POSTINGESTIVE SORTING OF PARTICLES WITHIN THE SEA SCALLOP *PLACOPECTEN MAGELLANICUS* (GMELIN)

by

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This thesis is accepted.

Dean of Graduate Studies

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Dedication

This thesis is dedicated to the memory of my brother, Reuben Nathaniel Smith,

June 14, 1974 - July 6, 1997.

Abstract

Suspension-feeding bivalves feed on a mixture of particles which fluctuate dramatically in quality and quantity over time. Bivalves are adapted to deal with such a diet by sorting particles both before and after ingestion to increase the energy value of the food ingested and to reduce energy losses associated with digestion. Preingestive selection has been well studied but postingestive sorting in intact bivalves has been confirmed for only a few species. Theoretically, postingestive selection may occur by preferentially directing certain particles to the digestive gland for intracellular digestion or by retaining certain particles within the gut longer than others to enhance extracellular digestion. However, the role of particle characteristics in postingestive selection has not been established. The objectives of this research were to determine if the sea scallop, *Placopecten magellanicus* (Gmelin), has the ability to sort particles within the gut and to determine the effects of particle characteristics (both physical and chemical properties) on particle processing. Experiments with radiolabelled algae and beads were used to confirm the ability of scallops to sort particles within the stomach. The role of particle characteristics was examined by feeding scallops mixtures of particles of different sizes, densities or chemical properties and comparing their gut retention times. Scallops retained larger beads (20 μm) longer than smaller beads (5 μm), and lighter beads (1.05 g ml^{-1}) longer than denser beads (2.5 g ml^{-1}). Furthermore, beads coated with protein were retained longer than uncoated beads and live algae were retained longer than dead algae. The ability of scallops to pass small, dense particles

through the gut quickly may be beneficial as most suspended inorganic material is small and dense. Also, the ability to distinguish between particles on the basis of subtle chemical cues would enable scallops to preferentially retain particles of higher food quality longer than those of poor quality. These abilities would work in concert in the natural environment to allow scallops to increase the amount of energy derived from their food supply while minimizing energetic costs associated with digestion. This research constitutes the first quantitative analysis of the effects of particle size, density and chemical composition on particle processing within intact bivalves.

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Chapter I: General Introduction

I. 1. Energetics

Bivalves play an important role in many estuarine and coastal environments, often dominating the macrofauna in such areas. Many species of bivalves are also commercially important as cultured and/or harvested species. An understanding of the energetics of these animals is necessary in order to comprehend energy flow and productivity of benthic ecosystems and to increase productivity of commercial species (Griffiths and Griffiths 1987).

Modelling bivalve energetics involves using the following basic equation which describes net energy exchange in an individual organism:

$$C = P + R + F + U$$

where C (consumption), is the energy equivalent of the food intake; P is the energy incorporated into reproductive products or growth; R is the energy equivalent of metabolic heat losses; F is the energy content of the ingested material that is lost as faeces; and U is the energy lost as secretions or excretions (urine, mucus). The objective for the animal is to balance gains from the environment against metabolic losses in order to have surplus energy to allocate to somatic growth and reproduction (Bayne and Newell 1983).

In their natural environment, benthic suspension-feeders are presented with a

mixture of particles that vary significantly in concentration and composition over time (Widdows et al. 1979; Berg and Newell 1986; Smaal et al. 1986; Fegley et al. 1992; Bayne 1993). Particle selection is one strategy used by suspension-feeding bivalves to enhance the quality of food and optimize energy gain. Bivalves may be able to control the energy content of their diet (C), through particle selection prior to ingestion. After ingestion selection may have an impact by reducing metabolic losses (R), since less digestive effort is wasted on indigestible or poor quality particles. Postingestive selection may also reduce faecal losses (F) as the energy content of the processed material may be more efficiently extracted if digestive effort is concentrated on the higher quality fraction.

This research deals with the capabilities of lamellibranch bivalves to sort particles postingestively. The capability of one species of lamellibranch bivalve to select particles within the gut will be studied and the particle properties upon which selection is based will be investigated.

I. 2. Suspension Feeding

Suspension-feeding bivalves feed by drawing water into the mantle cavity and across their gills. Beating cilia on the gills and mantle create a pressure gradient which draws water in through the inhalant siphon. Particles within the water are captured by the gill filaments, and are passed to the ventral ciliated grooves or the dorsal ciliated tracts of the gills. The particles are then transported to the labial palps either as mucus-

bound particle strings or suspended in particle slurries. The palps disperse the bound particles and either reject them as pseudofaeces or pass them along to the mouth where they are ingested (Ward 1996). Food passes into the stomach, then on through the intestine and is expelled as faeces.

There are three points along this pathway from particle capture to production of faeces where particle selection may occur. These are: on the gills, on the labial palps, and within the stomach (Figure 1).

I. 2. i. Selection on the Gills

Selection on the gills is mainly by size; most bivalves cannot retain particles that are much smaller than the gill ostia. For a number of suspension-feeding bivalves retention efficiency declines rapidly for particles less than 2 μm (Mohlenberg and Riisgard 1978); most bivalves cannot retain particles smaller than 1.5 μm in diameter (Griffiths and Griffiths 1987). There is evidence that some bivalves may be able to regulate their retention efficiency (Haven and Morales-Alamo 1970; Bayne et al. 1977; Palmer and Williams 1980; Cranford and Gordon 1992; Stenton-Dozey and Brown 1992; Barillé et al. 1993; MacDonald and Ward 1994). Some species are able to use their gills to sort particles on the basis of their chemical or nutritional properties as well (Ward et al. 1998).

I. 2. ii. Selection on the Palps

The labial palps are also capable of sorting particles on the basis of their

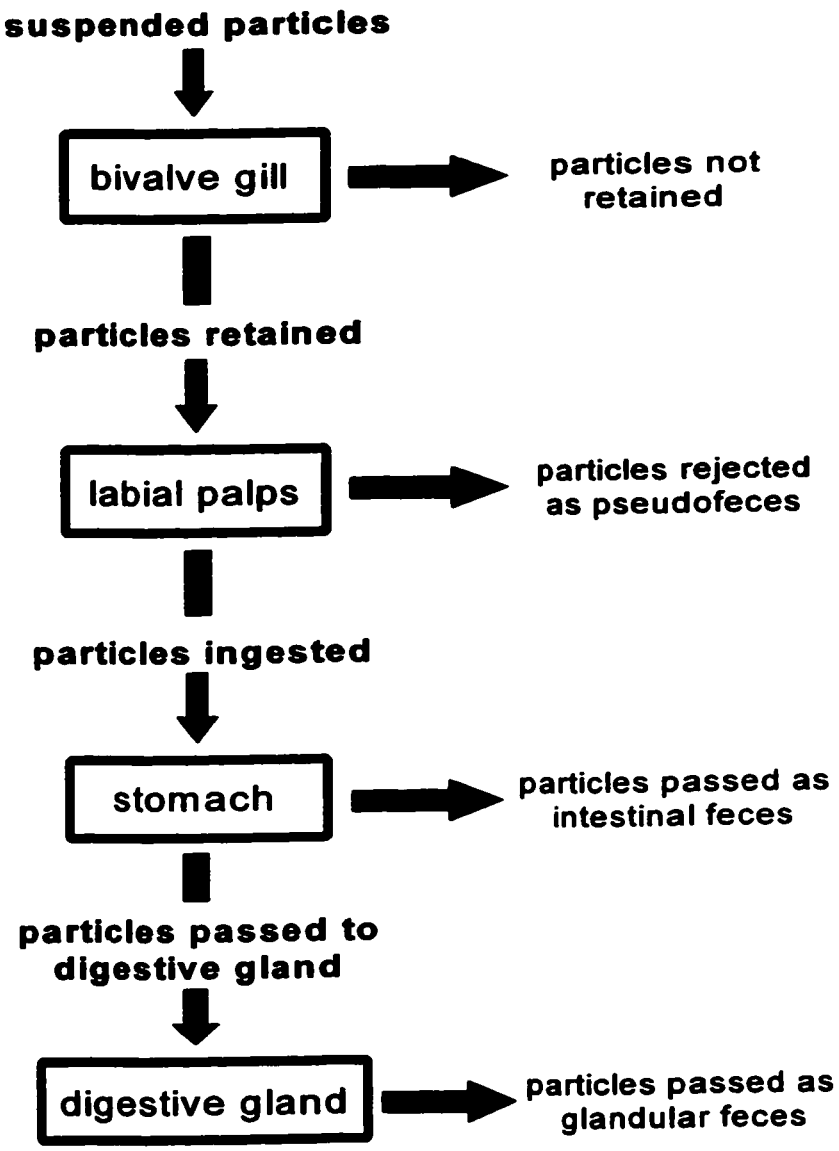

Figure 1: Schematic diagram of the pathway of food particles through a suspension-feeding bivalve.

chemical or nutritional properties (Kiorboe and Mohlenberg 1981; Newell and Jordan 1983; Ward and Targett 1989; Cranford and Gordon 1992; Iglesias et al. 1992; Navarro et al. 1992; Urban and Kirchman 1992; Bayne et al. 1993; MacDonald and Ward 1994; Hawkins et al. 1996; Iglesias et al. 1996; Ward and MacDonald 1996). The palps disperse the mucus-bound particles and sort them. Many bivalves will preferentially select more nutritious particles for ingestion and reject poorer quality ones. The rejected particles are then expelled through the inhalant siphon as pseudofaeces (Newell and Jordan 1983; Ward et al. 1994; Ward and MacDonald 1996).

This ability to manipulate the quality of food ingested may be of great benefit to the animal by enhancing the energy value of its local food supply. This benefit is most pronounced when selection is acting upon high concentrations of poor quality diets (Navarro and Iglesias 1993).

I. 2. iii. Selection within the Stomach

Suspension-feeding bivalves may be able to enhance the energy value of their diet after ingestion through selection mechanisms within the stomach. It is thought that some bivalves can differentiate between particles within the stomach on the basis of their chemical or physical properties and preferentially digest those particles that give the most nutritional benefit (Bricelj et al. 1984; Shumway et al. 1985; Lopez and Levinton 1987; Bayne 1993; Wang and Fisher 1996). This postingestive selection is the subject of this research project and will be discussed in detail in the following sections.

I. 3. Stomach Structure and Function

I. 3. i. Stomach Structure

The stomach of lamellibranch (or polysyringian) bivalves is complex (Figure 2). Typically the stomach is a sac with the esophagus entering anteriorly. The midgut leaves posteriorly accompanied by the style sac which may be separate from the midgut or may communicate with it through a narrow slit. The crystalline style (a flexible rod of muco-protein) originates within the style sac and projects across the stomach abutting onto the gastric shield. A dorsal hood originates from the roof of the stomach and opens dorsally or to the left. This hood varies in size and shape in different species from a narrow tapering pocket to a large pouch. Ducts from the digestive gland, which surrounds the stomach, open onto the right and left walls of the stomach; those on the left may be clustered and open into an embayment called the left pouch. There are also four main ciliary tracts and several ciliated grooves. The left and right duct tracts which lead to the duct openings on the left and right walls respectively, are bounded by two low narrow ridges, the major and minor typhlosoles, which originate near the midgut opening. A ciliated groove is associated with the major typhlosole and is called the intestinal groove. Three types of caeca may also be found in the bivalve stomach: the sorting caecum, the duct caeca and the appendix (Reid 1965).

I. 3. ii. Stomach Function

The function of the bivalve stomach has been inferred from its morphology and

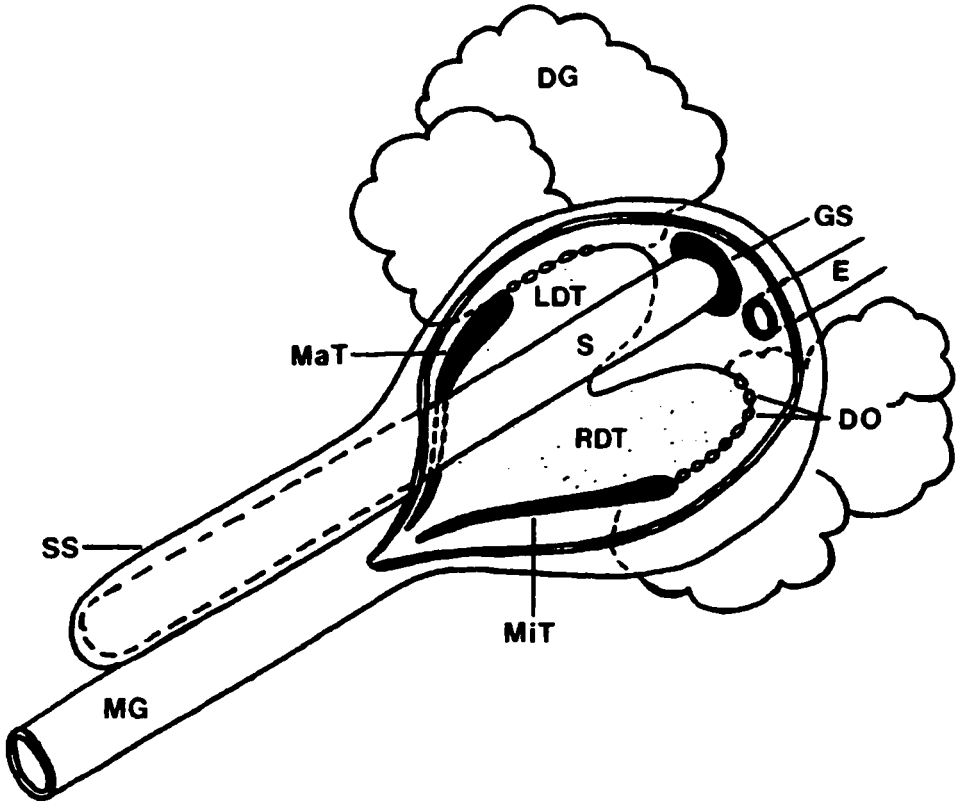

Figure 2: Simplified diagram of a typical polysyringian bivalve stomach. DG = digestive gland, DO = duct openings, E = esophagus, GS = gastric shield, LDT = left duct tract, MaT = major typhlosole, MiT = minor typhlosole, MG = midgut, RDT = right duct tract, S = style, SS = style sac. Dorsal hood is not shown (after Reid 1965).

histology, and through observing the movements of the cilia in dissected but still living animals (Yonge 1923; Reid 1965; Purchon 1977; reviewed by Purchon 1987).

The style sac is lined by ciliated cells and the movements of these cilia cause the rotation of the style. As food enters the stomach in a mucous string, the string is wound around the rotating style. As the style rotates it triturates the particles against the chitinous gastric shield. The style slowly dissolves, releasing enzymes which participate in extracellular digestion. The movements of the style and the beating of the various ciliated tracts set up a circulation of fluids within the stomach. Large particles are recirculated until they are broken down. The ciliated tracts mentioned above are typically formed into a complex pattern of folds and ridges to create sorting areas; small heavy particles fall into the folds of the tracts and are carried to the intestinal groove where they are passed into the midgut and become faeces (Figure 3). Lighter particles are passed along the ridges of the tracts towards the digestive diverticula. Ducts of the digestive gland consist of ciliated primary ducts at the entrance branching into secondary ducts and then to blind-ending tubules where intracellular digestion occurs. Long cilia found on one side of the primary ducts beat toward the stomach; this creates a counter-current flow which draws in particles from the stomach along the top of the duct while expelling waste material along the bottom (Reid 1965; Purchon 1977; Morton 1983).

In more complex stomachs the digestive diverticula are gathered into clusters and open as a group into caeca. Also the end of the major typhlosole is drawn out into a tongue which may protrude into the openings of these caeca. Within the caeca the

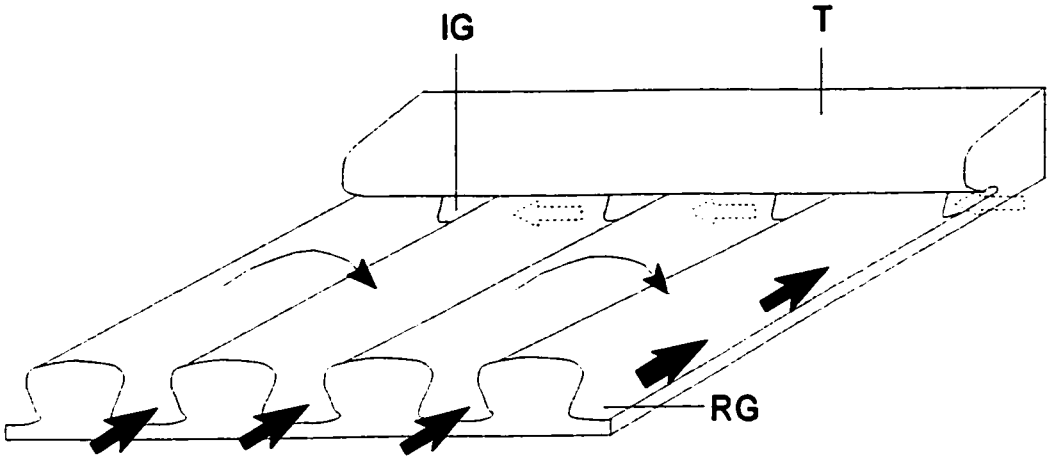

Figure 3: Portion of a ciliated tract showing ridges and grooves. IG = intestinal groove, RG = rejection groove, T = typhlosole. Thin arrows indicate movement of light particles, solid arrows represent movement of small heavy particles, stippled arrows indicate direction of movement within the intestinal groove (after Reid 1965).

tongue frequently forms a tube within a tube, sending a branch into each diverticular aperture. The inner tube is the incurrent passage into the diverticulum carrying sorted materials for intracellular digestion; the outer tube is an excurrent passage carrying waste materials from the digestive cells to the intestinal groove (Reid 1965; Purchon 1977; Morton, 1983) (Figure 4).

Digestion in the polysyringia is mainly intracellular, occurring within the cells of the digestive gland, although some extracellular digestion does occur in the stomach and absorption occurs across the walls of the intestine (Reid 1965; Purchon 1977; Morton 1983). It is believed that food passed directly through to the intestine will result in faeces that have been poorly digested (intestinal faeces), while food processed in the digestive gland will produce well-digested waste material (glandular faeces) (Widdows et al. 1979). Furthermore intestinal faeces should be produced sooner after feeding than glandular faeces (Foster-Smith 1975; Bricelj et al. 1984; Decho and Luoma 1991).

I. 3. iii. Stomach Types

Stomach structure across the Bivalvia is relatively uniform but some variation does occur (Morton 1983). Purchon (1956, 1957, 1958, 1960) has classified bivalve stomachs into five types. Types I and II belong to the protobranch and septibranch bivalves respectively, while types III, IV and V belong to the lamellibranch bivalves. Types III, IV and V are also known as the polysyringian bivalves, those bivalves with many ducts of the digestive gland opening into the stomach. Within the polysyringian bivalves, Type III stomachs are characterized by a narrow tongue extending from the

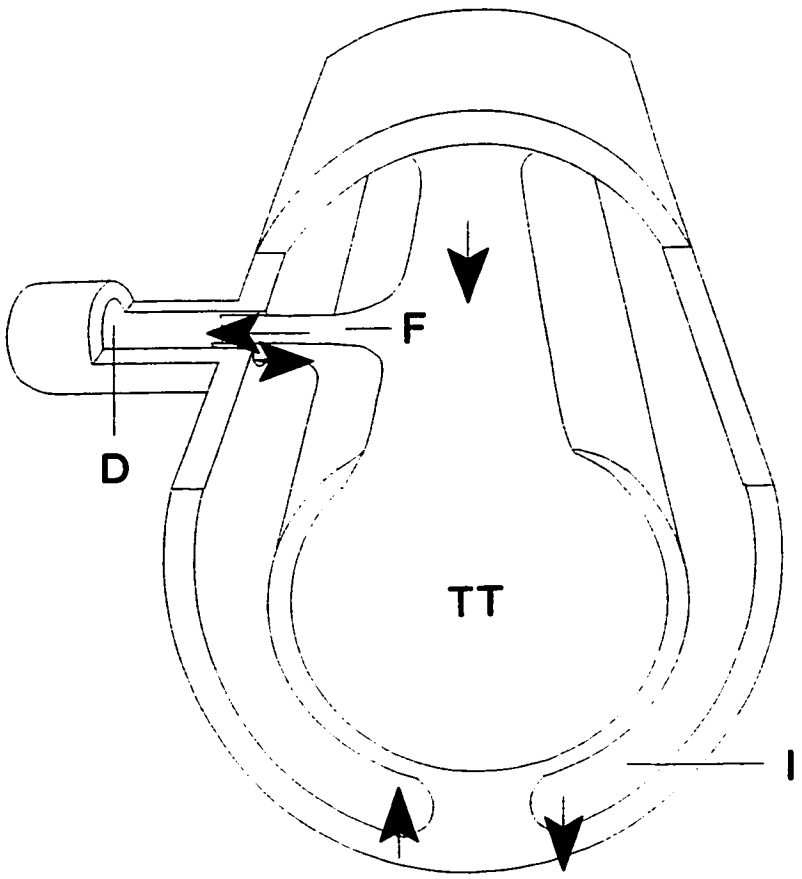

Figure 4: Diagram showing the tube within a tube arrangement of the tongue of the major typhlosole. D = duct aperture, F = flare of the typhlosole tongue, I = intestinal groove, TT = tongue of the major typhlosole. Arrows indicate the direction of movement of food particles (after Reid 1965).

major typhlosole to the left side of the stomach, while Type IV stomachs lack a tongue. In the Type V stomach the major typhlosole is drawn out into two broad tongues which enter the right and left duct caeca. Other differences are seen in the position and extent of the sorting areas (ciliated tracts).

Amongst the polysyringian stomach types, certain features are considered more advanced. These are: extensive development of the sorting areas, gathering together of the digestive gland duct orifices into clusters, development of deep embayments of the stomach wall into which the ducts open and the development of a relationship between the tongue of the major typhlosole and such embayments (Purchon 1957). These features have been interpreted as implying an enhanced sorting ability to prevent large or otherwise undesirable particles from entering the digestive gland ducts (Purchon 1957, 1958, 1960). However this interpretation has not been tested.

I. 4. Postingestive Selection

The structure of the stomach with its ciliated tracts and ducts suggests that selection does occur here and observations on dissected animals indicate that the ciliated tracts can sort heavy from light particles. However, observations on dissected structures may be unreliable as dissection interferes with fluid dynamics and may disrupt current flow. For example, dissection has been known to cause feeding structures to function abnormally (Ward 1996). Aside from these observations on dissected bivalves, a few studies have found evidence for postingestive selection in

intact bivalves (Hughes 1977; Bricelj et al. 1984; Wang and Fisher 1996; Gagnon and Fisher 1997). However, there have been few attempts to isolate which factors prompt postingestive selection, whether they be physical or chemical.

Postingestive selection may occur either by the retention of some particles longer than others in the stomach so that extracellular digestion has more time to act, or by directing some particles to the digestive gland for intracellular digestion. Theoretically, bivalves should retain the higher quality fraction of a diet for further processing and reject the poorer quality or indigestible fraction to the intestine (Lopez and Levinton 1987). This would minimize the energy lost by attempting to digest indigestible material. In either case gut retention times (GRTs) can be used to indicate if sorting has occurred. Studies on postingestive selection typically use GRTs to indicate sorting within the gut (Bricelj et al. 1984; Decho and Luoma 1991; Wang and Fisher 1996; Gagnon and Fisher 1997).

Bricelj et al. (1984) found evidence for postingestive selection in the hard clam, *Mercenaria mercenaria*. Using the ^{51}Cr:^{14}C twin tracer technique, they found that two algal species known to be utilized with different efficiencies by the clam were passed through the gut at different rates. An alga known to be utilized very poorly was passed through much quicker than one known to be used efficiently. This was interpreted as indicating that *M. mercenaria* can distinguish between different algal species within the gut and select the more digestible species for processing through the digestive gland while the other simply passes through to the intestine. Furthermore, it was found that ^{51}Cr passed through more quickly than ^{14}C, even though algal cells were labelled with

both markers. This was explained by noting that ^{51}Cr binds to the more indigestible cell parts, such as the cell wall, while ^{14}C is incorporated into the cell contents. Possibly the cells were disrupted within the stomach and the more digestible parts carried to the digestive gland for intracellular digestion while the less digestible parts were rejected to the midgut (Bricelj et al. 1984).

Wang and Fisher (1996) found evidence for postingestive selection in the blue mussel, *Mytilus edulis*. In this study radiolabeling was used to measure GRTs and assimilation efficiencies (AE's) of several species of algae and glass beads. Each algal diet was labelled with several different trace elements. These researchers found that the GRTs varied amongst the different algal species with indigestible chlorophytes being expelled fastest. This indicated that *M. edulis* is capable of distinguishing between different algal species in the gut. Similar to what was observed by Bricelj et al. (1984), this study also found that, with the exception of the chlorophytes, the AE's of all of the trace elements were significantly correlated with their cytoplasmic penetration within each algal cell for all algal species. This was interpreted as indicating that as algal cells break up within the stomach, the cytoplasm with its associated trace elements is processed in the digestive gland and the relatively indigestible cell wall with its trace elements undergoes only extracellular digestion in the stomach before being expelled as faeces.

Gagnon and Fisher (1997) provided evidence for selection by chemical properties. They fed *Mytilus edulis* radiolabelled inorganic particles both coated with organics and uncoated, and found that the labels associated with the coated particles

were retained longer on average than the labels of the uncoated particles. However, Decho and Luoma (1991) found no evidence of selection between bacterial food and latex beads fed to two bivalve species, *Potamocorbula amurensis*, a suspension-feeder, and *Macoma balthica*, a deposit-feeder capable of suspension-feeding. Although they found evidence for a glandular pathway and an intestinal pathway, there was no difference in the gut retention times of the beads and bacteria.

In all of these studies the materials being traced were always presented to the animals separately, never in combination. Presenting marker particles at different times is a common method of testing for particle selection (Bricelj et al. 1984; Decho and Luoma 1991; Wang and Fisher 1996; Gagnon and Fisher 1997); however, if there is no choice of particles given, there may be no reason for selection to occur. If the bivalve has any control over selection, it may not select unless presented with a mixture of desirable and less desirable particles. However the most recent study on postingestive selection (Penry 2000) found that *Potamocorbula amurensis* did not distinguish between 9 and 44 μm beads when they were presented simultaneously, although some individuals appeared to pass the two sizes of particles at different rates.

The above-mentioned studies provide some support for the presence of postingestive selection in at least two species of bivalves. However, there has been little attempt to isolate which factors prompt selection. These factors may be physical (size and/or density) or chemical, or some combination of physical and chemical properties. Combinations of physical and chemical properties might come into play, for example, during digestion of algae. The biochemical structure of the cell wall would determine if

mechanical and chemical breakdown within the stomach would release the cytoplasm, at which point selection might be based on the physical nature of the cell components (fluid vs. solids).

I. 5. Objectives

The objectives of this research were to investigate the ability of a lamellibranch bivalve to sort particles within the stomach and to determine which factors are the basis for selection.

Placopecten magellanicus (Gmelin), the sea scallop, was chosen as the study animal because it is readily obtained and easy to work with in the laboratory. This species is commercially important as it is harvested in the Northwest Atlantic, from Virginia to Newfoundland, including the Bay of Fundy (Naidu 1991). There is ongoing research into the possibility of culturing this species (Dadswell 1989; Naidu 1991; Miron et al. 1996; Harvey et al. 1997; Barbeau et al. 1998; Frechette et al. 2000; Penney and Mills 2000). Much is known about the ecology and physiology of this animal (Thompson 1977; MacDonald and Thompson 1985a, 1985b, 1986a, 1986b; MacDonald et al. 1987; Thompson and MacDonald 1990; Brand 1991; MacDonald and Bayne 1993) including its feeding physiology (Beninger et al. 1992; Ward et al. 1992; Cranford and Gordon 1992; Cranford and Hargrave 1994; MacDonald and Ward 1994). Preingestive selection has been well-established in the sea scallop (Shumway et al. 1985; MacDonald and Ward 1994). Scallops have a type IV stomach (Purchon 1987).

The ability of *Placopecten magellanicus* to sort particles within the stomach is covered in Chapter II. Selection by physical properties (size and density) is discussed in Chapter III. Selection on the basis of chemical properties is covered in Chapters IV and V.

Chapter II: Postingestive Selection, Beads vs. Algae

II. 1. Introduction

Postingestive selection has been observed in at least two species of bivalves. Bricelj et al. (1984) found selection in the hard clam *Mercenaria mercenaria* while the blue mussel *Mytilus edulis* has also shown postingestive selection (Wang and Fisher 1996; Gagnon and Fisher 1997). Postingestive selection in the sea scallop, *Placopecten magellanicus* has not been studied specifically. The only study in which postingestive selection in *P. magellanicus* has been implied is Cranford et al. (1998). This study found that two sizes of polystyrene beads were passed through *P. magellanicus* at different rates, however, whether this is evidence for postingestive selection is questionable. The particles were presented separately at a 2 h interval during which time the animals were feeding on ambient food of fluctuating quality and quantity. Moreover, the purpose of using the beads was simply to estimate the gut passage time of the ambient food present in the water column at the time the markers were presented, not to test for postingestive selection.

In most studies on postingestive selection, marker particles are fed to bivalves separately and the gut retention times of the markers are subsequently compared (Bricelj et al. 1984; Decho and Luoma 1991; Wang and Fisher 1996; Gagnon and Fisher 1997). However in their natural environment bivalves feed on a heterogenous

mixture of inorganic and organic particles of various sizes and densities (Widdows et al. 1979; Berg and Newell 1986; Smaal et al. 1986; Fegley et al. 1992). In order to test the ability of bivalves to sort particles under more natural conditions marker particles should be presented to the bivalves simultaneously. Bivalves may not retain one type of particle longer than another if no opportunity for choice is given.

The objective of this study was to determine if the sea scallop *Placopecten magellanicus* has the ability to sort particles within the stomach. This was tested by presenting scallops with a mixture of two very distinct particles, beads and algae, of similar size and shape. Theoretically, postingestive selection may occur in two ways, by retention of one type of particle within the gut longer than another so that digestion has more time to act or by directing certain particles to the digestive gland for intracellular digestion. The second method of selection was monitored in this study by dissecting the scallops at intervals after feeding and comparing the ratio of the two marker particles in the stomach to those in the digestive gland and faeces.

II. 2. Materials and Methods

II. 2. i. Collection and Holding of Animals

Scallops (*P. magellanicus*, 52 -83 mm shell height) were collected by divers near Deer Island, New Brunswick (45°00'12"N, 66°55'30"W). Scallops were transported to the University of New Brunswick, Saint John Campus (UNBSJ), where they were held in a recirculating seawater tank (salinity 32 ‰, temp. 14°C). The

scallops were fed *Dunaliella tertiolecta* and were held for 1 week before the experiment.

II. 2. ii. Preparation of Markers

The dinoflagellate, *Prorocentrum minimum* (Pavillard) Schiller (clone CCMP1329, Provasoli-Guillard National Center for Culture of Phytoplankton) (a rounded algae of 12 - 19 μm diameter), was grown in F/2-Si media under lights (18 h light : 6 h dark) at 25 °C. When the culture had reached 6000 cells ml^{-1}, 1 μCi per 100 ml of media of ^{14}C in the form of sodium bicarbonate ($NaH^{14}CO_3$), was added to the culture. The algae were grown in the presence of ^{14}C for four days. An identical culture was grown alongside but not radiolabelled in order to check cell concentrations. After four days the algae were filtered onto 1 μm nylon filters, washed with filtered seawater to remove any unassimilated ^{14}C and resuspended in filtered seawater.

Beads made of divinylbenzene (DuPont NEN®) (16 - 18 μm) labeled with ^{51}Cr (500 μCi) were mixed with identical unlabeled beads, suspended in filtered seawater and stirred overnight.

II. 2. iii. Experimental Design

Twenty-seven scallops were placed individually in 1 L beakers within a water bath (temp. 13-14 °C) 1 h before commencing the experiment. Radiolabelled algae and beads were added to twenty-four of the beakers to a final concentration of 10 000 particles ml^{-1} (5000 particles ml^{-1} of each); the remaining three scallops were not fed.

The scallops were allowed to feed on the radiolabelled particles for 1 h. Water samples (3 ml) were taken at the beginning and end of the hour and subsequently analyzed for ^{14}C and ^{51}Cr to measure the removal of particles from the water, however clearance rates were not measured. After the 1 h exposure time the radioactive water was replaced with natural seawater supplemented with *Dunaliella tertiolecta*.

The three scallops that had not been fed were frozen and dissected immediately (t = 0 h) to serve as controls. Three scallops were then frozen at each of the following times, t = 0.5, 1, 1.5, 2, 2.5, 3, 4, and 5 h and subsequently dissected.

II. 2. iv. Dissection, Treatment and Analysis of Tissues

The right valve of each scallop was removed, then the animal was immediately immersed in liquid nitrogen for 30 s, removed, and allowed to partially thaw for several minutes. The right stomach wall was sliced open and the frozen stomach contents were scooped out. The entire digestive gland was also removed and any faeces produced were collected.

The stomach contents and faecal samples were placed in separate scintillation vials and solubilized with 1 ml of Scintigest (Fisher Co.). The digestive glands were treated with 5 ml of Scintigest. All of the samples were subsequently homogenized and sub-samples were taken of the digestive gland homogenate.

Samples were analyzed for ^{51}Cr using a Sodium Iodide Crystal Well Detector equipped with a Canberra Series 20 Multichannel Analyser. In addition to gamma emissions, ^{51}Cr produces a beta signal which overlaps with that of ^{14}C, therefore the

samples were stored for four ^{51}Cr half-lives (half-life = 27.7 days) to allow ^{51}Cr to decay before ^{14}C was measured. ^{14}C was measured on a Beckman LS 6500 liquid scintillation counter using the scintillation cocktail described in McMaster et al. (1992).

Radioactivity was measured in counts per minute. The counts were corrected for background noise by subtracting counts from samples from the animals which were not exposed to radiolabelled algae or beads. Samples whose counts were below 15 CPM for C^{14} and/or 10 CPM for Cr^{51} were omitted from further analysis as counts this low were deemed unreliable. The ratio of ^{14}C:^{51}Cr was calculated for all samples.

II. 2. v. Statistical Analysis.

Most of the data were normally distributed; when data were not normal, nonparametric statistics were used. A paired t-test (SigmaStat statistical software, Jandel Scientific version 4.0) was used to test for significant differences in the amounts of ^{14}C and ^{51}Cr in the water samples over the 1 h exposure period while the Wilcoxon signed ranks test (SAS 1989) was used to test for changes in the ^{14}C:^{51}Cr ratio. The ^{14}C:^{51}Cr ratios from the first 2.5 h of samples (t = 0.5, 1, 1.5, 2, and 2.5 h) and the last 2.5 h (t = 3, 4, and 5 h) were pooled within locations (water, stomach, gland and faeces) in order to increase *n*. A one-way repeated measures ANOVA was then used to test for significant differences in the ratios between the locations (SigmaStat version 4.0). If significant differences between the locations were found ($P < 0.05$), a Tukey test was used to determine where the differences lay (SigmaStat version 4.0). Pearson correlation was used to look for trends in ^{14}C:^{51}Cr ratios over time (SigmaStat version

4.0).

II. 3. Results

II. 3. i. Water Samples

The absolute amounts of radioactivity for both ^{14}C (paired $t_{(2),23} = -5.597$, $P < 0.001$) (Figure 5a) and ^{51}Cr (paired $t_{(2),23} = -10.948$, $P < 0.001$) (Figure 5b) dropped significantly over the 1 h exposure period indicating that the particles were disappearing from the water. The ratio of ^{14}C:^{51}Cr increased significantly (paired $t_{(2)20} = 2.225$, $P < 0.05$) over the 1 h exposure period indicating that beads were being removed from the water faster than the algae (Figure 5c).

II. 3. ii. Stomach Samples

When the ^{14}C:^{51}Cr ratios from samples collected during the first 2.5 h after exposure are pooled within locations they show that initially the ^{14}C:^{51}Cr ratio within the stomach was significantly higher than the average ^{14}C:^{51}Cr ratio within the water (mean ratio of t0 and t1) ($F_{3,13} = 24.6$, $P < 0.001$) (Figure 6a). Pooled ratios from t 2.5 - 5 h show that the ^{14}C:^{51}Cr ratio in the stomach had decreased and was no longer significantly different from the ratio in the water the scallops had been exposed to (Figure 6b). There was a significant negative correlation between the ^{14}C:^{51}Cr ratio in the stomach and time $r = -0.564$, $n = 22$, $P < 0.01$) (Figure 7a), indicating that the algae were being removed from the stomach faster than the beads.

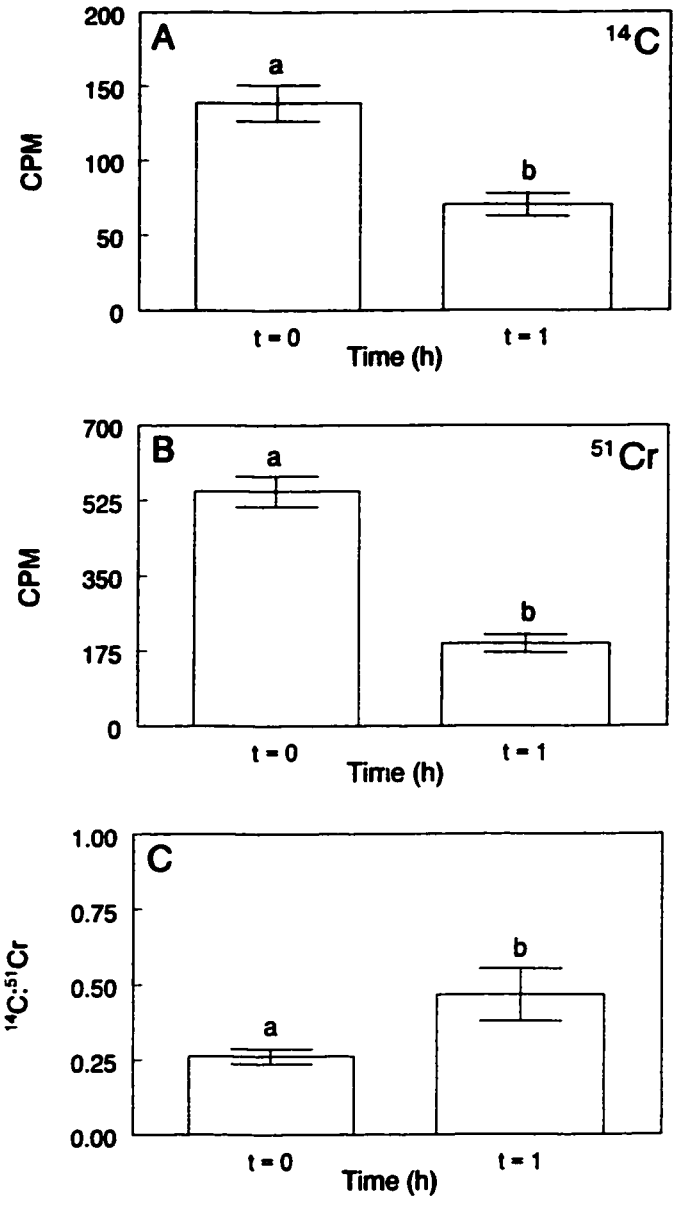

Figure 5: (A) Counts per minute (CPM) ($\bar{x} \pm$ S.E.) of ^{14}C within the water samples collected at the beginning (t = 0 h) and end (t = 1 h) of the 1 h exposure period ($n = 24$). (B) Counts per minute (CPM) ($\bar{x} \pm$ S.E.) of ^{51}Cr within the water samples collected at the beginning and end of the 1 h exposure period ($n = 24$). (C) Ratio of algae to beads ($^{14}C:^{51}Cr$) ($\bar{x} \pm$ S.E.) at the beginning and end of the 1 h exposure period ($n = 21$). Significant differences are denoted by different letters above the error bars.

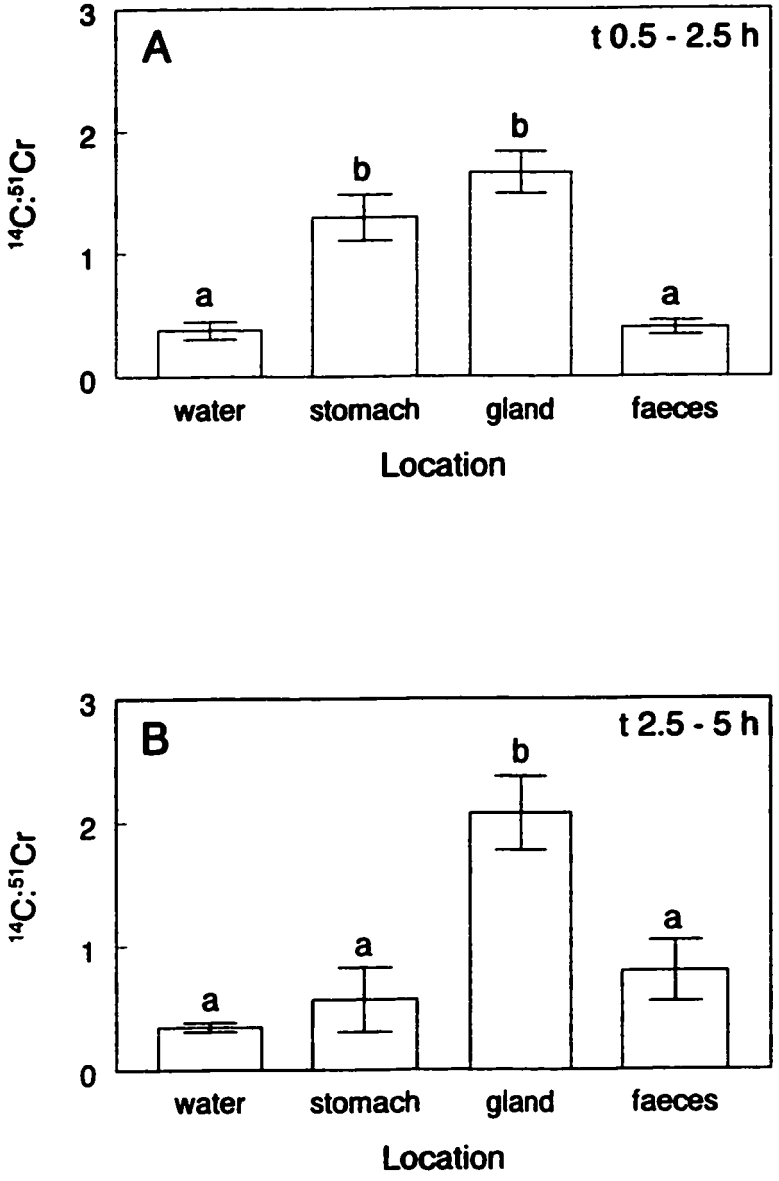

Figure 6: Ratio of algae to beads ($^{14}C:^{51}Cr$) ($\bar{x} \pm$ S.E.) over location. (A) Pooled results from scallop stomach (n = 13), digestive gland (n = 13) and faecal (n = 6) samples collected between 0.5 and 2.5 h and the corresponding mean water samples (n = 14) collected during the exposure period. (B) Pooled results from scallop stomach (n = 9), digestive gland (n = 9) and faecal (n = 7) samples collected between 2.5 and 5 h and the corresponding mean water samples (n = 9) collected during the exposure period. Significant (α = 0.05) differences are denoted by different letters above the error bars.

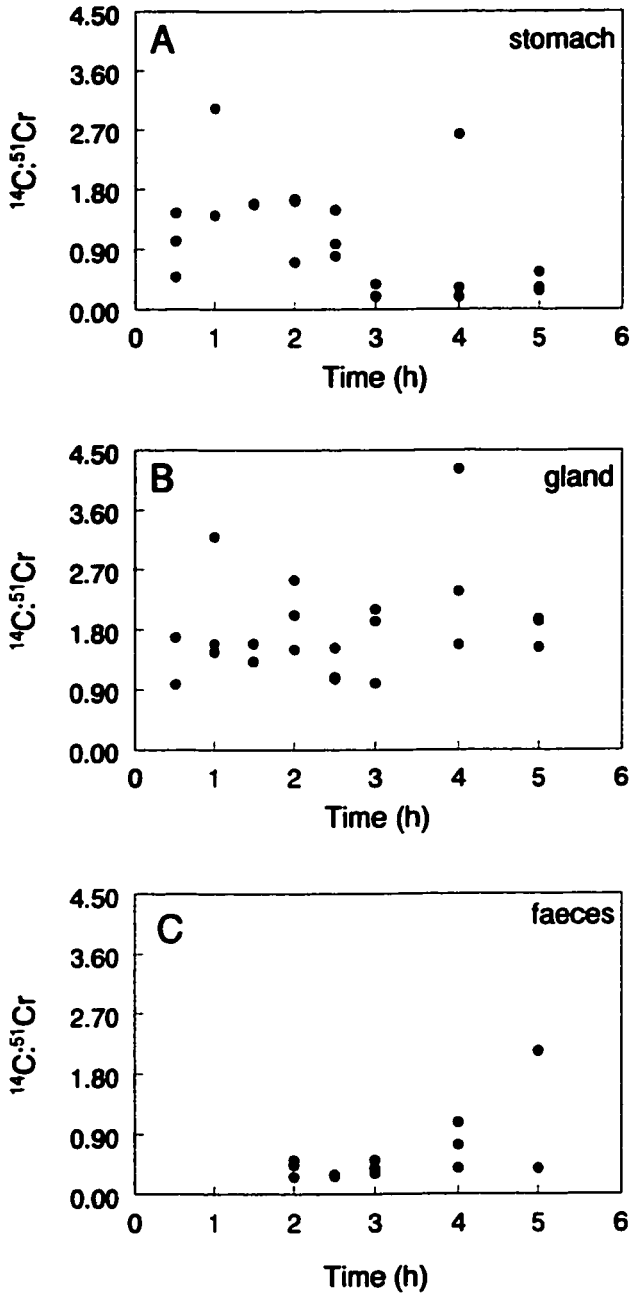

Figure 7: Scatterplots of $^{14}C:^{51}Cr$ ratios over time within the scallop (A) stomach ($n = 22$), (B) digestive gland ($n = 22$), and (C) faeces ($n = 13$). The $^{14}C:^{51}Cr$ ratio within the stomach was negatively correlated with time ($r = -0.56$).

II. 3. iii. Gland Samples

Initially the pooled ^{14}C:^{51}Cr ratio within the digestive gland (t 0.5 - 2.5 h) was not significantly different from the ratio within the stomach (Figure 6a). However later results (t 2.5 - 5 h) show the ^{14}C:^{51}Cr ratio in the stomach was significantly lower than that in the gland ($F_{3,8} = 18.4$, $P < 0.001$) (Figure 6b). There was no correlation between ^{14}C:^{51}Cr ratio in the gland and time $r = 0.216$, $n = 22$, $P > 0.05$) (Figure 7b).

II. 3. iv. Faecal Samples

Radiolabelled faeces began to be produced by 1.5 h after feeding. Initially, the pooled ^{14}C:^{51}Cr ratio in the faeces was lower than that within the stomach and digestive gland (Figure 6a). The faeces ratio increased slightly over time (Figure 6a and b) but there was no significant correlation with time $r = 0.516$, $n = 13$, $P > 0.05$) (Figure 7c). As the ratio in the stomach decreased, the faecal and stomach ratios became similar (Figure 6b).

II. 4. Discussion

The decline in algae and beads over the 1 h exposure period (Figure 5a and 5b) indicates that both types of particles were being removed from the water. However, the increase in the ^{14}C:^{51}Cr (algae:bead) ratio shows the beads were removed from the water faster over the 1 h exposure time than the algae (Figure 5c). The faster removal of beads may have been due to settling out or to preingestive selection for beads by the scallops.

However, the initial algae:bead ratio in the stomach was higher than the average ratio in the water (Figure 6a), which indicates that the increase in the algae:bead ratio in the water must have been due to settling out of beads, not preingestive selection for beads. Furthermore, the higher ratio in the stomach also suggests that algae may have been preferentially ingested by the scallops. Preingestive selection by scallops has been well documented. *Placopecten magellanicus* is known to sort inorganic and organic particles on the labial palps, preferentially ingesting organic particles and rejecting inorganic particles as pseudofaeces (Shumway et al. 1985; Cranford and Gordon 1992; MacDonald and Ward 1994; Bacon et al. 1998). Although there was no evidence of pseudofaeces production in this study, research by Ward et al. (1993) using endoscopy has shown that pseudofaeces are produced intermittently by many bivalves regardless of particle concentration. However, they may not be produced in large enough quantities to be readily noticed. Another explanation for the high ratio in the stomach initially is postingestive selection preferentially removing beads from the stomach to the faeces (see below). It is possible that preingestive selection for algae and postingestive selection against beads in the stomach may have been occurring simultaneously.

Changes in the initial algae:bead ratio, whether due to settling out or preingestive selection, do not pose a problem for this study as it is the resulting ratio within the stomach that is used to determine if postingestive selection occurred. The algae:bead ratio in the stomach was slightly lower than that in the gland during the first 2.5 h although not significant statistically (Figure 6a). The majority of individual scallops had a higher ratio in the gland than within the stomach (not shown). The ratio

in the stomach declined over time indicating that the algae were being removed from the stomach faster than the beads (Figure 7a). This drop in the stomach ratio combined with a slight (but not significant increase) in the gland ratio (Figure 7b) result in a significant difference between the stomach and gland (Figure 6b). These algae do not show up in the faeces therefore it appears that the algae were being selectively transferred to the digestive gland. It is likely that the ratio in the gland did not increase greatly over time because the algae were digested and the ^{14}C was taken up by the gland cells and absorbed into the circulatory system (Mathers 1972; Newell and Langdon 1986). The faecal samples are a combination of all faeces produced by the animal up to the time it was dissected. Therefore the later samples are a combination of faeces produced over the entire 5 h sampling period which makes them difficult to interpret. However, the low ratio in the early faecal samples probably represents the initial rejection of beads in the stomach to the faeces. The faeces ratio increased slightly (but not significantly) over time (Figure 7c), which may represent the production of unsorted material from the stomach. Unsorted material may have been produced at this point if the digestive gland was full. In any case the faecal samples remained low in ^{14}C indicating that the removal of algae from the stomach was not directed towards the faeces.

This interpretation is consistent with the theory that bivalves direct more digestible particles to the digestive gland for intracellular digestion. Previous studies have found that bivalves retain more digestible algae longer than others (Bricelj et al. 1984; Wang and Fisher 1996) and preferentially retain particles with an organic coating

longer than those without a coating (Gagnon and Fisher 1997).

The sorting of particles within the stomach observed in this study may be a passive phenomenon based on physical properties of the particles. Visual analysis of scallop stomach contents indicated that *Prorocentrum minimum* tests tend to break apart in the stomach. The released cell contents are presumably lighter than the beads and are therefore more likely to be caught up into the circulation of stomach fluids and drawn into the digestive gland. Wang and Fisher (1996) have shown that absorption efficiency is positively correlated with cytoplasmic penetration of a radioisotope label into a cell; radioisotopes that label only the cell wall are not absorbed as efficiently as those that label the cell contents. Gut retention time is also positively correlated with absorption efficiency which means that cell contents are retained longer than the cell wall. Bricelj et al. (1984) found similar results using algae labelled with both ^{14}C and ^{51}Cr. They found that *Mercenaria mercenaria* retained ^{14}C longer than ^{51}Cr. This result was interpreted as indicating that when the algal cells ruptured, the cell contents were carried to the digestive gland while the cell wall was rejected to the intestine, because ^{14}C is incorporated intracellularly while ^{51}Cr adsorbs to the cell wall and membranes.

The dramatic decline in the ratio within the scallop stomach over time provides evidence that *Placopecten magellanicus* is capable of postingestive selection, sorting organic and inorganic particles within the stomach. These results support those of studies on other bivalve species where different marker particles were presented separately (Bricelj et al. 1984; Wang and Fisher 1996). However, the factors on which this selection is based are not known. Although the marker particles were initially of

similar size and shape, the algae broke apart within the stomach, and the physical properties of the algae and beads were no longer the same.

Now that the ability of *Placopecten magellanicus* to sort particles within the gut has been established, the next step is to isolate the particle variables and test each individually. The most straightforward variables to test are the physical properties of size and density. The role of particle size and density in particle processing will be examined in the following chapter.

Chapter III: Selection by Physical Properties, Beads of Different Sizes and Densities[1]

III. 1. Introduction

The ability to sort particles within the gut may benefit bivalves by reducing the amount of energy expended attempting to digest indigestible or poor quality particles (Navarro and Iglesias 1993). Theoretically, rejected particles would be passed directly from the stomach to the intestine, while more desirable particles would be retained in the stomach for further extracellular digestion or directed into the digestive gland for intracellular digestion. Physical properties of the food particles may play a role in this process.

Particle size may be one factor used by bivalves to sort particles in the stomach. Observations on dissected bivalve stomachs indicate that very large particles do not enter the digestive tubules but are recirculated until they are broken down or expelled (Purchon 1977). At the other end of the scale, very small particles may also be rejected as they tend to fall into the rejection grooves of the sorting tracts if they are heavy enough; otherwise, they remain in suspension in the stomach fluid (Reid 1965). Here

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[1] The research presented in Chapter III has been published: Brillant, M.G.S. and MacDonald, B.A. 2000. Postingestive selection in the sea scallop, *Placopecten magellanicus* (Gmelin): the role of particle size and density. J. Exp. Mar. Biol. Ecol. 253:211-227.

we see the influence of another physical factor: density. Dense particles may be more likely to settle out onto the sorting tracts and be subject to size or density selection.

The benefit of rejecting large particles is clear: large particles would clog the digestive tubules. If large particles cannot be broken down by extracellular digestion in the stomach, their organic content is unavailable to the animal and therefore they should be expelled. Rejection of small particles may or may not be beneficial to the animal. Although suspended silt particles tend to be small in size (<10 μm) (Grant et al. 1997), many algal species are also small. Depending on the animal's habitat, there may be considerable overlap in the size distributions of these two types of particles. Rejection of dense particles may be beneficial to the bivalve since inorganic particles tend to be more dense than organic ones (Cranford et al. 1998).

Aside from observations on dissected animals, there have been few attempts to determine whether or not the physical properties of particles play a role in postingestive selection. Hughes (1977) compared the size distribution of natural sand particles extracted from different regions of the digestive system of *Abra tenuis*, a deposit-feeding Tellinacean. He found the size distributions of particles in the majority of the stomach and the intestine did not differ significantly from that of the particles ingested. However, particles at the entrance to the digestive tubules were significantly finer than those ingested, indicating some limited sorting by size had taken place. Cranford et al. (1998) found that two sizes of microspheres were passed at different rates through the gut of the sea scallop, *Placopecten magellanicus*. Penry (2000) fed two sizes of beads to *Potamocorbula amurenesis* simultaneously and found that on average the bivalves

did not sort the different sizes within the gut.

Most previous studies on postingestive selection have not attempted to isolate the properties on which particle selection is based. Bricelj et al. (1984) compared the gut retention times (GRTs) of a chrysophyte (4 μm) to that of two chlorophytes (<4 μm) and two cyanobacteria (≤2 μm) in *Mercenaria mercenaria*. Differences in the GRTs of the various food types were attributed to chemical selection but may have been due to size differences as well. Decho and Luoma (1991) compared the GRT of 15 μm latex beads to that of bacteria (which are considerably smaller) in two species of clams and found no significant difference in the averages GRTs. Wang and Fisher (1996) found no correlation between absorption efficiency (which is correlated to GRT) and algal size when mussels (*Mytilus edulis*) were fed algae ranging in size from 2 μm to 40 μm. In all of these cases, selection by size has been complicated by other factors such as the chemical properties of the different food types. Another complication is that, with the exception of Penry (2000), the different particle types were not fed to the test bivalves simultaneously; there was no choice given. If the animal has any control over selection by physical factors, it may not select unless presented with a mixture of desirable and less desirable particles.

Although observations on dissected bivalves indicate density may be a factor in sorting in the bivalve stomach (Reid 1965), this has not been studied in intact bivalves. There is a need to elucidate the role that size and density of particles play in postingestive selection in intact bivalves.

The main objectives of this component of the study were to determine whether

Placopecten magellanicus can sort particles within the gut solely on the basis of size or of density. In addition, selection by size in mussels (*Mytilus edulis*) and soft-shelled clams (*Mya arenaria*) was also tested in an attempt to compare selection abilities in polysyringian bivalves with different stomach types. *P. magellanicus* has a type IV stomach, *M. edulis* has a Type III stomach, while *M. arenaria* has a Type V stomach (Purchon 1987). The size of the digestive ducts and tubules within the digestive gland of *P. magellanicus* was also examined to determine the upper size limit for particles entering the digestive gland and to see if there is any correlation between scallop size and tubule size.

III. 2. Materials and Methods

III. 2. i. Selection by Size

Collection and Holding of Animals

Scallops (*P. magellanicus*, 46 - 73 mm shell height) were collected by divers near St. Andrew's, New Brunswick (45°02'55"N, 67°02'55"). The animals were held in a flow-through tank at the Department of Fisheries and Oceans Station in St. Andrew's for up to three days before they were transported to either the Huntsman Marine Science Centre (HMSC) in St. Andrew's or to UNBSJ. At HMSC scallops used in flow-through experiments were held in a flow-through tank supplied with seawater from nearby Passamaquoddy Bay (salinity 31‰, temp. 9 - 9.5°C). At UNBSJ, scallops used in static experiments were held in a recirculating seawater tank (salinity 32‰,

temp. 12°C). Scallops held in the recirculating tank were fed cultured algae, *Dunaliella tertiolecta*. Animals were held for at least 1 week prior to experimentation.

In addition to scallops, soft-shelled clams (*Mya arenaria*, 46.2 - 57.9 mm shell length), and blue mussels (*Mytilus edulis*, 49.5 - 61.7 mm shell length) were used in one experiment. Clams were collected from intertidal mud flats in St. Andrews, N.B. (45°04'33"N, 67°03'58"W) and mussels were collected from the lower littoral zone at Green's Point, N.B. (45°02'17"N, 66°53'33"W). These animals were transported to HMSC and held under the conditions described above.

In total, four experiments were run to test selection by size; two of these were static experiments conducted at UNBSJ while the other two used a flow-through system and were conducted at HMSC.

Bead Mixtures

A mixture of equal numbers of three sizes of polystyrene beads (5, 10, and 20 μm; PolySciences Inc.) was prepared in filtered seawater 24 h prior to each experiment. Algae (*D. tertiolecta*) were added just before the experiment to make the mixture more palatable to the animals and to stimulate feeding. The bead/algal mixture was presented to each animal at a final concentration of 10 000 particles ml^{-1} (2500 particles ml^{-1} of each bead size plus algae). The exception was static experiment 1, in which equivalent volumes of four sizes of polystyrene beads (5, 10, 20 and 40 μm) plus algae were fed to the animals to a final concentration of 20 000 particles ml^{-1}. Both equal numbers and equal volumes were used to see if there was any effect of the volume of particles

presented to the animals. When equal numbers of particles were fed to the scallops, the larger beads would have made up the bulk of the material processed by the scallops on a volume basis.

Static Experiments

The two static experiments were carried out in a raceway filled with recirculating seawater (temp. 12°C). Scallops were put on platforms constructed of plastic Petri dishes and craft sticks and placed in individual 1 L beakers (Figure 8). The beakers were positioned on submersible stir plates within the raceway, and the water in each beaker was stirred to keep the particles suspended and the water aerated.

Scallops were placed in the beakers 24 h prior to beginning the experiment. Twelve beakers were used altogether, with one beaker left empty as a control to correct for any particle settling. The bead and algae mixture was added to the beakers at the beginning of the experiment. Two of the eleven animals were used as controls and were fed only algae without beads. Scallops were exposed to the beads for 1 h then the bead mixture was replaced with natural seawater supplemented with algae (*D. tertiolecta*). Faeces were collected at regular intervals, every 2 h for the first 12 h, at 6 h intervals until 36 h after exposure, at 12 h intervals until 60 h, then at 24 h intervals for up to 160 h post exposure.

Clearance rates were measured during the 1 h exposure period to ensure that the scallops were clearing the beads from the water. Water was sampled from the empty control beaker and the eleven specimen beakers at t = 0 h and at t = 1 h. The

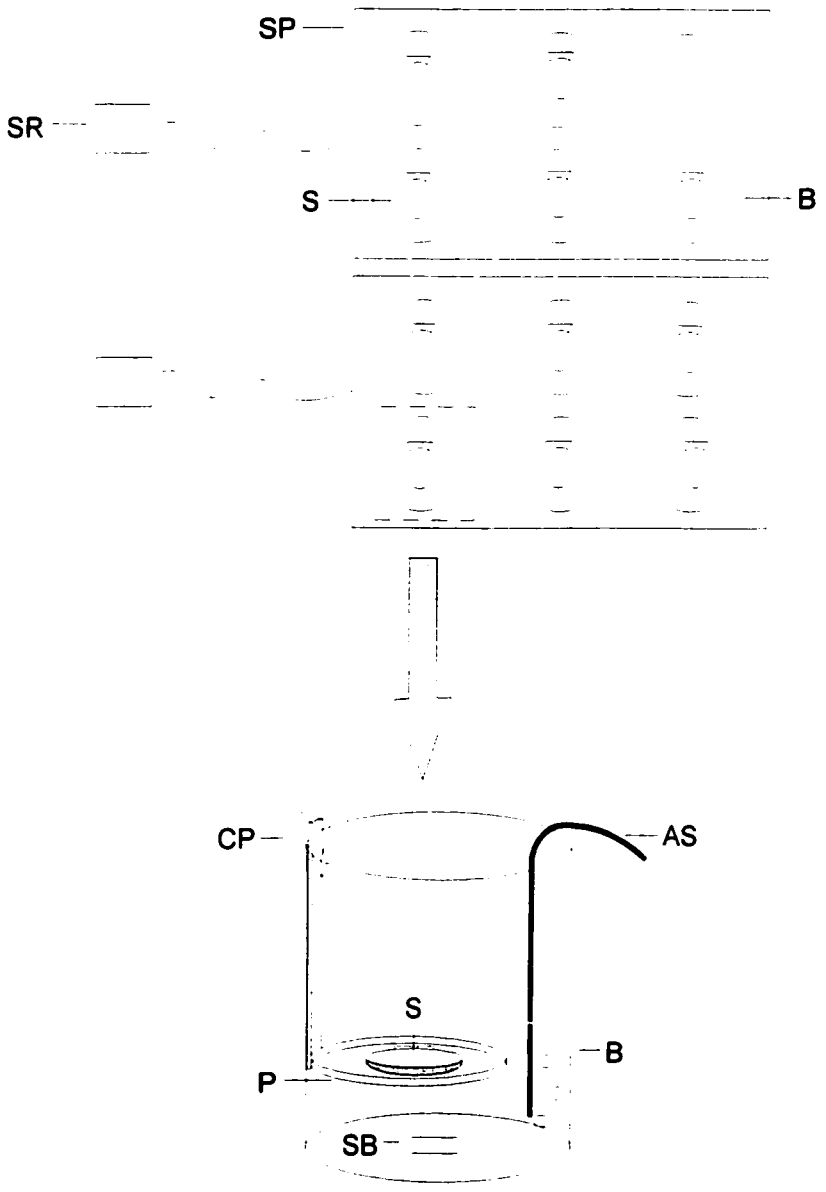


Figure 8: Diagram of static experimental set-up. AS = air supply, B = beaker, CP = clothes-pin, P = platform, S = scallop, SB = stir bar, SP = submersible stir plate, SR = stir regulator.

concentrations of particles in the water were measured with a Coulter multisizer fitted with a 100 μm aperture tube. Clearance rates (CR) for each individual animal were calculated using the following formula:

$$CR = V\, n^{-1}\, [((\log_e C_0 - \log_e C_t) - a)\, t^{-1}]$$

where V = volume (ml), n = number of animals per beaker, C_0 = initial concentration, C_t = concentration at time t, a = rate at which particles settle out of suspension. Settling rates were calculated using the following formula:

$$a = (\log_e C_0' - \log_e C_t')\, t^{-1}$$

where C_0' = initial concentration of control beaker, C_t' = concentration after time t in the control beaker (Coughlan 1969). Clearance rates were then standardized (CRs) for an individual scallop with a dry soft tissue weight of 1.0 g using the formula:

$$CRs = (Ws/Wo)^b \times CR$$

where Ws = standardized soft tissue dry weight, Wo = observed soft tissue dry weight, and b = a fitted parameter. The weight exponent value (b) has been determined for *Placopecten magellanicus* as 0.68 (MacDonald and Thompson 1986a).

Flow-through Experiments

Two experiments were conducted using a flow-through feeding system (Figure 9). Unfiltered seawater from Passamaquoddy Bay was pumped into a 20 L plastic bucket which served as a header tank. The bucket was equipped with an overflow pipe to maintain a constant head. Water was distributed to the specimen containers through Tygon tubing extending from the bottom of the bucket. The ends of the tubing were

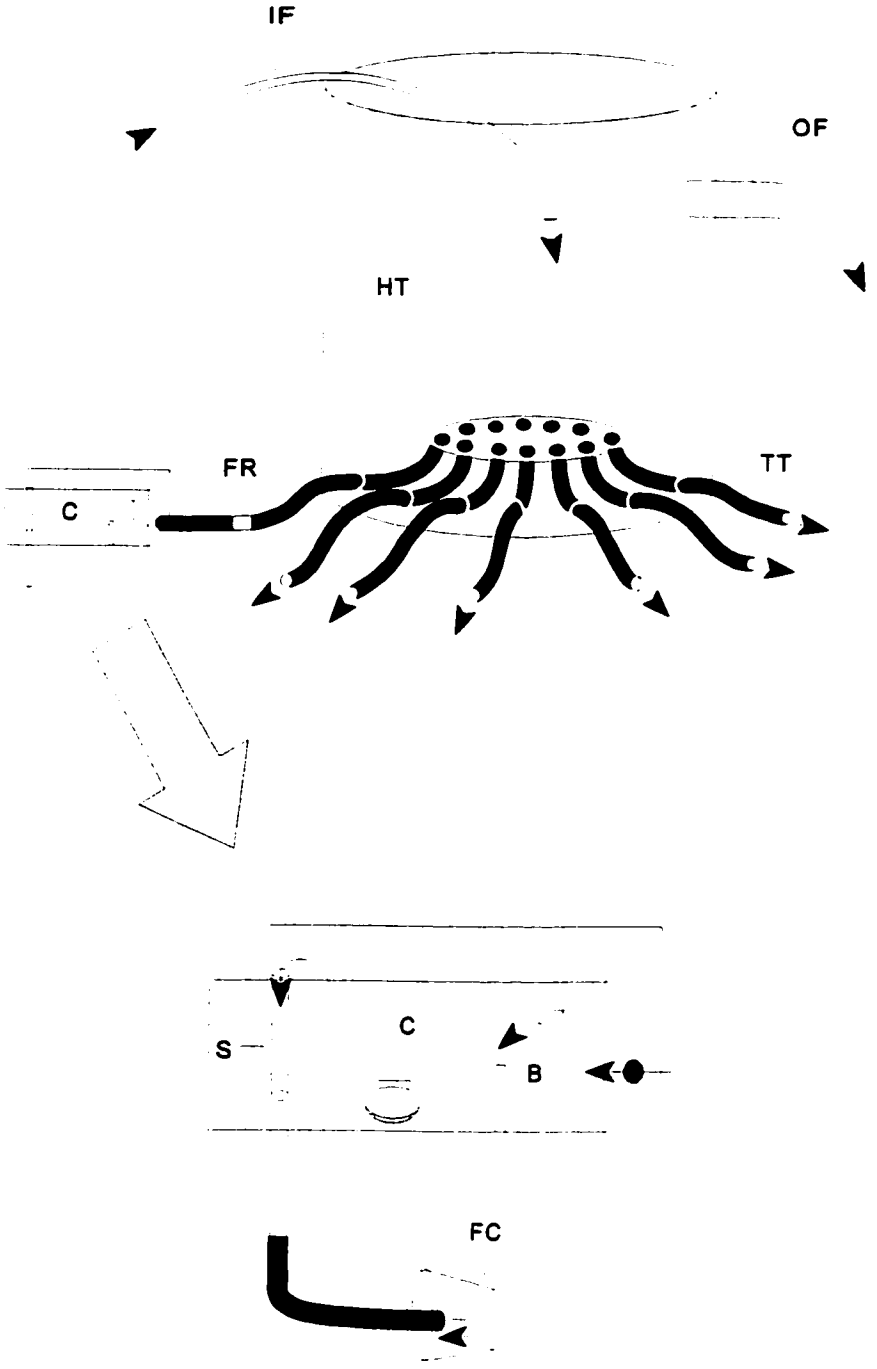


Figure 9: Diagram of flow-through experimental set-up. B = baffle, C = specimen container, FC = faeces collector, FR = flow restrictor, HT = header tank, IF = inflow line, OF = overflow pipe, S = standpipe, TT = Tygon tubing. Arrows indicate the direction of water flow.

secured inside the bucket through a Plexiglas disc, which held the ends upright about 10 cm off the bottom. Flow restrictors made of plastic plugs drilled with a 1.4 mm bit were placed along the tubes.

Two sizes of specimen containers were used. Containers for scallops were made out of 710 ml plastic containers (170 x 110 x 50 mm), while containers used to hold clams and mussels were made of 1 L plastic containers (170 x 80 x 105 mm). A horizontal inflow pipe was located at one end of the chamber, and a upright standpipe at the other. The upright standpipe passed through a hole drilled in the base of the container; the lower end of the standpipe was equipped with a faeces collector made of 120 ml plastic sample vials, the lid of which had the centre cut out and replaced with 100 μm mesh. The tube from the standpipe passed through the centre of the mesh screen. The vials were placed on their sides so that water flowing into them drained out through the mesh without interfering with the outflow from the standpipe. The specimen chambers also had a Plexiglas baffle, placed a few centimetres back from the inflow to ensure well-mixed and non-recirculating flow through the chamber. Animals were placed between the baffle and the standpipe.

Two flow-through experiments were carried out. Flow-through experiment 1 used ten scallops while flow-through experiment 2 was conducted using thirty animals: ten each of scallops, mussels (*Mytilus edulis*) and clams (*Mya arenaria*). The larger number of chambers in flow-through experiment 2 meant a lower flow rate, 178 ml min^{-1} in experiment 2 vs. 270 ml min^{-1} in flow-through experiment 1. Animals were placed in the chambers 24 h before the experiment began. Eleven chambers were used

for each of the three species, with one chamber left empty as a control. The bead and algae mixture was added to the chambers at the beginning of the experiment while the flow was shut off. After 1 h of exposure the flow was resumed to flush out the beads. Two of the ten animals were used as controls and were fed only algae without beads. Faeces were subsequently collected at 2 h intervals for the first 12 h, every 6 h until 36 h, then every 12 or 24 h up to 144 h after exposure.

Clearance rates were measured during the 1 h exposure period. The weight exponent value (b) for mussels is 0.73 (Winter 1977). This value has not been determined for *Mya arenaria*. When standardizing the clearance rates, the weight exponent for scallops (0.68) was chosen and used for the clams. This value has been used in previous work on clearance rates of *M. arenaria* (Bacon et al. 1998) and is comparable to weight exponent values reported for many bivalve species (Bayne and Newell 1983).

Particle concentration in the incoming water was measured over a tidal cycle by analyzing a water sample from the header tank every 2 h for 12 h. The amount and quality of material in the water was also measured every 2 h (see Appendix 1 for procedures and results).

Faecal Analysis

After the faeces were collected in 1.5 ml microcentrifuge tubes, they were centrifuged at 13 000 rpm for 6 min. The supernatant was pipetted off and discarded, and 1 ml of concentrated nitric acid was added to the pellet. The tubes were then

sonicated for 10 min and left overnight. The following day, the tubes were centrifuged again at 13 000 rpm for 6 min, the nitric acid was siphoned off and 1 ml of distilled water was added. The pellet was resuspended by shaking the tubes vigorously. The tubes were centrifuged once more at 13 000 rpm for 6 min. The distilled water was then siphoned off and replaced with 1.5 ml of 1 μm filtered seawater. The pellets were resuspended again by vigorous shaking and sonicated in an ice bath for 1 h. The resuspended material was then emptied into Coulter vials and diluted to 30 ml with filtered seawater.

The nitric acid was added to remove any organic material leaving only beads and any other inorganic particles. It was necessary to wash the samples to remove the acid as it disrupted the electrolytic balance of the suspension which, in turn, interfered with the particle size analysis on the Coulter multisizer.

Each faecal sample was analyzed on a Coulter multisizer fitted with a 100 μm tube to determine the size frequency distribution of the particles within the sample. If beads were present they could be detected as peaks at the appropriate size. An example size frequency distribution is shown in Figure 10; peaks can clearly be seen at 5 μm and 10 μm and a smaller peak around 20 μm. The approximate size range for each of the three bead sizes was determined by running a sample of beads in filtered seawater on the multisizer. The range in which 90% of the beads for each size fell was used as the window for further analysis.

In order to determine the number of beads present in each window, the background inorganic particles had to be subtracted. The absolute amount of faeces

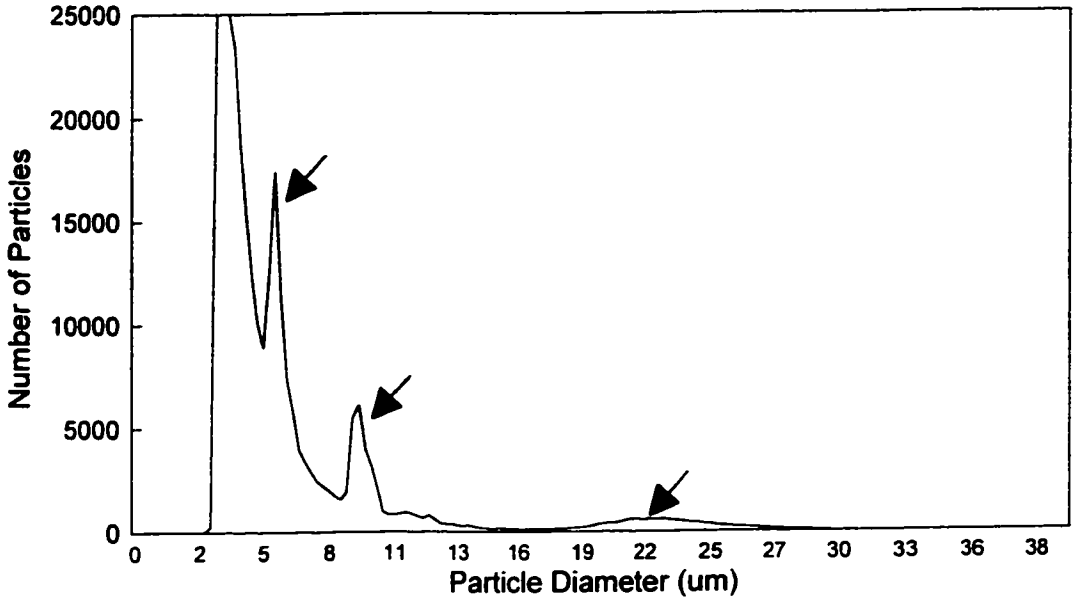


Figure 10: Representative particle size distribution of a faecal sample from *Placopecten magellanicus*. Arrows indicate the presence of 5, 10, and 20 μm beads. Particle size distribution was obtained using a Coulter multisizer equipped with a 100 μm aperture tube.

produced by each animal varied a great deal therefore the background faecal material (represented by the control animals' faeces) could not be subtracted directly; instead, percentage distributions were used. The size frequency distributions of the faecal samples were broken up into size categories, one surrounding each of the bead sizes, and counts within each category were converted to a percentage distribution. The size frequency distributions of faeces from control animals not fed beads were then subtracted from those of treated samples. The resulting positive percentages represented beads. These were summed and converted back to a bead count by multiplying by the total number of particles within that particular size category. The number of beads passed at each time interval were summed and used to determine the gut retention times.

III. 2. ii. Size of Digestive Tubules

Collection and Holding of Animals

Scallops (*Placopecten magellanicus*, 51 - 90 mm shell height) were collected by divers in the Letang Harbour near St. George, New Brunswick (45°03'32"N, 66°49'20"W). The scallops were transported to UNBSJ, where three of the animals were dissected immediately. The remaining animals were held in a recirculating seawater tank (salinity 32‰, temp. 10 °C) for 5 to 6 days before they were dissected.

Experimental Design

Twelve scallops were put into beakers placed on a submersible stir plate within

the recirculating seawater tank. The scallops were elevated on petri-dish platforms, a stir bar was placed in each beaker and aeration was supplied. The animals were fed 20 μm polystyrene beads and algae, *Isochrysis galbana*, Tahitian strain (T-iso), for 1 h, then transferred to natural seawater. Two scallops were dissected at each of the following intervals: t = 0.5, 1, 2, 5, 24, and 48 h.

Tissue Dissection, Processing, Sectioning and Staining

The digestive glands were dissected out of the animals and a block of tissue was removed from the left, right or anterior of the gland. The dimensions of the blocks were measured. The tissues were fixed in Bouin's solution for 48 h, then measured again to check for shrinkage and transferred to 70% ethanol, dehydrated in a graded series of ethanols and embedded in paraffin wax. Serial sections 6 μm thick were cut with a microtome and stained with Mallory's. The block of tissue was completely sectioned and ten portions of eight consecutive sections of the ribbons were chosen at fifty section intervals to make five slides for each scallop.

Since the dimensions of the tissue block could not be measured after it was embedded, a test was run to determine how much the wax itself shrinks as it hardens. A known volume of wax was pipetted into a paper container, allowed to harden and then measured by the volume of water it displaced.

The block dimensions before and after fixation were tested for normality using the Shapiro-Wilks test (Zar 1996) and for homogeneity using the F-Max test (Sokal and Rohlf 1981). All dimensions were normal and homoscedastic. Paired t-tests were then

used to compare size before and after fixation (SAS 1989).

Analysis of Sections

The digestive cells which make up the gland tubules pass through various stages of intracellular digestion synchronously; therefore, digestive gland tubules may be classified into four types. These are holding, absorptive, disintegrating and reconstituting tubules (Morton 1969; Langton 1975). All four types of tubules may be present within the gland at the same time, although one or another may predominate depending on the bivalve species. Tubules to be measured were chosen by selecting, from each slide, six tubules which appeared to be in the earliest stages of the digestive cycle (holding or absorptive stages). These stages are characterized by a large lumen surrounded by cuboidal digestive cells which, in the absorptive phase, have vacuoles at the distal regions (Mathers 1976). Diagrams and photomicrographs from previous studies on bivalve digestive glands indicate that the tubules usually have a circular external outline (Mathers 1976; Mathers et al. 1979; Morrison and Shum 1982; Syasina et al. 1997). Therefore only tubules which were roughly circular in outline, indicating that they had been sectioned at right angles, were measured. The lumens of the tubules were not perfectly round therefore two dimensions were measured: the narrowest dimension of the tubule lumen was called the width and the largest dimension was called the height. Altogether thirty tubules were measured from each animal. The sections were also examined for the presence and location of beads.

The null hypothesis was that there was no correlation between tubule size and

scallop shell size. Pearson correlation analysis was used to test for correlations between tubule size and scallop shell size (SAS 1989). Data were found to be normally distributed using the Shapiro-Wilk test prior to statistical comparisons (Zar 1996).

III. 2. iii. Selection by Density

Collection and Holding of Animals

Scallops (*P. magellanicus*, 66 - 80 mm shell height) were collected by scallop drag in the outer reaches of the Saint John Harbour, New Brunswick (45°12'00"N, 65°58'30"W). Scallops were transported to UNBSJ, where they were held in a recirculating seawater tank. Holding conditions were the same as those described earlier for the static size selection experiment. One static experiment was conducted at UNBSJ to test selection by density.

Bead Mixture

Ideally, selection by density would be tested using particles that are completely identical with the exception of their densities, however it was not possible to find two such particles which could be easily distinguished from each other in the faeces. The closest approach to this was to use two particles that were both inert. Ward and Targett (1989) found that when mussels were fed a mixture of silica and polystyrene microspheres treated with f/2 or f/20 media they did not exhibit preingestive selection. Therefore the decision was made to use polystyrene and glass beads of similar size but different densities.

A mixture of equal numbers of 8 μm glass beads (density 2.5 g ml^{-1}) (Duke Scientific) and 9 μm blue polystyrene beads (density 1.05 g ml^{-1}) (Polysciences Inc.) was prepared in filtered seawater 24 h prior to the experiment; algae (*Dunaliella tertiolecta*) were added the following morning. The mixture of equal numbers of glass beads, polystyrene beads and algae was added to the experimental chambers to a final concentration of approximately 10 000 particles ml^{-1}.

Experimental Design

The static set-up used to test selection by density was the same as that described in section II. 2. i (Figure 8). Scallops were placed in the beakers 24 h prior to beginning the experiment. Again twelve beakers were used, one as an empty control beaker. Two of the scallops were fed only *D. tertiolecta* while the other nine animals were fed the bead and algae mixture. The animals were exposed to the beads for 1 h, then the bead mixture was replaced with natural seawater supplemented with *D. tertiolecta*. Clearance rates were measured during the 1 h exposure period. Faeces were collected at intervals, as described for size experiments, for 168 h.

Faecal Analysis

The faeces were collected in 1.5 ml microcentrifuge tubes and treated with 0.1 ml of Coulter Dispersant Type IC. The faecal pellets were then broken up by stirring, shaken vigorously and sonicated for 1 h.

The beads were too close in size to be distinguishable on the multisizer

therefore the number of blue polystyrene beads and glass beads in each faecal sample were counted using a Levy counting cell under a binocular microscope equipped with phase contrast. Six subsamples were counted from each faecal sample.

III. 2. iv. Calculating Gut Retention Times

In order to standardize results, the time at which 50% (t_{50}) of the beads passed through the gut was used as the gut retention time (GRT). The t_{50} was estimated by plotting the cumulative percentage of beads passed at each time interval against time to get a cumulative egestion curve. The polynomial regression equation which best matched the curve was then fitted to the cumulative egestion curve (Figure 11). In some cases the data were log transformed to better fit the regression. R-squared values were at least 0.90 and usually greater than 0.95. GRT was estimated as the interpolated time at which 50% of the beads were egested.

This method of fitting a regression equation to the cumulative egestion curve to determine GRT has been used by Bricelj et al. (1984), Bayne et al. (1989) and Hawkins et al. (1990); however, in bivalve research, the time when 90 or 95% of a marker has passed (t_{90} or t_{95}) is commonly used as the GRT. In this study, small amounts of beads were still being passed at the end of the collection period. These final few beads may take much longer to pass than the bulk of the material; therefore, using a higher percentage as the GRT may inflate the retention time. Figures 12a and 12b show data from two animals where beads were passed much faster in one (Figure 12a) than in the other (Figure 12b). Using t_{95} they have similar GRTs, however, using t_{50} differentiates

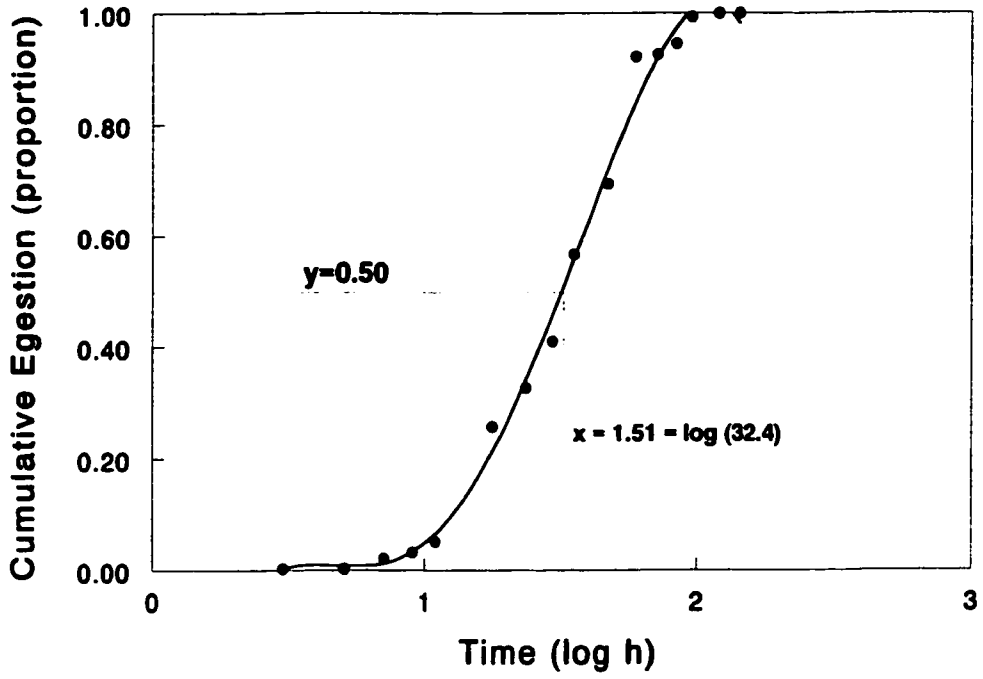


Figure 11: Representative cumulative egestion curve of 20 μm beads from one scallop. A polynomial regression equation is fitted to the data points and the time at which 50% of the beads were egested is interpolated. The x-axis data have been log transformed to better fit the regression.

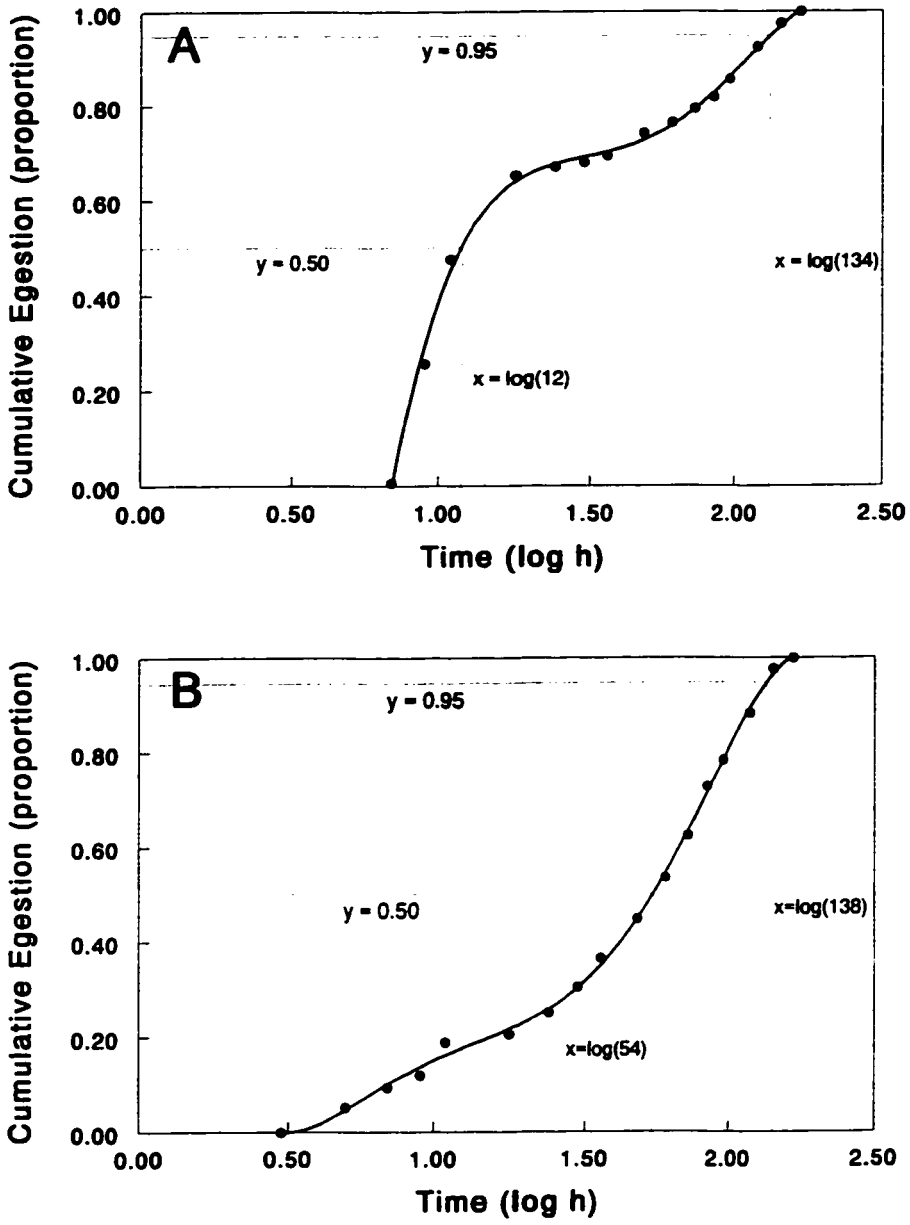


Figure 12: Cumulative egestion curves for two *Placopecten magellanicus*. (A) scallop with faster initial egestion rate and (B) scallop with a slower initial egestion rate. Note that the gut retention times are very similar at 95% cumulative egestion (134 h vs. 138 h), but are different at 50% cumulative egestion (12 h vs. 54 h).

between them. The median value (t_{50}) is occasionally used for bivalves (Scarratt 1994; Gagnon and Fisher 1997) and gastropods (Calow 1975), and is commonly used in finfish research (Noble 1973; Mills and Forney 1981).

Hawkins et al. (1990) found a linear relationship between gut retention time and soft tissue dry weights for *Mytilus edulis* and *Cerastoderma edule*. To determine if GRTs should be corrected for body size, GRTs and soft tissue dry weights for each experiment were tested for correlation using Pearson correlation analysis (SAS 1989). The data were found to be normally distributed using the Shapiro-Wilks test once they had been log-transformed (Zar 1996).

III. 2. v. Statistical Analysis

The null hypothesis for the selection experiments was that there was no effect of particle size or density on GRT. In the selection by size experiments the data were analyzed using repeated measures ANOVA. The data were tested for sphericity and if the sphericity requirement was not met, the Greenhouse-Geisser corrected *P*-value was used (SAS 1989). If significant differences between GRTs for different size beads were found ($P < 0.05$), a Student-Newman-Keuls test was used to determine where the differences lay (SAS 1989). In the selection by density experiments the average gut retention times for the beads of different densities were tested for significant differences using a two-tailed paired sample *t*-test (SAS 1989). GRTs were tested for normality using the Shapiro-Wilk test (Zar 1996) and homoscedasticity using the *F*-max test (Sokal and Rohlf 1981) prior to statistical comparisons. If necessary, log transformation

was performed.

III. 3. Results

III. 3. i. Clearance Rates

Results of clearance rate measurements are presented in Table 1. All clearance rates have been weight standardized for a bivalve of 1.0 g. Clearance rates for flow-through experiment 1 are negative although the animals were feeding and beads were subsequently found in all animals faeces.

III. 3. ii. Size of Digestive Tubules

Presence of Beads

The 20 μm polystyrene beads were clearly visible under the microscope when using phase-contrast. Beads could be seen within the primary ducts and tubules of the digestive glands of all of the animals fed beads (Figure 13). Beads were present within 30 min of feeding and were still present up to 48 h later. Although beads were present within the lumen of the digestive tubules, no beads were seen within the cells lining the tubules, indicating that the beads were not phagocytosed.

Size of the Tubules

The average dimensions of the tubule lumens were 31.1 ± 0.7 x 53.3 ± 0.8 μm (width ± S.E. x height ± S.E.). There were no correlations between tubule lumen width

Table 1. Clearance rates ($\bar{x}$ ± S.E.) of *Placopecten magellanicus*, *Mytilus edulis*, and *Mya arenaria* during 1 h exposure period to beads.

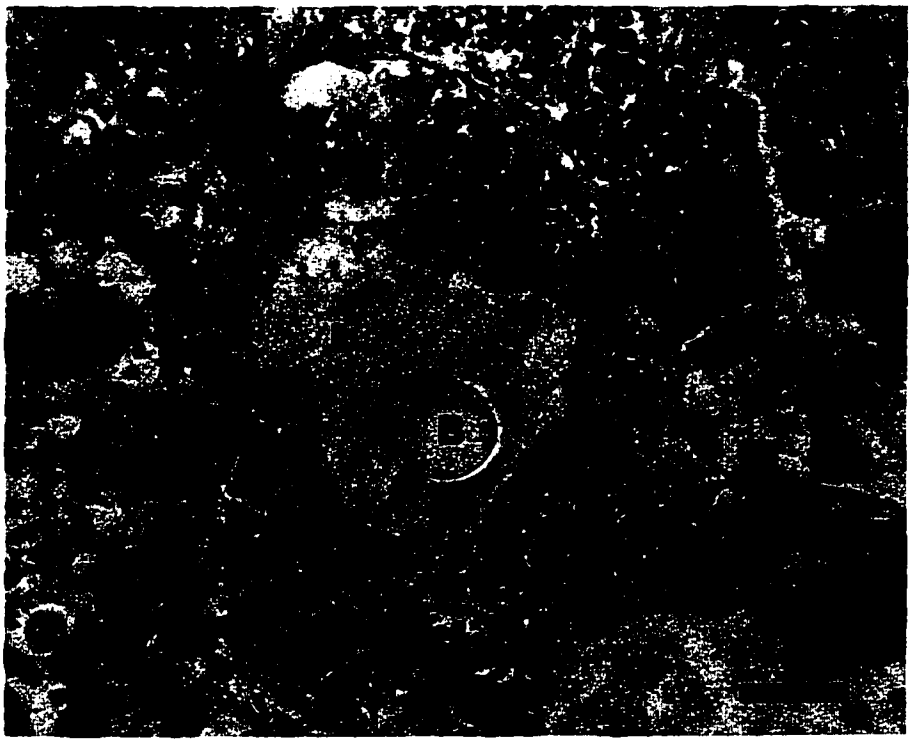
Experiment	Species	CR ($l\ h^{-1}g^{-1}$)
Size		
static 1	*P. magellanicus*	0.8466 ± .2849
static 2	*P. magellanicus*	0.4694 ± .0953
flow 1	*P. magellanicus*	-0.0962 ± .0405
flow 2	*P. magellanicus*	0.8109 ± .1521
	M. edulis	0.9255 ± .4005
	M. arenaria	1.0080 ± .2061
Density		
density 1	*P. magellanicus*	1.0230 ± .2379

CR = clearance rate

Table 2. Summary of Pearson correlation analyses relating scallop digestive gland tubule dimensions to scallop shell size.

Dimensions	*n*	*r*	*P*
tubule width x shell height	15	0.1354	0.6304
tubule width x shell width	15	0.1034	0.7138
tubule height x shell height	15	0.1645	0.5579
tubule height x shell width	15	0.1275	0.6508

Figure 13: Photomicrograph showing the presence of a 20 μm bead (B) within the lumen (L) of a digestive gland tubule of *Placopecten magellanicus*. Scale bar represents 20 μm.



or height and scallop shell width or height for the range of scallop sizes tested (51 - 90 mm shell height) (Table 2). This indicates that tubule size is independent of scallop size and that the digestive gland must grow by an addition of tubules not by an increase in the size of the tubules.

Effects of Fixation and Embedding

The only statistically significant change in the tissue block dimensions after fixation was an increase in the smallest dimension by 16%. The volume of the wax decreased by 10% upon hardening.

III. 3. iii. Patterns of Egestion

When the mean number of beads egested per hour is converted to a percentage and plotted against time some patterns in egestion can be seen. The egestion curves for scallops (Figures 14, 15, and 17) are characterized by one or two sharp peaks of egestion prior to 18 h followed by a gradual decline in the rate of egestion. Often there is another pulse of egestion around 24 - 32 h. Egestion in clams was erratic with several pulses of egestion throughout the collection period (Figure 16a). Mussels had a high initial rate of egestion followed by a steep decline (Figure 16b).

III. 3. iv. Gut Retention Times: Correlation with Body Size

There were no significant correlations between dry soft tissue weights and GRTs with the exception of the 5 μm beads in static experiment 2 (Table 3). When the

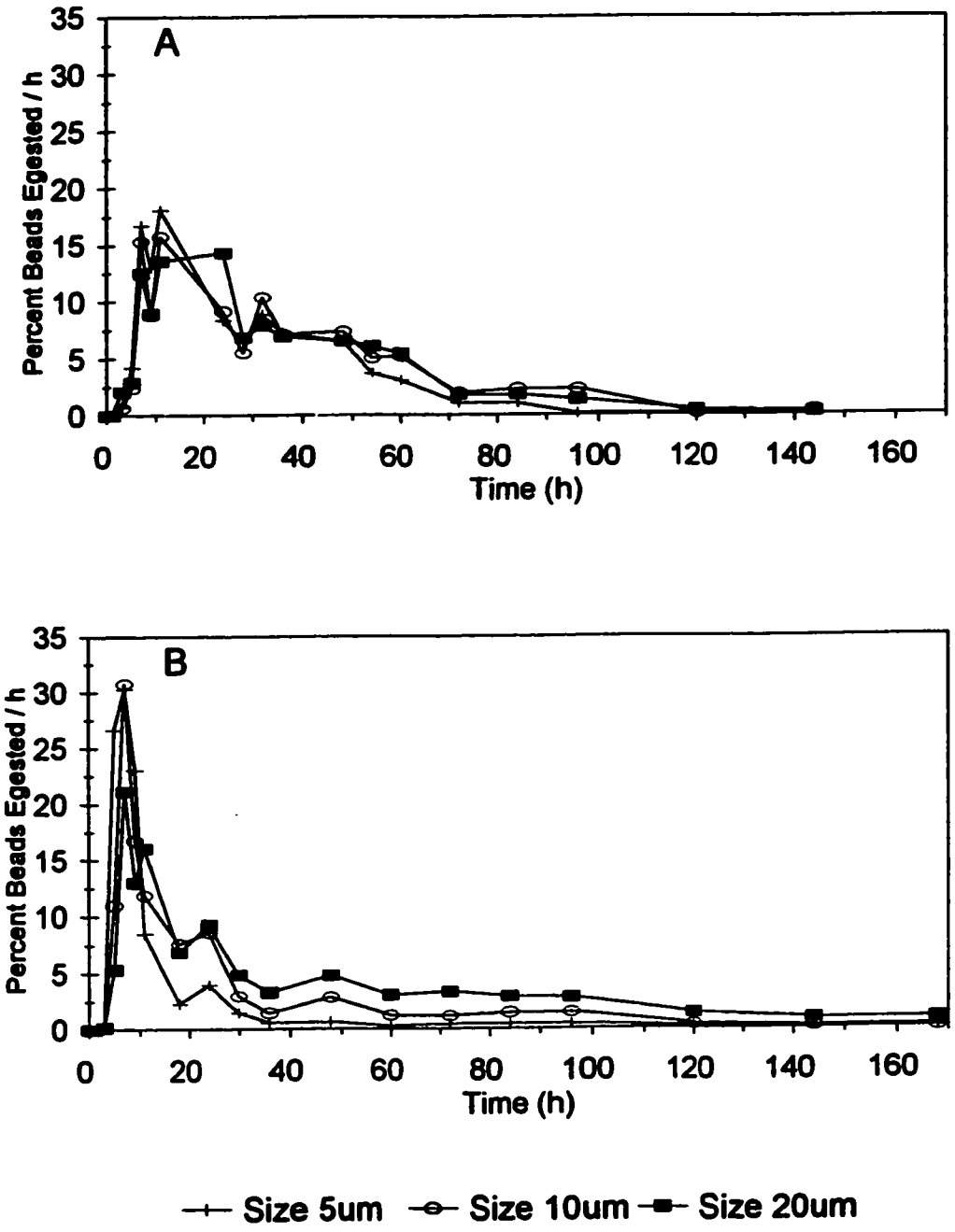


Figure 14. Mean egestion rate (number of beads egested per hour expressed as a percentage) of *Placopecten magellanicus* fed three sizes of polystyrene beads. (A) static experiment 1 ($n = 9$). (B) static experiment 2 ($n = 8$).

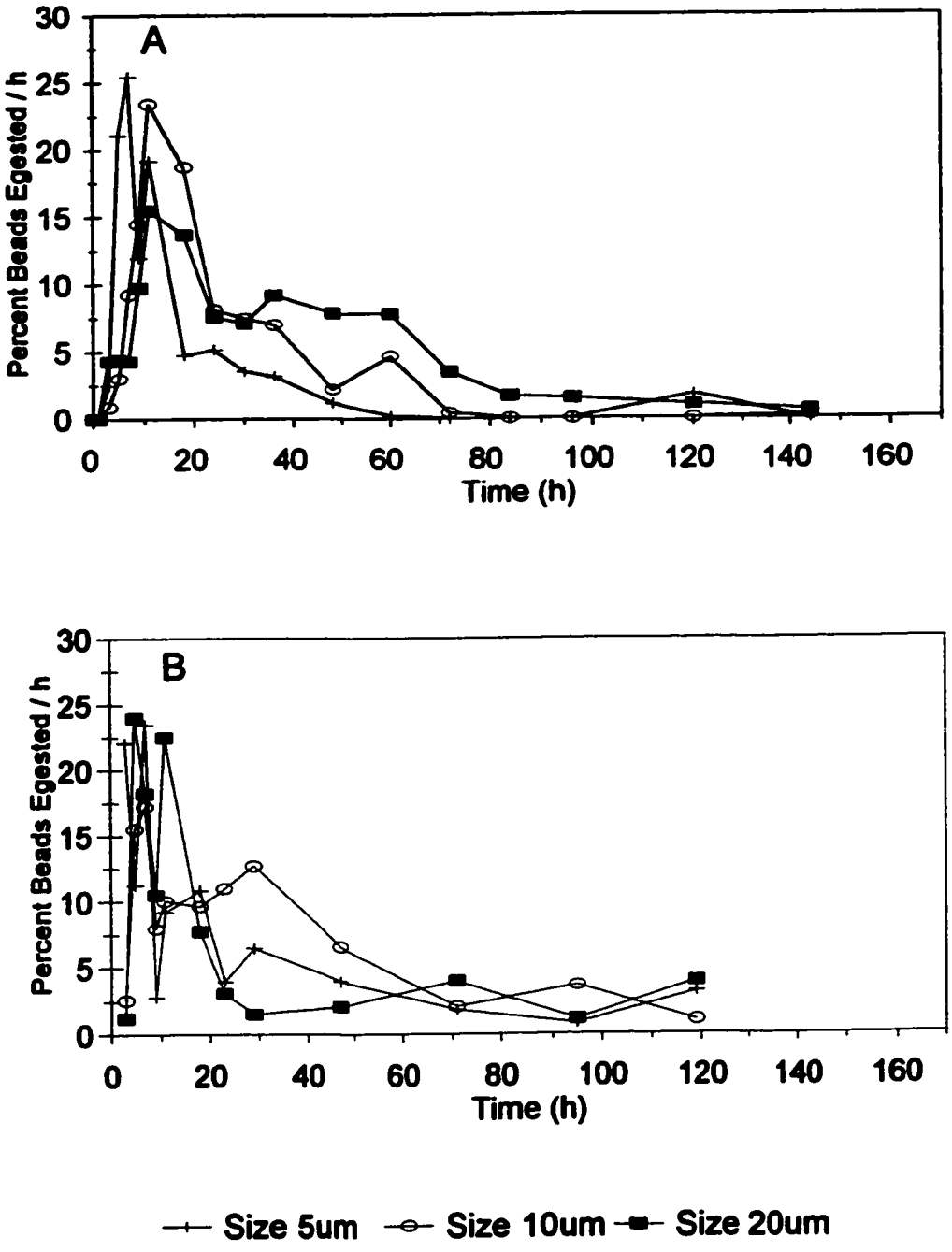


Figure 15. Mean egestion rate (number of beads egested per hour expressed as a percentage) of *Placopecten magellanicus* fed three sizes of polystyrene beads. (A) flow-through experiment 1 ($n = 8$). (B) flow-through experiment 2 ($n = 8$).

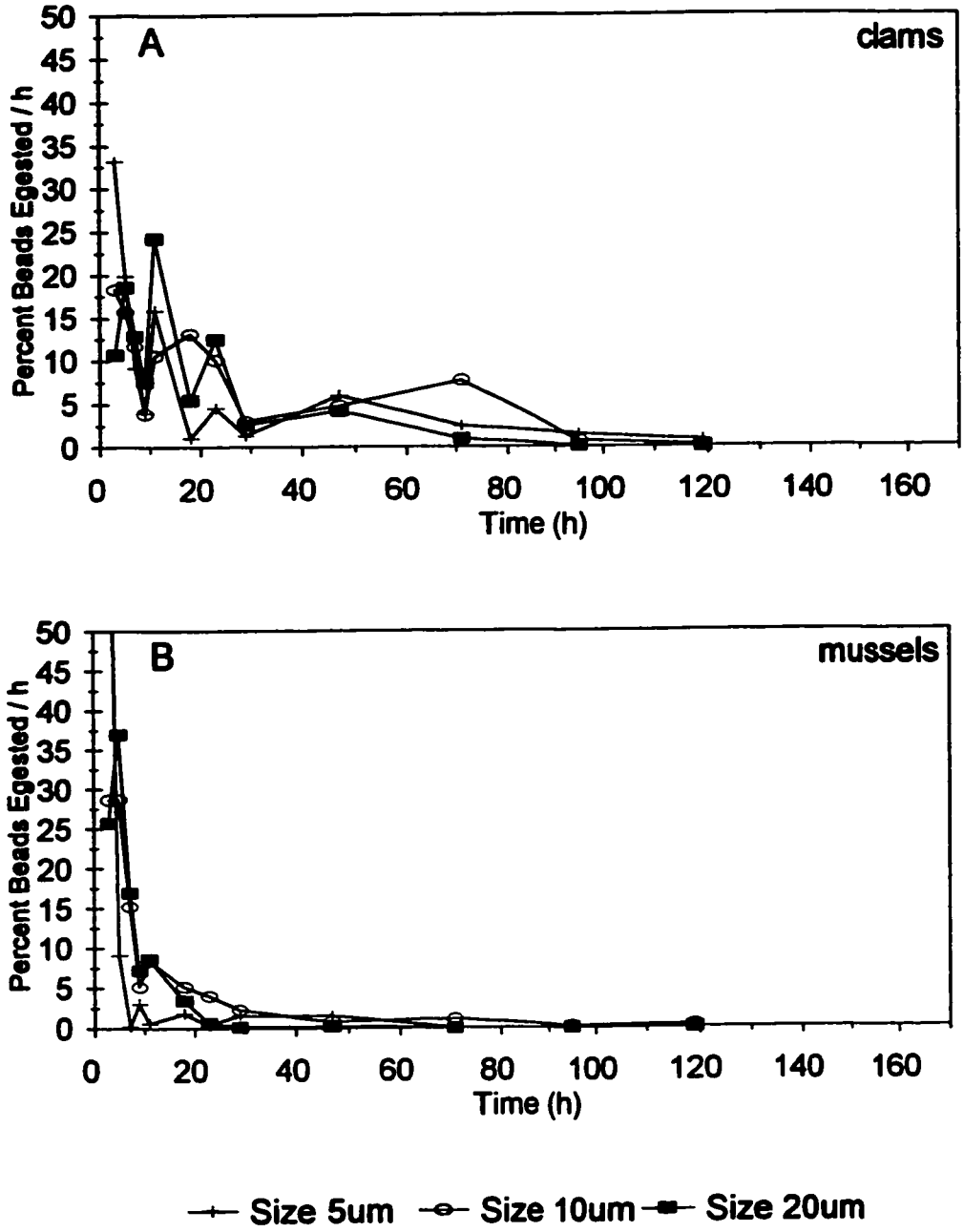


Figure 16. Mean egestion rate (number of beads egested per hour expressed as a percentage) of (A) *Mya arenaria* (n = 8) and (B) *Mytilus edulis* (n = 8) fed three sizes of polystyrene beads in flow-through experiment 2.

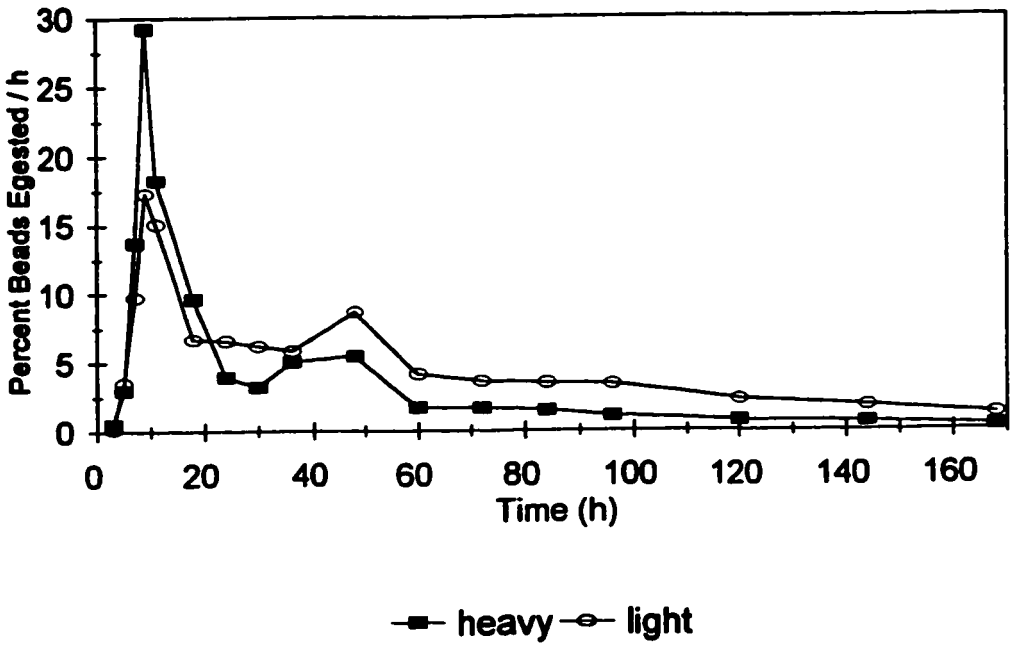


Figure 17. Mean egestion rate (number of beads egested per hour expressed as a percentage) of *Placopecten magellanicus* ($n = 9$) fed glass (heavy) beads of density 2.5 g ml^{-1} and polystyrene (light) beads of density 1.05 g ml^{-1}.

Table 3. Summary of Pearson correlation analyses relating gut retention time to soft tissue dry weight for *Placopecten magellanicus*, *Mytilus edulis*, and *Mya arenaria*.

Experiment	Species	Bead Type	*n*	*r*	*P*
static 1	*P. magellanicus*	5 μm	9	-0.2346	0.5434
		10 μm	9	-0.1271	0.7445
		20 μm	9	0.059	0.8801
static 2	*P. magellanicus*	5 μm	8	0.7057	0.0505*
		10 μm	8	-0.1141	0.7879
		20 μm	8	0.1969	0.6403
flow 1	*P. magellanicus*	5 μm	8	0.6264	0.0965
		10 μm	8	0.3841	0.3475
		20 μm	8	0.2134	0.6119
flow 2	*P. magellanicus*	5 μm	8	0.281	0.5002
		10 μm	8	0.1906	0.6511
		20 μm	8	0.3283	0.4272
	M. edulis	5 μm	8	-0.2121	0.6141
		10 μm	8	-0.1134	0.7891
		20 μm	8	-0.1703	0.6869
	M. arenaria	5 μm	8	0.2239	0.594
		10 μm	8	0.1433	0.735
		20 μm	8	0.2533	0.5449
density[†]	*P. magellanicus*	heavy	9	0.1444	0.711
		light	9	0.6165	0.077

* significant at $\alpha = 0.05$; [†] heavy = 2.5 g ml^{-1}, light = 1.05 g ml^{-1}

data were log transformed and retested, the results were the same (not shown). This indicates that either GRT is independent of bivalve size or the size range of bivalves used in these experiments (46 - 73 mm shell height) was small enough to reduce variation in GRTs resulting from body size. Therefore the decision was made not to attempt to correct GRTs for body size.

III. 3. v. Gut Retention Times: Selection by Size

Static Experiments

The mean GRTs of the 5, 10, 20 and 40 μm beads in static experiment 1 showed no significant difference ($F_{3,24} = 2.58$, $P = 0.13$) when all four bead sizes are included in the statistical analysis. When the 40 μm beads are omitted from the analysis to make the test comparable to the other experiments conducted with three sizes of beads, the existing difference between the mean GRTs of the three smaller sizes becomes statistically significant ($F_{2,16} = 13.77$, $P < 0.01$) (Figure 18a). There were significant differences between the average GRTs of 5, 10 and 20 μm beads in static experiment 2 ($F_{2,14} = 38.92$, $P < 0.01$) (Figure 18b). In both experiments the larger beads were retained longer than the smaller beads.

Flow-through Experiments

The mean GRTs of the 5, 10 and 20 μm beads in flow-through experiment 1 showed no significant differences ($F_{2,14} = 2.36$, $P = 0.17$) (Figure 19a). In flow-through experiment 2, the scallops did show a significant difference between average GRTs,

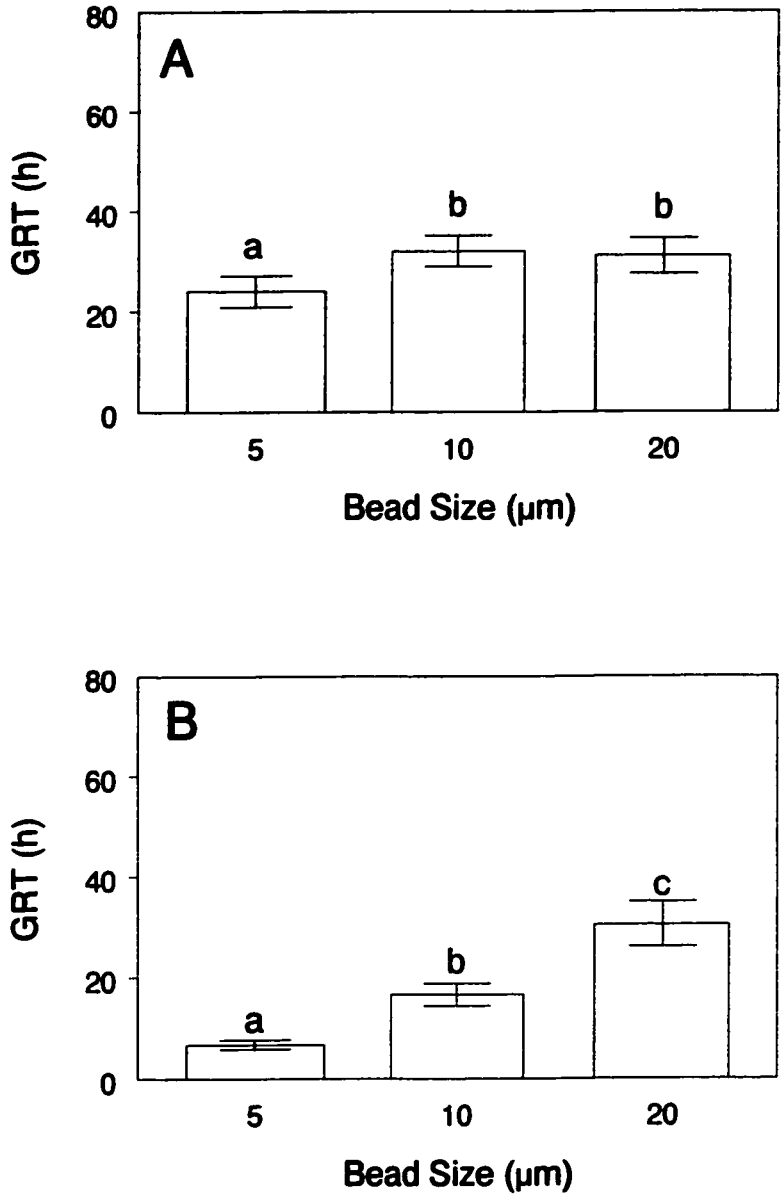


Figure 18: Gut retention times (GRT) ($\bar{x} \pm$ S.E.) of *Placopecten magellanicus* fed 5, 10, 20 µm beads (A) during static experiment 1 ($n = 9$), (B) during static experiment 2 ($n = 8$). Different letters above the error bars indicate a significant difference between mean gut retention times at $\alpha = 0.05$. In both experiments gut retention times for larger beads were significantly longer than for smaller beads.

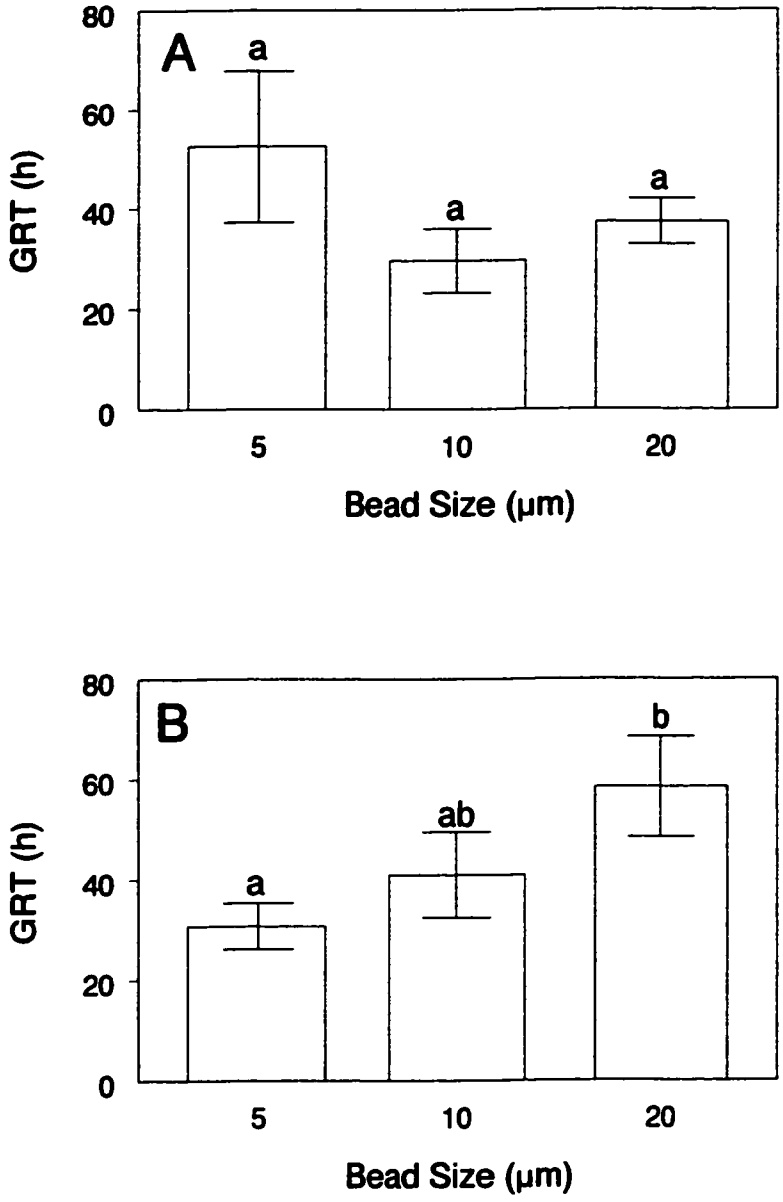


Figure 19. Gut retention times (GRT) ($\bar{x} \pm$ S.E.) of *Placopecten magellanicus* fed 5, 10, 20 µm beads (A) during flow-through experiment 1 ($n = 8$), (B) during flow-through experiment 2 ($n = 8$). Different letters above the error bars indicate a significant difference between mean gut retention times at $\alpha = 0.05$. In (B) gut retention times for larger beads were significantly longer than for smaller beads.

with the 20 μm beads again being retained significantly longer than the 5 μm beads ($F_{2,14} = 4.37$, $P < 0.05$) (Figure 19b). The clams and mussels did not show significant differences between the average GRTs of the three sizes of beads ($F_{2,14} = 1.57$, $P = 0.24$ and $F_{2,14} = 2.84$, $P = 0.09$, respectively) (Figures 20a and 20b).

III. 3. vi. Gut Retention Times: Selection by Density

Scallops exhibited significantly different GRTs between beads of different densities. The lighter polystyrene beads were retained longer than the heavier glass beads (paired $t_{(2),8} = -3.668$, $P < 0.01$) (Figure 21).

III. 4. Discussion

III. 4. i. Clearance Rates

Clearance rates were similar to those reported by other studies (Bayne et al. 1987; Lucas et al. 1987; Jørgensen and Riisgard 1988; Bacon et al. 1998) . Results from three of the five selection experiments gave scallop clearance rates within the range of values reported for *P. magellanicus* (Bacon et al. 1998), while the other two experiments gave clearance rates that were lower (Table 1). Negative clearance rates were obtained in flow-through experiment 1 although the scallops appeared to be feeding and beads were present in the faeces. This indicates that production of particles was occurring. Particle production as a result of cellular exfoliation is known to occur in scallops particularly when they are stressed (MacDonald et al. 1995; Potter et al.

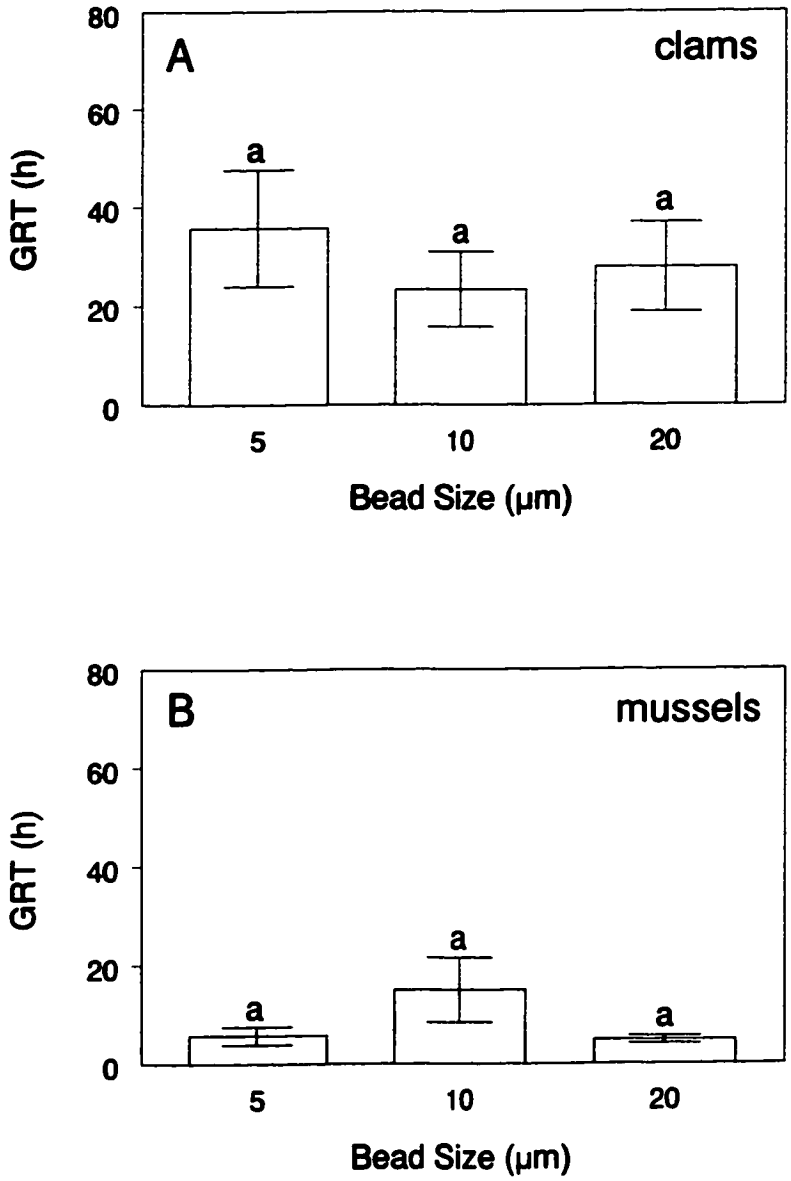


Figure 20: Gut retention times (GRT) ($\bar{x} \pm$ S.E.) of (A) *Mya arenaria* (n = 8) and (B) *Mytilus edulis* (n = 8) fed 5, 10, 20 µm beads during flow-through experiment 2. Identical letters above the error bars indicate no significant difference between mean gut retention times at $\alpha = 0.05$.

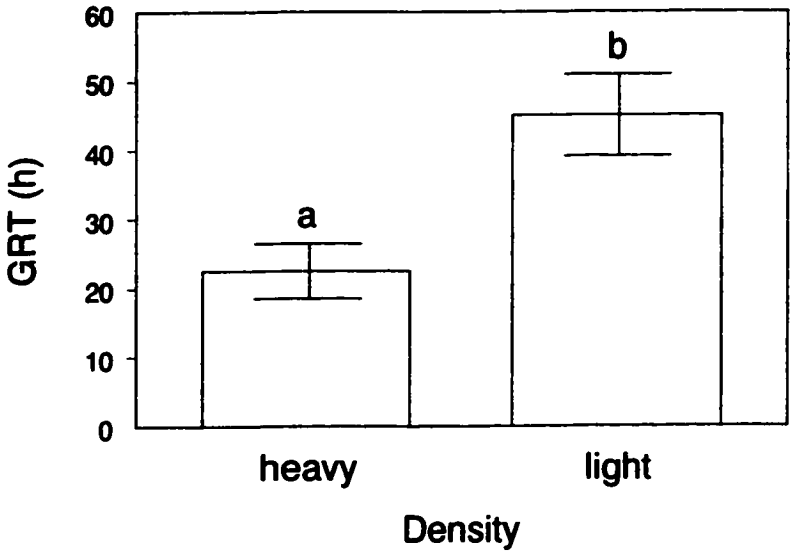


Figure 21: Gut retention times (GRT) ($\bar{x} \pm$ S.E.) of *Placopecten magellanicus* fed glass (heavy) beads of density 2.5 g ml^{-1} and polystyrene (light) beads of density 1.05 g ml^{-1}. Different letters above the error bars indicate a significant difference in mean gut retention times at $\alpha = 0.05$ ($n = 9$). GRTs of light beads were significantly longer than those of heavy beads.

1997).

Clearance rates determined for *Mya arenaria* were higher than that reported by some studies (Allen 1962; Wright et al. 1982) but fall within the range reported by Jørgensen and Riisgard (1988) and Bacon et al. (1998) (Table 1). Clearance rates for *Mytilus edulis* were lower than those reported by Newell et al. (1989), but fall within the range given by some authors (Bayne et al. 1987; Lucas et al. 1987) and are higher than those found by Wright et al. (1982) (Table 1).

Clearance rates vary depending on a number of factors including, food quality and quantity (Palmer 1980; Bacon et al. 1998), temperature (McLusky 1973; MacDonald and Thompson 1986), and flow rates (Wildish et al. 1992), making comparisons between different studies difficult. Despite the comparatively low clearance rates found in some experiments, beads were present within the faeces of all scallops in all experiments indicating that ingestion of beads did occur.

III. 4. ii. Size of Digestive Tubules

The dimensions of the scallop digestive tubule lumen were on average 31 x 53 μm. It was expected that fixation in Bouin's would cause the tissues to shrink, however this does not appear to be the case as the only statistically significant change in size was an increase in one of the dimensions of the tissue block by 16%. The volume of the wax in which the tissue blocks were embedded decreased by 10% upon hardening. Assuming that the tissues contracted along with the wax, each dimension would have decreased by approximately 3.45%. The combined effects of fixation and embedding

mean that the observed tissue dimensions may be overestimated by 13%. However, the digestive tubules are large enough to take in 20 μm beads even when this overestimation is taken into account. The average width of the tubules was less than 40 μm which indicates that the 40 μm beads used in static experiment 1 would not fit into the tubules.

Most studies on bivalve digestive glands do not report the dimensions of the digestive tubules. Palmer (1979) found a diameter of 60-70 μm for secondary ducts in *Arctica islandica*, however the diameter of the digestive tubules was not given. This value is slightly larger than the scallop digestive tubule dimensions found here.

The lack of a correlation between tubule and shell size (Table 2) indicates that growth of the digestive gland occurs by addition of tubules not by an increase in the size of the tubules. Furthermore, tubule size would have the same influence on size selection regardless of the size of the individual scallop.

There was a dual purpose for examining the gland, to ascertain if it was possible for the larger beads to fit into the tubules and to determine if the 20 μm beads do, in fact, enter the tubules. The presence of 20 μm beads within the tubules of the scallop digestive gland (Figure 13) confirms that the tubules are large enough to hold 20 μm beads and are therefore large enough to hold the 5 and 10 μm beads as well. Phagocytosis of the 20 μm beads however was not observed. The size of the 20 μm beads was roughly comparable to the size of the cells lining the tubules which indicates that these beads are too large to be phagocytosed. Selection in bivalves can happen either by retention of certain particles longer than others so that they undergo further

extracellular digestion or by directing certain particles to the digestive gland for intracellular digestion. Although 20 μm beads were visible within the tubules, they were not numerous and the observation that they were not phagocytosed indicates that directing larger beads to the digestive gland for intracellular digestion is probably not the strategy being used by scallops to retain large particles.

III. 4. iii. Egestion Patterns, Gut Retention Times, and Postingestive Selection

Patterns of Egestion

Decho and Luoma (1991) reported that *Potomocorbula amurensis* and *Macoma balthica* exhibit a two phase egestion pattern. Egestion began with a distinct initial pulse of marker followed by a lag period of very little marker in the faeces. Later there was a more prolonged and irregular release of marker. The authors interpreted the first phase as representing intestinal faeces and the second as glandular faeces. The length of time that each period lasted was much greater for *M. balthica* than for *P. amurensis*. Penry (2000) also studied egestion patterns in *Potomocorbula amurensis*. She found that some individuals (two-thirds of the animals studied) exhibited egestion patterns characterized by an initial pulse, a lag period and a later more prolonged period of egestion, although the lag period was not always distinct. However, the time scale over which the phases occurred was much greater than that described by Decho and Luoma (1991) for the same species (up to 80 h compared to 24 h). Penry also interpreted the two phases as representing intestinal and glandular faeces. Researchers studying the absorption of metals in the guts of *Mytilus edulis* (Wang et al. 1995), *Perna viridis*,

and *Ruditapes philippinarum* (Chong and Wang 2000) report egestion patterns that are characterized by an initial distinct peak which may or may not be followed later by several smaller pulses of metal egestion. This was the egestion pattern exhibited by *Mytilus edulis* in this study (Figure 16b). Otherwise the animals in this study did not produce the egestion patterns described above. Egestion curves for scallops were generally characterized by one or two initial peaks of beads before 18 h. These were sometimes followed by a later smaller pulse of beads occurring anywhere from 24 to 50 h, after which egestion usually declined gradually (Figures 14, 15, and 17). However the pulses were not separated by distinct lag periods of little to no egestion as described by previous researchers (Decho and Luoma 1991; Penry 2000).

It is difficult to interpret what these pulses of material represent. It is possible that the initial pulse(s) represent intestinal faeces with the later pulse representing glandular faeces. The absence of a lag period implies that in scallops, intestinal and glandular faeces are not well separated in time. Once glandular digestion has time to occur both types of faeces may be produced simultaneously. Furthermore, although previous researchers have interpreted the later prolonged egestion of faeces as glandular in origin, they may actually represent both glandular faeces and faeces that were not processed by the gland but were retained within the stomach for further extracellular digestion. The later pulse of material seen in this study probably does represent the production of some glandular faeces but it is likely that they are combined with intestinal faeces. The subsequent slow production of faeces could be a combination of both glandular and intestinal faeces or may just represent the slow release of particles

retained within the stomach.

Gut Retention Times

Comparisons among different studies on GRTs are complicated by variables such as quality and quantity of food, as well as the types of markers used. The method of defining GRT also varies among studies; some studies use the time at which 90 (t_{90}) or 95 (t_{95}) percent of the marker passes as the GRT, others use retention half-times (t_{50}), mean minimum gut passage times (t_0), or the time when egestion reaches a maximum (t_{max}) as the criterion.

The average GRTs (t_{50}) found in this study for *Mytilus edulis* of 4.8 - 14.9 h, are comparable with but in general slightly longer than those reported in previous studies on this species (Table 4). The average GRTs for *Mya arenaria* (23.3 - 35.7 h) found here are higher than those reported by Scarratt (1994) of 2 - 18 h, who also used t_{50} as the GRT (Table 4).

The GRTs found in this study for *Placopecten magellanicus* (6.8 - 58.4 h) are amongst the longest reported for any species (Table 4). The only other study on *P. magellanicus* where GRTs were measured is Cranford et al. (1998) who found GRTs of 4.4 h and 5.8 h using t_{90}. However, in Cranford et al. (1998) scallop faeces were only collected for 12 h after ingestion which may underestimate the GRT.

The long GRTs found in this study for all three species tested may be related to some aspect of the experimental design such as the amount of food available to the animals following exposure to the beads or the type of marker used. However, the

Table 4. Summary of bivalve gut retention time (GRT) studies.

Species	Authors	t_x*	GRT (h)
Cerastoderma edule	Hawkins et al. (1990)	t_{90}	~ 4 - 17
Cerastoderma edule	Navarro et al. (1994)	t_{95}	1.89 -6.42
Macoma balthica	Decho and Luoma (1991)	t_0	9.6
Macoma nasuta	Hylleberg and Galluci (1975)	t_0	1 - 9
Mercenaria mercenaria	Bricelj et al. (1984)	t_{90}	< 2 - 27
Mya arenaria	Scarratt (1994)	t_{50}	2 - 18
Mya arenaria	this study	t_{50}	23.3 - 35.7
Mytilus edulis	Hawkins and Bayne (1984)	t_{max}	3.71 - 9.75
Mytilus edulis	Bayne et al. (1987)	t_{90}	3.28 - 15.34
Mytilus edulis	Bayne et al. (1989)	t_{95}	1.81 - 3.23
Mytilus edulis	Hawkins et al. (1990)	t_{90}	~ 3 - 14.5
Mytilus edulis	Wang et al. (1995)	t_{90}	4.6 - 64.2
Mytilus edulis	Gagnon and Fisher (1997)	t_{50}	0.4 - 4.3
Mytilus edulis	this study	t_{50}	4.8 - 14.9
Perna viridis	Chong and Wang (2000)	t_{90}	4.0 - 15.8
Placopecten magellanicus	Cranford et al. (1998)	t_{90}	4.4 & 5.8
Placopecten magellanicus	this study	t_{50}	6.8 - 58.4
Potamocorbula amurensis	Decho and Luoma (1991)	t_0	0.86
Potamocorbula amurensis	Penry (2000)	t_{90}	2.5 - 97
Ruditapes philippinarum	Chong and Wang (2000)	t_{90}	3.3 - 35.4

* x = percent marker egested when gut retention time measured

consistently long GRTs exhibited by *P. magellanicus* despite variations in experimental design (e.g. static vs. flow-through, light vs. heavy beads) indicate that retaining food for long periods may be a digestive strategy of this species. The positive relationship between absorption efficiency and GRT is well documented (Bayne et al. 1984; Wang and Fisher 1996). Earlier studies have shown that *P. magellanicus* reduces clearance rates and increases pseudofaeces production to maintain a constant ingestion rate when seston concentration reaches 7 mg l^{-1} (Bacon et al. 1998). Also, MacDonald et al. (1998) found that scallops maintained the percent organic material in their faeces from 17-26 % although the seston on which the scallops were feeding varied from 25 - 80 % organics. Reducing ingestion rates and maintaining long GRTs may enable scallops to have relatively high absorption efficiencies even though food quality may be poor. However, other species show a similar feeding pattern of reducing clearance rates and/or increasing pseudofaeces production as seston concentration increases, including *Mya arenaria* (Bacon et al. 1998) and *Mercenaria mercenaria* (Bricelj and Malouf 1984) but do not have as lengthy GRTs as *P. magellanicus* (Bricelj et al. 1984; Scarratt 1994; this study).

Selection by Size

The gut retention times of the different bead sizes indicate that scallops are capable of selection on the basis of particle size. In only one of the experiments (flow-through exp 1) did scallops exhibit no selection (Figure 19a). This experiment also had negative clearance rates (Table 1), which may be an indication that the animals were

stressed and therefore behaved abnormally. Alternatively this variability may be normal; it is known that *Placopecten magellanicus* has the ability to select particles prior to ingestion but it does not always do so (MacDonald and Ward 1994). Static experiment 1 showed a trend towards retention of large particles longer but statistically the difference was not significant when all four treatments (bead sizes) were included. Eliminating the largest beads (40 μm) from statistical analysis indicated a strongly significant difference between the remaining beads ($P < 0.01$). Eliminating the largest bead was justified as this was the only experiment where this size bead was used; also, since beads were fed to the scallops in equal volumes instead of equal numbers as in other experiments, the actual number of 40 μm beads fed to the scallops was low (~30 beads ml^{-1}) and, therefore, measuring the amount of these beads in the faeces was more prone to error.

When selection was observed, the consistent trend was that larger beads (20 μm) were passed through the digestive tract at a slower rate than smaller beads (5 μm) (Figures 18 and 19b). This indicates that the larger beads were retained in some way, possibly they were selected for attempted intracellular digestion and carried to the digestive gland. However, this explanation is unlikely as it would be maladaptive for scallops to send particles that are too large to be phagocytosed into the gland where they might potentially clog the tubules. It is more likely that these large, light beads became caught up and remained in the recirculating fluid within the stomach because they were too large to fall into rejection tracts and were light enough to remain in suspension. Some 20 μm beads must have inadvertently been carried into the digestive

gland as they do appear in the pulse of material presumed to be glandular in origin seen in the egestion curves (Figures 14 and 15a). Also, the sections made of scallop digestive glands described previously indicate that some 20 μm beads do enter the digestive gland (Figure 13). In nature, retention of large light particles would be more adaptive, as particles with these characteristics are not likely to be inert and totally resistant to digestion as these polystyrene beads were. The preferential retention of large particles may be beneficial as large suspended particles are more likely to be organic. This is because inorganic particles tend to be denser than organic (Muschenheim 1987) and large dense particles tend to settle out of suspension quickly (Grant et al. 1997; Cranford et al. 1998). Kreeger and Newell (in review) have found that a large benthic diatom (15 μm) is utilized more efficiently by the ribbed mussel (*Geukensia demissa*) (up to 93% assimilation efficiency) than a smaller phytoplankton (5 μm) (up to 79% assimilation efficiency). Also, large organic particles contain more organic material per unit than smaller organic particles and may therefore be a more efficient food source.

The smaller 5 μm beads passed through the digestive tract faster; these small particles probably fell into the rejection grooves on the sorting tracts and were carried to the intestine (Figure 3). In nature this rejection of smaller particles may be beneficial. Most inorganic particles ingested by the scallop are likely to be small since, as mentioned, small inorganic particles such as silt tend to be resuspended more frequently and remain in suspension longer than larger inorganics (Grant et al. 1997; Cranford et al. 1998). This process might save energy by preventing attempted intracellular digestion of these indigestible particles. However, many algal species are also small;

rejection of small algae might not be a problem if they do not form a significant portion of the diet. If small algae are a significant component of the available food, selection on the basis of chemical properties may play a role in preventing the rejection of these particles (see Chapters IV and V). Also, if the algae are fairly digestible, the cell walls will probably be broken down by extracellular digestion in the stomach. The cell contents would then be carried to the digestive gland for intracellular digestion (Bricelj et al. 1984).

Observations on dissected structures may be unreliable as dissection interferes with the integrity of currents and has been found to cause bivalve feeding structures to function abnormally (Ward 1996). However, overall the results are in agreement with observations made on dissected bivalve stomachs. Reid (1965) noted that large, light particles and masses of particles were recirculated in the stomach while heavy particles small enough to fit into the sorting grooves were rejected to the intestine. Unfortunately, "small", "large", "heavy", and "light" were not defined by Reid.

Other studies give results which differ from those found here. Cranford et al. (1998) fed two sizes of beads (6 and 10 μm) to *Placopecten magellanicus*, and found that the smaller beads were passed at a slower rate than the larger beads; no statistical tests were reported to determine if the difference was significant. The two sizes of beads were presented to the scallops separately with the smaller beads first then the larger beads after a 2 h interval; this staggered exposure may have an influence on gut passage times of the beads. Also the study was not designed to test for postingestive selection by size, rather the beads were presented as tracers to measure the gut passage

time of the ambient food available to the scallops. In another study, Hughes (1977) sampled particles from different areas of the stomach of *Abra tenuis* and found particles at the entrance to the digestive tubules were significantly smaller than those ingested, indicating sorting by size had occurred with smaller particles directed to the digestive gland. *Abra tenuis* is a deposit-feeder; possibly deposit-feeding bivalves have a different strategy for size selection than suspension-feeders. Finally, Penry (2000) fed two sizes of polystyrene beads (9 and 44 μm) simultaneously to Asian clams (*Potamocorbula amurensis*). She found that overall there were no significant differences in gut passage times of the two sizes of beads. However some clams passed the 44 μm beads faster while approximately 28% of the clams passed the 9 μm beads faster. Penry attributed the slower passage of larger beads seen in some clams to clogging of the digestive gland tubules by the large beads. Penry reported that qualitative observations on the glands indicated that 44 μm beads entered and sometimes packed the tubules. However, the dimensions of the tubules were not reported. It is unlikely that clogging of the tubules by large beads would explain the observations made in the present study. The 20 μm beads used were not large enough to clog the tubules (Figure 13) and large numbers of beads were not found in the tubules or the ducts leading to them. Although the possibility of the 40 μm beads used in experiment 1 entering the tubules was not tested, the measurements of the tubule lumen indicate that the 40 μm beads would not fit into the tubules.

It was not possible to compare the size selection capabilities of different species of bivalves because clams and mussels did not exhibit selection in this study. From the

four experiments conducted on scallops it is clear that selection by size is not always present. Therefore, no conclusions on the size selection capabilities of mussels and clams can be made on the basis of the one experiment conducted (Figure 20a and 20b).

Selection by Density

Scallops are also capable of selection on the basis of density. Heavier particles (2.5 g ml^{-1}) passed through the digestive system faster than lighter particles (1.05 g ml^{-1}) (Figure 21). Heavy particles are more likely to fall onto the sorting tracts. If the particles are small enough, they will end up in the rejection grooves and be carried to the intestine (Figure 3). Lighter particles are more likely to remain in suspension. Rejection of dense particles may be beneficial to the bivalve since organic particles tend to be lighter than inorganic particles (Muschenheim 1987). Although it is acknowledged that composition may be a confounding factor in this experiment, evidence discussed earlier, that bivalves do not distinguish between untreated polystyrene and silica beads preingestively (Ward and Targett 1989), indicates that it is not an issue. Also the dramatic difference in densities between the glass and polystyrene beads used here (1.05 g ml^{-1} vs. 2.5 g ml^{-1}) is probably much more of a factor than any differences in surface characteristics.

The tendency for heavy particles to be rejected combined with the observed tendency of scallops to reject smaller particles may help to ensure that small dense particles, such as silt, are removed from the stomach. Silt has little nutritional value aside from adsorbed debris and surface bacteria which can be removed by extracellular

digestion in the stomach (Hylleberg and Gallucci 1975; Hughes 1977). Meanwhile larger, lighter organic particles are more likely to be retained. Retention of particles would give more time for extracellular digestion to act and increases the chances that the particles will enter the digestive gland for intracellular digestion.

In order to test selection solely on the basis of physical properties, the particles used in the experiments described in this chapter were completely inert and without nutritional value. In nature few particles encountered by scallops would be completely devoid of nutritional value, therefore selection on the basis of chemical properties may also play a role in digestion. The ability of scallops to detect chemical differences amongst particles within the gut and the impact, if any, of that capability on particle processing will be examined in the following two chapters.

Chapter IV: Selection by Chemical Properties, Protein-Coated vs. Uncoated Beads

IV. 1. Introduction

Many bivalves, including scallops, have the ability to sort organic from inorganic particles on the gills and labial palps (Newell and Jordan 1983; Cranford and Gordon 1992; Iglesias et al. 1992; Hawkins et al. 1996; MacDonald and Ward 1994; Ward and MacDonald 1996). One study has confirmed that *Mytilus edulis* can sort particles before ingestion solely on the basis of chemical properties (Ward and Targett 1989). Gagnon and Fisher (1997) have provided evidence that particle chemistry can also influence gut passage times in *M. edulis*, which implies postingestive selection. The objective of this component of the study was to determine if particle chemistry has a role in postingestive selection in *Placopecten magellanicus*.

In order to isolate the effect of particle chemistry from that of physical properties, several factors must be considered when choosing test particles. 1) The particles should appear chemically distinct but physically identical to the bivalve. This includes not only size, shape and surface characteristics but integrity as well. The particles must either remain intact within the bivalve stomach or break down at the same rate, otherwise physical factors such as density and size differences between the cell wall fragments and cell contents may come into play (Bricelj et al. 1984). This

precludes the use of two algal species as even the most similar algae will have subtle differences in size, shape, surface characteristics and digestibility. 2) Preferably the particles should be presented simultaneously and therefore must be easily distinguishable from one another by the researcher. 3) The test particles must also be detectable and quantifiable in the faeces. In this study these criteria were satisfied by utilizing two colours of fluorescent polystyrene beads, one of which had been coated with a protein.

IV. 2. Methods

IV. 2. i. Selection of Particles

The design of this experiment was to feed fluorescent beads of two colours which were identical in size, density and composition to scallops. One of the two bead types would be coated with an organic substance. The faeces would then be analyzed using flow cytometry to determine the gut retention times. Attempts were made to coat the beads 'in house' with a readily available protein such as gelatin or bovine serum albumin (BSA), because pre-coated beads available from manufacturers are expensive, sizes are generally very small and the only coatings available are mammalian antibodies. Several techniques to coat beads with proteins were attempted including passive adsorption and covalent-binding protocols (Polysciences 1991a; Polysciences 1991b; Bangs Laboratories 1998a; Bangs Laboratories 1998b). However, these attempts were mostly unsuccessful and when binding was achieved, the protein coat was unstable. Therefore pre-coated beads were obtained from a manufacturer. It was

assumed that the immune system of scallops would not respond to mammalian antibodies in the gut and would treat them simply as protein.

It was not possible to measure the integrity of the coating throughout the feeding process. Preliminary experiments indicated that the background protein level of the stomach contents was very high and overwhelmed any protein signal from the beads. Therefore, isolating beads from the stomach contents to measure the remaining protein coat was not possible. Due to the expense of the coated beads, further attempts to deal with this problem were foregone and the assumption was made that the protein coat was strong enough to remain intact until the beads reached the stomach where selection would occur. This assumption could not be tested and would only be proven correct by a difference in gut retention time between these otherwise identical beads.

IV. 2. ii. Collection and Holding of Scallops

Scallops (*Placopecten magellanicus*, 66-83 mm shell height) were collected by scallop drag near Maces Bay, New Brunswick (45° 04' N, 66° 40' W). The animals were held in a cage suspended from the Dipper Harbour wharf until they were transported to UNBSJ where they were held in a recirculating seawater tank (salinity 32‰, temp. 11 °C) for 3 days prior to experimentation. Scallops were fed cultured algae, *Isochrysis galbana* (strain T-iso), while being held.

IV. 2. iii. Bead Mixture

The beads used were fluorescent yellow polystyrene beads (excitation max. 490

nm, emission max. 515 nm), coated with a protein (goat anti-mouse IgG) and uncoated fluorescent pink beads (excitation max. 573 nm, emission max. 598 nm) (Bangs Laboratories Inc.). The mean diameters of the beads were 5.5 and 5.7 μm respectively and the density was 1.1 g ml^{-1}. Equal numbers of protein-coated yellow and uncoated pink polystyrene beads were presented directly to each scallop at an attempted final concentration of 20 000 beads ml^{-1}.

IV. 2. iv. Experimental Design

The experimental set-up used was similar to that described in section III. 2. i (Figure 6). Scallops were placed in the beakers 24 h prior to beginning the experiment (temp. 11 °C). The experiment was conducted twice; in total eighteen scallops received a mixture of yellow and pink beads, one scallop received only yellow beads, one scallop received only pink beads, four scallops acted as controls for background fluorescence and received only *Isochrysis galbana*; empty beakers acted as controls for particle settling and received both yellow and pink beads. The animals were exposed to the beads suspended in filtered seawater for 1 h, then removed to natural seawater. Throughout the remainder of the experiments, scallops were fed *I. galbana* supplied by peristaltic pump. Faeces were subsequently collected every hour for 12 h, then every 6 h for the next 24 h, then every 12 h until 144 h post exposure.

Water samples were taken at the beginning and end of the exposure period to measure clearance rates. The water samples were analyzed on a Coulter multisizer and clearance rates calculated as in section II. 2. i.

IV. 2. v. Faecal Analysis

Faecal samples were treated with Coulter Dispersant Type IC and homogenized to break up the pellets. Immediately prior to analysis each faecal sample was drawn through a 30 G needle ten times to fully break apart any clumps present. The samples were then run for 2 min on a Becton Dickson FACScan flow cytometer equipped with a 488 nm argon ion laser. The flow cytometer had three detectors which detected fluorescence of different wavelengths: FL1 detected wavelengths of 530 ± 30 nm, FL2 detected 585 ± 42 nm, and FL3 detected wavelengths 650 nm and above. A fourth detector detected forward light scatter (FSC). Forward light scatter is proportional to particle surface area, therefore this detector measured the relative size of the particles. The detectors were set to logarithmic mode, providing four decades of signal detection. The yellow beads were detectable on the both the FL1 and FL2 detectors while the pink beads were detectable only on the FL2 detector. The two emissions did not overlap therefore the region where each type of bead fell could be mapped and the number of events within each region determined (Figure 22). Due to time constraints the faeces from one scallop (#5, fed both types of beads) were not analyzed.

IV. 2. vi. Calculating Gut Retention Times

The time at which 50% of the beads passed through the gut (t_{50}) was used as the gut retention time (GRT). The t_{50} was estimated by plotting the cumulative percentage of pink or yellow beads passed at each time interval (indicated by the number of events counted in 2 min) against time to get a cumulative egestion curve. The data were log

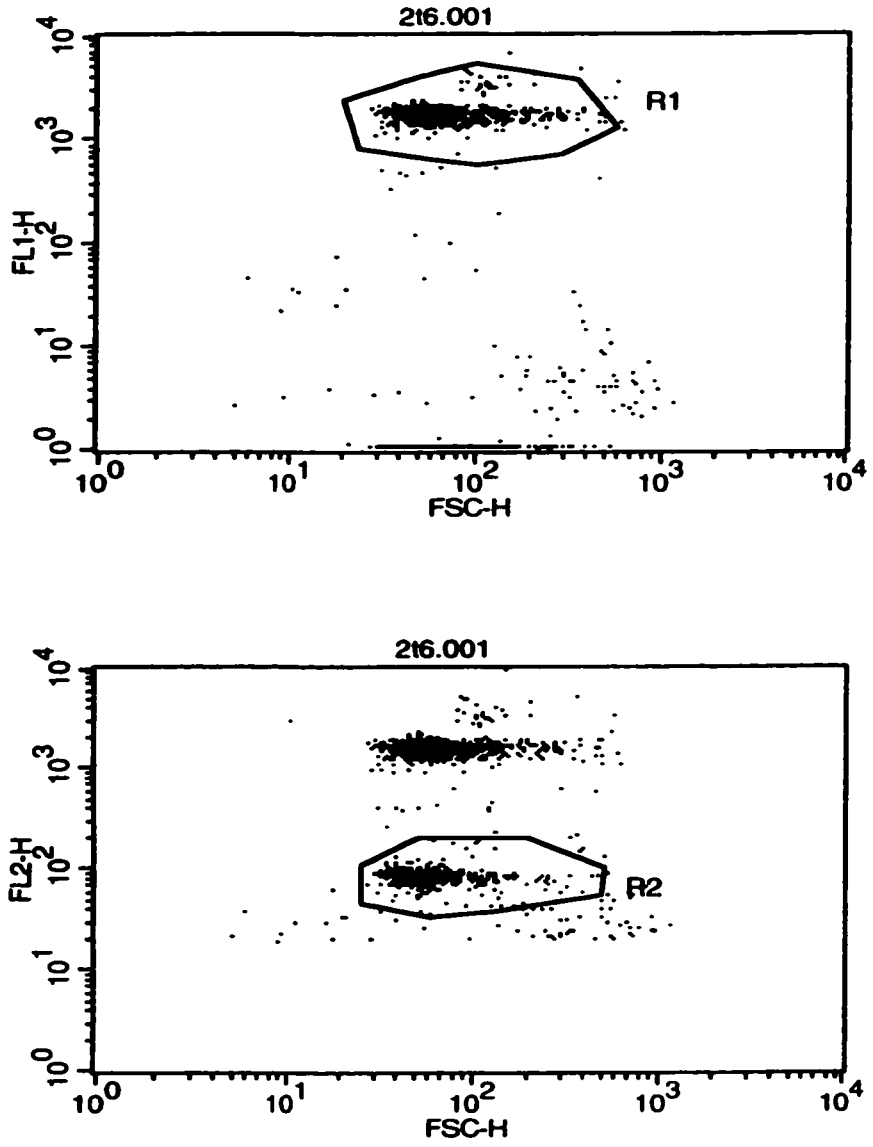


Figure 22: Flow cytometer printouts of a faecal sample from a scallop fed fluorescent yellow (excitation max. 490 nm, emission max. 515 nm) and pink beads (excitation max. 573 nm, emission max. 598 nm). Axes represent light intensity. FL1-H = fluorescence detector 1, FL2-H = fluorescence detector 2, FSC-H forward scatter detector (sizing parameter), R1 = region 1, R2 = region 2. R1 encompasses the region where the coated yellow beads fluoresce while R2 corresponds to the area where the uncoated pink beads fluoresce. Note that although the yellow beads are detected by both the FL1 and FL2 detectors they are clearly distinguishable from the pink beads which are only picked up by the FL2 detector.

transformed and the polynomial regression equation which best matched the curve was determined. R-squared values were always greater than 0.90, and usually greater than 0.97. GRT was estimated as the interpolated time at which 50% of the beads were egested. To determine if GRTs should be corrected for body size, correlations between GRTs and scallop soft tissue dry weights were tested using Pearson correlation analysis.

IV. 2. vii. Statistical Analysis

The null hypothesis for this study was that the presence or absence of a bead coating had no effect on GRT. The alternate hypothesis was that the GRT of uncoated beads (pink) would be shorter than that of coated beads (yellow). The average GRTs for the two types of beads were tested for significant differences using a one-tailed paired t-test. GRTs were tested for normality and homoscedasticity prior to statistical comparison. If the data were not normal, the Wilcoxon signed ranks test was used. All statistical tests were performed using SigmaStat statistical software (Jandel Scientific, version 4.0).

IV. 3. Results

The average standardized clearance rate for this experiment was $0.15 \pm .05$ lh^{-1} ($\bar{x} \pm$ S.E.). Gut retention times ranged from 5.4 - 34.0 h. There were no correlations between soft tissue dry weights and GRTs for pink beads ($r = 0.217$, $n = 17$, $P = 0.403$) or for yellow beads ($r = 0.124$, $n = 17$, $P = 0.635$). When the data were log transformed

and the tests repeated, the results were the same. This indicates that either there is no correlation between scallop size and GRT or that the scallops used (66-83 mm shell height) were similar enough in size to eliminate any size effect. Therefore the GRTs were not corrected for scallop size.

The average egestion curve for both experiments is characterized by one peak of both types of beads at 9 h and another peak of just coated beads at 12 h. Egestion of uncoated beads declines rapidly after 9 h, while coated beads are egested more gradually (Figure 23).

The majority of the scallops in experiment 1 retained the coated yellow beads longer than the uncoated pink beads (7 out of 9) (Figure 24a). However, due to the extremely long retention of uncoated beads by one animal (scallop #6) (Figure 24a), there was no statistically significant difference between the mean gut retention times (paired $t_{(1),8} = -1.155$, $P = 0.44$). The Dixon test was used to confirm that #6 was an outlier (Kanji 1993). When #6 is removed there is a strongly significant difference in the gut retention times (paired $t_{(1),7} = -2.701$, $P = 0.016$), with the yellow coated beads retained longer (Figure 25a). In experiment 2, only half of the scallops retained the coated beads longer, the remainder either did not select, or retained uncoated beads longer (Figure 24b). There was no significant difference in GRTs even when an outlier (confirmed by Dixon test) was removed (scallop #15) (Figure 25b) (paired $t_{(1),6} = -1.206$, $P = 0.14$). When results from both experiments are pooled there is a significant difference between the mean GRTs with the coated beads retained longer than the uncoated beads (paired $t_{(1),14} = -2.624$, $P = 0.01$) (Figure 25c).

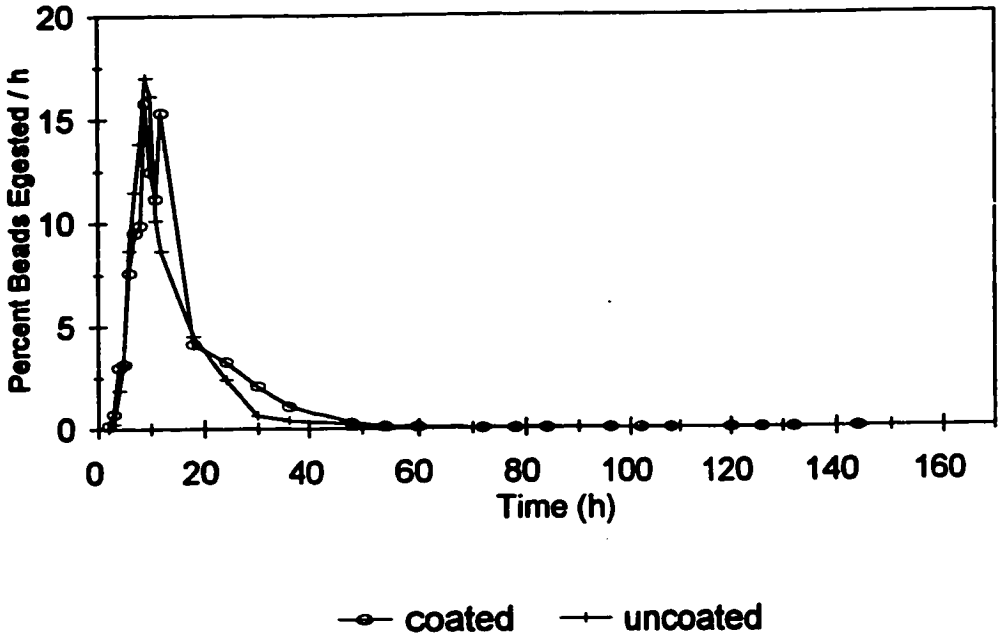


Figure 23. Mean egestion rate (number of beads egested per hour expressed as a percentage) of *Placopecten magellanicus* (n = 15) fed uncoated beads and beads coated with protein.

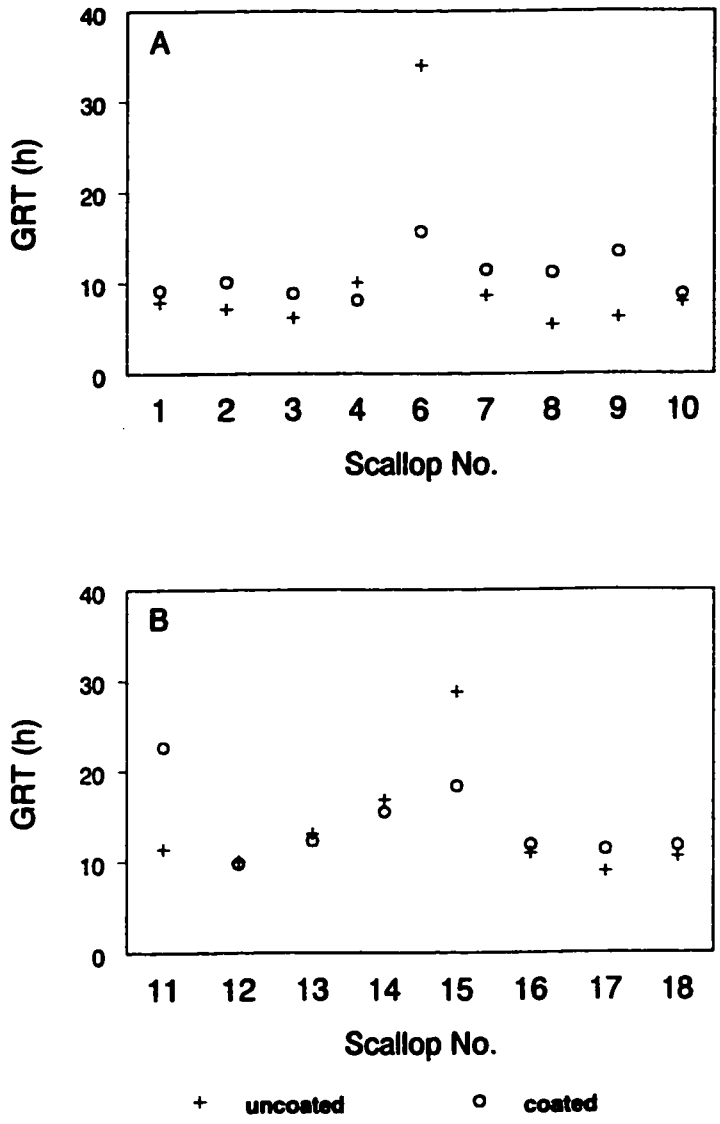


Figure 24: Scatter plots of GRTs of uncoated (+) and coated (◦) beads. (A) experiment 1 ($n = 9$), (B) experiment 2 ($n = 8$). Note the long GRT values for uncoated beads for scallops #6 and #15.

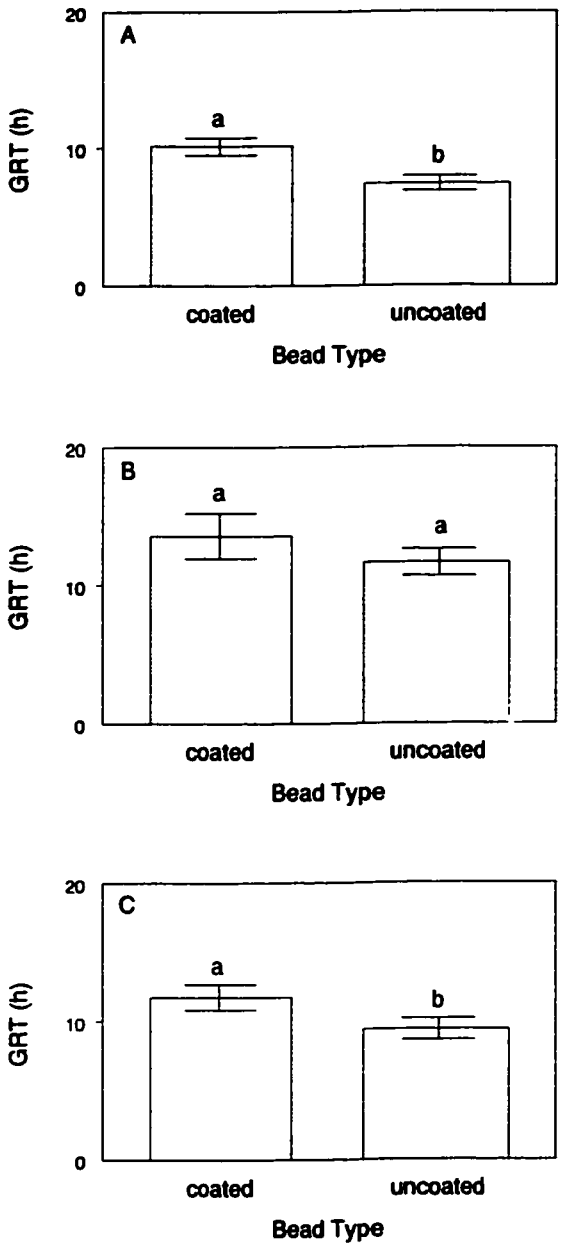


Figure 25: Gut retention times ($\bar{x} \pm$ S.E.) of *Placopecten magellanicus* fed protein-coated and uncoated beads. (A) experiment 1 ($n = 8$), (B) experiment 2 ($n = 7$), and (C) experiments 1 and 2 pooled ($n = 15$). Different letters above the error bars denote a significant difference at $\alpha = 0.05$. In (A) and (C) the coated beads were retained significantly longer than the uncoated beads.

IV. 4. Discussion

The egestion pattern produced by the scallops in these experiments differs from that seen in earlier experiments testing size and density. The initial pattern is similar to that observed before, there being two pulses of egestion before 18 h. However, the later pulse between 24 - 50 h is absent (Figure 23). This pattern is reflected in the relatively short gut retention times measured in this experiment (5.4 - 34 h). If the final pulse seen in the size and density experiments does represent glandular faeces (Figures 14, 15, and 17), its absence here may indicate that very few of the beads in these experiments were processed in the digestive gland. However, it is possible that the decrease in the slope of the egestion curve for coated beads between 20 and 40 h represents the production of some glandular faeces (Figure 23). Sorting of the two bead types is indicated by the absence of uncoated beads from the second peak and is reflected in the different gut retention times of the two bead types.

The differential gut retention times of the two types of beads indicate that scallops have the ability to sort particles within the stomach solely on the basis of their chemical properties. If there was no sorting of particles within the stomach, the two bead types would have been egested at the same time. The majority of the scallops in experiment 1 had the same pattern of egesting the uncoated beads before the coated (Figure 24a). Selection was not as pronounced in the second experiment, although the mean GRT for coated beads was longer than that of uncoated beads (Figure 25b). Overall, when the two experiments were combined to increase *n* there was a significant

difference in the GRTs of the two bead types (Figure 25c) with the coated beads retained longer.

There are a few possible explanations for the apparent discrepancy between the two experiments. First, there is no reason to believe that selection by chemical properties is a 'hard-wired' behaviour of bivalves that is always exhibited. Therefore the differences may be due to individual variation or experimental conditions. Although the experiments were conducted consecutively, subtle differences in temperature or feeding conditions at the holding site might have influenced selection. Variability in bivalve behaviour has been noted before in preingestive selection studies (MacDonald and Ward 1994). The differences may also be due to the condition of the beads themselves. Although the same batch of beads was used for both experiments, by the second experiment they were older and had been handled more. It is possible that bacterial contamination of the beads could have resulted in degradation of the protein coat.

Despite the variability exhibited it is clear that scallops have the capability of distinguishing between two physically identical particles solely on the basis of their chemical properties. These results are consistent with evidence found by Gagnon and Fisher (1997) for postingestive selection in *Mytilus edulis*. The authors were studying the bioavailability of sediment-bound metals to filter-feeding invertebrates. Sediments were radiolabelled with heavy metals and some were coated with marine fulvic acids. The two types of particles were presented to the mussels separately and the gut retention times measured. They found that sediments with an organic coating were

retained longer than uncoated sediments. As a consequence of the longer gut retention times, more metals were absorbed from the coated sediments than the uncoated sediments.

The difference between the average retention times for coated and uncoated beads was significant but not large (11.7 vs. 9.4 h) (Figure 25c). Gagnon and Fisher (1997) found similar results in their study: coated sediments were retained on average approximately 1.5 h longer than uncoated sediments. This is reasonable since the organic coating can be removed simply by extracellular digestion in the stomach (Hylleberg and Gallucci 1975; Hughes 1977).

The implications of the ability to distinguish between a particle with an organic coating and one without to a bivalve in nature are difficult to ascertain. In its natural environment it is unlikely a bivalve would encounter many uncoated particles, as most particles in seawater have an organic coating (reviewed in Hunter 1983; Johnson 1974). However, the confirmation of chemosensory abilities within the digestive system has obvious implications for digestion of other types of particles. For example, it would be beneficial to the bivalve to be able to distinguish between poor quality refractory organic material and high quality algal material, or between different algal species, some of which may be toxic. The ability of scallops to detect such subtle differences between organic materials is the focus of the following chapter.

Chapter V: Selection by Chemical Properties, Dead vs. Live *Chlorella*

V. 1. Introduction

The greatest test of a bivalve's selection abilities is to present it with two physically identical naturally-occurring organic particles with only subtle chemical cues to distinguish between them. To my knowledge this has not previously been tested postingestively or preingestively. Studies have found selection between different species of algae preingestively (Shumway et al. 1985) and postingestively (Bricelj et al 1984; Wang and Fisher 1996) but these studies are confounded by differences in algal size, shape, surface characteristics and digestibility.

It is always preferable to conduct experiments on feeding and digestion using natural food particles that the study animal might normally encounter. Unfortunately, attempts to eliminate confounding factors often necessitate the use of manufactured particles where size, density and chemistry can be tightly controlled. The intention of this study was to utilize a natural particle that could be treated in some way to make it chemically different from its natural form yet remain physically identical. Several options were considered including, growing an alga both autotrophically and heterotrophically, growing an alga under different nutrient regimes, or using dead and live forms of an alga with a tough cell wall. Heterotrophic rearing of *Tetraselmis* was

attempted but was unsuccessful. Utilizing different nutrient regimes was discarded because changes in nutrient availability often cause changes in algal cell size as well as cell wall structure which in turn influences digestibility (Flynn et al. 1996; Van Donk et al. 1997). The third option of using dead and live forms of an alga was possible by utilizing the green alga *Chlorella*. *Chlorella* was chosen as the test particle because it meets the criteria set out in chapter IV. *Chlorella* has a thick cellulose cell wall which meant that it could be treated in some way to make it chemically distinct from live, healthy *Chlorella* yet remain physically intact. The first objective of this study was to establish the suitability of *Chlorella* as a test particle for chemical selection. The second objective was to test postingestive selection by chemical properties by simultaneously feeding the two forms of *Chlorella* to scallops. Selection was detected by comparing the gut retention times of the two forms of *Chlorella*.

V. 2. Methods

V. 2. i. Effect of Heat Treatment on *Chlorella*

Chlorella (strain CCMP 1227, Provasoli-Guillard National Center for Culture of Phytoplankton) was grown in f/2 media under 24-h light. This alga is a football-shaped green alga, with a length of about 5 μm; its equivalent spherical diameter (measured on a Coulter multisizer) is ~ 3.8 μm. Preliminary experiments indicated that heat treatment worked better to kill the algae than freezing. To test the effect of heat treatment, cultures of *Chlorella* were boiled for 10 min, then placed in 24-h light. Tests

for chlorophyll a, Carbon, Hydrogen, Nitrogen, and integrity within the scallop stomach were conducted on live cultures and cultures which had been dead for 5 days (5-d dead *Chlorella*).

Chlorophyll a

Samples of *Chlorella* cultures were centrifuged at 3000 G for 10 min at room temperature, the supernatant was removed and the cells resuspended in 80% methanol. Chlorophyll was extracted by stirring the algae in methanol for 10 min at 60°C in the dark (Afi et al. 1996). The cells were removed by centrifugation and the supernatant retained. The methanol was then allowed to evaporate in the dark and the pigments were resuspended in 90% acetone. Absorbance was measured at 750 and 664 nm on a Beckman DU-640 spectrophotometer to determine turbidity and chlorophyll a. The sample was then acidified with 1N HCl and absorbance was measured at 750 and 665 nm to determine turbidity and pheopigment-corrected chlorophyll a. Pheopigment-corrected chlorophyll a was calculated using Lorenzen's modified monochromatic equation (Lorenzen 1967):

$$C_a = 26.7(\text{Abs } 664_b - \text{Abs } 665_a)$$

where, C_a = concentration of chlorophyll a in the extract solution (mgl^{-1}), Abs 664_b = sample absorbance at 664 nm (minus absorbance at 750 nm) measured before acidification, and Abs 665_a = sample absorbance at 665 nm (minus absorbance at 750 nm) measured after acidification. The concentration of chlorophyll a (mgl^{-1}) in the whole water sample (C) was calculated using the following equation:

$$C = C_a V_E DF V_S^{-1} CL^{-1}$$

where, V_E = volume of extract (l), DF = dilution factor, V_S = volume of sample (l), and CL = optical path length of cuvette (cm).

Carbon, Hydrogen, and Nitrogen

To analyse carbon, nitrogen, and hydrogen content in the algal samples, samples of the *Chlorella* culture were filtered onto nylon filters then scraped off into pre-ashed aluminum pans and dried at 80°C until analysis. The dried samples were analyzed on a Perkin-Elmer Series II 2400 CHNS/O analyser.

Integrity Within the Scallop Stomach

In order to see if heat treatment might cause the algal cell wall to break apart faster in the scallop stomach, both live and 5-d dead *Chlorella* were fed to scallops and the stomach contents observed. To mimic how the algae would be treated prior to a selection experiment, *Chlorella* cultures were centrifuged for 10 min and resuspended in filtered seawater. Eight scallops were placed in beakers within a recirculating seawater tank (salinity 32‰, temp. 10°C). Either live *Chlorella* (four scallops) or 5-d dead *Chlorella* (four scallops) were added to the beakers to a final concentration of ~ 5000 cells ml^{-1}. The scallops were allowed to feed for 1 h and then the water was replaced with filtered seawater. Two scallops (one fed live algae and one fed dead algae) were dissected at each of 0.5, 1, 2, and 3 h after algae was added to the beakers. The stomach contents were removed and immediately examined and photographed.

Statistical Analysis

The null hypotheses were that none of the variables measured (chlorophyll a, C, H, and N) changed after *Chlorella* was heat treated. C, H and N were compared between fresh and 5-d dead *Chlorella* using a two-tailed paired t-test. Chlorophyll a content was expected to decrease after heat treatment; therefore, a one-tailed t-test was used to compare live and dead *Chlorella*. The data were tested for normality prior to statistical comparisons. All statistical tests were performed using SigmaStat statistical software (Jandel Scientific, version 4.0).

V. 2. ii. Selection by Chemical Properties

Collection and Holding of Scallops

Scallops (*Placopecten magellanicus*, 57-83 mm shell height) were collected by divers at two locations near Deer Island, New Brunswick (44°59'50"N, 66° 55'50"W and 44°58'24"N, 66°54'48"W). The animals were transported to the laboratory where they were held in a recirculating seawater tank (salinity 32‰, temp. 11°C) for up to 1 week. Scallops were fed cultured algae, *Isochrysis galbana* (strain T-iso), while being held.

Preparation of Algae

Initially, two radioisotopes were used: ^{14}C was used to label dead algae while tritium (^{3}H) was used to label live algae. However when the faeces were analyzed it was discovered that the ^{14}C signal overlapped that of tritium to such an extent that the

tritium signal was undetectable. Therefore, the decision was made to use only one label; scallops would still be fed both algal forms simultaneously but only one form at a time would be labelled.

The green alga *Chlorella* (strain CCMP 1227, Provasoli-Guillard National Center for Culture of Phytoplankton) was grown in f/2-Si media under 24-h light at 22°C. When the culture had reached ~160 000 cells ml^{-1}, it was split into two flasks and ^{14}C in the form of sodium bicarbonate ($NaH^{14}CO_3$) was added to one of the flasks (0.03 μCi per ml of media). The algae were grown in the presence of ^{14}C for 5 days then both of the flasks were boiled for 10 min and left in 24-h light for 4 days. Meanwhile a second culture was grown, split into two cultures, ^{14}C was added to one and both were allowed to grow for a further 4 days. On the day prior to the experiment, the radiolabelled cultures were centrifuged for 10 min and resuspended in sterile filtered seawater to remove any unassimilated ^{14}C. The two unlabelled cultures were also centrifuged for consistency.

Algal Mixtures

For each experiment three mixtures of algae were prepared, i.e. equal numbers of (1) ^{14}C-labelled live and unlabelled dead algae, (2) unlabelled live and ^{14}C-labelled dead algae and (3) unlabelled live and unlabelled dead algae (background control).

Experimental Design

The set-up used was similar to that described in Section III. 2. i. (Figure 8).

Scallops were placed in individual aerated 1 L beakers 24 h prior to beginning the experiment (temp. 11 °C). The water in each beaker was replaced with fresh filtered seawater 1 h before the experiment. A mixture of algae was added to each beaker at the beginning of the experiment to a final concentration of 20 000 cells ml^{-1}. The experiment was conducted twice: in total nine scallops received mixture (1), nine received mixture (2), four scallops acted as controls for background radioactivity and received mixture (3) and empty beakers acted as controls for particle settling and received either mixture (1) or (2). The scallops were allowed to feed on the algae for 2 h. Faeces were collected every hour for 12 h, then at 6 h intervals for the next 24 h, following which the faeces were collected every 12 h until 144 h. Scallops were fed a mixture of live and dead unlabelled *Chlorella* for the first 24 h following the exposure period, then *Isochrysis galbana* for the remainder of the experiment.

Water samples were taken at the beginning and end of the exposure period to measure clearance rates. Clearance rate calculations were modified from Coughlan (1969) by replacing particle concentrations with counts per minute (CPM). Clearance rates (CR) were then standardized (CRs) for an individual scallop with a dry soft tissue weight of 1.0 g (see section II. 2. i for equations).

Faecal Analysis

Faecal samples were treated with 0.25 ml of tissue solubilizer (Scintigest Fisher Co.) and incubated at 60 °C for 2 h, sonicated for 5 min then left to incubate overnight at room temperature. ^{14}C in the faecal and water samples was measured on a Beckman

LS 6500 liquid scintillation counter using Scintisafe as the scintillation cocktail. Radioactivity was measured in counts per minute. The counts were corrected for background noise by subtracting counts from the control scallops not fed radiolabelled algae.

Calculating Gut Retention Times

The time at which 50% of the algae passed through the gut (t_{50}) was used as the gut retention time (GRT). The t_{50} was estimated by plotting the cumulative percentage of radioisotope passed at each time interval against time to get a cumulative egestion curve. The data were log transformed and the polynomial regression equation which best matched the curve was determined. R-squared values were all greater than 0.90, and usually greater than 0.97. GRT was estimated as the interpolated time at which 50% of the radioisotope was egested. To determine if GRTs should be corrected for body size, correlation between GRT and scallop soft tissue dry weight was tested using Pearson correlation analysis.

Statistical Analysis

The null hypothesis for this study was that algal treatment had no effect on GRT; the alternate hypothesis was that the GRT of poorer quality dead algae would be shorter than that of live algae. The average GRTs for the two types of *Chlorella* were tested for significant differences using a one-tailed t-test. GRTs were tested for normality and homoscedasticity prior to statistical comparison. All statistical tests were

performed using SigmaStat statistical software (Jandel Scientific, version 4.0).

V. 3. Results

V. 3. i. Effect of Heat Treatment on *Chlorella*

The chlorophyll a content of heat-treated *Chlorella* dropped significantly by 5 days after heat treatment ($t_{(1),4} = 52.726$, $P < 0.001$) (Figure 26a). Both carbon and nitrogen content of *Chlorella* also dropped significantly by 5 days after heat treatment ($t_{(2),4} = 3.133$, $P = 0.035$ and $t_{(2),4} = 3.106$, $P = 0.036$). Hydrogen content declined as well but not significantly ($t_{(2),4} = 1.75$, $P = 0.155$) (Figure 26b). *Chlorella* cells remained intact after heat treatment but the cytoplasm appeared granular (Figure 27a) and eventually lost its green colour and turned brown. After scallops were fed the algae, live and 5-d dead *Chlorella* were visible as intact cells within their stomach contents. The algae were still present and intact in the stomachs up to 3 h after feeding (Figure 27b).

V. 3. ii. Selection by Chemical Properties

Results from the two experiments were pooled to increase *n*. The average standardized clearance rate for this experiment was 0.1677 ± 0.0172 lh^{-1} ($\bar{x} \pm$ SE). Gut retention times ranged from 5.8 - 16.8 h. There were no correlations between GRT and soft tissue dry weight ($r = -0.134$, $n = 18$, $P = 0.596$). When the data were log transformed and the test repeated, the results were the same. This indicates that either

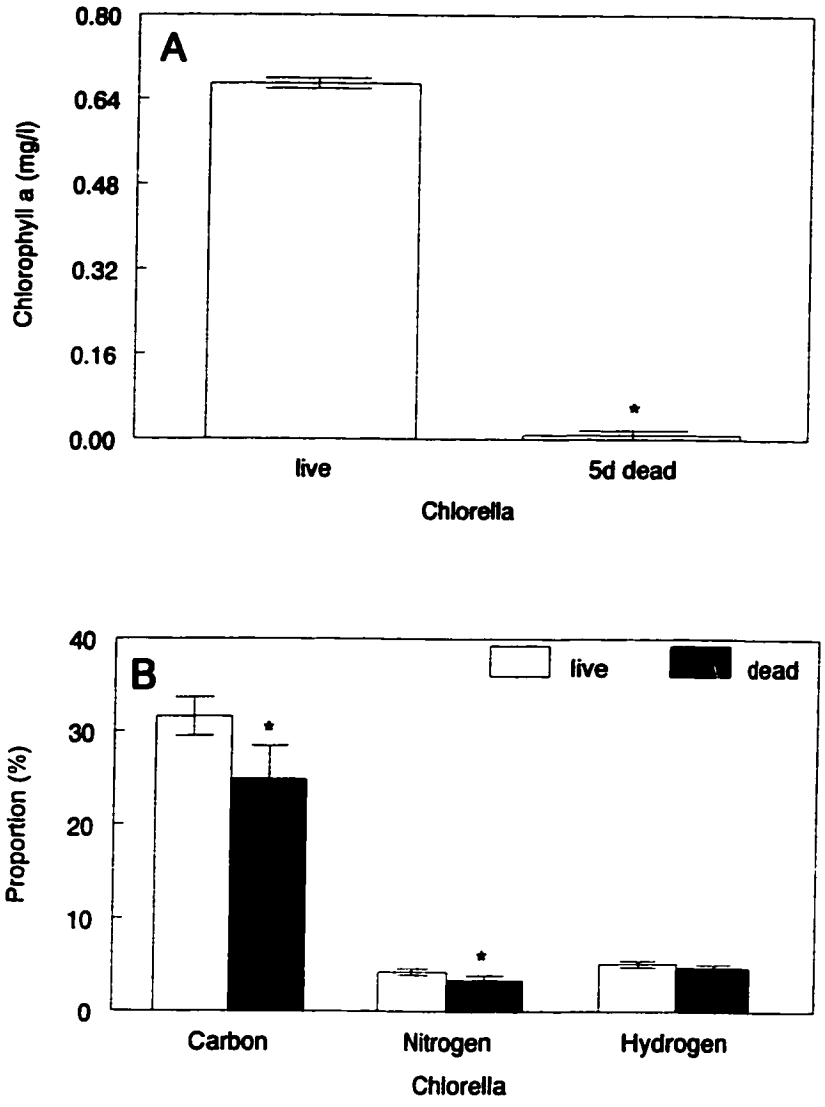
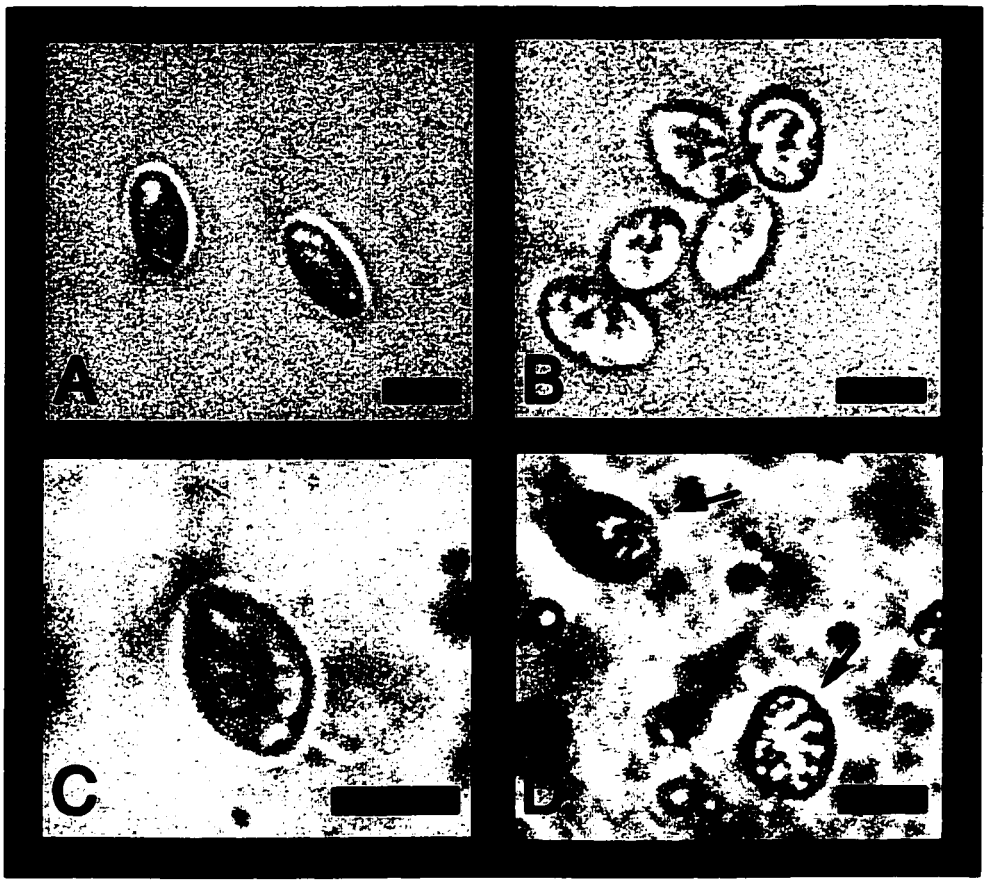


Figure 26: (A) Pheopigment-corrected chlorophyll a content ($\bar{x} \pm$ S.E.) of live and heat-treated, 5-day dead *Chlorella*. (B) Percent content of carbon, nitrogen, and hydrogen in live and heat-treated, 5-day dead *Chlorella*. Asterisk (*) above error bars indicates the values for the dead algae are significantly different from those for the live algae at $\alpha = 0.05$.

Figure 27: Photomicrographs of (A) live *Chlorella*, (B) 5-d dead *Chlorella*, (C) live *Chlorella* in the stomach contents of *Placopecten magellanicus* 3 h after feeding, and (D) 5-d dead *Chlorella* (indicated by arrows) in the stomach contents of *Placopecten magellanicus* 3 h after feeding. Note that the algae are recognizable and appear intact. Scale bar represents 5 μm.


A
B
C
D

there is no correlation between scallop size and GRT or that the scallops used (57-83 mm shell height) were similar enough in size to eliminate any size effect. Therefore, the GRTs were not corrected for scallop size.

The average egestion pattern was similar to that exhibited by scallops in Chapter IV. There are two peaks prior to 18 h, the first composed mostly of dead *Chlorella* and the second mostly of live *Chlorella*. Egestion then declines sharply for the dead algae but there is a small pulse between 20 - 40 h of live *Chlorella* (Figure 28).

There was a significant difference between the GRTs of live algae and dead algae ($t_{(1),13} = 2.114$, $P = 0.027$) (Figure 29). Live *Chlorella* were retained significantly longer than dead *Chlorella*. Three scallops were omitted from statistical analysis because they egested very little radioisotope in their faeces.

V. 4. Discussion

Heat treatment proved to be an effective method of diminishing *Chlorella* quality while retaining its physical integrity. The quality of *Chlorella* changed significantly by 5 days after heat treatment; chlorophyll a content dropped dramatically and the total content of carbon and nitrogen also declined (Figure 26). Chlorophyll a and nitrogen content are often used as indicators of food quality in ingestion studies (Newell and Jordan 1983; Cranford and Gordon 1992; MacDonald and Ward 1994; Ward and MacDonald 1996). Despite this decline in quality, the physical integrity of the algae did not change detectably. The algae remained intact within the scallop

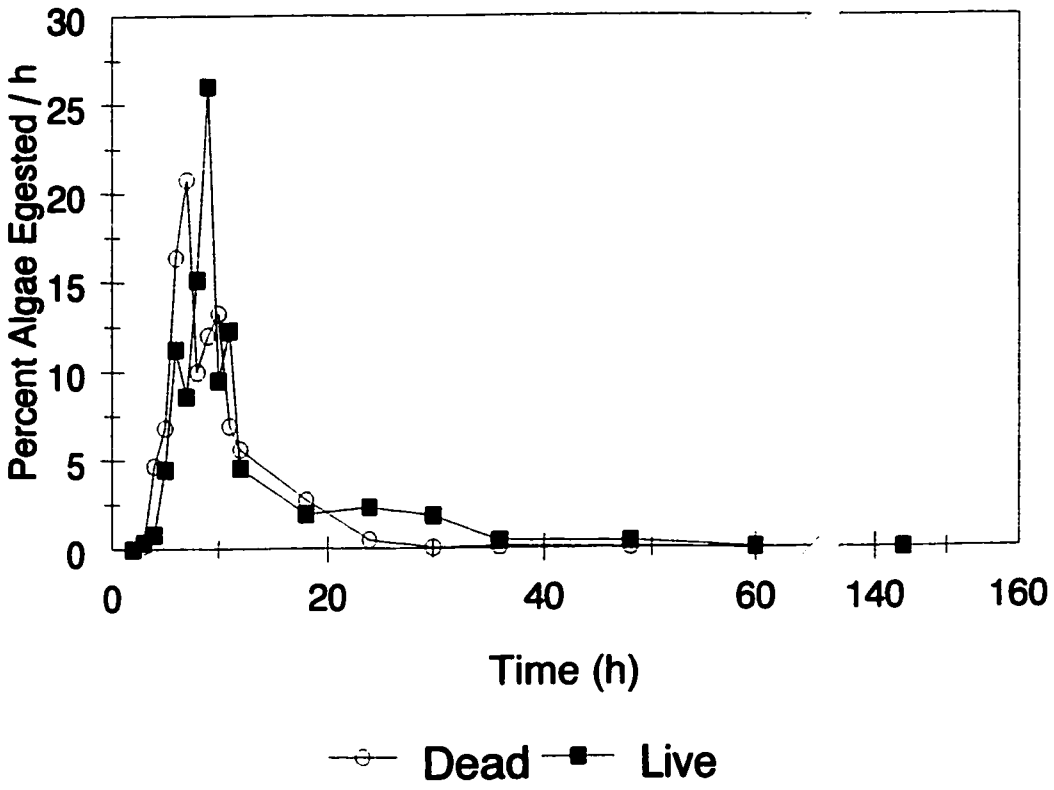


Figure 28. Egestion rate (amount of radioisotope egested per hour expressed as a percentage) of *Placopecten magellanicus* fed dead *Chlorella* (n = 8) and live *Chlorella* (n = 7) radiolabelled with ^{14}C. The x-axis has been broken from 65 to 135 h to clarify egestion patterns during the first 60 h.

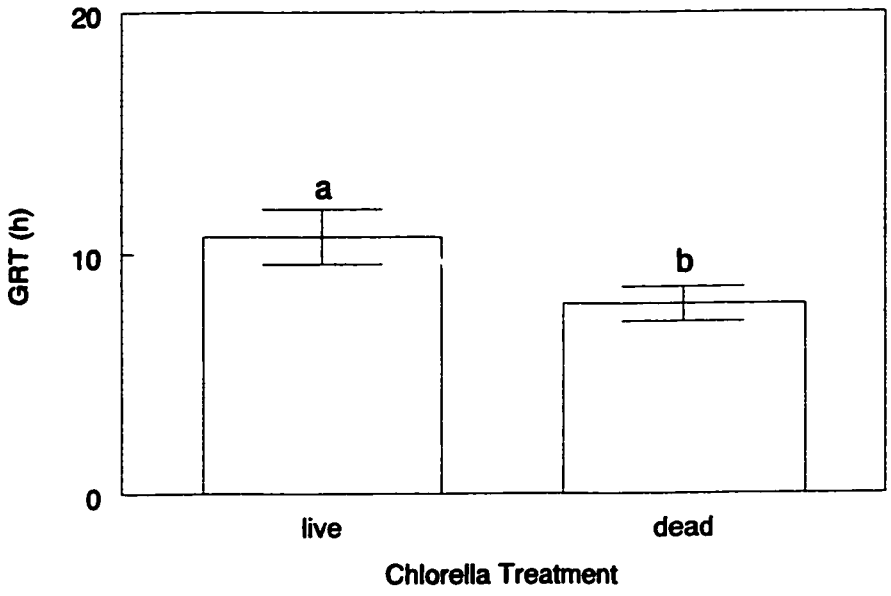


Figure 29: Gut retention times ($\bar{x} \pm$ S.E.) of scallops fed live ($n = 7$) and heat-treated, 5-day dead ($n = 8$) *Chlorella*. Different letters above the error bars indicate a significant difference in GRTs at $\alpha = 0.05$. Live algae were retained longer than dead algae.

stomach for at least 2- 3 h after ingestion (Figure 27d). This is probably a result of the algae's thick polysaccharide cell wall (Corre et al. 1996). Afi et al. (1996) treated *Chlorella* to a similar heat shock and, by using transmission and scanning electron microscopy, found the cell walls were not disrupted by the treatment. Some strains of *Chlorella* have a trilaminar sheath made of algaenans in their cell walls which makes them extremely resistant to enzymes and detergents (Atkinson et al. 1972; Corre et al. 1996). These features made *Chlorella* an appropriate test particle to examine postingestive selection by chemical properties. However, *Chlorella* is not necessarily a good food source for bivalves; the thick cell wall which enables manipulation of its chemical composition also makes it resistant to digestion. This quality may be reflected in the relatively short gut retention times of 5.8 - 16.8 h measured in this study.

Despite these deficiencies, *Chlorella* was ingested by the scallops. The egestion pattern of *Chlorella* is similar to that seen previously in Chapter III. The curve is characterized by two initial peaks and a small third pulse of live *Chlorella* only (Figure 28). The first peak is dominated by dead algae while the second is composed primarily of live algae. These two peaks probably represent intestinal faeces, while the third pulse may represent some small production of glandular faeces. Sorting of the two algae is evidenced by their separate peaks of egestion as well as the prolonged egestion of live *Chlorella.*

The scallops were able to distinguish between the poorer quality dead cells and the live cells, and passed them at different rates (Figure 29). The dead algae were egested more quickly than the live algae; this pattern indicates that the cell wall was not

weakened by the heat treatment. Bricelj et al. (1984) found that when cells break down in the stomach the cell contents are retained longer than the cell wall. If the cell walls of the dead *Chlorella* had been weakened by the heat treatment, they would have been more likely to break apart in the stomach than the live *Chlorella*. The light cell contents of the dead algae would have been drawn into the digestive gland and therefore retained longer than the intact live algae. The fact that live algae were retained longer than dead algae indicates that this did not happen.

The different GRTs of dead and live *Chlorella* imply that *P. magellanicus* was able to detect the chemical differences between the live and dead algal cells within the stomach and process them accordingly. The method of this detection is unclear. It is unlikely that the cells broke down quickly enough (if at all) in the gut to allow the scallop to detect the overall chemical composition of the algae. It is more probable that algal decay resulted in changes in surface chemistry and a decline in algal exudates. Live *Chlorella* would have been actively metabolizing and may have been producing exudates (Hammer and Brockmann 1983) that could be detected by the scallop. Ward and Targett (1989) have shown that algal ectocrines influence preingestive feeding behaviour in *M. edulis*. Feeding research on copepods has revealed that some species of copepods can select between algae of the same species but with different growth rates (Cowles et al. 1988) and will clear live algae faster than dead algae (Paffenhöfer and Van Sant 1985). Copepod researchers have postulated that elevated phytoplankton growth rates result in a larger 'phagostimulating envelope' of amino acids or other secreted materials around algal cells which copepods can detect (Cowles et al. 1988).

Perhaps scallops have chemosensory cells within the gut which can detect exudates produced by live algae.

Unfortunately, there are no studies directly comparable to this one. Other postingestive selection studies where algae were used did not attempt to control for algal size, shape, and digestibility (e.g. Bricelj et al. 1984; Wang and Fisher 1996). However, the results of this study support the conclusion of Chapter IV, that scallops can sort particles by their chemical properties. Furthermore, this study has shown for the first time that the digestive tract of scallops is capable of detecting subtle differences between dead and live organic material. The ability to distinguish between particles on the basis of subtle chemical cues may be beneficial to scallops by enabling them to retain organic particles of higher quality while rejecting poorer quality organic particles. This ability may also enable scallops to detect toxic algae in the gut and process or void them quickly to prevent the accumulation of toxins.

Chapter VI: General Discussion

VI. 1. Summary of Results

This research is the first thorough examination of the ability of one species of bivalve (*Placopecten magellanicus*) to sort particles within the gut. This research is unusual in that: 1) postingestive selection has been studied quantitatively in intact bivalves; 2) the role of different particle characteristics in postingestive selection has been clarified by tightly controlling test particle variables; and 3) the importance of choice in selection experiments has been acknowledged by always presenting two or three types of test particles simultaneously to the bivalves.

The results of this research can be summarized as follows:

1) scallops can sort algae and similar sized beads in the stomach, preferentially directing the algae to the digestive gland;

2) scallops can sort particles by size in the gut, passing smaller particles through the digestive tract faster than larger particles;

3) scallops can sort particles by density, egesting heavier particles faster than lighter particles;

4) scallops can distinguish between beads coated with protein and uncoated beads, egesting uncoated beads faster; and

5) scallops can distinguish between dead and live algae, passing dead algae through the digestive system faster than live algae.

VI. 2. Implications of Results

These finding indicate that *Placopecten magellanicus* can utilize both physical and chemical particle characteristics to sort particles within the gut and process them accordingly. These factors were tested in isolation, but in nature bivalves feed on a heterogenous mixture of inorganic and organic particles of varying sizes, densities, and nutritional values. We can infer what may happen in the natural environment from the selection abilities that have been demonstrated, combined with a knowledge of stomach structure and function.

In nature, scallops feed on a changing mixture of particles that vary in quantity and quality. Basically, what is available to bivalves is a mixture of algae (both diatoms and flagellates), detritus (such as macrophyte or phytoplankton debris), and silt, usually with an adsorbed organic component. Much of this material probably occurs as organic-mineral aggregates (Grant and Bacher 1998). Preingestive selection will improve the quality of the food ingested somewhat (Newell and Jordan 1983; Ward et al. 1994; Ward and MacDonald 1996) but it is far from 100% efficient. Therefore, what enters the stomach is a mixture of algae, detritus and mineral particles. All will be mixed about in the stomach by the rotation of the style and the beating of cilia. Extracellular digestion will begin and particle aggregates will be broken apart. Dense particles, such as silt and clay, will tend to settle out onto the ciliated tracts, fall into rejection grooves and be carried to the intestine via the intestinal groove. Although only one size of bead was tested in the density selection experiment, it is very likely that all sizes of dense particles would settle out in the stomach. Dense particles that are too large to fit into the

rejection grooves would not be ingested very often as dense particles > 20 μm are resuspended only intermittently by storm events and do not remain in the water column for long (Grant et al. 1997; Cranford et al. 1998). Large, light particles, such as detritus and large algae, will be recirculated until they begin to break down through extracellular digestion, then the smaller dense pieces such as cell walls will tend to fall into the rejection tracts and be carried to the intestine. The lighter breakdown products such as cell contents will be drawn into the entrance of the digestive gland and carried to the tubules where intracellular digestion will occur. Simultaneous with sorting by physical properties, selection by chemical properties will be occurring. Inorganic particles without an organic coating will be processed faster than particles with a coating. Live algae will be sorted from dead organic matter and will be preferentially retained. The chemosensory abilities of the scallop digestive system will probably also be used to egest toxic and/or noxious algae quickly. Wastes from materials shunted directly to the intestine will constitute intestinal faeces. Later wastes produced from glandular digestion will be excreted along with the remains of materials retained within the stomach for prolonged extracellular digestion.

VI. 3. Postingestive Selection in Other Animals

Comparisons of these finding with those of other molluscs and even other groups of animals help to indicate whether postingestive particle sorting is a widespread phenomenon and to clarify the role that sorting can play.

Particle processing in the gut of prosobranch gastropod veligers has been

described by observing food particles as they pass through these transparent animals (Fretter and Montgomery 1968). The stomach structure is similar to that of lamellibranch bivalve larvae but not as well-developed as that of adult bivalves. The researchers described the movement of several types of algae and indigestible inorganic particles. Digestible plant cells are subjected to enzymatic and mechanical treatment as cilia rotate the particles against the gastric shield. This treatment either weakens the cell wall so that digestive enzymes can penetrate it, or fragments it. The digestive diverticula pulsate to draw the stomach contents into direct contact with the digestive cells where the contents of the algal cells but not the cell walls are absorbed. When veligers are fed only inorganic particles of no food value, the particles are not retained but pass very rapidly to the intestine for egestion. The digestive diverticula do not pulsate to draw in the inorganic particles. If algae are ingested along with the inorganic particles, they are separated from the inorganics and retained near the openings to the digestive gland until the rest of the stomach is emptied and digestion proceeds. These qualitative observations of particle sorting in gastropod larvae indicate that particle processing is complex and well-developed at even the earliest feeding stages of some suspension-feeding molluscs.

The anatomy of the adult gastropod alimentary system suggests that sorting of particles also occurs here. Carriker (1946) described the stomach or pylorus as "... composed of a complicated system of folds and passages and counter ciliary currents and functions as a filter which permits only the soluble and finer food particles to pass into the liver. It shunts the undigested residues from the gizzard into the prointestine."

Gastropods produce both intestinal and glandular faeces (called the gizzard string and liver string respectively) (Calow 1975).

Research on some ampharetid polychaetes (Self and Jumars 1978; Penry 1989) indicates that they also may have the ability to sort particles within the gut. These deposit-feeding polychaetes have a ventral ciliated gutter in their digestive tracts that acts as a "bypass shunt" directing particles of higher specific gravity to the anus faster than the bulk of the remaining food.

The structure of the amphipod foregut has been interpreted as having a sorting function. The posterior chamber of the foregut has ventrolateral ridges along its sides. These ridges bear setae which intermingle at the midline effectively separating the posterior chamber into dorsal and ventral cavities. The dorsal cavity holds food particles and leads directly to the midgut. Fine particles and fluids are filtered through the setae into the ventral cavity, which leads to the openings of the midgut glands (digestive glands) (Shyamasundari 1981; Coleman 1992).

Some mammals also utilize postingestive selection. Rabbits produce two types of faeces, hard and soft. When hard faeces are bring formed in the gut, partially digested food leaving the cecum (where most fermentation occurs) is separated by peristaltic and antiperistaltic movements so that soluble fibre and fine particles (< 0.3 mm) are preferentially returned to the cecum. Larger particles move to the distal colon and become the hard faeces. Because of this process, food particle size affects fibre digestion efficiency and rate of passage in rabbits (Garcia et al. 1999).

These examples illustrate that sorting by physical properties occurs in a wide

variety of animals, both vertebrate and invertebrate. Sorting by chemical properties is less well known which may be because it is more difficult to study.

VI. 4. Potential Sources of Variability Amongst Experiments

The gut retention times measured throughout this research varied a great deal both within and between experiments (Table 5). Variability in bivalve feeding behaviour is well known amongst bivalve researchers: "A striking feature of any experimental study of bivalve feeding is the wide variability between individuals in all aspects of their feeding behaviour." (Bayne 1998). Therefore the wide range of GRT's found in this study are not entirely unexpected, however it is useful to examine some factors which may influence this variability.

Time of year is known to influence various aspects of bivalve feeding behaviour including, gut retention times, clearance rates and assimilation efficiencies (MacDonald and Thompson 1986a; Bayne et al. 1987; Kreeger and Newell in review). This is related to the overall suppression of metabolic activities by low water temperatures, as well as changes in food availability and the demands of gametogenesis. Sea scallops in the Bay of Fundy spawn in late August to late September. Therefore it might be expected that scallop feeding activity would be highest in spring and summer when gametogenesis is occurring. However, there was no seasonal pattern apparent in the gut retention times measured throughout this study (Table 5). Experiments conducted at the same time of year (size selection experiment 3 and live vs dead algae experiment; selection by density experiment and coated vs uncoated bead experiments) show very different

Table 5. Summary of the range of gut retention times found for the selection experiments conducted on *Placopecten magellanicus*.

Factor Tested	Exp No.	Test Particle	GRT's (h)	Month
selection by size	1	5 µm polystyrene	8 - 37	July
		10 µm polystyrene	16 - 49	
		20 µm polystyrene	16 - 43	
	2	5 µm polystyrene	2 - 11	Sept
		10 µm polystyrene	5 - 25	
		20 µm polystyrene	9 - 55	
	3	5 µm polystyrene	8 - 125	Nov
		10 µm polystyrene	8 - 48	
		20 µm polystyrene	21 - 64	
	4	5 µm polystyrene	17 - 51	June
		10 µm polystyrene	17 - 83	
		20 µm polystyrene	19 - 101	
selection by density	1	8 µm glass	9 - 37	March
		9 µm polystyrene	12 - 66	
coated vs uncoated beads	1	5.5 µm coated polystyrene	8 - 13	Feb
		5.7 µm uncoated polystyrene	5 - 10	
	2	5.5 µm coated polystyrene	9 - 22	Feb/Mar
		5.7 µm uncoated polystyrene	9 - 17	
dead vs live algae	1 & 2	3.8 µm live *Chlorella*	8 - 17	Nov/Dec
		3.8 µm dead *Chlorella*	6 - 12	

GRT's. It is unclear from this study what effect, if any, season has on the GRT's of scallops.

The scallops used in the various experiments for this study were often collected from different locations (although within each experiment the animals were always collected from the same site). Ideally all experiments would have been conducted on animals from the same source population however, technical difficulties made this impossible. This does not affect the main objective of this research, but it may be responsible for some of the variability in GRT's found amongst the different experiments. The scallops may have been physiologically pre-adapted to the feeding conditions at their local sites. Studies have shown that blue mussels will adjust their absorption efficiencies over several days when exposed to a changed food supply and may adjust their GRT's as well (Bayne et al. 1987; Bayne et al. 1993). Bayne et al. (1984) found that mussels from a site with a high quality food supply had higher clearance rates, shorter GRT's and lower absorption efficiencies than individuals from a site with poorer quality food. However, Bayne et al. (1987) found no marked difference in GRT's of mussels from two sites with very different food supplies. The Bay of Fundy has large tides resulting in a well-mixed body of water but some local variability in suspended material almost certainly exists depending on the bottom type and local sources of seston. Unfortunately little is known about the local feeding conditions that the scallops used in this research would have experienced at their original sites. Clearly more research is needed to clarify the role that local food supply has on the GRT's of bivalves in general and of *Placopecten magellanicus* in the Bay of Fundy in particular.

A detailed analysis of the influence of season (including water temperature and reproductive condition) and local food supply was beyond the scope of this study (see VI. 5. Future Research). These factors almost certainly influence overall GRT's but whether they affect postingestive selection is unknown. The main objective of this research was to establish the ability of *Placopecten magellanicus* to sort particles within the stomach and to determine the role of particle characteristics in this process. Utilizing scallops from different sites and at different times of year for different experiments did not interfere with this objective because within each experiment these sources of variability were eliminated by using animals collected at the same time and from the same location.

VI. 5. Future Research

This research has clarified the role that physical and chemical properties of particles play in postingestive selection yet there remains a need to explore how these processes combine in the natural environment to enable scallops to utilize a mixed diet most efficiently. Gut retention time experiments could be conducted utilizing natural diets where different components of the diet were traced each time the tests were run, or utilizing multiple tracers simultaneously. These studies would also determine the typical GRTs of various food types for scallops. This information is important for absorption efficiencies studies; typically absorption efficiency is measured by comparing the nutritional content of the faeces to the food ingested. When absorption efficiency is measured on a fluctuating diet, knowledge of GRTs is necessary to ensure

that the faeces are collected at the appropriate time and are the remains of the food which was measured.

It would also be useful to know under what circumstances postingestive selection is utilized. The results of this research suggest that selection may not always occur. Preingestive selection is believed to occur only once a threshold concentration of food is reached. Experiments could be conducted testing combinations of organic and inorganic food in different proportions and in different quantities. The effects of starvation, time of year, reproductive condition, temperature and habitat (local food supply) could also be evaluated.

The mechanisms of selection in *Placopecten magellanicus* also remain unclear. Although it is apparent that scallops retain large light particles longer, where the beads are held during this time is not. I have theorized that large plastic beads are recirculated within the stomach contents with some inadvertently entering the digestive gland. Dissection and histology could be used to clarify this. Natural analogs (large light refractory particles) might be used to elucidate whether this behaviour is an artifact of the experimental conditions (artificial particles) or if it actually happens in nature.

The mechanism for selection on the basis of chemical properties is unknown. Presumably some cells within the stomach have chemosensory abilities. The bivalve nervous system in general has not been well-studied. Bullock and Horridge reported in 1965 that most of the visceral organs of bivalves are not richly innervated. However, recent work by Smith et al. (1998) has shown that there are catecholamine-containing cells in the intestinal epithelium of *Placopecten magellanicus*. To my knowledge the

presence or absence of chemosensory cells in the stomach has yet to be investigated in bivalves.

Finally, it is necessary to study these processes in bivalves other than *Placopecten magellanicus*. The strategies of different bivalves for postingestive sorting would be very interesting to know. Preingestive research has shown that bivalves can be very diverse in their feeding strategies. Infaunal bivalves are able to ingest high concentrations of low quality seston better than epifaunal bivalves. For example, although both *Mya arenaria* (infaunal) and *Placopecten magellanicus* (epifaunal) reduce their clearance rates as the concentration of seston rises, *P. magellanicus* produces more pseudofaeces and therefore does not ingest as much material as *M. arenaria.* (Bacon et al. 1998). It is not unreasonable to hypothesize that bivalves that tend to inhabit areas with high loads of suspended sediments may have different particle processing strategies than bivalves like *P. magellanicus* that inhabit relatively clear waters. Furthermore, deposit-feeders would likely have very different approaches to particle sorting than suspension-feeders because the diet of deposit-feeders consists almost entirely of sediment particles.

VI. 6. Conclusions

This research has indicated that *P. magellanicus* has well-developed abilities to sort particles within the digestive tract. Scallops can distinguish particles both by physical (size and density) and chemical properties (presence of an organic coating, living vs. detrital material) and process them accordingly. These sorting processes all

work in concert to enable scallops to efficiently process a diet that is extremely variable in quality, and presumably to extract the most energy from the available food while minimizing energy losses associated with digestion.

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Appendix 1: Water Quality

Introduction

The purpose of collecting these data was to measure the amount and quality of particulate material in the water supplied to the animals in the two flow-through experiments conducted at the Huntsman Marine Science Centre (HMSC). The way particles are treated in the gut may be affected by what the animals subsequently ingests.

Materials and Methods

Three 50 ml water samples were taken from the header tank every 2 h for 10 h. The samples were run on the Coulter multisizer equipped with a 100 μm aperture tube for 120 s to determine the number of particles present in the incoming water over the tidal cycle.

Three 600 ml water samples were taken every 2 h from the header tank for 10 h. The water was then filtered onto pre-ashed and pre-weighed 1.6 cm Whatman GF/C filters and rinsed with 3% ammonium formate. A blank filter was also processed along with the sample filters by washing with just ammonium formate. The filters were dried for 24 h at 80°C and weighed to determine dry weight (Strickland and Parsons 1972):

$$\text{dry weight (mg l}^{-1}) = (W_2\text{-}W_1\text{-}B_1)\ V^{-1}$$

where W_1 = filter weight (mg), W_2 = filter weight plus dry particulate material (mg), V = volume of water filtered (l), and B_1 = blank correction factor:

$$B_1 = \text{dried blank filter weight - initial blank filter weight (mg)}$$

The filters were then ashed at 450°C for 12 h and reweighed. Percent organic content of the water was calculated using the following formula:

$$\%\ \text{organics} = [[\text{dry weight} - (W_3 - W_1 - B_2)\ V^{-1}]\ (\text{dry weight})^{-1}] \times 100$$

where W_3 = filter weight plus ashed particulate material (mg) and B_2 = blank correction factor:

$$B_2 = \text{ashed blank filter weight - initial blank filter weight (mg).}$$

Results and Discussion

During Experiment 1 the amount of material in the water varied from 1.78 mg l^{-1} to 3.41 mg l^{-1}. The number of particles fluctuated from roughly 12 000 to 19 500 particles ml^{-1}. Percent organics ranged from 14 to 30 %. Particle concentrations were highest on the falling tide; the quality of the material also increased on the falling tide.

Suspended material during Experiment 2, ranged from 2.23 mgl^{-1} to 19.70 mgl^{-1}. Particle concentrations varied from approximately 16 000 to 89 000 particles ml^{-1}. Water quality ranged from 19 to 27 % organics. Particle concentrations and weights were again highest on the falling tide, but quality was lowest.

According to Greenberg and Amos (1983) the concentration of suspended

material at the mouth of the Bay of Fundy, where the Passamaquoddy Bay is located, normally ranges between 1 and 2 mg l^{-1}, although higher concentrations are not uncommon. Scallops used in these experiments were collected near HMSC and would be accustomed to suspended material concentrations in the area.

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