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# Validation of the flow-through chamber (FTC) and steady-state (SS) methods for clearance rate measurements in bivalves

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## Summary

To obtain precise and reliable laboratory clearance rate (filtration rate) measurements with the 'flow-through chamber method' (FTC) the design must ensure that only inflow water reaches the bivalve's inhalant aperture and that exit flow is fully mixed. As earlier recommended these prerequisites can be checked by a plot of clearance rate (CR) *versus* increasing through-flow (Fl) to reach a plateau, which is the true CR, but we also recommend to plot percent particles cleared *versus* reciprocal through-flow where the plateau becomes the straight line CR/Fl, and we emphasize that the percent of particles cleared is in itself neither a criterion for valid CR measurement, nor an indicator of appropriate 'chamber geometry' as hitherto adapted in many studies. For the 'steady-state method' (SS), the design must ensure that inflow water becomes fully mixed with the bivalve's excurrent flow to establish a uniform chamber concentration prevailing at its incurrent flow and at the chamber outlet. These prerequisites can be checked by a plot of CR *versus* increasing Fl, which should give the true CR at all through-flows. Theoretically, the experimental uncertainty of CR for a given accuracy of concentration measurements depends on the percent reduction in particle concentration ($100 \times P$) from inlet to outlet of the ideal 'chamber geomety'. For FTC, it decreases with increasing values of P while for SS it first decreases but then increases again, suggesting the use of an intermediate value of P. In practice, the optimal value of P may depend on the given 'chamber geometry'. The fundamental differences between the FTC and the SS methods and practical guidelines for their use are pointed out, and new data on CR for the blue mussel, *Mytilus edulis*, illustrate a design and use of the SS method which may be employed in e.g. long-term growth experiments at constant algal concentrations.

## Introduction

Precise and reliable measurements of the filtration rate of mussels are essential in many studies dealing with bioenergetics, energy budgets and growth of mussels, and in mathematical modeling studies of e.g. bio-mixing and optimal design of mussel farms. Over the years, many attempts have been made to determine the filtration rates of filter-feeding mussels and other bivalves, but the different methods used have often caused difficulties leading to conflicting data due to dissimilar experimental conditions or methods (Riisgård, 2001a,b,c). Although much of this dissonance has gradually been resolved through convergence and standardization of the methodology (e.g. Petersen et al., 2004; Riisgård, 2004; Filgueira et al., 2006), it still seems appropriate to suggest methodical improvements and guidelines.

The widely used 'flow-through chamber method' (FTC) for clearance rate measurements in mussels was validated by Filgueira et al. (2006) using a specific chamber geometry and by making a clearance rate *versus* flow rate plot as per Riisgård (1977, 2001a). For the chamber geometry employed, Filgueria et al. (2006) found that a maximum of 20% depletion value was appropriate and apparently well justified as a guideline for future use of this specific chamber design. In the present comment we stress that the depletion depends on chamber design.

Another method less frequently used for clearance measurement in mussels is the 'steady-state method' (SS), but here we stress that both methods and the corresponding equations for clearance rate (CR) can not be used in the same kind of chamber. Filgueira et al. (2006) tested both equations and found that the FTC equation was 'the correct equation' for their chamber. The same equations have also been used and tested by Petersen et al. (2004) and Pascoe et al. (2009); however, there seems to be some degree of uncertainty concerning the fundamental differences between the 2 methods and the prerequisites for their use.

The earlier literature dealing with measurement of filtration rates in mussels has been reviewed previously (Riisgård, 2001a). The purpose of the present work is primarily to give some improved methodical guidelines for obtaining optimal filtration rate data, based on combined theoretical considerations and evaluation of experimental data from more recent studies using the FTC and SS methods. Thus, we first state the governing equations for clearance rate and its experimental uncertainty, then we examine by way of examples from

recent publications and new experimental data the prerequisites for optimizing precision and reliability. Finally, we point out that fundamental differences between the two methods and their equations have hitherto not been sufficiently recognized.

## Theoretical considerations

### Equations of clearance rate

Applying the conservation of mass to the flow diagrams shown in Figs. 1 and 2 for the the FTC and the SS methods, respectively, leads to the following equations for the clearance rate CR (volume of water cleared of suspended particles per unit of time) as function of through flow Fl (volume of water flowing through the chamber per unit of time) and concentrations at inlet and outlet of the chamber, $C_i$ and $C_o$, respectively,

$$\text{FTC method:} \quad CR = Fl \times (C_i - C_o)/C_i \qquad (1)$$

$$\text{SS method:} \quad CR = Fl \times (C_i - C_o)/C_o . \qquad (2)$$

These results assume steady state, principles of optimal flow, and 100% efficient particle retention (all particles are large enough to be retained with an efficiency of 100% , see Møhlenberg and Riisgård, 1978; Riisgård, 1988; Cognie et al., 2003; Beninger et al., 2004). For eqn 1 this implies: (i) no recirculation of water already filtered, (ii) only flow of chamber inlet concentration should enter mussels, and (iii) chamber exit flow is fully mixed at point (M) of Fig. 1 (see also Larsen, 2001). eqn 2 is based on: (i) no recirculation of water already filtered, (ii) only fully mixed chamber flow should enter mussels, and (iii) mussel exit flow is fully mixed with inflow at point (M) of Fig. 2.

For both the FTC and the SS methods, a constant algal concentration at mussel inflow can be maintained during the experiment, for the former advantageously specified by the inlet condition but for the latter to be determined *a posteriori* from the data and eqn 2. Both methods are suitable for reliable measurements of the filtration rates in mussels if all prerequisites are fulfilled.
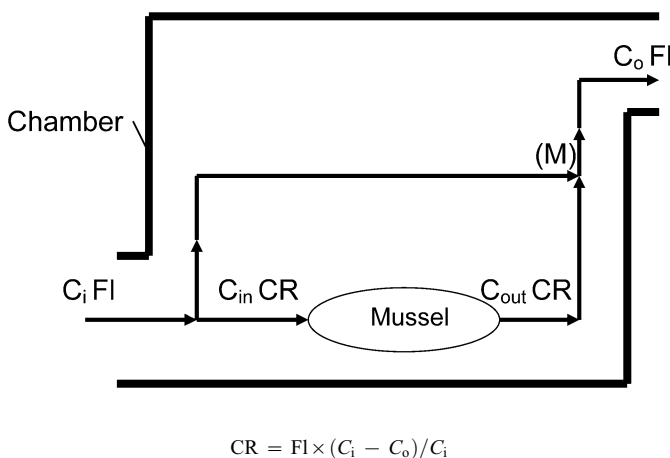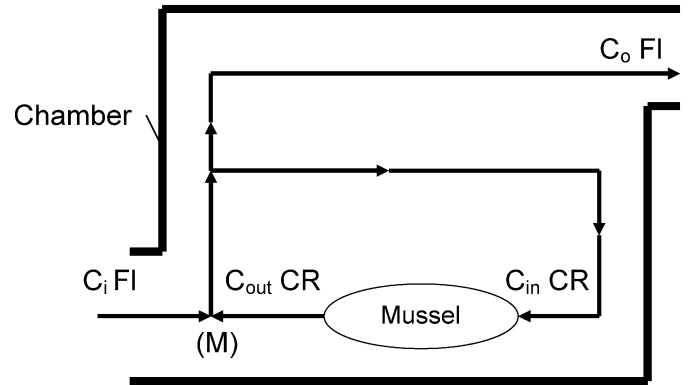


$$CR = Fl \times (C_i - C_o)/C_i$$

**Fig. 1. Diagram of the flow-through chamber (FTC) method.** (M) denotes point of perfect mixing. $C_i$ and $C_o$ denote concentration of chamber inflow and outflow, respectively, $C_{in}$ ($= C_i$), and $C_{out}$ ($= 0$ for 100% retention) the concentration of inhalant and exhalant flows, respectively, Fl the chamber through-flow, and CR the clearance rate given by Eq.(1).



$$CR = Fl \times (C_i - C_o)/C_o$$

**Fig. 2. Diagram of the steady-state (SS) method.** (M) denotes point of perfect mixing. $C_i$ and $C_o$ denote concentration of chamber inflow and outflow, respectively, $C_{in}$ ($= C_o$) and $C_{out}$ ($= 0$ for 100% retention) the concentration of inhalant and exhalant flows, respectively, Fl the chamber through-flow, and CR the clearance rate given by Eq.(2).

For the FTC method, inspection of Fig. 1 shows that as long as $Fl \leq CR$ all chamber inflow is ideally cleared of particles, so $C_o = 0$ and eqn 1 gives $CR = Fl$. This leads to a way of checking the proper conditions by plotting calculated clearance values *versus* increasing values of through-flow rates (Riisgård, 2001c; his Fig. 1). For smaller flow rates, data tend to depart little from the line given by $CR = Fl$, but above a certain critical flow rate the clearance values depart from the line and form a plateau. Only at flow rates above the critical level will clearance rates obtained by 100% efficient retention of particles and eqn 1 be representative of the true clearance rate of the mussel (CR= filtration rate or pumping rate). For an ideal chamber geometry, the deflection point is given by $Fl = CR$ (i.e. exactly all the inflowing water is filtered), but measurements are usually made at higher values of Fl.

For the SS method, Fig. 2 shows that $C_o > 0$ for any positive value of chamber through-flow $Fl > 0$, so eqn 2 should give the correct value of CR for an ideal chamber. This leads to a way of checking the proper conditions by plotting calculated clearance values *versus* increasing values of through-flow rates. Aside from experimental scatter of data this should lead to the same constant value of CR for all values of Fl, but measurements are usually made for values large enough to give a moderate reduction in concentration.

### Experimental uncertainty of clearance rate

Using standard analysis of accumulation of errors it is possible to estimate the experimental uncertainty in CR, given the uncertainty of the measured concentrations $C_i$ and $C_o$. Introducing for simplicity the relative reduction of concentration from chamber inlet to outlet, $P \equiv CR / Fl = 1 - C_o / C_i$, the relative uncertainty on P stemming from 2 measurements of concentration becomes (e.g. Meyer, 1975),

$$\delta P/P = [(1-P)/P]\left[(\delta C/C_i)^2 + (\delta C/C_o)^2\right]^{1/2}$$
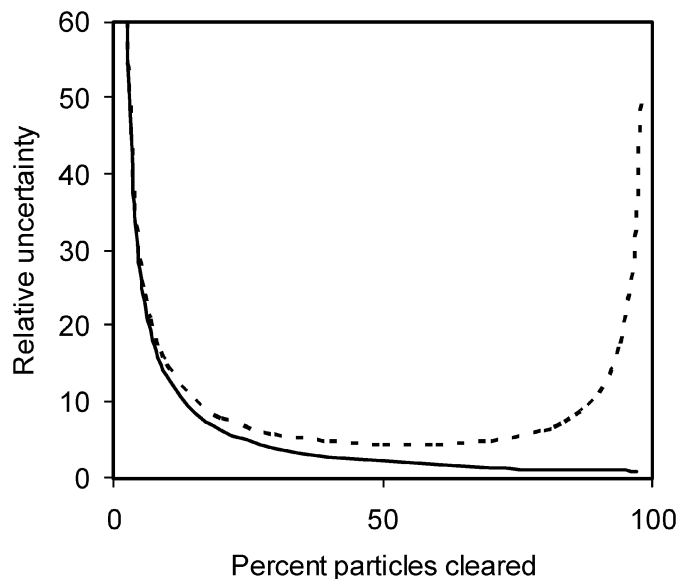$$= [(1 - P)/P]\left[1 + (1 - P)^{-2}\right]^{1/2} \delta C/C_i, \qquad (3)$$

**Fig. 3. Relative uncertainty in clearance rate for given uncertainty in inlet concentration, (δCR/CR) / (δC/C$_i$), versus percent particles cleared, 100 × P = 100 × (C$_i$ − C$_o$)/C$_i$ for the FTC (solid) and the SS (dashed) methods.**



**Fig. 4. Flow-through chamber (FTC) method.** Percent particles cleared, 100 × P = 100 × (C$_i$ − C$_o$)/ C$_i$, versus chamber through-flow, Fl. Full line: logarithmic fit (Filgueira et al., 2006; their Fig. 5, re-plotted); dashed line: hyperbolic function, CR$_m$/Fl, for ideal chamber and CR$_m$ = 78.87 ml min$^{-1}$.

assuming the measurements to be statistically independent and that standard deviations satisfy δC$_i$ ≈ δC$_o$ ≈ δC. It follows from eqn 3 that the relative uncertainty of the measured clearance rate, which applies to the FTC method, becomes

FTC method:    $\delta CR/CR = (1/P)\left[1 + (1 - P)^2\right]^{1/2} \delta C/C_1$,    (4)

ignoring any uncertainty in measuring the chamber through-flow Fl.

For the SS method, CR/Fl = 1/(1−P) − 1 according to eqn 2, leading to

SS method:    $\delta CR/CR = (1/P)\left[1 + (1 - P)^{-2}\right]^{1/2} \delta C/C_1$.    (5)

For a given accuracy of concentration measurements the relative uncertainty, (δCR/CR)/(δC/C$_i$), versus percent reduction of measured concentration (i.e. percent particles cleared), 100 × P, from Eqs. (4) and (5) is shown in Fig. 3 for the FTC and the SS methods. For FTC, it decreases with increasing values of P while for SS, it first decreases (almost as for FTC) but then increases again. When the percent reduction becomes large the relative uncertainty on CR becomes small for FTC because P approaches unity, but large for SS because C$_o$ approaches zero. In both cases, Fig. 3 suggests the use of an intermediate value of P, but in practice the optimal value of P may depend on the given 'chamber geometry'. As an example, in the range of 5 to 30% reduction in concentration, the uncertainty decreases from 27.6 δC/C$_i$ to 4.1 δC/C$_i$ for FTC, corresponding to a decrease from about 55 to 8% if the concentrations are measured with an accuracy of δC/C$_i$ = ±2%. The corresponding decrease for SS is not much different in this range, being from 58 to 12%.

## Discussion

### Influence of percent particles cleared

Over the years, many different values have been suggested for the percent reduction of particle concentration from inlet to outlet of
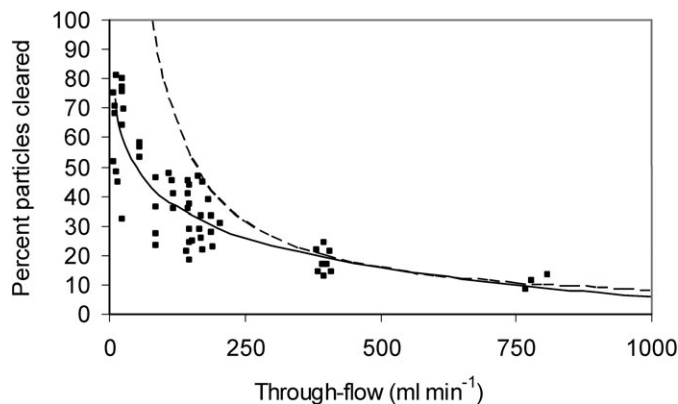
FTCs for optimal chamber performance (Cranford and Gordon, 1992; Smaal and Widdows, 1994; Hawkins et al., 1996; Hawkins et al., 1999; Newell et al., 2005; Filgueira et al., 2006; Pascoe et al., 2009). Although the percentage reduction is a convenient experimental parameter, no general value can be recommended for all types of chambers, with different size and shape used for one or many bivalves with different shell length. But it may be objectively assessed to what degree a certain reduction in concentration may be valid for a given flow-through chamber by making the CR–Fl plot as explained above.

Another useful plot for FTCs involves percent of particles cleared (100 × P = 100 × (1 − C$_o$ / C$_i$) = 100 × CR$_m$ / Fl) versus chamber through-flow (Fl), as used by Filgueira et al. (2006; their Fig. 5) who correlated their data by a logarithmic regression line which, however, has no theoretical basis. Re-plotted here in Fig. 4 along with the hyperbola (CR$_m$ / Fl) which represents the 'true' clearance rate (using CR$_m$ = 78.87 ml min$^{-1}$ from Filgueira et al., 2006; their Fig. 4) this appears only to be reached for reductions below about 20%. Data for higher percentage reduction (i.e. lower through-flow) in general show large scatter and deviate from the appropriate relation (dashed line in Fig. 4). This shows that the 'geometry' of the cylindrical chamber may not be optimal for low through-flow (e.g. allowing recirculation), which is also evident from the fact that here all CR values fall below the line CR = Fl (Filgueira et al., 2006; their Fig. 4). However, one may visualize a chamber with ideal 'geometry', for which reductions approaching 100% should still be valid, indicating that percentage reduction is neither a criterion for valid CR measurement, nor an indicator of appropriate 'chamber geometry'. It may be noted that the plot in Fig. 4 is not convenient for determining CR$_m$ which would require a family of hyperbolas corresponding to different values of CR$_m$ to find the true one. In place we recommend to plot percent of particles cleared versus reciprocal through-flow (1/Fl) in which CR$_m$ is readily determined as the steepest slope of a straight line (CR$_m$/Fl) above all data points as shown in Fig. 5. This line corresponds to the plateau of true clearance in the earlier recommended plot of clearance versus through-flow.

The great concern among researchers for not exceeding a certain maximal value of the reduction in particle concentration from inlet to outlet, in order to obtain valid results, may be
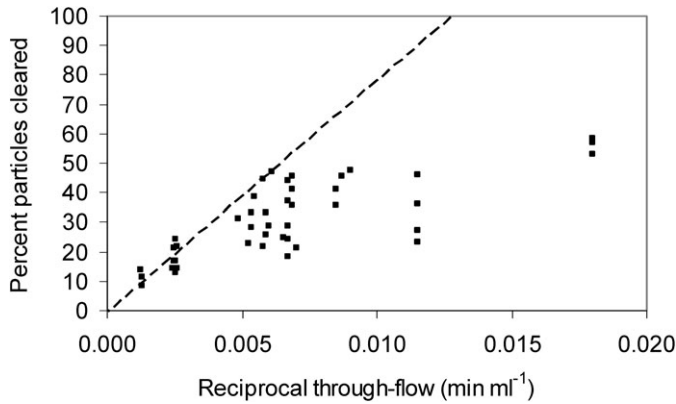
**Fig. 5. Flow-through chamber (FTC) method.** Percent particles cleared, $100 \times P = 100 \times (C_i - C_o)/C_i$, *versus* reciprocal chamber through-flow, $Fl^{-1}$. Dashed line: true clearance rate $CR_m/Fl$ based on $CR_m = 78.87$ ml min$^{-1}$ bounding data for $Fl > 50$ ml min$^{-1}$ from Filgueira et al. (2006; their Fig. 5, re-plotted).

explained by the fact that increasing reduction in concentration implies a reduction of the through-flow. Such a reduction may present difficulties in satisfying the requirements mentioned above, notably those of flow control and mixing.

### Using the right equation

eqn 2 instead of eqn 1 was presented by Hildreth and Crisp (1976) as a 'corrected formula' to overcome problems of recirculation for calculation of clearance rate of bivalves when using the flow-through chamber method. But this statement is not correct. Although there may at first seem to be a superficial similarity between eqn 1 and eqn 2 the fundamental differences between the two methods should be realised: eqn 1 is based on principles of optimal flow and no recirculation of once filtered water whereas eqn 2 is based on principles of steady-state and momentary mixing of all exhalant water in the whole water volume of the flow-through chamber. Hildreth and Crisp (1976) stated that their approximation 'would be helped by some artificial stirring', although this was not done in their own experiments. When using eqn 1 Smaal and Widdows (1994, p. 264) adjusted the through-flow rate so that the outflow particle concentration was 'not more than 30% below the inflow concentration, in order to use the inflow concentration as an estimate of the concentration in the chamber'. However, if this could not 'be achieved, thorough mixing in a larger chamber is required and then the internal concentration is assumed to be presented by the outflow concentration', and Eq. (2) should be used. Thus uncertainty about the proper preconditions for using eqn 1 and eqn 2 have become manifest. In an intercalibration exercise conducted by Petersen et al. (2004) the clearance rate as a function of chamber through-flow rate was estimated by both eqn 1 and eqn 2 using the same data. It was found that 'when eqn 2 is used, estimates of CR are independent of flow rate up to a certain level' (Petersen et al., 2004; Fig. 3 therein), and using eqn 2 'it is thus assumed that the geometry of the chamber allows for steady state and total mixing of the water' (Petersen et al., 2004, p. 192). In a later validation of the flow-through chamber method, Filgueira et al. (2006) stated that 'because both methods [i.e. eqn 1 and eqn 2] could be used in the same kind of chamber' - which is not completely correct - but having built a chamber not
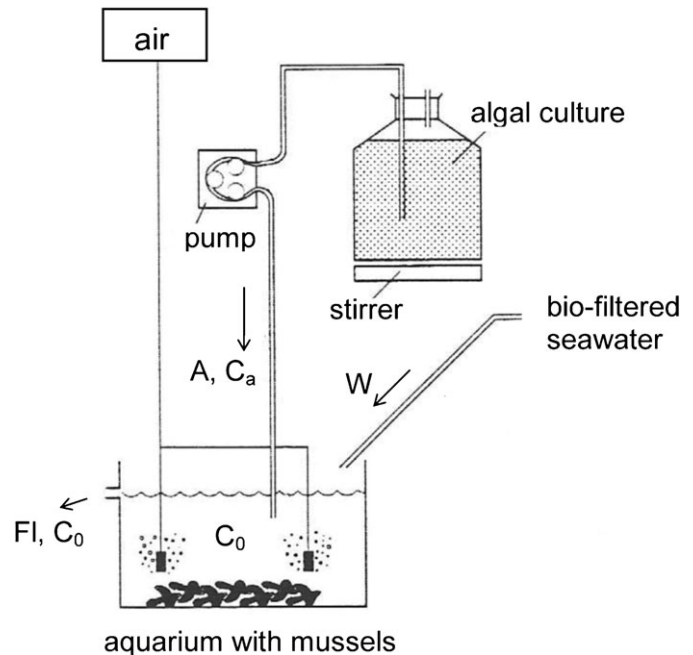


**Fig. 6. Experimental set up for performing steady-state experiments with mussels at defined algal concentration.**

knowing if the conditions match eqn 1 or eqn 2 it was 'necessary to validate the chamber for discerning between the correct equation to use'. Thus, by estimating the clearance rate as a function of chamber through-flow rate using the same data in both eqn 1 and eqn 2 (Filgueira et al., 2006; Fig. 7 therein), the authors concluded that eqn 1 is 'the correct equation for the flow-through chamber method', implying that eqn 2 is wrong. Likewise, Pascoe et al. (2009; Fig. 5A therein) compared clearance rate values as a function of through-flow rate derived from both eqn 1 and eqn 2, and the authors found that eqn 1 'is the better representation of true CR'. These examples indicate that the fundamental differences between the two methods and their equations have not always been sufficiently recognized.

### Example: Validation of steady-state (SS) method

Within a certain range of algal concentrations *Mytilus edulis* is continuously filtering with a constant rate (e.g. Riisgård, 2001d). But below a critical algal concentration between about 0.5 and 0.9 µg chl $a$ l$^{-1}$ *M. edulis* closes its valves (Riisgård et al., 2006; Pascoe et al., 2009), and further, while the mussel may filter at a constant rate at a given concentration, it will reduce its rate once the stomach is full (cf. 'saturation reduction', Riisgård, 2001b; Riisgård et al., 2011). When a group of *M. edulis* is continuously filtering in an aquarium (Fig. 6) with well-mixed seawater to which is added a suitable amount of algal cells from a culture at a constant rate (A) by means of a dosing pump, and further, with a constant through-flow due to inflowing particle-free seawater at a constant rate (W), the average clearance rate of one mussel (CR) can be calculated as (Riisgård and Randløv, 1981; Poulsen et al., 1982; Clausen and Riisgård, 1996; Riisgård et al., 2011):

$$CR = (A \times C_a - Fl \times C_o)/(n \times C_o), \qquad (6)$$

where $Fl = A + W$ is the chamber outflow, $n$ the number of actively-filtering mussels, $C_a$ and $C_o$ the algal concentration in
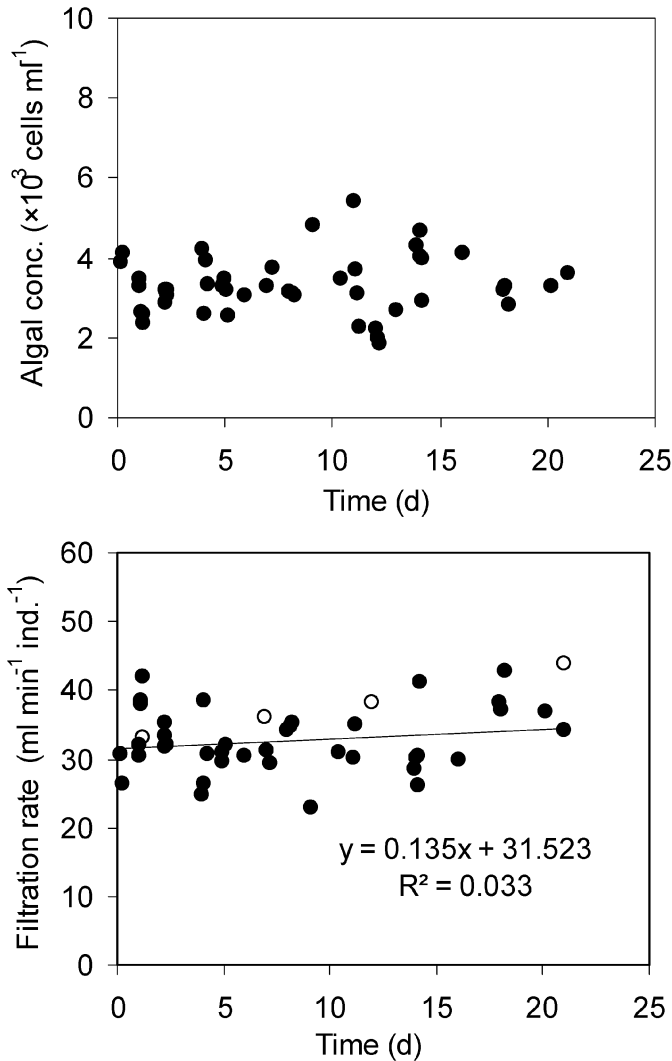
**Fig. 7. *Mytilus edulis*.** (Upper) Long-term steady-state experiment with a group of 25 mussels. Measured concentration of algal cells (*Rhodomonas salina*) in the aquarium (Fig. 6) during the experiment. The mean concentration was 3314 ± 734 cells ml$^{-1}$. (Lower) Estimated clearance rate (= filtration rate) of mussels during steady-state experiment according to Eq.(6), identical to SS method Eq.(2) (filled symbols). The regression line and its equation are shown. For comparison, clearance rates estimated from measured shell length according to Kiørboe & Møhlenberg (1981) are also shown (open symbols).

added culture and mussel aquarium, respectively. Noting that a mean concentration of total inflow can be calculated as $C_i = C_a \times A/Fl$, eqn 6 reduces to eqn 2. In this case − although the flow diagram deviates from that of Fig. 2 − the described setup illustrates one approach to implement the SS method and ensuring full mixing and steady-state.

In order to test the steady-state method, experiments with *Mytilus edulis* were conducted on a group of mussels in a 16 l aquarium with through-flowing filtered seawater (14 ℃). A dosing pump supplied the aquarium holding the experimental mussels with a suspension of pure algae (*Rhodomonas salina*) which were kept homogeneous by strong mixing with 4 air stones (Fig. 6). The through-flow ensured that the entire water volume in the aquarium was exchanged every 15 h. The algal concentration was measured by means of an electronic particle counter (Elzone 5380) several times a day.

The algal concentration measured during a 21-day steady-state experiment with a group of 25 mussels (mean shell length 31.9 ± 1.3 mm) along with the estimated clearance rate using eqn 6 is shown in Fig. 7. For comparison, clearance rates estimated from measured shell length (*L*) using the 'suction method' formula (Kiørboe and Møhlenberg, 1981; see also Riisgård, 2001a; Table 1 therein): CL(l h$^{-1}$) = 0.0012*L*(mm)$^{2.14}$ are also shown. The mean-individual estimated steady-state clearance rate was 32.6 ± 4.7 ml min$^{-1}$ during the experiment period where, however, mean shell length slightly increased, as also reflected in a tendency of increasing clearance rate during the 21-day experiment.

To further test the SS method, the clearance rate was also during the long-term steady-state experiment measured by following the exponential reduction in algal concentration after stoppage of the algal dosing pump and the through-flow of seawater, whereupon algal cells were added two times to reestablish the initial steady-state concentration. The slope of regression lines (*b*) in a semi-ln plot for the reduction in algal concentration with time were used to determine the clearance rate as: CR = *Vb/n*, where *V* = volume of water in aquarium, *n* = number of mussels. The mean clearance rate was estimated at 36.1 ± 3.5 ml min$^{-1}$, in good agreement with the SS method. Finally, the mean initial dry weight (*W*) of the soft parts of the mussels (125 ± 17 mg, measured in a control group) was used to calculate the clearance rate according to the 'suction method' formula (Møhlenberg and Riisgård, 1979; their Table 1): CR (l h$^{-1}$) = 7.45*W*(g)$^{0.66}$, and the calculated rate was 31.5 ml min$^{-1}$, in good agreement with the mean clearance rate obtained by means of the SS method (although it should be remembered that the relationship between shell length and body size, i.e. the 'condition index', is not constant, but varies during the year and from population to population, Dare, 1976; Riisgård, 2001a; Filgueira et al., 2008). The main cause of the variation in the data shown in Fig. 7 is believed to be due to difficulties with keeping the algal concentration constant in the algal cultivation flask from which the aquarium was supplied with algae. Replacement of the cultivation flask with an algal chemostat with a constant supply of algal cells in the same growth phase would eliminate much of the present variation in data. Nevertheless, the example shows that reliable clearance rates over an extended period of time may be obtained by the SS method using the simple set-up shown in Fig. 6.

### Concluding remarks

Careful use of clearance-rate methods leading to valid data are essential for many bivalve aquaculture and environmental studies, such as controlled feeding and growth studies of suspension-feeding bivalves in breeding systems, depletion of phytoplankton in mussel-raft cultures, effectiveness of mussel bio-filtration of effluents from marine fish-cage aquacultures, and grazing impact and bio-mixing of mussel-culture beds. The present note has reviewed the requirements for optimal design and operation of the FTC and SS methods according to the flow diagrams of Figs. 1 and 2 such that the governing Eqs. (1) and (2) apply and lead to correct results for the clearance rate on the assumption of 100% retention of food particles. Tests to verify proper operation are suggested for use of Eqs. (1) and (2) and involve examination of data acquired at increasing rates of chamber through-flow. Additional considerations of experimental uncertainty (Fig. 3), assuming the validity of Eqs. (1) and (2),

suggest it advisable to ensure a certain minimal reduction in concentration from chamber inlet to outlet of both types of chambers, of the order of 20 to 30%.

All types, shapes and sizes ('geometry') of chambers may not optimally satisfy the requirements at all rates of through-flow and for that reason it is necessary to perform the recommended tests to determine a suitable through-flow rate and to verify the appropriateness of the 'chamber design'. A number of allusions to 'optimal chamber geometry' have been made here, but how this is more exactly put into practice depends on e.g. the size, shape and number of the bivalve(s) to be placed in the chamber, for examples, see Walne (1972), Vahl (1972, 1973a,b), Riisgård (1977), Palmer and Williams (1980), Filgueira et al. (2006).

Obviously, the flow-through chamber (FTC) and steady-state (SS) methods are not limited to mussels, but may be extended to oysters, scallops (e.g. Vahl, 1973b; Walne, 1972; Palmer and Williams, 1980), and other suspension-feeding bivalves. Especially the SS method may prove to be be useful in future studies of infaunal bivalves (e.g. Møhlenberg and Kiørboe, 1981) and other zoobenthic suspension feeders such as e.g. the polychaete *Nereis diversicolor* (Vedel and Riisgård, 1993), the ascidian *Ciona intestinalis* (Petersen et al., 1995), and the amphipod *Corophium volutator* transferred to glass tubes or allowed to bury themselves in natural sediment (Riisgård and Schotge, 2007). The very precise conditions in such laboratory studies are never fully encountered in the natural environment and extrapolation of measured clearance rates may be risky, and therefore, the need to 'intercalibrate' with *in situ* type studies must be underlined.

## Competing Interests

The authors declare no competing interests.

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