



Observations on the measurement and interpretation of clearance rate variations in suspension-feeding bivalve shellfish

P. L. Pascoe, H. E. Parry, A. J. S. Hawkins*

Plymouth Marine Laboratory, Prospect Place, The Hoe, Plymouth PL1 3DH, UK

ABSTRACT: Mussels *Mytilus edulis* (35 ± 2 mm shell length) were fed on cultured *Isochrysis galbana* in a flow-through system, and measures were undertaken to quantify separate effects of inflow cell concentration, outflow cell concentration, percentage reduction (i.e. recycling), and flow rate and/or feeding history on clearance rate measures (CR; l h⁻¹). Findings identify the following features of physiological regulation: (1) a lag phase in the feeding response to changes in food concentration, of around 30 min; (2) a trigger level in algal cell (chlorophyll [chl] *a*) volume/concentration (4000 cells ml⁻¹, 0.5 µg l⁻¹ chl *a*) below which filtering in most mussels ceases; (3) saturation reduction, or satiation, resulting in reduced CR and valve closure after feeding for over 2 h at 30 000 or more cells ml⁻¹ (~6 µg l⁻¹ chl *a*); and (4) at food levels between Features 2 & 3, CR increases to maximal rates (ca. 2 to 2.5 l h⁻¹ ind.⁻¹ or 6 to 7.5 l⁻¹ h⁻¹ g⁻¹ dry weight). Our findings also help resolve uncertainties associated with the measurement and interpretation of clearance rate variations. These uncertainties are associated with: differences that result from analyses of feeding responses to different components of the available seston; whether variations in seston availability are physiologically relevant; analyses of individual shellfish as compared with average responses computed for >1 ind.; and comparisons between short-term and integrated measures. We think it is important to consider whether measures of feeding are really required per individual in real time, and we stress that population averaging and temporal integration are required for the scaling up of results when simulating ecological interrelations between shellfish populations and their environments.

KEY WORDS: Filter feeding · Suspension feeding · Clearance rate · *Mytilus edulis* · Bivalve shellfish · Flow-through · Physiological regulation · Supply rate

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

The current understanding of filter feeding and its measurement in bivalve shellfish is beset by a number of controversies, and several reviews have summarised the current knowledge with a view to resolving these controversies (Jorgensen 1996, Bayne 1998, Riisgård & Larsen 2000, 2001, Riisgård 2001a,b). Riisgård (2001b) highlighted methodological shortcomings and possible misinterpretations of data, suggesting guidelines to help obtain reliable data for measures of the rate with which suspension-feeding bivalves remove particles from seawater (clearance rate, CR). Conclusions on the through-flow chamber method were collated in an ap-

pendix by Larsen (2001). These guidelines stimulated further work on the comparison of 3 different methods of measuring CR (Petersen et al. 2004), resulting in more comment and debate (Bayne 2004, Petersen 2004, Riisgård 2004). More recently Filgueira et al. (2006) again addressed the design and validation of the flow-through chamber method for CR measurements.

There is general recognition of the need to better understand the intriguing complexity of feeding processes in bivalve shellfish, which shows remarkable regulation and morphological adaptation of particle processing mechanisms within and between species, respectively (e.g. Beninger & St-Jean 1997, Beninger et al. 1997, 2008). Here, we address some key environ-

*Corresponding author. Email: ajsh@pml.ac.uk

mental factors and behavioural issues affecting variations in CR that are observed within species. Using mussels fed on cultured algae in a flow-through system, experiments were undertaken to quantify separate effects of inflow cell concentration, outflow cell concentration, percentage reduction (i.e. recycling) and flow rate and/or feeding history on CR measurements. Notable reductions or cessation of feeding were observed at both low (below trigger level) and high (through satiation) food concentrations. The interpretation of results is intended to help researchers ensure that the measures they use are 'fit for purpose', including whether averaging and integration may be required to simulate ecological interrelations between shellfish populations and their environments.

MATERIALS AND METHODS

Mussels *Mytilus edulis* L., at shell lengths of 35 ± 2 mm, were collected at Great Bull Point ($50^{\circ} 36.8' N$, $3^{\circ} 26.0' W$), near the mouth of the Exe estuary, SW England (Hilbish et al. 2002). After removal of any epibiotic growth, individual animals were numbered and then maintained in a 7 l vessel linked to a 3000 l system of circulating seawater at $14 \pm 1^{\circ}C$ and 34 ± 1 psu for a period of 10 d before the experiments. During this time and between experiments, mussels were fed on a maintenance ration (approximately 2000 cells ml^{-1}) of the microalga *Isochrysis galbana*. In the feeding experiments, 14 ind. were each placed in a chamber within a flow-through raceway system as described by Hawkins et al. (1996) to measure CR (litres of water cleared of particles h^{-1}) over separate ranges of algal cell concentration, water flow rate and percentage reduction in food particles, as water passed from inflow to outflow. Mussels were orientated with their inhalant apertures towards the incoming flow. The volume of the chambers was approximately 200 ml, and experimental flow rates through each chamber ranged from 30 to 400 $ml\ min^{-1}$, which for comparative purposes equate to maximal current speeds of $<0.003\ m\ s^{-1}$ through those chambers, measured with a Valeport 800-175 electro-magnetic current meter. As particle sedimentation within the mussel chambers was found to be negligible, 2 'control' chambers were maintained without mussels. At prescribed intervals, 20 ml aliquots from the outflows of the 2 control chambers, and from chambers containing an individual mussel, were analysed using a Coulter Multisizer II (100 μm diameter orifice) with Coulter Accucomp software. Both the total number and volume of particles between 3 and 6 μm equivalent spherical diameter, which spanned the size range of *I. galbana*, were measured and recorded in replicate 0.5 ml samples. We

concede that using an algal monoculture as food may produce different responses from those using natural seston, but it was chosen here as a more consistent and comparable food source to study the experimental variables.

Clearance rate was determined by 2 equations that are in common use:

$$CR = Fl \times [(C_i - C_o)/C_i] \quad (1)$$

where Fl is the flow through the chamber, and C_i and C_o are the number of particles per millilitre within the inflow and outflow, respectively, and

$$CR = Fl \times [(C_i - C_o)/C_o] \quad (2)$$

where the outflow concentration is used as the denominator, and is normally used in steady-state conditions (Hildreth & Crisp 1976, Riisgård 2001b). Concentrations measured in the outflows from control chambers were used as the inflow concentration to chambers containing experimental mussels. To avoid errors associated with potential recycling, we have normally restricted calculations of CR to mussels effecting a reduction in particles between inflow and outflow of 5 to 30% (Hawkins et al. 1999). It should be noted that, for analytical purposes, greater reductions have in some cases deliberately been induced here.

Experiments were designed to resolve any separate effects of inflow cell concentration, outflow cell concentration, percentage reduction (recycling) and/or flow rate (current speed) on CR. To achieve this, 3 experimental phases were undertaken:

(1) Expt 1. To confirm the time course in response of CR to stepwise increases in food supply (cell concentration), CR was measured simultaneously in 14 mussels after 5, 30, 60 and 180 min following a change from the maintenance ration to between 4000 and 200 000 cells ml^{-1} . At each cell concentration, flow rates were adjusted to give between 5 and 30% reduction in algal cell concentration between inflow and outflow.

(2) Expt 2. To resolve the effects of flow rate, percentage reduction (recycling) and outflow cell concentration on CR, a series of experiments was undertaken using constant inflow concentrations (5, 10, 20, 30 and 50 000 cells ml^{-1}), and flow rates were varied to give mean percentage reductions in cell concentration between inflow and outflow of between 5 and 65%. On the basis of findings from our first experimental phase above, and as explained in the results, CR measures were standardised after 30 min under each condition.

(3) Expt 3. To assess the relative importance of inflow concentration versus outflow concentration, mussel chamber outflow concentrations were maintained as close as possible to 10 000 cells ml^{-1} , applying the same flow rates as in Experiment 2 above, and varying the algal dosing rate (inflow concentration) to give the

same percentage reductions in cell number as had been recorded at each flow rate during Experiment 2.

Because feeding history may affect the feeding responses of bivalve shellfish, care was taken to limit the time that each experimental mussel was exposed to high food rations, thus minimising any satiation that may have led to reduced CRs. On a day to day basis, experiments administering high rations were alternated with those at low rations. At each sampling point, individual mussels that were closed and not feeding, or which were producing pseudofaeces, were noted.

Chlorophyll *a* (chl *a*) was measured in filtered aliquots from the outflows of each control tray. Samples were collected on separate 47 mm Whatman GF/F filters that were frozen at -20°C before using standard procedures for acetone extraction and fluorometric analysis (Holm-Hansen et al. 1965).

Following feeding experiments, all soft tissues were excised from each mussel and dried at 60°C before weighing to a constant total soft tissue dry weight (mean \pm 2 SE: 0.168 ± 0.019 g). Clearance rates presented here were not weight standardised. Instead, the stated mean total soft tissue dry weight affords the opportunity for others to do so at their discretion.

To resolve any effects of inflow or outflow cell concentrations, percentage reduction (recycling) and/or flow rates, the differences between mean CR measures were first tested for statistical significance with 1-way analysis of variance (ANOVA), applying a Bonferroni correction (Sokal & Rohlf 1995). Upon any suggested

difference, specific pair-wise comparisons were undertaken using a Tukey's honestly significant difference test. These analyses were carried out using SYSTAT 11 (Systat Software Inc.).

RESULTS

Experiment 1

The effects on CR of stepwise increases in *Mytilus edulis* cell concentrations after 5, 30, 60 and 180 min following change from the maintenance ration to between 4000 and 200 000 cells ml^{-1} are summarised in Fig. 1. These CR values were measured under conditions when cell reductions ranged from 5 to 30%, and were computed using Eq. (1), with inflow concentration as the denominator. Findings illustrate that reduced average clearance rates were often associated with lower numbers of actively feeding animals. At low cell concentrations averaging <4000 cells ml^{-1} (less than ~ 0.5 μg chl *a* l^{-1}), CR remained at <1 l h^{-1} throughout the time course of up to 3 h, when low proportions of the mussels were actively feeding. At cell concentrations of 5000 to 200 000 cells ml^{-1} (~ 0.6 to 25 μg chl *a* l^{-1}), CR increased to values of between 1.93 and 2.38 l h^{-1} after 30 min, compared to <1 l h^{-1} below 4000 cells ml^{-1} ($p < 0.01$). 'Saturation reduction' occurred after 180 min at high concentrations as evidenced by a higher CR of >2 l h^{-1} at 30 000 cells ml^{-1} , compared with <1.5 l h^{-1} at >100 000 cells ml^{-1} ($p < 0.05$).

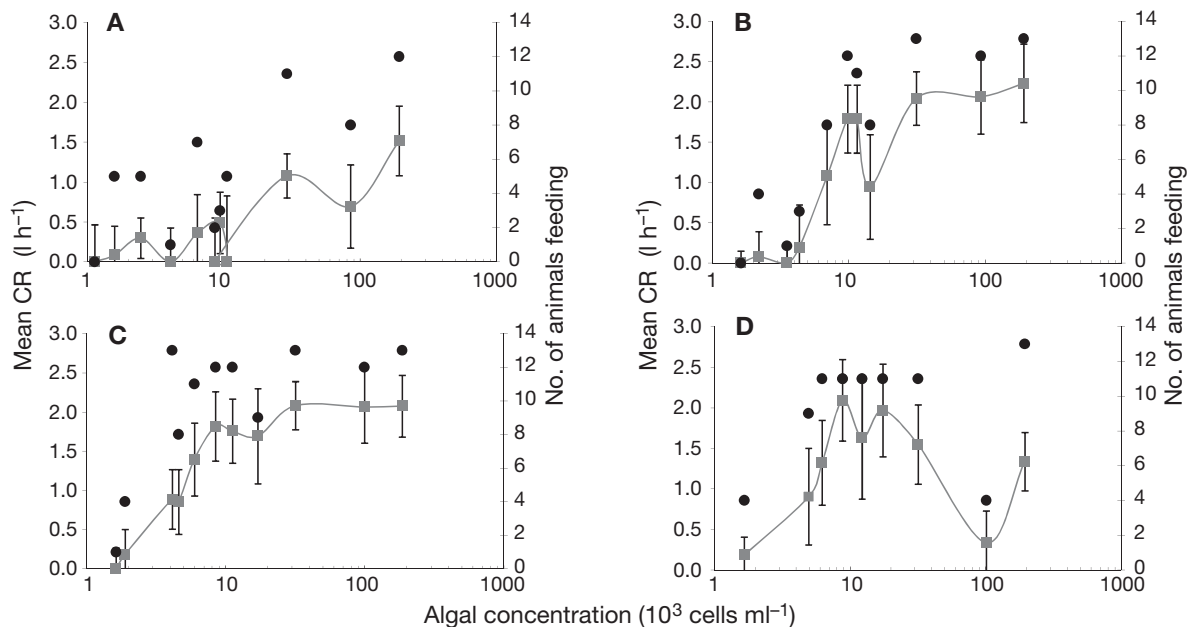


Fig. 1. *Mytilus edulis*. Relationships between clearance rate (CR; mean \pm 2 SE) and algal cell concentration after different time intervals at each food ration. (A,B,C,D) Responses after 5, 30, 60 and 180 min at each ration, respectively. Data are for all animals, including those not feeding. (●) number of animals feeding

Experiment 2

On the basis of the above results, further CR measures were standardised to 30 min after any experimental change in feeding conditions. Fig. 2 illustrates all CR measures for which the percentage reduction in cell concentration between inflow and outflow was >5%, and shows that average CR increases with cell concentration from 5000 (mean = 1.58) to around 20 000 cells ml⁻¹ (mean = 2.14) and then decreases to around 1.5 at higher concentrations of about 50 000 cells ml⁻¹. CR values at the lowest 3 concentrations were all significantly different from each other ($p < 0.01$), and that at 20 000 cells ml⁻¹ was significantly different from all others ($p < 0.01$). Fig. 3 shows how, for given flow rates, CR calculated using Eq (1) increased with percentage reduction in cell concentration be-

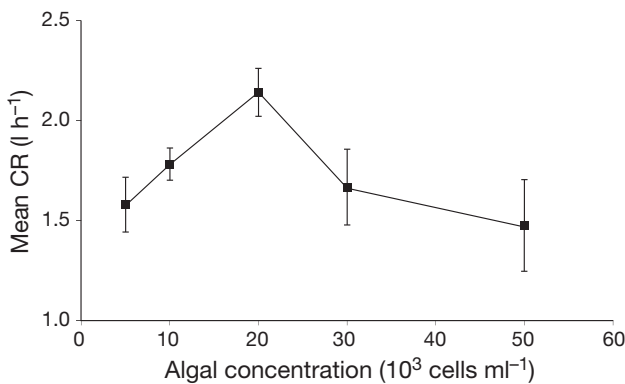


Fig. 2. *Mytilus edulis*. Relationships between clearance rate (CR) and algal cell concentration showing mean values (± 2 SE) at each nominal concentration for all animals (with a >5% reduction in particles between inflow and outflow) and at all flow rates from Experiment 2

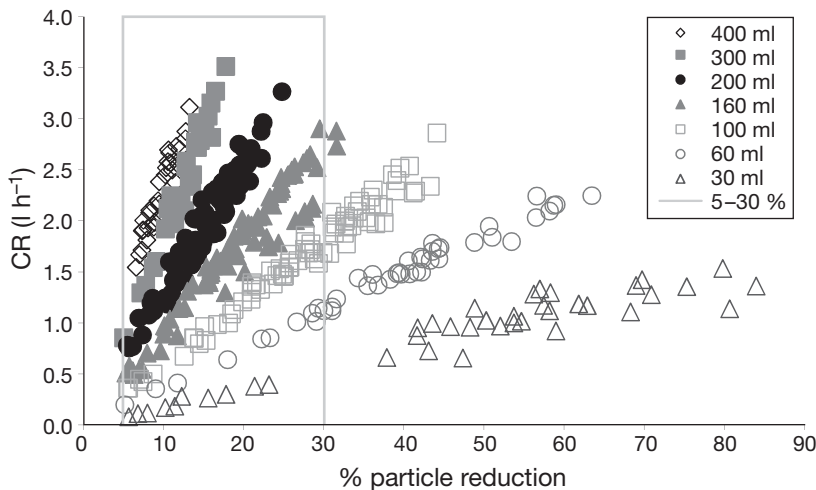


Fig. 3. *Mytilus edulis*. Clearance rate (CR) versus percentage particle reduction, plotted for all animals at each nominal flow rate (shown in the legend, ml min⁻¹). The box shows values lying between 5 and 30% reduction in particle numbers

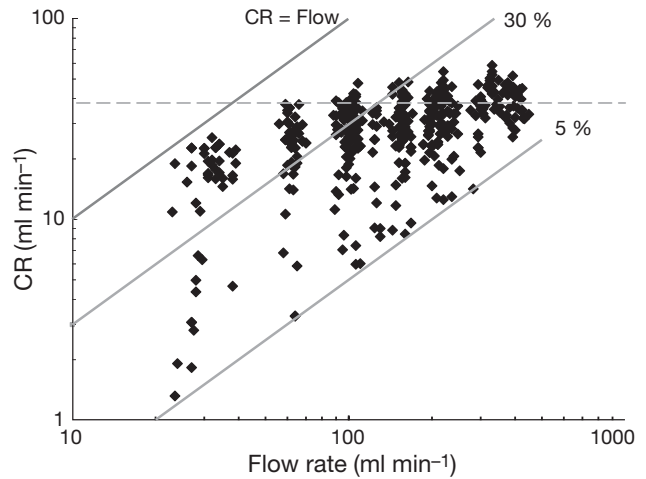


Fig. 4. *Mytilus edulis*. Relationships between clearance rate (CR) and flow rate following Riisgård (2001b), to validate the flow-through chamber design. Lines are plotted to show CR (i.e. flow rate), and the limits of 5 and 30% reduction in particles. Horizontal dashed line: theoretical CR (2.28 l h⁻¹) derived for the size of *M. edulis* used here, from the equation $CR = 7.45W^{0.66}$, where W is g dry soft tissue (Møhlenberg & Riisgård 1979)

tween inflow and outflow. Flow rate appeared to be the dominant parameter, as slopes of relations between CR and percentage reduction increased with increasing flow. Also, at low flow rates, percentage reductions were high, whilst CR remained low. The same data plotted according to Riisgård (2001b) show CR values approaching a plateau as flow rates rise above 150 ml min⁻¹, at which point virtually all values fall between the lines representing 5 and 30% reductions in particles (Fig. 4). At these higher flows of >150 ml min⁻¹, the maximal recorded average CR of around 38 ml min⁻¹ (or 2.28 l h⁻¹) is similar to that predicted by Møhlenberg & Riisgård (1979) for *Mytilus edulis* of this size.

Experiment 3

Fig. 5 illustrates relationships between CR and flow rate or percentage reduction according to inflow and outflow concentrations, including any differences resulting from the use of Eqs. (1) versus (2). Results are compared for feeding conditions with constant inflow (Fig. 5A & B) and outflow (Fig. 5D & C) concentrations, all of about 10 000 cells ml⁻¹. Fig. 5 Panels A & B show that at low flow rates, differences between cell concentrations in inflows and outflows (percentage reductions) were high due to likely recycling of

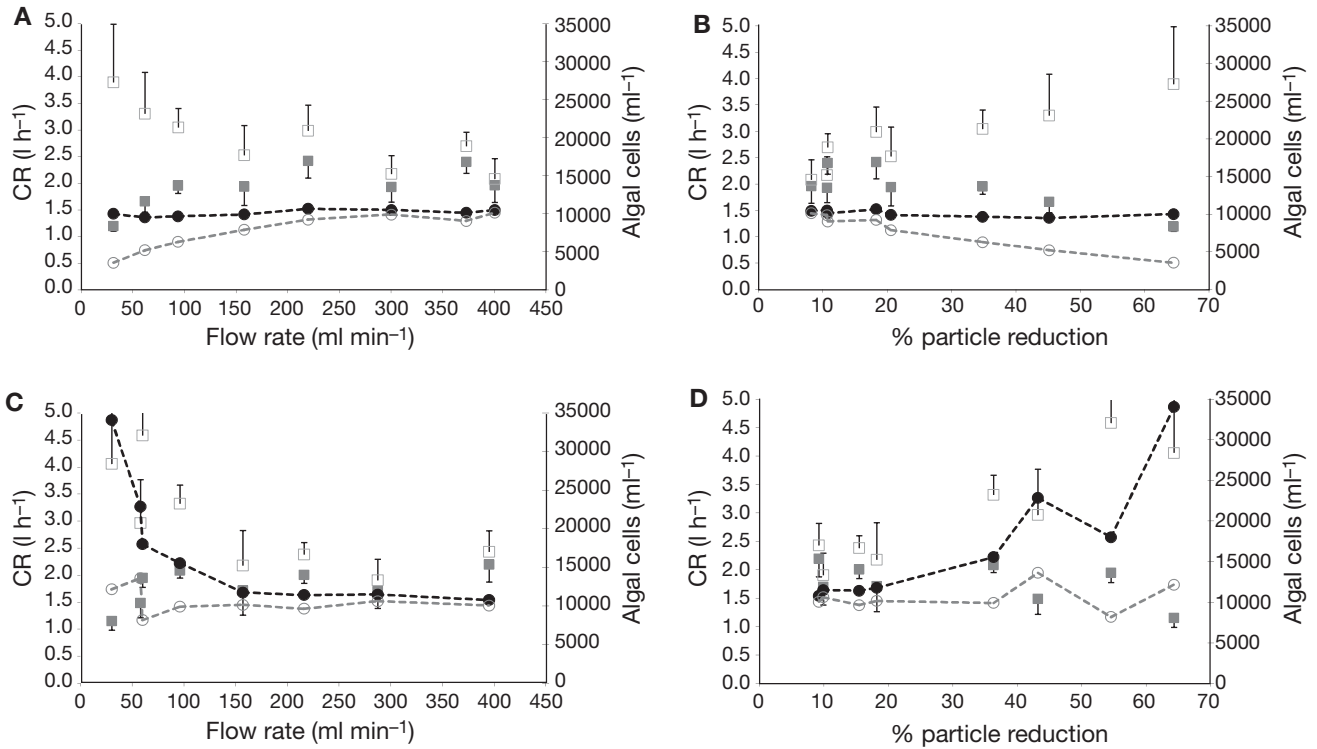


Fig. 5. *Mytilus edulis*. Relationships between clearance rate (CR; mean \pm 2 SE) and both flow rate and percentage reduction in particles, for 2 separate conditions: (A,B) maintaining a constant inflow concentration of 10 000 cells ml⁻¹ and (C,D) maintaining a constant outflow concentration of 10 000 cells ml⁻¹. CR values were derived from both Eq. (1) (■) and Eq. (2) (□), and cell concentrations are shown for both inflows (●) and outflows (○)

the water, which results in CR calculated using Eq. (2) being much greater than CR calculated using Eq. (1). This may highlight the lack of ‘steady state’ conditions in this case. Also, the latter may be depressed, as the concentration of algal cells falls towards the trigger level. At high flow rates, percentage reductions were very low, and, consequently, there is little difference between CR calculated using Eqs. (1) & (2). In this case, the animals are filtering at maximal rates, but they are only clearing a small percentage of the total supply, as most of the flow is bypassing them (see Larsen 2001). As the formulae would suggest, differences in CR values resulting from the 2 equations are clearly proportional to the percentage reduction. For example, given a reduction of 20%, CR calculated using Eq. (2) will be 20% higher than CR derived from Eq. (1).

Results from feeding conditions with constant outflows at about 10 000 cells ml⁻¹ (Fig. 5D & C) are similar to those with constant inflow concentrations (Fig. 5A & B), since, although inflow concentrations at low flow rates are markedly different, the percentage reductions, and hence CR values calculated using either Eq. (1) or (2), are similar whether measured with constant outflows or inflows. The same data plotted against elapsed time show that CR calculated using

Eq. (1) was inversely related to inflow concentration, and again illustrates a dependency related to flow rates <150 ml min⁻¹ (Fig. 6). The effect of feeding history is also illustrated here, as CR values are lower and more variable after feeding at higher inflow concentrations and for a longer time.

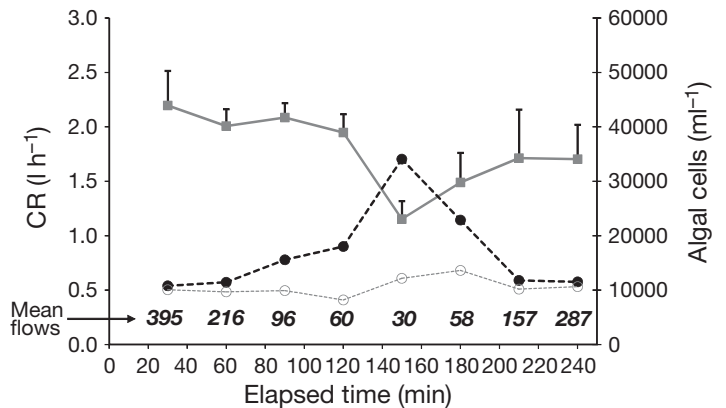


Fig. 6. *Mytilus edulis*. Interrelationships between mean clearance rates (CR; mean \pm 2 SE) derived from Eq. (1) (■) and algal cell concentrations against time elapsed from food change (i.e. Expt 3). A constant outflow concentration of 10 000 cells ml⁻¹ was maintained. Cell concentrations are shown for both inflows (●) and outflows (○), and flow rates for each measure are shown in **bold italic**

DISCUSSION

Our findings are consistent with 'physiological regulation' of bivalve filter feeding, according to indicators of such regulation suggested by Riisgård (2001a,b): (1) a lag phase was evident in the feeding response to changes in food concentration, that lag being between 5 and 30 min in the present study, when mussels were acclimated to a low food ration of approximately $2000 \text{ cells ml}^{-1}$ giving $0.25 \mu\text{g l}^{-1}$ chl *a*; (2) a trigger level was reached in algal cell (chl *a*) concentration, below which filtering in most mussels ceases, and which, in this study, appeared to be around $0.5 \mu\text{g l}^{-1}$ chl *a*; (3) saturation, or satiation, was reduced, resulting in reduced clearance rates and valve closure, after feeding for some time at high food rations, evidenced here after feeding for $>2 \text{ h}$ at $\geq 30\,000 \text{ cells ml}^{-1}$ ($\sim 6 \mu\text{g l}^{-1}$ chl *a*); and (4) at suitable flow rates and food levels between Points 2 & 3, CR increased to maximal rates, unless affected by other environmental conditions. There has been uncertainty over whether the transitions from no feeding to maximal CR, both at low and very high cell concentrations, entail abrupt stepwise events or a more gradual change resulting in a bell-shaped response of CR versus cell concentration. We suggest that different observations will result from measures based on instantaneous particle depletion by individuals, as compared with measures that average the responses of $>1 \text{ ind.}$, thus disguising significant inter-individual variability. Alternative methods may temporally integrate CR and other responses over longer time periods when measured as particle depletion within static seawater volumes (Widdows 1985, Petersen et al. 2004) or through the collection of faecal biodeposits (Cranford & Hargrave 1994, Hawkins et al. 1996, Cranford & Hill 1999, Navarro & Velasco 2003, Velasco & Navarro 2005). To illustrate this point, Fig. 7 compares differences between individual responses to food concentration in 3 mussels (Fig. 7A) with the averaged values for those same individuals (Fig. 7B).

It is clear that considerable variation in CR values can be produced by different methods of measurement. Problems have been recognised and recommendations advanced for the optimization and standardization of those methods, addressing chamber geometry, flow rate and the relative definitions of filtration rate and clearance rate (Riisgård 1977, Møhlenberg & Riisgård 1979, Widdows 1985, Riisgård 2001b). In the present study, one aim has been to re-assess our flow-through system as used by Hawkins et al. (1996, 1999, 2002) according to Riisgård's (2001b) suggested protocol. Figs. 3, 4 & 5 illustrate how—providing the normal limits of flow ($>150 \text{ ml min}^{-1}$) and percentage reductions (5 to 30%) are met—measures made with our system are entirely consistent with the guidelines pro-

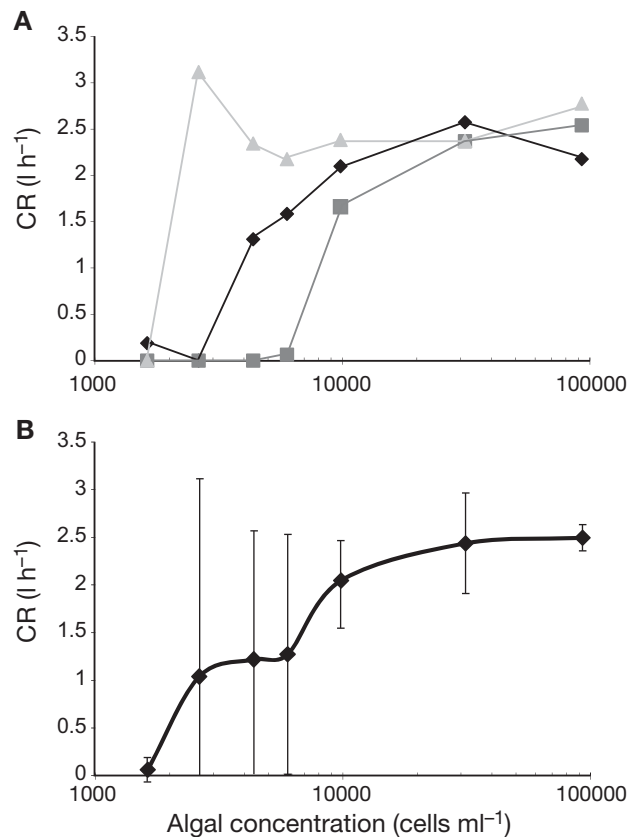


Fig. 7. *Mytilus edulis*. Relationships between clearance rate (CR,; mean $\pm 2 \text{ SE}$) and algal cell concentration, standardised to 30 min following changed feeding conditions, showing: (A) variation in 3 individual animals and (B) the mean values at each concentration for the same 3 animals

posed. Indeed, maximal CR values of around 2.5 l h^{-1} described here for mussels of 0.168 g mean dry weight and $35 \pm 2 \text{ mm}$ shell length compare favourably with related measures of 2.29 l h^{-1} from $\text{CR} = 7.45W^{0.66}$ (Møhlenberg & Riisgård 1979) and 2.42 from $\text{CR} = 0.0012L^{2.14}$ (Kjørboe & Møhlenberg 1981), where W and L are dry soft tissue weight and shell length, respectively.

Since Riisgård's (2001b) review, further methodological investigations have included inter-calibration by Petersen et al. (2004), comparing the flow-through method, the biodeposition method and the indirect (or clearance) method, which further stimulated criticism and debate (Bayne 2004, Petersen 2004, Riisgård 2004). Although this inter-calibration (Petersen et al. 2004) still left some uncertainty over the correct methodology and measurements, their suggested maximal CR of 3.6 l h^{-1} is, in fact, close to estimates from early published equations, e.g. $\text{CR} = 7.45W^{0.66}$ (Møhlenberg & Riisgård 1979). Widdows (1985) showed that the different methods (static, flow-through and steady-state) and their relevant equations should produce similar CR values. Also, as we have highlighted above, the dif-

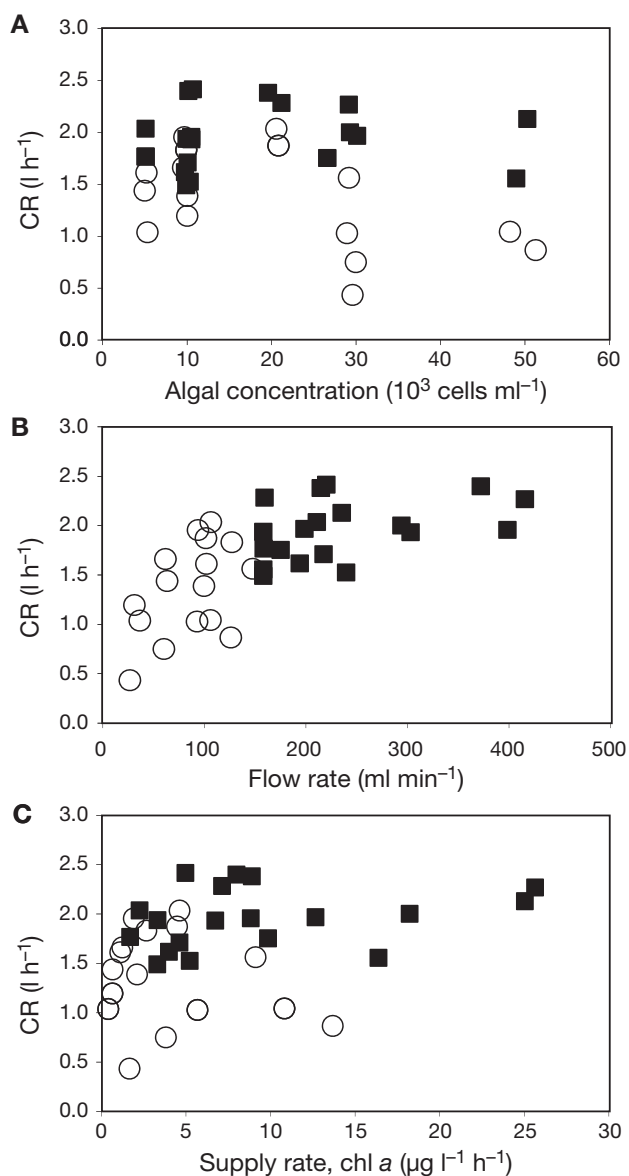


Fig. 8. *Mytilus edulis*. Mean clearance rates (CR) of up to 14 animals, at each flow rate and each inflow algal concentration, expressed as a function of: (A) inflow algal concentration, (B) flow rate and (C) supply rate ($\mu g\ chl\ a\ l^{-1}\ h^{-1}$). (\square) flow rates of $>150\ ml\ min^{-1}$; (\bullet) flow rates of $<150\ ml\ min^{-1}$

ference in CR values using Eqs. (1) & (2) for a flow-through system is proportional to the percentage reduction in particles. A clear validation of 2 different flow-through systems was made by Filgueira et al. (2006). They investigated the fluid dynamics in individual cylindrical experimental (flow-through) chambers (ICEC) and then, with *Mytilus galloprovincialis*, established that the system complied with the requirements for accurate CR measurements as suggested by Riisgård (2001b). Their proposed protocol for validation—identifying phases where CR is dependent on flow rate, where it is independent of flow, and the tran-

sitional phases—allows simple analysis of any flow-through chamber, even those used in previous studies. An average of 20% (range 13 to 25) reduction in particles is recommended for valid measurements, and our Eq. (1) is deemed correct for the flow-through chamber method. Accuracy and validity of the method relies to some extent on a suitable ratio of animal size to chamber size, and the suggested scaling of the chamber by reference to the allometric length–volume relationship of mussels is both simple and practical. Filgueira et al. (2006) also validate a mesocosm system by comparison with results from the ICEC, so, overall, their work represents a major step forward in the debate on measurement of clearance rates in bivalves.

Figs. 5 & 6 show the difference between CR values using the 2 equations, and confirm that Eq. (1) is the better representation of true CR. However, care needs to be taken over the limits of flow rate and percentage reduction, and also feeding history. Results presented here validate our methods for measurement of clearance rates, and confirm that guidelines for the limits of percentage particle reduction suggested by some previous authors were correct (Hawkins et al. 1999, 2002, Larsen 2001, Filgueira et al. 2006).

Our findings for *Mytilus edulis* complement those illustrating regulatory feeding behaviour in the New Zealand green-lipped mussel *Perna canaliculus* (Hawkins et al. 1999) and the Chinese scallop *Chlamys farreri* (Hawkins et al. 2002). CR has been observed to increase with the availability of natural suspended sediments in turbid environments, up to experimental maxima of as much as about $80\ mg\ total\ particulate\ matter\ l^{-1}$ (e.g. Hawkins et al. 1996). However, particles suspended in turbid environments are dominated by silt, when chl *a* may only be present at up to about $3\ \mu g\ l^{-1}$ (e.g. Iglesias et al. 1992, Newell & Shumway 1993, Hawkins et al. 1996). As reported here for *M. edulis*, it is notable that maximum CRs recorded in species from temperate latitudes typically average up to about $8\ l\ h^{-1}\ g^{-1}$ dry soft tissue and are most commonly observed in mussels feeding on cultured unicellular algae at concentrations affording up to about $5\ \mu g\ chl\ a\ l^{-1}$ (e.g. Møhlenberg & Riisgård 1979, Riisgård 1988, Clausen & Riisgård 1996, Jørgensen 1996, Hawkins et al. 1997). Alternatively, at higher concentrations of cultured unicellular algae, CR has frequently been observed to decrease in a negative exponential relation to increasing food availability (Winter 1978, Griffiths 1980, Iglesias et al. 1992, Arifin & Bendell-Young 1997, Hawkins et al. 1997, 1998, Yukihiro et al. 1998, Denis et al. 1999). This is again consistent with observations for *M. edulis* here, when saturation reduction, or satiation, resulted in reduced CRs and valve closure after feeding for $>2\ h$ at $30\ 000$ or more cells ml^{-1} ($\sim 6\ \mu g\ chl\ a\ l^{-1}$) (Fig. 1).

Three main points may be emphasised. Firstly, CR in suspension-feeding shellfish is highly flexible. Reductions from maximal rates occur, when costs of feeding (e.g. Widdows & Hawkins 1989) may outweigh potential returns, both at low particle availabilities and upon satiation at high food availabilities, when coincident rates of ingestion and absorption are nevertheless maintained, that is, until such elevated concentrations are reached to incur physical inhibition (e.g. clogging) of the feeding mechanism. Secondly, adjustments in CR are best understood in relation to the utilisable organic component of available seston, rather than the total suspended load; whether that utilisable component is measured as chlorophyll, as a marker for living organic matter, or as the remaining detrital organic matter, which is increasingly understood to play an important role in the nutrition of bivalve shellfish (Navarro et al. 2009, this Theme Section). Thirdly, responsive adjustments in CR are most evident at low availabilities of that utilisable component. Chl *a* may reach as much as $150 \mu\text{g l}^{-1}$ in dense blooms of algae within unmixed eutrophic waters. Yet, maximal CR in *Mytilus edulis* in the present study was observed at $<6 \mu\text{g chl a l}^{-1}$. Similarly, maximal CR in *Chlamys farreri* occurred at about $5 \mu\text{g chl a l}^{-1}$, this species experiencing between about 1 and $20 \mu\text{g chl a l}^{-1}$ during culture in China (Sun et al. 1996). More striking still, maximal CR in *Perna canaliculus* occurred at $<2 \mu\text{g chl a l}^{-1}$ (Hawkins et al. 1999), normally experiencing between about 0.5 and $5 \mu\text{g chl a l}^{-1}$ during culture in New Zealand (Gibbs & Vant 1998). On this basis, variations in availability of suspended organic matter are of greatest physiological consequence in suspension-feeding bivalves whilst over the lower end of observed natural ranges, when responses are higher per unit change in food abundance and have the highest proportional impacts upon net energy balance (Hawkins et al. 1999).

A further advance in this field might be to formulate the relationship between the effects of both flow and food concentration on CR, by using the product of the two as units of food supply, provision, or availability, expressed as grams of chl *a* or carbon per litre per hour. Fig. 8 shows these relationships, both separately and combined as the supply rate, using data derived in the present study for chl *a* content in *Isochrysis galbana*. Over a wide range of supply rates, all the CR measures that we have judged to be valid in this study, i.e. those measured at flow rates of $>150 \text{ ml min}^{-1}$, lie within a limited range of from 1.5 to $2.5 \text{ l}^{-1} \text{ h}^{-1}$, averaging about $2 \text{ l}^{-1} \text{ h}^{-1}$ or $6 \text{ l h}^{-1} \text{ g}^{-1}$ when standardised according to dry soft tissue weight, and which are similar to the near-maximal or 'autonomous filtration' condition suggested by Riisgård (2001a) for moderate food levels.

In any continued quest for accuracy, it is important to consider what measures of feeding are intended for, and whether they are 'fit for purpose'. For example, we often measure feeding in individual bivalves and ignore the variation between individuals by taking mean values for given conditions. Extrapolating these data for use at the population or ecosystem level, for example, in modelling growth rates and impacts of bivalve feeding, is a significant step requiring some understanding of interactions between neighbouring individuals, the hydrodynamics of the system and, of course, temporal variations in food quantity and quality. In this context, there is a wealth of literature concerning the effects of flow or current velocity on bivalve feeding in nature (Wildish & Kristmanson 1997, Dolmer 2000, Newell et al. 2001, Widdows et al. 2002). It is worth emphasising that most or all of this research relates to relatively high current velocities (flows), including associated effects of external and internal pressure fields on the ciliary pump, neither of which are normally relevant to the effects of flow rate measured using a flow-through chamber system. These studies, whether using field measurements or attempting realistic simulations in a flume, usually involve flows well above 0.05 m s^{-1} , whereas maximal flow through our chambers was measured at $<0.003 \text{ m s}^{-1}$.

Recently, Saurel et al. (2007) examined the feeding behaviour of mussel beds in the Menai Strait in relation to multiple environmental factors. Based on *in situ* video measurements of valve gape area (VA) over 2 tidal cycles, they concluded that mussel feeding was regulated solely by chl *a* concentration measured at 1 m above the mussel bed (0.6 to $2.5 \mu\text{g l}^{-1}$), and not by flow velocity, changes in suspended particulate matter, or the presence of predators. However, at times of low flow during slack water, concentrations of chl *a* decreased to levels below the threshold for maximal CR and mussels consequently reduced their feeding activity. In fact, Saurel et al. (2007) estimated that maximal CR (VA of 80 to 100%) occurred for only ca. 45 min d^{-1} , suggesting that optimal feeding conditions are rarely experienced in the Menai Strait. Suggestions are made, with reference to the review by McKindsey et al. (2006), as to how these and other suitable *in situ* measurements may facilitate assessment and modelling of carrying capacity. Other work on *in situ* measurements has assessed the use of valve gape or exhalent siphon area as proxies for pumping rate in bivalves and propose these as useful methods for following the dynamic changes and physiological adaptations during natural bivalve feeding (Riisgård et al. 2003, 2006, Maire et al. 2007). Many of these field studies emphasise that CR varies greatly under natural conditions, such that dynamic modelling is required to assess ecological interrelations between bivalve sus-

pension feeders and their environment. Dynamic feeding responses have increasingly been established using the biodeposition method, which, through separate coincident collections of both true faeces and pseudofaeces and in contrast to measures of CR based solely upon particle depletion, not only integrates over time, but also enables calculations of ingestion rate and absorption efficiency (Hawkins et al. 1996, 2002, Cranford et al. 1998, Cranford & Hill 1999). Measured responses may then be used to simulate each physiological component of growth (Hawkins et al. 2002), for use in integrated ecological modelling (Ferreira et al. 2007, 2008).

Acknowledgements. The authors thank Professors P. G. Beninger and H. U. Riisgård, and 1 anonymous reviewer, for constructive suggestions and corrections that significantly improved this contribution.

LITERATURE CITED

- Arifin Z, Bendell-Young LI (1997) Feeding response and carbon assimilation by the blue mussel *Mytilus trossulus* exposed to environmentally relevant seston matrices. *Mar Ecol Prog Ser* 160:241–253
- Bayne BL (1998) The physiology of suspension feeding by bivalve molluscs: an introduction to the Plymouth 'TRO-PHEE' workshop. *J Exp Mar Biol Ecol* 219:1–19
- Bayne BL (2004) Comparisons of measurements of clearance rates in bivalve molluscs. *Mar Ecol Prog Ser* 276:305–306
- Beninger PG, St-Jean SD (1997) Particle processing on the labial palps of *Mytilus edulis* and *Placopecten magellanicus* (Mollusca: Bivalvia). *Mar Ecol Prog Ser* 147:117–127
- Beninger PG, Dufour SC, Bourque J (1997) Particle processing mechanisms of the eulamelibranch bivalves *Spisula solidissima* and *Mya arenaria*. *Mar Ecol Prog Ser* 150:157–169
- Beninger PG, Valdizan A, Decottignies P, Cognie B (2008) Impact of seston characteristics on qualitative particle selection sites and efficiencies in the pseudolamelibranch bivalve *Crassostrea gigas*. *J Exp Mar Biol Ecol* 360:9–14
- Clausen I, Riisgård HU (1996) Growth, filtration and respiration in the mussel *Mytilus edulis*: no evidence for physiological regulation of the filter-pump to meet nutritional needs. *Mar Ecol Prog Ser* 141:37–45
- Cranford PJ, Hargrave BT (1994) *In situ* time-series measurement of ingestion and absorption rates of suspension-feeding bivalves: *Placopecten magellanicus*. *Limnol Oceanogr* 39:730–738
- Cranford PJ, Hill PS (1999) Seasonal variation in food utilization by the suspension-feeding bivalve molluscs *Mytilus edulis* and *Placopecten magellanicus*. *Mar Ecol Prog Ser* 190:223–239
- Cranford PJ, Emerson CW, Hargrave BT, Milligan TG (1998) *In situ* feeding and absorption responses of sea scallops *Placopecten magellanicus* (Gmelin) to storm-induced changes in the quantity and composition of the seston. *J Exp Mar Biol Ecol* 219:45–70
- Denis L, Alliot E, Grzebyk D (1999) Clearance rate responses of Mediterranean mussels, *Mytilus galloprovincialis*, to variations of flow, water temperature, food quality and quantity. *Aquat Living Resour* 12:279–288
- Dolmer P (2000) Feeding activity of mussels *Mytilus edulis* related to near-bed currents and phytoplankton biomass. *J Sea Res* 44:221–231
- Ferreira JG, Hawkins AJS, Bricker SB (2007) Management of productivity, environmental effects and profitability of shellfish aquaculture—the Farm Aquaculture Resource Management (FARM) model. *Aquaculture* 264:160–174
- Ferreira JG, Hawkins AJS, Monteiro P, Moore H and others (2008) Integrated assessment of ecosystem-scale carrying capacity in shellfish growing areas. *Aquaculture* 275:138–151
- Filgueira R, Labarta U, Fernandez-Reiriz MJ (2006) Flow-through chamber method for clearance rate measurements in bivalves: design and validation of individual chambers and mesocosm. *Limnol Oceanogr Methods* 4:284–292
- Gibbs MM, Vant WN (1998) Seasonal changes in factors controlling phytoplankton growth in Beatrix Bay, New Zealand. *NZJ Mar Freshw Res* 31:237–248
- Griffiths RJ (1980) Natural food availability and assimilation in the bivalve *Choromytilus meridionalis*. *Mar Ecol Prog Ser* 3:151–156
- Hawkins AJS, Smith RFM, Bayne BL, Héral M (1996) Novel observations underlying the fast growth of suspension-feeding shellfish in turbid environments: *Mytilus edulis*. *Mar Ecol Prog Ser* 131:179–190
- Hawkins AJS, Smith RFM, Bougrier S, Bayne BL, Héral M (1997) Manipulation of dietary conditions for maximal growth in mussels, *Mytilus edulis* L., from the Marennes-Oléron Bay, France. *Aquat Living Res* 10:13–22
- Hawkins AJS, Smith RFM, Tan SH, Yasin ZB (1998) Suspension-feeding behaviour in tropical bivalve molluscs: *Perna viridis*, *Crassostrea belcheri*, *Crassostrea iradelei*, *Saccostrea cucullata* and *Pinctada margarifera*. *Mar Ecol Prog Ser* 166:173–185
- Hawkins AJS, James MR, Hickman RW, Hatton S, Weatherhead M (1999) Modelling of suspension-feeding and growth in the green-lipped mussel *Perna canaliculus* exposed to natural and experimental variations of seston availability in the Marlborough Sounds, New Zealand. *Mar Ecol Prog Ser* 191:217–232
- Hawkins AJS, Duarte P, Fang JG, Pascoe PL, Zhang JH, Zhang XL, Zhu M (2002) A functional simulation of responsive filter-feeding and growth in bivalve shellfish, configured and validated for the scallop *Chlamys farreri* during culture in China. *J Exp Mar Biol Ecol* 281:13–40
- Hilbish TJ, Carson EW, Plante JR, Weaver LA, Gilg MR (2002) Distribution of *Mytilus edulis*, *M. galloprovincialis*, and their hybrids in open-coast populations of mussels in southwestern England. *Mar Biol (Berl)* 140:137–142
- Hildreth DJ, Crisp DJ (1976) A corrected formula for calculation of filtration rate of bivalve molluscs in an experimental flowing system. *J Mar Biol Assoc UK* 56:111–120
- Holm-Hansen O, Lorenzen CJ, Holmes RW, Strickland JDH (1965) Fluorometric determination of chlorophyll. *J Cons Int Explor Mer* 30:3–15
- Iglesias JIP, Navarro E, Alvarez Jorna P, Armentia I (1992) Feeding, particle selection and absorption in cockles *Cerastoderma edule* (L.) exposed to variable conditions of food concentration and quality. *J Exp Mar Biol Ecol* 162:177–198
- Jørgensen CB (1996) Bivalve filter feeding revisited. *Mar Ecol Prog Ser* 142:287–302
- Kjørboe T, Møhlenberg F (1981) Particle selection in suspension-feeding bivalves. *Mar Ecol Prog Ser* 5:291–296
- Larsen PS (2001) A note on through-flow chamber method, Appendix 1. In: Riisgård HU (ed) On measurement of fil-

- tration rates in bivalves—the stony road to reliable data: review and interpretation. *Mar Ecol Prog Ser* 211:275–291
- Maire O, Amouroux JM, Duchêne JC, Grémare A (2007) Relationship between filtration activity and food availability in the Mediterranean mussel *Mytilus galloprovincialis*. *Mar Biol (Berl)* 152:1293–1307
- McKindsey CW, Thetmeyer H, Landry T, Silvert W (2006) Review of recent carrying capacity models for bivalve culture and recommendations for research and management. *Aquaculture* 261:451–462
- Møhlenberg F, Riisgård HU (1979) Filtration rate, using a new indirect technique, in thirteen species of suspension-feeding bivalves. *Mar Biol (Berl)* 54:143–148
- Navarro JM, Velasco LA (2003) Comparison of two methods for measuring filtration rate in filter feeding bivalves. *J Mar Biol Assoc UK* 83:553–558
- Newell CR, Shumway SE (1993) Grazing of natural particulates by bivalve molluscs: a spatial and temporal perspective. In: Dame RF (ed) *Bivalve filter feeders in estuarine and coastal ecosystem processes*. Springer-Verlag, Berlin, p 85–148
- Newell CR, Wildish DJ, MacDonald BA (2001) The effects of velocity and seston concentration on the exhalant siphon area, valve gape and filtration rate of the mussel *Mytilus edulis*. *J Exp Mar Biol Ecol* 262:91–111
- Petersen JK (2004) Methods for measurement of bivalve clearance rate—hope for common understanding. *Mar Ecol Prog Ser* 276:309–310
- Petersen JK, Bougrier S, Smaal AC, Garen P, Robert S, Larsen JEN, Brummelhuis E (2004) Intercalibration of mussel *Mytilus edulis* clearance rate measurements. *Mar Ecol Prog Ser* 267:187–194
- Riisgård HU (1977) On measurement of the filtration rates of suspension feeding bivalves—in a flow system. *Ophelia* 16:167–173
- Riisgård HU (2001a) Physiological regulation versus autonomous filtration in filter-feeding bivalves: starting points for progress. *Ophelia* 54:193–209
- Riisgård HU (2001b) On measurement of filtration rates in bivalves—the stony road to reliable data: review and interpretation. *Mar Ecol Prog Ser* 211:275–291
- Riisgård HU (2004) Intercalibration of methods for measurement of bivalve filtration rates—a turning point. *Mar Ecol Prog Ser* 276:307–308
- Riisgård HU, Larsen PS (2000) A comment on experimental techniques for studying particle capture in filter-feeding bivalves. *Limnol Oceanogr* 45:1192–1195
- Riisgård HU, Larsen PS (2001) Minireview: ciliary filter feeding and bio-fluid mechanics—present understanding and unsolved problems. *Limnol Oceanogr* 46:882–891
- Riisgård HU, Kittner C, Seerup DF (2003) Regulation of opening state and filtration rate in filter-feeding bivalves (*Cardium edule*, *Mytilus edulis*, *Mya arenaria*) in response to low algal concentration. *J Exp Mar Biol Ecol* 284: 105–127
- Riisgård HU, Lassen J, Kittner C (2006) Valve-gape response times in mussels (*Mytilus edulis*)—effects of laboratory preceding-feeding conditions and *in situ* tidally induced variation in phytoplankton biomass. *J Shellfish Res* 25: 901–911
- Saurel C, Gascoigne JC, Palmer MR, Kaiser MJ (2007) *In situ* mussel feeding behavior in relation to multiple environmental factors: regulation through food concentration and tidal conditions. *Limnol Oceanogr* 52:1919–1929
- Sokal RR, Rohlf FJ (1995) *Biometry*. WH Freeman, New York
- Sun Y, Song Y, Cui Y, Fang J, Sun H, Yang Q, Yin L (1996) Primary productivity and the affecting factors in Sanggou Bay. *Mar Fish Res* 17(2):31–40
- Velasco LA, Navarro JM (2005) Feeding physiology of two bivalves under laboratory and field conditions in response to variable food concentrations. *Mar Ecol Prog Ser* 291: 115–124
- Widdows J (1985) The effects of stress and pollution on marine animals. Physiological procedures. In: Bayne BL, Brown DA, Burns K, Dixon DR and others (eds) *Praeger*, New York, p 161–178
- Widdows J, Hawkins AJS (1989) Partitioning of rate of heat dissipation by *Mytilus edulis* into maintenance, feeding and growth components. *Physiol Zool* 62:764–784
- Widdows J, Lucas JS, Brinsley MD, Salkeld PN, Staff FJ (2002) Investigation of the effects of current velocity on mussel feeding and mussel bed stability using an annular flume. *Helgol Mar Res* 56:3–12
- Wildish D, Kristmanson D (1997) *Benthic suspension feeders and flow*. Cambridge University Press, Cambridge
- Winter JE (1978) A review of the knowledge of suspension-feeding in lamellibranchiate bivalves, with special reference to artificial aquaculture systems. *Aquaculture* 13: 1–33
- Yukihira H, Klumpp DW, Lucas JS (1998) Comparative effects of microalgal species and food concentration on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima* (Bivalvia:Pteriidae). *Mar Ecol Prog Ser* 171:71–84

Submitted: May 15, 2008; Accepted: September 12, 2008

Proofs received from author(s): November 28, 2008