

# **Shellfish Culture and Particulate Matter Production and Cycling: Laboratory Calibrations Report**

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## Abstract

Clearance rate (CR,  $\text{l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ ) and biodeposit production rate (BPR,  $\text{mg}\cdot\text{gdrywt}^{-1}\cdot\text{d}^{-1}$ ) were measured for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* in a series of flow-through laboratory experiments. To determine the potential effect of diet concentration on CR and BPR for each species, experimental trials were run at three different algal cell concentrations representing the range of particle concentrations measured *in situ* at bivalve farm sites in BC. Relationships between CR and endogenous factors (shell length, tissue dry weight, gender, gonad development) and between CR and algal cell concentration were investigated for the 3 species using statistical analyses. Tissue dry weight was negatively correlated with CR for all 3 species and proved to be a significant factor influencing CR. For all 3 species, CR was significantly higher in trials using medium algal cell concentrations (19,000 to 30,500  $\text{cells}\cdot\text{ml}^{-1}$ ) than at high algal cell concentrations (35,000 to 46,500  $\text{cells}\cdot\text{ml}^{-1}$ ), although results for *M. edulis* and *M. galloprovincialis* from trials using high algal cell concentrations may have been confounded by a size bias (random use of larger specimens) in the high concentration trials. Tissue dry weight of *C. gigas* was negatively correlated with BPR and accounted for 25% of the variance in BPR. No comparable, statistically significant linear relationship was found for BPR and size of either *M. edulis* or *M. galloprovincialis*. The range of algal cell concentrations used in this study did not significantly affect BPR in the 3 bivalves. All results, including inter-specific comparisons of CR and BPR, are discussed in light of the effects of endogenous and exogenous factors on CR and BPR reported in the literature, particularly the effects of food quality and quantity in field versus laboratory studies. Sinking rates were measured for biodeposits collected from *C. gigas*, *M. edulis* and *M. galloprovincialis* fed an algae diet in the laboratory. No visible pseudofeces were produced by any of the species and no significant difference was found in the sinking rates of biodeposits of the 3 species. The effects of width and density of biodeposits on sinking rate, and subsequent dispersion potential, are discussed.



## Introduction

Enhanced biodeposition under bivalve culture is well documented (Dählback and Gunnarsson, 1981; Tenore *et al.*, 1982; Jaramillo *et al.*, 1992; Hatcher *et al.*, 1994) but few studies have attempted to relate enhanced biodeposition to bivalve feeding physiology. For example, although the deposition of feces and pseudofeces, collectively termed biodeposits, may play an important role in pelagic-benthic coupling, few studies have paid attention to the dynamics of biodeposition. In particular, little is known about biodeposit quality (Navarro and Thompson, 1997), biodeposit production rates (Kautsky and Evans, 1987), and dispersion potential (Miller *et al.*, 2002; Giles and Pilditch, 2004; Harstein and Stevens, 2005). Research that focuses on the production rates of bivalve biodeposits is relatively uncommon for many cultured bivalve species and yet, the need for additional research in this area, particularly in light of the recent interest in depositional models (Chamberlain *et al.*, 2006), is clear.

Endogenous factors suggested to affect biodeposit production in filter feeding bivalves include size, age and spawning (Haven and Morales-Alamo, 1966; Tsuchiya, 1980; Callier *et al.*, 2006). Exogenous factors with potential to affect biodeposit production rates include quality and quantity of diet (Widdows *et al.*, 1979; Robinson and Langton 1980; Iglesias *et al.*, 1992; Hawkins *et al.*, 1998; Urrutia *et al.*, 2001; Callier *et al.*, 2006). However, when attempting to identify endogenous and exogenous factors that affect production of bivalve biodeposits, it must be remembered that these factors are potentially influencing feeding behaviour in general. Biodeposit production rate and clearance rate, for example, may be linked in filter feeding bivalves. Clearance rates of bivalves are not constant within a species or a population and, even for an individual, are variable dependent on the balance between the same endogenous and exogenous factors that may affect biodeposit production (for review, see Barnes, 2006). Understanding how these factors may affect clearance rate is critical to understanding the role of filter feeding bivalves in coastal ecosystems from a phytoplankton (resource) depletion perspective. However, more importantly for this study, is to investigate the potential link between this information and biodeposit production rate and the usefulness of this investigation from an environmental carrying capacity perspective.

The lack of information noted earlier for biodeposit production rates is also applicable to biodeposit sinking rates and factors affecting this rate (Callier *et al.*, 2006). Because bivalve biodeposits typically differ from the naturally-occurring seston particles in aggregate particle size and shape, organic content and cohesive properties (Haven and Morales-Alamo, 1966, Kautsky and Evans, 1987; Dame, 1993, 1996), repackaging of seston into biodeposits induces a downward vertical flux of material from the water column to the seabed. It has been suggested that bivalve feces and pseudofeces settle out at rates up to 40 times that of nonaggregated particles (Kautsky and Evans, 1987; Widdows *et al.*, 1998). In order for ecosystem models to make accurate predictions of benthic loading and subsequent effects on the local



environment, a better understanding of bivalve biodeposition dynamics, particularly parameterisation of biodeposit sinking rates, quality, and erosion characteristics, is required (Henderson *et al.*, 2001). Particle sinking rate is a dominant parameter requiring characterization in all particle tracking models (Chamberlain *et al.*, 2006). Sinking rates of bivalve biodeposits are poorly estimated by simple sinking rate equations such as Stoke's law, as has been demonstrated by Chamberlain (2002) and Giles and Pilditch (2004). Hence, experiments are required to obtain estimates of the sinking rates of these particles. Unfortunately, data on sinking rates of bivalve biodeposits are relatively rare and most available data, generated only recently, are relevant to mussels (Chamberlain *et al.*, 2006).

In this study, clearance rate (CR) and biodeposition production rate (BPR) were measured for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* in laboratory experiments using a flow-through system. Bivalves were fed three different algal cell concentrations (calculated to replicate the range of particle cell concentrations recorded at field sites) and endogenous factors, including shell length, tissue dry weight, condition index, gender and gonad index were recorded. Relationships between endogenous factors or algal cell concentration and clearance rate or biodeposit production rate were examined and tested for significance using statistical analyses. For each species, the relationship between clearance rate and biodeposit production rate was explored statistically. Inter-specific comparisons of clearance rate and biodeposit production rate were also investigated and tested statistically. Sinking rates for biodeposits produced by *C. gigas*, *M. edulis* and *M. galloprovincialis*, maintained in the laboratory and fed an algae diet, were measured. Sinking rates were compared for the three species and also related to biodeposit size.



## Methods

### Clearance Rate and Biodeposit Production Rate

#### Collection and Maintenance of Bivalves

*Crassostrea gigas* specimens were obtained from commercial tray culture in Baynes Sound (E. Vancouver Island) and Ladysmith, BC (6 oysters). *Mytilus edulis* and *Mytilus galloprovincialis* specimens were obtained from commercial long-line and tray culture in Gorge Harbour (Cortes Island), with a small number of mussels (2 *M. edulis* and 6 *M. galloprovincialis*) collected from commercial tray culture in Baynes Sound. All bivalves were collected randomly, maintained in ambient seawater and immediately transported to the Centre for Shellfish Research (CSR). *M. edulis* and *M. galloprovincialis* specimens collected were relatively uniform in size with shell lengths ranging from 55.81 to 91.12 mm and 54.72 to 89.6 mm, respectively. *C. gigas* shell lengths, in contrast, ranged from 66.4 to 159.0mm. Mussel and oyster experiments were run from February 23 to June 13, 2005 and from August 15 to September 14, 2005, respectively, with coordinated collecting trips.

Upon arrival at the CSR, mussels and oysters were cleaned gently to remove epibiotic growth and then maintained in holding tanks prior to experimentation. Seawater temperature in the holding tanks ranged from 10 to 15 °C (February to April 2005) and 11.5 to 14.2 °C (May to October, 2005); salinity was stable at 32.5 ppt. Seawater in the holding tanks was filtered to 1µm; the tanks were supplied with air and the water was changed daily. The majority of mussels and oysters were used 2 days after collection; bivalves held for 4 days (maximum) were given a spot batch feed of Tahitian *Isochrysis galbana* on day 2. Hence, all experiments were preceded by a 2-day, bivalve depuration period.

#### Experimental Design

Set-up: Clearance rates and biodeposit production rates were determined using an “octopus” set-up described in Bayne (1976) (Fig. 1). Two header tanks (A, B Fig. 1) contained a combined volume of 760 litres of seawater. The 2 header tanks and the constant header tank (D, Fig. 1) were supplied with air for the duration of each trial (C, Fig. 1, is air manifold). Flow rate to 8 experimental chambers (G, Fig. 1) was controlled by a “feeding” manifold (E, Fig. 1); water flowed through 3/16” internal diameter flexible tubing terminating with a 90° fitting positioned to direct water flow toward the pedestal base (see Trials below). The experimental chambers were constructed out of 4-litre transparent, plastic, food-storage containers. Outflow from the top of the experimental chambers was through 3/4” internal diameter stiff, thick-walled tubing attached to a grommet with an O-ring. Outflow from the chambers and overflow from the constant header tank (F, Fig. 1) directed seawater into a trough (H, Fig. 1) connected to the lab floor drain.

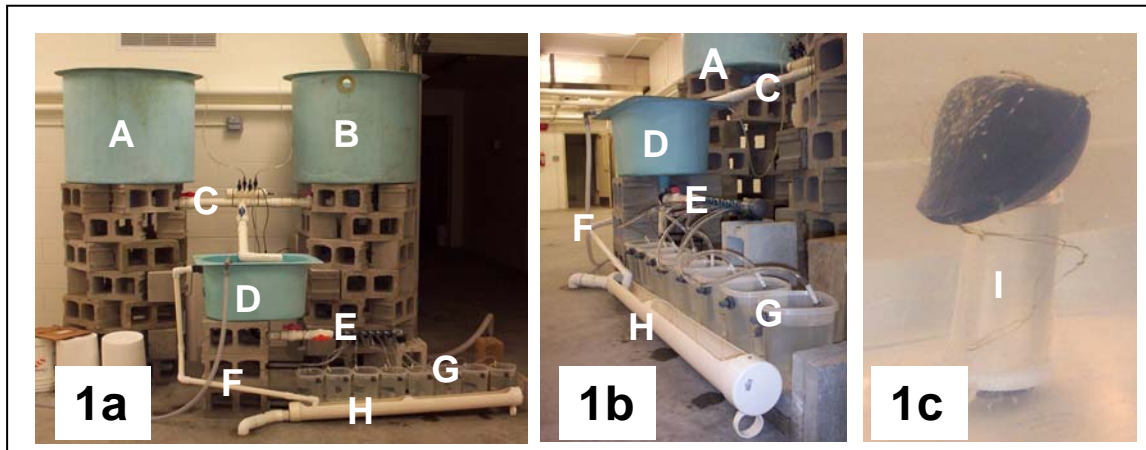


Figure 1. Clearance rate and biodeposition rate experimental set-up. 1a. Front view of flow-through system; 1b. Side view of chambers; 1c. Mussel on the pedestal.

FIGURE KEY	
A = Head Tank I	F = Overflow (constant head tank to drain)
B = Head Tank II	G = Experimental Chambers
C = Air Manifold	H = Drain Trough
D = Constant Header Tank	I = Pedestal
E = Feeding Manifold	

The combined header tanks volume of 760 litres allowed for a flow rate of  $170 \text{ ml}\cdot\text{min}^{-1}$  through each of 8 experimental chambers for  $\sim 7$  h. The flow rate through the chambers was sufficient to limit settlement of algae during the experimental period (also, see Calculation of Clearance Rates below).



### Experimental Trials:

Twenty-four hours prior to the start of the trial, mussels were attached to a pedestal using cyanoacrylate cement and then returned to the holding tanks where byssus threads were produced. At the start of each trial, the pedestals with attached mussels were removed from the holding tank and attached to the bottom (center) of the experimental chambers using Velcro (Fig. 1c). Oysters were either balanced on top of the pedestal (adhesive was not required) or, if too large for the pedestal, placed on the bottom of the experimental chamber. To test the water flow path within the chamber, dye was injected into the chamber inflow at flow rates of 60, 100, 140 and 170 ml·min<sup>-1</sup>. With the pedestal situated in the middle of the chamber, the inflowing water was found to flow past the inhalant siphon of the mussel immediately after coming into contact with the base of the pedestal (Bayne, 1976). A pedestal was not required for large oysters because the inflow water flowed immediately past the siphon.

Before starting each trial, the entire system was rinsed with freshwater. Following rinsing, the header tanks were filled with 1µm-filtered seawater. Background particle counts in replicate (20 ml) samples taken from the header tanks, prior to inoculation with algae, were determined using a Beckman Coulter Counter Multisizer III equipped with a 100 µm aperture tube. In all cases, background cell counts (data not shown) were negligible in comparison to algae cell concentrations following inoculation.

Algae cultures for all trials were obtained from the CSR algae culture facility. Oysters were fed a combination of Tahitian *Isochrysis galbana* and *Chaetoceros gracilis*, with an average composition of 34% and 64%, respectively. Mussels, in 6 out of 9 trials, were fed a combination of Tahitian *I. galbana* and *C. gracilis*, with an average composition of 45% and 55%, respectively; in 1 trial, mussels were fed 100% Tahitian *I. galbana*. In the remaining 2 trials, mussels were fed a 29%:71% combination of Tahitian *I. galbana*: *Phaeodactylum tricornutum*, respectively. The change in diet composition for mussels was unavoidable due to unexpected fluctuations in the algae available from the culture facility for some of the experiments.

Algae densities in batch culture columns were determined using a hemacytometer and the Coulter Counter. Filtered seawater in the header tanks was then inoculated with algae culture (species combined as detailed above) in volumes calculated to yield the following total algal cell concentrations, which spanned the range of cell counts in seasonally variable field samples: 1) “low” - 10,000 to 12,500 cells·ml<sup>-1</sup>; 2) “medium” - 19,000 to 30,500 cells·ml<sup>-1</sup>; 3) “high” - 35,000 to 46,500 cells·ml<sup>-1</sup>. Algal cell concentrations following inoculation were verified (and particle size frequency distribution from 2.6 µm to 60 µm measured) with the Beckman Coulter Counter Multisizer III. As a quality check, hemacytometer counts were performed on the header tanks prior to, and after, inoculation with algae. For comparison with the literature, algae cell densities were converted to mg·ml<sup>-1</sup> using conversion factors for the



individual algal components provided in Brown (1991). Conversions are as follows: 1) “low” – 0.60 to 0.75 mg·l<sup>-1</sup>; 2) “medium” – 1.13 to 1.87 mg·l<sup>-1</sup>; 3) “high” – 2.13 to 3.07 mg·l<sup>-1</sup>.

Following inoculation of the header tanks, and verification of the algal cell concentrations, flow to the experimental chambers was initiated. For each experimental trial, 2 of the 8 experimental chambers were randomly selected as controls (no bivalves); bivalves were placed in the remaining 6 chambers. Bivalves were then allowed to acclimate to experimental conditions (algae-inoculated seawater at 170 ml·min<sup>-1</sup> flow rate) for 60 minutes. During the acclimation period, particle concentration and size were measured in the constant head tank and in the control chamber outflows to ensure that the particle concentration and composition remained stable. At 60 minutes ( $T_0$  for the experiment), the first experimental samples were collected from the outflow of the 8 chambers and from the constant head tank. Throughout the, typically, 6-hour duration of each trial, water samples (20ml) were collected every 30 minutes from the constant head tank (inflow =  $C_{in}$ ) and the outflows of the eight chambers (outflow =  $C_{out}$ ). Clearance rates were calculated for each set of samples collected, yielding time-series data points for each trial. At the 170 ml·min<sup>-1</sup> flow rate, the 2 header tanks were usually empty after 7 hours (1-hour acclimation + 6-hour trial). Immediately after turning point flows off, biodeposits were collected (see below) and bivalves were maintained in the experimental chambers overnight (14 - 19 hours) without flowing water and additional biodeposits produced were collected the following morning (see below).

The period during which mussel experiments were conducted (late February through mid-June) included the typical spawning periods for *M. edulis* and *M. galloprovincialis* in BC. *M. edulis* spawning typically occurs in March and April, dependant on environmental conditions. However, animals do not necessarily release all gametes in one spawning event and a second spawning in June (second reproductive peak) may occur depending on environmental conditions. In this study, 17.2% of *M. edulis* spawned during experiments. *M. galloprovincialis* typically spawns during February/March and in this study, 6.5 % of the individuals of this species spawned during experiments. During spawning, experimental chambers became cloudy and an increase in the concentration of particles corresponding in size to gametes was observed using the Coulter Counter. The trials in which mussels spawned were usually terminated and the data not used. However, if the spawning was not extensive, sampling was resumed once the chamber waters were completely free of gametes. In all cases, the trials that included spawning were tagged for specific examination of the data. However, no pre-spawning or post-spawning effects on clearance rates were observed based on the water samples collected. Oysters, in the area from which experimental specimens were collected, usually spawn in June and July (B. Dixon, pers. comm.). The oyster experiments were run from mid-August to mid-September and no oysters spawned during experiments. Surprisingly, however, observations of the gonad (see gonad index) demonstrated that oysters used in the experiments had not already spawned but contained mature gonad, suggesting an unusually late spawning period.



### Calculation of Clearance Rates

Clearance rates of filter feeding bivalves are generally calculated using some modification of the following equation:

$$CR = \frac{(C_{in} - C_{out})}{C_s} \times Q \quad (1) \text{ (Hildreth and Crisp, 1976)}$$

Where  $C_{in}$  and  $C_{out}$  are particle concentrations flowing into and out of the chambers, respectively, and  $Q$  is the flow rate (e.g.  $\text{ml}\cdot\text{min}^{-1}$ ).  $C_s$  is the particle concentration surrounding the bivalve, which is extremely difficult to measure directly and is generally assigned the same value as either  $C_{in}$  or  $C_{out}$ . Clearance rates (prior to incorporating tissue dry weight) in this study were calculated using  $C_s = C_{in}$ , as per equation (2).

$$CR = \frac{(C_{in} - C_{out})}{C_{in}} \times Q \quad (2) \text{ (Riisgård, 2001)}$$

There is much ongoing debate regarding which concentration,  $C_{in}$  or  $C_{out}$ , best represents  $C_s$ . The debate stems from the issue of dilution of the inflowing water stream as it enters the flow chamber given that water in the flow chamber may be recirculated (and re-filtered by the bivalve) prior to flowing out of the chamber. If the flow chamber water is well mixed and can be considered to be at steady state, then the particle concentration surrounding the bivalve will be more closely approximated by  $C_{out}$  than  $C_{in}$  (Hildreth and Crisp 1979; Petersen *et al.* 2004). In these scenarios, using the particle concentration flowing into the chamber ( $C_{in}$ ) as the denominator would clearly underestimate the clearance rate of the bivalve due to re-filtration of seawater (Petersen *et al.*, 2004), particularly in volume-limited experimental chambers (Hildreth and Crisp, 1976; Jorgensen, 1976; Winter, 1978; Newell, 1979). However, if the geometry of the flow chamber facilitates optimal flow-through and does not allow recirculation (and therefore re-filtration of water) then  $C_s$  is more closely approximated by  $C_{in}$  than  $C_{out}$  (Riisgård, 2001). Hence, most researchers agree that the geometry (size and shape) of the flow chamber, in addition to the flow rate, are of great importance in determining whether  $C_{in}$  or  $C_{out}$  is used as the denominator of the equation used to calculate clearance rate.

#### 1.

In designing this experiment, the above debate was considered and dye tests were used to monitor the inflowing water stream. Mussels and small oysters were mounted on pedestals to insure that the inflowing water passed the bivalve's siphon as a stream. Little dilution of the dye stream was observed as it passed through the chamber. In addition, flow rate used was consistently  $170 \text{ ml}\cdot\text{min}^{-1}$ . Bayne (1976) reported that a flow rate of  $60 \text{ ml}\cdot\text{min}^{-1}$  was sufficient to reduce the error (resulting from using  $C_{in}$  as equivalent to  $C_s$ ) to a negligible value. Riisgård (2001) plotted clearance rate versus flow rate for *M. edulis* experiments and demonstrated that, using equation (2), clearance rate = flow rate at low flow rates and that clearance rates are only representative of true filtration rate at flow rates above a critical level



where clearance values form a plateau. Based on the data plotted, the latter was suggested to be 30-40 ml·min<sup>-1</sup> and this clearance rate plateau was maintained up to flow rates of approximately 400 ml·min<sup>-1</sup> (Riisgård, 2001).

Note that in equations (1) and (2), CR is used to mean clearance rate in l·h<sup>-1</sup>. However, as noted earlier, all clearance rates in this study were adjusted for tissue dry weight and, as a result, CR throughout this report refers to clearance rate in l·h<sup>-1</sup>·gdrywgt<sup>-1</sup>.

### Biodeposit Production

Biodeposits produced during the experimental trials were collected, after termination of the trials, for determination of biodeposit production rates. After water flow was terminated and biodeposits collected, bivalves were left in the chambers overnight, with no water flow, and additional biodeposits produced were collected the following morning for comparative purposes only. All biodeposits were collected using an electronic pipetter (IBS Pipetboy) and a 25ml plastic pipette and then placed into pre-labeled, screw-top 50ml plastic centrifuge tubes. Biodeposit samples were centrifuged at 3000 rpm (4-5 °C) for 10 minutes in a Centra CL3R Thermo IEC and then dried at 60 °C until constant weight. Preliminary observations suggested that the dried samples were hygroscopic due to residual salts and that this influenced the “dry” weight of the samples, particularly those with very low weights. Hence, “dried” samples were washed to remove salts. Samples were re-suspended with 20 ml distilled water and re-centrifuged at 3000 rpm for 10 minutes; the washing process was repeated 3 times. To accommodate the lengthy washing steps, some dried and weighed biodeposit samples were stored frozen, and then thawed, prior to washing. All washed samples were dried at 60 °C until constant weight, dry weights were recorded and then samples were maintained frozen (-4 °C) for later analysis of %organics and %carbonates. The results of the latter analyses are not presented; biodeposit weights, in many cases, were so low that the post-ignition sample weights were below accurate detection limit of the analytical balance (0.1 mg).

### Bivalve Measurements

Bivalve shell length, width, height, internal volume, wet weight of whole animal and soft tissues, plus dry weight of soft tissues were recorded for each specimen following termination of the trial. Shell dimensions were measured using digital calipers; internal shell volume was determined through the difference in water displacement between the whole animal and the shell only (Higgins, 1938). Once the bivalve was opened, but prior to removal of the soft tissues, gender was recorded and the gonad was assessed visually. Most bivalves could be identified as male or female but several individuals were of indistinct gender due to no visible gonad; bivalves classified as “indistinct gender” had a gonad index of 0-1 (see following). *M. galloprovincialis* spawns most of the gonad, leaving a very thin layer of mantle and post-spawn recovery in these animals is very slow. Significant mortality is associated with *M.*



*galloprovincialis* spawning in intensive culture situations in BC. Because of this, and observations in the literature regarding the potential effect of reproductive status on bivalve feeding behaviour, data on the developmental stage of the gonad were recorded. The gonad was assigned a value (gonad index) of 1-5 based on a set of physical characteristics indicative of the gonad development stage as follows: (1) gonad thin, opaque or no gonad; (2) gonad small, some colour, no definition (definition = granular texture with visible tubules); (3) gonad medium, coloured, no definition; (4) gonad plump, coloured, minor definition; (5) gonad granular with tubules, plump with milky consistency; and (6) gonad has started being resorbed. Following observation of the gonad, the soft tissue was removed from the shell and dried at 60° C until constant weight.

Condition indices for bivalves are reviewed by Crosby and Gale (1990). Condition indices of the bivalves in this study were determined using the following equation from Walne (1970):

$$CI = \frac{\text{dry weight (g)} \times 1000}{\text{internal volume (ml)}} \quad (3)$$

Biodeposit production rate (BPR) in this study is expressed as  $\text{mg} \cdot \text{gdrywt}^{-1} \cdot \text{d}^{-1}$ , where biodeposits are measured as mg dry weight and the rate calculation uses the duration of the feeding trial plus acclimation period (as a fraction of a day). Biodeposit production rates per individual bivalve ( $\text{mg} \cdot \text{ind}^{-1} \cdot \text{d}^{-1}$ ) are included in the data tables (see Results) only for comparison and are not used for statistical analyses.

### **Biodeposit Sinking Rate**

Biodeposits used to determine sinking rates were collected from *Mytilus galloprovincialis*, *Mytilus edulis* and *Crassostrea gigas*. Five *M. galloprovincialis* and 5 *M. edulis* were obtained from commercial long-line culture in Deep Bay, BC on April 13, 2005 and 5 *C. gigas* were obtained from commercial tray culture in Ladysmith, BC on April 21, 2005. The animals were transferred, in ambient seawater, to the CSR and acclimated for 5 days in a large, flow tank at 10 to 15 °C and salinity of 32.5 ppt. Animals were spot batch fed twice daily during the acclimation period and then depurated for twenty-four hours immediately prior to feeding and biodeposit production. Bivalves were isolated into chambers, as per the CR/BPR experiments above and fed a mixture of Tahitian *Isochrysis galbana* (47% of mixture) and either *Pavlova lutherii* or *Chaetoceros gracilis* (53% of mixture) for 4.5 hours. Using a hemacytometer, final algal concentrations in the chambers were 65,000 cells·ml<sup>-1</sup> (*M. galloprovincialis*), 50,000 cells·ml<sup>-1</sup> (*M. edulis*) and 45,000 cells·ml<sup>-1</sup> (*C. gigas*). Flow-rate for each feeding trial was constant; flow rates for the different feeding trials varied between 200 and 500 ml·min<sup>-1</sup>.



Following the feeding trials, biodeposits were collected using a 20 ml plastic pipette with a rubber bulb and then gently ejected into a pre-labeled glass Petri dish. Biodeposits were observed using a dissecting microscope and the general shape plus length and width were recorded. Most biodeposits were photographed using a Zeiss Stemi SV 11 dissecting scope and digital camera for later measurement. Biodeposits were then removed from the Petri dish with a small metal spatula, weighed, and placed in a pre-labeled glass vial in the refrigerator (3-4 °C) until used in sinking experiments later the same day.

Biodeposit sinking rates were measured in a cylindrical tank (1.22 m height, 0.75 m diameter) filled with 1 µm-filtered seawater that was allowed to reach stable (room) temperature prior to trials (18 °C; 32.5 ppt). Each individual biodeposit was placed gently onto the surface of the water at the center of the tank. The time for the biodeposit to travel 1.12 m, from a 10 cm mark until contact with the tank bottom, was recorded. Only sinking times for non-fragmented biodeposits were recorded. Sinking rates were calculated using sinking times and distance traveled. Observations on descent patterns of the biodeposits were recorded.

### **Bivalve Measurements**

Following collection of the biodeposits, bivalves were removed from the chamber and wet weights (whole animals) were recorded. Bivalves were then stored in the freezer (-4 °C) in pre-labeled plastic bags. Within 48 hours, bivalves were removed from the freezer and shell length, width and depth of each individual measured using digital calipers. Bivalve shells were opened and wet weight of soft tissues recorded. Following drying at 60 °C to constant weight (~72 hours), dry weight of soft tissues was recorded.

### **Data Processing and Statistical Analyses**

Table showing mean clearance rates were prepared to simplify presentation of the results of trials that used different algal cell concentrations and different species. Means in these tables were calculated as follows: the clearance rates measured for each individual bivalve over the course of the 6-hour time series experimental trials were grouped and a mean CR calculated for that individual bivalve for that trial. These mean clearance rates for each individual bivalve were then used to calculate a mean CR for each species for each algal cell concentration used.

Means tables for biodeposit production rates use the biodeposit production of each individual bivalve during the 6-hour feeding trials. The second biodeposit production rate calculated for each bivalve, using biodeposit production during the overnight depuration period, were measured for comparative purposes



only (biodeposit production rates during depuration were 20-25% of the feeding trial rates) and are not discussed further.

Exploratory analyses of the simple relationships between endogenous (shell length, tissue dry weight, condition index, gender and gonad index) and exogenous factors (diet composition, algal cell concentration) and clearance rate were done using Pearson correlations. Non-numeric factors (i.e. gender, diet type, categorized algal cell concentrations) were arbitrarily coded for ease of analysis. Gonad index (1 to 6) was simplified for analyses as follows: (0-1) gonad thin, opaque or no gonad; (2-4) gonad developing, some level of colour and zero to minor definition; (5) gonad granular with tubules, plump with milky consistency; and (6) gonad has started being resorbed. Although not shown, all these relationships were also plotted to examine non-linear relationships not evidenced by correlations. In some cases, when non-linear relationships were suggested, exploratory ANOVAs were done with categorized data (i.e. gender, diet type) to examine differences between means. It was assumed for this purpose that variance characteristics were similar for all categories within each analysis, but it is recognized that ANOVA is relatively robust with respect to deviations from normality, particularly for 2-tailed tests (Zar, 1999). These exploratory analyses were done in order to examine the potential confounding influence of experimental variables on the outcome of the original experimental design.

Factors were not combined into larger multi-factor analyses because of the likelihood that some factors were not independent, or were covariates. Therefore, for clarity of interpretation, all analyses were based on 2-factor comparisons. Inferential tests of hypotheses for which the experiments were originally designed were done using regressions (where linear relationships were expected), or ANOVAs, where non-linear relationships or categorized data were collected (c.f. low, medium, high algae cell concentrations). Paired t-tests were used to test for inter-specific differences in CR, BPR and biodeposit sinking rates. All methods used were based on standard normal statistical procedures as outlined in Zar (1999).

The experimental hypotheses were;

*Ho: Clearance rates were the same for all algal cell concentrations\**

*Ho: Clearance rates were the same for all three bivalve species*

*Ho: Biodeposition rates were the same for all algal cell concentrations\**

*Ho: Biodeposition rates were the same for all three bivalve species*

*Ho: There is no relationship between clearance rates and biodeposition rates\**

*Ho: Biodeposit sinking rates were the same for all three bivalve species*

Hypotheses with \* were tested for each species of bivalve. Sub-hypotheses generated from the above general hypotheses were tested as described earlier.



## Results

### Clearance Rate

Clearance rate (CR) data for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis*, for the algal cell concentration trials, have been summarized (means +/- s.d.) in Table 1. CR mean values were calculated as described in Methods; all clearance rates are expressed as per unit body weight. Also summarized in Table 1 are data for tissue dry weight and shell length of all bivalves used in the clearance rate experiments. Analyses involving clearance rate data (correlations, regressions, ANOVAs) used all values in the data set (i.e. data from all time points, for all individual bivalves in the time series experimental trials).

Riisgård (2001) cited the failure of some studies to measure maximum filtration rates of the experimental bivalves as a factor affecting the reliability of some studies. In this study, clearance rates were relatively consistent for each bivalve throughout each time series trial and, as a result, maximum clearance rates fell well within the variance around the mean CR. Regardless, all analyses were repeated using maximum clearance rates (results not shown) but, in all cases, results of statistical analyses were identical to those obtained using clearance rate data.

Table 1. Clearance rates for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* in laboratory experiments.

Species	Algal Cell Concentration Group*	Sample Size (n)	Mean CR (l·h <sup>-1</sup> ·gdrywt <sup>-1</sup> ) (+/- s.d.)	Tissue Dry Wgt (g)	Shell Length (mm)
<i>Crassostrea gigas</i>	Medium	21	1.90 (+/- 0.79)	4.45 (+/- 2.26)	92.79 (+/- 23.14)
	High	15	1.23 (+/- 0.58)	7.4 (+/- 4.39)	121.13 (+/- 35.11)
<i>Mytilus edulis</i>	Low	6	2.19 (+/- 0.78)	1.18 (+/- 0.38)	60.06 (+/- 3.12)
	Medium	13	3.50 (+/- 0.72)	1.07 (+/- 0.2)	62.29 (+/- 4.38)
	High	8	2.15 (+/- 0.60)	2.04 (+/- 0.8)	72.6 (+/- 9.44)
<i>Mytilus galloprovincialis</i>	Low	7	5.15 (+/- 2.2)	0.75 (+/- 0.41)	64.68 (+/- 9.22)
	Medium	12	4.12 (+/- 1.59)	0.95 (+/- 0.33)	72.99 (+/- 7.34)
	High	8	2.46 (+/- 0.88)	1.62 (+/- 0.41)	76.38 (+/- 3.39)

\*Food concentration groups: low - 10,000 to 12,500 cells·ml<sup>-1</sup>; medium - 19,000 to 30,500 cells·ml<sup>-1</sup>; high - 35,000 to 46,500 cells·ml<sup>-1</sup>.

### *Crassostrea gigas*

*C. gigas* experiments, unlike the mussel experiments discussed below, were run only at medium and high algal cell concentrations. When used in oyster experiments, the low concentrations of algal cells (e.g. 10,000 to 12,500 cells·ml<sup>-1</sup>) resulted in time series cell counts in oyster chamber outflow water that were



below detection capability. Preliminary trials revealed that approximately  $19,000 \text{ cells}\cdot\text{ml}^{-1}$  was the minimum diet concentration for *C. gigas* which, for the flow-through experimental design employed, would yield a measurable cell concentration in outflow water.

Clearance rates for *C. gigas* used in these experiments (both algal cell concentration categories, all time series data points) ranged from 0.24 to  $3.88 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ . Tissue dry weight for individual oysters ranged from 1.67 to 15.19g and shell length ranged from 66.4 to 159.0 mm. Of the randomly selected oysters used in this study, 55.6% were female, 36.1% male and 8.3% of indistinct gender. Gonad indices of experimental oysters were observed to fall into the following categories: 0-1 (13.9%), 5 (41.7%) or 6 (44.4%). Hence, the majority of *C. gigas* in this study had mature gonads. Condition indices of individual oysters ranged from 76 to  $298 \text{ g}\cdot\text{ml}^{-1}$ . Mean CR, tissue dry weight and shell length for the different experimental groups are summarized in Table 1.

An exploratory Pearson correlation table was constructed for preliminary examination of possible linear relationships between endogenous factors (tissue dry weight, shell length, condition index, gender and gonad index), exogenous factors (algal cell concentration) and clearance rate (Table 2). Oyster shell length and tissue dry weight are shown to be strongly, positively correlated (0.80); this is expected as the two factors are not independent. Both shell length and tissue dry weight are strongly negatively correlated with clearance rate (-0.70 and -0.81, respectively). There is a moderate positive correlation between gonad index and condition index and, again, these two factors are not independent; animals of greater tissue mass (including gonad) generally have higher condition indices (as per the equation of Walne, 1970) and animals with larger gonads generally have higher gonad indices. The moderate negative correlation between gender and gonad index can be attributed to the fact that female oysters, rather than males or those of indistinct gender, were randomly coded with the lowest gender code (1) combined with the fact that virtually all female oysters had the highest gonad index (6).

Table 2. Pearson correlations for *Crassostrea gigas* clearance rate experiment data.

	Gender	Gonad Index	Shell Length	Tissue Dry Weight	Condition Index	Time Series	Clearance Rate
<b>Gender</b>	1.00						
<b>Gonad Index</b>	<b>-0.52</b>	1.00					
<b>Shell Length</b>	0.35	-0.45	1.00				
<b>Tissue Dry Weight</b>	0.07	0.04	<b>0.80</b>	1.00			
<b>Condition Index</b>	-0.01	<b>0.56</b>	-0.34	0.00	1.00		
<b>Time series</b>	-0.01	-0.04	-0.01	-0.05	-0.03	1.00	
<b>Clearance Rate</b>	-0.01	-0.06	<b>-0.70</b>	<b>-0.81</b>	0.03	0.09	1.00
<b>Algal Cell Concentration</b>	-0.06	-0.04	0.30	0.28	-0.30	-0.08	-0.32



The strong negative correlations between CR and tissue dry weight, and between CR and shell length, suggest that larger oysters have a lower clearance rate (per g dry weight) than smaller oysters. At tissue dry weights greater than 8 g, *C. gigas* clearance rates show markedly less variation with increase in tissue dry weights and appear to level off at approximately  $1 \text{ l} \cdot \text{h}^{-1} \cdot \text{g dry weight}^{-1}$  (Fig. 2). The null hypothesis that clearance rate and oyster tissue dry weight were not linearly related was tested by regression analysis, which revealed a significant relationship ( $p < 0.000$ ) explaining 61% of the variance in clearance rates ( $R^2 = 0.61$ ). Similarly, regression revealed a significant linear relationship between CR and oyster shell length ( $R^2 = 0.48$ ,  $p < 0.000$ ), although shell length appears to be a slightly poorer predictor of CR than tissue dry weight. Bivalve tissue dry weight is clearly an important factor in determining CR.

The relationship between oyster clearance rate and algal cell concentration was examined by regression ( $H_0(1a)$ ) and ANOVA ( $H_0(1b)$ ):

*H<sub>0</sub>(1a): There is no linear relationship between C. gigas clearance rate and algal cell concentrations.*

Results of the regression revealed that, although significant ( $p < 0.000$ ), the relationship between CR and algal cell concentration explains very little of the variance (15%) in clearance rates ( $R^2 = 0.15$ ).

*H<sub>0</sub>(1b): Clearance rate of C. gigas is the same for medium and high algal concentrations*

ANOVA results revealed that clearance rates for oysters fed on medium algal cell concentrations were significantly higher than those for oysters fed on high algal cell concentrations ( $F = 58.3$ ,  $p < 0.000$ ) but, as illustrated in Table 1, the difference between mean clearance rates for these two categories of algal cell concentration is relatively small and the physiological significance is questionable.

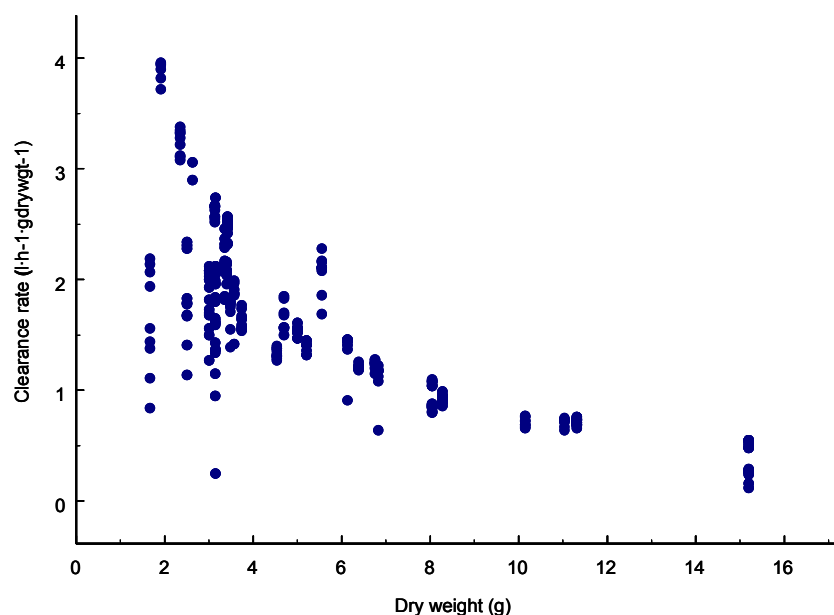


Figure 2. *Crassostrea gigas* clearance rate ( $\text{l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ ) versus dry tissue weight (g).

#### *Mytilus edulis* and *Mytilus galloprovincialis*

While all oyster clearance rate experiments used a diet of the same algal composition, *M. edulis* and *M. galloprovincialis* experiments used, unavoidably, 3 diets that differed in algal species composition (see Methods). Clearance rates (all algal cell concentrations, 3 diet compositions, all time series data points) of *M. edulis* ranged from 1.19 to  $4.62 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ , while those of *M. galloprovincialis* ranged from 1.13 to  $9.16 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ . Tissue dry weights for individual *M. edulis* and *M. galloprovincialis* ranged from 0.68 to 3.21 g and from 0.33 to 2.18 g, respectively; shell length ranged from 55.81 to 91.12 mm (*M. edulis*) and from 54.72 to 89.6 mm (*M. galloprovincialis*). Of the randomly selected *M. edulis* used in this study, 59.3% were female, 22.2% male and 18.5% of indistinct gender. *M. galloprovincialis* were 44.4% female, 22.2% male and 33.3% indistinct gender. Gonad indices of experimental *M. edulis* were observed to fall into the following categories: 0-1 (14.8%), 2-4 (59.3%) and 5 (25.9%); those for *M. galloprovincialis* fell into the following gonad index categories: 0-1 (48.15%), 2-4 (48.15%) and 5 (3.7%). Hence, the majority of *M. galloprovincialis* had spawned previous to the experiments, while 25% of *M. edulis* were ready to spawn. Condition indices for *M. edulis* and *M. galloprovincialis* ranged from 42 to  $95 \text{ g}\cdot\text{ml}^{-1}$  and 19 to  $94 \text{ g}\cdot\text{ml}^{-1}$ , respectively. Mean CR, tissue dry weight and shell length for the different experimental groups for each *Mytilus* species are summarized in Table 1.

Exploratory Pearson correlation tables (Tables 3 and 4), as for oyster data above, were used for preliminary examination of linear relationships between endogenous factors, exogenous factors and



clearance rates. Similar to results for *C. gigas*, shell length and tissue dry weight are strongly, positively correlated (0.90) for *M. edulis* (Table 3). Also similar to *C. gigas* results, both factors are moderately negatively correlated with clearance rate although tissue dry weight is more strongly correlated (-0.66) with clearance rate than is shell length (-0.52) (Table 3). Table 4 reveals a moderate, positive correlation between shell length and tissue dry weight (0.45) for *M. galloprovincialis*, reflecting a potentially different growth physiology from *M. edulis*. Tissue dry weight is moderately negatively correlated with clearance rate (-0.59) for *M. galloprovincialis*.

For both *Mytilus* species, tissue dry weight and condition index are positively correlated: moderately for *M. edulis* (0.53) and strongly for *M. galloprovincialis* (0.85). As observed for oysters, tissue dry weight is a component of the equation used to calculate condition index (Walne, 1970) and bivalves with high tissue weight usually have a high condition index. Dry tissue weight and condition index, for both mussel species, have a moderate negative correlation with clearance rate; larger mussels (which have higher condition indices) have a lower clearance rate (per gdrywgt) than smaller mussels and hence, mussel size, particularly tissue dry weight, is an important factor to consider when studying clearance rates.

Table 3. Pearson correlations for *Mytilus edulis* clearance rate experiment data.

	Diet Composition	Gender	Gonad Index	Shell Length	Tissue Dry Weight	Condition Index	Time series	Clearance Rate
Gender	-0.18	1.00						
Gonad Index	0.13	-0.41	1.00					
Shell Length	0.47	-0.21	0.29	1.00				
Tissue Dry Weight	<b>0.61</b>	-0.25	0.26	<b>0.9</b>	1.00			
Condition Index	0.32	-0.14	-0.14	0.22	<b>0.53</b>	1.00		
Time Series	0.09	-0.13	0.23	-0.02	-0.01	-0.11	1.00	
Clearance Rate	-0.33	-0.05	0.10	<b>-0.52</b>	<b>-0.66</b>	<b>-0.57</b>	0.19	1.00
Algal Concentration	0.11	-0.25	0.09	<b>0.61</b>	<b>0.55</b>	0.11	0.00	<b>-0.13</b>

Table 4. Pearson correlations for *Mytilus galloprovincialis* clearance rate experiment data.

	Diet Composition	Gender	Gonad Index	Shell Length	Tissue Dry Weight	Condition Index	Time Series	Clearance Rate
Gender	0.09	1.00						
Gonad Index	0.19	-0.44	1.00					
Shell Length	0.53	0.52	-0.13	1.00				
Tissue Dry Weight	<b>0.74</b>	0.16	0.34	<b>0.45</b>	1.00			
Condition Index	0.49	-0.06	0.38	-0.01	<b>0.85</b>	1.00		
Time Series	0.08	0.09	-0.07	0.09	-0.03	-0.08	1.00	
Clearance Rate	<b>-0.55</b>	-0.03	-0.24	-0.23	<b>-0.59</b>	<b>-0.48</b>	0.05	1.00
Algal Concentration	<b>0.70</b>	0.21	0.10	0.43	<b>0.60</b>	0.38	-0.03	<b>-0.44</b>

For both *M. edulis* (Table 3) and *M. galloprovincialis* (Table 4), there is a notable positive correlation between tissue dry weight and diet composition (0.61 and 0.74, respectively). The 3 diets of different



algal composition were randomly coded 1-3 for correlations. Examinations of the data reveal that mussels randomly chosen for diet #3 were larger than those in other diet groups. Therefore, the weak and moderate negative correlations between diet composition and clearance rates for *M. edulis* and *M. galloprovincialis* respectively, are likely reflecting the size bias in diet group #3. As observed for *C. gigas*, large mussels have lower clearance rates than small mussels (see later in this section). Since the clearance rate correlations were stronger for dry weight than for diet, it was concluded that these two factors were covariates, and that tissue dry weight rather than diet composition is likely the more important factor affecting clearance rate.

There are moderate positive correlations between algal cell concentration and mussel tissue dry weight for both species (Tables 3 and 4) and between algal cell concentration and shell length for *M. edulis* (Table 3). For both mussel species, examination of the data reveals that over 50% of the trials using high algal cell concentrations were run with diet #3 and hence, the correlation between algal cell concentration and mussel tissue dry weight is also reflecting the size bias noted in diet group #3 discussed above. The dominance of diet #3 in trials with the high algal cell counts is apparent in the positive correlation (0.70) between diet composition and algal cell concentrations observed for *M. galloprovincialis*. The moderate, negative correlation between algal cell concentration and clearance rate for *M. galloprovincialis* (Table 4) shows that algal cell concentrations were less confounded by diet type than in *M. edulis*.

The relationship between clearance rates and tissue dry weights for both species of mussel is illustrated in Figures 3 and 4. At tissue dry weights greater than 2 g, *M. edulis* clearance rates show markedly less variation with increase in tissue dry weights and appear to level off at approximately  $2 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$  (Fig. 3). *M. galloprovincialis* clearance rates are much more variable than those of *M. edulis* at low dry tissue weights but show a similar trend to those of *M. edulis*, leveling off (at approximately  $2 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ ) at dry tissue weights greater than 1.6 g (Fig. 4). Null hypotheses that clearance rates were not linearly related to tissue dry weights were tested for both *Mytilus* species. Not surprisingly, regressions revealed that the relationship between tissue dry weight and clearance rate is significant for both *M. edulis* and *M. galloprovincialis* ( $p < 0.000$  for both species) and that tissue dry weight explains 43 % and 35% of the

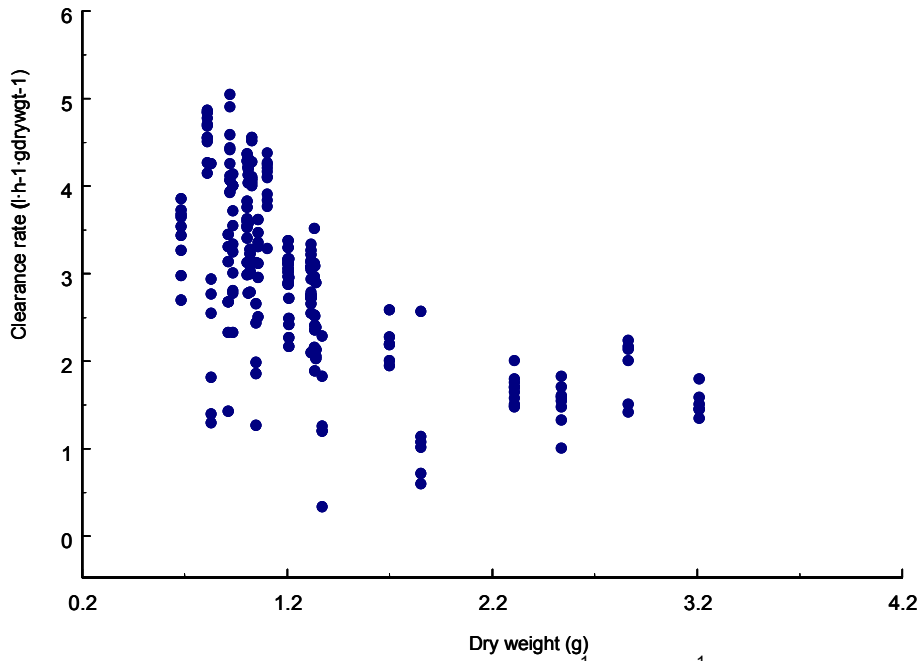


Figure 3. *Mytilus edulis* clearance rate (l·h<sup>-1</sup>·gdrywtg<sup>-1</sup>) versus dry tissue weight (g).

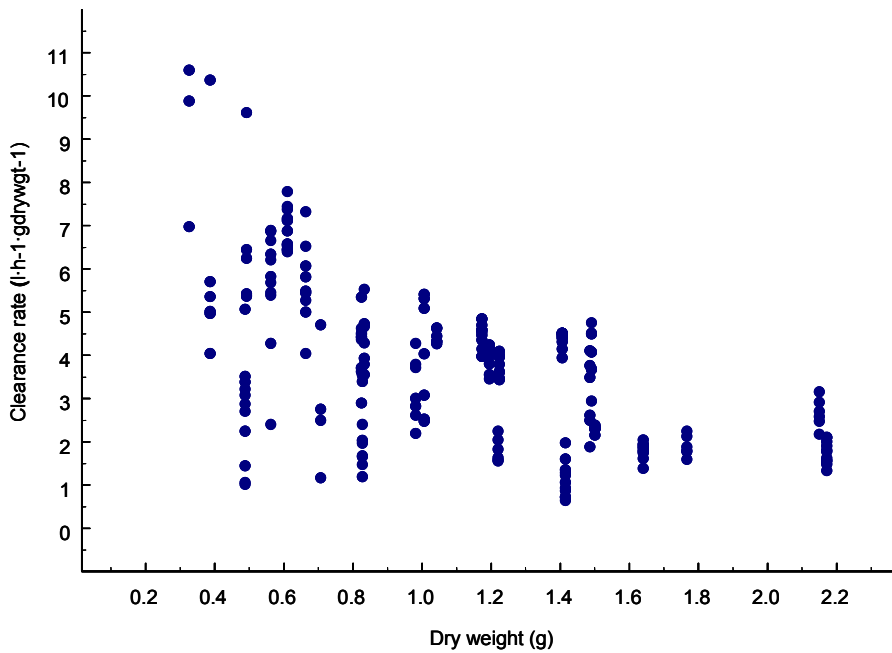


Figure 4. *Mytilus galloprovincialis* clearance rate (l·h<sup>-1</sup>·gdrywtg<sup>-1</sup>) versus dry tissue weight (g).



variance in clearance rates in *M. edulis* and *M. galloprovincialis*, respectively. The relationship between shell length and clearance rate was also found to be significant for both *Mytilus* species ( $p < 0.000$  for both species), but shell length explained only 27 % and 5% of the variance in clearance rate in *M. edulis* and *M. galloprovincialis*, respectively. Tissue dry weight appears, therefore, a better predictor of clearance rate than shell length. As noted for *C. gigas*, bivalve tissue dry weight is clearly an important factor in determining CR.

The relationship between mussel clearance rates and algal cell concentrations was examined by regression and ANOVA designed to test the null hypotheses:

*Ho(3a): Clearance rates for M. edulis and M. galloprovincialis were not linearly related to algal cell concentrations*

Results of the regression revealed that, although significant ( $p < 0.000$ ), a linear relationship between clearance rate and algal concentration for *M. galloprovincialis* explains very little of the variance in clearance rates ( $R^2 = 0.19$ ). The relationship between clearance rate and algal concentration for *M. edulis* was not significant ( $p = 0.06$ ,  $R^2 = 0.01$ ). For both regressions, the linear fit is unconvincing and this is confirmed by ANOVA results below.

Algal cell concentrations were grouped into low, medium and high concentrations to test:

*Ho(3b): Clearance rates for M. edulis and M. galloprovincialis were the same for low, medium and high algal cell concentrations.*

ANOVA results for *M. edulis* and *M. galloprovincialis* are summarized in Table 5. *M. edulis* fed with medium algal cell concentrations had clearance rates significantly higher than those of *M. edulis* fed either the low ( $F = 81.76$ ,  $p < 0.000$ ) or high ( $F = 164.02$ ,  $p < 0.000$ ) algal cell concentrations. Clearance rates for *M. edulis* fed the low or high algal concentrations were not significantly different ( $F = 0.96$ ,  $p = 0.33$ ). The significant decrease in CR between *M. edulis* fed medium and high algal cell concentrations is likely confounded due to the size bias (larger animals) associated with the high concentration trials.

ANOVA results (Table 5) reveal that *M. galloprovincialis* fed high algal cell concentrations had clearance rates significantly lower than those fed at either medium ( $F = 58.24$ ,  $p < 0.000$ ) or low ( $F = 53.6$ ,  $p < 0.000$ ) algal cell concentrations. Clearance rates of *M. galloprovincialis* fed medium and low algal concentrations were not significantly different from each other ( $F = 2.37$ ,  $p = 0.13$ ). However, as noted for *M. edulis*, interpretation of the significance of this result must consider the size bias (larger animals) noted for the trials using high algal cell concentration.

Table 5. *Mytilus edulis* and *Mytilus galloprovincialis*. ANOVA results: clearance rate versus algal cell concentration.

<i>Mytilus edulis</i>	F	P	<i>Mytilus galloprovincialis</i>	F	P
<b>Algal Concentration Category</b> low vs med vs high	92.769	0.000	<b>Algal Concentration Category</b> low vs med vs high	31.612	0.000
low vs med	81.76	0.000	low vs med	2.367	0.126
med vs high	164.020	0.000	med vs high	58.243	0.000
low vs high	0.956	0.331	low vs high	53.60	0.000

The above results suggest that for the 3 bivalve species studied, algal cell concentration is a less important factor in CR in these experiments than is tissue dry weight. However, more research, using bivalves of uniform size in all experimental trials, is required to prove this for the *Mytilus* species.

#### Inter-specific Comparison of Clearance Rates

Paired t-tests were used to examine differences in mean clearance rate per animal between the three species of bivalve used in these experiments (data pooled across all variables for each species). Results are summarized in Table 6. *C. gigas* mean clearance rates were significantly lower than those of *M. edulis* and *M. galloprovincialis*. Examination of the means for experimental groups in Table 1 reveals that clearance rates for both *Mytilus* species were usually at least twice those of *C. gigas*. Mean clearance rates of *M. edulis* were significantly lower than those of *M. galloprovincialis*. Although this may not appear to be the case at high algal concentrations (Table 1), in fact the relationship between CR for *M. edulis* and *M. galloprovincialis* is linear, with very little deviation from the relationship (data plots not shown), suggesting that algal cell concentration does not have any notable effect on this relationship. The trend in clearance rates for the 3 species may reflect inter-specific, physiological differences but, interestingly, it also reflects the comparative size of the bivalves (Table 1). Additional research is required to differentiate between these possibilities.

Table 6. Results of paired t-tests: inter-specific differences in mean clearance rates.

Species	Mean Clearance Rate	
	t	p
<i>Crassostrea gigas</i> & <i>Mytilus edulis</i>	-5.640	<0.001
<i>Crassostrea gigas</i> & <i>Mytilus galloprovincialis</i>	-6.611	<0.001
<i>Mytilus edulis</i> & <i>Mytilus galloprovincialis</i>	2.671	0.01



### **Biodeposit Production Rate**

Biodeposit production rate data for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* have been summarized (means +/- s.d.) in Table 7. Both biodeposit production rate per individual bivalve and biodeposit production rate per g tissue dry weight (BPR) are presented; statistical analyses were conducted only on the latter, however, because exploratory analyses showed that, as observed for CR, there was a strong relationship with size of animal. Data on biodeposit production rate per individual bivalve are presented in some literature, however, and were included for comparative purposes. Also summarized in Table 7 are corresponding data for tissue dry weight and shell length of all bivalves used in the experiments. All biodeposit production rate mean values in Table 7 are based on the biodeposits produced by each individual bivalve during the experimental clearance rate trials.

Table 7. Biodeposit production rates for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* in laboratory experiments. Mean +/- std. dev.

Species	Food Concentration Group*	Sample Size (n)	Biodeposit Production Rate (mg-gdrywt <sup>-1</sup> -d <sup>-1</sup> )	Biodeposit Deposition Rate/Individual (mg-ind <sup>-1</sup> -d <sup>-1</sup> )	Tissue Dry Weight (g)	Shell Length (mm)
<i>Crassostrea gigas</i>	Medium	22	6.68 (+/- 4.29)	24.1 (+/- 10.5)	4.25 (+/- 2.34)	92.11 (+/- 22.8)
	High	16	8.55 (+/- 5.65)	46.4 (+/- 14.57)	7.9 (+/- 4.68)	123.81 (+/- 35.57)
<i>Mytilus edulis</i>	Low	3	16.11 (+/- 21.79)	14.24 (+/- 17.29)	1.25 (+/- 0.54)	59.79 (+/- 2.88)
	Medium	10	11.66 (+/- 10.02)	11.62 (+/- 7.91)	1.06 (+/- 0.21)	61.47 (+/- 4.66)
	High	3	20.62 (+/- 13.9)	23.69 (+/- 12.05)	1.23 (+/- 0.18)	65.11 (+/- 1.66)
<i>Mytilus galloprovincialis</i>	Low	6	18.26 (+/- 13.37)	13.7 (+/- 12.04)	0.75 (+/- 0.41)	64.68 (+/- 9.22)
	Medium	11	14.8 (+/- 8.76)	14.2 (+/- 11.12)	0.95 (+/- 0.33)	72.99 (+/- 7.34)
	High	3	24.37 (+/- 7.45)	32.0 (+/- 11.36)	1.33 (+/- 0.28)	77.95 (+/- 5.34)

\* Food concentration groups: low - 10,000 to 12,500 cells·ml<sup>-1</sup>; medium - 19,000 to 30,500 cells·ml<sup>-1</sup>; high - 35,000 to 46,500 cells·ml<sup>-1</sup>

#### *Crassostrea gigas*

Biodeposit production rates (BPR) for individual *C. gigas* ranged from 1.97 to 18.18 mg-gdrywt<sup>-1</sup>-d<sup>-1</sup>. Biodeposit production rate, per individual oyster, ranged from 3.6 to 71.52 mg-ind<sup>-1</sup>-d<sup>-1</sup>. For a Pearson correlations table of all bivalve factors, other than BPR, see Table 2; Pearson correlations for BPR and endogenous/exogenous factors are listed in Table 8. Both shell length and tissue dry weight show moderate negative correlations with biodeposit production rate. The trend for biodeposit production rates to decrease as bivalve size increases is similar to the relationship found for *C. gigas* clearance rate and size. The negative correlation between BPR and tissue dry weight may appear to conflict with the means



data in Table 7. However, it must be remembered that the data in Table 7 is grouped according to a *a priori* assignment of concentration categories while the correlation is based on the BPR of individual oysters. In addition, the high variances around the mean biodeposit production rates in Table 7 indicate a broad range of values. In fact, when plotted (not shown), the data for BPR for the 2 algal cell concentration categories shows a very similar range in values within each category.

Table 8. Pearson correlations for *Crassostrea gigas* biodeposit production rate data.

Factor	Biodeposit Production Rate
Gender	-0.14
Gonad Index	0.15
Shell Length	<b>-0.54</b>
Tissue Dry Weight	<b>-0.54</b>
Condition Index	-0.06
Algal Concentration	0.19

The null hypothesis that there was no linear relationship between tissue dry weight or length and biodeposit production rate was tested by regression. The relationship between BPR and dry weight was found to be significant ( $p < 0.000$ ). However, tissue dry weight accounted for only 25% of the variance in BPR ( $R^2 = 0.25$ ). There was also a significant relationship between BPR and oyster shell length ( $R^2 = 0.26$ ,  $p < 0.000$ ), suggesting that the two size measures are comparable predictors of BPR in *C. gigas*.

The relationship between oyster biodeposit production rate and algal cell concentration was examined by regression and ANOVA designed to test the null hypotheses *Ho(5a)* and *Ho(5b)*:

*Ho(5a): Biodeposit production rate for C. gigas was not linearly related to algal cell concentrations*

Results of the regression revealed no significant relationship between biodeposit production rate and algal concentration ( $R^2 = 0.00$ ,  $p = 0.45$ ).

*Ho(5b): Biodeposit production rate for C. gigas was the same for medium and high algal cell concentrations*

ANOVA results revealed that biodeposit production rates for oysters fed on high algal concentrations were significantly higher than those for oysters fed on medium algal concentrations ( $F = 1.36$ ,  $p < 0.05$ ). However, examination of the means ( $\pm$  s.d.) in Table 7 and data plots (not shown) reveals a lot of scatter in the data that is supported by the low F value. This is clearly shown in the non-significant relationship between biodeposit production rates and actual (not categorized) cell concentrations (see regression results above).

Results of the *C. gigas* biodeposit production experiments suggest a similar relationship between tissue dry weight and BPR as was observed for tissue dry weight and CR, that is BPR decreases with increasing



oyster size. While BPR for oysters fed high algal cell concentrations was significantly higher than those fed medium algal cell concentrations, the difference between the biodeposit production rates is extremely small (Table 7) and the significant result is due to the large sample size rather than any physiologically relevant difference in means.

#### *Mytilus edulis* and *Mytilus galloprovincialis*

BPR for individual *M. edulis* and *M. galloprovincialis* ranged from 1.3 to 41.12 mg·gdrywt<sup>-1</sup>·d<sup>-1</sup> and 1.99 to 45.00 mg·gdrywt<sup>-1</sup>·d<sup>-1</sup>, respectively. Biodeposit production rates, expressed as per individual mussel, ranged from 2.40 to 37.20 mg·ind<sup>-1</sup>·d<sup>-1</sup> for *M. edulis* and from 2.42 to 44.94 mg·ind<sup>-1</sup>·d<sup>-1</sup> for *M. galloprovincialis*. Mean values for BPR, BPR expressed per individual mussel, tissue dry weight and shell length are listed in Table 9.

For exploratory Pearson correlations of all *M. edulis* and *M. galloprovincialis* factors, other than BPR, see Tables 3 and 4, respectively. *M. edulis* tissue dry weight, as observed for *C. gigas*, is moderately negatively correlated with biodeposit production rate (Table 9). Hence, *M. edulis* biodeposit production rates (per g tissue dry weight) follow the same trend as *M. edulis* clearance rates (per g dry tissue weight): decreasing with increasing bivalve size. This relationship between biodeposit production rate and tissue dry weight does not occur for *M. galloprovincialis* (Table 10), although a moderate negative correlation was found between CR and tissue dry weight for *M. galloprovincialis*, as reported earlier.

Table 9. Pearson correlations for *Mytilus edulis* biodeposit production rate data.

Factor	Biodeposit Production Rate
Gender	0.07
Gonad Index	<b>-0.62</b>
Shell Length	-0.16
Tissue Dry Weight	<b>-0.49</b>
Condition Index	-0.35
Algal Concentration	0.11
Diet Composition	-0.25

Table 10. Pearson correlations for *Mytilus galloprovincialis* biodeposit production rates.

Factor	Biodeposit Production Rate
Gender	-0.12
Gonad Index	0.10
Shell Length	0.27
Tissue Dry Weight	0.09
Condition Index	-0.06
Algal Concentration	0.11
Diet Composition	0.11



Gonad index appeared to be an important factor for *M. edulis* BPR, since there was a negative correlation (-0.62) between these two factors (Table 11). The null hypothesis that there was no linear relationship between these factors was tested using regression. *M. edulis* gonad index and biodeposit production rate showed a significant relationship ( $p=0.01$ ) with gonad index accounting for 35% of the variance in BPR. Thus, gonad index may be a contributory factor in BPR. Alternatively, it may be a covariate of a different, unmeasured factor affecting BPR. This relationship was not apparent for *M. galloprovincialis*.

Tables 9 and 10 show no strong linear relationship between gender and biodeposit production rate for either species of *Mytilus*, but examination of data plots suggests that *M. galloprovincialis* females have a somewhat lower biodeposit production rate than males or specimens of indistinct gender. An ANOVA test of the null hypothesis that BPR was the same for all *M. galloprovincialis* genders revealed that biodeposit production rate was significantly lower in females than either males ( $F=5.34$ ,  $p=0.05$ ) or indistinct specimens ( $F=8.03$ ,  $p=0.01$ ). This was the only gender-specific, physiological difference found in these experiments. There is no intuitive reason to expect this difference, suggesting that gender may have been a covariate of some other, unmeasured, factor of importance.

The null hypotheses that tissue dry weight, and shell length, were not linearly related to BPR was tested by regression. The hypothesis could not be rejected for *M. edulis* for either dry weight ( $R^2=0.18$ ;  $p=0.06$ ) or shell length ( $R^2=0.00$ ,  $p=0.56$ ). Similarly, the null hypothesis could not be rejected for *M. galloprovincialis* for either tissue dry weight ( $R^2=0.00$ ;  $p=0.71$ ) or for shell length ( $R^2=0.02$ ,  $p=0.26$ ).

The relationship between *M. edulis* BPR and algal concentration was examined by regression and ANOVA designed to test the null hypotheses;

*Ho(6a): Biodeposit production rate of M. edulis (or M. galloprovincialis) was not linearly related to algal concentrations.*

Results of the regressions revealed that the null hypothesis could not be rejected for *M. edulis* ( $R^2=0.00$ ,  $p=0.69$ ) or *M. galloprovincialis* ( $R^2=0.00$ ,  $p=0.65$ ).

*Ho(6b): Biodeposit production rate of M. edulis (or M. galloprovincialis) was the same for low, medium and high algal concentrations.*

ANOVA results suggest that BPR for *M. edulis* and *M. galloprovincialis* fed on the 3 different algal concentrations were not measurably different ( $F=0.58$ ,  $p=0.58$  and  $F=1.08$ ,  $p=0.36$ , respectively), since the null hypothesis could not be rejected for either species.

In summary, the above results suggest that, during these experiments and particularly for the *Mytilus* species, biodeposit production rate is erratic. Tissue dry weight was a factor affecting BPR in *C. gigas*,



although only accounting for 25% of the variance in BPR. Algal cell concentration, over the range of concentrations used in this study, was not a factor affecting BPR in the 3 bivalves.

### Inter-specific Comparison of Biodeposit Production Rates

Paired t-tests were used to examine differences in biodeposit production rates per animal between the three species of bivalve used in these experiments (data pooled across all variables for each species). Results are summarized in Table 11. *C. gigas* biodeposit production rates were significantly lower than those of *M. edulis* and *M. galloprovincialis*. Biodeposit production rates of *M. edulis* and *M. galloprovincialis* were not significantly different. Examination of the mean data in Table 7 reveals that biodeposit production rates of both *Mytilus* species were, as noted earlier for clearance rates, usually greater than double those of *C. gigas*, regardless of algal cell concentration. The trend in clearance rates for the 3 species may reflect inter-specific, physiological differences but, interestingly, it also reflects the comparative size of the bivalves (Table 1). Additional research is required to differentiate inter-specific, physiological differences.

Table 11. Results of paired t-tests: inter-specific differences in biodeposit production rates.

Species	Biodeposit Production Rate	
	t	p
<i>Crassostrea gigas</i> & <i>Mytilus edulis</i>	3.074	<0.005
<i>Crassostrea gigas</i> & <i>Mytilus galloprovincialis</i>	4.698	<0.001
<i>Mytilus edulis</i> & <i>Mytilus galloprovincialis</i>	0.433	>0.5

### Clearance Rate and Biodeposit Production Rate

Simple Pearson correlations between rates measured are summarized in Table 12. Clearance rate and biodeposit production rate were not strongly correlated in the 3 species tested in these experiments. As noted earlier, biodeposit production rates were erratic in these experiments. It is possible that the 6-hour feeding trials (plus the 1-hour acclimation period), although of sufficient duration to yield consistent clearance rates for an individual bivalve, may have been of insufficient duration to yield consistent biodeposit production for a given species and algal cell concentration.

Table 12. Pearson correlations for clearance rate and biodeposit production rate for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis*.

<b><i>Crassostrea gigas</i></b>	<b>Clearance Rate</b>	<b>Biodeposit Production Rate</b>
<b>Clearance Rate</b>	1.00	
<b>Biodeposit Production Rate</b>	0.27	1.00
<b><i>Mytilus edulis</i></b>	<b>Clearance Rate</b>	<b>Biodeposit Production Rate</b>
<b>Clearance Rate</b>	1.00	
<b>Biodeposit Production Rate</b>	0.21	1.00
<b><i>Mytilus galloprovincialis</i></b>	<b>Clearance Rate</b>	<b>Biodeposit Production Rate</b>
<b>Clearance Rate</b>	1.00	
<b>Biodeposit Production Rate</b>	0.08	1.00

### **Biodeposit Sinking Rate**

In laboratory experiments, the biodeposits produced by *C. gigas*, *M. edulis* and *M. galloprovincialis* appeared to be entirely fecal material (noticeable pellets and/or strings) with no clearly recognizable pseudofeces component. The descent patterns of biodeposits of all 3 species were observed to be consistently straight in virtually all experiments, with very little drift away from the center of the water column. Typically, the biodeposit ribbons fell on a horizontal plane. On several occasions the ribbons fell vertically and it was observed that in these cases, one end of the biodeposit appeared to be weighted more than the rest of the ribbon, usually through folding over itself at the end. In a few trials, the biodeposits fragmented upon entry into the water column and the sinking times of these fragments were not recorded.

Table 13. Biodeposit sinking rates for *Crassostrea gigas*, *Mytilus edulis* and *Mytilus galloprovincialis* in laboratory-based experiments. \* n=26

<b>Species</b>	<b>Sample Size (n)</b>	<b>Biodeposit Sinking Rate (cm·s<sup>-1</sup>)</b>	<b>Biodeposit Width (cm)</b>
<i>Crassostrea gigas</i>	26	0.6 (+/- 0.1)	0.11 (+/- 0.2)
<i>Mytilus edulis</i>	25	0.6 (+/- 0.2)	0.2 (+/- 0.0)
<i>Mytilus galloprovincialis</i>	22	0.7 (+/- 0.3)*	0.21 (+/- 0.1)

Average biodeposit sinking rates were virtually identical for all three species (Table 13). *Crassostrea gigas* and *Mytilus edulis* biodeposit sinking rates were 0.6 +/- 0.1 cm·s<sup>-1</sup> and 0.6 +/- 0.2 cm·s<sup>-1</sup>, respectively. Average rate for *M. galloprovincialis* was 0.7 +/- 0.3 cm·s<sup>-1</sup>. Biodeposit sinking rates for



each species were remarkably consistent, as evidenced by the small standard deviation values (Table 13). Not surprisingly, results of paired t-tests (not shown) demonstrated no significant differences between biodeposit sinking rates of the three species ( $p > 0.15$ ). *C. gigas* biodeposits, although produced by larger bivalves, were approximately half as wide as those of *M. edulis* and *M. galloprovincialis* (Table 13).



## Discussion

### Clearance Rate

Of the endogenous factors that may potentially affect bivalve CR, including size, spawning condition, and general condition, only size significantly influenced CR in our experiments. Bivalves with greater tissue dry weights had higher clearance rates per individual but lower clearance rates per unit body weight. The relationships between both clearance rate per individual and clearance rate per unit body weight (CR) are well documented in the literature (for review see Bayne, 1976). Larger bivalves may have greater CR per individual because they have larger ctenidia that facilitate greater amounts of water flow and that offer a larger capture area for suspended particles (Hughes, 1969; Bayne, 1976). There are numerous examples of allometric equations presenting clearance rate per individual as a function of bivalve size (tissue dry weight or shell length) in the literature (see Riisgard, 2001 for review) and for some bivalves, e.g. *Mytilus californianus*, the relationship between clearance rate per individual and tissue dry weight is adequately described by a power function for a wide range of body weights (Bayne, 1976). For some bivalve species, however, a power curve fits the data for small animals only. In these allometric equations (e.g.  $CR = aW^b$ ), the size exponent (b) generally decreases as animal size increases (Rao, 1953; Segal *et al.*, 1953; Thompson and Bayne, 1974). For example, the weight exponent for *M. edulis* less than 1 g tissue dry weight was determined as 0.39 but it decreased for larger animals (Bayne, 1976). There is a reduction in the growth rate of the gill in larger (heavier) *M. edulis* (Vahl, 1973; Bayne, 1976) and it has also been observed that the activity of gill cilia is reduced in old specimens of *M. edulis* and *Modiolus modiolus* (Schlieper *et al.*, 1958; Winter, 1973). Both gill size and reduction in cilia activity may contribute to the reduction in the rate of increase in filtration activity observed for larger animals. Lower clearance rates (per unit body weight) in larger bivalves than smaller bivalves, as observed in this study and reported in the literature, may be related to physiological status (Bayne, 1976; Denis *et al.*, 1999). Increased energy demands associated with the development and ripening of gametes (Bayne and Widdows, 1978; Grant *et al.*, 1993; Hatcher *et al.*, 1997) may cause a negative impact on clearance rate as energy is moved away from feeding and food processing and into reproduction. Although clearance rates in *Crassostrea gigas* have been shown to be affected by gametogenesis (Bougrier *et al.*, 1995), and to be particularly depressed during the later stages of gametogenesis (Lefebvre *et al.*, 2000), no clear relationship between *C. gigas* gonad index and CR was found in this study. Cranford and Hill (1999) suggested, for *M. edulis*, that low *in situ* feeding rates were generally associated with reproductive demands but this was not evident for *M. edulis*, or *M. galloprovincialis*, in this study.

The decrease in clearance rates per unit body weight with increasing bivalve size, in combination with CR leveling off at a specific tissue dry weight, which is shown for the 3 bivalve species in this study, is also reported in the literature for *M. edulis* and *M. californianus* (see Bayne, 1976 for review). Bayne (1976) plotted CR versus tissue dry weight for *M. edulis* and *M. californianus* and, in the resulting curves, CR



leveled off at a tissue dry weight between 1 and 2 g for *M. edulis*, and between 8 and 10g for *M. californianus*; these curves are similar to those shown in Figures 3 and 4 for *M. edulis* and *M. galloprovincialis*, respectively, and in Figure 2 for *C. gigas*.

Exogenous factors that may affect CR in bivalves include salinity, temperature, diet quality and diet quantity. Salinity and temperature were relatively stable in this study and the variation in diet composition (3 different combinations of algae) for the mussel experimental trials did not have a significant independent effect on CR. However, algal cell concentration had a significant, but small, effect on *C. gigas* CR. *C. gigas* fed medium algal cell concentrations (19,000 to 30,500 cells·ml<sup>-1</sup>; 1.13 to 1.87 mg·l<sup>-1</sup>) had a significantly higher CR than *C. gigas* fed high algal cell concentrations (35,000 to 46,500 cells·ml<sup>-1</sup>; 2.13 to 3.07 mg·l<sup>-1</sup>). While *C. gigas* CR in this study were generally lower than those reported in the literature, most of the very high rates reported were recorded in oligotrophic waters or areas with generally low food availability. For example, Dupuy *et al.* (2000) relate a high CR in *C. gigas* (11.8 l·h<sup>-1</sup>·gdrywtg<sup>-1</sup>) in an oligotrophic lagoon to the low concentration of suspended particulate matter (0.65mg·l<sup>-1</sup>). Fiala-Medioni *et al.* (1983) measured *C. gigas* CR at 5.7 l·h<sup>-1</sup>·gdrywtg<sup>-1</sup> with 10,000 cells/ml; the authors consider 10,000 cells·ml<sup>-1</sup> a high concentration of algae. The CR measured by Fiala-Medioni *et al.* (1983) is considered a low CR for *C. gigas* and is comparable to others reported in the literature for *C. gigas* fed similar “high” algae concentrations (Deslous-Paoli and Jousset, 1987; Bourgrier *et al.*, 1997; Dupuy *et al.*, 1999). The lower *C. gigas* CR in our experiments may be attributed to the comparatively higher cell concentrations used and potentially, to the mature gonad in most of the specimens. However, additional research using *C. gigas* specimens at all stages of gonad development is required to test the latter hypothesis.

Clearance rates for *M. edulis* in this study are within the range of *M. edulis* CR reported in the literature for a variety of studies using algae culture or natural seston (Widdows *et al.*, 1979; Lucas *et al.*, 1987; Smaal and Widdows, 1994; Widdows *et al.*, 1995; Hawkins *et al.*, 1996). Although the relationship between *M. edulis* CR and algae cell concentration was confounded by a size bias towards larger animals in the high cell concentration trials, the significant increase in CR between *M. edulis* fed low (10,000 to 12,500 cells·ml<sup>-1</sup>) and medium (19,000 to 30,500 cells·ml<sup>-1</sup>) algal cell concentrations, given no size bias in those groups, is considered a valid relationship. Given the comparatively high algal cell concentrations used in this study, it is unlikely that this result reflects a climb in CR up to a maximum rate which is maintained, or decreases, with additional increases in algal cell concentration as described by Riisgård and Randløv (1981) and Winter (1973). In fact, the increase in *M. edulis* CR with increasing cell concentration appears to contradict the results of Winter (1973) which showed that clearance rates of *M. edulis* decrease as algal cell concentrations increase from 10,000 to 40,000 cells·ml<sup>-1</sup> (Winter, 1973). It must be noted, however, that the increase in *M. edulis* CR observed in this experiment is small, however, and is of questionable physiological significance.



The results of field studies on the response of *M. edulis* CR to an increase in food (seston) quantity are difficult to compare with laboratory studies using algal culture. Field studies report large variability in *M. edulis* CR, using natural seston, even when quantity of seston were similar (e.g. Cranford and Hill, 1999) and this variability may be partially due to the silt versus algae components of the diet. Higher concentrations of silt do not appear to have a depressing effect on CR and, in fact, several milligrams of silt in suspension may be a condition for *M. edulis* to fully exploit clearance potential (Kiorboe *et al.*, 1980). Hence, CR response to increased food quantity must be considered in light of the silt and algae content.

Although no statistically significant effect of algal cell concentration on CR was found in this study, and the size bias towards larger animals in the high cell concentration trials must be considered, mean CR of *M. galloprovincialis* did decrease slightly with increasing algal cell concentration. *Mytilus galloprovincialis*, based on a recirculating flume experiment with algal cultures, demonstrated low CR ( $0.2$  to  $0.4 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ ) with high cell concentration ( $8500$  to  $11000 \text{ cells}\cdot\text{ml}^{-1}$ ) and much higher CR ( $0.5$  to  $2.5 \text{ l}\cdot\text{h}^{-1}\cdot\text{gdrywt}^{-1}$ ) when cell concentration was low ( $1300$  to  $5800 \text{ cells}\cdot\text{ml}^{-1}$ ) (Denis *et al.*, 1999). In this study, the lowest algal cell concentration used,  $10,000 \text{ cells}\cdot\text{ml}^{-1}$ , corresponded to the highest concentration used by Denis *et al.* (1999). While the general trend of CR decreasing with increasing cell concentration agrees with the literature, the CR measured in this study were an order of magnitude higher, for comparable cell density, than those reported by Denis *et al.* (1999). However, Fisher *et al.* (1996) report CR of  $0.2$  to  $2.0$  per individual mussel at  $27,400 \text{ cells}\cdot\text{ml}^{-1}$  which are comparable to the clearance rates measured in this study.

This study supports findings reported in the literature that clearance rate (per unit body weight) decreases as bivalve tissue dry weight increases for *M. edulis* and provides new information for *C. gigas* and *M. galloprovincialis*. The decrease in CR with increasing algal cell concentration for *C. gigas* agrees with results from comparable experiments on other filter feeding bivalves reported in the literature, although clearance rates in this study were generally lower than those reported in the literature. The lower clearance rates may be due to the comparatively high algal cell concentrations used or to the reproductive state of the *C. gigas* specimens used (most were ready to spawn).

*M. edulis* CR measured in this study are well within the range of CR values reported in the literature for this species. The increase in CR with increasing cell concentration for *M. edulis* that occurred from low to medium algae cell concentrations is unexpected given contrary results reported in the literature but the measured increase in CR is very small and of questionable physiological significance. *M. galloprovincialis* CR in this study were comparable to those reported in 1 of 2 studies found in the literature. Although clearance rates for *M. galloprovincialis* were highly variable and demonstrated no statistically significant relationship with algal cell concentration, a trend for CR to decrease with increasing



algal cell concentration was observed in the data. For both *M. edulis* and *M. galloprovincialis* trials, the potential effect of high algal cell concentration on CR was confounded by a size bias towards larger animals in the trials using high algal cell concentrations and additional research is required to clarify the effects of bivalve size versus food quantity at high algal concentrations.

Regardless of algal cell concentration, *C. gigas* mean clearance rates were significantly lower than those of *M. edulis* and *M. galloprovincialis*. Mean clearance rates of *M. edulis* were significantly lower than those of *M. galloprovincialis*. This observed trend in CR for the 3 bivalve species is difficult to compare with results from literature studies which used a variety of experimental conditions and none of which compared clearance rates, in the same experiment, for these 3 bivalves. While the trend in clearance rates for the 3 species may reflect inter-specific physiological differences, it also reflects the comparative size of the bivalves. Additional research is required to differentiate between these possibilities.

### **Biodeposit Production Rate**

Biodeposit production rates for *C. gigas* in this study (3.6 to 71.52 mg·ind<sup>-1</sup>·d<sup>-1</sup>) were comparable to the rate reported for individual *C. gigas* in Japan (diet undefined) (Ito and Imai, 1955) and were shown to increase with increasing oyster weight. An increase in biodeposit production rates, per individual oyster, is reported by Haven and Morales-Alamo (1966); in laboratory studies, large specimens (33 g wet weight) of *C. virginica* were demonstrated to produce twice and four times more feces and pseudofeces, respectively, than smaller oysters (11 g wet weight). Biodeposit production rates per unit body weight (BPR) of *C. gigas* decreased with increasing oyster tissue dry weight, similar to the relationship noted for *C. gigas* tissue dry weight and CR, and oyster tissue dry weight accounted for 25% of the variance in BPR. While comparable data for *C. gigas* are unavailable in the literature, similar results have been reported in the literature for *M. edulis* (see below).

Results similar to those obtained for *C. gigas* in this study were reported for *M. edulis* by Tsuchiya (1980), who demonstrated that although positively correlated to body size, biodeposit production per unit body weight was higher in small mussels than in large mussels. Similarly, Callier *et al.* (2006) reported that *in situ* biodeposition by 0+ (11 to 14 months old) and 1+ (23 to 26 months old) *M. edulis* cohorts was a function of mussel size: the 1+ mussels produced, on average, 1.6 times more biodeposits than the 0+ mussels. In contrast, the amount of biodeposits produced per unit body weight was greater for smaller mussels than for larger mussels. The



linear relationship between biodeposit production ( $\text{mg}\cdot\text{gdrywt}^{-1}\cdot\text{d}^{-1}$ ) and mussel tissue (dry wgt) reported by Callier *et al.* (2006) was temporally variable but consistently negative. This result is not surprising, given that physiological rates are an allometric function of body size and thus decline with the relative body surface area available for oxygen diffusion, which decreases with respect to body size as the organism grows (Hawkins and Bayne, 1992). Biodeposit production rates of *M. edulis* in this study are comparable to those reported by Cranford and Hill (1999), as ranging from 20 to 80  $\text{mg}\cdot\text{gdrywt}^{-1}\cdot\text{d}^{-1}$  seasonally. However, BPR for *M. edulis* in this study were lower than those of *in situ M. edulis* of comparable size, reported by Callier *et al.* (2006); the slightly higher BPR measured in the latter study may be related to diet quantity and quality, as discussed below. Although, as observed for *M. edulis* by Callier *et al.* (2006), *M. edulis* BPR in this study was negatively correlated with tissue dry weight, the linear relationship was not significant. It is hypothesized that because *M. edulis* used in this study were one age cohort, unlike those of Callier *et al.* (2006), there was insufficient variation in size to significantly effect BPR. Biodeposit production rates of *M. galloprovincialis* in this study demonstrated no linear relationship with tissue dry weight which again, may be partially due to the limited size range of specimens.

Because exogenous factors, such as food quality and quantity, have been reported to affect biodeposit production, comparison of data with those in the literature must incorporate the potential influence of these factors. Food quality has been demonstrated to affect the proportion of feces and pseudofeces, as well as biodeposit production, in many bivalves; for example, increasing silt and clay content in seston resulted in an increase in pseudofeces production by the cockle, *Cerastoderma edule*, and the clam, *M. mercenaria* (Robinson and Langton 1980; Iglesias *et al.*, 1992; Urrutia *et al.*, 2001). Hence, biodeposit production rates obtained in this study, which used pure algae diets, may be expected to be lower than those obtained at comparable concentrations of natural seston *in situ*, due to the difference in diet composition (food quality). This may partially explain the lower biodeposit production rates measured in this study compared to those measured *in situ* for *M. edulis* by Callier *et al.* (2006) and for *C. virginica* by Haven and Morales-Alamo (1966); in both cases, however, food quantity may have also played a role (see below).



The effects of food quantity on biodeposit production rates of marine bivalves have been reasonably well studied but results are far from homogenous. In this study, the range of algal cell concentrations used, which reflects the seasonal range of food concentrations at the field study sites, had no obvious effect on the BPR of *M. edulis* and *M. galloprovincialis*. BPR of *C. gigas* was significantly higher for oysters fed high algae concentrations than those fed medium algae concentrations but, as noted in the results section, examination of the data suggests that this may be due to the large sample size rather than any physiologically relevant difference in means. Haven and Morales-Alamo (1966) reported similar results based on a major study of biodeposition by *C. virginica*:

no statistical correlation was found between seasonal variation in production of feces and pseudofeces and seasonal variation in total seston. Also similar to the results of this study are those of Hawkins and Bayne (1992), who showed that *M. edulis* excretion (including biodeposit production) may vary greatly over small periods of time (8 h) without any apparent relationship with exogenous influences. Based on a seasonal *in situ* study of *M. edulis*, Cranford and Hill (1999) reported weight-specific fecal production rates, comparable to those reported in this study, and for which daily seston abundance and composition, and environmental parameters, could explain only 28% of the variation in the bivalves' daily ingestion rates and, by inference, a comparable percentage of variation in associated daily fecal production rates. Cranford and Hargrave (1994) reported that fecal production rate for *Placopecten magellanicus* were poorly correlated with seston flux.

Despite the results discussed above, several studies report an increase in biodeposit production for filter feeding bivalves in response to increasing concentrations of food. For example, *C. gigas* and *M. edulis* have been reported to maintain relatively high clearance rates even when seston concentrations increase (Hawkins *et al.* 1998) through increased production of pseudofeces. Tsuchiya (1980) observed that *M. edulis* produced more pseudofeces, but a constant amount of feces, when food concentrations increased and that production rate of mussel biodeposits increased with increased flow rate. Such a response to flow is reasonable considering that the food supply to filter feeders is a function of both concentration and flow velocity (Muschenheim, 1987; Cahalan *et al.*, 1989). Callier *et al.* (2006) found that *in situ* biodeposit production of *M. edulis* was greater towards the end of August than in the middle of August and suggested that this difference may be due to differences in seston concentration, which ranged from 9.0 to 27.4 mg·l<sup>-1</sup> from the middle to end of August.

The apparent dichotomy of results in regard to the effect, or no effect, of food concentration on BPR is likely due to the specific food concentrations used and threshold values for pseudofeces production in the bivalves. For example, Hawkins *et al.* (1998) used very high seston concentrations, ranging from 20 to 90 mg·l<sup>-1</sup>, to demonstrate the almost linear relationship between increasing food concentrations and filtration rate plus rejection (pseudofeces production) rate. The concentration of suspended particulate material at which *M. edulis* first produces pseudofeces is approximately 4.5 to 5 mg·l<sup>-1</sup> (Widdows *et al.*,



1979). Hence, it seems likely that in our study and others in which field-based algae concentrations are used, the threshold for pseudofeces production is not reached and as a result, the relationship between algae cell concentration and bivalve BPR is less predictable. The comparatively small, albeit realistic, range of algae concentrations used in this study may have contributed to the failure to detect a relationship between food concentration and feces production.

Although individual biodeposit production rates were higher for individual *C. gigas* than for individual mussels, biodeposit production rates per unit body weight were significantly lower for *C. gigas* than for *M. edulis* and *M. galloprovincialis*, regardless of food concentration. BPR for *M. edulis* and *M. galloprovincialis* were not significantly different from each other. Similar results were reported for *M. edulis* and *C. virginica* by Tenore and Dunstan (1973): for all food concentrations tested, *M. edulis* had a higher biodeposition rate per unit body weight than *C. virginica*. The trend in biodeposit production rates for the 3 species in our study may reflect inter-specific, physiological differences but may also reflect the relationship between bivalve size and BPR discussed above. As observed for CR, additional research on BPR is required to differentiate inter-specific, physiological differences

#### **Clearance Rate and Biodeposit Production Rate**

For each of the 3 bivalve species in this study, CR and BPR demonstrated no linear, or other visibly obvious, relationship. This may be largely a result of the highly variable biodeposit production rates measured in this study. It might be anticipated, *a priori*, that CR adjusts to maintain a maximal ingestion rate and that biodeposit production rate would remain relatively constant, using algae diets, if filtration rate is constant. An increase in silt content of the diet, or very high algae concentrations (see below), would be expected to increase biodeposit production rate through increased pseudofeces production, but neither scenario is applicable to this study. Additional research, modified as noted earlier in the discussion, may help to clarify the relationship between CR and BPR.



## Biodeposit Sinking Rate

A better understanding of bivalve biodeposition dynamics, particularly parameterisation of biodeposit sinking rates, quality, and erosion characteristics, is required in order for ecosystem models to make accurate predictions of benthic loading and subsequent effects on the local environment (Henderson *et al.*, 2001; Section 4). Particle sinking rate is a dominant parameter requiring characterization in all particle tracking models (Chamberlain *et al.*, 2006). The sinking rates of particles depend on several properties of the particles including size, density and porosity and are often based on variations of Stokes Law (Hendricks and Eganhouse, 1992; Cromey *et al.*, 1998). However, Stokes Law assumes that particle density and shape are similar across all size ranges and this is not an assumption that is valid for bivalve fecal particles. As a result, bivalve biodeposit sinking rates are poorly estimated by simple sinking rate equations such as Stoke's law, as has been demonstrated by Chamberlain (2002) and Giles and Pilditch (2004). Experiments are required, therefore, to obtain estimates of the sinking rates of these particles. Unfortunately, data on sinking rates of bivalve biodeposits are relatively rare and most relevant data has been generated only recently (Chamberlain *et al.*, 2006). For example, despite a thorough literature search, no data on the sinking rates of feces and/or pseudofeces of *Crassostrea gigas* or *Mytilus galloprovincialis* were found. A limited number of recent studies report sinking rates for feces and pseudofeces of *M. edulis*, as well as for *Perna canaliculus* and *Atrina zelandica*.

In this study, pseudofeces could not be visually detected in the biodeposits, which consisted largely of fecal pellets and strings. The bivalves were fed algal diets and, therefore, production of pseudofeces would be less likely than if fed a combination of algae and silt, as occurs in natural seston (Robinson and Langton, 1980; Iglesias *et al.*, 1992; Urrutia *et al.*, 2001). However, pseudofeces may be produced by these bivalves when fed high concentrations of algae. The production of pseudofeces by filter feeding bivalves in media of high phytoplankton concentration is well documented in the literature (Loosanoff and Engle, 1947; Dean, 1957; Galtsoff, 1964; Jorgensen, 1966). In this experiment, algae concentrations ranged from 2.46 to 2.61 mg·l<sup>-1</sup>. The concentration of suspended particulate material at which *M. edulis* first produces pseudofeces is approximately 4.5 to 5 mg·l<sup>-1</sup> (Widdows *et al.*, 1979). Hence, it seems unlikely that in this study pseudofeces were produced by *M. edulis*, although comparable thresholds for pseudofeces production for the other two species could not be found in the literature. Surprisingly, Callier *et al.* (2006) observed no pseudofeces when *M. edulis* were fed natural seston *in situ* at concentrations of suspended particulate material ranging from 9.0 to 27.4 mg·l<sup>-1</sup> but the authors suggest that undetected pseudofeces may have been present in low quantities in the flocculated, sedimented material.

Mean sinking rate of the biodeposits of *C. gigas*, *M. edulis* and *M. galloprovincialis* in this study were not significantly different from each other. Although sinking rates of fecal pellets of *M. edulis* (4 cm shell length, slightly smaller than *M. edulis* in this study) fed natural seston have been reported to settle at velocities <0.5 cm·s<sup>-1</sup> (Chamberlain, 2002; Chamberlain *et al.*, 2001), the sinking rates in this study are



comparable to those reported by Callier *et al.* (2006) for biodeposits of *M. edulis* (3 to 7 cm shell length) fed natural seston. The range in sinking rates of *M. edulis* biodeposits ( $0.27$  to  $1.81 \text{ cm}\cdot\text{s}^{-1}$ ) obtained in the study by Callier *et al.* (2006) was larger than in this study and may be due to the range of algae and silt in the natural seston diet used in the former study. Food quality affects the density of biodeposits and, hence, sinking rate (Chamberlain *et al.*, 2006). For example, biodeposit sinking velocities are known to vary significantly with diet for the green-lipped mussel, *Perna canaliculus* (Giles and Pilditch, 2004). Sinking velocities of fecal pellets produced by *P. canaliculus* on an algae-dominated diet were approximately 3 to 4 times lower than those of pellets produced on the natural or silt-dominated diets. Sinking velocities of pseudofeces produced on the algae-dominated diet were approximately 3 to 5 times lower than those of pseudofeces produced on the natural diet and 5 to 10 times lower than those of pseudofeces produced on the silt-dominated diet. Hence, pseudofeces produced on the natural and algae-dominated diets settled 50 to 70% slower than feces produced on the same diet, potentially because of their fluffy texture, but sinking velocities of silt-dominated diet feces and pseudofeces were similar (Giles and Pilditch, 2004). Similar results have been reported for *Atrina zelandica*, a filter-feeding, benthic pinnid bivalve (up to 30 cm) common in New Zealand (Powell, 1979; Cummings *et al.*, 1998). Despite no significant differences in biodeposit shape or size (length or width), biodeposits from *A. zelandica* fed phytoplankton and silt had a sinking velocity ( $3.0 \pm 1.3 \text{ cm}\cdot\text{s}^{-1}$ ) twice that of biodeposits from *A. zelandica* fed only phytoplankton ( $1.7 \pm 0.8 \text{ cm}\cdot\text{s}^{-1}$ ). Hence, fecal pellets (Chamberlain, 2002; Giles and Pilditch, 2004) or biodeposits (Miller *et al.*, 2002; Callier *et al.*, 2006) from mussels fed on diets with high silt content sank more rapidly than those from mussels fed on diets high in algae or natural diets. A similar relationship between sinking rate and diet was found for mussel pseudofeces (Giles and Pilditch, 2004).

Sinking rates reported for *A. zelandica*, above, are high in comparison to rates reported for other bivalves but *A. zelandica* specimens used were considerably larger (18.5 to 26 cm) than those used by most other studies (Miller *et al.* 2002). While it has been suggested that fecal pellet length is more a function of current speed than mussel morphology, it has been documented that larger mussels produce larger (wider) fecal pellets (Giles and Pilditch, 2004; Callier *et al.*, 2006) and larger (greater surface area) pseudofeces (Giles and Pilditch, 2004) than smaller mussels. The width of feces and pseudofeces may positively affect sinking rates. Callier *et al.* (2006) reported that fecal pellet width (0.3 to 1.8 mm in width) was better correlated with fecal pellet sinking rate for *M. edulis* than were either surface area or length of fecal pellets. Width of fecal pellets of *M. edulis* ( $0.20 \pm 0.00 \text{ cm}$ ) and *M. galloprovincialis* ( $0.21 \pm 0.01 \text{ cm}$ ) used in this study were relatively consistent. *C. gigas* fecal pellets, although approximately half as wide ( $0.11 \pm 0.2 \text{ cm}$ ) as those of either *Mytilus* species, sank at a comparable velocity. Based on the range of *M. edulis* fecal pellet widths reported by Callier *et al.* (2006), it is possible that the difference in widths observed in this study was not sufficient to result in different sinking rates. In addition, the trend



for larger bivalves to produce wider biodeposits, which have faster sinking rates, may not be applicable to inter-specific comparisons.

The sinking rates for bivalve biodeposits reviewed above emphasize that these rates, and hence biodeposit dispersal and flux to the benthos, depend on available diet and bivalve size and thus, could differ significantly between locations, seasons and populations of different size structure. As a result, biodeposit sinking rates based on laboratory studies must be applied to field conditions within the context of diet and bivalve size. Obviously, however, biodeposit dispersal is also dependent on the flow environment, which may be dominated by bi-directional, wave and tidal currents interacting with bottom topography; estimating the length scales for dispersal of biodeposits is complicated in the marine environment. Miller *et al.* (2002) found a weak, but significant, negative relationship between sinking velocity of *A. zelandica* biodeposits and distance downstream of the bivalves, suggesting that other variables (e.g. current flow) were important in addition to sinking velocity. Furthermore, flow modification due to farm structures is only just being quantified (Plew *et al.*, 2005).

A small variation not only in biodeposit sinking velocity, but also in current velocity or water column depth, may have a significant impact on the extent of biodeposit dispersion (Giles and Pilditch, 2004). For example, Callier *et al.* (2006) demonstrated great differences in the potential dispersion of fecal pellets from two *M. edulis* cohorts, 0+ and 1+. An average summer current speed of  $5.5 \text{ cm}\cdot\text{s}^{-1}$  was used with sinking velocity data and distance between the mussel lines and the bottom. For the 0+ cohort, the average sinking velocity was  $0.79 \text{ cm}\cdot\text{s}^{-1}$  and the distance between the 0+ mussel lines and the bottom was 1 to 3.5 m; hence, the initial horizontal dispersion from the lines was estimated between 7 and 24.4 m. In contrast, with the same current speed, wider fecal pellets from the larger 1+ mussels sank at an average velocity of  $0.97 \text{ cm}\cdot\text{s}^{-1}$  and the distance below 1+ mussel lines was between 0 and 1.3 m. Hence, the initial horizontal dispersion from the lines was estimated to be between 0 and 7.4 m. The current velocity reached  $18 \text{ cm}\cdot\text{s}^{-1}$  during strong wind events, however, so that the estimated dispersion of fecal pellets from the 0+ and 1+ mussels was as much as 79.7 and 24.1 m, respectively (Callier *et al.*, 2006). This example also demonstrates that, despite the relatively slow sinking speed and even for deep sites, the time for material to reach the bed will be relatively rapid (minutes) compared to time-scales of variability important for modelling larger-scale transport processes (e.g. tides) (Harstein and Stevens, 2005).



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