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COMMENT

Comparisons of measurements of clearance rates in bivalve molluscs

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The recent paper by Petersen et al. (2004) is a useful contribution to the debate on the relative merits of techniques which measure the clearance rates (CR) of suspension feeding bivalve molluscs. However, one of their conclusions should not be taken as generally (i.e. universally) true, viz. that the 'bio-deposition method' underestimates CR relative to the 'particle-clearance method'.

In a pilot experiment for a study on the genetically based variability in feeding behaviour of the Pacific oyster *Crassostrea gigas* (Bayne et al. 1999), we compared these 2 methods. The oysters were from controlled crosses between inbred lines. Ten juvenile oysters from each of 3 crosses (mean dry flesh weight 0.226 ± 0.041 mg, mean ± SD) were placed into shallow trays in the seawater system at Bodega Marine Laboratory, California, USA, and dosed with an experimental diet of a mixture of *Isochrysis galbana* (T-Iso) cells and natural silt suspended in seawater at a flow rate of 150 ml min^{-1} and particle concentration of 6.2 ± 1.9 mg l^{-1} (mean ± SD). The silt was surface sediment from the oyster-growing area, filtered between 150 and 10 µm and left to stand for 60 min before decanting.

When the oysters were seen to be discharging both true faeces and pseudofaeces, the trays were cleaned of these bio-deposits. Then, over 2 periods of 60 min each, faeces and pseudofaeces were collected quantitatively, filtered onto washed and weighed GF/C filters, dried to constant weight at 60°C, then ashed at 450°C for 4 h before cooling in a desiccator and weighing again. Samples of food collected in trays not containing oysters were treated similarly. CR was calculated as in Hawkins et al. (1996):

$$CR = (IRR + IER)/PIM$$

where IRR is the inorganic matter rejected as pseudofaeces, IER is the inorganic matter in the true faeces, and PIM is the inorganic matter present as food. The average of the 2 estimates was used for statistical analysis. This is the bio-deposition method (Iglesias et al. 1998).

The same oysters were then placed in 3 l beakers in flowing seawater with the same experimental diet. Beakers without oysters were set up to control for natural sedimentation. After a period of stabilization to these conditions, the flow of water was stopped and water samples taken at 10 min intervals for 1 h for particle counts by CoulterCounter. A duplicate run was then performed after a further period of 60 min in flowing seawater with added particles, and CR was calculated according to Coughlan (1969):

$$CR = \ln(C_0 - C_t) \times V$$

where C_0 and C_t are particle concentrations per litre at time zero and after 1 h, respectively, and $V = 3$ l, i.e. the volume of water used. All the raw particle counts were first plotted to confirm log-linear decline over the experimental period, and individual CR was noted as the average of the 2 measurements. This is the particle-clearance method.

There were significant differences in CR between families (Table 1; for a discussion of equivalent findings in the main experiment, see Bayne et al. 1999); *t*-tests (for a repeated measures design) on data standardized to a dry flesh weight of 250 mg $ind.^{-1}$ indicated no difference between the 2 measures of CR for the data set as a whole ($t = 1.46$, df = 29; $p > 0.05$). Within Family A, the difference between the 2 measures was significant, but not within Families B and C. Note that CR were higher for individuals in Family A than for the other oysters, and that estimates of CR by the biodeposition method, in this family only, were on average 82% of measures by the particle-clearance method.

Oysters are particularly suited to measurements by the bio-deposition technique because the deposits of pseudofaeces and true faeces are spatially separated in the experimental trays, so that accurate quantitative

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Table 1. *Crassostrea gigas.* Clearance rates (l h^{-1}; mean ± SD, n = 10 ind.) by oysters (mean size: 0.25 g dry flesh weight) from 3 families, measured at 18°C using the bio-deposition and particle-counting methods. Values for *t* and p are for paired comparisons between methods within families; data were log-transformed for normality. Oysters in family A were hybrids from 2 inbred lines B and C (Bayne et al. 1999)

Family	Bio-deposition	Particle counts	$t_{(df=9)}$	p
A	2.75 ± 0.57	3.34 ± 0.62	2.48	<0.05 to >0.01
B	1.81 ± 0.42	1.75 ± 0.50	0.42	>0.05
C	2.03 ± 0.50	1.98 ± 0.77	0.63	>0.05

sampling is possible. This is less true of mussels *Mytilus edulis*, requiring the use of an artificial barrier (e.g. 'a cover slip positioned on a ball of putty'; Petersen et al. 2004) to separate the 2 types of deposit; this introduces a potential error (the possibility of a disturbance to feeding behaviour) not present in experiments with oysters.

In the pilot experiment referred to here it was clear that collection of the bio-deposits, by careful pipeting, should be done frequently. Pseudofaeces are less compact than true faeces and subject to dispersion, however slight, under the conditions of water flow employed. It is not advisable, therefore, to leave the deposits over a period of 2 h before sampling, as in Petersen et al. (2004); any loss of material from the pseudofaeces will result in an underestimation of true CR. In our pilot experiment we sampled after 1 h. In subsequent experiments we have attempted to sample the bio-deposits every few minutes, concluding that the 18% mismatch between techniques (cp. differences reported by Petersen et al. 2004 of 56 to 72%) was probably due in large part to a failure to sample pseudofaeces quantitatively when CR, and rates of deposition, were high.

As Petersen et al. (2004) correctly point out, the bio-deposition technique is uniquely suited for use in the field, and yields much useful information, in addition to CR, on feeding behaviour. By careful attention to detail (flow rates and the pattern of flow over the animal, the geometry of the experimental chamber, animal orientation within the chamber, frequent collection of deposits), there is nothing to prevent the use of this procedure in studies of bivalves feeding under natural situations in the field. And it is by such field-oriented studies, using natural particulates, that we can best expect to understand the full complexities of their feeding behaviour.

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