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CLEARANCE RATES OF *VILLOSA IRIS* (BIVALVIA: UNIONIDAE) FED DIFFERENT RATIONS OF THE ALGA *NEOCHLORIS OLEOABUNDANS*

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ABSTRACT

We investigated effects of algal cell concentration and mussel size (shell length) on the clearance rate (CR) of the rainbow mussel, *Villosa iris*. Mussel were either batch-fed a single ration of algae for 24h, or were fed three different algal rations that were replenished every hour for 3 hours. Mean CR of *V. iris* batch fed a single ration (1.3×10^6 c/mL, 8.8 mg/L) of algae (*Neochloris oleoabundans*) decreased with time and the concomitant decline in cell concentration, but never reached zero. As length increased, so did clearance rate ($p<0.0001$). Pseudofeces were produced by all individuals in the first three hours of feeding, and were irregularly produced as algal cell concentration dropped later in the test.

Mussels fed the lowest ration (0.34 mg dry wt/L) maintained elevated CRs over time with no production of pseudofeces; CR of mussels fed the middle ration (1.02 mg dry wt/L) decreased with time, and produced pseudofeces – intermittently. CR's of mussels fed the high ration (3.4 mg/L) increased with time, and produced a large amount of pseudofeces throughout the test. Following the premise that the optimum ration yields greatest net particle ingestion without incurring sorting costs of pseudofecal production, we estimated that *V. iris* would require 2.8 mg dry wt of algae (4.2×10^8 cells of *N. oleoabundans*) on a daily basis, based upon CR's measured for the middle ration in this study.

KEY WORDS Freshwater mussels, clearance rate, algal ratio

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INTRODUCTION

Freshwater mussels of the Unionacea are among the most widespread bivalves (Banarescu, 1990). Where mussels are present, they often comprise a significant proportion of the benthic biomass (Strayer et al., 1994; Newton et al., 2011), and play important roles in particle removal, nutrient cycling, and in structuring benthic species assemblages in lakes and streams (Howard & Cuffey, 2006; Vaughn & Spooner, 2006; Atkinson et al., 2010; Allen et al., 2012). Thus, their decline may be adversely affecting aquatic ecosystem integrity. To improve our understanding of the effects of suspension-feeding populations of freshwater mussels on aquatic ecosystems, we need quantitative information on various feeding processes. Additionally, conservation efforts to restore populations through propagation and culture require a better understanding of freshwater mussels' feeding physiology and the effect of particle concentration on feeding rates in order to develop cost-effective feeding regimes that meet the animals metabolic demands.

The bulk of information on bivalve feeding physiology has been collected on commercially significant marine bivalves (clams, mussels, and oysters). In marine bivalves, clearance rate (CR) generally increases with increasing particle concentration (number of cells or dry weight of cells per unit volume) to a maximum and then progressively declines (Foster-Smith, 1975; Bayne et al., 1976; Riisgard, 1991); thereby, regulating particle retention rate and the amount of material available for ingestion (Winter, 1978; Navarro & Winter, 1982; Navarro et al., 1992) as well as avoiding excessive pseudofecal production and energy costs associated with sorting (Jorgensen, 1990). Paterson (1984) observed this pattern in the freshwater mussel, *Elliptio complanata*, as did Roper and Hickey (1995) in *Hyridella menziesi*. Pusch et al. (2001) and Vanderploeg et al. (1995) found that the natural seston concentrations in their studies, however, did not saturate the clearance rate capacity of their unionid mussels. Bricelj and Malouf (1984) suggested that a bivalve's success in maximizing its energy gain in a turbid environment depends on a combination of two features: a high selection efficiency pre-ingestion which may prevent significant loss of nutritious food material in pseudofeces (Kiorboe & Mohlenberg, 1981), and producing copious amounts of pseudofeces to reject bulk excess or irritating material and to preferentially reject undesirable particles to improve quality of material ingested. These bivalves would be better adapted to cope with high suspended loads than other species, which control ingestion mainly by reducing clearance rate (Winter, 1970; Foster-Smith, 1975). Indeed, Ward and MacDonald (1996) showed that some bivalve species demonstrate high plasticity in how they respond to a broad range of suspended particle concentrations, by maintaining both high and low CR, high pseudofecal production, and utilizing pre-ingestion selection capabilities to select for desired food items otherwise diluted by non-nutritive material in turbid environments. They suggested that the ability of a species to compensate for increased suspended particle concentrations depends on the capacity adaptations of the species. For example, species that typically reside in low turbid environments were unable to compensate for increased particle concentrations and demonstrated high mortality and poor growth (Cranford & Gordon, 1992). Gascho Landis et al. (2013) recently reported that high suspended particle concentrations resulted in reproductive failure for freshwater mussels. Indeed, researchers have looked at several factors known to affect clearance rate in bivalves, such as flow rate, temperature, particle size and concentration, body size and reproductive phase (Kryger & Riisgard, 1988; Tankersley & Dimock, 1993; McCall et al., 1995; Vanderploeg et al., 1995; Spooner & Vaughn, 2008). Clearance rates of freshwater mussels exposed to a variety of particle concentrations, however, needs further examination.

The rainbow mussel, *Villosa iris*, is a small-sized mussel (< 70 mm) commonly found in small rivers in riffle-glide environments, and has a wide distribution in the St. Lawrence, upper Mississippi, Ohio, Tennessee and Cumberland River basins. It is bradytictic (long-term brooder), which generally spawns in late summer. Gravid females hold their glochidia (larvae) over the winter in their marsupial gill area until spring when the glochidia are released to encyst on a suitable host-fish, where metamorphosis into a juvenile mussel is completed. While the conservation status of the *V. iris* is presently of no concern, the Tennessee River system contains a significant number of endangered freshwater mussel species. Many of these endangered species also are small-sized, long-term brooders that inhabit similar environments as *V. iris*. Differences in feeding physiology and the ability of a suspension-feeder to adapt to changes in seston concentrations may contribute to niche partitioning within a bed of mussels (Vanden Byllaardt, 2011), and may explain why one species is imperiled and the other is not within the same drainage. Nevertheless, until empirical data on feeding requirements of endangered species are available, we propose to use data from this study of *V. iris* as a guide for the development of captive care protocols for endangered *Villosa sp*. and *Epioblasma sp*. of freshwater mussels. Our objectives were to evaluate the clearance rate of *V. iris* over 24 h from a single batch-feeding, investigate the effect of algal cell concentration (ration) on clearance rate, and estimate the algal cell concentration to feed mussels on a daily basis that could meet their presumed energy balance in captivity.

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METHODS

Clearance rates of *V. iris* were measured in two experiments. In the first experiment (Single Ration Test, SRT), mussels were fed a single ration of algae, and clearance rates (mL/h) and algal cell concentrations (c/mL) were monitored for 24 h. In the second experiment (Multiple Ration Test, MRT), clearance rates (mL/h/g dry tissue weight (dtw) of our standard-sized mussel) were measured for mussels fed one of three algal rations; these rations were maintained for three 1-h feeding periods. Calculation of clearance rates is described later in this paper. Although clearance rate, filtration rate, and pumping rate are sometimes used interchangeably, they measure different physiological functions. According to Bayne et al. (1993) clearance rate is the "rate at which water pumped by the animal is cleared of particulate matter by filtration (mL/h)"; filtration rate is the "rate at which seston or particles are removed from suspension (mg/h)"; pumping rate (mL/h) is the total volume of water that is pumped through the gills and is usually higher than the CR. In this paper we determined the clearance rate of *V. iris* from the clearance of suspended material according to Coughlan (1969).

Mussel acclimation and algae culture

Twelve male V. iris (shell length 37-52 mm, mean $\pm$ SD = 43.4 $\pm$ 3.8 mm) were collected from Copper Creek, Scott Co., Virginia, U.S.A. in June, 1997 for use in the single ration test. We collected 30 male mussels (shell length 37-51 mm) in February, 1998 for the multiple ration test. We measured shell length from the anterior to posterior ends of the shells. Mussels were transported to the laboratory in 10L of aerated river water in a cooler. Mussels were then acclimated from field temperatures (17°C in summer and 12°C in winter) at 1.2 °C.h^{-1} to laboratory temperatures of 20°C. Mussels collected in the summer (SRT) were acclimated overnight at ambient temperatures of 20°C; they also were batch fed 1 x 10^6 c/mL (6.8 mg dry wt/L) of Neochloris oleoabundans and allowed to feed for 24h. Mussels collected for the MRT were collected at river temperatures of 11°C. After a 3h transport to the laboratory, temperatures in the cooler of mussels reached 14°C. Mussels then were held individually in 250 mL containers without food, and acclimated from 14 to 20°C at 1.2 °c.h^{-1} for 5 h to the new temperature regime.

We selected the green alga *N. oleoabundans* for this study because it was shown to be suitable for rearing mussels (Gatenby et al., 1997; Patterson, 1998). Algae were grown in *Neochloris* media (Gatenby et al., 1997) under continuous white fluorescent light (photon flux: 35 $\mu E \cdot m^{-2} \cdot s^{-1}$) at 20 + 1°C. Some mussels clear particle sizes of 5-10 µm more efficiently than smaller particles (< 5 µm) (Paterson, 1986; Miura & Yamashiro, 1990; Tankersley & Dimock, 1993). We harvested *N. oleoabundans* during log phase growth when the algae ranged 5-10 µm (average ca. 6.2 µm) in diameter. Ten 100 mL aliquots of algae were dried for 8 h at 90°C to calculate dry weight.

Experimental procedure

Single ration test

Following acclimation, mussels were transferred to individual aerated chambers containing 250 mL (pH 8.0) of a 1:1 mix of well water and dechlorinated city water. They were then batch fed 1.3 x 10^6 c/mL (8.8 mg dry wt/L) of *N. oleoabundans*. We collected 10 mL water samples after 1, 2, 3, 5, 8, and 24 h, and reduction in particle concentration was determined. Algal cells were not replenished after each sampling interval, simulating an aquaculture practice of feeding mussels only once per day. We determined particle concentration at each sampling using a Coulter Counter, Model ZM, aperture 100 µm. The production of feces and pseudofeces was visually observed and noted for each mussel during the first 8 h of the test.

Clearance rate calculation

Clearance rates (CR) in mL/h for the single ration test were calculated using the following equation (Coughlan 1969):

$$CR = \{V \cdot (n.t)^{-1}\} \cdot [\ln(conco \cdot [concf]^{-1})]\}$$

where V = volume (mL) of feeding chamber, n = 1 mussel in each feeding chamber, t = duration of sampling interval, and $conc_o$ and $conc_f$ are cell concentration at the beginning and end of each sampling interval, respectively.

Because we intended to return these test animals to the creek, we did not calculate CR on a dry weight basis.

Statistical analyses

Clearance rate data were log-transformed prior to analysis to stabilize the variance. We used repeated measures analysis of variance (rmANOVA) to examine clearance rates over time (Proc Mixed, SAS version 8.2, SAS Institute, Cary, North Carolina), with shell length included as a covariate. This allowed us to determine if there was an effect of mussel size on CR, while also examining the CR trend in time. The least squares (LS) means for clearance rates (i.e., the CR means adjusted for mussel size) were compared.

Multiple ration test

In preliminary tests, we observed decreased feeding activity (reduced valve gape, closed apetures) with increased time in the laboratory. We began the multiple ration test, therefore, immediately upon acclimating the mussels to laboratory temperatures. Following acclimation, mussels were transferred to individual aerated chambers containing 250 mL (pH 8.0) of a 1:1 mix of well

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water and dechlorinated city water. Ten individuals (per ration) were fed one of three rations (cell concentration) of *N. oleoabundans*: ration A was $5.0x10^4$ c/mL (0.34 mg dry wt/L), ration B was $1.5x10^5$ c/mL (1.02 mg dry wt/L), and ration C was $5.0x10^5$ c/mL (3.4 mg dry wt/L). The experiment lasted 3 h and was initiated when each mussel exhibited shell gape and apertures were visible. Particle concentrations were measured at the end of each 1 h period using a Coulter Counter (Model ZM), and any algal cells that were cleared were replaced. The production of feces and pseudofeces was not quantified by dry weight; however production of pseudofeces and feces were visually observed and noted for each mussel.

Clearance rate calculation

In the multiple ration test, we calculated hourly weight-specific clearance rates as mL/h/dry tissue weight of standard mussel used in this test (see CR calculations below); hereafter, referred to as mL/h/g_{std}. All clearance rates were adjusted for animal weights using an allometric relationship because the magnitude of most physiological responses is dependent on the size of the animal. Thus, clearance rate was expressed in terms of the average weight of the animal used in this experiment to avoid extrapolating to sizes not adequately represented in our investigation (Kreeger & Newell, 2001). This allometric relationship between size and clearance rate was derived by least squares regression of the log_e-transformed dry tissue weight and the loge-transformed raw clearance rates. All mussels were randomly assigned to each treatment. We assumed that there was equal distribution of mussel sizes in each treatment as was represented in the total sample size. The average dry tissue weight for all of the mussels was 0.289 g, and this was considered to be the standard animal weight. The calculation of CR, therefore, was as follows:

$$CR = \text{antilog}\,[(\ln CR_{raw}) + \{b \times ((\ln dtw_{std\ mussel}) - (\ln dtw_{rep.}))\}]$$

where Cr_{raw} = raw clearance rates (mL/h); b = the allometric weight exponent (0.854), which was generated by regression of the pooled CR and size data used in the MRT; dtw_{std} = 0.289 g, mean dry tissue weight of standard-sized mussel (standard-sized mussel = mean dry weight of 30 mussels used in the MRT); $dtw_{rep.}$ = dry tissue weight (g) of replicate mussel. Three algae-only controls demonstrated that no algae settled out of suspension ($p<0.01$).

Statistical analyses

We compared mussel clearance rates between the 3 hourly feeding periods within each treatment and among treatments. First, we re-examined the relationship between shell length (ranging 37-51mm) and clearance rate, expressed as both mL/h and mL/h/g_{std} (0.289 g standard-sized mussel), using simple linear regression. We examined whether the effect of shell length on clearance rate was sufficiently removed when clearance rates were expressed on a per weight basis (for all rations combined). Having determined that the size effect was removed, we analyzed the effect of algal ration (cell concentration) and time on CR that were weight-corrected by allometry (see CR Calculations; CR = mL/h/g_{std}). We used a two-factor repeated measures analysis of variance (two-way rmANOVA) to determine the time (within subjects) and treatment (between subjects) effects on Ln-transformed CR data (Proc Mixed, SAS, version 8.2, SAS Institute, Cary, NC). The Tukey-Kramer honestly significant difference (HSD) multiple comparison test was used to identify treatment differences within time periods. In order to estimate the amount of algae to feed mussels on a daily basis, the effect of algal ration on total amount of organic material cleared in 3 hours was evaluated using One-way ANOVA followed by Duncan's Multiple Range Test (DMRT) that tested for between treatment differences. The number of algal cells was log-transformed to stabilize the variance.

RESULTS

Single ration test

Repeated measures analysis of variance (rmANOVA) showed that clearance rates decreased with time ($p = 0.0001$) (Figure 1); there also was a significant covariate effect of shell length on clearance rate ($p=0.0001$), indicating that the relationship between CR and time was not independent of shell length. Further examination of the adjusted (LS) means indicated a significant non-linear relationship for CR with time ($p=0.001$). This relationship is described by the following equation (Figure 1; the predicted points from the relationship are represented by Ps and the actual observed values are represented by 0s.):

$$LS_CR = \beta_1 + \beta_2 / \beta_3 + time$$

where β_1=2.78, β_2=1.63, and β_3=0.33.

Thus, CR slowed down with time and the concomitant decline in cell concentration, but never reached zero (Figure 1). The covariate estimate for length was 0.1042 ($p<0.0001$), indicating that as length increased, however, so did clearance rate. For example, mean algae concentration had dropped over ten-fold in the chambers with the three largest mussels (54-57 mm), and the concentration dropped seven-fold in the chambers holding the smaller-sized (37-44mm) mussels. Pseudofeces were produced by all individuals in the first three hours of feeding, and were irregularly produced as algal cell concentration dropped later in the test. Clearance rates at 24h ranged 18.2 – 24.8 mL/h and approximately 6.5 mg dry wt of algae was cleared in 24 h.

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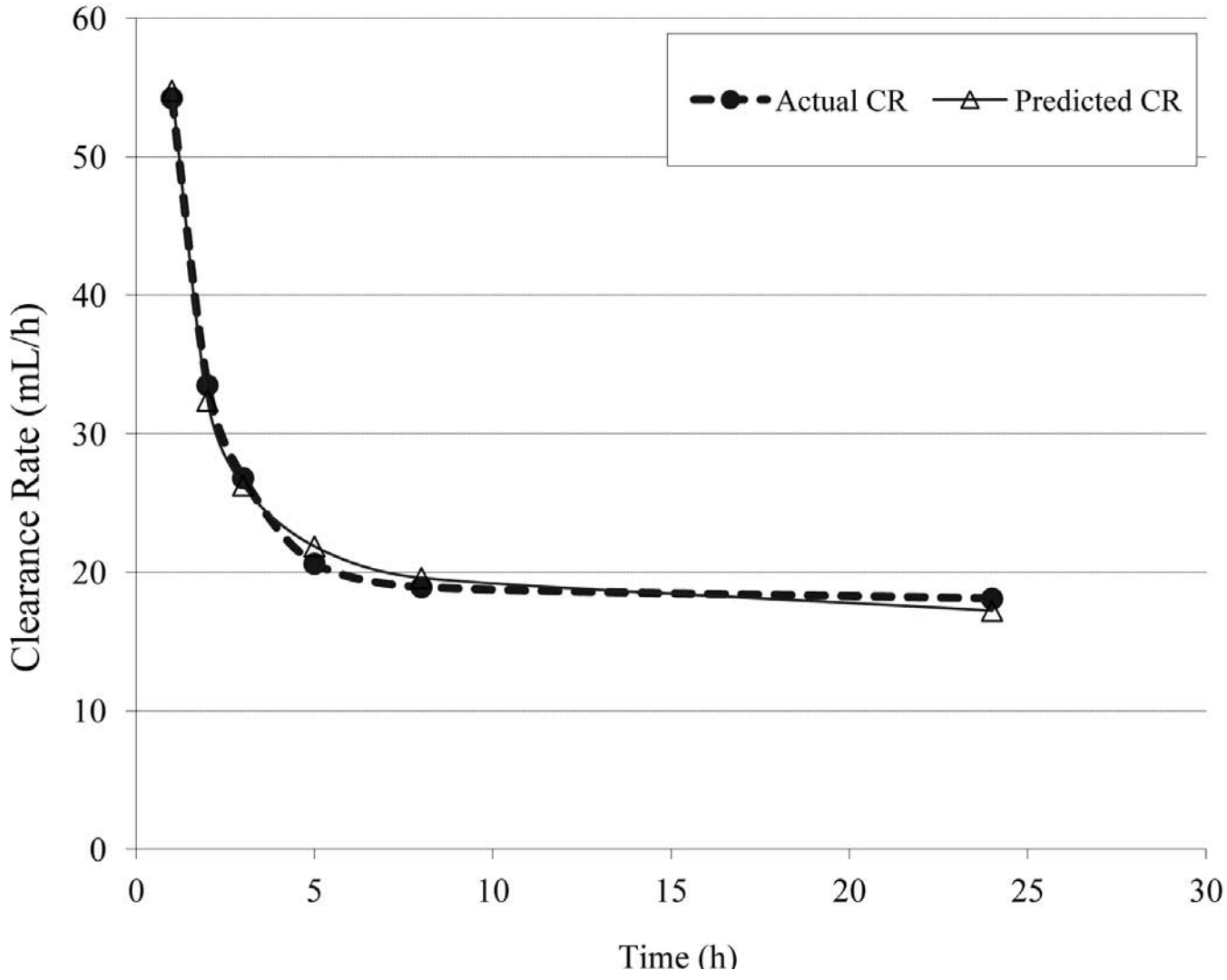


FIGURE 1

Change in clearance rate over time for Villosa iris fed a single ration (1.3 x 106 algal c/mL) of algae. This relationship is described by the following equation:

$$LS_CR = \beta_1 + \beta_2 / \beta_3 + time;$$

the predicted points from the relationship are represented by Δ's and the actual observed values are represented by •'s.

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Multiple ration test

Linear regressions showed that clearance rates expressed as mL/h were dependent on shell length ($p = 0.012$); however, clearance rates that were weight corrected by allometry (expressed as $mL/h/g_{std}$) showed no significant relationship with shell length ($p = 0.448$). The effect of length on clearance rate was, therefore, removed when clearance rates were expressed on a weight-specific basis, and subsequent analyses were performed on these values.

The rmANOVA showed that there was a significant interaction effect of treatment with feed hour (time) ($p=0.0035$), which then precluded the main effects of either factor alone. In other words, the main effect of time was not independent of the main effect of treatment. So, we looked at the interaction of the two rather than the effect of either alone.

The Tukey-Kramer HSD indicated that in hours 1 and 2 there was a significant difference between ration C and rations A and B (Table 1). Three hours after initiation there was a difference between ration A and B, but there was no longer a difference between rations B and C, nor between A and C. Mussels fed ration A maintained elevated clearance rates through time. Mean clearance rate of mussels fed ration B significantly decreased with time, and mean clearance rate of *V. iris* fed ration C significantly increased with time. In addition, the total number of cells cleared by V. iris during the entire 3h feeding period was significantly different among treatments (Table 2; $p<0.05$). Mussels fed the highest ration (C) cleared the greatest amount of algae followed by mussels fed ration B and then ration A. A greater percentage of the available algae, however, was cleared from ration A than from rations B and C (Table 2). All mussels in ration C produced pseudofeces during all 3 feeding times. We did not observe production of pseudofeces by mussels fed ration A; however, we observed intermittent production of pseudofeces in ration B, which also varied between mussels during the 3 h test. In other words, not all mussels in ration B produced pseudofeces all the time. We estimated an average ingestion rate during the 3 h period at 0.05 mg/h for Ration A and 0.15 mg/h for Ration B based upon CR and total number of cells removed from suspension. We could not estimate ingestion rate for Ration C because we did not quantify pseudofeces.

DISCUSSION

Bivalve clearance rates are a function of physiological and environmental factors including gill type, body size, body condition, temperature, current speed, particle size, particle type and concentration (Winter, 1970, 1978; Walne, 1972; Way et al., 1989; Vanderploeg et al., 1995; Spooner & Vaughn, 2008). The interplay between clearance rate and ingestion also depends on the digestibility and nutritional value of the diet. For example, in Willows' (1992) model for optimal digestive investment, energy costs for particle removal, sorting, and digestion are balanced by the energy gained from the food type. He cautions that at low concentrations of food, energetic costs of filtration and digestion become a significant component of the overall energy budget, and will determine the upper limit for sustained clearance rate. At very high particle concentrations, bivalve clearance rates may decline which would reduce excessive pseudofecal production and energy costs associated with sorting (Jorgensen 1990). Indeed, suspension-feeding marine bivalves have been shown to be highly adaptive with the ability to regulate clearance rates within a range of suspended particle concentrations (Ward & MacDonald, 1996). Our work with *V. iris* suggests that freshwater mussels are equally as adaptive as their marine counterparts.

Single ration test

Villosa iris fed a dense suspension of algae (1.3×10^6 c/mL, 8.8 mg/L), produced an abundance of pseudofeces within the first hour, with pseudofecal production declining over time with the decrease in algal cell concentration. *Villosa iris* initially cleared at a high rate, lowered clearance rate by over 56 % within 2 h, and between 8 and 24 h, clearance rates appeared to level off presumably to maintain particle ingestion rate. Greater clearance during the first feeding hour may have reflected an empty gut, with all or most food from the previous acclimation period having been assimilated or passed through the gut. Higgins (1980) reported an increase in clearance rate when unfed oysters were re-introduced to food. The provision of food to starved *Mytilus edulis* also stimulated an increase in filtration and O2 consumption, followed by a reduced clearance rate upon satiation of the digestive system (Thompson & Bayne, 1974; Bayne et al., 1976; Riisgard, 1991). Alternatively, *V. iris* may have lowered its clearance rate to avoid excessive sorting costs as demonstrated by other suspension feeding bivalves.

Multiple ration test

Very low food concentrations can lead to shell closure, reduced clearance rate, and reduced metabolism (Riisgard & Randlov, 1981; Jorgensen et al., 1986). Indeed, several species of marine bivalves have been shown to reduce their feeding activity in the laboratory when particle concentrations dropped by 50% of initial concentration (Bricelj & Malouf, 1984; Higgins, 1980; Navarro & Winter, 1982). In ration A, *V. iris* maintained elevated CRs such that on average, 59% of the particles were removed each hour. The moderate decrease in CR

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in the third feeding hour could indicate that feeding activity was altered in response to the particle concentration dropping by over 50% of the starting concentration. It is unclear whether these elevated CRs (mean CR=145.5 mL/h/g) at low rations would be maintained beyond 3 h as high CR's have added energy costs and could result in lower scope for growth. We estimated that mussels in ration A ingested an average of 0.05 mg/h.

In ration B, we suspect that *V. iris* maximally cleared (160.2 mL/h/g) in the first hour also as a result of food deprivation during acclimation, which was followed by a decrease in clearance rate presumably to regulate particle ingestion in accordance with the replenished ration. This relationship between clearance rate and ingestion rate following gut satiation is common among bivalves (Hornbach et al. 1984; Way, 1989; Riisgard, 1991). Virtually no pseudofeces were produced by mussels fed Ration B; therefore, we estimated their average ingestion rate during the 3 h period at 0.15 mg/h, which presumably yielded a positive and balanced energy rate.

Paradoxically, clearance rates of *V. iris* fed at the highest concentration (3.4 mg/L) increased over time. Copious amounts of pseudofeces were produced over time, but they were not quantified. It is plausible that *V. iris* regulated ingestion following the strategy proposed by Bricelj and Malouf (1984) when exposed to high particle concentrations, by increasing clearance rate and pseudofecal production. Thompson and Bayne (1972) concluded that *M. edulis* suffered nutritive stress when feeding for long periods in high concentrations of suspended particles. *Villosa iris* was fed on a highly nutritious diet; thus, it is unclear whether they would suffer nutritive stress over a longer period of high ration maintenance (of a nutritious diet). The energetic gains or losses of this ration would depend on the costs of maintaining high CR and high pseudofecal production over a long period.

Winter (1978) suggested that the greatest cell density found to not produce pseudofeces was the optimum food concentration at which the costs of filtration activity were reduced to a low-energy consuming level and all algal cells cleared were ingested. For *V. iris*, this "pseudofeces threshold" appeared to be near 1.02 mg/L of *N. oleoabundans* where only a small amount of pseudofeces was observed. We estimated the average ingestion rate during the 3 h period for mussels at 0.15 mg/h (or 3.6 mg dry wt per 24h). Thus, a lower CR combined with more energy (mg dry wt of organic material) gained (cleared) by mussels fed 1.02 mg/L than mussels fed 0.34 mg/L presumably yielded the better energy investment. We believe that ration C is suboptimal to the lower rations because mussels could have greater energetic costs associated with high CR, sorting and pseudofeces production. As well, the continuous production of pseudofeces implied an excess of food was provided, and this would not be cost-effective for a hatchery. Similarly, mussels fed the single ration (8.8 mg dry wt/L) cleared approximately 6.5 mg dry wt of algae in 24 h, but they too produced an abundance of pseudofeces early on and then intermittently throughout the test. We assumed that excess algae allocated to the production of pseudofeces were not ingested. Therefore, 6.5 mg dry wt of algae in 24 h would exceed that which is necessary to support this mussel's condition in the lab, and not economical for a hatchery.

We concluded that 1.02 mg/L of *N. oleoabundans* would best balance the energy needs of V. iris in a laboratory-setting. At the hourly CR's measured for the middle ration in this study, *V. iris* would require 3.6 mg dry wt of algae (4.2 x 108 cells of N. oleoabundans) on a daily basis. These results were consistent with latter findings on the effect of particle concentration on the carbon budget (unpublished data), and our estimates of total particles ingested based upon four components that we found accounted for all of the 14C activity ingested by mussels. In our study of the C-budget, ingestion went down with increasing cell concentration, and we hypothesized that excess material removed from suspension and directed to the production of pseudofeces was not ingested.

Besides animal size and species, differences in CR of suspension-feeding bivalves also have been attributed to the quality of the test diet (Newell & Jordan, 1983; Kreeger & Newell, 2001). Kryger and Riisgard (1988) suggested that algal diets are higher in quality than natural seston quality. We compared our results with results for other freshwater mussels regardless of whether seston, laboratory algae, or bacteria was the food source (Table 3). Because food quality is known to affect CR, we only included studies that had an organic component in their "test diets", such as algae or natural seston. We extrapolated CR data reported as mL/g/min to mL/g/ h and data reported as mL/h we converted to mL/g/h if the bivalve dry tissue weight was reported. As expected, *V. iris* fed at any algal ration had a total mean CR greater than another small-sized mussel, *Toxolasma texasense* fed bacteria at 1-2 x 107 c/mL. The CR of *V. iris* fed algae was similar, however, to the CR of larger-sized (>80 mm) mussels, *Anodonta anatina* and *Unio tumidus*, fed a high concentration of seston (Table 3). In addition, *A. anatina*, *U. crassus*, *U. pictorum*, *U. tumidus* and *Lampsilis siliquoidea* fed algae showed CRs ten-fold greater than similar sized mussels fed natural seston, and nearly ten-fold greater than the *V. iris* fed algae in this study (Patterson, 1984; Kryger & Riisgard, 1988; Vanderploeg et al., 1995; Pusch et al., 2001) (Table 3). We attributed these differences in CR to differences in diet quality (animals feed at a higher rate on

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higher quality food), the size and physiological status of the animal, and the environmental tolerances of the species (Silverman et al., 1997). Clearance rates in bivalves can be underestimated due to experimental conditions that do not simulate the habitat of infaunal bivalves, and methodology used to quantify CR (Riisgard, 2001). Thus, the clearance rates reported here could very well be different than that which would be observed under natural conditions. The complex relationships between diet quality, ration, mussel species, physiological or reproductive status and CR needs further examination in order to understand the effect that suspension-feeding mussels have on aquatic ecosystems, and in order to design a feeding regime that is appropriate to the nutritional needs of a suite of freshwater mussel species.

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TABLE1

Mean hourly clearance rates (mL.h^{-1}.g^{-1}) of *Villosa iris* for 3 feeding periods and at 3 algae rations: Ration A = 5x10^4 c.mL-1, Ration B = 1.5x10^5 c.mL^{-1}, and Ration C = 5.0x10^5 c.mL^{-1}. Mean CR + SD (of mean LN CR) in columns (Ration) followed by the same letter are not statistically different by Tukey-Kramer HSD; means within feed hours followed by the same upper case letter also are not significantly different by Tukey-Kramer HSD, α = 0.05.

Time Interval (h)	Ration A	Ration B	Ration C
1	108.6 ± 0.9 a, A	160.2 ± 0.5 a, A	29.9 ± 1.4 a, B
2	181.1 ± 0.4 a, A	143.4 ± 0.6 a, A	56.6 ± 0.6 a, b, B
3	146.9 ± 1.2 a, A	42.4 ± 1.8 b, B	86.2 ± 0.9 b, A, B

TABLE 2

Mean total algal cells (mg dry wt) cleared, and the percent of available algae cleared by *Villosa iris* at three different algal rations during all three feeding hours. Algal rations: Ration A = 5x10^4 c/mL, Ration B = 1.5x10^5 c/mL, and Ration C = 5.0x10^5 c/mL. Means + SD (LN cells cleared) within a column followed by the same letter were not significantly different (α = 0.05).

Ration	Mean Algal Cells (mg/h) Cleared per mussel	Mean Percent of Total Available Algae Cleared
A	7.3 x 10^6 cells ± 0.38 (0. 05 mg/h)	59.2%
B	2.2 x 10^7 cells ± 0.43 (0.15 mg/h)	54.9%
C	3.9 x 10^7 cells± 0.57 (0.26 mg/h)	30.7 %

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TABLE 3

Comparison of clearance rates (CR) from *Villosa iris* (this study) with those of other unionid mussels fed organic diets.

Species	Food Source	Ration	CR ($mL \cdot h^{-1} \cdot g^{-1}$)	Bivalve Dry wt (g)	References
Anodonta anatina	*Chlorella vulgaris*	1.2 x 10^4c/mL	827-923	3.141	1
Unio crassus	*C. vulgaris*	1.2 x 10^4 c/mL	1233-1532	2.7	1
Unio pictorum	*C. vulgaris*	1.2 x 10^4 c/mL	1060-1525	3.017	1
Unio tumidus	*C. vulgaris*	1.2 x 10^4 c/mL	875	2.4	1
Anodonta anatina	seston	8-19 mg/L	170-620	3.5	2
Unio tumidus	seston	8-19 mg/L	150-581	2.5	2
Villosa iris	*N. oleoabundans*	5.0 x 10^4 c/mL (0.34 mg/L)	181.1	0.289	3
Villosa iris	*N. oleoabundans*	1.5 x 10^5 c/mL (1.02 mg/L)	143.3	0.289	3
Villosa iris	*N. oleoabundans*	5.0 x 10^5 c/mL (3.4 mg/L)	56.6	0.289	3
Villosa iris	*N. oleoabundans*	1.3 x 10^6 c/mL	18.2 – 24.8 mL/h	na	3
Villosa lienosa	bacteria	1-2 x 10^7c/mL	393.6	0.913	4
Toxolasma texasense	bacteria	1-2 x 10^7c/mL	33.8	0.454	4
Cyclonaias tuberculata	bacteria	1-2 x 10^7c/mL	1150	0.67	4
Lampsilis ovata	bacteria	1-2 x 10^7c/mL	354	1.180	4
Elliptio dilatata	bacteria	1-2 x 10^7c/mL	459.6	1.080	4
Elliptio complanata	5 μm beads, lake water	1 x 10^3c/mL	ca. 300	6-7cm (length)	5
Elliptio complanata	2.02 μm beads in 0.45 μm filtered seston	1-3 mg/L	10.9 mL/h	na	6
Lampsilis siliquoidea	*Chlamydomonas* sp.	2.5 mm^3/L	1450	1.5-2.1	7
Actinonaias ligamentina	green and diatom algal mix	89.7 mg C/L	ca. 3*		8*
Lampsilis cardium	green and diatom algal mix	89.7 mg C/L	ca. 4		8
Truncilla truncata	green and diatom algal mix	89.7 mg C/L	ca. 8		8
Quadrula pustulosa	green and diatom algal mix	89.7 mg C/L	ca. 4		8
Amblema plicata	green and diatom algal mix	89.7 mg C/L	ca. 3		8
Fusconaia flava	green and diatom algal mix	89.7 mg C/L	ca. 3		8
Megalonaias nervosa	green and diatom algal mix	89.7 mg C/L	ca. 1		8
Obliquaria reflexa	green and diatom algal mix	89.7 mg C/L	ca. 4		8

(1) Kryger & Riisgard (1988). (3) This study. (5) Paterson (1984). (7) Vanderploeg et al. (1995).
(2) Pusch et al. (2001). (4) Silverman et al. (1995 and 1997). (6) Leff et al. (1990). (8) Spooner & Vaughn (2008).

*Clearance rates of mussels acclimated to 25°C were reported in bar graphs; thus, we approximated CR values based on unit scales in graphs

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