THE REGULATION OF PARTICLE TRANSPORT WITHIN THE VENTRAL GROOVE OF THE MUSSEL GILL (Mytilus Edulis) IN RESPONSE TO VARIATIONS IN ENVIRONMENTAL CONDITIONS

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THE REGULATION OF PARTICLE TRANSPORT WITHIN THE VENTRAL GROOVE OF THE MUSSEL GILL (*MYTILUS EDULIS*) IN RESPONSE TO VARIATIONS IN ENVIRONMENTAL CONDITIONS

by

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ABSTRACT

In order to understand thoroughly the feeding processes of the blue mussel, *Mytilus edulis*, in response to the variable environmental conditions it experiences in nature, it is important to examine individually the different components that comprise its feeding system. The ciliated ventral food groove represents one of these primary components, within which the majority of food particles trapped by the gill are transported to the labial palps and gut. The ability of mussels to adjust food transport rates within this groove could serve as an important feeding regulatory mechanism in response to variations in the environment.

Using video endoscopy, mucous strand velocities in the ventral groove of mussels *Mytilus edulis* were determined over variable time periods and in response to short-term manipulations of ambient particle concentration, temperature and particle type. Mucous strand velocities decreased with increasing ambient particle concentration and particle load on the gill, and also when particle type was switched from algae to sediment. This evidence supports the hypothesis that *M. edulis* possesses compensation mechanisms involving particle transport at the level of the ventral groove cilia to deal with short-term changes in the environment. Furthermore, mucous strand velocity in the ventral groove increased when the ambient temperature of mussels acclimated to 4.5°C was increased to 15°C. This response is consistent with standard physiological responses of ciliary systems to changes in temperature. Changes in velocity over time were also observed, but there was no consistent pattern.

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CHAPTER 1 - INTRODUCTION

1.1 Background

Blue mussels (*Mytilus edulis*) live in estuarine and coastal areas where they experience variable temperatures and concentrations of algal cells, detritus and silt. There are many published studies concerning the rates at which suspension feeding bivalves process various qualities and quantities of particles in different environmental conditions (e.g. Bayne et al., 1976; Winter, 1978; Navarro & Iglesias, 1993). *Mytilus edulis* is important as an aquaculture species, and it is abundant in all major oceans in the world where it is known to contribute significantly to nutrient recycling and resuspension (Asmus & Asmus, 1993; Dame, 1993). For these reasons, there has been interest in determining how environmental factors affect different aspects of suspension feeding such as pumping rate, clearance rate, selection efficiency, ingestion rate, rates of feces and pseudofeces production, and absorption efficiency. The adaptations of suspension feeders to different conditions have proven to be very complex and diverse, and most responses occur in conjunction with adjustments within the pallial system.

Since the pallial system has proven to be difficult to study *in vivo* due to the presence of an opaque shell, early experiments on particle transport velocities or ciliary beat frequencies, determined stroboscopically, were done with excised fragments of gills, damaged specimens or juveniles (e.g. Gray, 1931; Hirasaka et al., 1957; Hoshi & Hoshiyama, 1963; Dral, 1967; Jørgensen, 1975; Stefano et al., 1977; Catapane et al., 1981; Jørgensen & Ockelmann, 1991). Recently, the technique of video endoscopy has been employed, thus allowing *in vivo* observations of the bivalve feeding system and providing researchers with a tool which has already helped resolve some long-standing controversies concerning particle transport on the bivalve gill (e.g. Ward et al., 1993). Endoscopy provides an excellent opportunity to address further questions concerning transport rates of particles by suspension feeders.

There are many factors which may potentially influence the velocity of particles on bivalve gills including the temperature of the surrounding seawater, the body size and feeding history of the animal, and the density, volume and type of cells to which the animals are exposed. Ward et al. (1991, 1993) give mean particle velocities in the ventral groove of *M. edulis* at two and three particle concentrations, respectively; Ward et al. (1994) report mean particle velocities on different areas of the gills of the oyster, *Crassostrea virginica*; and Beninger et al. (1992) give velocities in the dorsal arch of the scallop, *Placopecten magellanicus*, at three different particle concentrations. Levinton et al. (1996) report velocities on the palp proboscides and ctenidia of the deposit-feeder *Yoldia limatula* and the ctenidia of *Macoma* spp.. Others have measured the transport rates of particles by the frontal cilia along the ctenidial filaments of different species (e.g. Ward et al., 1991; Tankersley, 1996). These studies represent the bulk of data collected for particle velocities on the gills of suspension feeders determined *in vivo* with the endoscope. Little attempt has been made to determine whether particle transport rates vary according to environmental conditions, or how any resulting changes may relate to or affect other parts of the feeding system of *M. edulis*.

In general, there are two ways of viewing the feeding processes of bivalves: 1- as ciliary level responses, and 2- as system level responses. The ciliary level represents the basic unit of response to changes in the environment and is determined by the physical properties of the cilia responsible for pumping water and entraining and transporting particles. These responses may be direct physiological responses or indirect responses arising from feedback information originating in other compartments of the feeding system. If particle processing by the labial palps or the gut is a rate-limiting step, there may be a feedback mechanism to the gills which causes a decrease or increase in particle transport or collection. This process would be an example of an indirect ciliary level response. Alternatively, the decrease in ciliary beat in response to a change in ambient temperature represents a direct ciliary level response.

A system level response represents the innate physiological processes of regulation of feeding behaviour by suspension feeders in order to maximize net energy gain in response to environmental changes. The bivalve feeding pathway may be viewed as a stepwise chain of components (Fig. 1.1), and a system level response involves the adjustment of one or more feeding compartments in order to maintain homeostasis during environmental change. For example, bivalves feeding at high particle concentrations could adopt one or more of the following behavioural responses to regulate ingestion: decrease clearance rate, increase production of pseudofeces, or decrease time spent feeding (Foster-Smith, 1975a). Since feeding responses should be observed and interpreted using a combination of these levels, the present work will involve feeding system responses as well as ciliary responses of mussels to variations in the environment.

The following four sections are included to provide necessary descriptive information regarding the feeding system of *M. edulis* and the dynamics of mucociliary transport. The terms and concepts developed here will be used in later chapters to help explain the results of this study.

1.2 Description of feeding processes of *Mytilus edulis*

Mytilus edulis has a filibranch ctenidium, i.e. nonplicate (flat) and homorhabdic (filaments all of one type). The ctenidia are folded into large, W-shaped structures made up of inner and outer demibranchs, each composed of descending and ascending lamellae, suspended on each side of the body from a ctenidial axis (Bayne et al., 1976; Gosling, 1992; Fig. 1.2). The ctenidia divide the mantle cavity into inhalant and exhalant chambers. The two lamellae forming each demibranch are joined together by interlamellar connective tissue junctions, and adjacent filaments which comprise the lamellae are connected loosely by ciliated discs (Morton, 1979). Lateral cilia located along the sides of each filament (Fig. 1.3) are arranged in continuous rows and create the inhalant and the exhalant currents that enter and leave via the inhalant and exhalant

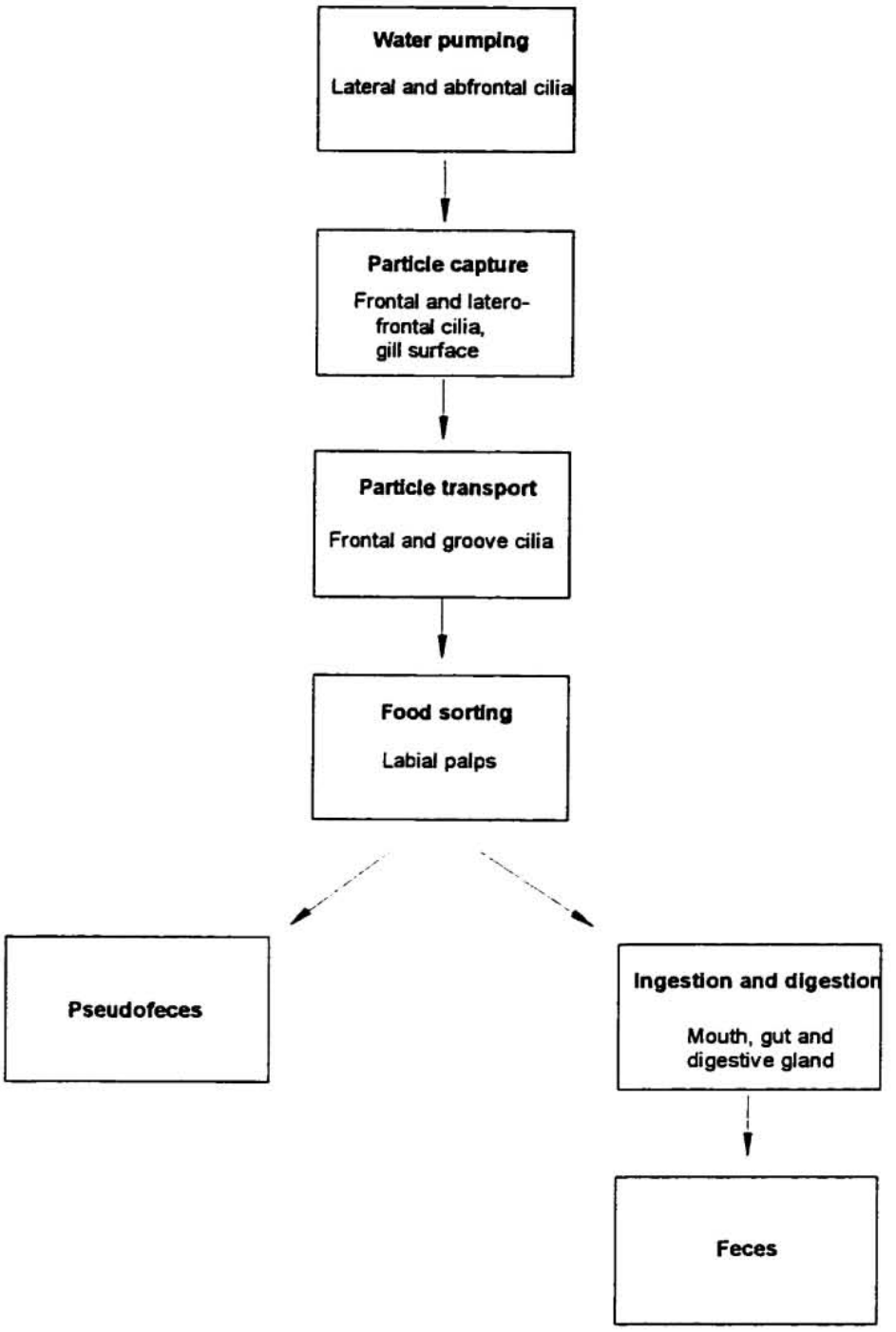

Figure 1.1. Flow chart showing the different compartments in the suspension feeding and digestive systems of *Mytilus edulis*.

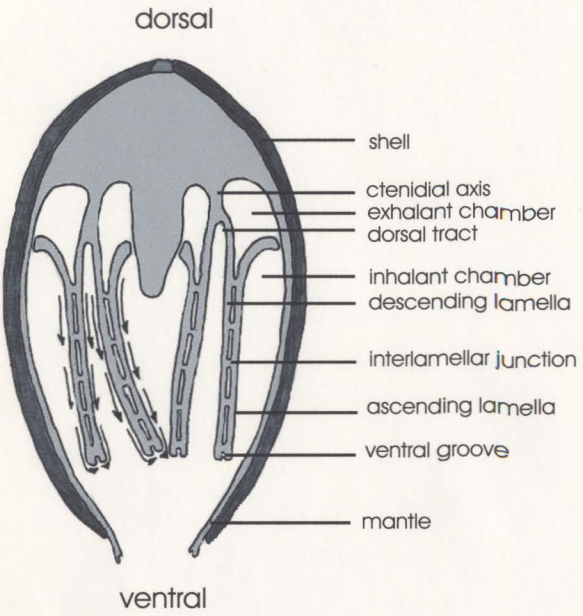

Figure 1.2. Diagrammatic cross-section of the gills of *Mytilus edulis*. Arrows indicate the direction of particle transport on the gill filaments. Redrawn from Bayne et al., 1976.

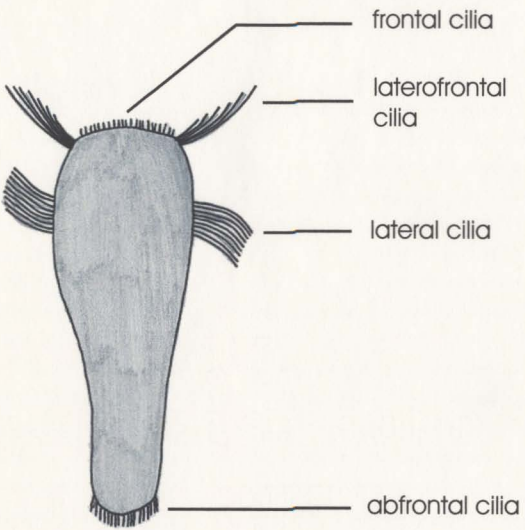

Figure 1.3. Diagrammatic cross-section of one filament showing the four different types of cilia on the gills of *Mytilus edulis*.

siphons, respectively. There is also evidence that suggests that the abfrontal cilia act in concert with the lateral cilia and contribute to the water flow in mussels (Jones et al., 1990).

Water enters the inhalant siphon, which spans the entire ventral surface of the mantle, and passes through the ostia in the ctenidial surface. Particles are captured on the filaments, with the aid of latero-frontal cirri, and frontal cilia located on the crests of the filaments (Fig. 1.3) move the food particles within a fine mucous layer or in direct contact with the frontal cilia ventrally to one of four ciliated food grooves (Ward et al., 1993; Ward, 1996). Two dorsal ciliated tracts (Fig.1.2) may also serve as secondary transport paths for particles, but evidence shows that little material is transported here in *Mytilus edulis*, even at high particle concentrations (Ward et al., 1993). Filtered water passes into the exhalant chamber and exits the mantle cavity via an exhalant siphon that is smaller than, and dorsal to, the inhalant siphon.

Particles arriving at the ventral groove can enter through one of three tracts (Foster-Smith, 1975b). Tract 1 represents the most direct route from the filament surface to the deepest part of the ventral groove, tract 2 allows particles entry midway between the deepest part of the groove and the crest of each filament, and tract 3 on the crest of each filament allows entry to the outermost part of the ventral groove (Figs. 1.4 & 1.5). Using a window technique to view the pallial system of *M. edulis* (i.e. a hole was cut in the shell and a glass cover slip glued over it), Foster-Smith (1975b) observed that the smallest mucous strings enter the ventral groove from the frontal surface of the filaments via tract 1, larger mucous strings via tract 2 and the largest via tract 3; the three tracts thus form a graded system of entry. He also observed that mussels could decrease entry of mucous strands into the groove by inclining the filament tips forward and blocking off tracts 1 and 2. The purpose of this graded entry with respect to mucous string size is unclear, since the majority of captured particles eventually arrive at a ventral groove, and all mucous strings reach the labial palps for selection or rejection, regardless of the size of the mucous strand (Foster-Smith, 1975b). The mucous strings are drawn out of the

Figure 1.4. Side view of one filament of *Mytilus edulis* showing the three tracts taken by particles to arrive in the ventral food groove. Redrawn from Foster-Smith, 1975b.

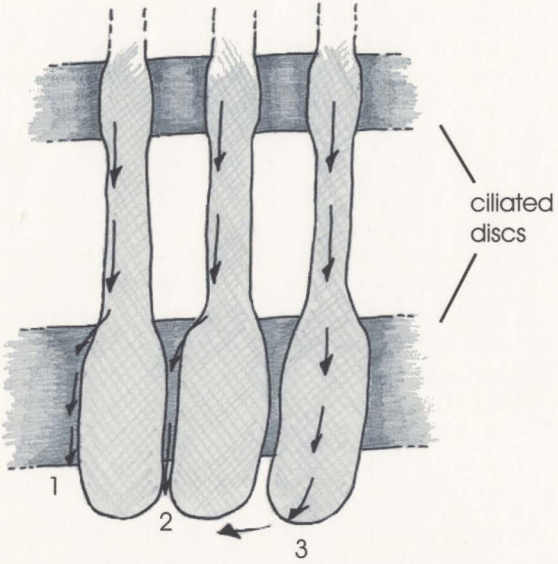

Figure 1.5. Frontal view of the three tracts leading from the filaments to the ventral grooveof *Mytilus edulis*. Arrows represent paths of particles. Redrawn from Foster-Smith, 1975b.

ventral grooves by the labial palps, which then disperse the particles for selection by the mechanical action of the dorsal margin and ridged palp surface (Ward, 1996).

Studies using video endoscopy have confirmed that particles are captured and transported on the gills via mucociliary processes in the ventral grooves and hydrodynamic processes in the dorsal tracts of the gills of some bivalves (Ward et al., 1993), but mucociliary transport is predominant on the gill of *Mytilus edulis*. In this species, the ventral food groove is the primary route by which food is carried within cohesive mucous strings to the labial palps, although some particles do travel within the dorsal tracts when animals are exposed to high particle concentrations. Pseudofeces are removed from the infrabranchial chamber at the dorsal edge of the inhalant siphon by ciliary action of the mantle and entrainment with the exhalant current (Morton, 1983). Pseudofeces represent material filtered from suspension but rejected by the labial palps before ingestion, and are produced by suspension feeders in order to remove excess particles in dense suspensions (Foster-Smith, 1975a; Beninger et al., 1995) or to allow for particle selection by removing inorganic or other non-nutritious particles and leaving nutritious organic particles behind for ingestion (Kiørboe & Møhlenberg, 1981; Ward et al., 1997).

1.3 Structure and function of cilia

Since the cilium is the basic functional unit of particle transport on the bivalve gill, information about the innervation and activity of cilia can be related to particle velocity data obtained using the endoscope. Since cilia govern the pumping rate and particle transport capabilities of the gill, any alteration to ciliary activity would have an impact on suspension feeding. Most of the research to date deals with mammalian cilia, and the relatively small amount of work on bivalves involves lateral cilia (e.g. Aiello, 1960, 1970; Paparo, 1972; Catapane at al., 1981; Murakami & Machemer, 1982; Jørgensen et al., 1990; Jørgensen & Ockelmann, 1991; Prins et al., 1991), although some focus has been on the frontal cilia (e.g. Nomura & Tomita, 1933; Hirasaka et al., 1957; Hoshi & Hoshiyama, 1963). Little mention is made of ventral groove cilia, but presumably they resemble

frontal cilia rather than lateral cilia in their morphology and activity since ventral and frontal cilia have similar functions (i.e. transport of particles within mucus).

All cilia move water or mucus via a recovery stroke, in which the cilium moves in a curved path bent close to the epithelial surface, and an effective or propulsive stroke, where the cilium swings through an erect arc perpendicular to the epithelial surface. As a cilium moves in its recovery stroke, the entrained fluid around it comes into contact with other cilia and stimulates them to initiate movement that will minimize contact between adjacent cilia. It is in this manner that a metachronal wave is propagated, whereby the beating of many cilia is organized into coordinated waves (Sleigh et al., 1988). The main contribution of metachronism to the functional efficiency of ciliary systems is probably to maintain a regular flow (Sleigh & Aiello, 1972), which is more important in water-propelling than mucus-propelling systems.

According to Sleigh (1982) and Sleigh et al. (1988), mucus-propelling cilia have important morphological and functional distinctions from water-propelling cilia. Mucus-propelling cilia are shorter, ~5 to 7 μm in length, and display intermittent and irregular metachronism, as opposed to water-propelling cilia which are 10 to 20 μm long and exhibit more regular and continuous metachronism. Although these are both important adaptations that increase the efficiency of transport by each type of cilium, it seems that the two ciliary types respond similarly to changes in their environment. For example, lateral cilia, which propel water, have been shown to increase in beat frequency in response to increased temperature (Catapane et al., 1981; Jørgensen & Ockelmann, 1991; Prins et al., 1991) as have mucus-propelling frontal cilia (Gray, 1929; Hirasaka et al., 1957; Hoshi & Hoshiyama, 1963).

Water-propelling cilia transport homogeneous fluid, whereas mucus-propelling cilia are covered by a two-layer fluid consisting of a low-viscosity periciliary fluid closest to the epithelial surface, and an outer viscoelastic mucous layer which is penetrated by the cilia tips during the effective stroke (Sanderson & Sleigh, 1981). Although most of the information concerning the structure and function of mucus-propelling cilia is derived

from mammalian cilia transporting mucus at a liquid/air interface, the same type of propulsion is also found in aquatic animals having no liquid/air interface above the mucus (Sleigh, 1989). Beninger et al. (1997) recently confirmed that this two-layer fluid model for mucociliary transport applies to the gills of *Mytilus edulis*. As a general rule, if the beat frequency of the cilia is increased, the tip velocity of the effective stroke also increases, both resulting in a higher velocity of the mucus (Sleigh, 1982).

Changes in particle transport may reflect changes in the activity of frontal cilia (i.e. alterations in beat frequency, form or pattern of beat, or metachronal coordination among cilia) (Murakami, 1989), the pattern of frontal ciliary currents, or the quantity and viscosity of the traveling mucus (Tankersley, 1996). Beat frequency and pattern of beat are controlled by cellular activities such as changes in membrane potential (Eckert, 1972), and the cellular activities are in turn regulated by nervous and humoral control (see section 1.4). Metachronism is a product of mechanical interaction among cilia and is therefore independent of cellular mechanisms (Murakami, 1989).

1.4 Control of cilia

Paparo (1972) succeeded in tracing branchial nerve fibres from the visceral ganglion to the ciliated epithelium of the gill filaments, and other researchers observed cessation in beating of cilia after severing the branchial nerve, thus suggesting that cilia are under nervous control (Gray, 1929; Aiello, 1960). Nervous control of ciliary activity could therefore mediate some observed effects on suspension feeding due to changes in the ambient environment such as temperature, particle type and particle concentration. Further research has determined that the lateral, frontal and latero-frontal ciliated cells are innervated and regulated by serotonergic and dopaminergic neurons originating in the central nervous system (Aiello, 1960, 1970; Catapane et al., 1978). Increased levels of serotonin, which can be stimulated by factors such as increased temperature, result in an increase in the beating rates of lateral cilia (Catapane et al., 1981). In addition, ciliary activity varies at different locations on the gill at one time (Dral, 1968), and numerous

adjacent filaments are known to be innervated by the same nerve bundle (Aiello and Guideri, 1965). This evidence suggests that the *Mytilus* gill has the capacity for local control over the various ciliary systems. Ciliary beating is ultimately dependent on the availability of ATP and consequently on the rates of glycolysis and respiration, which are in turn stimulated by serotonin (Aiello, 1970). Modulated calcium concentrations serve as intracellular messengers to regulate ciliary activity (Machemer & Sugino, 1989).

1.5 Mucus structure and rheology

Mucus is a non-Newtonian fluid, and thus there are special implications for its movement by cilia. For example, as the forces applied by cilia increase, the viscosity of mucus decreases, thereby facilitating its movement (Sleigh et al., 1988). At the same time, mucus is viscoelastic and tends to return to its original shape after being stretched, and according to Sleigh et al. (1988), viscous effects dominate at low beating frequencies whereas elastic effects dominate at high frequencies. These properties have important but not easily understood consequences concerning the capture and transport of particles by mucus. The rheology of mucus, which involves the stress, strain and shear rate generated by ciliary action, and the viscoelastic properties of mucus are critical factors in mucociliary transport (Verdugo, 1982). Vogel (1994, p 20) avoided dealing with any non-Newtonian fluids, and states that rheology is "...perhaps the messiest and least understood branch of fluid mechanics."

Mucus consists of a concentrated mixture of glycoproteins (containing mucopolysaccharides), proteoglycans, lipids and other components that extend to form a highly cross-linked viscoelastic gel (Blake & Sleigh, 1974; Litt et al., 1976). Mucus secretions are very complex because they share both fluid-like and solid-like properties that are constantly altered. The viscoelastic cohesion of a string of mucus tends to keep the entire string moving together as a unit, unlike water, even if sections of cilia are inactive (Sleigh, 1989).

Mucus in the ventral groove of *Mytilus edulis* is composed of equal mixtures of acid-dominant and neutral-dominant mucopolysaccharides, resulting in an intermediate viscosity mucus compared with the highly viscous mucus, composed solely of acid-dominant mucopolysaccharides, produced by some other bivalves (Beninger et al., 1993). All filtered material is transported to the labial palps in this intermediate viscosity mucus within the ventral grooves of *M. edulis*.

1.6 Objectives

The present study focuses on one compartment of the feeding system of *Mytilus edulis* - the ventral groove. This area of the gill is important because the majority of food particles captured by the gills are transported along one of four ventral grooves before being ingested as food or rejected as pseudofeces. The purpose of this study is to determine if and how food particle velocities in the ventral groove of *Mytilus edulis* change in response to different environmental factors. Data from the literature are reviewed briefly to help explain changes in the rates of food transport in the ventral groove over time and in response to short-term manipulations of ambient particle concentration, temperature (in both acute and acclimated situations) and particle type.

CHAPTER 2 - METHODS

2.1 Collection and maintenance of mussels

Blue mussels (*Mytilus edulis*) were collected at Bellevue, Trinity Bay, Newfoundland, by SCUBA divers in August 1995 and March 1996 and were kept in flowing seawater at the Ocean Sciences Centre, Logy Bay. Since physiological rate functions of bivalves are known to be affected by body size (e.g. Winter, 1973; Widdows et al., 1979; Navarro & Winter, 1982), a relatively small size range of mussels was selected (~ 6-8 cm length). Mussels were batch fed two to four times per week with *Isochrysis* sp. cultured in f/2-Si medium (Guilliard, 1983) at ~17°C. Each mussel was scrubbed clean of epiphytes and either labeled with plastic tags using Super Glue or placed on a labeled plastic tray. Shell length and width were recorded, and Velcro strips were glued onto the right or left valve of each mussel, depending on the experimental setup used, for subsequent experiments. During winter, mussels were transferred from the flow-through water tables to 50 L refrigerated tanks in order to keep the animals acclimated to the appropriate temperature.

2.2 Video setup

Experiments that required the use of an endoscope were set up according to Ward et al. (1991). An Olympus endoscope fitted with a 1.7mm diameter optical insertion tube (OIT) was attached to an optical zoom adapter (maximum magnification ~150x, Schölly Fiberoptic) and a charge-coupled-device colour (Sanyo) or black and white camera (Cohu 6500)(Fig. 2.1). A Hi 8 video recorder and a monitor were connected to the camera, and light was provided by a fibre optics cold light source. The endoscope and camera were mounted on a micromanipulator (Fine Science Tools) and metal stand so that the position and angle of the endoscope could be adjusted.

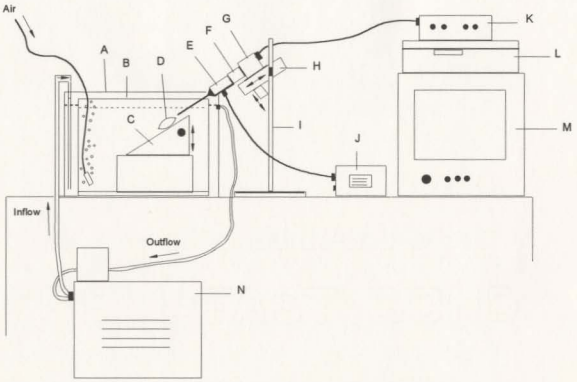

Figure. 2.1. Static system setup and videotaping equipment used in particle concentration and temperature experiments with *Mytilus edulis*. (A) outer tank containing coolant water and connected with temperature control unit, (B) inner experimental chamber containing seawater and algal suspension, (C) adjustable plastic stand, (D) mussel, (E) endoscope, (F) zoom adaptor, (G) camera head, (H) micromanipulator, (I) support stand, (J) light source, (K) camera controls, (L) Hi 8 recorder, (M) monitor, (N) temperature control unit.

2.3 Particle concentration and temperature experiments (static system)

2.3.1 Setup

The majority of the particle concentration and temperature experiments were carried out in an aerated static system (Fig. 2.1). For each preparation, one mussel was attached with Velcro to an adjustable plastic stand that rested inside a 10L container. A seawater/algae mixture within the container was aerated and maintained at a constant temperature using a cooling unit (Neslab Instruments). Each animal was allowed to graze down a predetermined concentration of *Isochrysis* sp. (ranging from 6 to 188 cells μl^{-1}) in the 10L container for 30 to 60 minutes before videotaping was initiated. Control experiments without mussels demonstrated that the algal cells did not sink in the experimental chamber.

2.3.2 Markers

To serve as marker particles during an experiment, approximately 200 µl of fluorescent yellow plastic chips (2-10 µm diameter) suspended in seawater were added to the experimental container after the insertion of the OIT into the pallial cavity of a mussel. Fluorescent yellow particles showed the highest contrast against the dark brown background of algae-rich mucus traveling along the gill filaments, as compared with red, orange or pink fluorescent particles, thus allowing for accurate mucous strand velocity measurements (see section 2.8). Particle concentration versus time plots later showed that the small amount of marker added had a negligible effect on the total particle concentration. As an alternative to plastic chips, if a particularly clear and undistorted image was obtained after initial insertion of the OIT, small drops of 20 or 25 µm diameter fluorescent yellow microspheres (Fluoresbrite Plain Microspheres, Polysciences) suspended in seawater were added via a pipette directly to the inhalant siphon to serve as calibration beads during subsequent image analysis of filaments (see section 2.7). After microspheres had been in the ventral groove for 5-10 minutes, the less expensive plastic chip suspension was pipetted into the experimental tank.

2.3.3 Videotaping

In order to observe the same region of the gill during all experiments, the OIT was always inserted into the posterior region of the shell gape (Fig. 2.2). This region proved to be less sensitive to disturbances from movements of the OIT than more anterior regions of the mantle. Videotaping was initiated once a suitable image was obtained and the animal appeared to be feeding normally (i.e. wide shell gape with extended mantle and siphons). No attempt was made to prop the shell valves open once the mussel began feeding, and if the animal was disturbed enough to close down on the OIT, videotaping was ceased until it relaxed again. By allowing the mussel to close when disturbed, the number of disturbance times could be recorded and thereby provide an indication of whether the mussel was feeding normally during each experiment. Adjustment of the endoscope using the micromanipulator was minimized in order to decrease the possibility of disturbing a mussel during an experiment. If a mussel was disturbed too often (e.g. greater than ~10 times in one hour), that session was not included in subsequent analyses.

Repeated experiments using the same group of mussels were completed in order to determine the variance of mucous strand velocities within and between individuals. Forty-five particle concentration experiments were completed using 15 different animals (Table 2.1), although many more were attempted. Failure of an experiment was generally due to intermittent feeding of a mussel or its sensitivity to the presence of the OIT. Of the 43 static system experiments, 16 were run at temperatures of 4 to 6°C, the remainder at 14 to 15°C. These temperatures lie within the natural temperature range of *Mytilus edulis* in Newfoundland waters (0° - 20°C) and are easy to maintain in the laboratory. To allow for ample acclimation time, all mussels were maintained at the appropriate temperature for at least three weeks prior to each experiment, although they typically require only 14 days to physiologically and biochemically adapt to a change in temperature (Bayne et al., 1976).

Experiment 45 was the only experiment in which algae were added to the suspension while the mussel was grazing; in all other static system experiments the mussel was allowed to graze down the suspension without replacement, mainly because of the difficulties involved in monitoring the particle concentration and maintaining a suitable

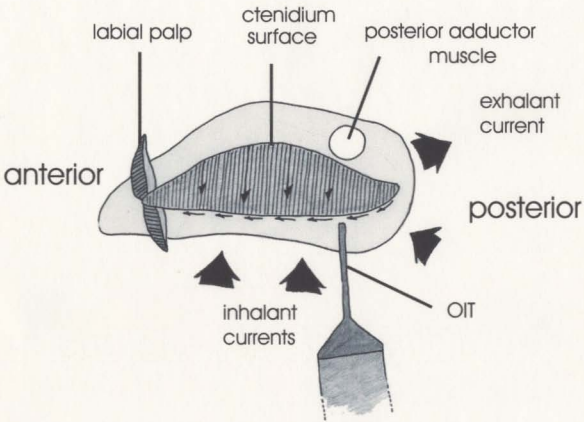

Figure 2.2. Position of OIT in the pallial system of *Mytilus edulis* during filming of the ventral groove. Small arrows indicate the movement of particles on the ctenidial surface.

Table 2.1: Experimental conditions to which mussels (*Mytilus edulis*) were subjected during particle concentration and acclimated temperature experiments in a static or flow-through system (experiments 35 and 51 were the only two performed in a flow-through system). Two columns are included to differentiate concentrations during the entire experiment with concentrations during ventral groove videotaping (there was normally a 20-45 minute feeding period before the start of videotaping). Experiment 23 took place over a long time period and was videotaped in three parts, therefore three particle concentration ranges, clearance rates and particle loads are given, although they are all derived from the same preparation. Experiment 45 was the only static system experiment in which algae were replaced when 30% of the suspension had been removed by the mussel. Three particle concentration ranges, clearance rates and particle loads are also listed for this experiment. Exp = experiment #, Mus = animal #, T = temperature, N = number of velocity measurements taken, Part. conc.(cr) = particle concentration range over which the clearance rate was calculated, Part. conc. (video) = particle concentration range during videotaping, Clear. rate = clearance rate, Load = particle load, S size = relative thickness of mucous strand (arbitrary units, values 1-4), Prev. fed = previous feeding history of each animal (1 = not previously feeding; 2 = previously feeding, see section 2.8, page 33 for explanation), Exp. time = duration of experiment.

Exp	Date	Mus	T °C	N	Part. conc. part μl^{-1} (cr)	Part. conc. part μl^{-1} (video)	Clear. rate ml min^{-1}	Load part min^{-1} $*10^6$	S size	Prev fed	Exp. time min
1	Sep23'95	18	14	42	65-11	34-15	144.0	3.6	2	1	120
2	Oct2'95	4	14	33	26-10	16-10	78.9	1.0	2	1	110
3	Oct12'95	22	14	20	30-27	29-27	14.4	0.1	1	1	100
4	Oct13'95	9	14	9	11-6	11-7	116.0	1.0	1	1	90
5	Oct23'95	9	14	66	97-36	63-36	95.0	4.8	2	1	105
6	Oct24'95	24	14	38	117-63	74-49	82.3	5.1	2	1	120
7	Oct25'95	22	14	43	125-44	72-45	82.7	4.8	2	1	120
8	Nov1'95	18	14	50	148-109	119-101	29.5	3.2	4	1	105
9	Nov6'95	22	14	25	170-133	100-134	32.6	4.6	4	1	80
10	Feb7'96	24	14	13	125-97	107-100	22.0	2.3	4	1	100
11	Feb15'96	9	14	29	136-73	105-73	62.0	5.6	4	1	90

Table 2.1 (cont.)

Exp	Date	Mus	T °C	N	Part. conc. part μl^{-1} (cr)	Part. conc. part μl^{-1} (video)	Clear. rate ml min^{-1}	Load part min^{-1} $*10^{6}$	S size	Prev fed	Exp time min.
12	Feb16'96	22	14	25	152-47	95-47	130.0	8.7	3	1	90
13	Feb20'96	24	14	18	75-48	61-48	50.0	2.7	2	1	90
14	Mar5'96	18	14	28	94-22	46-22	127.0	4.3	2	1	120
15	Mar6'96	4	14	11	127-93	126-111	38.0	4.5	3	1	90
16	Mar14'96	4	14	20	81-27	51-30	124.0	5.0	2	1	90
17	Mar15'96	16	14	20	79-27	47-28	122.0	4.4	2	1	90
18	Mar19'96	16	14	12	232-165	170-147	62.1	9.8	2	1	90
19	Mar20'96	24	14	17	253-140	188-144	64.8	11.0	3	1	90
20	Apr3'96	30	6	11	66-54	59-55	25.4	1.5	1	1	80
21	Apr4'96	31	6	21	73-37	53-37	72.3	3.3	3	1	105
22	Jun26'96	9	6	14	120-102	109-103	13.6	1.4	2	1	90
23	Jul11'96	9	6	28	136-85	111-90	44.3	4.5	3	2	350
				11	85-45	63-52	64.1	3.6			
				22	45-13	33-13	88.3	2.3			
24	Jul14'96	9	6	28	123-79	105-82	49.6	4.7	2	1	90
25	Jul15'96	16	6	11	124-77	86-66	81.8	6.4	3	2	80
26	Jul18'96	9	6	21	103-65	84-68	52.8	3.9	3	1	90
27	Aug5'96	9	6	13	83-63	73-63	37.7	2.6	2	2	80
28	Aug6'96	16	6	18	120-78	107-79	74.6	6.9	3	2	80
29	Aug6'96	9	6	19	22-13	19-14	84.5	1.4	2	2	80
30	Aug7'96	16	6	20	17-9	13-9	96.7	1.1	2	2	80

Table 2.1 (cont.)

Exp	Date	Mus	T °C	N	Part. conc. part μl^{-1} (cr)	Part. conc. part μl^{-1} (video)	Clear. rate ml min^{-1}	Load part min^{-1} $*10^6$	S size	Prev fed	Exp time min.
31	Aug12'96	16	6	25	84-56	82-57	91.1	6.4	3	1	80
32	Aug12'96	24	6	9	80-56	69-56	87.1	5.5	3	1	60
33	Aug13'96	24	6	19	44-21	31-21	100.0	2.6	3	2	80
34	Aug14'96	22	6	5	31-20	24-21	99.2	2.2	2	2	50
35	Sep3'96	9	15	22	145	145	71.9	10.4	3	2	140
45	Jan11'97	30	4	8	32-22	24-22	90.0	2.1	2	2	130
				12	30-23	30-24	106.0	2.9			
				5	34-18	33-30	113.0	3.6			
51	Mar15'97	30	4	35	6	6	75.1	0.43	2	1	120
52	Aug24'95	15	15	18	35-14	19-14	97.7	1.6	2	1	100
54	Aug30'95	18	14	25	30-10	16-10	96.9	1.2	1	1	110
55	Sep7'95	19	14	34	28-8	16-9	112.0	1.3	1	1	110
56	Sep9'95	23	14	14	24-21	22-21	12.6	0.28	1	1	90
57	Sep13'95	10	14	38	28-7	14-7	117.0	1.2	1	1	110
58	Sep13'95	8	14	5	35-20	23-21	54.6	1.2	1	1	90
59	Sep15'95	26	14	14	24-15	18-15	49.1	0.83	1	1	80
60	Sep19'95	23	14	32	19-5	10-5	118.0	0.87	1	1	110

image on the video monitor at the same time. Many of the animals remained healthy and seemed to adapt to the presence of the OIT over long periods of time. Some of these mussels were used for many months in different experiments in addition to mussels brought in at a later date.

2.3.4 Clearance rates

Twenty ml samples of seawater were taken at ~15 minute intervals beginning when an animal was first placed in the experimental container and continuing until the experiment was terminated (the duration of most of these experiments ranged from 1.5 to 2 hours, depending on the behaviour of the mussel). Any feces or pseudofeces produced by a mussel during an experiment were removed with a pipette in order to prevent resuspension of biodeposits. Samples taken from the experimental chamber were counted (size range 3 to 64 μm diameter) with a Multisizer (Coulter Electronics) fitted with a 100μm orifice tube.

To determine the clearance rate of each animal, the slope of the regression of natural logarithm of particle concentration versus time was multiplied by the volume of the experimental container. If the clearance rate changed during the duration of an experiment, the longest linear section of the regression line was used to calculate the clearance rate for that animal (one clearance rate per experiment was necessary for clarity and for statistical purposes). The particle concentration range over which each clearance rate was determined is recorded in Table 2.1.

2.4 Time effect experiments

2.4.1 Setup

A flow-through system was used to determine whether there were long term time effects on the velocity of mucus in the ventral groove (Fig. 2.3). With this system, the ambient particle concentration could be maintained for many hours and the clearance rate of the mussel and particle velocities on the ventral groove recorded. A 1.3L experimental

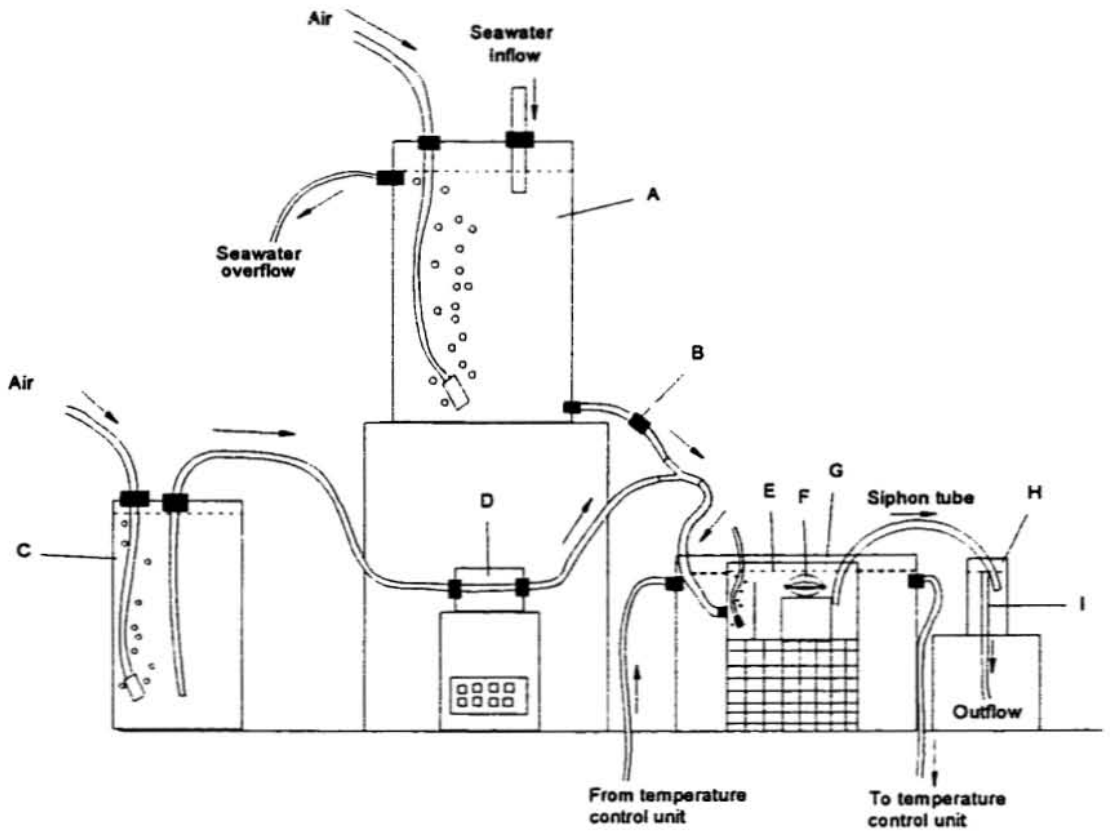

Figure 2.3. Flow-through system setup used in temperature, time and particle quality experiments with *Mytilus edulis*. See Fig. 2.1 for endoscope and videotaping equipment. (A) header tank, (B) fow restrictor, (C) algal culture, (D) peristaltic pump, (E) experimental chamber, (F) mussel, (G) outer tank containing coolant and connected to temperature control unit, (H) siphon cup, (I) standpipe.

container was connected to a 20L header tank continuously filled with aerated seawater and to a separate 10L carboy containing fluorescent marker particles in a dense culture of *Isochrysis*. The flow of seawater from the header tank to the experimental tank was controlled with different sized flow restrictors (plastic plugs with small holes drilled through the center), and the aerated algal culture was added to the system at specific rates using a peristaltic pump (Minipuls 3, Mandel Scientific). The outflow from the experimental chamber consisted of a siphon hose that was connected to a cup containing seawater, and a standpipe that maintained the water level in the experimental chamber. Flow velocities through the system were calculated using the time taken to fill a graduated cylinder with seawater from the outflow tube.

After using the apparatus a few times, it was noticed that the mussels adapted to the presence of the marker particles after being exposed to them for 2 to 3 hours. The animals were able to separate the marker particles from the algae-rich mucous strings traveling within the ventral groove and relocate them to the outer edges of the groove where the particles were susceptible to dislodgment by feeding currents, thus making it difficult or impossible to determine the mucous string velocities. In order to counteract this effect, two carboys were used: one with marker particles added, and one without. For time periods in which the ventral groove was not being videotaped during an experiment (see section 2.4.2 for details), the carboy which contained only algae was connected to the flow-through system, whereas the carboy which also contained marker particles was connected to the system only during videotaping periods. This procedure prevented the animals from adapting to the markers and creating this 'marker particle shunt'. The experimental chamber was immersed in a refrigerated water bath, and an aerator was placed next to the seawater inlet to equilibrate O_2 levels and resuspend the algae before the water reached the mussel on the other side of a baffle (Fig.2.3).

2.4.2 Videotaping

Each mussel was propped at an angle in the experimental chamber using a small plastic stand to allow for insertion of the endoscope at an angle of approximately 45°

(Velcro was not used during flow-through experiments). Animals were positioned so that the inhalant siphon faced the seawater inlet (i.e. facing the opposite direction from animals in the static system experiments). Once a flow rate was achieved which prevented each mussel from removing more than 30% of the suspended cells (flow rate ranged from 150 to 429 ml min^{-1}), and the desired particle concentration was obtained, a 15 to 20 minute adjustment period followed until the mussel's shell gape permitted entry of the OIT and videotaping was initiated.

Nine time effect experiments, each lasting from ~3 to 48 hours, were successfully completed (Table 2.2). During each experiment, the ventral groove was videotaped for 15 to 20 minutes, after which the mussel was left to feed in the absence of the OIT for ~45 minutes to 2 hours before OIT reinsertion. For some of the very long experiments, mussels were left to feed undisturbed in the experimental setup for 5 to 14 hours between videotapings. In order to determine if there were any feeding history effects, the mussel in experiment 40 was allowed to feed on a predetermined particle concentration for 23 hours prior to videotaping, whereas animals in experiments 41 and 42 were kept without food for 2 days prior to videotaping.

2.4.3 Clearance rates

In order to determine the clearance rate of each mussel, twenty ml samples of seawater were taken from the inflow, siphon and outflow locations during each experiment. According to Hildreth and Crisp (1976), the calculation of the clearance rate for a bivalve in a flow-through system should take into account the dilution effect of inflowing water as it enters the experimental tank. To do this, one must determine the particle concentration in the area immediately surrounding the mussel, which in this case is the siphon area, and apply the equation:

$$CR = \frac{C_i - C_o}{C_s} * FR \qquad \text{Equation 2.1}$$

Table 2.2: Experimental conditions to which mussels (*Mytilus edulis*) were subjected during time effect experiments in a flow-through system. See Table 2.1 legend for descriptions of column headings. Particle concentration and clearance rate values are means with standard deviations in brackets. The mussel in experiment 40 had been feeding for 23 hours before filming, and experiments 40, 41 and 42 are partitioned since clearance rates or particle concentrations changed slightly during the experiments.

Exp	Date	Mus	T °C	N	Particle conc. part μl^{-1} (std dev)	Clearance rate ml min^{-1} (std dev)	Load part ml^{-1} $*10^6$	S size	Prev fed	Exp. time hours
36	Sep4'96	9	15	43	162(6.22)	69.0(11.1)	11.2	3	2	5.8
37	Sep10'96	9	14	25	96.3(3.85)	58.5(19.4)	5.6	2	2	5.8
38	Jan6'97	32	5	13	119(11.8)	67.7(18.5)	8.1	2	1	8
39	Jan7'97	30	4	21	11.5(1.83)	76.7(34.5)	0.88	2	1	8
40	Sep19'96	24	13	59	42.6(4.88)	122.7(20.34)	5.2	2	2	48
				60	34.8(3.66)	148(30.8)	5.2			
41	Oct1'96	9	13	73	34.5(3.21)	110(28.0)	3.9	2	1	28
				43	27.5(3.21)	55.3(16.1)	1.3			
42	Nov6'96	30	8.5	20	122(6.44)	51.1(12.4)	6.2	2	1	14
				55		34.4(9.85)	4.2			
49	Feb12'97	33	4	28	4.47(0.31)	59.9(36.1)	0.27	2	1	3.6
50	Feb17'97	33	3.5	24	27.4(1.63)	54.1(16.0)	1.5	2	1	2.8

where CR= clearance rate (ml min^{-1}), C_i= inflow concentration (particles μl^{-1}), C_o= outflow concentration (particles μl^{-1}), C_s= concentration in the siphon area (particles μl^{-1}), and FR= flow rate (ml min^{-1}). In the flow-through experiments carried out in this study, the particle concentration at each mussel's siphon was very similar to the particle concentration measured in the outflowing seawater, and since the outflow concentrations tended to be less variable than siphon concentrations, the following modified equation was used for all clearance rate calculations:

$$CR = \frac{C_i - C_o}{C_o} * FR \qquad \text{Equation 2.2}$$

2.5 Particle quality experiments

2.5.1 Setup

Two particle quality experiments were done using the flow-through system described in section 2.4 (Table 2.3). Animals were fed algal suspensions for 3 hours and then fed an inorganic sediment suspension for the remainder of each experiment. In experiment 46, the sediment suspension was introduced into the experimental chamber with a peristaltic pump after disconnecting the supply of algae, but much of the sediment settled within the tubing before reaching the mussel, even with high pumping rates. To avoid sediment settling in the tubing in the second experiment, sediment was suspended in seawater in large temperature controlled tanks and was transferred directly to the experimental header tank when a particle quality change was required. This setup resulted in a shorter route between the sediment suspension tank and the experimental chamber and also permitted vigorous stirring of the sediment within the header tank, which in turn helped to keep the sediment suspended for longer periods. By running this system without a mussel present, a settling rate of 6.5% of the total sediment particles traveling to the experimental chamber was determined (additional stirring within the experimental chamber

Table 2.3: Experimental conditions to which mussels (*Mytilus edulis*) were subjected during particle quality experiments using a flow-through system. See Table 2.1 legend for descriptions of column headings. Particle concentration and clearance rate values are means with standard deviations following in brackets. Experiment 48 is partitioned since clearance rates changed slightly.

Exp	Date	Mus	T °C	N	Particle conc. part μl^{-1} (std dev)	Clearance rate ml min^{-1} (std dev)	Load part min^{-1} $*10^6$	S size	Prev fed	Exp. time hours
46	Feb3'97	33	3.5	26	7.11(0.85)	64.24(30.74)	0.44	2	1	6
48	Mar19'97	30	2.5	22	8.42(0.74)	151(50.8)	1.3	2	1	7
				36		113(27.3)	0.98			

was not possible since the resulting vibrations disturbed the feeding mussel and made videotaping impossible). Clearance rate measurements were therefore corrected for particle settlement.

2.5.2 Sediment

Sediment was taken from the upper 10 cm of a core sample from 250 m depth in Conception Bay, Newfoundland, and dried at 60°C. The sediment was then combusted in a muffle furnace at 420°C for 6 hours, cooled and ground with a mortar and pestle before being resuspended in seawater. The size frequency distribution of the final suspension was similar to that of *Isochrysis* (i.e. mode near 3μm diameter).

2.6 Acute temperature change experiments

Two temperature experiments were done using the flow-through system in which the animals used were acclimated to 4 - 5°C and then exposed to 14 - 15°C during feeding (Table 2.4). After 2 hours of feeding at the lower temperature (ambient seawater temperature), seawater in the experimental header tank was replaced with heated water. It took ~20 minutes for the temperature change to be completed in the experimental chamber, and although the mussels' mantle gape decreased during the change, both mussels continued to feed normally. Particle concentration measurements necessary for clearance rate calculations were recorded continuously during the entire experiment.

2.7 Gill filament measurements

Image analysis software (Mocha, Jandel Scientific) was used to take filament width measurements directly from the videotapes of ventral grooves made during experiments in which fluorescent microspheres were used. By capturing a video frame with a non-distorted image of a microsphere adjacent to the ventral groove filaments, the microsphere could be used as a reference point with which to measure the filament widths. Since the image analysis method of measuring filaments is a novel technique, these on-screen

Table 2.4: Experimental conditions to which mussels (*Mytilus edulis*) were subjected during accute temperature experiments using a flow-through system. See Table 2.1 legend for descriptions of column headings. Particle concentration and clearance rate values are means with standard deviations following in brackets. Both experiments are partitioned since clearance rates changed when the ambient temperature was altered.

Exp	Date	Mus	T °C	N	Particle conc. part μl^{-1} (std dev)	Clearance rate ml min^{-1} (std dev)	Load part min^{-1} $*10^6$	S size	Prev fed	Exp. time hours
43	Jan25'97	30	4	47	29.8(3.84)	112(41.3)	3.3	2	1	5
			15	49		34.0(22.4)	1.0			
44	Jan27'97	32	5	23	11.8(0.77)	147.2(30.45)	1.7	3	1	4
			14	38		133.7(41.44)				

measurements of filaments from living mussels were later compared with post-mortem measurements of filaments using a dissecting microscope and calibrated ocular micrometer.

2.8 Video analysis and variables

Freeze-frame analyses of the video recordings were used to determine the velocity of each mucous strand traveling within the ventral groove of a demibranch. When a distinct fluorescent marker particle that was firmly lodged within a mucous strand (i.e. showing consistent movement within the ventral groove and not affected by mantle currents) was observed, the velocity of the particle was calculated from the number of frames required for it to travel across the tips of a known number of filaments. Since the video speed was 30 frames per second (NTSC standard), and filament size was previously determined using image analysis, the velocity of the particle in was easily calculated. Particle velocity measurements were taken from each videotape at ~2 minute intervals, when possible, and the corresponding 'time spent feeding' was calculated using video time and real time comparisons with pauses taken into consideration.

Because particle velocity measurements were taken at smaller intervals than the particle concentration measurements, it was necessary to calculate the particle concentration values at all times using plots of ln particle concentration vs time. The following equation was used to determine the particle concentrations that corresponded to each video measurement in the static system experiments:

$$C_t = C_o * e^{-gt} \qquad \text{Equation 2.3}$$

where C_t= particle concentration (particles μl^{-1}) at time t (minutes), C_o= particle concentration (particles μl^{-1}) at time 0, and g= slope of the ln concentration vs time plot. This equation was not required for the flow-through experiments since the particle concentration was kept constant. The particle load (particles min^{-1}), or total amount of food material moving on the gill per unit time, corresponding to each velocity

measurement was determined by multiplying the clearance rate (ml min^{-1}) by particle concentration (particles μl^{-1})*1000.

Since the size of a mucous strand within the ventral groove changed between, and sometimes within, experiments, the relative size of each strand was also recorded. Four categories were used to indicate strand thickness: 1= thin, 2= medium, 3= thick, and 4= extended (Figs. 2.4 - 2.7). Category 4 describes situations where the outside edges of the mucous strand were extending beyond the ventral groove and obscuring the filaments. It was not possible to measure strand width directly using image analysis because sections of the mucous strands were often hidden by the contraction of the wide filament tips at the ventral groove. Since food particles are entrained within mucus while traveling in the ventral groove, the terms 'mucus strand or string velocity', 'particle velocity' and 'transport rate within the ventral groove' are synonymous and are used interchangeably throughout this thesis.

Some of the mussels were observed to be feeding in their holding tanks before an experiment, therefore the previous feeding condition of each animal was recorded as either: 1 - mussel had not been feeding before the start of the experiment, or 2 - mussel had been feeding prior to the experiment. Also, since experiments were performed over a period of twenty consecutive months, it was necessary to test whether there was a seasonal or month effect on particle velocity, therefore each experiment was assigned to the appropriate month (1 - 20) and the variable 'month' included in subsequent analyses. Table 2.5 lists the response variables that were included in the statistical analyses discussed in section 2.9.

2.9 Statistical analyses

Since nearly all of the experiments in this study were carried out using the same basic protocol during the first 120 minutes (i.e. individual mussels fed with *Isochrysis* sp. at variable particle concentrations and temperatures), it was possible to pool the acute temperature, acclimated temperature, particle quality, time effect and particle

Figure 2.4. Ventral groove of *Mytilus edulis* containing a thin mucous strand.

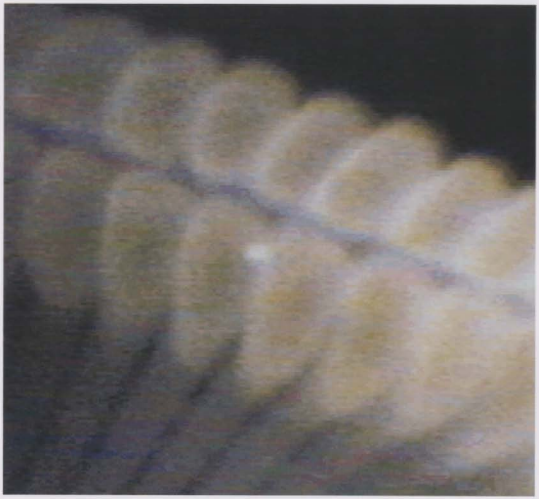

Figure 2.5. Ventral groove of *Mytilus edulis* containing a medium mucous strand with one marker particle visible.

Figure 2.6. Ventral groove of *Mytilus edulis* containing a thick mucous strand and two microspheres.

Figure 2.7. Ventral groove of *Mytilus edulis* with a mucous strand extending over the filament tips.

Table 2.5: Variables considered in the statistical analyses of pooled particle velocity data in *Mytilus edulis*.

Variable	**Abbreviation**	**Type of variable**
lag for particle velocity	lag	continuous
particle concentration	conc	continuous
clearance rate	cr	continuous
particle load	load	continuous
time	time	continuous
temperature	temp	categorical
mussel #	mus	categorical
previous feeding history	prev	categorical
month	month	continuous

concentration experiments in order to determine an overall particle velocity model (see Tables 2.1 - 2.4 for details of individual experiments). Restricting this pooled data set to time spent feeding less than 120 minutes kept time effects to a minimum; long-term time effects are addressed separately. It was assumed that using a static or flow-through system in different experiments would not affect the basic feeding physiology of the mussels.

Data from all experiments, with the exception of experiment 40, were included in the pooled statistical analyses. Experiment 40 was a long-term experiment in which a mussel was allowed to graze on an algal suspension for 23 hours before videotaping. Since the standard procedure during the first 120 minutes of feeding was modified, this experiment was eliminated from the final model. The remaining data were divided into one of two temperature groups: one group consisting of 32 experiments conducted in a temperature range of 8.5 to 15°C (mean 14°C), and the second group consisting of 28 experiments conducted in 3 to 6 °C seawater (mean 4.5 °C).

A general linear model (GLM) was used to test the effects of the numerous categorical and continuous explanatory variables (Table 2.5) and their interactions on the mucus velocities in the ventral groove. Because all the data collected were derived from time series, significant autocorrelation of the residuals (detected by the Durbin-Watson test or the Arima procedure in the SAS statistical package) resulted when applying any model to the pooled data. Since association of residuals violates one of the major assumptions of the GLM, various techniques were employed to remove the autocorrelation from the analyses. The most successful procedure for this particular data set involved creating a new variable consisting of lagged values of the response variable, y_{t-1}, which resulted in a model of the type:

$$\mathbf{y}_t = \beta_0 + \beta_1 * \mathbf{x}_t + \beta_2 * \mathbf{y}_{t-1} + \varepsilon_t \qquad \text{Equation 2.4}$$

(Myers, 1990). By including this new variable in the GLM, the association of the residuals was taken into account and therefore corrected for in the analyses.

Separate analyses of individual experiments using regression analysis, ANOVA and correlation analysis were also completed in addition to the overall particle velocity model. Many of these models also incorporate a 'lag' term to eliminate autocorrelation of the error terms.

CHAPTER 3 - RESULTS

3.1 Gill filament measurements

Filament width measurements at the ventral margins of live mussels determined using image analysis were smaller than those obtained from freshly dissected animals using a stereoscope, even after an underestimation factor was applied to the image analysis measurements (Table 3.1). This factor of ~7 % represents the difference between measurements of the diameters of calibration particles using image analysis software and the actual size of the calibration particles provided by Polysciences (25 μm). The underestimation factor was applied to the grand mean of the image analysis filament width measurements to give a corrected mean of 83.8 μm, which was 9.8 % lower than the grand mean for stereoscope measurements (92.9 μm).

3.2 Mucous strand size

Particle velocity decreased with increasing mucous strand size (Fig. 3.1), and as strand size increased so did the particle load on the gill (Fig. 3.2). Since strand size was correlated with particle load, it was decided that only the quantitative and non-subjective variable 'load' would be used in the final statistical model (section 3.3).

Table 3.1: Summary of filament width measurements at the ventral groove of *Mytilus edulis* using both image analysis and a stereoscope with calibrated micrometer. Mussel = animal #, N = number of filament measurements per mussel.

Image analysis:

Mussel	N	Mean (μm)	Standard deviation
18	45	68.93	4.16
16	52	81.68	7.16
9	221	82.48	14.24
22	60	80.28	6.27
24	31	78.31	4.7
	Grand mean:	**78.34 μm** * 7% = 5.48	
	Corrected:	5.48 + 78.34 = **83.8 μm**	

Stereoscope:

Mussel	N	Mean (μm)	Standard deviation
3	8	101.8	0
5	16	82.9	10.4
12	15	93.3	11.4
24	5	106	4.18
22	5	105	5.0
32	5	80	3.6
30	5	83	4.5
31	5	93	4.5
9	5	105	5.0
16	5	86	4.2
33	5	86	4.2
	Grand mean:	**92.9 μm**	

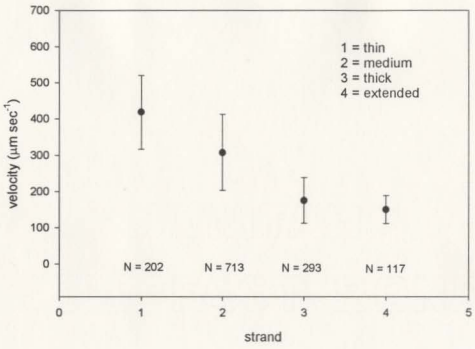

Figure 3.1. The relationship between particle velocity and mucous strand size in the ventral groove of *Mytilus edulis*. The mean and standard deviation for particle velocity in each strand size category are plotted, and sample numbers are included. Strand size and velocity are significantly correlated ($r^2 = 0.44$, $n = 1325$, $p < 0.0001$).

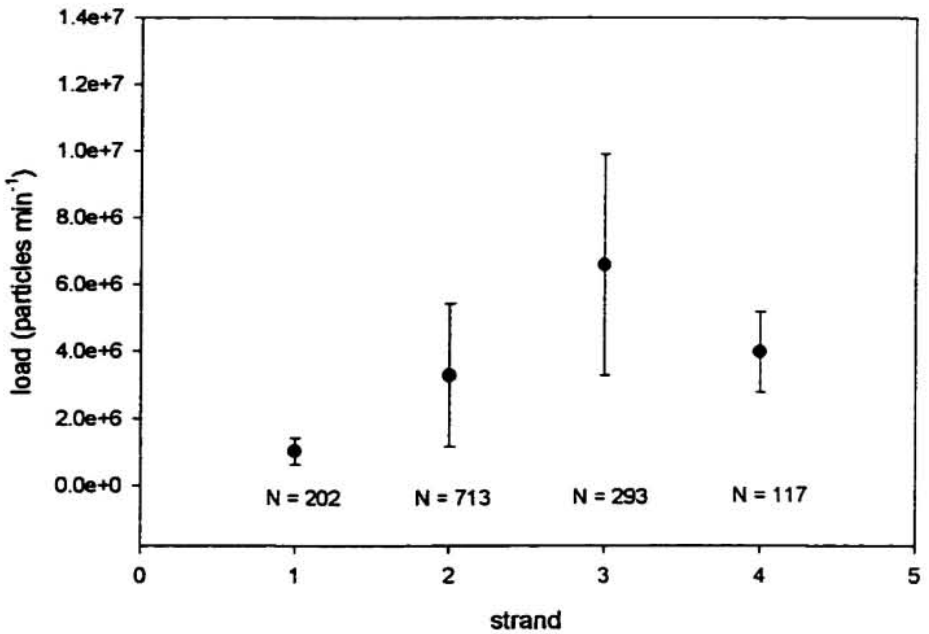

Figure 3.2. The relationship between particle load and mucous strand size in the ventral groove of *Mytilus edulis*. The mean and standard deviation for particle loads in each strand size category are plotted, and sample numbers are included. Strand size and particle load are significantly correlated ($r^2 = 0.53$, $n = 1325$, $p < 0.0001$).

3.3 Statistical analyses

3.3.1 Final model

After eliminating variables that had insignificant effects on particle velocity ($p > 0.05$), the following terms were included in the final model:

$$\textbf{velocity} = \beta_0 + \beta_1\textbf{*lag} + \beta_2\textbf{*temp} + \beta_3\textbf{*conc} + \beta_4\textbf{*mus} + \beta_5\textbf{*load} + \beta_6\textbf{*conc*mus} + \beta_7\textbf{*load*temp} + \beta_8\textbf{*load*mus} + \beta_9\textbf{*temp*mus} + \varepsilon \quad \text{Equation 3.1}$$

where $n = 1324$, $r^2 = 0.94$ and $p < 0.0001$ (see Table 3.2 for p values of individual variables). Since not all the mussels were tested in both temperature groups, a second analysis was performed including only those animals for which ventral groove strand velocities were measured at both temperatures (# 9, 16, 22, 24, and 30). A similar but more simple model resulted from this data set:

$$\textbf{velocity} = \beta_0 + \beta_1\textbf{*lag} + \beta_2\textbf{*conc} + \beta_3\textbf{*temp} + \beta_4\textbf{*mus} + \varepsilon, \quad \text{Equation 3.2}$$

where $n = 826$, $r^2 = 0.94$ and $p < 0.0001$ (see Table 3.2 for individual p values). The variables 'temp', 'conc' and 'load' within Equations 3.1 and 3.2 are examined individually and in more detail in the following sections. The variable 'mus' is not examined further since its significance in these models is probably due to individual variation. The insignificance of the variable 'month' eliminates the possibility of seasonal effects and indicates that the mussels used remained healthy over the experimental time period, and the insignificance of 'prev' and 'time' indicates that velocities did not depend on previous feeding history or vary significantly in 120 minutes.

3.3.2 Particle load

In general, decreasing mucous strand velocities were found with increasing particle load (Fig 3.3). The curvilinear equation gave the best fit to particle velocity

Table 3.2: P values associated with each significant explanatory variable in the final GLM (Equations 3.1 and 3.2).

Equation 3.1:

variable	df	p
lag	1	<0.0001
concentration	1	<0.0001
temperature	1	<0.0001
mussel	15	<0.0001
particle load	1	0.0042
concentration*mussel	15	0.0040
load*temperature	1	0.0027
load*mussel	8	<0.0001
temperature*mussel	4	<0.0001

Equation 3.2:

variable	df	p
lag	1	<0.0001
concentration	1	0.0022
temperature	1	<0.0001
mussel	4	0.0002

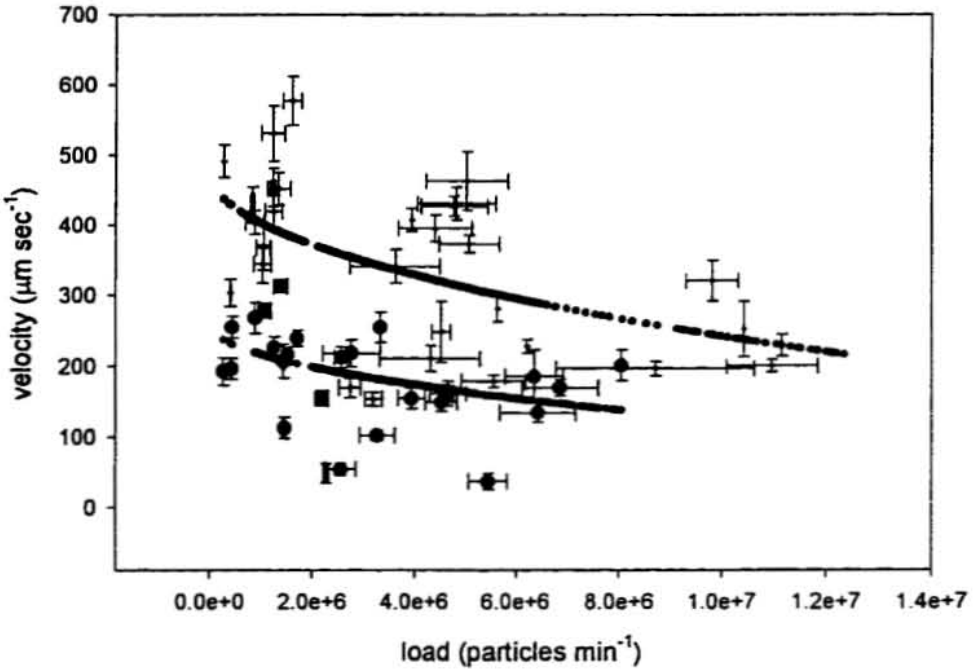

Figure 3.3. The relationship between particle velocity in the ventral groove of *Mytilus edulis* and particle load. (+) = 14°C temperature group, (●) = 4.5°C temperature group. Each point on the graph represents one experiment, and the error bars are standard deviations. The raw data in the 14°C temperature group were fit to the equation: $velocity = 477 - 0.074\sqrt{load}$, n = 831, $r^2 = 0.20$. The data from the 4.5°C temperature group was fit to: $velocity = 260 - 0.043\sqrt{load}$, n = 497, $r^2 = 0.20$. The predicted curve from each model overlays the data in each temperature group.

vs particle load data in each temperature group and resulted in the following models:

$$15°C \text{ group:} \quad velocity = 477 - 0.074\sqrt{load} \qquad \text{Equation 3.3}$$

where n = 831 and $r^2 = 0.20$.

$$4.5°C \text{ group:} \quad velocity = 260 - 0.043\sqrt{load} \qquad \text{Equation 3.4}$$

where n = 497 and $r^2 = 0.20$.

Both correlation coefficients were significant ($p < 0.05$), therefore the slopes for both curves were significantly different from zero. Velocities in the 14 °C group were higher than those in the 4.5 °C group, corresponding to a Q_{10} of 2. The two components comprising particle load (particle concentration and clearance rate) were analyzed separately to determine the main source of the decreasing particle velocity trend.

3.3.3 Particle concentration

Particle velocities decreased with increasing particle concentration, with generally higher velocities in the 14°C group than in the 4.5°C group (Q_{10} of 1.7 - 2) (Fig. 3.4). A similar relationship was shown even when animals not tested at both temperatures were excluded from the analysis. The same equation used in the previous section, $y = a + b\sqrt{x}$, was applied to the data in each temperature group:

$$15°C \text{ group:} \quad velocity = 532 - 27.4\sqrt{conc}, \qquad \text{Equation 3.5}$$

where n = 831 and $r^2 = 0.49$.

$$4.5°C \text{ group:} \quad velocity = 264 - 10.3\sqrt{conc}, \qquad \text{Equation 3.6}$$

where n = 497 and $r^2 = 0.31$.

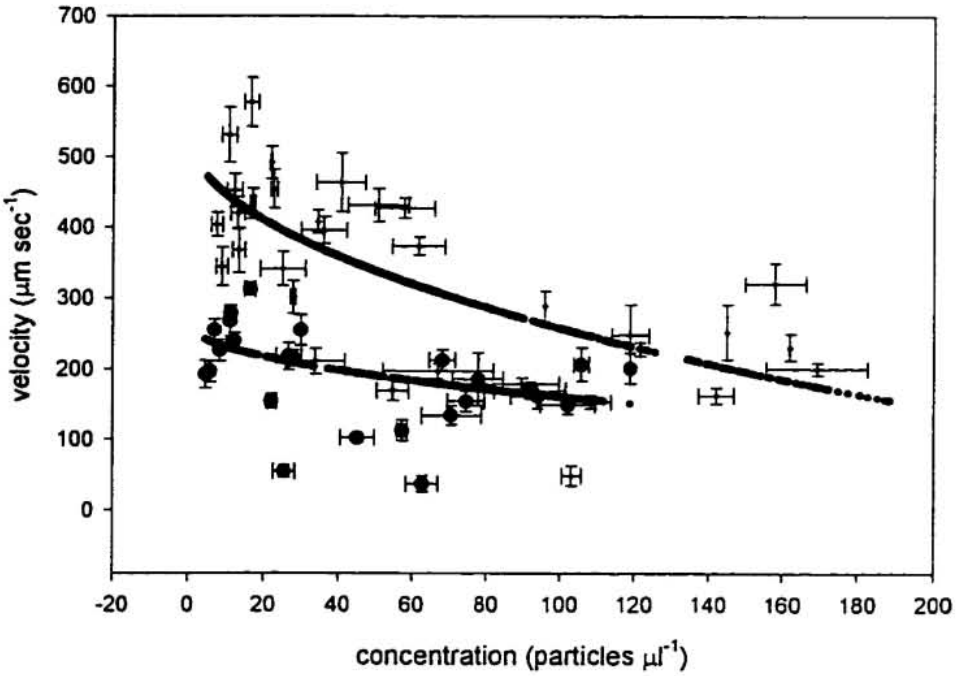

Figure 3.4. The relationship between particle velocity in the ventral groove of *Mytilus edulis* and ambient particle concentration. (+) = 14°C temperature group, (●) = 4.5°C temperature group. Each point on the graph represents one experiment, and the error bars are standard deviations from the means. The raw data in the 14°C temperature group were fit to the equation: $velocity = 532 - 27.4\sqrt{conc}$, n = 831, $r^2 = 0.49$, and data in the 4.5°C group were fit to: $velocity = 264 - 10.3\sqrt{conc}$, n = 497, $r^2 = 0.31$. The predicted curve from each model overlays the data in each temperature group.

3.3.4 Clearance rate

Although many of the animals altered their clearance rates, sometimes considerably, during an experimental run, when all other variables were taken into account clearance rate did not significantly affect the particle velocities on the ventral groove (Stepwise regression including all continuous variables, n = 1324, p = 0.92; ANOVA with categorical terms 'temp' and 'mus' included, p = 0.72). As mentioned, clearance rate was not included in the final general linear model (Equation 3.1) for this reason. Although no relationship exists between velocity and clearance rate, clearance rates can be separated into two temperature groups (Q_{10} of 2) (Fig. 3.5).

Since the product of clearance rate and particle concentration equals particle load, the relationship between clearance rate and particle concentration was analyzed. Clearance rates tended to decrease with increased particle concentration (Fig. 3.6), although the temperature effect on clearance rate (Fig. 3.5) is eliminated when particle concentration is taken into account (GLM with 'lagcr' term included, n = 1324, p = 0.97 for temperature term and interaction term).

3.3.5 Time effect experiments

With a few exceptions, particle velocities remained fairly constant over the first 120 minutes of each experiment (see Figs. 3.7 and 3.8 for examples). Of the 57 experiments carried out, 33% showed significant changes in particle velocity over this time, whereas 67% did not (see Table 3.3 for regression statistics).

Figures 3.9 to 3.17 depict flow-through experiments varying from 3 to 48 hours in duration (see Table 2.2 for experimental details). Figures were plotted on similar velocity scales to allow comparisons between experiments, and although both minutes and hours were used to label the time axes, depending on the duration of each experiment, conversions of the time end points were given to aid comparisons. No consistent pattern was apparent for mussels feeding over long time periods, although velocities often changed gradually over time.

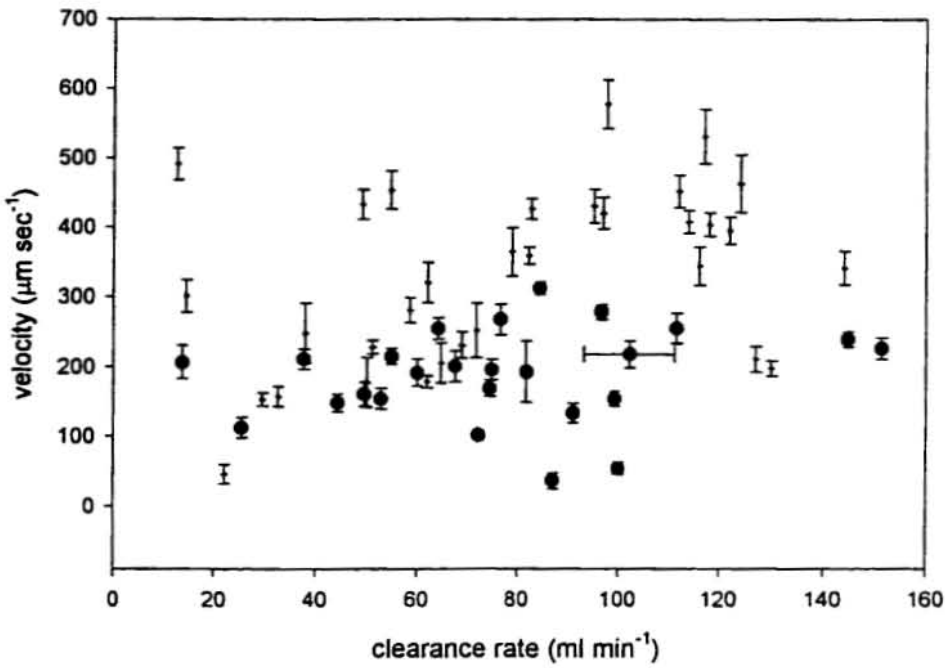

Figure 3.5. The relationship between particle velocity in the ventral groove of *Mytilus edulis* and clearance rate. (+) = 14°C temperature group, (●) = 4.5°C temperature group. Each point on the graph represents one experiment, and the error bars are standard deviations from each mean.

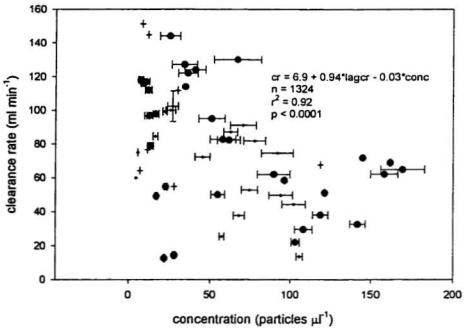

Figure 3.6. The relationship between clearance rate of *Mytilus edulis* and particle concentration. (●) = 15°C group, (+) = 4.5°C group. Each point represents one experiment, and the error bars are standard deviations from each mean. Clearance rate declines significantly with increased concentration (regression analysis with 'lagcr' term included).

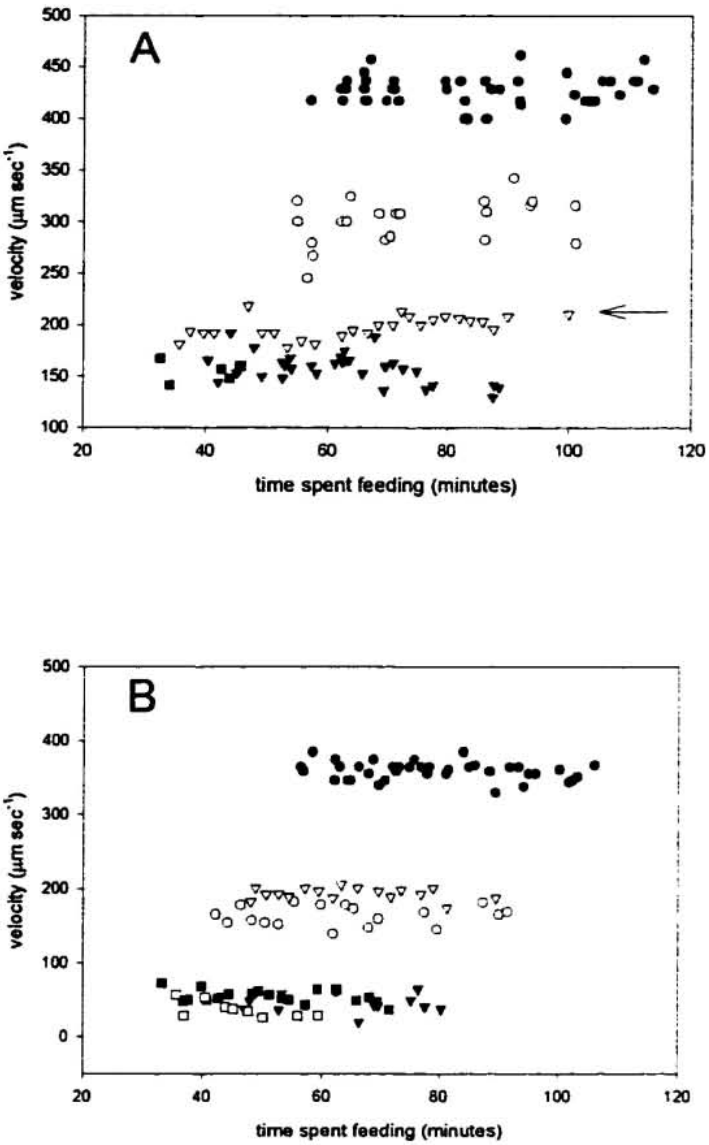

Figure 3.7: A. The change in particle velocity in the ventral groove of mussel #22 over time in static system experiments. (●) Experiment 7, (○) Experiment 3, (▽) Experiment 12, (■) Experiment 34, (▼) Experiment 9. Arrow indicates a significant linear regression (see Table 3.3 for regression results).

B. The change in particle velocity in the ventral groove of mussel #24 over time in static system experiments. (●) Experiment 6, (○) Experiment 13, (▽) Experiment 19, (■)Experiment 33, (▼) Experiment 10, (□) Experiment 32.

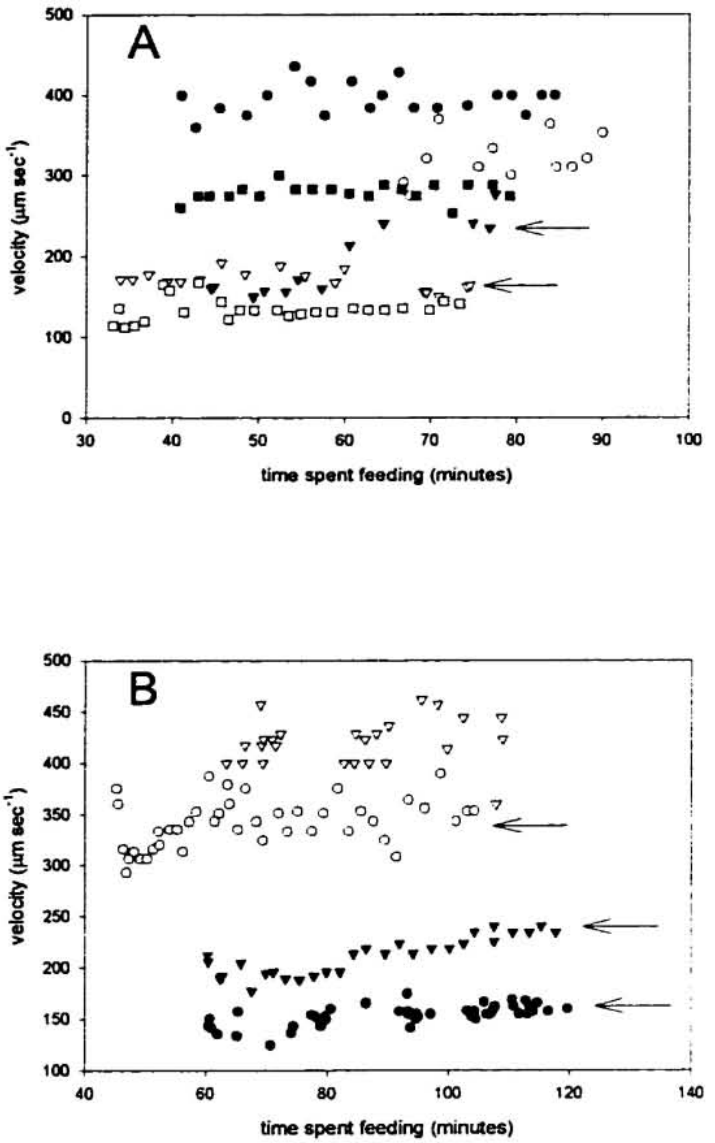

Figure 3.8: A. The change in particle velocity in the ventral groove of mussel #16 over time in static system experiments. (●) Experiment 17, (○) Experiment 18, (▽) Experiment 28, (■) Experiment 30, (▼) Experiment 25, (□) Experiment 31.

B. The change in particle velocity in the ventral groove of mussel #18 over time in static system experiments. (●) Experiment 8, (○) Experiment 1, (▽) Experiment 54, (▼) Experiment 14. Arrows indicate significant linear regressions (see Table 3.3 for regression information).

Table 3.3: Summary of static system experiments having significant linear regression slopes for time (< 120 minutes) vs particle velocity in the ventral groove of *Mytilus edulis*. Some of the equations have a 'lag' term included in order to remove significant autocorrelation of the error terms in the model.

Experiment	n	p	r^2	Equation
1	42	0.002	0.27	vel = 204 + 0.32*lag + 0.40*time
8	50	<0.0001	0.44	vel = 119 + 0.37*time
12	25	<0.0001	0.66	vel = 91 + 0.46*lag + 0.24*time
14	28	<0.0001	0.75	vel = 112 + 0.20*lag + 0.67*time
15	11	0.0004	0.77	vel = 1.01 + 3.45*time
24	28	<0.0001	0.55	vel = 84 + 0.27*lag + 0.61*time
25	11	0.0003	0.78	vel = 13.4 + 3.01*time
26	21	<0.0001	0.88	vel = 82.3 + 1.09*time
28	18	0.015	0.32	vel = 195 - 0.45*time
36	43	0.010	0.20	vel = 185 + 0.14*lag + 0.17*time
42	11	0.042	0.38	vel = 181 + 1.18*time
43	26	<0.0001	0.75	vel = 173 + 0.45*lag - 0.43*time
46	26	0.001	0.36	vel = 234 + 0.39*time
48	13	0.008	0.48	vel = 212 + 0.27*time
49	28	<0.0001	0.79	vel = 158 + 0.50*time
55	34	0.001	0.29	vel = 390 + 0.82*time
57	38	<0.0001	0.56	vel = 404 + 1.74*time
59	14	0.007	0.46	vel = 513 - 1.59*time
60	32	0.001	0.30	vel = 404 + 1.74*time

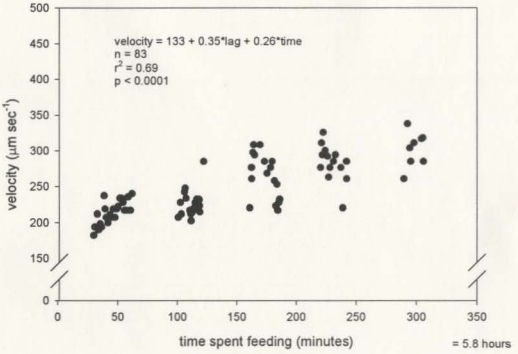

Figure 3.9. The relationship between particle velocity in the ventral groove of mussel #9 and time spent feeding in flow-through experiment #36. The particle concentration was 162 ± 6.22 SD particles μl^{-1}, and the mean clearance rate was 69 ± 11 SD ml min^{-1}. The velocity increases significantly over time (regression analysis with 'lag' term included).

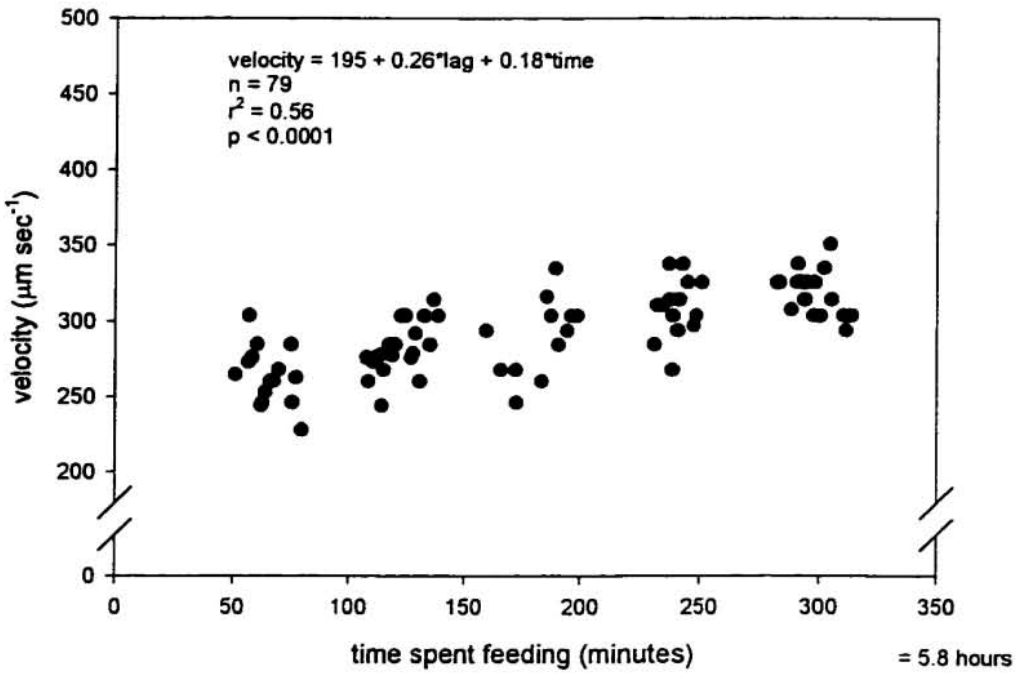

Figure 3.10. The relationship between particle velocity in the ventral groove of mussel #9 and time spent feeding in flow-through experiment #37. The particle concentration was 96 ± 3.9 SD particles μl^{-1}, and the mean clearance rate was 59 ± 19 SD ml min^{-1}. The velocity increases significantly over time (regression analysis with 'lag' term included).

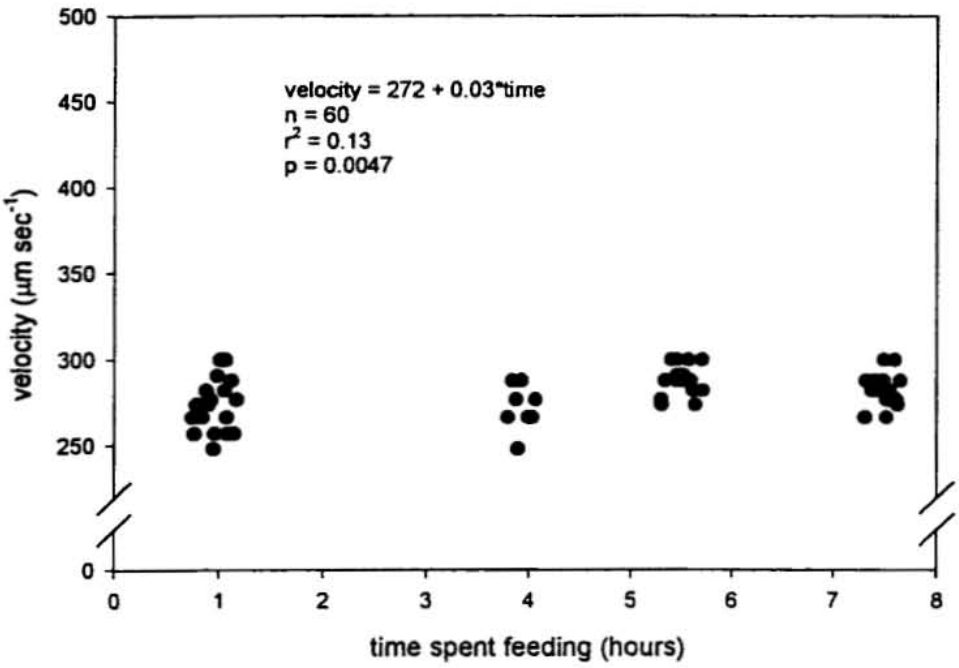

Figure 3.11. The relationship between particle velocity on the ventral groove of mussel #30 and time spent feeding in flow-through experiment #39. The particle concentration during this experiment was 12 ± 1.8 SD particles μl^{-1}, and the mean clearance rate was 77 ± 35 SD ml min^{-1}. The velocity increases significantly over time (regression analysis).

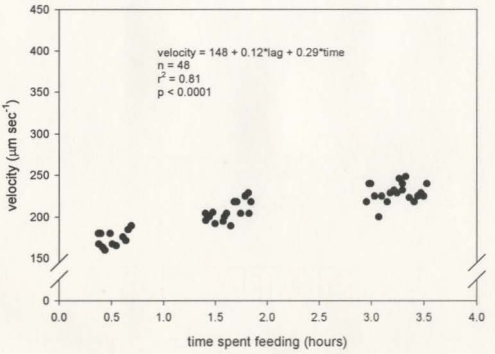

Figure 3.12. The relationship between particle velocity on the ventral groove of mussel #33 and time spent feeding in flow-through experiment #49. The particle concentration was 4.5 ± 0.3 SD particles μl^{-1}, and the mean clearance rate was 60 ± 36 SD ml min^{-1}. The velocity increases significantly over time (regression analysis with 'lag' term included).

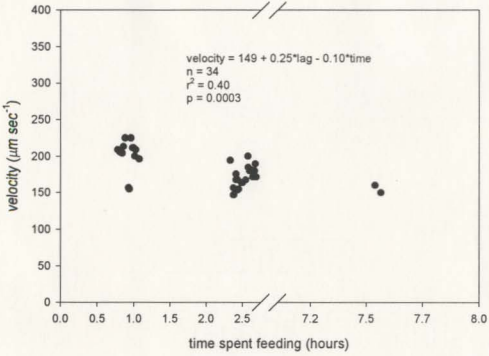

Figure 3.13. The relationship between particle velocity on the ventral groove of mussel #32 and time spent feeding in flow-through experiment #38. The particle concentration was 119 ± 11.8 SD particles μl^{-1}, and the mean clearance rate was 68 ± 19 SD ml min^{-1}. The velocity decreases significantly over time (regression analysis with 'lag' term included).

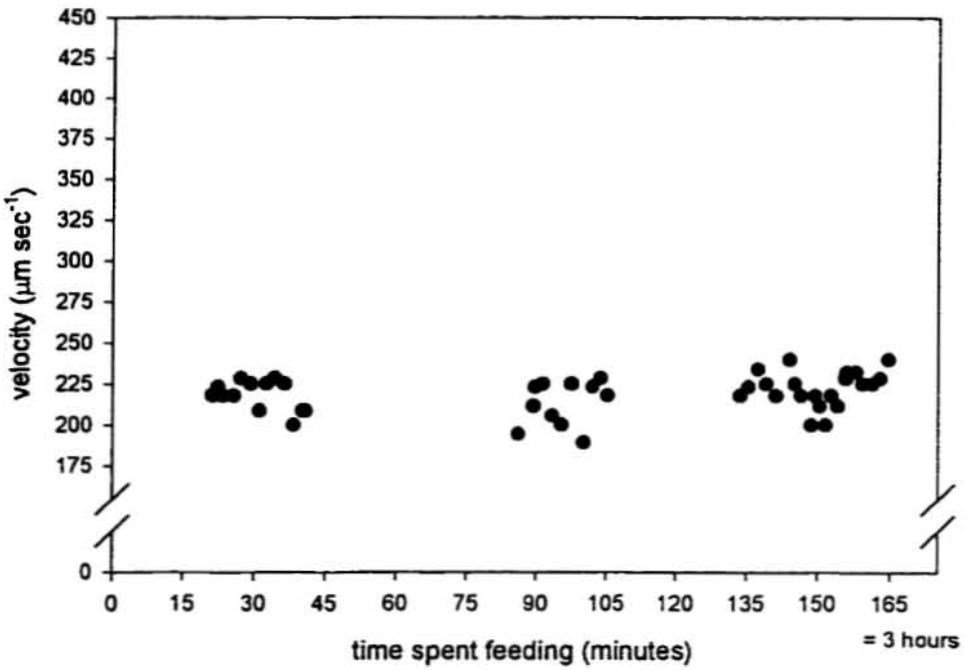

Figure 3.14. The relationship between particle velocity on the ventral groove of mussel #33 and time spent feeding in flow-through experiment #50. The particle concentration was 27 ± 1.6 SD particles μl^{-1}, and the mean clearance rate was 54 ± 16 SD ml min^{-1}. The velocity does not vary significantly over time (regression analysis with 'lag' term added, n = 45, p= 0.13).

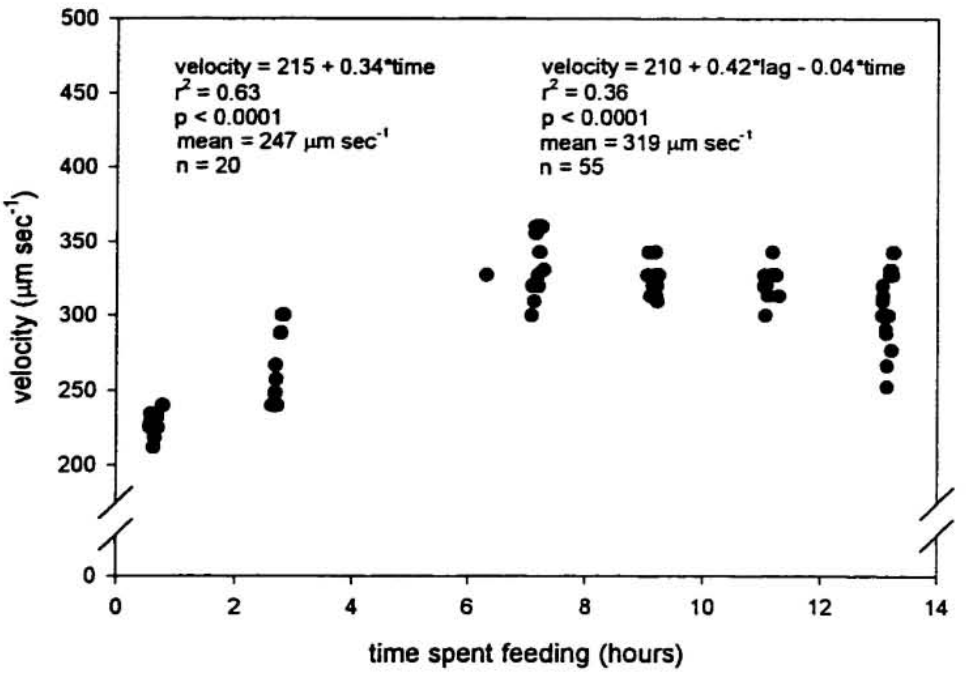

Figure 3.15. The relationship between particle velocity on the ventral groove of mussel #30 and time spent feeding in flow-through experiment #42. The particle concentration during the entire experiment was 122 ± 6.44 SD particles μl^{-1}, and the mean clearance rate was 51 ml min^{-1} ± 12 SD for the first 6 hours and 34 ml min^{-1} ± 9.9 SD for 6 to 14 hours spent feeding. The mean velocity in the first part of the experiment varies significantly from that of the second ($p = 0.0002$), and the regression slopes in each section are significantly different from one another, as shown by the significant p value ($p < 0.0001$) for the interaction term (ANCOVA with 'lag' term included to eliminate autocorrelation of errors, $n = 75$, $r^2 = 0.83$).

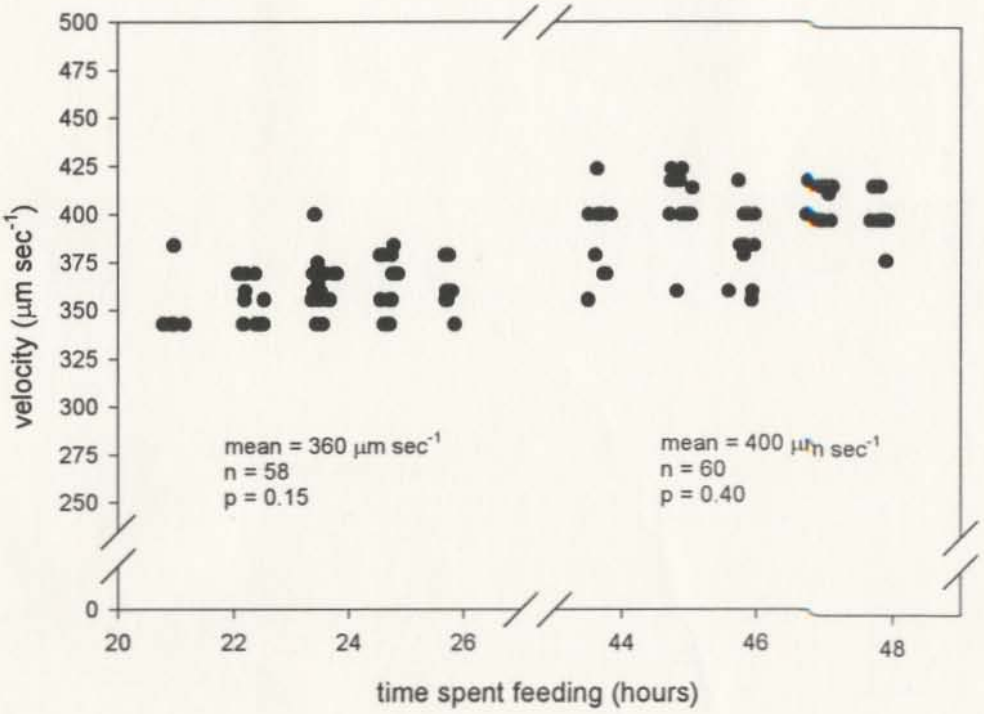

Figure 3.16. The relationship between particle velocity on the ventral groove of mussel #24 and time spent feeding in flow-through experiment #40. The particle concentration was 43 ± 4.9 SD particles μl^{-1} during the first half of the experiment and 35 ± 3.7 SD particles μl^{-1} for the second half, and the mean clearance rate was 123 ± 20.3 SD ml min^{-1} and 148 ± 30.8 SD ml min^{-1} for the first and second halves, respectively. Velocity does not vary over time in each four hour section (regression analysis, p values given in figure), but the mean velocity in the first half of the experiment varies significantly from that of the second half (One-way ANOVA, n = 119, $r^2 = 0.59$, $p < 0.0001$).

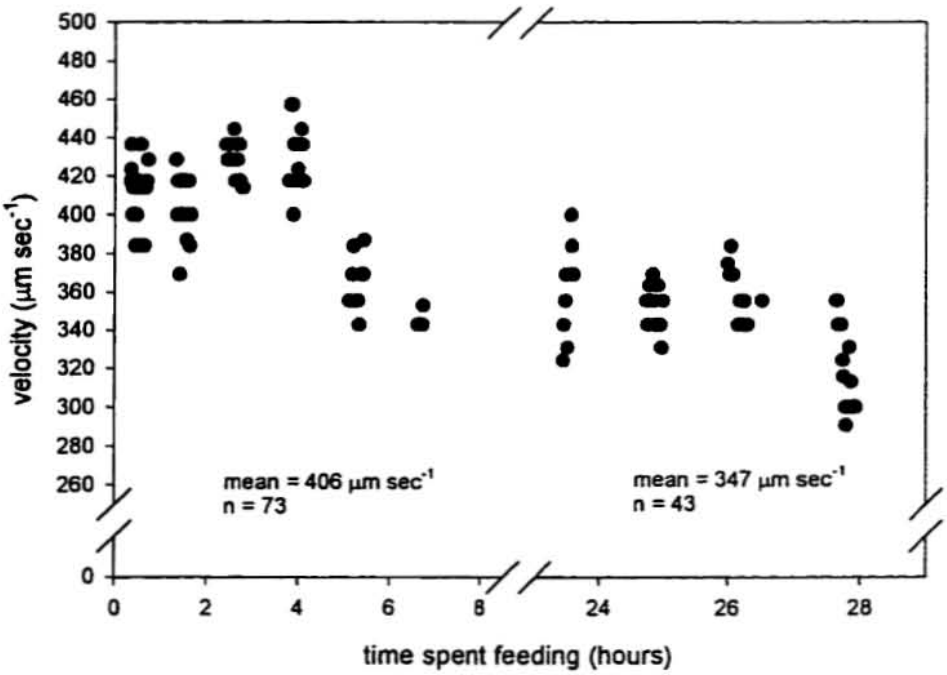

Figure 3.17. The relationship between particle velocity on the ventral groove of mussel #9 and time spent feeding in flow-through experiment #41. The particle concentration was 35 ± 3.2 SD particles μl^{-1} during the first half of the experiment and 28 ± 3.2 SD particles μl^{-1} for the second half, and the mean clearance rate was 110 ± 28 SD ml min^{-1} and 55 ± 16 SD ml min^{-1} for the first and second halves, respectively. The mean velocity in the first half of the experiment varies significantly from that of the second half (Two-way ANOVA with 'lag' term included, n = 116, $r^2 = 0.74$, $p < 0.0001$).

Velocity increased significantly over 5.8 hours in experiment 36 (Fig. 3.9), over 5.8 hours in experiment 37 (Fig. 3.10), over 8 hours in experiment 39 (Fig. 3.11), and over 4 hours in experiment 49 (Fig. 3.12). In contrast, velocity decreased significantly over 8 hours in experiment 38 (Fig. 3.13) and did not vary significantly over 3 hours in experiment 50 (Fig. 3.14). In experiment 42 (Fig. 3.15), velocity increased significantly for the first 6 hours and then decreased slightly over the next 8 hours. Experiments 40 and 41 involved videotaping a mussel for periods of 28 to 48 hours after time zero. Experiment 40 showed a rise in particle velocity over a long time period (Fig. 3.16), while experiment 41 displayed a decrease in particle velocity over a long time period (Fig. 3.17).

3.3.6 Particle quality experiments

Figures 3.18 and 3.19 depict the response of particle velocity in the ventral groove when particle type was changed from algal cells to inorganic sediment (see Table 2.3 for experimental details). Particle velocities in the presence of sediment were lower than those for algae in both experiments. For experiment 46 (Fig. 3.18), an ANCOVA with the term 'lag' included ($n = 107$, $r^2 = 0.59$) indicated that the mean velocities in each of the two sections differed significantly from one another ($p < 0.0001$ for particle type term), and that the slopes differed significantly from one another ($p < 0.0001$ for interaction term). In this experiment, particle velocity decreased significantly over time in the inorganic sediment suspension but remained constant in the algal suspension (see Fig. 3.18 for regression statistics), indicating that the mussel was feeding at a steady state before the particle quality manipulation was executed.

Since the data in experiment 48 resulted in an irregular plot, a different model was used to show that the velocities were significantly affected by particle type (two-way ANOVA with 'lag' term included, $n = 58$, $r^2 = 0.70$, $p = 0.05$ for particle type term). More interesting is the rapid drop in particle velocity after the addition of inorganic sediment to the suspension, and then the appearance of a slow recovery curve to reestablish steady state at a level slightly below that achieved in the algae section (Fig. 3.19).

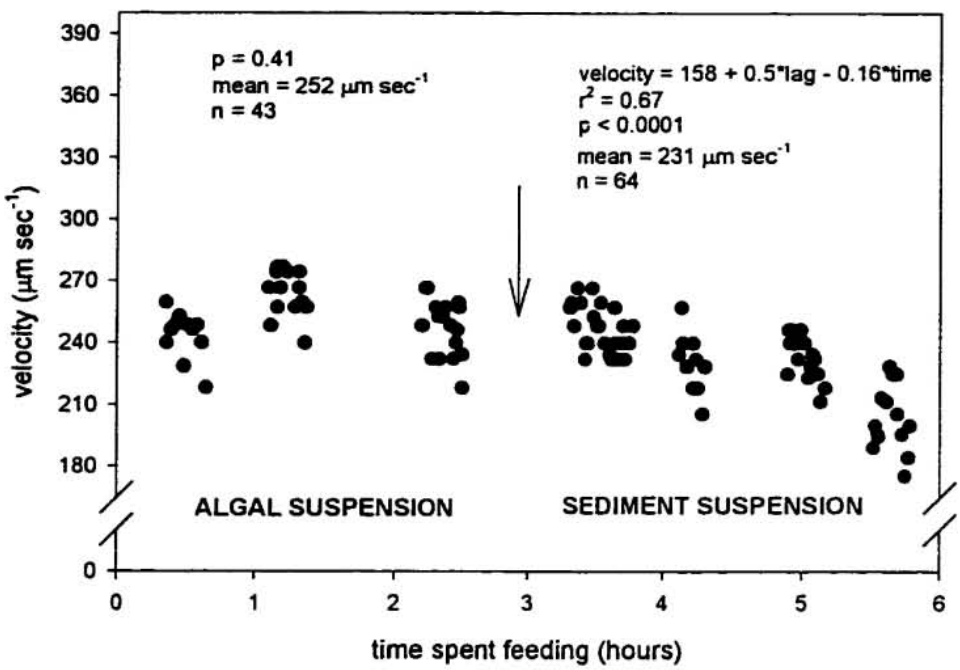

Figure 3.18. The relationship between particle velocity on the ventral groove of mussel #33 and time spent feeding in the algae to inorganic sediment experiment #46. The particle concentration was 7.1 ± 0.85 SD particles μl^{-1}, and the mean clearance rate was 64 ± 31 SD ml min^{-1}. The mussel was feeding on an algal suspension before the time indicated by the arrow and on an inorganic sediment suspension afterwards. Velocities on the left side of the arrow do not change over time (regression analysis with 'lag' term included, p = 0.41), whereas velocities on the right side vary significantly over time (regression analysis with 'lag' term included).

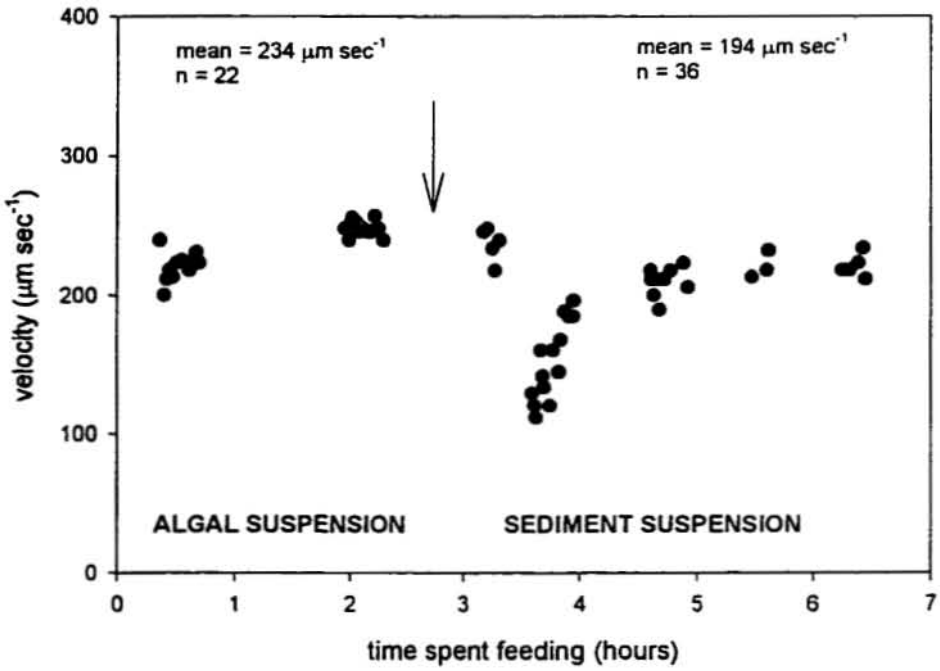

Figure 3.19. The relationship between particle velocity on the ventral groove of mussel #30 and time spent feeding in the algae to inorganic sediment experiment #48. The particle concentration was 8.4 ± 0.74 SD particles μl^{-1}, and the mean clearance rate was 151 ± 50.8 SD ml min^{-1} in the algal suspension section of the experiment and 113 ± 27.3 SD ml min^{-1} in the sediment suspension section.

3.3.7 Acute responses to temperature changes

Figs. 3.20 and 3.21 show the results of the two experiments in which the temperature was suddenly increased while a mussel was feeding (see Table 2.4 for experimental details). Particle transport velocities derived in the 4°C seawater were significantly lower than those in the 15°C seawater (ANCOVA with 'lag' term included: Experiment 43: $n=96$, $r^2=0.89$, $p<0.0001$ for temperature term, Experiment 44: $n = 61$, $r^2=0.94$, $p<0.0001$ for temperature term), and the acute Q_{10} for both experiments was 1.7. The regression slopes in each temperature section also differed significantly from one another in both experiments ($p = 0.0033$ for interaction term in experiment 43, and $p < 0.0001$ for interaction term in experiment 44).

In experiment 43, particle velocities changed significantly over time for both temperatures (see Fig. 3.20 for regression statistics), but velocity changed with time only in the higher temperature in experiment 44 (Fig. 3.21). In both experiments, the variation in particle velocities was much greater at the higher temperature (coefficients of variation 4.7 - 8.3% at 4°C and 10.1 - 12.6% at 15°C).

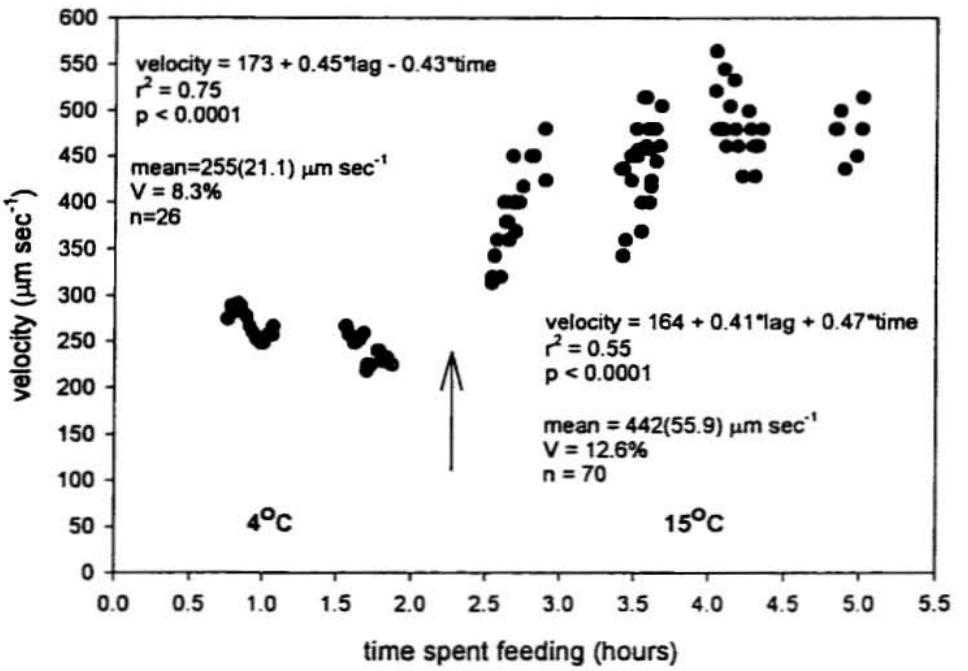

Figure 3.20. The relationship between particle velocity on the ventral groove of mussel #30 and time spent feeding in temperature experiment #43. The particle concentration was 29.8 ± 3.84 SD particles μl^{-1}, and the mean clearance rate was 112 ± 41.3 SD ml min^{-1} for the first two hours of the experiment in 4°C seawater (previous to arrow) and 34 ± 22 SD ml min^{-1} in 15°C seawater (after arrow). Velocities change significantly over time in each temperature (regression analysis with 'lag' term included). The mean, standard deviation, coefficient of variation (V) and sample number for each temperature section are given.

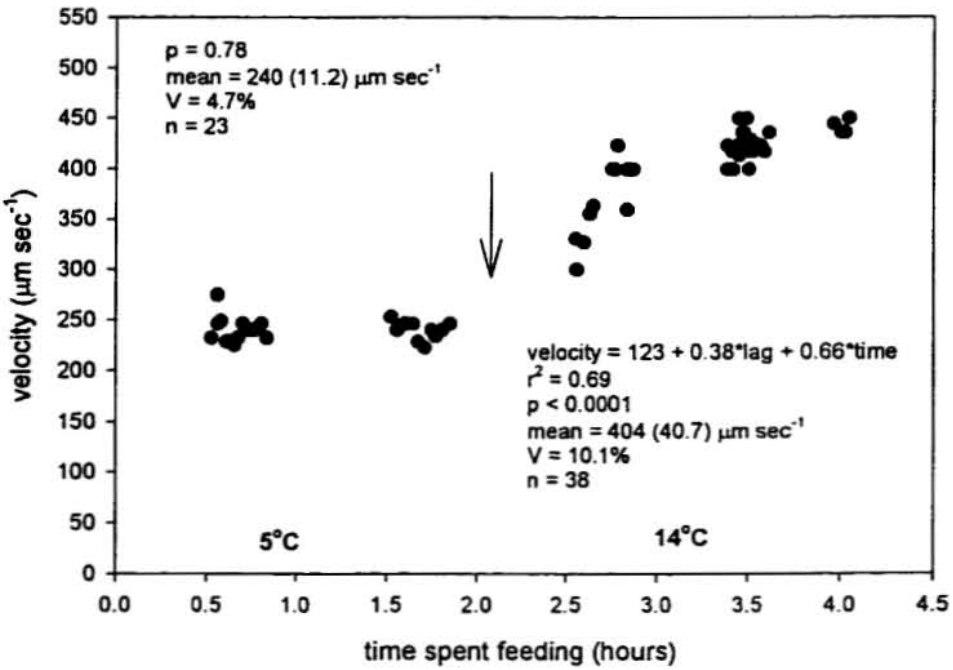

Figure 3.21. The relationship between particle velocity on the ventral groove of mussel #32 and time spent feeding in temperature experiment #44. The particle concentration was 12 ± 0.77 SD particles μl^{-1}, and the mean clearance rate was 147 ± 30.5 SD ml min^{-1} for the section previous to the arrow (@ 5°C) and 134 ± 41.4 SD ml min^{-1} after the arrow (@14°C). Velocities vary significantly over time in 14°C but not in 5°C (regression analysis including 'lag' term in the14°C equation). The mean, standard deviation, coefficient of variation (V) and sample number for each temperature section are given.

CHAPTER 4 - DISCUSSION

4.1 Gill filament measurements

Filament measurements of *Mytilus edulis* available in the literature were all made on flat sections of the gill, where the filaments are narrow and inter-filamental distances are substantial (e.g. Dral, 1967; Jones et al., 1992); therefore, direct comparisons of filament widths at the ventral groove with published values are not possible. Jones et al. (1992) found that filament widths and inter-filamental distances increased with shell length for mussels ranging from 10 to 80 mm in length. Because the filaments in *M. edulis* widen at the ventral margins so that the inter-filamental distance is zero in an undisturbed animal, I used the regression equations in Jones et al. (1992) to calculate the filament widths and inter-filamental distances of the mussels used in the present study (shell length 60 to 83 mm). Theoretically, the sum of the filament width and the inter-filamental distance on the frontal surface of the gill is equal to the width of the filaments at the ventral groove. This calculated range of filament widths at the ventral groove, 72 to 83 μm, is within the range of the means determined by image analysis (78.3 and 83.8 μm) in this study, but is lower than the mean obtained by measurements under the stereoscope (92.9μm) (Table 3.1).

The difference between measurements derived from the two methods in the present study may be attributable to several causes. First, filaments of freshly killed mussels may have relaxed slightly, thus appearing larger than when intact. Alternatively, the widths of filaments in live mussels may have been consistently underestimated due to the relatively low resolution of the computer system used to perform the image analyses. This possibility is supported by the 7% underestimation factor calculated from the remeasured calibration particles. Finally, variation among individuals and populations is also an important factor when considering morphological measurements (Jones et al., 1992).

4.2 Mucous strand size

With a seven-fold increase in particle load on the gill, the strand size changed from strand width category 1 to 3 (Figs. 2.4, 2.5, 2.6 & 3.2) rather than remaining the same width at all loads by becoming more densely packaged with increasingly cohesive mucus. Strand size category 4, which represents mucous strands that are extending outside the ventral groove, is a surprising exception to this trend since one would expect extended mucous strands at the highest particle loads. Foster-Smith (1978) also noted that mucous strings in the ventral groove became thicker and less compact as the amount of material collected by the gill increased. He also observed that the thickness of the mucous string was what determined the position along the ventral groove that each string was transferred to the palps (i.e. small mucous strings were transferred to the palps in a more anterior position on the ventral groove than large strings), as well as the path of the string once it arrived at the palps. New evidence suggests that it is the positions of the dorsal folds of the labial palps, which in turn depend on the mussel's ingestive capacity, that determine the destination of each string (Beninger & St. Jean, 1997). Tankersley (1996) observed that mucous strings increased in diameter as they moved anteriorly and approached the labial palps, but this phenomenon would not be observed in this study since all measurements were taken at approximately the same location on the gill.

As the mucous strand increases in size, the traveling velocity along the ventral groove also decreases (Fig. 3.1). Since increased strand sized is correlated with higher particle loads, possible reasons for this general trend are discussed in section 4.3.

4.3 Particle concentration, particle load and clearance rate

There have been numerous studies of the feeding physiology of suspension feeding bivalves in response to varying particle concentrations (e.g. Foster-Smith, 1975a; Schulte, 1975; Theisen, 1977; Widdows et al., 1979; Kiørboe et al., 1980; Bricelj & Malouf, 1984; Smaal et al., 1986; Sprung & Rose, 1988; Bayne et al., 1989; Riisgård, 1991; Iglesias et al., 1992; Navarro et al., 1992; Bayne et al., 1993). One relatively consistent finding is

that clearance rates of suspension feeding bivalves decrease with increased particle concentrations above a certain range, a trend that was also found within a concentration range of 6 to 188 cells μl^{-1} in the present study (Fig. 3.6). This behaviour can allow for regulation of food intake by maintenance of a constant particle clearance at high particle concentrations (Winter, 1973; Navarro & Winter, 1982). In addition to regulating ingestion, the reduction in clearance rate may prevent overloading of the gill, or it may be a response to 'suboptimal conditions' (Smaal et al., 1986; Navarro et al., 1992). It is unclear how bivalves detect the ambient particle concentration, but it may be a tactile response to particle load on the gills or a distance response via chemoreception (Ward et al., 1992).

Increased pseudofeces production was also observed at higher particle concentrations, a process which also serves as a regulatory mechanism in addition to, or instead of, decreasing clearance rates (Bricelj & Malouf, 1984; Navarro et al., 1992). Because pseudofeces production is often associated with selection for more nutritional particles, mussels fed with unialgal diets, where selection is irrelevant, would theoretically conserve more energy by decreasing clearance rates rather than increasing pseudofeces production. On the other hand, mussels exposed to a natural seston diet would benefit more from increasing pseudofeces production, which may result in ingested material that is organically enriched. The rates and amounts of pseudofeces production were not determined in the current study, but the observed ability of *M. edulis* to reduce clearance rate and/or produce pseudofeces in response to increased particle concentrations reflects two adaptations to deal with the turbid environment in which it often lives (Bricelj & Malouf, 1984).

Particle velocities in the ventral groove were found to decrease by ~50% in response to a fifteenfold increase in ambient particle concentration at 14°C, and velocities decreased by ~40% with an elevenfold increase in concentration at 4.5°C (Fig. 3.4). Particle velocity measurements fall within the ranges published for mucociliary areas of the pallial system (Table 4.1). The most common purpose for estimating transport rates on

Table 4.1. Some examples of particle velocities on the frontal surface and ventral groove of bivalves in different studies. Starred reference involved invasive techniques to obtain measurements.

Velocity ($\mu m\ sec^{-1}$) ± SD	Particle concentration (particles μl^{-1})	Temp (°C)	Area of gill	Species	Reference
210 - 600	not provided	5 - 20	ventral groove	*Mytilus edulis*	Jørgensen, 1975 *
399 ± 41	7.6	12 - 15	ventral groove	*Mytilus edulis*	Ward et al., 1991
421 ± 22	10	12 - 15	ventral groove	*Mytilus edulis*	Ward et al., 1991
215 ± 20	5 - 7	4 - 5	ventral groove	*Mytilus edulis*	Ward et al., 1993
224 ± 11	11 - 13	4 - 5	ventral groove	*Mytilus edulis*	Ward et al., 1993
292 ± 56	22 - 24	4 - 5	ventral groove	*Mytilus edulis*	Ward et al., 1993
50 - 310	6 - 115	4.5	ventral groove	*Mytilus edulis*	present study
60 - 570	8 - 170	14	ventral groove	*Mytilus edulis*	present study
320 ± 65	6.5	12 - 15	frontal surface	*Mytilus edulis*	Ward et al., 1991
380 ± 95	10 - 20	12	ventral margin	*Placopecten magellanicus*	Beninger et al., 1992
185 ± 151	> 20	12	ventral margin	*Placopecten magellanicus*	Beninger et al., 1992
115 ± 13	5 - 7	4 - 5	ventral groove	*Mya arenaria*	Ward et al., 1993
137 ± 32	11 - 13	4 - 5	ventral groove	*Mya arenaria*	Ward et al., 1993
123 ± 9	22 - 24	4 - 5	ventral groove	*Mya arenaria*	Ward et al., 1993
245 - 567	23	9 - 18	frontal surface	*Pyganodon cataracta*	Tankersley, 1996

bivalve gills in previous studies was to determine whether mucociliary or hydrodynamic processes were involved in particle transport. For this reason, these authors used very small particle concentration ranges; therefore no conclusions regarding the effects of particle concentration on particle velocity were drawn from these studies. Species such as *Placopecten magellanicus* utilize hydrodynamic processes in the dorsal tracts to transport particles in addition to mucociliary processes within the ventral tracts, and particle velocities involved in the former are generally much higher (413 - 8,115 $\mu m\ sec^{-1}$) than those in ventral grooves or tracts (Ward et al., 1993, 1994; Table 4.1). Determination of transport rates in this context has provided researchers with a tool to increase understanding of the function of different areas of the pallial system.

Increased particle loads resulted in decreased velocities in the ventral grooves of *M. edulis* (Fig. 3.3). Since increased particle concentrations resulted in increased particle loads in the present study, possible ciliary level responses to both of these conditions are discussed in conjunction with one another. The load effect on velocity was not as pronounced (coefficient of determination 20%) as the particle concentration effect (coefficients of determination 31% and 49%), resulting in the elimination of the variable 'load' from the final GLM (Equation 3.2) when only those experiments in which animals tested at both temperature groups were included. Since particle load is the product of clearance rate and particle concentration, clearance rate effects may mask the effects of load on particle velocity. The absence of a clearance rate effect on particle velocity may be due to the similar effects of particle concentration on clearance rate and particle velocity. Since clearance rate and particle velocity both decrease in response to increased particle concentration (see Figs. 3.4 and 3.6), any clearance rate effect could easily be masked.

The decrease in particle velocity at increased particle concentrations and loads may be a result of a feedback mechanism originating at the labial palps or the gut in order to regulate ingestion. In conjunction with decreased pumping rates due to decreased activity of lateral and/or abfrontal cilia, decreased transport rates on the ventral groove would

decrease the input rate of food to the labial palps and gut, therefore allowing more time for processing in these areas. Unlike the scallop and oyster, the blue mussel does not redirect food particles to other tracts of the gill as a means of transporting food at different velocities (Ward et al. 1993). For example, Ward et al. (1994) suggested that oysters may handle gill overloading by transporting more particles in the ventral tracts, where transport is slower than in dorsal tracts. By directing food to the slower tracts, oysters are slowing down the transfer of particles to the next feeding compartment and preventing overloading. *Mytilus edulis* does not utilize the dorsal tracts, even at high particle concentrations (Ward et al., 1993), but a decrease of particle velocity in the ventral groove may serve a similar purpose of slowing down particle delivery to the palps and mouth.

Alternatively, the decrease in particle velocity due to increased particle concentration and load may be a result of the groove cilia lacking the ability to compensate for the increased volume of cohesive mucus needed to transport the higher particle load. Abfrontal cilia (Stommel & Stevens, 1988) and lateral cilia (Murakami & Machemer, 1982) in *Mytilus* can be activated by Ca-dependent mechanical stimuli. The presence of mucus acts as a mechanical stimulator of ciliary beating and can initiate activity as well as accelerate it (Sleigh et al., 1988). Theoretically, an increased particle load would lead to increased resistance to movement by cilia. To compensate for this increased resistance, an individual cilium can increase its force and frequency of beat, and other cilia can also be recruited to increase the propulsive force. Since the number of cilia in motion at one time depends on the force needed to move the mucus, as the required force increases with load, more cilia commence beating to add enough propulsive effort to move the mucus (Sleigh, 1982). If the load is increased beyond the point at which all cilia have been recruited and each cilium is exerting the maximum force possible (depending on the amount of ATP available), the increasing load may result in backward-bending forces on cilia during their effective stroke. The greater the backward bend in a cilium the lower

its efficiency of propulsion due to its distorted angle (Sleigh, 1982), as may be the case when a high particle load in the ventral groove results in reduced mucus velocity.

Other behavioural adaptations by the mussel gill to regulate ingestion in the presence of high particle concentrations have previously been observed, such as the detachment of the ventral margins from the mantle to create a shunt for particles to pass through unfiltered, and the ability of the ventral groove to open and close (Dral, 1968). These responses, together with the ability to alter particle transport rates in the ventral groove, suggest the presence of neuronally controlled feedback mechanisms.

4.4 Temperature effects

Many researchers have studied the effects of temperature on the feeding physiology of suspension feeders (e.g. Schulte, 1975; Jørgensen et al., 1990; Loo, 1992; van Erkom Schurink & Griffiths, 1992; Podolsky, 1994), and it is well known that the physiological activity of bivalves is closely related to temperature. *Mytilus edulis* has the ability to tolerate a large range in ambient temperature and has been known to survive temperatures from –9 to 27°C (Newell, 1989). In the present study, particle velocities in the ventral groove increased abruptly when the ambient seawater temperature of mussels acclimated to 4.5°C was increased to 14°C (Fig. 3.4). Mussels acclimated to 14°C also showed higher particle velocities than mussels acclimated to 4.5 °C. The Q_{10} of 2 derived in these temperature experiments corresponds well with literature values regarding feeding physiology of mussels (Catapane et al., 1981; Jørgensen et al., 1990; Jørgensen & Ockelmann, 1991). Clearance rates of acclimated mussels were also generally higher in the warm temperature group (Fig. 3.5); acute temperature effects on clearance rates were not tested. Mussels are known to increase their clearance rates in response to an acute increase in temperature between 5 and 20 °C and vice versa (Walne, 1972; Widdows, 1976). Since the lateral and abfrontal ciliary systems responsible for water pumping, and thus clearance rate, respond in this manner to a change in temperature, it is not surprising that ventral groove cilia would have a similar response to temperature changes, which directly affect cell metabolism.

Although most of the literature concerning temperature effects on suspension feeders focuses on filtration, respiration, ingestion and absorption rates, a few scientists have attempted to examine particle velocities or ciliary beat frequencies on the gill surface (e.g. Aiello, 1960; Jørgensen, 1975; Stefano et al., 1977; Jørgensen & Ockelmann, 1991). For example, Jørgensen (1975) used an eyepiece micrometer to measure particle velocities along the demibranchs of *M. edulis* that were anaesthetized and had severed adductor muscles. He observed an increase in the rate of transport of small particles when the temperature was increased from 5°C to 20°C in the presence of a stimulatory neurotransmitter. Similarly, Stefano et al. (1977) discovered a linear relationship between temperature and lateral ciliary beating rates (beats min^{-1}), although they provided no regression equation for comparison. These authors concluded that *Mytilus edulis* possesses a neuronal mechanism sensitive to temperature which alters levels of serotonin, a neurotransmitter which in turn affects ciliary beating rates (Prins et al., 1991). Other researchers have found that frontal cilia are also directly influenced by temperature (Hirasaka et al., 1957; Hoshi & Hoshiyama, 1963), but since these data were collected using excised gill pieces they do not represent the behaviour of fully innervated gill tissue.

In addition to its physiological effects, temperature can also affect ciliary transport mechanically by influencing the viscosity of mucus (Machemer, 1972; Podolsky, 1994). In his study to determine the degree to which each process (physiological or mechanical) in suspension feeding echinoderm larvae was affected by temperature changes, Podolsky (1994) found that a change in viscosity alone accounted for at least half of the decrease in feeding rates at a low temperature. Ciliary beat frequency, and therefore transport rate, decreases with increased mucus viscosity (Machemer, 1972; Winet & Blake, 1980; Winet et al., 1982), probably due to reduced velocity in the effective stroke or reduced penetration of the tips of cilia into the viscous mucous layer (Sleigh et al., 1988). The influence of viscosity on cell membrane properties via changes in internal concentrations of divalent cations may also affect ciliary activity (Machemer, 1972). In addition,

metachronal coordination has been shown to change with increased viscosity (Machemer, 1972).

Jørgensen et al. (1990) found a positive relationship between pumping rates of *Mytilus edulis* and temperature which was linearly correlated with the temperature-determined decrease in water viscosity, suggesting that viscosity is the sole effector of velocity changes in the lateral cilia. Willows (1992) opposed this conclusion and stated that physiological traits such as clearance rate show an acute response to temperature changes based on the temperature dependence of metabolism. The observed influence of temperature on particle transport rates in the ventral groove probably results from a combination of the two processes.

4.5 Time effects

Many of the long-term experiments showed significant changes of particle velocity over time, but not in a consistent pattern. Most of these changes occurred after 120 minutes spent feeding in any given experiment, indicating that ventral groove cilia tend to alter their activity gradually over time without requiring the presence of any known stimulus. The results of the particle quality and non-acclimated temperature experiments demonstrate that the cilia have the ability to alter their activity very abruptly. In addition, feeding history does not appear to affect the changes in particle velocity over time.

Time effects are known to occur in other aspects of suspension feeding physiology. Schulte (1975) observed that clearance rates in *Mytilus edulis* decreased during the duration of his particle concentration experiments, with variations in filtration activity being more extreme at lower concentrations than at higher concentrations. In contrast, Riisgård (1991) found decreases in clearance rate of *M. edulis* over 8 hour periods at high particle concentrations (15 particles μl^{-1}) but not at lower concentrations (2-6 particles μl^{-1}). Although Beninger et al. (1992) reported no velocity changes on the scallop gill over time at a particular particle concentration, they did note that at higher concentrations (20-32 particles ul^{-1}) particle movement and gill behaviour were similar to those described for individuals at medium concentrations (10-20 particles ul^{-1}) for long periods of time.

4.6 Particle type

Changing particles from algae to inorganic sediment has a direct effect on ventral groove ciliary activity. The two experiments involving changes in particle type from algae to inorganic sediment both showed decreases in particle velocity upon the addition of sediment, although one mussel showed a much more abrupt response than the other (Figs. 3.19 & 3.20), indicating that there can be individual variations for each response. The decrease in velocity upon the addition of inorganic sediment to the suspension may be due to the increase in density of the sediment particles effecting a greater weight on the ventral groove. A similar explanation has been postulated regarding particle selection on the gills of suspension feeders, but has been argued against since such a density-dependent mechanism must operate on one plane and results in downward forces on particles, whereas bivalves are not consistently oriented with respect to gravity in nature (Newell & Jordan, 1983). Furthermore, since the pallial system operates at very low Reynolds numbers, gravitational forces are assumed to be insignificant, therefore it is unlikely that the specific gravity of particles is an important factor in controlling particle selection (Jørgensen, 1989). If the decrease in velocity within the ventral groove is the result of a ciliary level response, it is probably due to the increased mass of the sediment particles. Even when clearance rates remain the same upon switching particle types, the increased mass of sediment compared with algal cells would result in increased backward forces, and more effort via ciliary action would be required to maintain forward movement of the mucous strand.

Alternatively, the decrease in particle velocity as a result of the addition of sediment may be a response initiated by sensory cells in the gill, perhaps sensory cells similar to those responsible for selecting particles on the labial palps. It is known that mussels are able to regulate ingestion by selecting among particles of different quality and varying their rates of feeding in response to changing composition of seston (Shumway et al., 1985; Bayne et al., 1989; Navarro & Iglesias, 1993). The efficiency of selection and the proportion of material rejected as pseudofeces both depend on the composition and quantity of the seston (Bayne et al., 1993). Although it is generally agreed that most

particle selection occurs at the labial palps, there is evidence supporting particle selection at the gills in some bivalve species (Beninger & St. Jean, 1997; Ward et al., 1997). Although absolute evidence for the presence of sensory cells is still needed, Ward &Targett (1989) postulated that particles may be selected for on the gill due to their shape, electrical charge, or chemical properties.

Finally, decreased velocity due to the influx of inorganic sediment particles may involve a feedback response originating from the labial palps or gut. Since mussels seem to require 2-12 days for the digestive enzymes to respond to a change in diet (Bayne et al., 1993), altering particle transport on the gill may serve as one of many possible short-term responses (in addition to altering clearance rates and/or rates of pseudofeces production) to an abrupt change in diet.

To my knowledge, no other researchers have attempted to discover whether different particle types are transported along one part of an intact gill surface at different velocities. Stevens (1987) used excised gill pieces from scallops to test the effects of silt on gill crawling speed and found that increased silt concentrations, as well as decreased particle sizes (< 10 μm), resulted in a decreased gill piece velocity. This finding can not be generalized to suspension feeding behaviour since it is highly unlikely that enervated gill tissue responds in the same manner as intact and fully innervated tissue.

Particle quality or type has been shown to affect other aspects of feeding physiology in bivalves (e.g. Kiørboe et al., 1980; Bricelj et al., 1984; Newell et al., 1989; Iglesias et al., 1992; Bayne et al., 1993). Winter (1975) and Kiørboe et al. (1981) observed that the feeding activity of *M. edulis* was stimulated by the addition of low concentrations of silt to an ambient algal suspension, whereas the opposite effect was found in oysters (Loosanoff & Engle, 1947). Increasing additions of clay to suspension resulted in decreased retention efficiency, filtration rate and ingestion rate in scallops, although the same effects were not produced with similar concentrations of algae (Cranford & Gordon, 1992), therefore inorganic particles appear to influence feeding mechanisms and ingestion selectivity in some suspension feeders.

4.7 Summary

1 - Velocities of food particles entrained in mucus within the ventral groove of *Mytilus edulis* decreased in response to increased concentrations of *Isochrysis* sp..

2- Switching the ambient algal suspension to inorganic sediment resulted in a decrease in mucous strand velocity in the ventral groove.

3- An increase in ambient temperature from 4.5°C to 14°C resulted in a rapid increase in mucous strand velocity.

4- Mucus velocity in the ventral groove changed over time, but no consistent pattern was observed.

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