samples were run through a 10 min. gradient of 80% methanol, 15% Milli-Q water and 5% ion pairing solution to 70% methanol and 30% acetone. Fluorescent emission was detected by a Gilson Model 121 detector and displayed as a standard chromatogram (Fig. 2.1). Pigments were identified by comparisons with known standards obtained from Sigma Chemical Company or TLC extraction.

Results and Discussion

The presence or absence of chlorophyll *b* and its component breakdown products in each sample is listed in Table 2.1. In most samples, chlorophyll *b* was present in either trace amounts, or could not be detected at all. This includes the seston sample, and initial (0h) faecal samples, which indicates that background levels of chlorophyll *b* were very low. Clams C and D did not pump during the period of exposure to *T. suecica*, and therefore did not show any chlorophyll *b* in subsequent faecal samples. However, two clams did show presence of b-pigments in their faeces: clam A at 4 h after exposure to *T. suecica*, and clam E at 24 h. (It should be noted that the 24 h sample could have been voided from the gut at any time between 4 and 24 h after delivery of the marker). Furthermore, both of these samples showed breakdown of chlorophyll *b* in the form of phaeophytin b, indicating that the *T. suecica* cells were partially degraded in the gut. Some *T. suecica* was rejected as pseudofaeces, but this is probably due to the high concentration of *T. suecica* administered to the clams.

From this experiment it was concluded that *M. arenaria* will ingest and digest *T. suecica* cells, making this alga appropriate for use as a GRT marker.

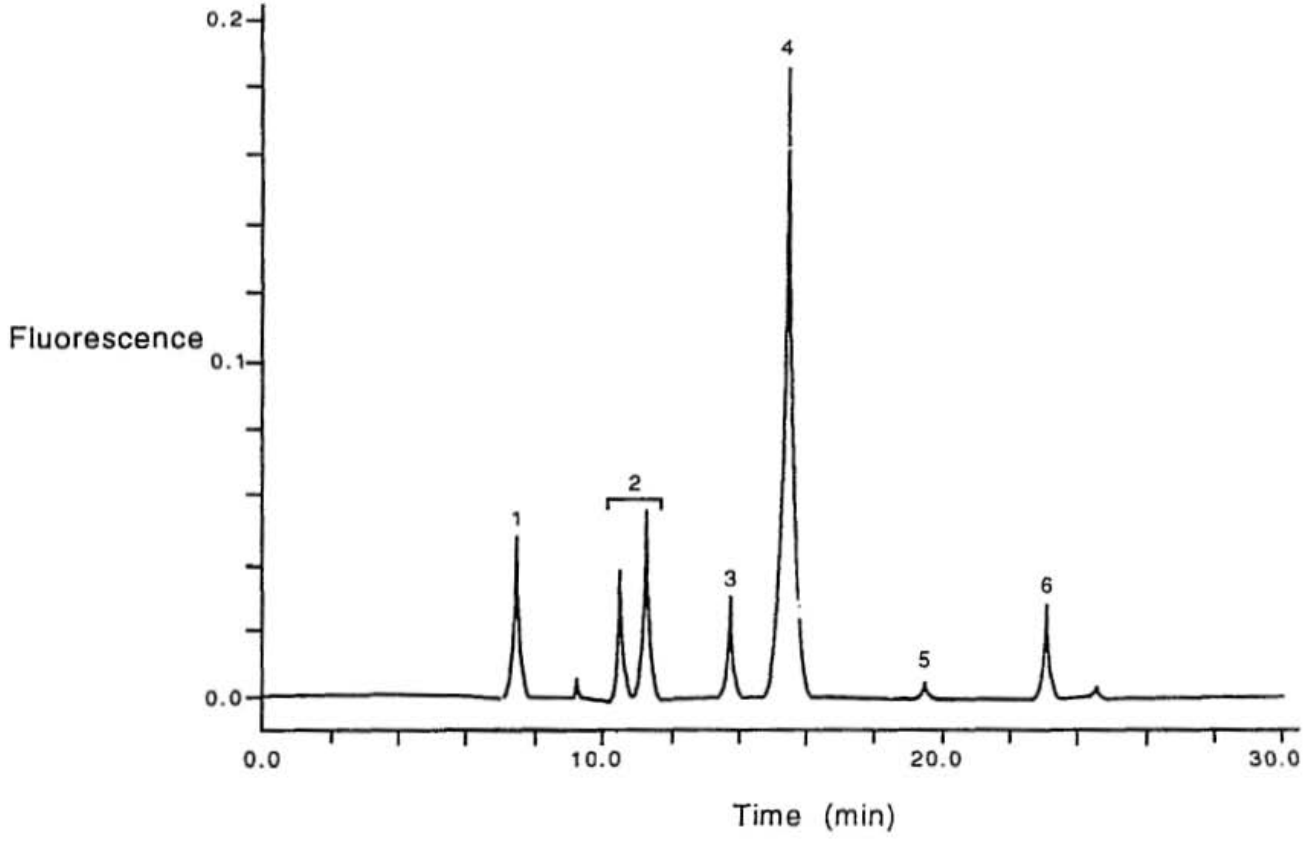

Fig. 2.1: Example of a chromatogram showing pigment composition of faeces from a clam feeding on a mixture of *C. mulleri* and silicon dioxide particles, as well as a 30 min pulse of *T. suecica* and silicon carbide particles. Peaks can be identified as: 1-chlorophyll c, 2-phaeophorbide a, 3- chlorophyll b, 4-chlorophyll a, 5- phaeophytin b, 6-phaeophytin a.

Table 2.1: Presence of chlorophyll *b* and its component breakdown products in faecal samples collected from *M. arenaria* exposed to a 45 min pulse of *Tetraselmis suecica* .

Sample	Chlorophyll b	Breakdown of b	Comments
A - 0h	Trace	Absent	little background b-pigment
B - 0h	Trace	Absent	little background b-pigment
E - 0h	Trace	Absent	little background b-pigment
E - 1h	Absent	Absent	no *Tetraselmis*
B - 2h	Absent	Absent	no *Tetraselmis*
C - 2h	Absent	Absent	no *Tetraselmis*
D - 2h	Absent	Absent	no *Tetraselmis*
A - 4h	Trace	High	degraded *Tetraselmis*
B - 4h	Trace	Absent	no *Tetraselmis*
E - 4h	Trace	Absent	no *Tetraselmis*
E - 24h	High	High	*Tetraselmis* cells present
PF	High	Absent	pseudofaeces is *Tetraselmis*
Seston	Trace	Absent	little background b-pigment

2.2.2. Ingestion and Digestion of Silicon Carbide by *M. arenaria.*

The purpose of the following experiment was to determine whether *M. arenaria* can ingest silicon carbide (SiC) particles. Detection of particles was done from a visual examination of the faecal pellets: SiC is grey in colour and easily distinguished from otherwise naturally brown pellets.

Methods

All the following experiments were conducted in the flow-through system shown in Fig. 2.2. A 20 l plastic bucket served as a header tank. Seawater was filtered to 100 µm and flow to the header tank was maintained at 4 l min^{-1} throughout the course of each experiment. Seawater was distributed simultaneously to the experimental containers through 1 cm internal diameter tygon tubing. The free ends of these lines were secured inside the header tank through a 15 cm diameter Plexiglas disc, raised about 9.5 cm above the bottom of the bucket. Flow through these lines into the experimental containers was controlled by plastic plugs drilled with a 0.5 mm bit. This resulted in a standardized flow of 100 - 120 ml min^{-1} to each container, predetermined to be an appropriate rate for *M. arenaria* : see Appendix A. A constant water head pressure was maintained by ensuring that water continuously exited via the overflow outlet.

Experimental containers were made from 1.0 l plastic containers (170 x 80 x 110 mm, Fig. 2.2). A 1 cm internal diameter inflow tube was passed through a hole drilled through the wall of the container, 3 cm above the base. A 0.5 cm internal diameter standpipe was passed through the base with its upper end 1 cm below the rim of the container. Joints were sealed with silicone where necessary. A four-pronged plastic "holder" for positioning the clams in the natural upright position was secured by a plastic screw through the base of the container, 4.5 cm forward of the standpipe. Finally, a 5.5 cm tall Plexiglas baffle was affixed with silicone 4.3 cm back from the inflow, to ensure a non-recirculating flow through the container.

Preliminary experiments with this flow-through system showed that there was no significant difference in particle concentration between all experimental containers. It is

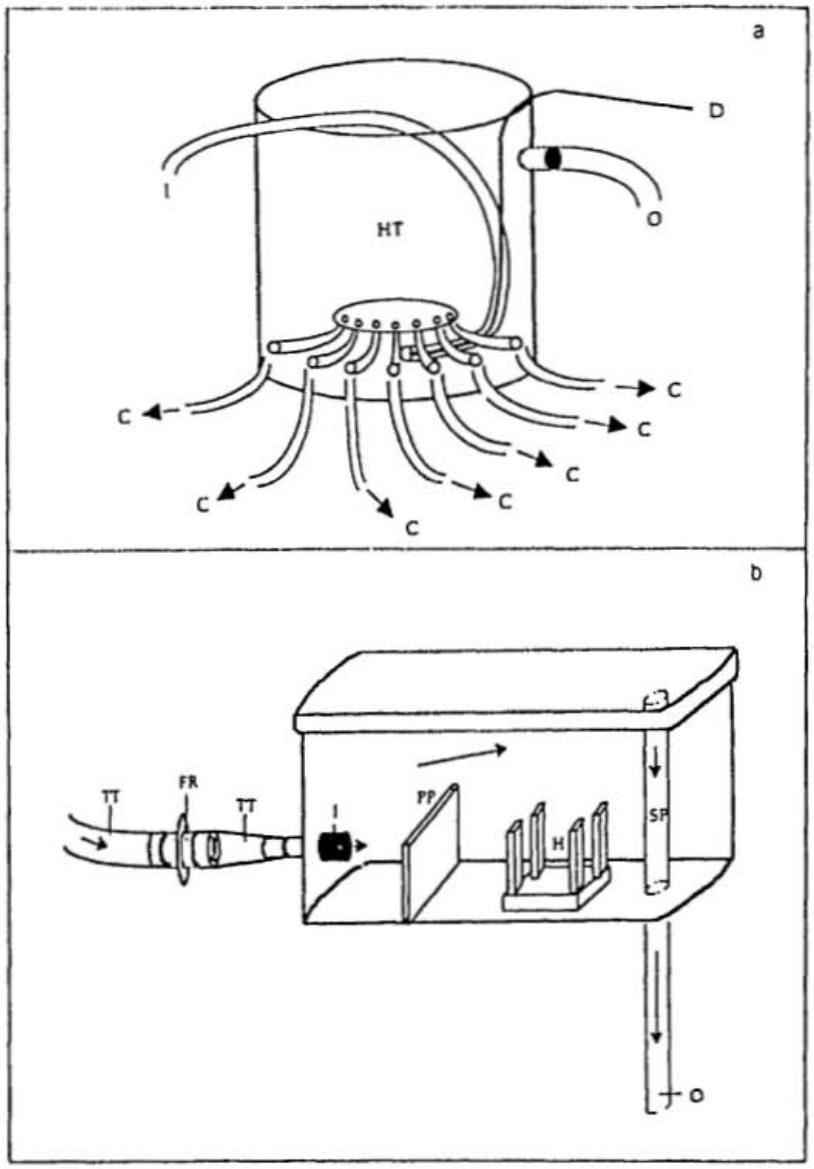

Fig. 2.2: Internal and external features of the experimental apparatus. A) Features of the flow-through header tank. HT = header tank, I = inflow, O = overflow, D = diet inflow, C = experimental containers (n = 16 maximum). B) Features of the experimental containers. TT = tygon tubing, FR = flow restrictor, I = inflow, PB = Plexiglas baffle, H = holder for clam, SP = stand pipe, O = outflow. Arrows indicate the direction of seawater flow.

therefore reasonable to assume that in these experiments, mixing in the header tank was sufficient to ensure that identical suspensions were delivered to all individual containers.

In this experiment, five clams were held in individual 1.0 l plastic pots in the flow-through apparatus at ambient temperature. Clams were allowed to adjust to these conditions for 24 h, before a few drops of SiC in suspension (at 1.6×10^6 particles ml^{-1}) were introduced into the water above the incurrent siphons of 4 clams. The fifth served as a control (no SiC added). Clams were allowed to feed undisturbed for 24 h before faecal pellets were visually examined for the presence of SiC.

Results

After 24 h, all clams exposed to the SiC particles produced grey faecal pellets, indicating that SiC was ingested and voided from the gut. There were also grey pseudofaeces in some containers, which were more loosely bound than faecal pellets and floated at the surface. The containers also had a light film of grey particles across the bottom, presumably from SiC settling out of the water column. This film was easily distinguished from the grey faecal pellets.

From this experiment, it was concluded that SiC is ingested by *M. arenaria*, and could therefore be used as a marker for GRT.

2.2.3. Development of a Procedure for Quantifying SiC Marker Particles in Faecal Pellets

Although the presence of SiC in faecal pellets could be detected visually by their grey colour, it was necessary to develop a method for quantifying the amount of SiC particles present in each sample. Three possible methods were explored, 1) microscopy, in which SiC particles would be individually counted, 2) ash weight analysis, in which faecal samples containing SiC would have a higher inorganic content, and 3) particle count analysis, in which the SiC particles would be quantified from a size distribution of particles in each sample.

Methods

Five clams, labelled A-E, were placed in individual 1 l plastic containers in the flow through apparatus described in section 2.2.2. Clams were allowed to adjust to the experimental conditions for 24 h, before SiC marker particles were delivered to the clams at a concentration of approximately 5000 particles ml^{-1} for 30 min. This was achieved by pouring a pre-determined amount of SiC particles in suspension into the top of the header tank. The inflow line to Clam A was plugged during this time, to prevent exposure to SiC and thus act as a control. After 30 min feeding, the flow rate of the incurrent seawater line was increased to flush marker particles out of the system quickly through the overflow line (see Fig. 2.2), and the line to clam A was unplugged. Faeces samples were collected at 6, 12, and 24 h after delivery of the marker particles. This experiment was repeated 3 times, with examination of the faecal pellets for SiC by microscopy, ash weight analysis and particle size analysis respectively.

Faeces samples collected for microscope analysis were pipetted into 1.5 ml Eppendorf tubes filled with 1.0 µm filtered seawater, sonicated for 15 min to break up mucus-bound clumps, filtered onto Whatman GF/C filters, and examined at 10X on a dissecting microscope.

Samples collected for ash weight analysis were filtered onto pre-ashed and weighed Whatman GF/C filters, rinsed with 3% ammonium formate, placed in an oven at 80°C and dried to constant weight. Filters were then heated at 450°C for 6 h to combust organic compounds, cooled in a desiccator to room temperature, reweighed, and the proportion of organic compounds in each sample determined. Organic content was also determined for samples of pure SiC.

Samples collected for analysis of the particle size distribution were pipetted into 1.5 ml Eppendorf tubes filled with 1.0 µm filtered seawater, and sonicated for 30 min to disperse the particles. Each sample was then added to 250 ml of 1.0 µm filtered seawater and analyzed for 2 min on a Coulter Multisizer equipped with a 100 µm diameter orifice tube (which represents a volume of 4.5 ml). This produced a frequency histogram of particles between approximately 2 and 62 µm diameter for each sample. The SiC particles were found by Multisizer analysis to have a particle size distribution of about 4.7 to 17.9

µm (Fig. 2.3). Therefore, samples without SiC would have a "baseline" number of particles between 4.7 and 17.9 µm diameter. Faecal samples containing SiC would have a larger "peak" of particles in this size range. Furthermore, the amplitude of the 4.7 - 17.9 µm peak is an indication of how many SiC particles are present in the sample.

Results

Microscopy: SiC particles were visible on the filters as shiny grey chips. However, counting the number of chips on each filter proved to be difficult and laborious. It was decided that this was not an efficient way to quantify the SiC marker particles in each sample.

Organic content: The underlying theory to this analysis is that more SiC present in a sample should lower its organic weight content). Clam A (control) did not produce sufficient faeces for this analysis, so four fresh faecal pellets were taken from clams in the holding tank (where they had been exposed to the same water temperatures and seston load as those in the flow-through apparatus) as samples of faeces without SiC. These had a mean of 23.2 % organic content by weight (SD = 8.3). Three grey pellets produced by clams exposed to SiC were analyzed, and found to have a mean of 18.1% organic content (SD = 1.5). Two samples of pure SiC were also analyzed, and found to have a mean organic content of 9.75% (SD = 2.5). The organic content of faeces from clams exposed to SiC and control samples did not differ significantly (independent t-test, $t = 1.024$, $df = 5$, $p = 0.353$). This may have been because the seston had a low organic content, or because the clams had a high AE for organic compounds. It was concluded that ash-weight determination is not a reliable method for quantifying the amount of SiC present in faecal pellets, for two reasons. First, a large amount of faecal material is needed for an accurate analysis by weight, and not all clams produced sufficient faecal material in the course of this experiment. Second, for analysis by this method, it is essential that a constant AE be maintained by each clam throughout the experiment. Otherwise, observed fluctuations in the proportion of organics due to changing AE could erroneously be interpreted as fluctuating amounts of SiC. Clams may well adjust AE over short time periods.

Particle size analysis: Little faecal material was produced during the 24 h period, so faecal pellets from clams exposed to the SiC particles were pooled into one

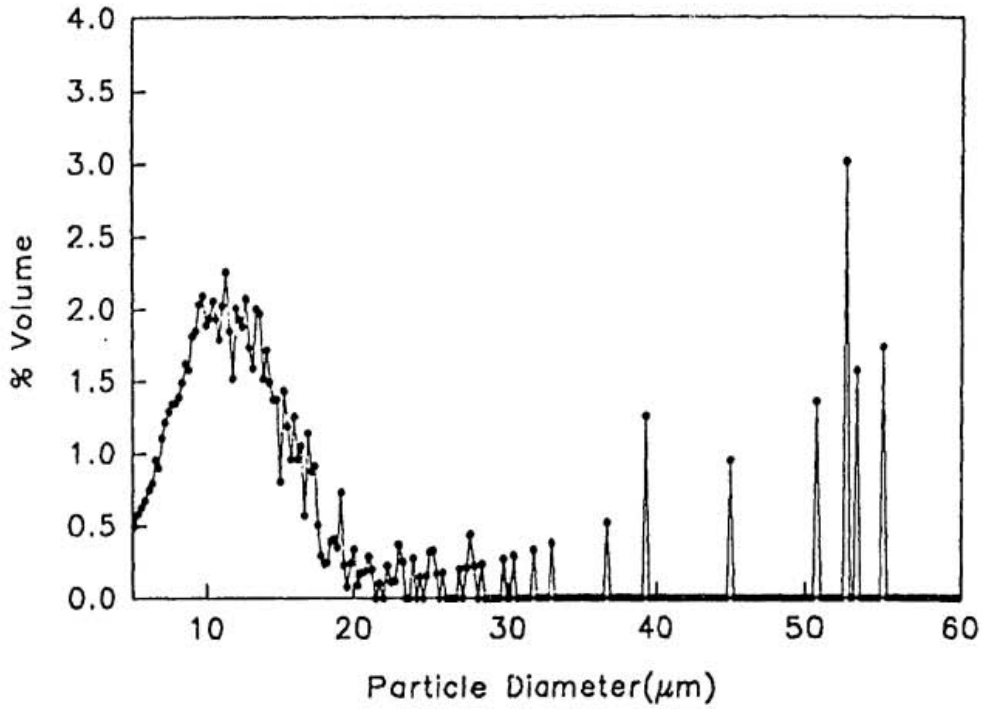

Figure 2.3: Size distribution of suspended SiC particles as determined by Coulter Multisizer. The SiC peak ranges from approximately 4.7 to 17.9 μm.

sample. Likewise, faecal pellets from the one clam not exposed to the SiC particles were pooled into a second sample.

Seventy-four percent of the SiC particles were determined to be between 4.64 and 17.86 µm in diameter (Fig. 2.3), so the proportion of particles in this size range compared to the 2 to 62 µm size range scanned was calculated for each sample. Faeces from clams exposed to SiC showed a greater proportion of particles in this size range than either faeces from the clam not exposed to SiC or natural seawater (40.6% compared to 31.5% and 33.1% respectively). This was interpreted as evidence of the presence of SiC in the faeces of clams exposed to the pulse of SiC particles. It was concluded that SiC was most easily and accurately detected and quantified by use of the Coulter Multisizer to analyze the size distributions of particles in the faecal pellets.

It was necessary to modify the procedure described above in order to acquire a stronger, sharper SiC particle peak in the histograms. The following three sections of this chapter deal with the procedures developed to obtain a stronger SiC signal.

2.2.4. Acidification of Faecal Pellets to Enhance the SiC Particle Peak

The first step in enhancing the SiC particle peak in the particle size distributions was to remove as many other particles from the pellets as possible. The method employed was to treat faecal pellets with acid to dissolve any organic particles, leaving behind only those which could withstand this treatment, including SiC. This would facilitate detection of the SiC particle peak.

Methods

The flow-through apparatus described in section 2.2.2 was set up with 6 clams, each in individual containers: 3 were exposed to SiC particles and 3 (the controls) were not. For this experiment, the inflowing seawater was supplemented with a mixture of microalgae (*C. muelleri*), and silicon dioxide (SiO_2) particles (not to be confused with the silicon carbide marker particles) to stimulate the clams to produce more faecal material.

Chaetoceros muelleri was grown in 200 l cylinders at 22°C using constant illumination and F2+ medium.

Suspensions of algae and silicon dioxide were mixed separately in 60 l buckets to predetermined concentrations. A large stirbar was placed in the bottom of the bucket containing silicon dioxide. In addition, the silicon dioxide bucket was plunged manually every 1-2 h to limit settling. An airstone was placed in the algae bucket to ensure proper aeration and mixing. Particles from both buckets were delivered to the header tank by a peristaltic pump. The inflow lines from the peristaltic pump were attached to the larger seawater inflow line to facilitate mixing. A large stir-bar was also placed in the bottom centre of the header tank. The flow rate required by the peristaltic pump to produce the desired particle concentration was determined by the formula:

$$C_s = C_{ht} \times \frac{F_s}{F_{pp}} \qquad \text{Equation 2.1}$$

where C_s = the concentration of the stock culture, C_{ht} = the concentration required in the header tank, F_s = the flow rate in the main seawater inflow line (into the header tank), and F_{pp} = the flow rate through the peristaltic pump delivering stock particles to the header tank.

The artificial diet was delivered at a final concentration of 10,000 particles ml^{-1} of each particle type. Clams were allowed to adjust to these conditions for 24 h. SiC marker particles were then introduced at a concentration of approximately 10,000 particles ml^{-1} for 30 min. Lines to the 3 control clams were plugged during this time to prevent them from ingesting the SiC particles.

Two faecal samples from clams exposed to SiC particles, and two samples from clams not exposed to SiC particles, were pipetted separately into 1.5 ml Eppendorf tubes, covered with a few drops of concentrated nitric acid, and allowed to stand overnight. The tubes were centrifuged at 16,000 rcf for 3 min, the supernatant discarded, and the pellets washed once in distilled water. Pellets were then covered with 1.5 ml of 1.0 µm filtered seawater, sonicated for 10 min to de-aggregate the particles, added to 250 ml of 1.0 µm filtered seawater, and analyzed by Coulter Multisizer as described in section 2.2.3.

Results

Treatment with acid did not alter the size distribution of the SiC particles. Particle size distributions for the four samples are given in Fig. 2.4. One sample (Treatment 2) showed a higher proportion of particles between approximately 4 and 8 µm than the Treatment 1 and Control 1 samples, indicating the presence of SiC. This was further supported by visual observations of this sample, which was grey in colour unlike the others. It should be noted that the SiC particle peak is present only in the smaller half of its size fraction, i.e. the peak does not continue from 8 to approximately 18 µm as expected. This is probably due to settling of the SiC marker particles in the header tank, with the larger particles settling out first. In all subsequent experiments, the header tank was manually stirred during the period of marker particle introduction to keep as many particles as possible in suspension. The Control 1 sample also had a high proportion of particles between 3 and 8 µm diameter, but was not grey in colour. It was possible that the 10 min sonication time was not sufficient to fully disperse all particles in the samples (resulting in two extremely different particle distributions for the control samples, Fig. 2.5 A), so the following experiment was designed to investigate this.

2.2.5.Determination of the Minimum Sonication Time Required to Disperse all Particles in Faecal Pellets

Methods

Two faecal samples from the above experiment were selected for this analysis: one from a control clam, and the one known to contain SiC particles. Samples were sonicated for 60 min and analyzed by the Coulter Multisizer after 20 min, and thereafter at 10 min intervals. The particle count analysis was performed as described in section 2.2.3.

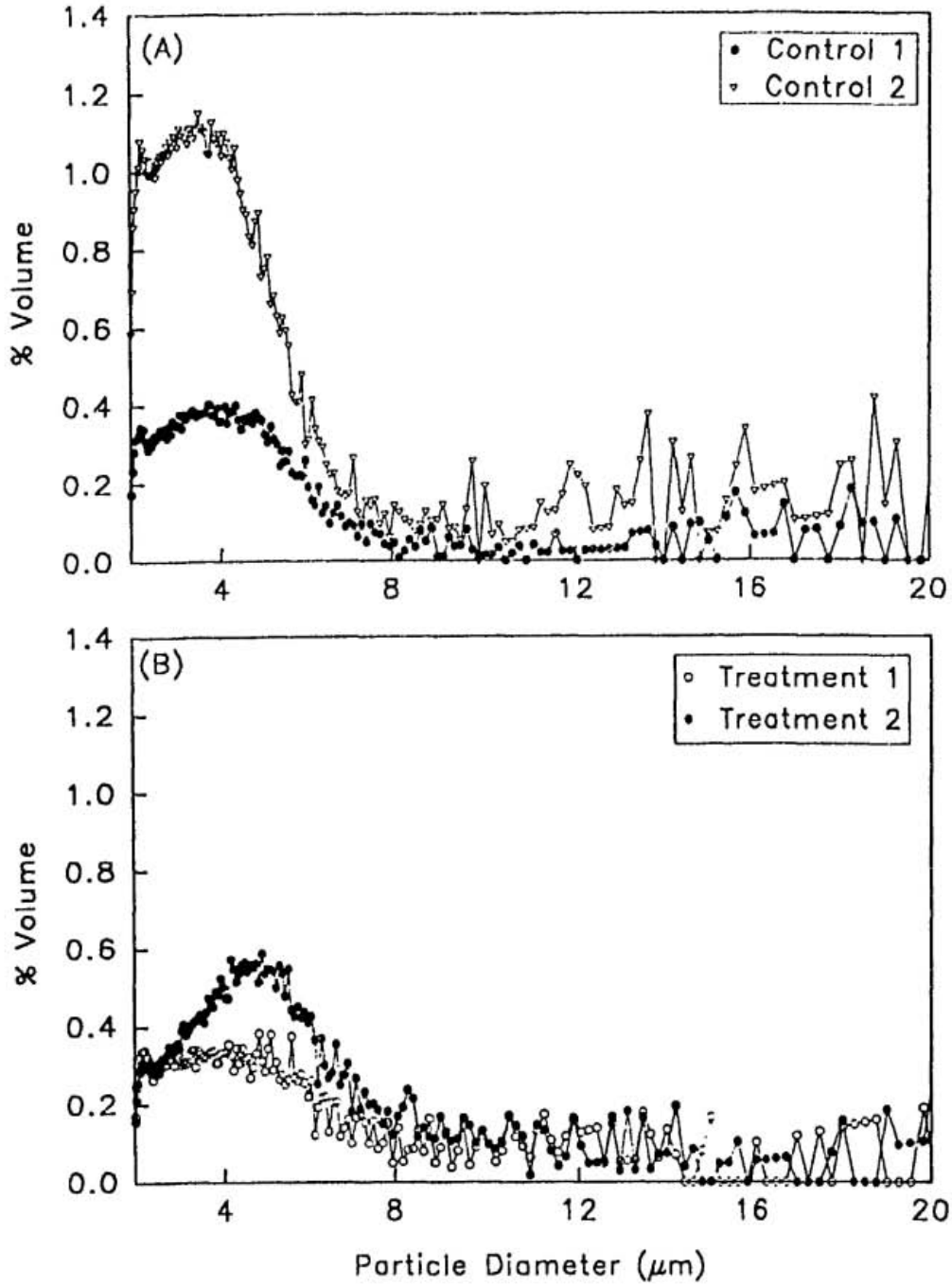

Figure 2.4: Particle size distributions of 4 faecal samples, two samples from clams not exposed to SiC (A), and two samples from clams exposed to SiC (B).

Results

The particle size distributions at the 20, 40 and 60 min. intervals are given in Fig. 2.5. In both samples, there is little change in the particle size distributions after about 40 min. Based on these graphs, a conservative decision was made to sonicate all samples for 60 min before analysis by the Coulter Multisizer.

2.2.6. Enhancement of SiC Response Peak by Sieving Particles

To define the SiC particle peak further, SiC particles were sieved using Nitex screens to retain only those particles between 10 and 15 µm diameter. This served two purposes: 1) by narrowing the size distribution from its previous range of approximately 4.7 - 17.9 µm, detection of the SiC particles is easier and more accurate, and 2) to render the size range of SiC more equivalent to that of the *Tetraselmis suecica* marker particle, with a size distribution of approximately 10 - 12 µm. Using the sieved particles did not affect any of the conclusions made from the preceding experiments using unsieved particles. Furthermore, sieving the particles, rather than just narrowing the particle size range on the Multisizer, increased the amplitude of the particle peak on the Multisizer, thus giving a stronger and more accurate signal.

2.3 Summary

The experiments included in this chapter have demonstrated the development of a new technique to assess the GRT of suspension-feeding bivalves. The technique makes use of 2 marker particles: *Tetraselmis suecica* and silicon carbide (SiC) to estimate the GRT of organic particles and inorganic particles respectively. *Tetraselmis suecica* is traced in faecal samples by identifying and quantifying its characteristic chlorophyll *b* components using high performance liquid chromatography. SiC particles were identified and quantified through particle size analysis on the Coulter Multisizer. SiC particles have a discrete size distribution, which is enhanced by sieving to retain only those between 10 and 15 µm. Thus, faecal samples with a peak of particles between 10 and 15 µm not present in control samples will indicate the presence of SiC. Furthermore, the amplitude of that peak is used

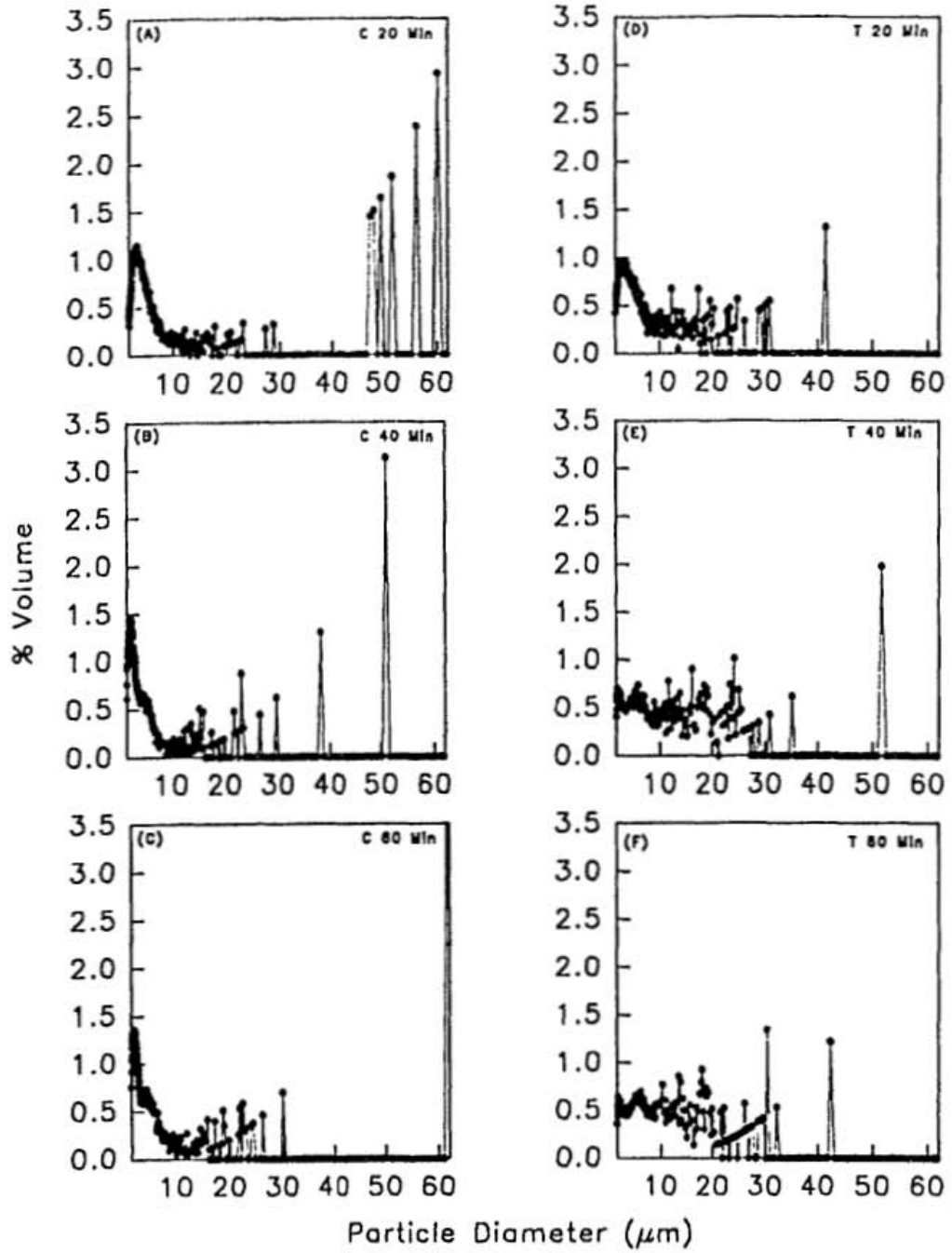

Figure 2.5: Particle size distributions of one faecal sample from a clam not exposed to SiC (A,B,C), and one faecal sample from a clam exposed to SiC (D,E,F), measured at 20, 40 and 60 minute intervals in the sonication bath.

as an indication of the quantity of SiC particles present. Detection of this peak is further assisted by elimination of extraneous particles through treatment with nitric acid.

This chapter has demonstrated the feasibility of each technique (organic and inorganic markers) when used separately. In the following chapter, these two marker particles will be used simultaneously to assess the GRT of organic and inorganic fractions in *M. arenaria* feeding on different quantities and qualities of food.

CHAPTER III

Pre- and Post-Ingestive Activity of the Soft-Shelled Clam, *Mya arenaria*, in Response to Different Food Regimes

3.1. Introduction

3.1.1. Importance of Studies of Feeding Behaviour

Studies of the feeding behaviour of bivalves are important in understanding physiological adaptations to environmental change. These, in turn, are important in understanding energy fluxes in the coastal marine ecosystem, and can be used to maximize bivalve growth efficiency in aquaculture (Newell et al. 1989) or other commercial ventures (Griffiths and Griffiths 1987). The purpose of this series of experiments was to investigate the feeding behaviour of the soft-shelled clam, *Mya arenaria*, in response to food suspensions differing both in the concentration and composition of particles.

Feeding behaviour is measured by a number of variables, some of which have been outlined in Chapter I. The most important of these are CR, IR, GRT, AE and particle selection at all points in the alimentary tract. Previous studies have determined some basic trends in these variables, particularly in relation to changes in the food supply. The next portion of this introduction will describe some of these trends, from both mathematical models and experimental results.

3.1.2. Evaluating Changes in Food Supply

Changes in food supply are generally evaluated in two ways. First, many researchers have investigated the effects of changes in the quantity of particles in suspension (particle load). This can be expressed in one of two ways: dry weight of particulate matter per unit volume of water, and number of particles per unit volume of water. The former method (usually in mg l^{-1}) is preferable for the purposes of comparing

results of different authors, because larger particles provide a higher volume and hence a higher particle load than an equivalent number of smaller particles (Foster-Smith 1975b, Griffiths and Griffiths 1987). For this reason, this study will express changes in the quantity of particles in suspension as changes in the particulate dry weight per litre of seawater.

Many studies have also identified the importance of relating feeding responses to changes in the quality of food in suspension (see reviews by Bayne and Newell 1983, Bayne et al. 1988). This is important when considering 1) the physiology and energy budget of a consumer experiencing variations in its food supply, and 2) the evidence that some bivalves are capable of sorting nutritionally rich from poor food particles at various points in the digestive tract (Bayne et al. 1984).

Food quality is most easily defined as the amount of organic matter per unit volume of particles (Bayne et al. 1987, Bayne et al. 1989). However, other factors have been identified as being important in defining quality, specifically 1) the size of the individual particles, 2) the balance in the diet between biologically inert and metabolizable fractions, and 3) the biochemical composition of this metabolizable fraction (Bayne et al. 1987). These three points were taken into consideration when choosing particles for the artificial diet. The diatom *C. muelleri* and inert silicon dioxide particles were chosen as the organic and inorganic fractions respectively. *Chaetoceros muelleri* has a size distribution between approximately 4 and 6 µm diameter, which would be retained by *M. arenaria* with 100% efficiency (Möhlenberg and Riisgård 1978). Furthermore, the biochemistry of a variety of *Chaetoceros* species has been studied, and this genus has been identified as a reasonably high quality food source (Enright et al. 1986). The silicon dioxide used has a size distribution from 1 to approximately 10 µm, most of which would also be retained with 100% efficiency by *M. arenaria* (Möhlenberg and Riisgård 1978). To adjust the balance between biologically inert (silicon dioxide) and metabolizable (diatom) fractions, the relative amounts of each particle can be varied when clams are exposed to a mixed suspension of the two. This study therefore defines food quality as the amount of particulate organic matter present, expressed as a proportion of total seston dry weight per litre seawater (Bayne et al. 1988). The higher the proportion of organic particles, the higher the "quality" of the food suspension.

3.1.3. Bivalve Feeding Responses to Changes in Dietary Quantity

Most bivalves, both suspension and deposit feeders, respond to an increase in the amount of food in suspension by reducing pumping rate and CR (Winter 1969, Foster-Smith 1975a, Widdows et al. 1979, Malouf and Bricelj 1989, review papers by Bayne 1976, Bayne and Newell 1983, Griffiths and Griffiths 1987, Bayne et al. 1989). Corresponding increases in GRT and AE are predicted by some models (e.g. Taghon 1981) but are not always observed. For example, in a variety of bivalve species, several authors have found a decrease in AE with increasing concentration of food (Thompson and Bayne 1974, Foster-Smith 1975a, Griffiths and King 1979, Griffiths 1980, and Möhlenberg and Kiørboe 1981). Furthermore, Bayne et al. (1989) found that *Mytilus edulis* decreases GRT in response to an increase in particle concentration.

3.1.4. Bivalve Feeding Responses to Changes in Dietary Quality

A model of bivalve feeding which predicts responses to changes in the quality of the food suspension was developed by Taghon (1981). This model predicts that the optimal response to a higher nutritional quality of food is to increase the rate of feeding, observed as a rise in CR. As CR increases, food is passed through the gut more quickly (decreased GRT) and is absorbed with less efficiency. This strategy may result in a greater gain of energy per unit of time, than that of attaining a higher AE but over a longer gut retention time (Bayne and Newell, 1983). Several studies have tested this hypothesis with conflicting results. Some studies have supported Taghon's (1981) model (Foster-Smith 1975a, Widdows et al. 1979, Bricelj and Malouf 1984), while others have not (Kiørboe et al. 1981). Furthermore, deposit feeders are predicted by some to adapt to poor food sources by increasing their rates of feeding (see review by Lopez and Levinton 1987).

Data on GRT and AE, in relation to the quality of food, are also conflicting. For GRT measurements, the results of Bayne et al. (1984, 1987, 1988), as well as the general response of deposit feeders (Bayne and Newell 1983) do not support the predictions of Taghon (1981). Absorption and/or digestion efficiencies measured by Vahl (1980), Hawkins et al. (1986), and Bayne et al. (1987, 1988) increase with poorer quality food. These observations are inconsistent with Taghon's (1981) predictions. Also, Foster-Smith (1975b) and Bricelj (1984) found no relation between AE and food quality.

3.1.5. Particle Selection by Suspension-Feeding Bivalves

As stated previously, some bivalves exhibit particle selection at various points along the alimentary tract. It has been suggested that the extent to which selection is invoked may depend on the food supply. If bivalves are food limited, they may be expected to select the most nutritional particles (i.e. those most likely to maximize net energy gain) for digestion, thereby reducing the amount of energy lost in processing indigestible or poor quality particles (Bayne et al. 1988). Furthermore, as the proportion of inorganic material in the seston increases, selective rejection of this indigestible fraction could counteract the "dilution" effect of organics by high inorganic loads (Widdows et al. 1979, Kiørboe and Möhlenberg 1981, Bayne et al. 1988, Iglesias et al. 1992). Apparent selection of organic particles by the labial palps has been documented in a variety of bivalve species (Kiørboe and Möhlenberg 1981, Newell and Jordan 1983) including *M. arenaria* and *M. edulis* (Kiørboe and Möhlenberg 1981), although other studies could not detect this ability in *M. edulis* (Foster-Smith 1975b, Widdows et al. 1979), or in *Chlamys islandica* (Vahl 1980).

3.1.6. Objectives

The objective of this component of the study was to assess the feeding behaviour of *M. arenaria* to artificial diets differing in both the quantity (i.e. particle load) and quality (i.e. proportion of organic content) of particulate matter in suspension. Specifically, the CR, IR, ingestion rate of organic material (IR_o), GRT of organic and inorganic components, and AE were monitored.

This study, therefore, represents a preliminary, exploratory approach to the question of how *M. arenaria* reacts to different food types. Results will be compared to those found in other species, and a feeding strategy for *M. arenaria* will be proposed.

3.2. Materials and Methods

3.2.1. Collection Site, Animals, Transport Conditions, and Holding Facilities

Soft-shell clams, *Mya arenaria* , approx. 40.0 - 51.3 mm in shell length (maximum distance between anterior and posterior margins, see Appendix C for morphological analysis of *M. arenaria*) were collected from the mid- and lower littoral zones at Riverhead, St. Mary's Bay, Newfoundland. Collections were made approximately every 4-6 weeks from January to June 1992. Clams were transported immediately on ice (trip duration = approx. 2 h) to holding facilities at the Ocean Sciences Centre, Memorial University of Newfoundland. There were few transport-related mortalities. Clams were held in the holding facility and feeding regime described in section 2.2 for not more than 3 weeks prior to experimentation. The holding tank was siphoned clean of faeces and debris every 1-2 weeks. A different set of clams was used for each experiment.

3.2.2. Preparation of Markers

Tetraselmis suecica was grown in 4 l flasks (constant illumination, 22°C, F2 medium), and harvested at a concentration of 1.2 - 1.8 x 10^6 cells ml^{-1}. Silicon carbide (SiC) was obtained in powdered form, suspended in distilled water, and sieved to retain only particles of 10 to 15 µm diameter (comparable to the 8 - 12 µm diameter size range of *T. suecica*). SiC particles were suspended in filtered seawater to a concentration of 0.8 - 1.3 x 10^6 particles ml^{-1}, and refrigerated in a 1.5 l airtight bottle.

3.2.3. Experimental Apparatus and Procedure

Seven days prior to experimentation, 18 clams were transferred from the holding tank to an "acclimation tray" (32 x 18 x 6 cm, flow rate = 1.5 l min^{-1}) in which the water temperature was raised by 1 - 2°C daily, from ambient temperature to 12°C. This rise in temperature did not adversely affect the behaviour of the clams (see Appendix B). A food suspension was prepared by mixing known concentrations of *C. muelleri* cells and silicon dioxide particles (not to be confused with the silicon carbide marker particles). This suspension was delivered to the clams at a concentration and an organic / inorganic dry weight ratio approximating that of the upcoming experiment.

All experiments were conducted in the flow-through system described in section 2.2.2. fitted with a total of 16 containers: the control container remained empty, three containers were used to measure absorption efficiencies (containers AE), and the remaining twelve containers were used to measure GRT and CR.

The three AE containers were fitted with plastic "pseudofaeces collectors", constructed from 1 l plastic containers. A 9.5 cm diameter hole was cut in the lid of each container and covered with a 100 µm Nitex screen (large enough to trap pieces of pseudofaeces without becoming clogged with seston) to retain particle clumps that exited the experimental containers via the standpipe. A small slit was cut in the centre of this screen for the standpipe to pass through. Temperature was maintained at 12°C in the experimental containers by passing the incurrent water line through a heat exchanger.

Twenty-four h prior to the start of each experiment, water flow to the header tank of the flow-through apparatus was started and each container allowed to fill. Pumping clams were selected from the acclimation tray and positioned in the holders with the siphons upward and the ventral margin (and incurrent siphon) facing towards the inflow. Elastic bands were stretched loosely around the holders to prevent the clams from slipping. Preliminary studies showed that the absence of sediment did not adversely affect feeding behaviour (see Appendix A).

When all clams were in place, delivery of the test diet was started. Algae and silicon dioxide diets were prepared and delivered to the flow-through apparatus as described in section 2.2.4, to final concentrations as described in section 3.2.4.

Clams were left undisturbed for 24 h to adjust to the experimental conditions. Thirty min prior to delivery of the GRT markers, the food suspension was temporarily shut off. Lines to the C, AE and six of the GRT containers (to act as controls) were plugged on the inside of the header tank to prevent exposure of clams in these containers to the markers. The main seawater inflow line was reduced to prevent overflow during the 30 min of exposure while a constant head was maintained (approx. 1.4 l min^{-1} flow). This minimized dilution of the marker by inflow water, while ensuring that flow rates in the

remaining six GRT containers were not reduced. Reduction of these flow rates would cause increased settling of the heavy SiC marker particles.

Tetraselmis suecica and SiC marker particles were delivered to the clams by pouring pre-determined amounts of each in to the header tank. Markers were introduced at a concentration and organic / inorganic ratio (by number rather than weight) similar to that of the *C. muelleri* / silicon dioxide diet. In order to maintain an approximately constant concentration of marker over the 30 min exposure period, markers were added in two equal amounts at 15 min intervals. Although there was dilution of the markers during each 15 min period, it was assumed that this would not significantly alter the precision of subsequent observations and analyses. The header tank was mixed manually during this time to keep as many SiC particles in suspension as possible.

After the second 15 min interval, the inflow line was reset to 4 l min^{-1} flow and 10 min later the lines to the AE and six GRT containers were unplugged, after ensuring that all markers were adequately flushed from the system. Flow was adjusted to maintain particle suspensions at their original values, and the bottom of each container was carefully siphoned clean of faeces, debris and settled marker without disturbing the clams.

Introduction of the marker was recorded as time zero, and experimental conditions were maintained for 24 h after this to ensure that conditions remained constant. Faeces samples were collected every 2 h for 12 h and again at 24 h to determine GRT. Particle concentrations in the water collected from the outflows were taken every 2 h for 10 h to calculate CR. Faeces samples were taken from each of the AE containers at 6 and 12 h to determine absorption efficiencies.

3.2.4. Experimental Design

Digestive processes of *M. arenaria* were tested in response to four different food suspensions, each consisting of *C. muelleri* and inert silicon dioxide particles. Three diets were mixed to 10 mg l^{-1} dry weight, at organic contents of 25%, 50% and 75% by weight. Comparison of these 3 diets tests the response of clams to changes in diet "quality". A fourth diet was mixed at 2 mg l^{-1} dry weight at an organic content of 50% by weight. When compared to the 10 mg l^{-1}, 50% organic content diet, this tests the clams' responses to two

different levels of diet "quantity". For simplicity, these food suspensions will be referred to from now on as the 10 mg 25%, 10 mg 50%, 10 mg 75% and 2 mg 50% diets. The actual particle concentration used to reach these seston loads are given in Table 3.1.

3.2.5. Clearance Rate and Ingestion Rate Measurements

Water was collected simultaneously for 90 s beneath each outflow standpipe to measure flow rates, and the number of particles with diameters between ca. 2 and 62 µm was determined in each sample with a Coulter Multisizer fitted with a 100 µm tube. Clearance rates were calculated for each individual using the formula:

$$CR = FR \frac{(C1 - C2)}{C1} \qquad \text{Equation 3.1}$$

where CR = clearance rate (l h^{-1}), FR = flow rate through the container (l h^{-1}), C1 = particles ml^{-1} in the inflow (measured by the Control container), and C2 = particles ml^{-1} in the outflow (Hildreth and Crisp 1976). Clearance rates were then standardized (CRs) for an individual with a dry soft tissue weight of 1.0 g using the relation:

$$CRs = (Ws / Wo)^b \times CR \qquad \text{Equation 3.2}$$

where Ws = standardized soft tissue dry weight, Wo = observed soft tissue dry weight, and b is a fitted parameters (MacDonald and Thompson 1986, Bayne et al. 1987). Since the weight exponent value (b) has not been determined for CR of *Mya arenaria*, a value of 0.68, that of *Placopecten magellanicus* in Newfoundland was arbitrarily chosen (MacDonald and Thompson 1986). This is a reasonable assumption, since values of b usually lie between 0.66 and 0.82 (Winter 1978, see also Bayne 1976, Griffiths and Griffiths 1987 for reviews).

Ingestion rate was calculated for each clam using the formula (Malouf and Bricelj 1989):

Table 3.1: Concentrations of *Chaetoceros muelleri* and silicon dioxide used to acheive the 4 experimental diet loads. Note that *C. muelleri* has approximately 72% organic content by weight.

Experimental Diet	C. muelleri (cells ml^{-1})	Silicon Dioxide (particles ml^{-1})
2 mg 50 %	2.074×10^4	2.704×10^3
10 mg 25%	5.225×10^4	2.519×10^4
10 mg 50%	1.037×10^5	1.352×10^4
10 mg 75%	1.633×10^5	---

$$IR = CR \times C \qquad \text{Equation 3.3}$$

where IR = ingestion rate of total material (mg h^{-1}), CR = clearance rate (l h^{-1}) and C = concentration of diet suspension (mg l^{-1}, see Table 3.1).

Ingestion rate was modified to IR_o using the formula:

$$IR_o = IR \times P \qquad \text{Equation 3.4}$$

where IR_o = ingestion rate of organic material (mg h^{-1}), IR = ingestion rate of total material (mg h^{-1}) and P = the mean proportion of organic material in the food (from Table 3.1). It should be noted that IR has not been corrected for pseudofaeces production. However, in all experiments little or no pseudofaeces was produced by the clams, and corrections in IR based on pseudofaeces production would have been negligible.

3.2.6. Gut Retention Time Measurements

Gut retention time analysis was divided into two components: analysis of the organic marker and analysis of the inorganic marker. For both estimates, faeces were collected with a pasteur pipette from the bottom of each experimental container and transferred into 1.5 ml Eppendorf disposable tubes. Whenever possible, all faecal material was collected. Seawater was removed, and samples were stored overnight at -20°C.

Organic Marker (O.M.):

Samples were thawed and analyzed for pigment content by the method outlined in section 2.2.1. (the pellet collected after extraction of pigments was stored frozen for analysis of the inorganic marker). Pigments were identified by comparisons with known standards. The amount of pigment present in the samples was then calculated by the area of each pigment peak. The amount of organic marker (O.M.) present in each sample was then calculated as:

$$\frac{(C_b + B_b)}{(C_c + B_c)} \qquad \text{Equation 3.5}$$

where C_b = chlorophyll *b*, B_b = breakdown products of chlorophyll *b*, C_c = chlorophyll *c*, and B_c = breakdown products of chlorophyll *c*. This formula standardizes the amount of b-type pigments (exclusive to *T. suecica* in this experimental design) as a ratio to the amount of c-type pigments (exclusive to *C. muelleri*), to correct for the variation in the weight of faecal samples. It should be noted that standardization by weight was not possible due to the confounding factor of the SiC marker particles, which were present in the faecal samples in varying amounts.

Inorganic Marker (I.M.):

For analysis of the SiC marker, the pellet collected after extraction of the pigments was covered with 3 drops of concentrated nitric acid, sonicated in an ice bath for 10 min, and left overnight to dissolve all organic matter. The suspended pellet was then centrifuged and the nitric acid removed. The pellet was washed once with distilled water, suspended in 1.5 ml of 1 µm filtered seawater, sonicated for 60 min, then added to 200 ml of 1 µm filtered seawater. This entire process did not alter the size distribution of the SiC particles. The size distribution of the suspension was then analyzed with a Coulter Multisizer. Approximately 4.5 ml of each sample was counted using a 100 µm tube. Presence of the SiC marker could be observed as an increase in the proportion of particles between 10 and 15 µm diameter in faeces sampled from clams exposed to the SiC marker particles. The relative amount of inorganic marker (I.M.) present in each faeces sample was calculated as:

$$I.M. = P_t - C_c \qquad \text{Equation 3.6}$$

where P_t = the proportion of particles between 10 and 15 µm in the treatment sample, C_c = the average proportion of particles between 10 and 15 µm in the control samples taken at the same time as the treatment sample.

3.2.7. Absorption Efficiency Measurements

Absorption efficiency was calculated by the method described by Conover (1966):

$$AE = \frac{(F-E)}{(1-E)F} \times 100 \qquad \text{Equation 3.7}$$

where F and E = the proportion of organics in the food and faeces respectively.

Faeces samples were collected from the bottom of each AE container at 6 and 12 h after delivery of the markers (no pseudofaeces were produced throughout all experiments). At least 5 water samples were collected throughout the course of each 48 h experiment. All samples were filtered through pre-ashed and weighed Whatman GF/C filters, rinsed with 3% ammonium formate and dried to constant weight at 80°C. Filters were then ashed at 450°C for 12 h, reweighed, and AE calculated for each sample.

3.2.8. Water Samples

Water samples were collected from the standpipe of the control container at frequent intervals to determine the weight and organic content of the food ration. Occasionally, seawater was also collected from the inflow line entering the header tank bucket (before mixing with the *C. muelleri* and silicon dioxide) to determine background seston levels. Water samples were filtered onto pre-ashed and pre-weighed Whatman GF/C filters, and dried at 80°C to constant weight. Ash weight was determined after combusting filters overnight at 450°C.

3.3 Results

3.3.1. Actual Diet Loads

The food rations delivered to the clams in each experiment are summarized in Table 3.2. Observed values for the dry weights (mg l^{-1}) are higher than those expected due to background levels of seston in the main seawater line. It was not possible to reduce background levels of seston by further filtering the seawater (to 1 or 10 µm), because this caused too great a reduction in the seawater inflow rate.

Table 3.2: Actual diet loads delivered to the experimental apparatus for each laboratory experiment.

Experiment	Dry Weight mg/l		% Organic Weight		n
	Mean	S.D.	Mean	S.D.	
10 mg 50%	15.4	1.183	48.2	4.119	5
10 mg 25%	13.7	4.576	23.5	3.603	7
2 mg 50%	5.5	1.885	43.9	15.286	5
10 mg 75%	16.6	5.542	61.7	7.099	5

Multiple comparison of means by Fisher PLSD and Sheffe-F tests were used to determine significant differences between the particulate dry weights and the percentage of organics in each food suspension (Table 3.3). There was no significant difference in the dry weights of the three 10 mg diets (10 mg 25%, 10 mg 50% and 10 mg 75%). However, the relative organic contents were significantly different between these three. Dry weights of the 10 mg 50% and 2 mg 50% diets were significantly different, but the relative organic contents of the two were not.

It was not possible to measure the actual concentration of the food suspension in the acclimation tray. Two methods were tried: 1) removing a small (20 ml) water sample from the tray, and 2) collecting a sample from the end of the seawater inflow line with lines from the food suspension stock buckets attached. The first method was unsuccessful because faecal material was invariably collected with the water sample, clogging the Coulter Multisizer aperture and affecting particle counts. The second method was also unsuccessful because in transferring the ends of the seawater and food lines from the acclimation tray to a water collection vial, the height differential between the header beaker and the ends of the lines would be shortened, thereby reducing gravitational flow and changing the final concentration of particles. Therefore, there is no information on the actual particle load delivered to the clams in the acclimation tray. However, visual observations showed clear differences in the particle loads of the four experiments (overall particle concentrations as well as the proportion of *C. muelleri* to silicon dioxide), and it is reasonable to assume that the diet delivered to the clams in the acclimation tray was comparable with the diet delivered in the experimental apparatus.

3.3.2. Use of Transformations and Non-Parametric Statistics

Continuous variables to be tested by ANOVA (CR, IR, IR_n, AE and GRT) were examined for normality and homogeneity of variance (Sokal and Rohlf 1969) by use of a Shapiro-Wilks test and F-Max test respectively. In all cases, at least one of these two tests gave significant p-values (meaning the data were unsuitable for this parametric statistic), so data were transformed (log [x+1]) and retested. Again, all cases yielded significant p-values, so non-parametric statistics are used throughout most of this study.

Table 3.3: Multiple comparison of means of particulate dry weights and percentages of organic content in each food suspension. Tests were made using a Fisher PLSD and Sheffe F-tests. An asterisk * indicates significance at $\alpha = 0.05$.

Diets Compared	Dry Weights		% Organics	
	Fisher	Sheffe	Fisher	Sheffe
10mg25% vs 10mg50%	5	0.20	10 *	8 *
10mg25% vs 10mg75%	5	1.00	10 *	20 *
10mg50% vs 10mg75%	5	0.09	11 *	2
10mg50% vs 2mg50%	5 *	6 *	11	0.2

3.3.3. Clearance Rate and Relation to Diet

Mean CR (standardized for 1 g soft tissue dry weight) was relatively constant over the 10 h of measurements in each experiment (Fig. 3.1) with the exception of the hour-10 measurement for the 10 mg 25% diet, at which point the CR dropped. This drop in CR was caused by either partial or complete closure of the siphon apertures in most clams. This may have been in response to the algae culture, which was starting to deteriorate in quality at this time (visible as a change in colour, clumping of the algal cells, and formation of a sticky film on the culture surface). From Fig. 3.1, it is evident that CR was affected by the diet on which the clams were feeding. This relation is illustrated more clearly with data for each experiment pooled in Fig. 3.2, showing that an increase in the proportion of organic content (= increased 'quality') resulted in a significant decrease in CR (Fig. 3.2 A, Kruskal-Wallis, H=16, df=2, p=0.0003), as did also an increase in food quantity (Fig.3.2 B, Wilcoxon Rank test, Z=2.981, n_2=12, p=0.003).

3.3.4. Ingestion Rate and Relation to Diet

Increases in both the quantity and quality of the food resulted in a decrease in IR (Fig. 3.3). This trend was significant for both differences in quality (Kruskal-Wallis, H=15, df=2, p=0.0005) and quantity (Wilcoxon Ranks, Z=2.824, n_2=12, p=0.005) of the food suspension delivered.

3.3.5. Ingestion Rate of Organic Material and Relation to Diet

Change in food quality did not significantly affect IR_o (Fig. 3.4 A, Kruskal-Wallis, H=15, df=2, p=0.933). There was also no significant difference in IR_o between the two diets of differing quantity (Fig. 3.4 B, Wilcoxon Rank, Z=-1.647, df=12, p=0.099).

3.3.6. Absorption Efficiency and Relation to Diet

Neither quality or quality of food significantly affected AE in *M. arenaria* (Fig. 3.5 A, Kruskal-Wallis, H=2, df=2, p=0.289, Fig. 3.5 B, Wilcoxon Ranks, Z=0.535, n_2=3, p=0.593).

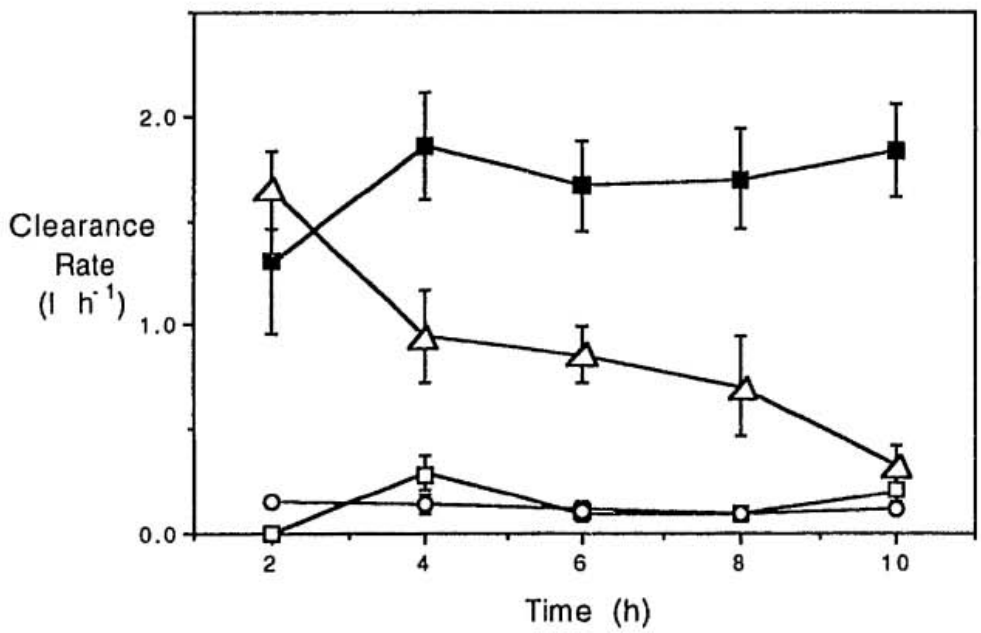

Fig. 3.1: Clearance rate of *M. arenaria* feeding on 4 different food suspensions over a 10 hour period: —○— 10 mg / l, 75% organics; —□— 10 mg / l, 50% organics; —△— 10 mg / l, 25 % organics; —■— 2 mg / l, 50% organics. Vertical bars are standard error of the means and n = 12 for each point.

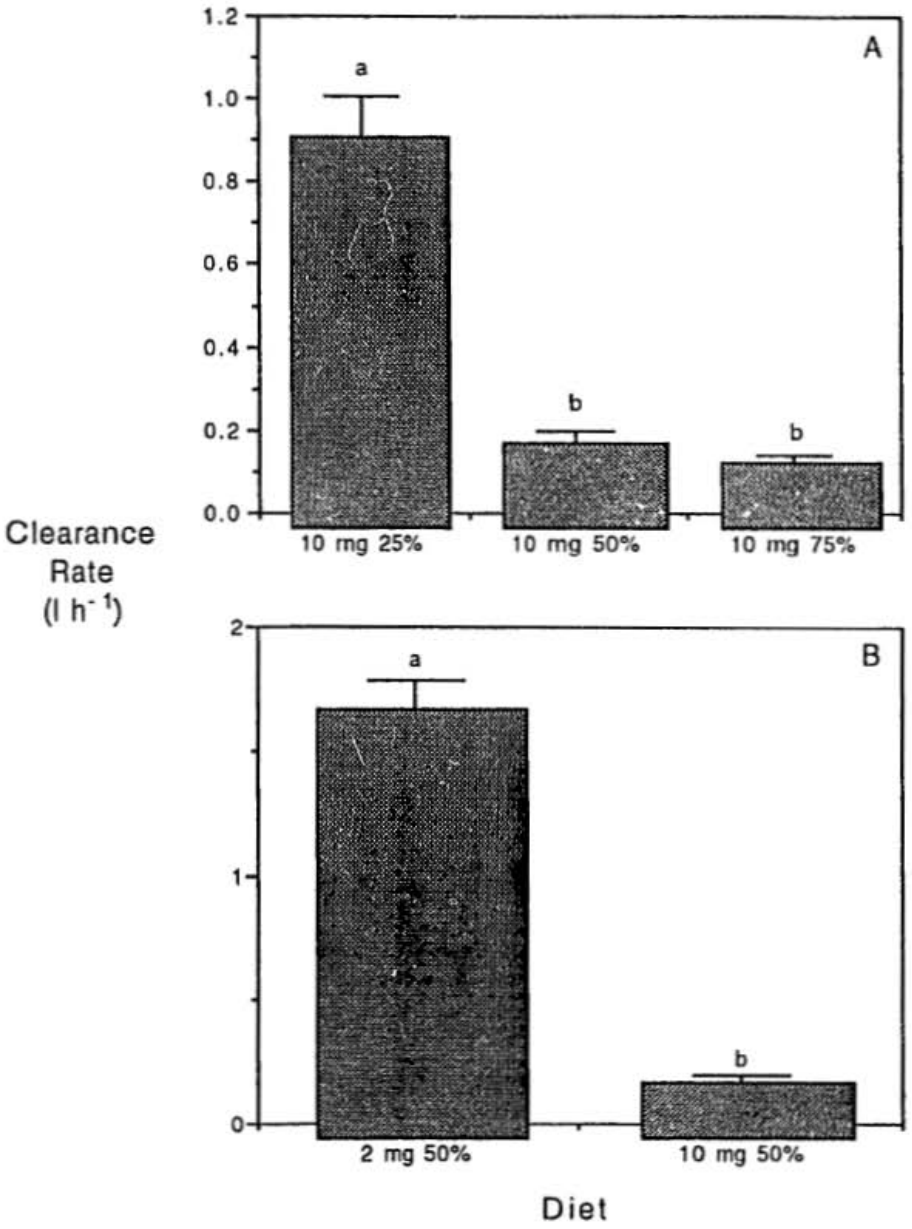

Fig. 3.2: Clearance rate of *M. arenaria* in relation to the quality (A) and quantity (B) of food in suspension. Error bars are the standard error of the mean, n = 60 for each. Different letters above each bar indicate significant difference at alpha = 0.05, STD test.

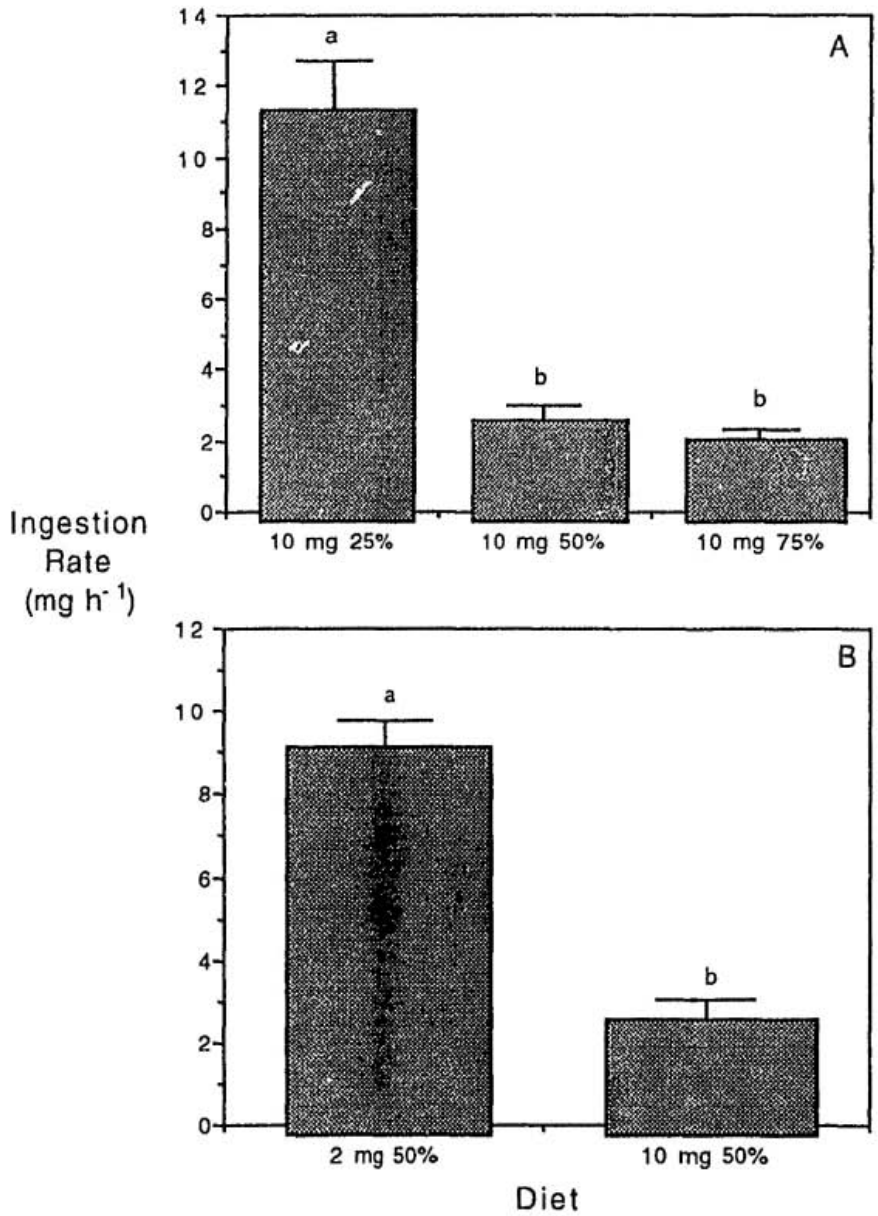

Fig. 3.3: Ingestion rate of *M. arenaria* in relation to the quality (A) and quantity (B) of food in suspension. Error bars represent the standard error of the mean, and n = 60 for each. Different letters above the bars indicate significant difference at alpha = 0.05, STD test.

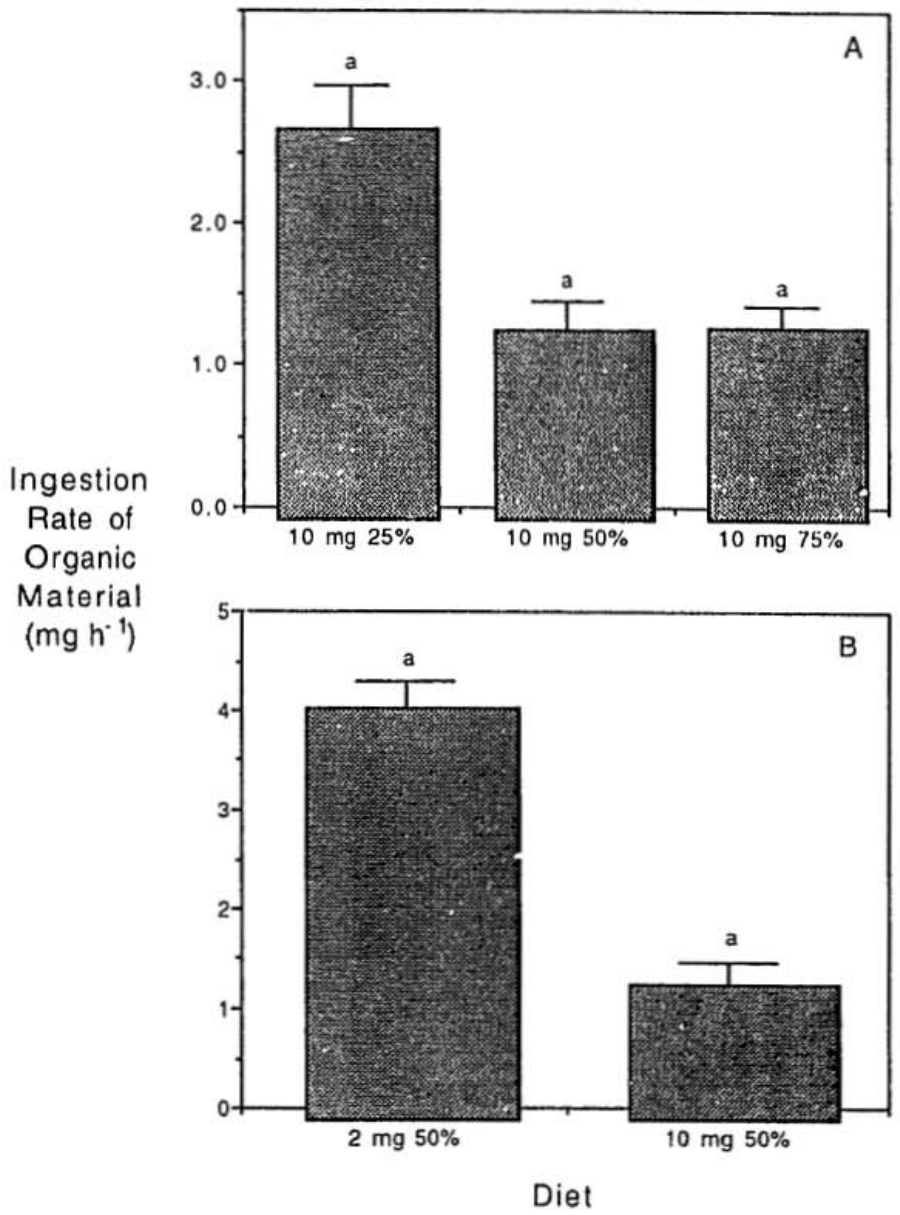

Fig 3.4: Rate of ingestion of organic material of *M. arenaria* in relation to the quality (A) and quantity (B) of food in suspension. Error bars are the standard error of the mean, and n = 60 for each diet. Different letters above the bars indicate significant difference at alpha = 0.05, STD test.

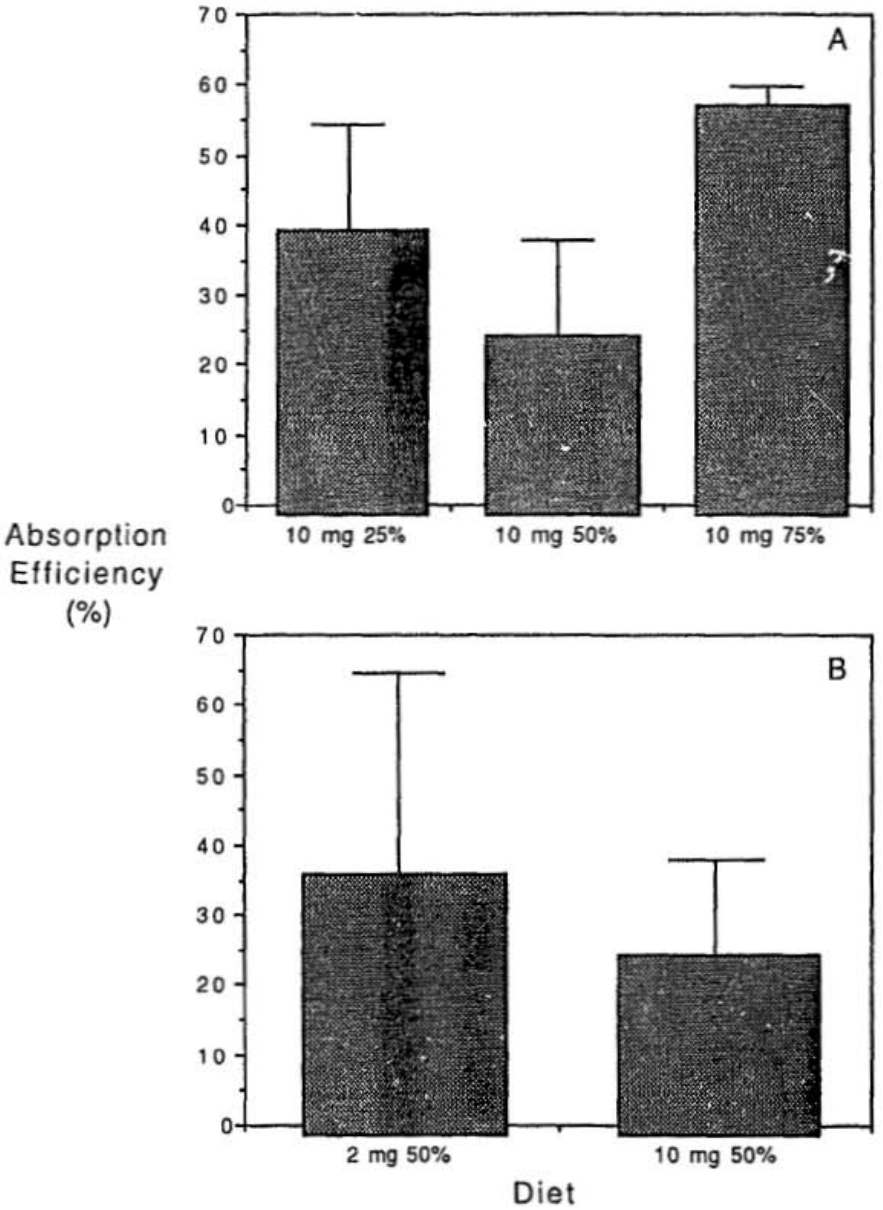

Fig. 3.5: Absorption efficiency of *M. arenaria* in relation to the quality (A) and quantity (B) of food in suspension. Error bars represent the standard error of the mean, and n = 6 for each diet. There was no significant difference between means of any diets at alpha = 0.05, STD test.

Three of the four diets had one significant outlier in the AE data, contributing to very high variance values. However, removal of these outliers does not affect any of the conclusions drawn above.

3.3.7. Gut Retention Time and Relation to Diet

The criteria to determine GRT is given in Appendix D, which is to use the time interval containing the median (50% of cumulative) O.M. or I.M. value as a measure of GRT (Nobel 1973, Mills and Fourney 1981, Cochran and Adelman 1982, Rice et al. 1983). It was not necessary to correct the GRT data set for size of clam (Hawkins et al. 1990) because plots of GRT vs dry soft-body mass (Fig. 3.6) showed no correlations. This indicates that the size range of clams used in these experiments was small enough to eliminate any variations in GRT resulting from body size.

The effect of quality of food on GRT of the organic fraction was significant (Fig. 3.7 A, Kruskal-Wallis, H=11.89, df=2, p=0.003), with GRT increasing above the 50% organic content level. Although the effect of food quality on the GRT of inorganics was not significant (Kruskal-Wallis, H=1.563, df=2, p=0.458), it did follow a similar increasing trend. In contrast, the GRT of organics decreased with increased quantity of food (Fig. 3.7 B, Wilcoxon Ranks, Z=2.264, n_2=6, p=0.024). The effect of quantity on the inorganic GRT followed the same trend but was again not significant (Wilcoxon Ranks, Z=.730, n_2=5, p=0.078). There were no significant differences between the mean GRT of organic and inorganic fractions for each diet (Table 3.4).

GRT of both the organic and inorganic fractions were further found to be inversely proportional to the of the amount of SiO2 in suspension (Simple Regression: R = 0.715, F = 23.002, df = 1/22, p = 0.0001 and R = 0.472, F = 6.013, df = 1/21, p = 0.023 for GRT of organic and inorganic fractions respectively; Spearman Correlation: Z = -3.979, N = 24, p = 0.0001 and Z = -2.059, N = 23, p = 0.0395 for GRT of organic and inorganic fractions respectively).

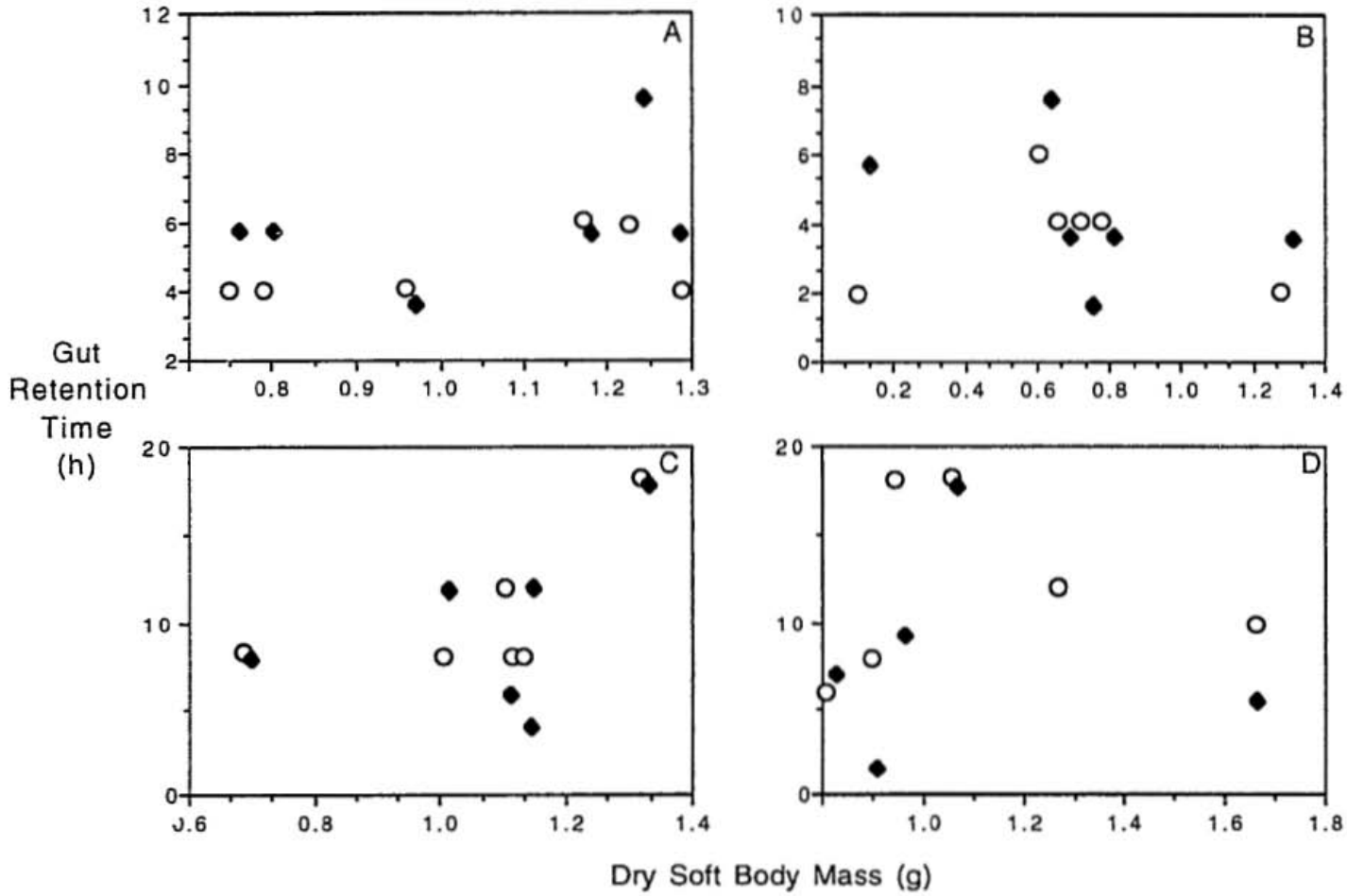

Fig. 3.6: Gut retention time vs dry soft body mass for clams feeding on 10 mg 50% (A), 10 mg 25% (B), 2 mg 50% (C) and 10 mg 75% (D) diets. There are no obvious allometric trends. O = GRT of organic fraction, ◆ = GRT of inorganic fraction.

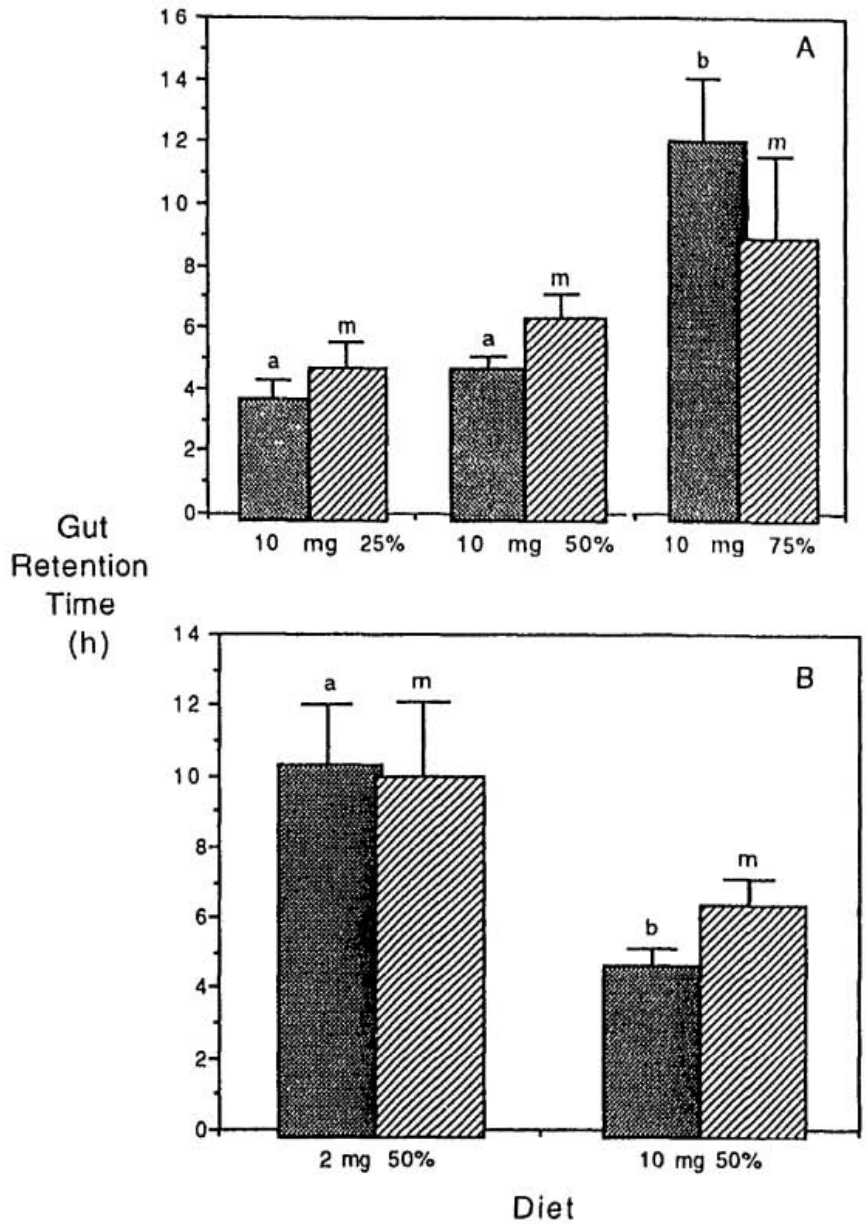

Fig. 3.7: Gut retention time of organic (solid bars) and inorganic (striped bars) dietary fractions in *M. arenaria* in response to differing quality (A) and quantity (B) of food in suspension. Error bars indicate the standard error of the means, and n = 6 for each diet. Different letters above bars indicate significant difference at alpha = 0.05, Wilcoxon Rank test. Note: there is no significant difference between organic and inorganic gut retention times within diets at alpha = 0.05, Wilcoxon Rank test.

Table 3.4: Values from Wilcoxon rank test (Z) and associated two-sided p-values (P) of gut retention time of organic vs inorganic fractions for *M. arenaria* feeding on each diet..

	10 mg 50%	10 mg 25%	2 mg 50%	10 mg 75%
Z	1.890	1.134	-0.378	-0.542
P	0.059	0.257	0.705	0.588

3.3.8. Summary of the Effects of Changes in Diet

A summary of the effects of changes in food, both in quantity and in quality, are given in Table 3.5. As the quantity of the food increases, CR, IR, IR_o and GRT decrease. All of these trends are significant at $\alpha = 0.05$ with the exception of the GRT of inorganic particles. There is no significant change in AE. As the quality of the food increases, CR, IR and IR_o decrease. All but the change in IR_o are significant at $\alpha = 0.05$. Gut retention time increases (for the organic component only) and there is no significant change in AE.

3.3.9. Relation between Gut Retention Time and Clearance and Ingestion Rates

The relationship between CR and GRT was assessed by two correlations (Fig. 3.8): one including the 10 mg 50% and 2 mg 50% data sets (changing quantity) and one including the three 10 mg data sets (changing quality). There was a significant inverse correlation between both the GRT of organic and inorganic fractions and the CR for the three 10 mg data sets ($Z = -2.965$, $N = 18$, $p = 0.003$ and $Z = -2.2026$, $N = 17$ and $p = 0.043$ respectively). Although there is no clear trend in the 10 mg 50% and 2 mg 50% data sets, the Spearman correlation coefficient indicated a significant direct correlation between GRT of the organic fraction and CR ($Z = 2.156$, $N = 12$, $p = 0.031$). This was not significant for the inorganic fraction ($Z = 0.579$, $N = 12$, $p = 0.56$).

Correlations between IR and GRT follow similar trends as those listed above for CR. There was a significant inverse correlation between both the GRT of organic and inorganic fractions and the IR for the three 10 mg data sets (Spearman correlation, $Z = -2.965$, $N = 18$, $p = 0.003$ and $Z = -2.026$, $N = 17$, $p = 0.0427$ respectively). There were no significant trends for GRT vs IR in the 10 mg 50% and 2 mg 50% data sets (Spearman correlation, $Z = 1.205$, $N = 12$, $p = 0.228$ and $Z = -0.374$, $N = 12$, $p = 0.703$ respectively).

3.3.10. Relation Between Clearance / Ingestion Rate and Absorption Efficiencies

Correlations of AE on both CR or IR were highly insignificant (Fig. 3.9, $Z = -0.163$, $N = 12$, $p = 0.87$ and $Z = 0.139$, $N = 12$, $p = 0.89$ respectively), contrary to suggestions by other studies (e.g. Calow 1975, Widdows 1978).

Table 3.5: Summary of the effect of change in quantity and quality of diet on clearance rates, ingestion rate, ingestion rate of organic material, gut retention time of organic and inorganic fractions, and absorption efficiency of *M. arenaria*. An asterisk * indicates significance at $\alpha = 0.05$, check previous sections for more detail.

	Increase Quantity	Increase Quality
Clearance Rate	decrease *	decrease *
Ingestion Rate	decrease *	decrease *
Ingestion Rate of Organics	decrease	decrease
GRT of Organics	decrease *	increase *
GRT of Inorganics	decrease	increase
Absorption Efficiency	no change	no change

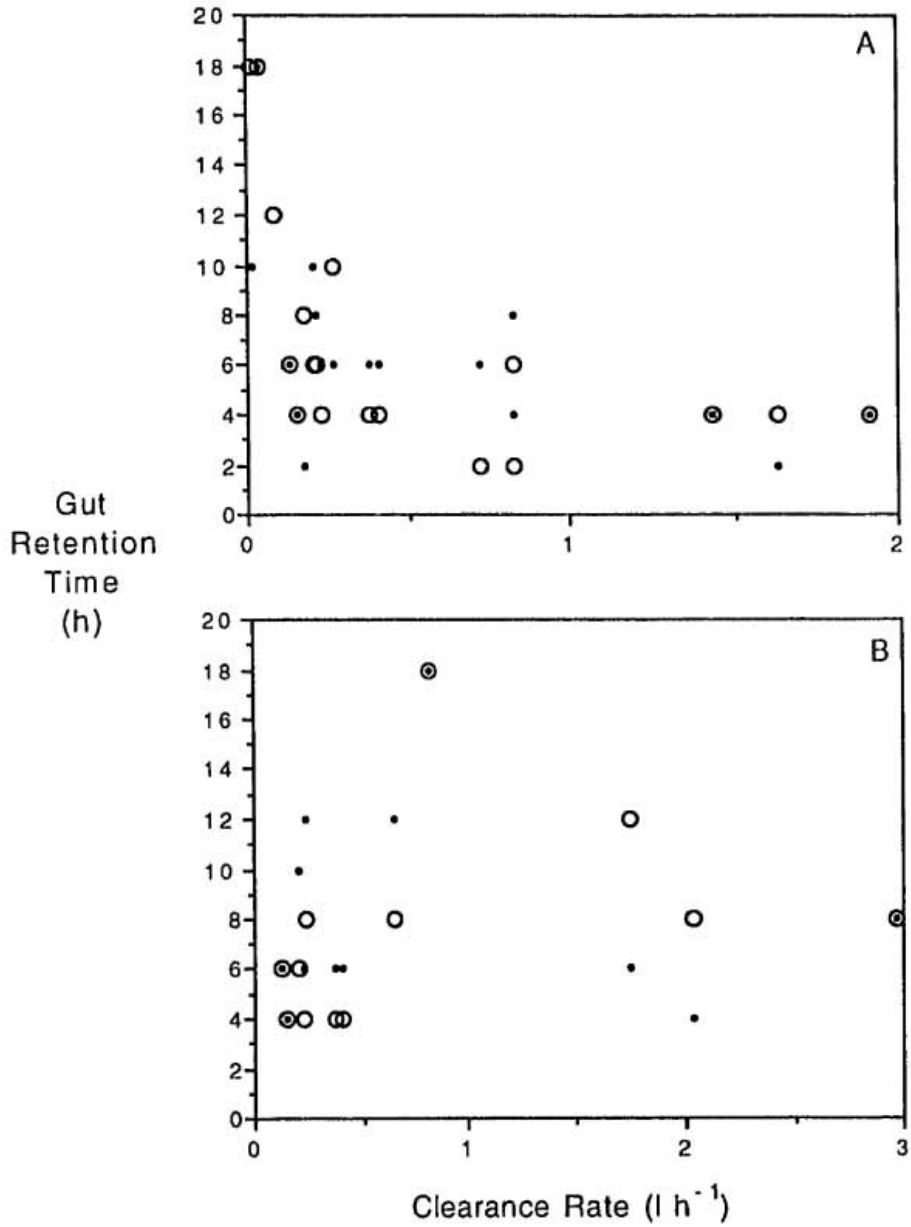

Fig. 3.8: Gut retention time of *M. arenaria* vs clearance rates for change in food quality (A) and quantity (B) data sets. Open circles represent the gut retention time of the organic fraction, solid circles represent the gut retention time of the inorganic fraction. Spearman correlation coefficients between gut retention times and clearance rates, and significance levels are given in the text.

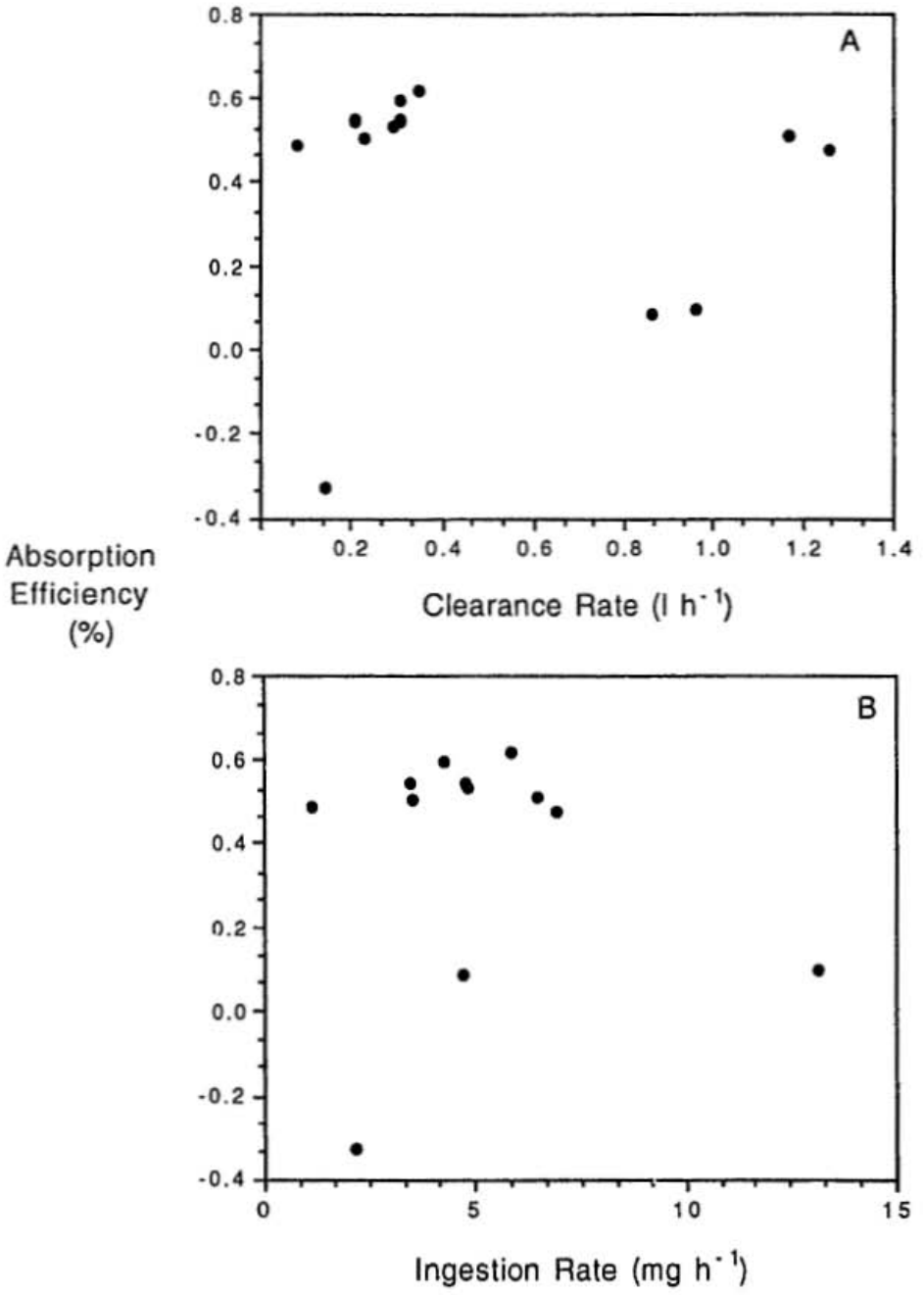

Fig. 3.9: Clearance rate (A) and ingestion rate (B) vs absorption efficiency of *M. arenaria*. There are no significant correlations.

3.3.11. Selection of Particles Within the Gut

Although there were no significant differences in the mean GRT of organic and inorganic fractions (Table 3.4), examination of the marker profile graphs for individual clams (see Appendix D, Fig. D.4 for an example of 2 individuals) clearly show that some clams were preferentially retaining one fraction over the other. The clams can therefore be subdivided *a posteriori* into three categories: those preferentially retaining the organic fraction longer, those retaining the inorganic fraction longer, and those showing no preferential retention. Clams were assigned to a category based on a comparison of their organic and inorganic GRT (="gut selection category"). Table 3.6 gives the frequency of each gut selection category for each diet. In all diets, 33.3% of the clams did not show any selection within the gut. Of those which did show selection within the gut, there is no apparent diet-based pattern to their type of selection.

To determine if the differences between the mean GRT of each gut selection categories were significant, a multiple comparison of means test was performed. The mean GRT of inorganic particles of each category were not significantly different (Wilcoxon Rank, α = 0.05, Table 3.7), however, the mean GRT of organic particles of the "inorganic" and "organic" gut selection categories were statistically significant at α = 0.05 (Wilcoxon Ranks, Table 3.7) and also between the "inorganic" and "none" gut selection categories at α = 0.10 (Wilcoxon Ranks, Table 3.7). This information is given graphically in Fig. 3.10.

If certain clams are indeed retaining one type of particle significantly longer within the gut, this could be indicative of an overall digestion strategy. Accordingly, CR and IR_o of the clams were compared based on their groupings by gut selection category. Multiple comparison of means by Wilcoxon ranks showed no significant differences in both CR and IR_o based on gut selection (Table 3.8). This analysis was repeated using only the data from the 10 mg diets (quality change data set) and data from the 2 mg 50% and 10 mg 50% diets (quantity change data set). There were no significant differences in CR and IR_o in either case (Tables 3.9 and 3.10).

Table 3.6: The following table summarizes the instances of preferential retention of particles within the gut (= gut selection category) of *M. arenaria*, sorted by diet. N = 6 for each diet, and determination of preferential retention is based on a comparison of GRT of organic and inorganic material for each individual.

	Gut Selection Category		
Diet	Organic	Inorganic	None
10 mg 25%	16.7%	50%	33.3%
10 mg 50%	0%	67.7%	33.3%
10 mg 75%	50%	16.7%	33.3%
2 mg 50%	33.3%	33.3%	33.3%

Table 3.7: Values from Wilcoxon rank test (Z) and associated two-sided p-values (P) for gut retention time of organic material (GRTo) and inorganic material (GRTi) comparisons among gut selection categories, for *M. arenaria*.

			Gut Selection Categories		
			Inorganic	Organic	None
Inorganic	GRTo	Z	0.000		
		P	1.000		
	GRTi	Z	0.000		
		P	1.000		
Organic	GRTo	Z	2.207	0.000	
		P	0.027	1.000	
	GRTi	Z	-0.089	0.000	
		P	0.276	1.000	
None	GRTo	Z	1.897	-0.271	0.000
		P	0.058	0.786	1.000
	GRTi	Z	0.813	1.051	0.000
		P	0.416	0.293	1.000

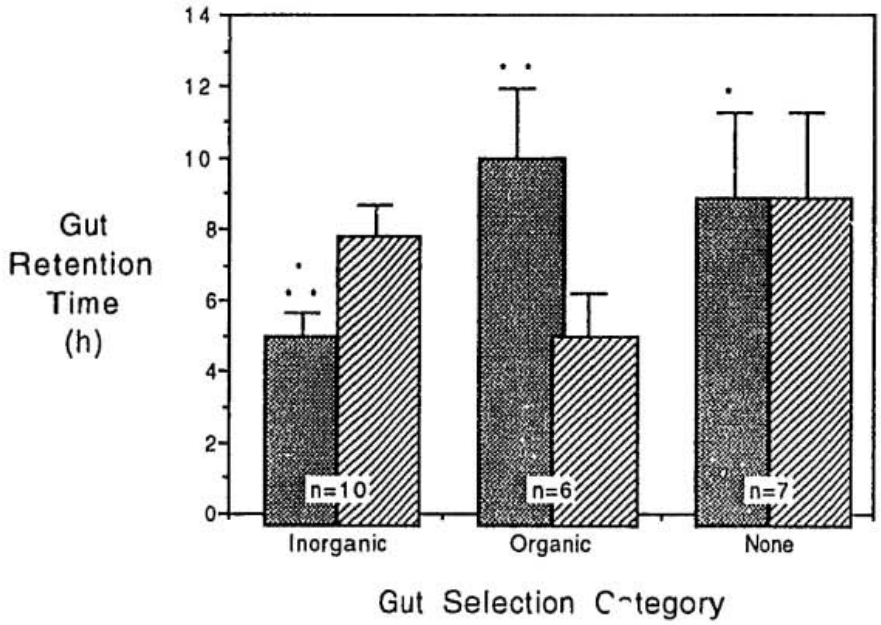

Fig. 3.10: Mean gut retention time of organic (solid bars) and inorganic (striped bars) particles in *M. arenaria* adopting one of three different gut selection strategies: preferential retention of inorganic particles, organic particles, or no selection. Error bars are standard error of the mean. * and ** above two bars indicates significant difference at alpha = 0.05 and 0.1 respectively, Wilcoxon Ranks test.

Table 3.8: Values from Wilcoxon rank test (Z) and associated two-sided p-values (P) for clearance rate (CR) and ingestion rate of organic material (IR_o) comparisons among gut selection categories, for *M. arenaria*.

			Gut Selection Categories		
			Inorganic	Organic	None
Inorganic	CR	Z	0.000		
		P	1.000		
	IR_o	Z	0.000		
		P	1.000		
Organic	CR	Z	0.943	0.000	
		P	0.345	1.000	
	IR_o	Z	0.524	0.000	
		P	0.600	1.000	
None	CR	Z	1.014	-0.105	0.000
		P	0.310	0.917	1.000
	IR_o	Z	0.845	-0.314	0.000
		P	0.398	0.753	1.000

Table 3.9: Values from Wilcoxon rank test (Z) and associated two-sided p-values (P) for clearance rate (CR) and ingestion rate of organic material (IR_o) comparisons among gut selection categories, 10 mg diets only (quality change data set), for *M. arenaria*.

			Gut Selection Categories		
			Inorganic	Organic	None
Inorganic	CR	Z	0.000		
		P	1.000		
	IR_o	Z	0.000		
		P	1.000		
Organic	CR	Z	-0.365	0.000	
		P	0.715	1.000	
	IR_o	Z	-0.730	0.000	
		P	0.465	1.000	
None	CR	Z	0.135	1.461	0.000
		P	0.893	0.144	1.000
	IR_o	Z	0.405	1.461	0.000
		P	0.686	0.144	1.000

Table 3.10: Values from Wilcoxon rank test (Z) and associated two-sided p-values (P) for clearance rate (CR) and ingestion rate of organic material (IR_o) comparisons among gut selection categories, 10 mg 50% and 2 mg 50% diets only (quantity change data set), for *M. arenaria*.

			Gut Selection Categories		
			Inorganic	Organic	None
Inorganic	CR	Z	0.000		
		P	1.000		
	IR_o	Z	0.000		
		P	1.000		
Organic	CR	Z	-1.342	0.000	
		P	0.180	1.000	
	IR_o	Z	1.342	0.000	
		P	0.180	1.000	
None	CR	Z	1.069	-1.000	0.000
		P	0.285	0.317	1.000
	IR_o	Z	0.535	-1.000	0.000
		P	0.593	0.317	1.000

3.4. Discussion

3.4.1. Effect of Variation in Nutritional Value of Individual Diets

There was considerable variation in the dry weight (mg l^{-1}) of the food suspensions and in the proportion of organic material in the food suspensions throughout the course of each experiment. This can be attributed to several factors. First, changing wind and wave conditions in Logy Bay frequently caused changes in the background particle counts of the seawater, as well as changes in the flow rate of the inflow seawater. Particle concentrations of the food suspension also varied as a result of settling of silicon dioxide particles in the stock suspension and throughout the experimental apparatus, as well as changes in concentration in the stock suspension of the algae, due to either continued growth or aging of the culture.

However, *M. arenaria* typically inhabits intertidal and estuarine environments (Hanks 1963, Hidu and Newell 1989), which are noted for having both temporal and spatial variability in the concentration and composition of suspended seston (Langdon and Newell 1990, Iglesias et al. 1992). Therefore, species such as *M. arenaria* should be adapted to cope with a fluctuating food supply, and the variation in food concentrations in each of the experiments in this study should not have adversely affected the clams.

Despite variability in the particle loads delivered to the animals within each experiment, all diets were significantly different from one another based on both total dry weight per litre and proportion of organic particles. Although some studies have recorded much higher particle loads above intertidal mud flats, such as between 200 - 500 mg l^{-1} (Hargrave et al. 1983) and as much as 3000 mg l^{-1} (Grant and Thorpe 1991), the values used in this study are well within the limits which could be expected in field situations (see Chapter IV).

3.4.2. Clearance Rates of *M. arenaria* and Relation to Diet

Clearance rates were determined using particles of 2 to 62 µm diameter only. However, this is probably a good estimate of the CR of all particles in the given food suspension. *Mya arenaria* retains particles of 4 µm in diameter with 100% efficiency, and those of 2 µm diameter with less than 25% efficiency. *Mya arenaria* does not clear bacteria from suspension (Wright et al. 1982). Furthermore, there was very little material in suspension above about 30 µm, and the seawater line was filtered to 100 µm.

Winter (1969) reported an average CR of 0.32 l h^{-1} for *M. arenaria* feeding on 40000 cells ml^{-1} *Chlamydomonas* sp. at 12°C. When standardized for a 1 g (dry tissue weight) animal with the same weight exponent used in the present study, this value becomes 0.394 l h^{-1} g^{-1}. This is greater than the average CR for clams feeding on pure *C. muelleri* algae recorded in the present study (0.123 l h^{-1} g^{-1}), but clams in the present study were grazing on a much higher concentration of approximately 150,000 cells ml^{-1}.

Wright et al. (1982) measured a CR of 0.43 l h^{-1} g^{-1} for *M. arenaria* feeding on 15 mg l^{-1} of semi-colloidal graphite particles. This is slightly lower that the CR value of 0.828 l h^{-1} g^{-1} measured in this study for clams feeding on 13.7 mg l^{-1} at 23.5% organic content, however the particles used in Wright's (1982) study were generally smaller (1-2.5 µm diameter compared to 0.5 - 15 µm used here) and therefore were likely retained with less efficiency.

Jørgensen and Riisgård (1988) recorded CR values of 0.7 - 3.0 l h^{-1} g^{-1} for *M. arenaria* feeding on *Dunaliella marina*. Allen (1962) measured CR of 0.8 l h^{-1} g^{-1} for *M. arenaria* feeding on *Phaeodactylum* at 17 - 18°C. Both are well within the range measured in this thesis study. However, neither of these studies gives the concentration of algae used, nor a description of how weight standardization was accomplished, and both studies use a different method for determining CR.

The values of CR recorded in this study are generally low in comparison to values reported for other species (see review by Malouf and Bricelj 1989). However, previous studies have frequently noted that *M. arenaria* tends to have a lower CR than other species of bivalves (Hughes 1969, Winter 1969). It has been suggested that infaunal species of

bivalves have a lower CR than epifaunal bivalves, perhaps as an adaptation to their more turbid environment (Allen 1962, Hughes 1969, Winter 1969, Malouf and Bricelj 1989).

The CR of individual clams fluctuated widely during the 10 h measurement period. This variability has been observed previously in *M. arenaria* (Jørgensen and Riisgård 1988), who attributed it to mechanical disturbance. *Mya arenaria* is extremely sensitive to mechanical stimuli and can respond by reducing valve gape, retracting the mantle, and withdrawing siphons slightly. Jørgensen and Riisgård (1988) noted that this response was more pronounced in clams held in aquaria without sediment, such as in this study. The CR of undisturbed clams averaged 3.0 l h^{-1} g^{-1} whereas the CR of clams in which valve gape had been reduced and the siphons and mantle were retracted averaged only 0.7 l h^{-1} g^{-1} (Jørgensen and Riisgård 1988). The fluctuations in CR observed in this thesis may also have been a means of regulating intake of food: when exposed to high concentrations of suspensions, *M. arenaria* pumps only intermittently causing IR to decline (Foster-Smith 1976b). Intermittent pumping has also been observed in continuously submerged *Crassostrea virginica* with no apparent correlation to tidal or diurnal rhythm (Epifanio and Ewart 1977).

Production of pseudofaeces was negligible in all experiments. This agrees with other studies: *M. arenaria* has a higher threshold for pseudofaecal production than other bivalves (Griffiths and Griffiths 1987), specifically measured at above 3.7 x 10^5 cells ml^{-1} (Shumway et al. 1985), 10 - 20 mg l^{-1} (Kiørboe and Möhlenberg 1981) and 100 - 119 mg l^{-1} (Grant and Thorpe 1991), all similar to or above the range used in the present study.

This study found that *M. arenaria* compensates for an increased concentration of both total and organic particulate matter by decreasing the rate of particle clearance. Grant and Thorpe (1991) found that *M. arenaria* reduces respiration rate when turbidity is high, and suggested that this may be analogous to the decreases in CR with increasing particle concentration observed in other species. This inverse relationship between CR and particle concentration is well documented in a variety of species including *Arctica islandica* (Winter 1969), *Mercenaria mercenaria* (Malouf and Bricelj 1989), *Cerastodema edule* and *Venerupis pullastra* (Foster-Smith 1975a), and *M. edulis* (Widdows et al. 1979) among others (see review papers by Bayne and Newell 1983, Griffiths and Griffiths 1987). However, this inverse relationship does not hold true for all studies: CR has also been

found to 1) increase with and 2) be independent of increasing particle concentration (see review paper by Griffiths and Griffiths 1987). These inconsistencies can be reconciliated with a 3-phase model of clearance rate response to increasing particle concentration (Winter 1978, Hummel 1985, see review by Bayne and Newell, 1983). This model suggests that bivalves first increase clearance rate in response to a particle concentration exceeding some low threshold level. Then, over optimum feeding levels, clearance rate remains relatively independent of food concentration. At concentrations above this "plateau region", there is a progressive decline in clearance rates as food concentration increases. If *M. arenaria* conforms to this three-phase model, then under the experimental conditions used in this study, the "plateau region" would occur at particle loads less than or equal to approximately 5.5 mg l^{-1}, the lowest particle concentration used in this study.

In this study, *M. arenaria* also increased CR in response to increased inorganic content of the diet. This is the opposite of what has been predicted and observed in some studies of suspension-feeding bivalves (Foster-Smith 1975a, Widdows et al. 1979, Newell 1981, Taghon 1981, Bricelj and Malouf 1984). Low levels of particulate inorganic matter may enhance feeding in *M. edulis* (Kiørboe et al. 1981). Since *M. arenaria* is an infaunal species inhabiting mud and sand flats, it may tolerate prolonged high levels of particulate inorganic matter better than *M. edulis*, which is an epifaunal species. Therefore, the enhanced feeding observed in *M. edulis* at low levels of particulate inorganic matter may be analogous to the enhanced feeding by *M. arenaria* observed here.

Although *M. arenaria* does not display the same relationship between CR and food quality as other suspension-feeding bivalves, the relationship is as predicted and observed for deposit-feeders (e.g. Gordon 1966, Conover 1978, Cammen 1980, see review by Lopez and Levinton 1987). *Mya arenaria* has been described as both a suspension- and deposit-feeder, since the position of its siphons at the sediment surface results in a great deal of sediment being ingested (Lopez and Levinton 1987). This type of dual feeding behaviour has also been described for other bivalves such as *Scrobicularia plana* (Earl 1975), *Tellina fabula* (Salzwedel 1979), and *Macoma balthica* (Hummel 1985). Deposit feeders are believed to compensate for living on a food source consisting of mostly inorganic material by processing large volumes of sediment (see review by Lopez and Levinton 1987). If this is achieved by increasing CR, then the increase in CR of *M.*

arenaria in response to increasing inorganic load observed in this study may be a deposit-feeding, as opposed to a suspension-feeding, response.

There is no difference in IR_o between diets of differing quantity or quality. Thus, *M. arenaria* appears to adjust CR to keep the intake of organic matter constant. Given their highly variable natural environment, animals capable of maintaining a constant amount of organic matter within the gut, irrespective of the "dilution" effect of inorganic matter (Widdows et al. 1979), may be at an advantage.

3.4.3. Absorption Efficiency of *M. arenaria* and Relation to Diet

Absorption efficiency is generally believed to be independent of body size (see Griffiths and Griffiths 1987), so for this study AE was not corrected for the size of clam. The AE of *M. arenaria* was generally around 50%, which is well within the range reported for other species (see review by Bayne and Newell 1983). It should be noted that the AE of one individual feeding on the 10 mg 50% diet was -33%, indicating possible "metabolic faecal loss" (Hawkins and Bayne 1985) in that individual.

Absorption efficiency did not vary significantly with the quantity of food in suspension. This agrees with observations on *Arctica islandica*, *Modiolus modiolus* (Winter, 1969) and *M. edulis* (Bayne et al. 1989). However, other studies have found a decrease in the efficiency of digestion and absorption with increasing food concentration in species such as *M. edulis* (Thompson and Bayne 1974, Foster-Smith 1975a), *Aulocomya ater* (Griffiths and King 1979), *Choromytilus meridionalis* (Griffiths 1980), and *Spisula subtruncata* (Möhlenberg and Kiørboe 1981).

Absorption efficiency was also independent of the quality of the food suspension. This is supported by Bricelj (1984), who found that addition of ashed silt to diets of *Pseudoisochrysis paradoxa* had no effect on AE of the hard clam *Mercenaria mercenaria*. Foster-Smith (1975b) found that the addition of alumina particles to suspensions of *Phaeodactylum* did not affect AE of *M. edulis*, *Cerastoderma edule*, and *Venerupis pullastra*. However, these three species were also capable of eliminating the alumina from the gut more rapidly than the algal fraction.

Other studies have found that AE decreases with increased concentration of particulate inorganic material (Vahl 1980, Hawkins et al. 1986, Bayne et al. 1987, see Bayne and Newell 1983 for a review). This is usually explained by the "dilution effect" of organics by increased inorganic material in the gut (Widdows et al. 1979), specifically in species which do not selectively reject inorganic material on the labial palps. Since *M. arenaria* did not produce any pseudofaeces in these experiments, one might expect to see a reduction in AE at high levels of inorganics. The fact that this did not happen may be attributable to a "grinding" effect of the inorganic material (Newell 1981, Bricelj and Malouf 1984, Enright et al. 1986) : *M. arenaria* may utilize inorganic material to assist in the mechanical breakdown of organic particles (although an actual mechanism for this has not been demonstrated). Thus, animals feeding on a food suspension with high inorganic content may be able to compensate for the "dilution" of organic particles by utilizing the inorganic fraction as an aid to digestion. This may also be the strategy of the surf clam *Spisula solidissima*, which shows an increase in consumption rate (like *M. arenaria*) and digestive efficiency in response to increased proportions of silt in the diet (Robinson et al. 1984).

In calculating AE by the Conover Ratio (Conover 1966), a number of assumptions are made: 1) there is no absorption of inorganics in the gut nor excretion of organic material with the faeces (Bricelj et al. 1984), 2) both organic and inorganic material pass through the gut at a similar rate, and 3) the bivalve exhibits non-selective feeding (Kiørboe and Möhlenberg 1981). In the case of *M. arenaria*, at least two of these assumptions appear to be violated: clams clearly secrete mucus with the faecal pellets, and there is evidence that the organic and inorganic fractions can pass through the gut at different rates by at least some individuals (this will be discussed in detail in section 3.4.4). Furthermore, there is evidence from other species that there is indeed absorption of inorganics within the digestive tract (Bricelj et al. 1984). Therefore, the only assumption made for the use of the Conover Ratio which is unequivocally met in this study is that of non-selective feeding, given that no pseudofaeces was produced by the clams. However, although the these assumptions have been violated, this is generally the case in experiments of this type. It is widely accepted that the Conover Ratio method has limitations, but that it is useful for comparative purposes.

There was also extremely high individual variation in AE in each of the diets, notably in the 10 mg 25%, 10 mg 50% and 2 mg 50% diets. This could simply be a result of violations of the assumptions discussed above, or, alternatively, indicative of different forms of digestion taking place in different clams. Bivalves are known to use two different forms of digestion: "intestinal" digestion which takes place extracellularly in the stomach and intestine with low AE, and "glandular" digestion taking place intra- and extracellularly in the digestive gland with higher AE (Widdows et al.1979, Bayne and Newell 1983, Decho and Luoma 1991). It is possible, given the high individual variation in most physiological parameters examined in this study, that some clams were adopting a strategy involving mainly intestinal digestion (with lower AE and possibly shorter GRT) while others used mainly glandular digestion (with higher AE and longer GRT). This subject will be discussed further in later sections of this chapter.

3.4.4. Gut Retention Time in *M. arenaria* and Relation to Diet

There are no literature values of GRT in *M. arenaria* to compare with those determined in this study. However, the range of retention times reported in this study (approximately 2 - 18 h) are comparable with those reported for other species (using a variety of measurements and analytical techniques) including *Macoma balthica* and *Potamocorbula amurensis* (Decho and Luoma 1991), *Mercenaria mercenaria* (Bricelj et al. 1984), *Cerastoderma edule* (Hawkins et al. 1990), *Choromytilus meridionalis*, *Perna perna*, and *Aulacomya ater* (Bayne et al. 1984), and *M. edulis* (Bayne et al. 1987, 1989, Hawkins et al. 1990).

In this study, *M. arenaria* decreased GRT in response to an increase in the quantity of food in suspension. A similar trend in *M. edulis* feeding on a mixed suspension of 2 algal species and ashed silt was reported by Bayne et al. (198°).

Conversely, *M. arenaria* increased GRT in response to an increase in the quality of food in suspension. This trend has also been reported for *Choromytilus meridionalis*, *Perna perna*, and *Aulacomya ater* (Bayne et al. 1984) as well as being the general trend predicted for deposit-feeders (Cammen 1980, Bayne and Newell 1983). This is not the relation suggested by Taghon (1981), who predicted mathematically that as the quality of the food increases, the optimal response should be to increase feeding rate with a

corresponding decrease in GRT. Interestingly, Bayne et al. (1987) found no change in GRT of *M. edulis* feeding on diets of differing quality, although the digestion efficiencies of the mussel did decrease significantly as food quality increased.

There appear to be two strategies for bivalves to adjust GRT when feeding on foods of decreasing quality (Bricelj et al. 1984). First, they can increase GRT in order to give the digestive processes longer to act upon the stomach contents, and thereby attempt to maximize nutrient and energy acquisition. Second, bivalves can decrease GRT, which represents a more conservative strategy, and may be of benefit when the food suspension is almost completely indigestible and not worth a great deal of energy expense. In this study, *M. arenaria* has adopted the second strategy (GRT of both organic and inorganic fractions were inversely proportional to the proportion and total amount of inorganic matter in suspension), which seems reasonable given the indigestible nature of the SiO_2 particles forming the majority of inorganic component of the food suspension. Furthermore, *M. arenaria* inhabits an unpredictable environment with large, short-term variations in food supply and composition. Given these conditions, it may be advantageous for clams to adopt an "opportunistic" feeding strategy. That is, clams should pass poor food material through the gut quickly, without wasting too much energy on digestion and absorption, since it is likely that a better food source will soon be presented.

Previous studies have suggested that particle selection may occur after ingestion, and it is generally predicted that poorer quality or indigestible fractions will be voided from the gut more quickly than the more nutritious fraction (Foster-Smith 1975a, Self and Jumars 1978, Bricelj et al. 1984, Lopez and Levinton 1987). Although there were no significant differences in the retention times of organic and inorganic fractions when animals within each treatment were pooled, individual clams clearly retained one fraction longer than the other. However, there are no clear links between this behaviour and the properties of the food suspension on which the clams were feeding. Furthermore, in all experiments, one third of the clams retained neither fraction longer than the other. It is therefore not possible to deduce why some clams show selection within the gut while others, feeding on identical food, do not, nor why some individuals preferentially retain the orga.. fraction, while others, under identical conditions, preferentially retain the inorganic fraction.

Preferential retention of the organic fraction in the bivalve gut has been demonstrated in a number of species including *M. edulis*, *Cerastoderma edule* and *Venerupis pullastra* feeding on a mixture of *Phaeodactylum* and alumina (Foster-Smith 1975a), and in *Mercenaria mercenaria* feeding on *Pseudoisochrysis paradoxa* labelled with ^{51}Cr and ^{14}C (Bricelj et al. 1984). Preferential retention of organics has also been demonstrated in bivalve veligers (see Robinson, 1983, for a review). Conversely, Decho and Luoma (1991) found no significant difference between the minimum GRT of ^{51}Cr-labelled bacterial cells and ^{51}Cr-labelled latex beads in both *Potamocorbula amurensis* and *Macoma balthica*, despite the beads being substantially larger (15 µm diam.) and of no nutritional value.

Throughout this study, individual *M. arenaria* often appeared to be separating faecal production into two components. This is consistent with the description of two forms of digestion: "intestinal" extracellular digestion in the stomach and intestine, and more prolonged "glandular" intracellular digestion in the digestive gland (Widdows et al. 1979). It has been proposed that the ratio of intestinal to glandular faeces increases with increasing IR, since not all material entering the stomach will be able to be processed in the digestive gland beyond a certain threshold level (= max. gut capacity), and that this is reflected in a decrease in AE (Thompson and Bayne 1974, Widdows et al.1979). Since it was not possible to quantify intestinal and glandular faeces separately in this study, the above relationship cannot be tested. However, observations of trends (i.e. bimodality in the GRT marker profile graphs (Appendix D) as a representation of separation of intestinal and glandular faeces) do not indicate any relationship between the production of glandular faeces and IR. The fact that there was often an overlap in the production of intestinal and glandular faeces in this study may be due to the relatively high concentrations of seston used: preliminary experiments made by Decho and Luoma (1991) also showed that at high food concentrations the production of intestinal faeces overlapped with faecal release from the digestive gland.

3.4.5. Summary of the Feeding Strategy of *M. arenaria* in Response to Changing Quantity and Quality of Suspended Particulate Material

In response to a 3-fold increase in food concentration (see Table 3.2), *M. arenaria* decreased CR by almost 9-fold, and therefore ingested less material, even though more

food was available. These clams with lower IR also had a shorter GRT (see Table 3.5). Gut volume is the product of IR and GRT (Bayne and Newell, 1983). Therefore, with ingesting food at a lower rate, and retaining the food for a shorter time, these animals had much less material in their guts (approx. 1 / 6 that of clams feeding on the 2mg 50% diet, based on mean IR and GRT values).

It is difficult to hypothesize exactly what the relative benefits and costs of this particular feeding strategy may be without a detailed energy budget for each case. Nevertheless, it seems plausible that clams feeding on the low quantity (2 mg 50%) diet are expending more energy in obtaining particles (higher IR despite lower food concentrations) but perhaps less energy in digesting them (clams on the 2 mg 50% diet maintained an AE equal to those feeding on the 10 mg 50% diet, but over a longer GRT thereby giving digestive enzymes longer to work). If the net energy saved in the digestive process (i.e. lower amounts of enzymes working over a longer time) is greater than that expended in filtering, this would be an effective feeding strategy for clams to adopt when experiencing low quantities of food in suspension. The actual role of enzymes in this process cannot be determined from the present study, but remains and interesting question for further research.

This feeding response can be contrasted to that identified for *M. edulis* feeding on differing quantities of seston (Bayne et al. 1989). Like *M. arenaria*, *M. edulis* shortened GRT and maintained a constant AE. However, *M. edulis* increased CR and IR, the opposite of that observed for *M. arenaria*. Therefore, the end result for both species was the same: maintenance of a constant AE. However, the mechanisms by which this was achieved is different for the two species. Also contrary to what has been suggested above for *M. arenaria*, the volume of food in the gut of the mussels was not related to the quantity of food in suspension, but to the organic content of that food (mussels feeding on low quality foods had larger gut contents, irrespective of the total food concentration). It is possible that each species encounters a different concentration and composition of seston in nature, given that one is infaunal and the other is epifaunal, and therefore their feeding strategies also differ.

The response of *M. arenaria* to changes in the quality of food in suspension is perhaps easier to interpret (see Table 3.5). As the proportion of organic material increases,

clams decreased CR and IR, so as to maintain a constant IR_o. Although GRT lengthened, AE remained unchanged. Therefore, clams regulated both their intake of food and GRT to keep their ingestion rate of organic material constant, and maintain AE levels.

A similar response to changes in organic content has been modelled mathematically for deposit-feeders and detritivores (Cammen 1980). In this model, the optimal response to an increase in organic content in the food is to decrease feeding rates with a corresponding increase in GRT and possibly AE (Bayne and Newell 1983). With the exception of the change in AE, these are the trends observed in *M. arenaria*. In contrast, Taghon (1981) predicted for suspension-feeders that the optimal response to an increase in food quality is to increase feeding rates with a subsequent decrease in GRT and AE. The relationships predicated by Taghon (1981) have been demonstrated in field studies with *Choromytilus meridionalis*, *Perna perna*, and *Aulacomya ater* (Bayne et al. 1984). However, in laboratory studies with *M. edulis*, increasing the quality of the food resulted in no major changes in CR and GRT, although AE did increase (Bayne et al. 1987).

An alternative explanation for why clams decreased CR and IR with increased quantity of food in suspension could be an inhibitory effect of the increased amount of SiO_2 in suspension. However, this hypothesis does not hold true when looking at the clams' responses to an increase in the proportion of SiO_2 in suspension (quality change data set), where CR and IR increased with the high inorganic (SiO_2) diet.

Since clams were allowed to adjust to the test food suspensions for 8 days prior to experimentation, it is not clear whether these adjustments can be made on a time-scale of hours or of days. It has been suggested that compensations for changes in the quality of food in suspension take place over a period of several days, and therefore are relevant only to longer, seasonal cycles of nutrient availability rather than short-term fluctuations such as tidal cycles and wind or wave effects (Bayne et al. 1988). In contrast, Grant and Thorpe (1991) found short-term adjustments in respiration rate and CR in *M. arenaria* exposed to fluctuations in particle concentrations, a strategy which enables clams to tolerate intermittent turbidity, but which caused starvation during long-term exposure at turbidity levels of 100-200 mg l^{-1}.

Regardless of the time scale of these adjustments, *M. arenaria* is successful in compensating for a wide variety of seston conditions: Emerson (1990) found that of 7 levels of sediment disturbance intensity, *M. arenaria* grew fastest at the maximum disturbance level tested (the top 10 mm of sediment disturbed for 10 s on a daily basis). Furthermore, Grant and Thorpe (1991) found that *M. arenaria* is capable of adjusting physiological rates in response to turbidity levels up to 2000 mg l^{-1}, although clams exposed to 100 - 200 mg l^{-1} for 30 days showed stress responses in terms of greatly decreased O:N ratios, and increased ammonia excretion.

In general, *M. arenaria* appears to be capable of adapting to changes in the quantity and quality of food in suspension, so as to maintain a constant absorption efficiency. The actual feeding response, however, is not the same as documented for mussels. This indicates a possible difference in feeding strategies between an infaunal and epifaunal species, perhaps due to differences in their natural habitat and food sources.

3.4.6. High Individual Variation

Throughout this study, a great deal of variability was observed in all physiological measurements. Although this study was designed to control for the effects of temperature, diet, previous feeding history, flow rate, animal size, and orientation of the clams with respect to the direction of water flow, there were other uncontrolled variables which could have contributed to the observed variation. These include the sex and reproductive condition of the clams, general health of the clams, and variations in genotype, among others. Although these factors may have some effect on the physiological variables tested, the variability observed probably resulted from the sporadic feeding behaviour observed throughout the study. Individuals would often stop feeding for several minutes to several hours for no apparent reason.

This sporadic feeding behaviour has also been observed in *Macoma balthica* (Decho and Luoma 1991), and may actually be a mechanism for processing the large quantities of seston common to an infaunal bivalve's diet. It is interesting to note that when the clams were exposed to the 10 mg 25% diet, the amount of variability in gut retention time was substantially reduced. This was the treatment in which the clams were most nutrient-limited in terms of the proportion of organic material in the diet. It is possible that the other diets

were nutritionally adequate, thus clams did not need to adopt any particular feeding strategy to meet metabolic requirements, and individual variation increased accordingly.

3.4.7. Avenues for Further Research

Throughout the course of this experiment, questions arose which were beyond the scope of the present study, but which would make interesting projects for further work. The first such question deals with the role of enzymes in the digestive process: do clams adopting different digestive strategies (particularly in the selection of particles within the gut), or feeding at different seston loads have different compositions or concentrations of enzymes in the digestive tract? A second question is the effect of tidal regime on the feeding behaviour of the clams. This study took intertidal clams (subtidal clams were not available from the sampling site) and subjected them to a regime of constant immersion (with at least 2 weeks acclimation before testing) to investigate feeding behaviour. Long-term monitoring of feeding behaviour and physiology might indicate whether clams retain any tidal feeding rhythms from their intertidal habitat once placed in a permanently submerged regime (this is assessed cursorily in the following chapter). It might also be interesting to repeat such feeding experiments with the field population's immersion / emersion cycle duplicated in the laboratory. One final question is the observed individual variation being indicative of different feeding strategies. Although this study has taken a preliminary look at this question (section 3.3.11), to more carefully test this hypothesis the same clams should be remeasured a number of times to ensure that their response is consistent and the results reproducible. Unfortunately this was beyond the scope of the present study.

CHAPTER IV

Field Measurements of Feeding Behaviour of *Mya arenaria*, in Platter's Cove, Terra Nova National Park, Newfoundland

4.1. Introduction

Numerous studies have identified the need for more field evaluation of feeding behaviour and physiology of bivalves (see review by Bayne et al. 1988). Feeding behaviour observed in laboratory studies, using artificial or mono-algal particulates as food sources, may not be representative of feeding behaviour in natural populations (Widdows et al. 1979). Laboratory studies can give precise information about a particular aspect of an animal's biology. However, that information may not be representative of behaviour in natural conditions. In contrast, field studies can provide more useful information, although the precision of measurements may be lower due to reduced control of experimental conditions (Aldrich 1989). It is therefore desirable to couple laboratory studies of feeding behaviour with observations made at natural population sites (Widdows et al. 1979, Bayne et al. 1988, Aldrich 1989).

The trends in feeding behaviour described in the previous chapter give insight into the mechanisms used by *M. arenaria* to compensate for changes in food composition. However, it was not known if the actual values of the calculated parameters (CR, GRT and AE) were representative of *M. arenaria* feeding on natural particle assemblages found above clam beds. The laboratory study used two particles (*C. muelleri* and SiO_2) to supplement seawater pumped from Logy Bay, Newfoundland (a rocky-bottomed, exposed site, quite unlike soft-bottomed, sheltered sites favourable to clam populations). Seston normally encountered by clams would have a markedly different composition.

Like infaunal deposit bivalves, *M. arenaria* ingests large amounts of resuspended sediments (Lopez and Levinton 1987) which would contain a large amount of indigestible inorganic material. However, these sediments are not without some nutritional value due to

associated microbes (see Lopez and Levinton, 1987, for a discussion of this theory) and detrital material. Inorganic sediments may also enhance digestion by assisting in the mechanical breakdown of food particles (Newell 1981, Bricelj and Malouf 1984, Enright et al. 1986). In addition to this layer of sediment, *M. arenaria* would ingest a wide variety of organic particles in suspension over the clam flat.

Feeding behaviour of *M. arenaria* in natural populations is confounded by two other factors: 1) many populations of *M. arenaria* are intertidal and therefore may have restricted periods of feeding, and 2) the intertidal and shallow subtidal zones populated by *M. arenaria* are extremely unpredictable, being exposed to many short-term fluctuations in particle assemblages caused by wind and wave conditions, effects of estuarine run-offs, and increased resuspension of particles with the rising tide.

This part of the study was designed to investigate the behaviour of *M. arenaria* feeding on seston pumped directly from the water column covering a *M. arenaria* population in Platter's Cove, Terra Nova National Park, Newfoundland. Experiments were conducted on two different days, one on a rising and one on a falling tide, in order to identify any possible inherent diurnal feeding rhythms.

4.2. Methods

4.2.1. Study Site and Animals

Field experiments were performed on August 5 and August 12, 1992, at Platter's Cove, Terra Nova National Park, Newfoundland. Approximately 20 *M. arenaria*, 47 - 60 mm in shell length, were collected from the mid- and lower littoral zones on August 4 and August 9. Clams were held in a pearl net tied to a stake positioned below the low tide mark until the morning of each experiment.

4.2.2. Experimental Apparatus

The flow-through apparatus used in the field was identical to that described in section 2.2.2, with the exception of the following modifications:

Seawater was pumped directly from Platter's Cove (intake depth ranged from approximately 15 - 45 cm) to the experimental apparatus located at the top of the beach. The inflow end of the line was placed on a flat stone several cm above the sediment. To remove any large pieces of debris, the seawater inflow was passed through a 100 µm screen before entering the header tank. On August 12, this mesh size was increased to 200 µm to reduce the frequency with which the screen clogged. A stir bar was omitted from the bottom of the header tank bucket, since the force of the inflow seawater was sufficient to keep the seston adequately mixed. Lids were placed over the header tank bucket and each experimental container for the majority of each day to prevent disturbance by rain.

4.2.3. Experimental Procedure

Two hours prior to the beginning of each experiment, clams were transferred from the pearl net to the experimental containers, and left undisturbed to adjust to experimental conditions. After 2 h, lines to the AE, C, and 6 GRT containers were plugged, and the gut marker particles (*T. suecica* and SiC) were poured into the header tank bucket to a concentration of 5000 particles ml^{-1} and 12,000 particles ml^{-1} each on August 5 and 12 respectively (more marker was given on August 12 because seston concentrations were noticeably higher). Procedures followed during this time were as outlined in section 3.2.3.

Introduction of the markers was recorded as time zero. Experimental conditions were thereafter maintained for 8 h. Samples were taken every hour for the next 5 h to determine CR. Faeces samples were taken hourly for 8 h for GRT analysis. Faeces samples for AE analysis were taken at 4 and 8 h after marker delivery commenced.

4.2.4. Clearance Rate Measurements

Approximately 100 ml of seawater was collected beneath each outflow standpipe into plastic specimen cups. Water samples were preserved in 1% Lugol's and 1% formalin fixatives to prevent changes in particle characteristics before concentrations could be determined. Background studies indicated that addition of the fixatives does not significantly alter particle counts in a sample (see Appendix E). Samples were analyzed on the Coulter Counter Multisizer within 4 days of the experiment, and CR was calculated as described in section 3.2.5.

4.2.5. Gut Retention Time Measurements

Samples collected for GRT analysis were kept on dry ice for a maximum of 8 h before being transferred to a freezer. Samples were kept frozen before being processed by the method described in section 3.2.6.

4.2.6. Absorption Efficiency Measurements

Faeces samples collected for calculation of AE were filtered immediately upon collection onto pre-ashed and pre-weighed Whatman GF/C filters. Filters were kept on dry ice for a maximum of 5 h before being transferred to a freezer, and kept frozen until subsequent analysis by the method described in section 3.2.7.

4.2.7. Water Samples

One to two litres of seawater were collected from the standpipe of the control container hourly for 8 h. Water samples were filtered immediately onto pre-ashed and pre-weighed Whatman GF/C filters and stored on dry ice until they could be transferred to a freezer. Filters were then analyzed by the method outlined in section 3.2.8.

4.3. Results

4.3.1 Seston Analysis

Microscopic examination of the preserved water samples collected from the outflow of the control chambers showed that on both days most of the seston was comprised of detritus. Living cells were either small flagellates or larger *Euglena*, although on the second day there were some live *Protoparadinium* (dinoflagellates). Other live species (at much lesser densities) included *Cryptomonas* sp. and *Nitzschia closterium* (diatoms) and choanoflagellates (heterotrophs). In general, very few live diatoms were present, although many empty diatom shells could be seen. There were also very few ciliates present, with the exception of a rare tintinnid. On August 5, there was a 10-fold increase in the number

of *Euglena* in the water 8 h after initiation of the feeding experiment, possible due to relocation of the water intake position at that time (necessary because of the changing tidal height). In general, the majority of the bivalves' diet in late summer at Platter's Cove appears to be flagellates and detrital matter.

The presence of large numbers of *Euglena sp.* noted above should not have affected the accuracy of the chlorophyll *b* GRT marker. Although *Euglena* does contain chlorophyll *b*, this would not have become apparent in faecal material until after *Euglena* had passed through the gut. Since *Euglena* was not present in the seston in large amounts until 8 h into the experiment (and thus at the final collection time), this does not present a problem. Furthermore, any traces of *Euglena*-derived chlorophyll *b* present in faecal sample prior to the hour-8 collection would be accounted for in the faeces collected from the control clams.

The proportion of organic matter in the seston varied greatly throughout the 8 h sampling period, and generally increased or decreased in an inverse relation to the total amount (dry weight) of seston present (Fig. 4.1). This suggests that the total amount of organic matter available to the clams was relatively constant, while the amount of inorganic matter (resuspended sediments) was more variable (Fig. 4.2). The main exception to this trend occurred with the 9-hour sample on Aug. 5, when there was a sharp increase in organic matter (Figs. 4.1 A and 4.2 A). This was due to the 10-fold increase in the numbers of *Euglena* flagellates present. This was also visible as a peak of particles approx. 15 µm in diameter (mode) in particle size distributions of water samples measured at that time.

Clams were exposed to significantly higher seston concentrations on August 12 compared to August 5 (independent t-test, $P < 0.005$). There was no significant difference in the percentage of organic matter (dry weight) between the two days (independent t-test, $p > 0.4$). The statistics for these analyses are in Table 4.1.

Water temperatures on both days averaged 14°C (SD = 0.6).

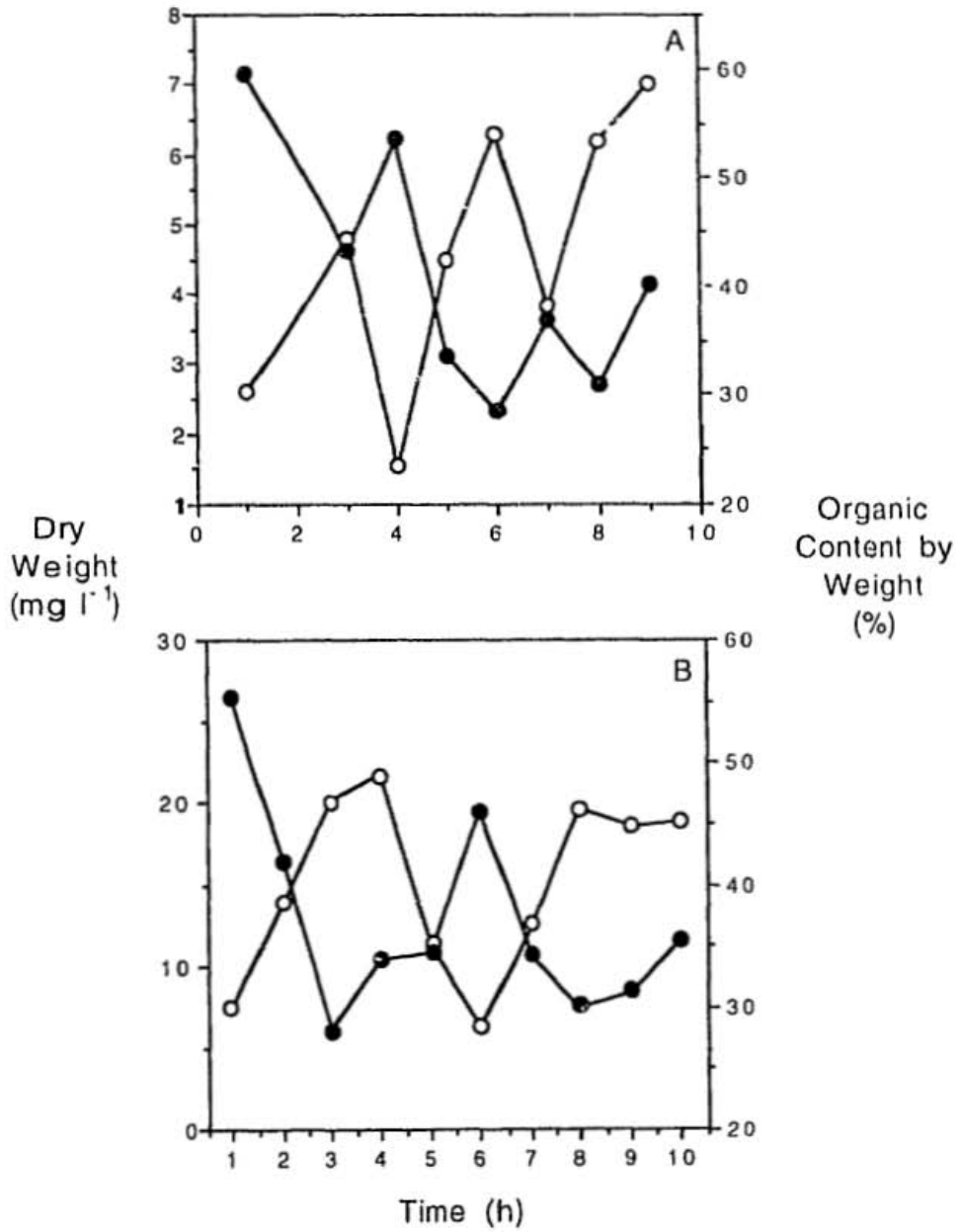

Fig. 4.1: Results of seston analysis at Platter's Cove, NF, on August 5 (A) and August 12 (B), 1992. Indicated are the total dry weight of the seston per litre of seawater (—o—) and percent organic content of the seston (—●—).

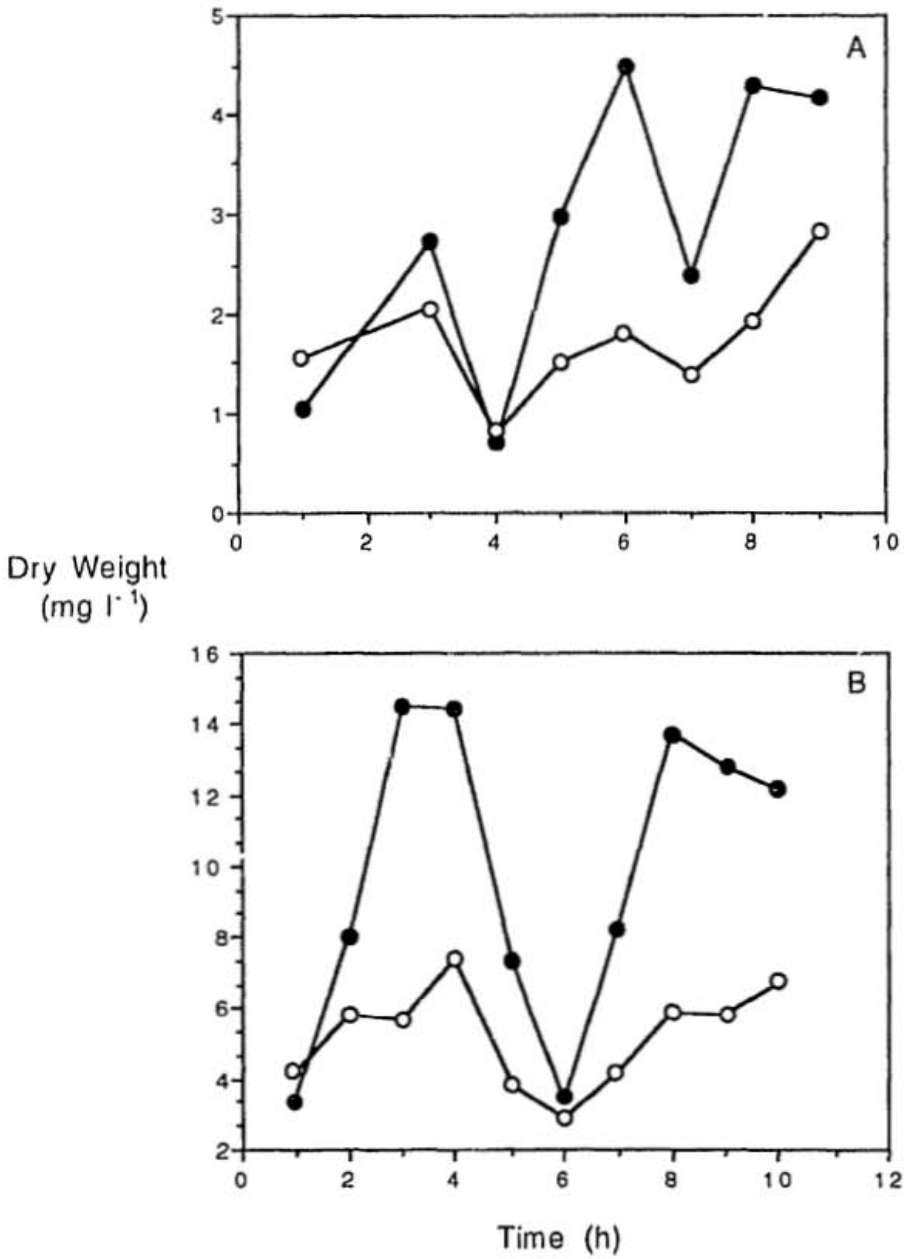

Fig 4.2: Results of seston analysis at Platter's Cove, NF on August 5 (A) and August 12 (B), 1992. Indicated are the amounts of organic (——o——) and inorganic (——●——) material present in 1 litre samples of seawater.

Table 4.1: Results of ash analysis of seston samples taken at Platter's Cove, Terra Nova National Park.

Date	n	Dry Weight mg/l (+- S.D.)	% Organic (+-S.D.)
Aug. 5	8	4.59 (+- 1.91)	40.8 (+-10.9)
Aug.12	10	15.04 (+- 5.55)	37.0 (+- 8.4)

4.3.2. Clearance Rate

Clearance rates (standardized for 1.0 g soft tissue dry weight) were relatively constant over the 5-hour measurement period of both days (Fig. 4.3), and were significantly higher on August 12 than on August 5 (independent t-test, $t = 18.86$, d.f. = 118, $p < 0.005$).

4.3.3. Absorption Efficiency

On both August 5 and 12, an insufficient amount of faeces was produced to accurately measure AE.

4.3.4. Gut Retention Time

Very few faeces samples were collected from the clams: 4 total on August 5, and 11 total on August 12. Therefore, GRT could not be determined by use of marker profile graphs as outlined in Appendix D. However, it is possible to draw some conclusions based on the chlorophyll *b* and SiC analyses.

Table 4.2 gives the proportions of chlorophyll *b* (O.M.) and SiC (I.M.) found in each faecal sample, calculated by equations 3.5 and 3.6 respectively. On August 5, only one control clam (C5) produced faeces. Of the other samples collected on that day, only one (from clam T4, taken at hour 5) showed a large increase in the amount of both O.M. and I.M. when compared to the control sample. This is strong evidence of the marker particles being present, and is supported by visual observations of that sample, describing it as strongly grey-green in colour as compared to the brown pellets produced by the control clam. Thus, a GRT of 5 h can be estimated for one clam from the August 5 experiment.

When the 5 faeces samples from control clams in the August 12 experiment were pooled, the mean values of O.M. and I.M. could then be compared to each of the individual O.M. and I.M. values of the faeces samples from treatment clams. If either value was significantly higher in the treatment individual, this could indicate presence of the gut

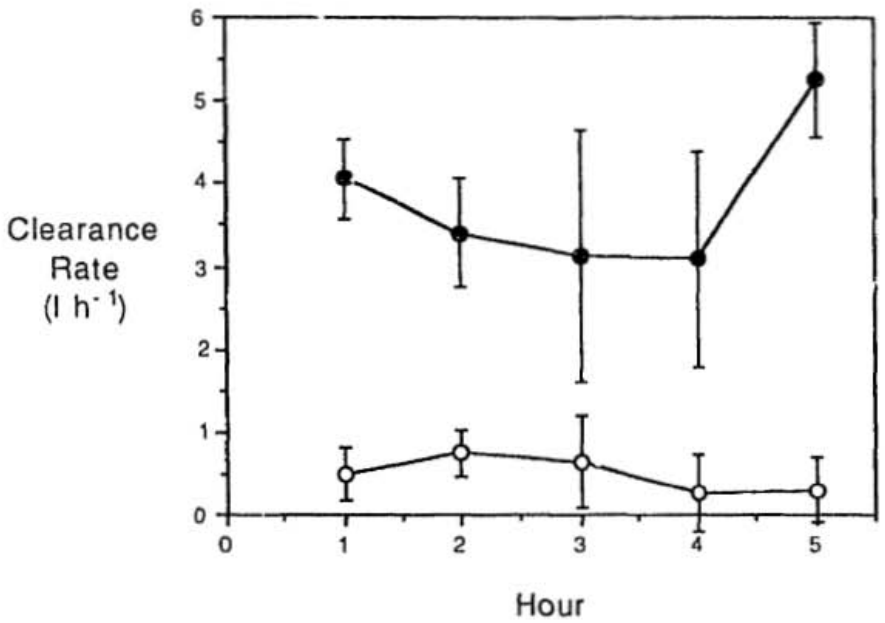

Fig. 4.3: Clearance rates of clams feeding on a natural particle assemblage at Platter's Cove, NF, on August 5 (——o——) and August 12 (——●——), 1992. Concentration of suspended particulate matter was approx. 4X greater on August 12 than on August 5. Vertical bars are standard deviations of the mean, n = 12 for each point.

Table 4.2: The relative amounts of organic chlorophyll b marker (O.M., Equation 3.5) and inorganic SiC marker (I.M., Equation 3.6) present in each faecal sample, collected from studies of *M. arenaria* at Platter's Cove, Terra Nova National Park. An asterisk (*) indicates significant difference from faeces samples taken from control individuals on the same day at $\alpha = 0.05$ (one-tailed t-test, t=2.812, d.f.=4).

Date	Clam	Time (h)	O.M.	I.M.
Aug. 5	C5	4	0.616	8.63
	T4	5	14.545	14.82
	T6	3	1.655	1.14
		8	1.678	0.96
Aug. 12	C3	3	0.392	1.33
	C6	2	0.000	2.76
		3	0.119	1.84
		6	0.372	1.20
		7	3.389	0.82
	T3	8	5.247 *	1.87
	T4	1	0.917	1.60
		2	0.221	1.19
		3	0.876	2.82
	T5	1	0.188	0.68
		8	0.786	0.95

marker. Only the hour-8 sample from clam T3 showed a significant increase in O.M., and no samples showed a significant increase in I.M. From this one can conclude that the organic GRT of clam T3 was approximately 8 h, but that the inorganic GRT cannot be determined.

4.4. Discussion

4.4.1. Seston Sampling Accuracy

One of the primary reasons for conducting field studies is to assess animal behaviour and physiology under more natural conditions. However, in studies of feeding physiology such as this one, it is important to understand what the natural food source is. This experiment assessed the particle load available to clams in Platter's Cove in early to mid August. Seston was composed of a wide variety of live cellular matter, detritus, and inorganic particles, and varied greatly in quantity and quality over short time periods due to shifting wind and wave conditions. The highly variable particle load observed in this study is characteristic of intertidal substrates (Fegley et al. 1992; Iglesias et al. 1992).

To accurately assess the seston utilized by *M. arenaria* at the Platter's Cove population, the seawater intake was placed on a flat stone several centimetres above the sediment surface. This prevented excess sediments being sucked up by the pump. When clams are feeding undisturbed in their natural habitat, their siphons are positioned with the aperture flush with the sediment surface. *Mya arenaria* will likely ingest a portion of sediment drawn into the siphons by the ciliary currents, however the amount of sediment ingested is likely far less than that drawn in by the pump if it were not placed on a stone. So although the water delivered to the clams in this experiment was taken a few centimeters above the layer on which they would normally have been feeding, it is a more accurate sample than if the seawater intake had been placed directly on the sediment.

4.4.2. Clearance Rate

Clearance rates of clams in this field study increased in response to increased food concentration. This contradicts the results of the laboratory study described in Chapter III, when increased food quantity depressed CR. However, two uncontrolled factors could account for this discrepancy. Clams used on Aug. 5, the ones with very low CR, were dug up only the previous evening, and could therefore have still been suffering from stress brought on by collection. Furthermore, the water pump used on Aug. 5 leaked oil for a few minutes at the beginning of the experiment. Although clams were exposed to a very small quantity of oil for only a few minutes, this could have disturbed them enough to cause them to shut down pumping rates for the remainder of the day: presence of oil does cause *M. arenaria* to decrease filtration rates over extended periods (Gilfillan et al. 1976). These two problems were rectified for the Aug. 12 experiment by collecting the clams three days prior to the experiment, and by using a different water pump.

Clearance rate values recorded on Aug. 12 were much higher than any CR measurements made in the lab (both data sets were standardized for 1.0 g soft tissue dry weight, and thus direct comparisons can be made). One possible explanation for this is that clams in the field study were compensating for reduced feeding times in their intertidal habitat by increasing CR (and IR) during periods of immersion. Although clams in the laboratory study did come from an intertidal population, they has been acclimated to a continuously submerged regime for at least 2 weeks before feeding measurements were made.

There is conflicting evidence from other studies for whether intertidal bivalves compensate for reduced periods of feeding by increasing feeding rates (relative to subtidal individuals) during periods of immersion. Early studies on this matter suggested that rhythmicity in feeding as well as higher filtration rates on submersion did occur in some intertidal bivalve species (see Bayne 1976 for a review). However, later studies (e.g. Griffiths and Buffenstein 1981, Widdows and Shick 1985) have found no compensatory feeding behaviour. None of these studies, however, dealt with *M. arenaria*. This remains an interesting question for further research.

4.4.3. Gut Retention Time

The biodeposits produced by clams in this study were of three types, as described by Brown (1986). Mucus-bound pellets were the most common type, looser pseudofaecal material was produced rarely, and unconsolidated sand and silt grains, which likely settled out in the mantle cavity (Bernard 1974, Brown 1986), occasionally appeared. Of these three types, the mucus-bound faecal pellets were collected for GRT analysis. Two clams produced faecal pellets containing marker particles in this study at a time within the range observed in the laboratory study described in Chapter III.

The difficulty in determining AE and GRT in the field can be attributed to the sporadic defaecation pattern of the clams. Although on Aug. 12 the clams had very high CR, they produced very few faecal pellets. This suggests that they had a much higher gut capacity than those clams used in the laboratory study, which produced substantially more faeces, even at lower seston concentrations. The apparently larger gut capacity of the clams used in this field study may reflect another intertidal adaptation: if food is available only intermittently, clams might pack the gut full of material prior to aerial exposure, and subsequent cessation of feeding. Indeed, on Aug. 12 when clams had high filtration rates and apparent high gut capacity, the tide was initially falling with low tide at approximately 2:45 pm (the experiment commenced at 10:00 am and continued until 7:30 pm, most of which time the clams would naturally have been on the exposed mudflats). Alternatively, this could be indicative of incomplete acclimation to the experimental apparatus: clams were given only 2 h to adjust to the flow-through apparatus in the field, compared to 24 h in the lab.

4.4.4. Avenues for Further Research

This field study was partially successful in assessing the feeding behaviour of *M. arenaria* outside the laboratory, and gives some insight into this species' feeding physiology. It has also accurately assessed the seston available to clams in Platter's Cove, NF, in early August. The most important considerations for future field studies with *M. arenaria*, in which behavioural or physiological measurements of individuals are made, include: 1) the need to limit physiological stress associated with removing clams from the sediment, or to perform measurements and collect faeces samples of *M. arenaria in situ*

(difficult given their muddy intertidal habitat), 2) the need to reconcile observations with tidal rhythms, which may affect their feeding behaviour and physiology, and 3) the need to overcome the problems in measuring GRT, given the clams' sporadic defaecation rates.

CHAPTER V

Evaluation of the New Gut Marker Technique

The dual marker technique developed in this thesis is based on several assumptions: 1) any differential treatment of the two markers within the bivalve gut is due primarily to their different organic contents, 2) that marker particles are captured and processed in the same way as the main components of the diet, and 3) if chlorophyll is absorbed in the gut, the rates of absorption are similar for chlorophyll *b* and *c* (and their component breakdown products).

With respect to assumption 1, the two marker particles were selected to be the same size to prevent differential rejection or selection based on particle size. It is therefore reasonable to assume that any differential treatment within the gut of the silicon carbide particles and *T. suecica* is due primarily to their surface properties and organic content.

Assumption 2 has several implications. It is possible that the *T. suecica* marker is ingested (or rejected) at a different rate than the *C. muelleri* cells: *T. suecica* is a much larger cell (10 µm vs 5 µm modal diameter), and may have different surface properties. However, pre-ingestive selection would not preclude the use of *T. suecica* cells as GRT markers, providing that some cells were indeed ingested.

If post-ingestive processing of the marker particles were to differ significantly from that of the natural diet, the technique may become less useful or less meaningful ecologically. Previous studies have shown that some bivalves are capable of separating an indigestible algal species in the gut from a more digestible algal species and eliminating it more rapidly (Bricelj et al. 1984, Shumway et al. 1985). If *M. arenaria* in this study were eliminating the *T. suecica* marker cells more rapidly than the *C. muelleri* cells, GRT would be underestimated. Shumway et al. (1985) have demonstrated that *M. arenaria* is capable of retaining and digesting a cryptomonad species in preference to dinoflagellates and diatoms, when fed a mixed diet of the three. Cryptomonads are soft-bodied, and probably easier to digest than either diatoms or dinoflagellates which possess hard frustules. Because the cell is covered with small scales, *T. suecica* may be difficult for bivalves to digest (Epifanio and Ewart 1977, Enright et al. 1986). It is uncertain how "digestible" this "scaled" species is

compared with *C. muelleri*, a diatom with a hard frustule, and thus whether they may experience differential treatment within the gut. Enright et al. (1986) found that juvenile oysters *Ostrea edulis* had higher growth rates when fed *Chaetoceros* spp. than when fed *Tetraselmis* spp., but were unable to determine whether this was due to poor digestibility of *Tetraselmis*, or due to its lack of the essential fatty acid 22:6ω3 (Langdon and Waldock 1981).

The third assumption is that chlorophyll *b* and its breakdown products are absorbed at the same rate as chlorophyll *c* and its derivatives. The fact that chloropigments are absorbed in the gut (Conover et al. 1986, Hawkins et al. 1986, Robinson et al. 1989, Abele-Oeschger and Theede 1991) does not affect the accuracy of this technique, since the amount of chlorophyll *b* marker is standardized as a ratio to chlorophyll *c*. However, if one form of chlorophyll is preferentially absorbed over the other, the technique becomes less accurate. Little is known about the relative absorption rates of the different chlorophyll pigments, although Abele-Oeschger & Theede (1991) found that the ratio of chlorophyll *a* : *c* in the faeces of the gastropod *Littorina littorea* feeding on *Fucus* was the same as in the gut, and slightly lower than in the food. This suggests that if the pigments are absorbed, absorption rates of the two forms of chlorophyll are similar since the ratio does not change during passage through the gut.

In general, one must use caution in using pigments as biogenic markers (Abele-Oeschger and Theede 1991). Many studies have used HPLC or fluorometric techniques in studies of bivalve physiology (e.g. Kiørboe and Möhlenberg 1981, Robinson 1983, Robinson et al. 1984, 1989; see Hawkins et al. 1986 for a review). However, there are drawbacks to these techniques in that chlorophyll molecules are "lost" while passing through the gut. This loss can occur through degredation to phaeopigments (Shuman and Lorenzen 1975, Hendry et al. 1987), by absorption in the gut (Conover et al. 1986, Hawkins et al. 1986, Abele-Oeschger and Theede 1991), or through degredation to non-fluorescing end products (Conover et al. 1986, Head 1992). The extent to which pigments are lost depends on a variety of factors including previous feeding history, rhythm, and diet composition (Head 1992) as well as duration of gut passage, animal size, form of faecal pellets, and nutritional state of the animals (Abele-Oeschger and Theede 1991).

The degredation of chlorophyll to phaeopigments has been compensated for in this study by identification and inclusion of many of the breakdown products as possible in the marker detection process. Loss of pigment to non-fluorescing end products and absorption would not significantly affect these results if the rate of pigment loss was constant throughout the experiment. Differential rates of pigment loss among individuals would be minimized by the week-long acclimation time and narrow size range of the animals. Furthermore, even if there were a significant difference in rates of pigment loss among individuals, this should not affect the accuracy of this study: only the GRT values (and not the pigment ratios) were compared between individuals.

The procedure used to quantify the organic marker (as a ratio of chlorophyll *b* to *c*) may not be the ideal method. In most studies of GRT, the absolute, cumulative amount of marker particles in the faecal samples is traced, and is not corrected for pellet size. However, in this particular study it was not possible to quantify the amount of chlorophyll *b* and its degradation products in this manner. In many instances, it was impossible to collect all faecal material produced in a given time interval, due to the production of tiny, fragmented faecal pellets which were scattered across the bottom of the container. In these instances, a representative proportion of faecal material was collected. To determine GRT by cumulative quantification of pigment, all faecal material must be collected. This was not always possible. Therefore, determining the amount of chlorophyll *b* in each sample as a ratio to the amount of chlorophyll *c* may not be ideal, but is a reasonable compromise. Future studies may wish to modify this procedure accordingly.

The dual-marker technique has the advantage of showing, simultaneously, differences in the processing by the bivalve digestive system of organic and inorganic components of the diet. This has been done previously by Bricelj et al. (1984) using algae spiked with double radioisotope tracers (^{51}Cr: ^{14}C). These authors found that ^{14}C was retained in the gut longer than ^{51}Cr, and proposed that the ^{51}Cr was bound to the indigestible cell wall and was voided from the gut more quickly than the ^{14}C incorporated into the cell cytoplasm. The technique presented here is slightly different in that the digestible and indigestible markers are not incorporated into the same particle, but are independent. This may facilitate separation of the two markers within the gut. It also reflects a significantly different objective: to examine the fate of organic and inorganic particles in a mixed suspension.

In conclusion, although there may some theoretical limitations to the use of *T. suecica* and SiC particles as markers for organic and inorganic food content, these do not negate their use. Furthermore, there are distinct advantages to this technique, including applications to flow-through systems and field-based projects. The technique is also sensitive enough to detect post-ingestive selection of particles by individual clams. Although the soft-shelled clam, *M. arenaria*, was used in this study, the double-marker technique could easily be adapted for use with other suspension-feeding bivalves: a portion of this technique has already been modified successfully to assess the GRT of *M. edulis* feeding on *Alexandrium tamarense* (Scarratt et al., 1993). The double-marker technique presented in this paper is therefore a viable alternative to those procedures currently used to measure GRT in suspension-feeding bivalves.

Appendix A

The Effect of Flow Rates and the Absence of Sediment on Clearance Rates of *M. arenaria*

Introduction

The purpose of this experiment was 1) to determine the point at which CR becomes independent of the rate of water flow through the experimental containers, and 2) to compare the behaviour of *M. arenaria* when supported in small plastic clamps as opposed to being in sand.

Many studies have reported that CR is directly proportional to flow rate when flow rate is low (due to recirculation of water within the containers), but that the two become independent as flow rate increases (Hildreth and Crisp 1976, Möhlenberg and Riisgård 1979). If CR is independent of flow rate, it can be calculated as:

$$CR = FR \frac{(C1-C2)}{C1} \qquad \text{Equation 3.1}$$

where CR = clearance rate ($l\ h^{-1}$), FR = flow rate ($l\ h^{-1}$), C1 = the concentration of particles in the inflow and C2 = the concentration of particle in the outflow (Hildreth and Crisp 1976). It was necessary to determine the flow rate at which CR of *M. arenaria* became independent of flow rates in the experimental apparatus.

There is also some concern over the effect on physiological rates of keeping an infaunal bivalve out of the sediment. Several studies have addressed this problem, and most have found that in short-term experiments, infaunal bivalves are not adversely affected by being out of the sediment (Newell 1977). However, infaunal bivalves kept out of sediment for prolonged periods of time (a few months) may experience muscle degeneration which leads to mortality (Chris Frantzi, pers. comm.).

Materials and Methods

The flow-through seawater apparatus used in this experiment was that described in section 2.2.2. A total of 12 containers were used in this experiment. Six were identical to those described in section 2.2.2, five with clams placed in each plastic holder and one empty to serve as a control. Another 6 containers were modified to contain 0-grade silica industrial sand. Five of these each had one clam positioned in the centre of the container with siphons facing upwards and the incurrent siphon facing towards the inflow. The sixth contained sand but no clam to serve as a control. Before use in this experiment, the sand was washed in tap water until the rinse water ran clear, then autoclaved for 30 min for sterilization. Clams were allowed to adapt to the experimental apparatus for 24 h prior to experimentation. This time was sufficient for clams placed in the sand to completely bury themselves. Seawater was filtered to 100 µm and maintained at 12°C throughout the experiment. Seston was supplemented with *C. muelleri* to a mean final concentration of 15.5 x 10^3 particles ml^{-1} (S.D. = 2415.4, n = 30).

Flow rate to each container was initially set at 44 - 59 ml min^{-1} (= 2.64 - 3.54 l h^{-1}), and then raised in three increments to a maximum of 246 - 310 ml min^{-1} (=14.76 - 18.60 l h^{-1}). Clams were allowed to adjust to each flow rate for a minimum of 3 h before CR measurements were made by the method described in section 3.2.5. At each flow rate, a minimum of three steady-state measurements of CR were made, with measurements being made at 30 min to 1 h intervals.

Results

If flow rate is low enough so that all particles in the water are filtered by the clam (i.e. C2 = 0 from Equation 3.1), then CR = flow rate. As flow rate increases and more particles are not filtered by the animal, C2 will increase and CR becomes less than FR. This relation is illustrated with data from this experiment in Fig. A.1, where CR is independent of flow rate at all points measured. Therefore, it was decided that a standard flow rate of 100 - 120 ml min^{-1} would be used in all experiments. At flow rates less than this some clams withdrew their siphons slightly and reduced valve gape, and at flow rates greater than this, there was a corresponding increase in variance between individuals (see Fig. A.1).

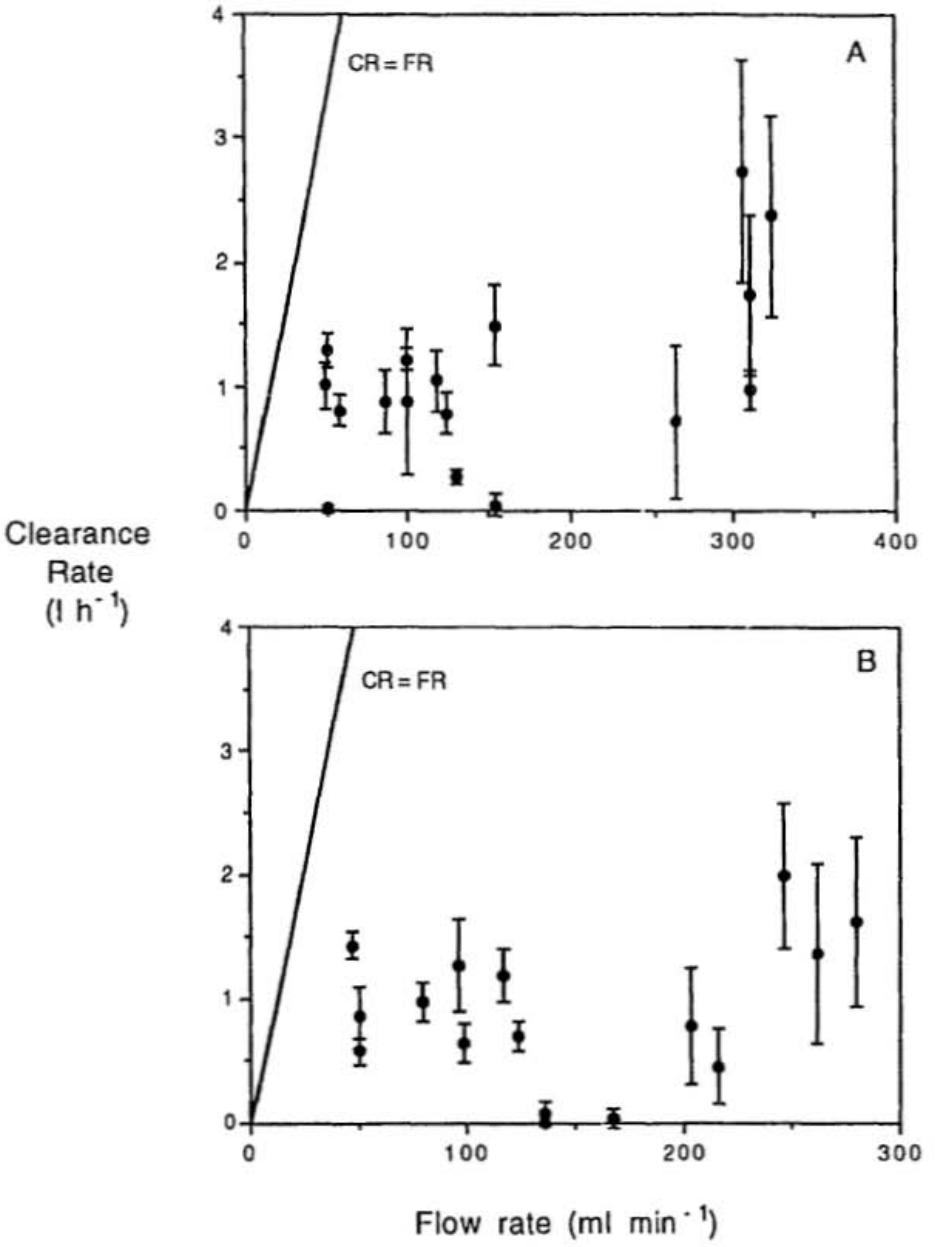

Fig. A.1: Clearance rate of *M. arenaria* held in holders (A) and in sand (B) in relation to flow rate of seawater through experimental containers. At all points measured, clearance rate is independant of flow rate. Error bars are standard deviations, and n = 5 for each point.

Clams held in plastic holders did not have different CR than those supported in sand (Fig. A.2). Therefore, it was decided that all subsequent experiments would be performed with clams held in the plastic clamps. This facilitated detection and collection of faecal particles, which proved more difficult from containers filled with sand.

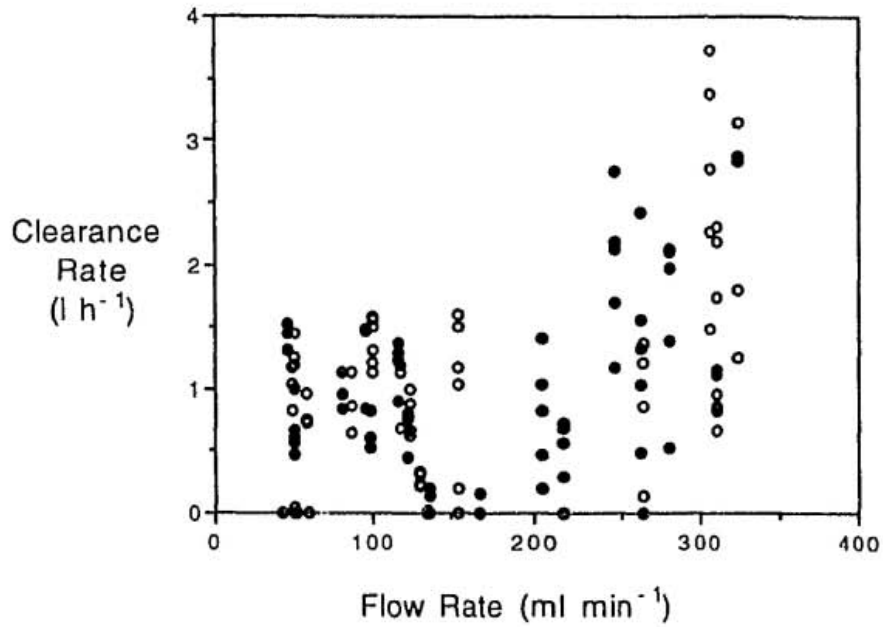

Fig. A.2: Clearance rates of *M. arenaria* held in plastic holders (○) and in sand (●). Absence of sediment does not affect clearance rates of *M. arenaria*.

Appendix B

Effect of Temperature on Physiology of *M. arenaria*

Introduction

The purpose of this experiment was to compare the physiology of clams in ambient 1°C seawater with those acclimated to seawater heated to 12°C. The natural seawater temperature in Newfoundland ranges greatly throughout the course of the year. Since the laboratory experiments described in this thesis spanned several months during which time the ambient temperature varied considerably, all experiments were conducted at one temperature. Twelve degrees Celsius was chosen because it represents an intermediate point (attainable by the Neslab heat exchanger) in the range (approx. -1 to 17°C) of temperatures measured in the seawater system throughout the year at the Ocean Sciences Centre. Laboratory experiments were started in the month of January, when ambient seawater temperature was 1°C. It was therefore necessary to see whether a substantial rise in seawater temperature has any adverse effects on the clams. It has been proposed that standard metabolic rates vary little with temperature, but active metabolic rates can be substantially affected by temperature (Lowe and Trueman 1972, Newell 1979).

Methods

Two experiments were run as outlined in section 3.2. The ambient seawater temperature at the time of collection was 1°C. In the first experiment, clams were adjusted to 12°C by increasing the seawater inflow temperature to the acclimation tray over the course of 7 days by a maximum of 2°C per day. Once 12°C was reached, clams were further allowed to acclimate for 2 more days before experiments were started. In the second experiment, clams were kept in 1°C seawater throughout the acclimation and experimentation periods. In both experiments clams were exposed to a supplemental diet of *C. muelleri* algae and inert silicon dioxide particles to a concentration of 3 mg l^{-1} at 50% organic content by weight.

Results and Discussion

Clams feeding at 12°C had a significantly higher CR (Fig. B.1, Wilcoxon Rank, Z=44, n_2=12, p=0.0001) and AE (Fig. B.2, Wilcoxon Rank, Z = -1.992, n_2=3, p=0.046) than clams feeding at 1°C on identical diet content. There was no significant difference in GRTs of organic and inorganic particles at either temperature (Wilcoxon Rank, Z=-0.962 n_2,=6, p=0.345 and Z=-1.414, n_2=6, p=0.180 for 12°C and 1°C respectively) so organic and inorganic GRT measurements for each experiment were pooled. The differences in GRT for clams at 12°C and 1°C were not significant (Mann-Whitney U, Z=-0.826, p=0.408).

The Q_{10} of the CR of *M. arenaria* in this experiment was calculated at 1.936. This is much higher that of 1.05 measured for pumping rates of *M. arenaria* by Lowe and Trueman 1972. However, the measurements made by Lowe and Trueman (1972) were made using thermistor probes and are therefore not analogous to the CR values calculated in this study. Furthermore, Lowe and Trueman raised the temperatures in their study at a rate of 1°C every 10 min, and thus responses by the clams do not represent acclimations but rather responses to acute temperature change (Malouf and Bricelj 1989). In comparison, clams in this study were exposed to changes in temperature at a much slower rate of 2°C per day.

The effect of temperature on CR has been studied extensively for *M. edulis*, and studies have generally shown that a change in temperature has little effect on CR (Widdows 1978, Widdows et al. 1979, Conover 1981). However, Newell and Bayne (1980) and Jørgensen et al. (1990) found that CR increased with increasing temperature for *Cerastoderma edule* and *M. edulis* respectively. This relation to temperature has been interpreted as an energy-conserving adaptation for feeding during the winter when concentrations of particles in the seston are generally low (Newell and Bayne 1980). However, Jorgensen et al. (1990) suggest that increases in clearance and pumping rates at higher temperatures are simply a result of changes in the viscosity of the water (lower temperatures cause increased viscosity, increased resistance to the cilia, and hence reduced pumping rates). This matter is still in dispute.

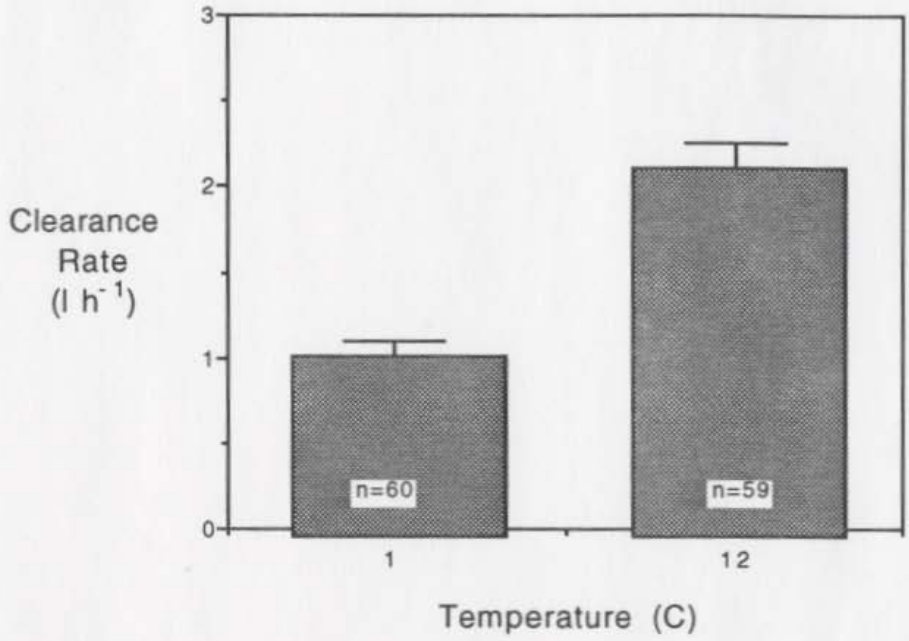

Fig. B.1: Effect of temperature on clearance rate of *M. arenaria* feeding on *Chaetoceros mulleri*. Error bars are the standard error of the mean.

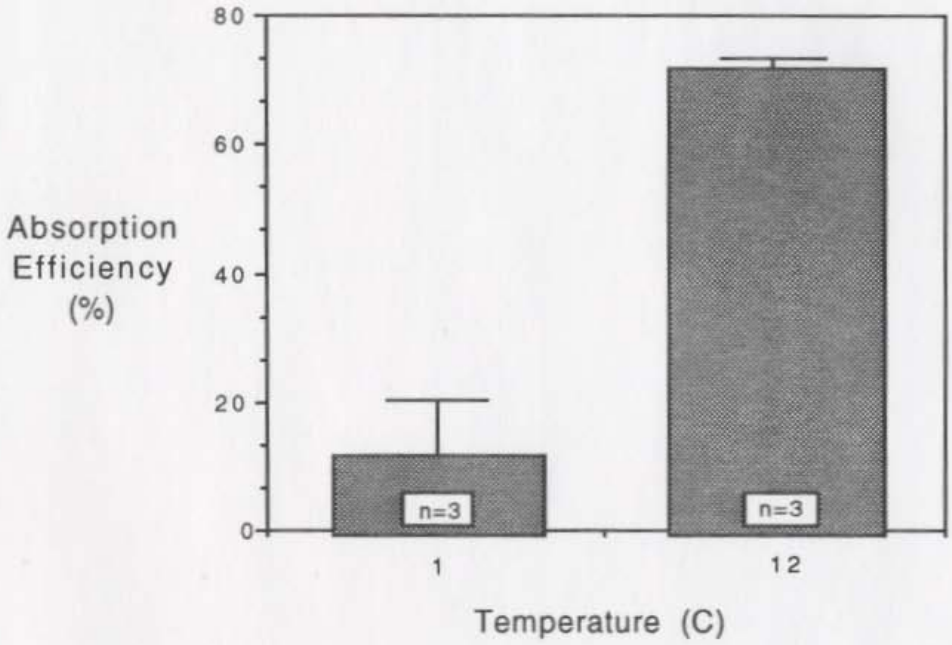

Fig. B.2: Effect of temperature on absorption efficiency of *M. arenaria* feeding on *Chaetoceros mulleri*. Error bars are the standard error of the mean.

Previous studies have also found contrasting results for the effect of temperature on AE. Like the results of this study, Winter (1969, 1977) and Elvin and Gonor (1979) found that AE was higher at higher temperatures. However, Bayne (1976) and Widdows and Bayne (1971) reported the opposite trend in *M. edulis*. Still other studies have found no relation between the two (Widdows 1978, Buxton et al. 1981). Unlike this study, Gilfillan et al. (1976) found assimilation ratios in populations of *M. arenaria* to decrease with increasing temperature. However, the assimilation ratios measured by Gilfillan et al. (1976) were determined, by radiolabelling of algae, as the ratio of dpm's present in the clam's tissues to dpm's cleared by the clams, and are therefore not strictly analogous to the AE measurements made in this study.

The GRT of *M. arenaria* was unaffected by the rise in temperature from 1 to 12°C. A similar result was documented by Hummel (1985) with *Macoma balthica*.

Although previous studies have determined a minimum thermal acclimation period of 14 days (e.g. Widdows and Bayne, 1971), it was not possible given our flow-through apparatus to grow enough *C. muelleri* algae to feed the animals for 2 full weeks. However, since the temperature adjustment was done slowly in this study (an increase of 1-2 °C each day, as opposed to complete, instantaneous changes in previous studies), and further acclimation was allowed for 2 days before testing, thermal shock should have been minimal at the time of experimentation.

Appendix C:

Morphological Information On *M. arenaria*

Introduction

Previous studies have generally found linear relations between shell length and tissue mass of a variety of bivalve species. The lengths and tissue masses of *M. arenaria* used in this study were measured to gain insight into the underlying morphological characteristics of each population, as well as for use in correcting certain physiological functions for body size.

Methods

Clams were shucked immediately upon completion of each laboratory experiment. When working at Platter's Cove, Terra Nova National Park, clams were frozen quickly with dry ice after each experiment, and kept frozen until return to the Ocean Sciences Centre where they were thawed and shucked. The left valve of each individual was measured with calipers from anterior to posterior tips to the nearest 0.1 mm. The dry soft-body weight of each clam was determined to the nearest 0.01 g by drying to constant weight at 80°C.

Results and Discussion

Fig. C.1 illustrates a positive relationship between shell length and dry tissue weight in clams from both the laboratory experiments (Riverhead population) and the field experiments (Platter's Cove population). Regression equations were calculated as:

Riverhead Population: $W = 2.176 \times 10^{-4} L^{2.208}$ $r^2 = 0.523$

Platter's Cove Population: $W = 1.208 \times 10^{-4} L^{2.278}$ $r^2 = 0.734$

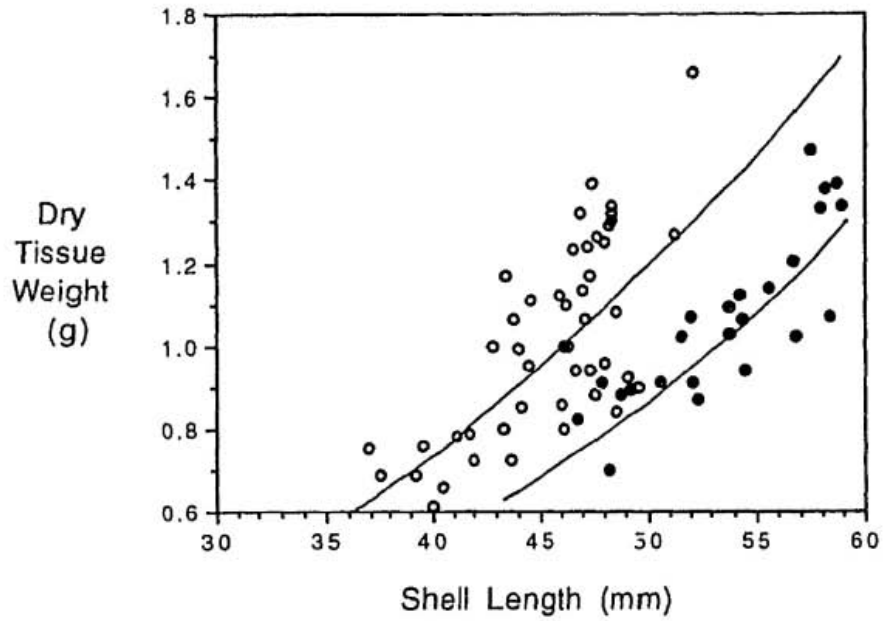

Fig. C.1: Relation between dry tissue weight and shell length in *M. arenaria* from Riverhead (o) and Platter's Cove (•) populations. Equations are:

Riverhead $W = 2.167 \times 10^{-4} L^{2.208}$

Platter's Cove $W = 1.208 \times 10^{-4} L^{2.287}$

These equations can be compared to those of Grant and Thorpe (1991) recorded for *M. arenaria* in Minas Basin, Nova Scotia, at $W = 2.32 \times 10^{-5} L^{2.68}$ and $W = 4.86 \times 10^{-2} L^{-0.58}$ in October and June respectively.

It should be noted that clams from Riverhead have a lower goodness of fit value than do clams from Platter's Cove. This can be attributed to two things: clams in the Riverhead sample were collected over many months, and therefore are more likely to show variation in weight / length ratios due to seasonal effects like gonadal ripeness. Furthermore, clams from Riverhead were more irregularly shaped.

Clams from the Platter's Cove population generally have a longer shell length per unit dry weight when compared to Riverhead clams. This is supported by visual observations: clams from Platter's Cove has longer more slender shells. Also, the shells from Platter's Cove were much thinner and more regularly shaped, whereas those from Riverhead were thicker and irregularly formed. These differences are most likely due to the differences in substrate type between the two locations: Platter's Cove has a soft, silty substrate whereas the Riverhead site consists mainly of coarse gravel.

Many studies have investigated the effects of substrate on growth and shell allometry of bivalves, including *M. arenaria* (Newell and Hidu 1982). In particular, *M. arenaria* has been observed to grow fastest in sand or sandy mud (Swan 1952, Newell and Hidu 1982). Also, shells from *M. arenaria* living in coarse sediments such as gravel have been described as rough, heavy and distorted (like those observed in clams from the gravelly Riverhead population) whereas those from sandy or muddy sediments are lighter and narrower (like those from the sandy Platter's Cove population) (Belding 1916, Swan 1952). Sediment type factors which may affect growth rate and shell morphology include 1) the effects of abrasion and chipping of the edges of the shell, 2) the position of the mantle in relation to the edge of the shell (increased abrasion could cause the mantle to retract, resulting in a thicker growth of the shell), 3) the degree of irritation caused by the substrate type, 4) the energy needs associated with a particular sediment type for activities such as maintaining a burrow, 5) the effect of sediment type on the physico-chemical environment of the clam, and 6) the position of the clam in the substratum (Swan 1952). Furthermore, muddy areas may be more nutrient-rich and provide a higher quality of food than gravelled areas.

Appendix D

Determination of a Criterion to Describe Gut Retention Time

Preliminary GRT Analysis

Scatter plots of organic marker (O.M.) and inorganic marker (I.M.) (see section 3.2.6 for details) against the time the sample was taken were constructed for each treatment clam. These, henceforward, will be referred to as "marker profile graphs". For each graph, the model $Y=\alpha Kte^{-Kt}$ (Bayne et al.1984) was fitted to the data using an iterative least squares procedure. GRT was then determined, by integration of the curve, as the point on the x-axis (Time) corresponding to 90% of the area under the regression line (Bayne et al. 1987, Hawkins et al. 1990). Fig. D.1 shows several examples of these graphs, two showing good fit to the regression model (Fig. D.1 A and B) and two showing poorer fit to the model (Fig. D.1 C and D). This model successfully described only 40% of the graphs - those with the maximum amount of marker passing through within the first 4 h. If the maximum peak occurred later, or if there was a double peak (i.e. bimodal distribution), the model was inappropriate. It should be noted at this point that, due to the non-linearity of the regression equation, there are no goodness of fit measures for these graphs. The appropriateness of the model was therefore determined by examination of residuals, and visual comparison of the regression line and the observed values.

To determine the average gut retention response for all clams within one diet treatment, the data from the marker profile graphs were pooled into a series of "pooled profile graphs", two examples of which are given in Fig. D. 2. To do this, values of both O.M. and I.M. were first standardized on a proportional scale, necessary because some clams ingested more marker particles than others. The mean standardized O.M. and I.M. values were then plotted as a function of the time at which those samples were taken. Again, the $y = \alpha Kte^{-kt}$ model was fitted to these data. The regression model adequately described 5 of the 8 pooled profile graphs. Error bars on the graphs were large due to the high amount of individual variation between clams. This method of analysis was also deemed unsuccessful.

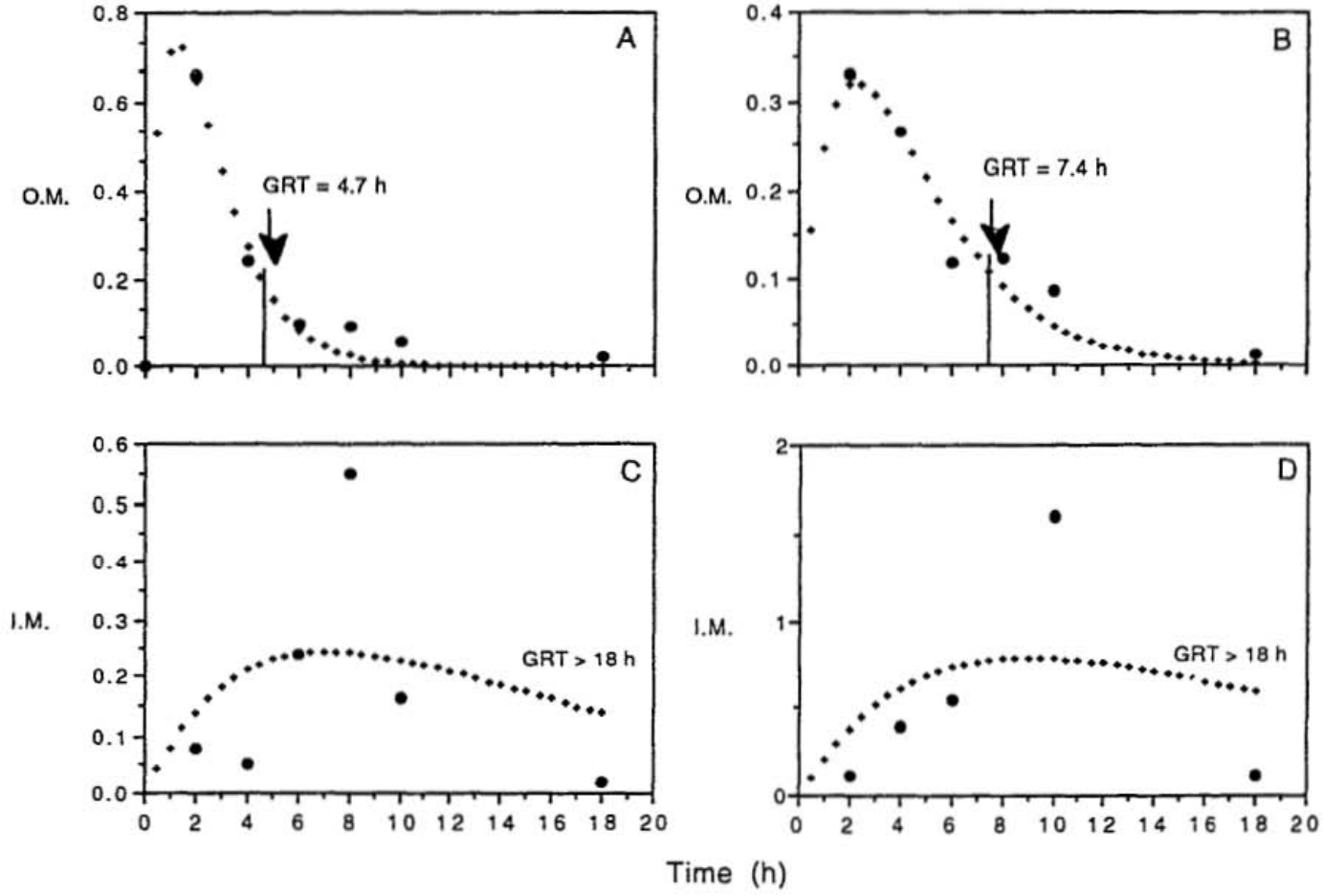

Fig. D.1: Example of marker profile graphs showing good (A and B) and poor (C and D) fit to the non-linear model described in Appendix D. Circles are observed values: crosses are fitted values.

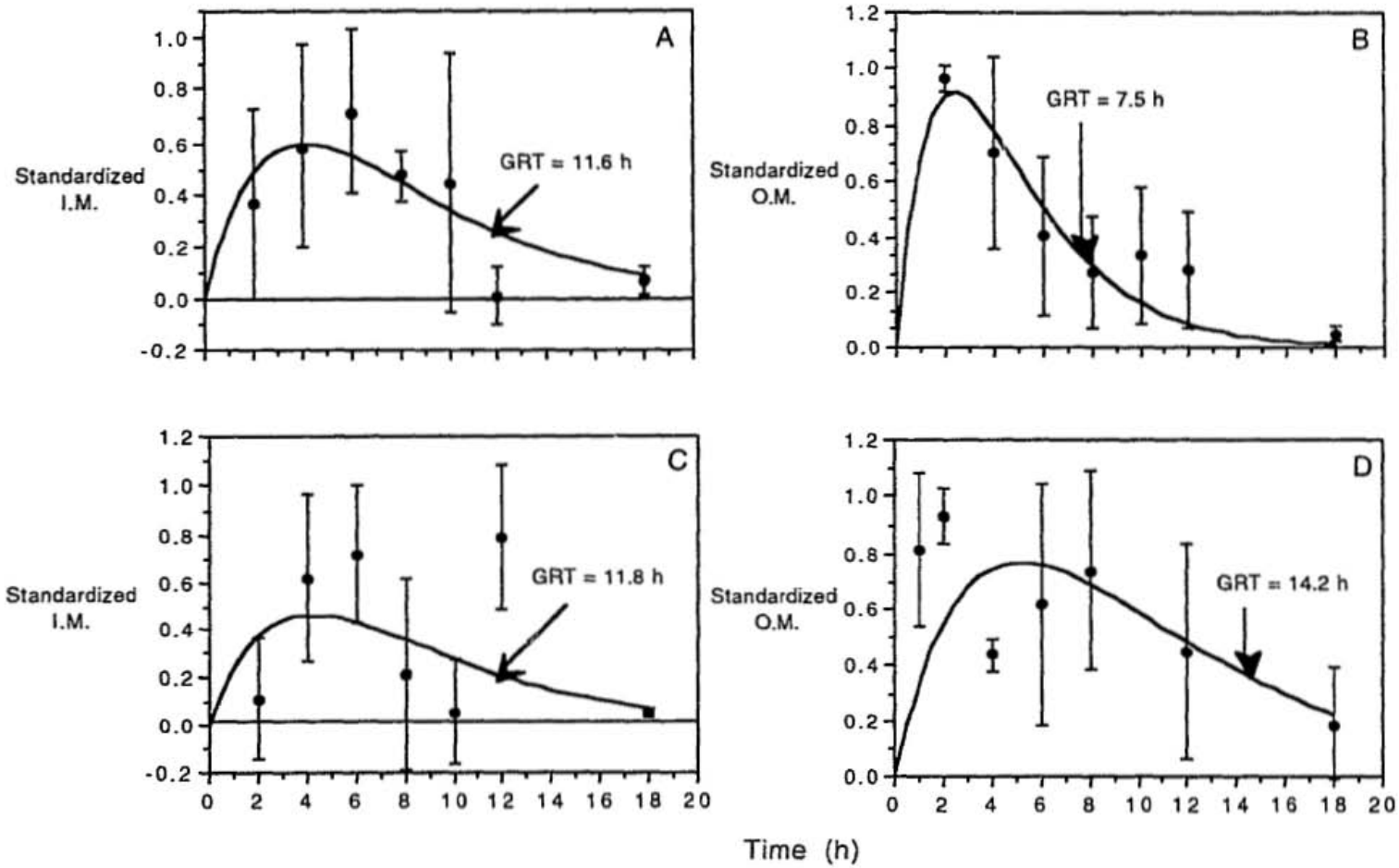

Fig. D.2: Examples of pooled profile gra[illegible] showing good (A and B) and poor (C and D) fit to the non-linear model described in Appendix D. n = 6 for each, error bars are standard deviations.

To successfully fit regression lines to all graphs, several different mathematical models would have to be used, often within one diet treatment. It was decided that another method for determining GRT would have to be used.

Secondary GRT Analysis

Two new criteria were proposed for the determination of GRT: 1) the time at which O.M. or I.M. reaches a maximum in the marker profile graphs, reconfigured as bar graphs, and 2) the medians of these graphs.

When the average GRT for each diet as determined by each of the above two criteria are compared, the trends are identical (Fig. D.3) with only slight differences in significance levels. Therefore, both the maximum peak and the median appear appropriate criteria for determining GRT. Since the maximum peak may not always be easy to identify (if there are two peaks of similar size, or if a peak spans more than one time interval), the median was chosen as the criterion for determining the GRT of each individual. Although median values have not commonly been used in the bivalve literature, this appears to be the common method in finfish research (e.g. Nobel 1973, Mills and Forney 1981, Cochran and Adelman 1982, Rice et al. 1983). Marker profile graphs and the corresponding GRT of two individuals using the median time interval are illustrated in Fig. D.4. In both Fig. D.4 A and B, the clam has a GRT of 4 h and 6 h for the organic and inorganic fractions respectively.

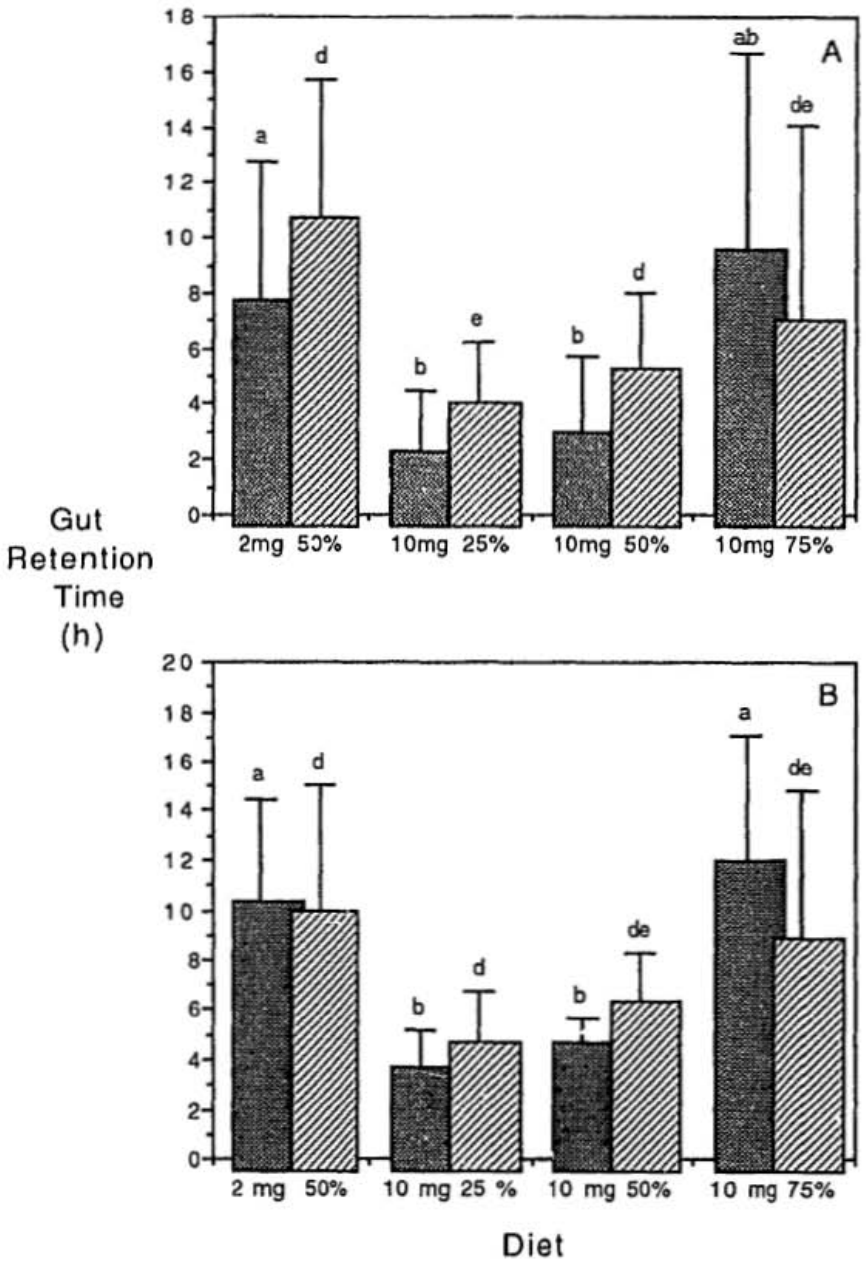

Fig. D.3: Gut retention time of organic (solid bars) and inorganic (striped bars) marterial determined by the time interval containing the median (A) and maximum (B) values. Vertical bars = standard errors, n = 6. Similar letters above each bar indicate no significant difference at alpha = 0.05, Wilcoxon Rank Test. Note: There were no significant differences in the gut retention times of organic and inorganic fractions within each diet at alpha = 0.05, Wilcoxon Rank Test.

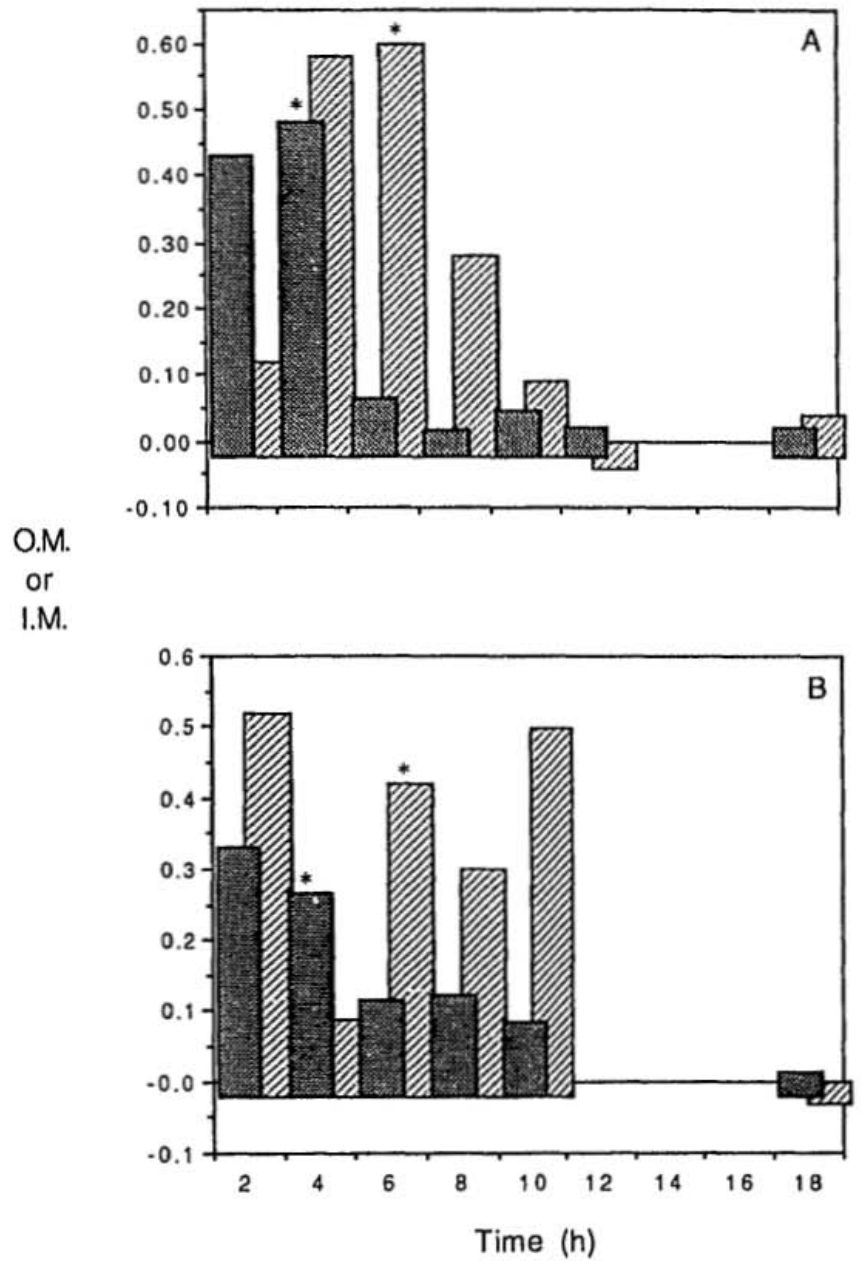

Fig. D.4: Examples of marker profile graphs of the amount of organic marker O.M. (solid bars) and inorganic marker I.M.(striped bars) in faecal samples collected from two *M. arenaria* (A and B) over an 18 h time period after a 30 min exposure to marker particles. An asterisk (*) indicates the bar containing the median value and hence the gut retention time designation. Note that both clams illustrated here retained the inorganic fraction longer than the organic fraction.

Appendix E

Effect of Lugols and Formalin Fixatives on Seawater Particle Counts

Introduction

The purpose of this experiment was to determine whether preservation of seawater samples in 1% Lugols fixative and 1% formalin significantly altered particle counts.

Methods

Two 100 ml seawater samples were collected from the main laboratory seawater lines. The concentration of particles in each sample was determined immediately with a Coulter Multisizer fitted with a 100 µm diameter orifice tube. One ml Lugols fixative was added to each sample, followed by 1 ml formalin after gentle mixing. Over the next 24 h, one sample was kept in the light, while the other was kept in darkness. The concentration of particles in each sample was determined 3.5 h and 24 h after addition of the fixatives.

Results

Addition of the Lugols fixative and formalin did not significantly change particle counts in either seawater sample (Table E.1, Paired T-tests, $\alpha = 0.05$)

Table E.1: Means and standard deviations of particle concentrations (particles ml^{-1}) of seawater samples before and after treatment with 1% each Lugols and formalin fixatives. There are no significant differences between initial and later concentrations of each sample.

Sample	Initial		3.5 h		24 h	
Dark	Mean	3845	Mean	3759	Mean	3880
	S.D.	168.2	S.D.	51.6	S.D.	131.8
	n	3	n	3	n	3
Light	Mean	2198	Mean	2176	Mean	2239
	S.D.	100.6	S.D.	243.5	S.D.	119.1
	n	3	n	3	n	3

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