

## Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalves

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Received 8 August 2000

The effect of varying algal cell concentration, salinity and body size on the filtration (FR) and ingestion rate (IR) of three species of cultivable Indian bivalves, (green mussel *Perna viridis*, the backwater oyster *Crassostrea madrasensis* and the shortneck clam *Paphia malabarica*) were investigated under laboratory conditions. Axenic cultures of the unicellular alga *Isochrysis galbana* were used in the test solutions. The filtration and ingestion capacities of the different species in the order of high to low was *Crassostrea* > *Perna* > *Paphia*. The differences in the FR and IR have been attributed to the epifaunal habitat of the first two species as compared to the infaunal habitat of the latter. Increasing algal cell concentrations resulted in escalating FR and IR until a threshold of  $10^5$  cells.ml<sup>-1</sup> in the case of *Crassostrea* and *Perna* and  $7.5 \times 10^4$  in the case of *Paphia*. However, at this concentration all the species showed production of pseudofaeces and therefore the critical cell concentration was one step lower to the threshold level. The FR and IR were significantly higher in larger bivalves and the peak was observed at the ambient natural salinities of the respective species tested.

Many bivalve species form subsistence fisheries, and recently, many of these are being used for mariculture in India<sup>1</sup>. Elsewhere, cultivable bivalves are also being considered as biofilters in eutrophicated shrimp/fish ponds with varying degrees of success<sup>2,3</sup>. Bivalves being filter feeders, the filtration and ingestion rates are parameters of considerable ecological and nutritional significance. Filter feeding behavior in bivalves is known to be highly responsive to fluctuations in both the abundance and composition of suspended seston<sup>4</sup>. Hence, information on the feeding behavior of cultivable bivalve species under laboratory and field conditions is vital in plotting the optimal food concentration to be supplied.

The filtering activity of bivalves is diverse, influenced by the concentration of phytoplankton, quality and size of food particles and size of the animal<sup>5</sup>. The physical parameters of the natural habitat like temperature, salinity and flow of water also affect the filtration rate<sup>6</sup>. Furthermore, above a threshold particle concentration, bivalves are able to regulate ingestion through rejection of excess particles as pseudofaeces. Thus, a knowledge of feeding habits which involves the determination of filtration and ingestion rates according to animal size, food concentration and salinity is important for understanding the nutritional biology of filter feeders such as bivalves and also to avoid

excess feeding under laboratory and hatchery conditions.

Filtration rate (FR) or clearance rate is defined as the volume of water filtered completely free of particles per unit of time and is also sometimes synonymously used as the pumping rate when all the particles entering the mantle cavity are completely retained by the gills<sup>7</sup>. The ingestion rate (IR) or feeding rate is defined as the number of algal cells an organism consumes per unit time<sup>8</sup>.

The FR and IR of temperate water bivalves have been particularly well documented<sup>4</sup>. Similar studies from the tropics and especially from India are few<sup>9,10</sup>. Therefore, experiments were designed to measure the influence of algal cell concentration, salinity and body size on the FR and IR of three species of cultivable bivalves viz., the green mussel (Bivalvia: Mytilidae) *Perna viridis* (Linnaeus), the edible oyster (Bivalvia: Ostreidae) *Crassostrea madrasensis* (Preston) and the shortneck clam (Bivalvia: Veneridae) *Paphia malabarica* (Chemnitz).

### Materials and Methods

The green mussel, *Perna viridis* (size group I – 64-67 mm and II – 100-105 mm), the edible oyster *Crassostrea madrasensis* (size group I – 65-70 mm and II – 100-105 mm) and the shortneck clam *Paphia malabarica* (size group I – 30-32 mm and II – 45-47 mm) were collected from an estuarine bivalve farm site in Ashtamudi Lake, Kollam, Kerala. All the collected

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animals were kept for acclimatization for 2 weeks in the laboratory at  $30 \pm 1$  °C and salinity 32 ppt in 10 liter plastic basins, with continuous air supply. The water was changed every second day and the animals were fed on axenic cultures of the microalga *Isochrysis galbana* (Parke) (Haptophyceae: Isochrysidaceae)

*Isochrysis galbana* (7  $\mu$  dia) from laboratory stock cultures were subcultured in 3 liter flasks using Walne's medium<sup>11</sup>. Since the experiments required considerable amount of alga, outdoor cultures were made in 15 liter translucent plastic buckets using 3 liter subculture as stock and a standard fertilizer mix as medium. The number of algal cells per milliliter of culture was counted by using a haemocytometer with improved Neubauer ruling. Desirable algal counts were obtained within 3-4 days.

The indirect method of determination of filtration and ingestion rates requires the measurement of the concentration of suspended particles at certain intervals of time. The filtered volume of water is an estimate or measure of the minimum volume (filtration rate) which the bivalve must have filtered in order to reduce the particle concentration to the observed values. The indirect method was preferred because of the relatively low degree of disturbance for the filtering bivalves during the experiment<sup>12</sup>. The experiments were designed to measure the effect of (a) different algal concentrations ranging from  $3 \times 10^4$  to  $1.25 \times 10^5$  cells.ml<sup>-1</sup>, (b) body size and mean dry tissue weight and (c) varying salinity on the filtration and ingestion rate of three selected species of bivalves (Table 1). Each experiment was carried out using three individuals each of mussel and oyster and five individuals each of shortneck clam in 5 liters of seawater in appropriate plastic basins. All treatments were replicated thrice.

All bivalves were acclimated in the containers at the required algal concentration and salinity before the experiment. Prior to each experiment, the animals were starved for at least 24 h. Aeration was not provided so as to prevent the artificial circulation of water in the basin. Before starting the experiment, the water was changed completely and fresh filtered seawater of the required salinity was added gently without much disturbance or stress to the animal. After the re-immersion, mussels opened their valves immediately, other bivalves took 15-20 min to open their valves and start filtration activity. After the valves were open, *Isochrysis galbana* cell suspension of desired concentration was added with least disturbance to the animals. Control basins containing algal sus-

pensions without animals were set up to correct any error, which might result from flocculation or reproduction of the algae during the experimental period.

At fixed intervals of time (every 30 min) algal samples were collected from the basins and the algal concentration was determined. Similarly, the faecal matter from the bottom of the basins was collected using a Pasteur pipette and examined under the microscope to record the production of pseudofaeces if any. The filtration rate (F, ml.h<sup>-1</sup>) was determined by the formula<sup>11,13</sup>:

$$F = V \times \frac{\log \text{conc } t_0 - \log \text{conc } t_1}{\log_e t} \times 60$$

where, V = volume (ml of algal solution used, here 5 l); conc t<sub>0</sub> = initial algal concentration and conc t<sub>1</sub> = algal concentration after t (time). Similarly the ingestion rate (I, cells.h<sup>-1</sup>. animal<sup>-1</sup>) was determined by the formula,

$$I = \frac{C_1 - C_2}{n t} \times V \times 60$$

where, C<sub>1</sub> = initial algal cell concentration; C<sub>2</sub> = final algal cell concentration after time t; t = duration of the experiment (min); V = volume of water and n = number of bivalves per replicate. The mean filtration and ingestion rates were determined from the replicate values for each treatment and results were expressed as  $\pm 2$  SE. The FR and IR data were analyzed using ANOVA for an asymmetrical factorial type experiment<sup>14</sup>. The analysis was done using the SPSS/PC software.

Table 1—Details of treatments made to test the FR and IR

Species		Size group (mm)	Salinity regimen (ppt)	Algal conc (cells.ml <sup>-1</sup> )
<i>Perna viridis</i>	1	64-67	15	$3 \times 10^4$
			25	$5 \times 10^4$
	2	100-105	25	$7.5 \times 10^4$
			32	$1 \times 10^5$
<i>Crassostrea madrasensis</i>	1	65-70	10	$3 \times 10^4$
			20	$5 \times 10^4$
	2	100-105	20	$7.5 \times 10^4$
			32	$1 \times 10^5$
<i>Paphia malabarica</i>	1	30-32	15	$3 \times 10^4$
			25	$5 \times 10^4$
	2	45-47	25	$7.5 \times 10^4$
			32	$1 \times 10^5$
				$1.25 \times 10^5$

**Results and Discussion**

The variation in filtration rate (litres.h<sup>-1</sup>.animal<sup>-1</sup>) of *Perna viridis* with change in algal cell concentration, salinity and body size are shown in Fig.1a. The FR increased with increasing algal cell concentration until 10<sup>5</sup> cells.ml<sup>-1</sup>, after which there was a rapid decline. Salinity had a significant influence (p < 0.05) on the FR with animals in 32 ppt showing higher FR. Similarly, larger animals had significantly (p < 0.05) higher FR than smaller size groups. In both the size groups 10<sup>5</sup> cells.ml<sup>-1</sup> algal concentration showed maximum FR, excepting smaller size mussels in 15 ppt which peaked at 7.5 × 10<sup>4</sup> concentration. But at this concentration, the presence of pseudofaeces was observed indicating that at 10<sup>5</sup> cells.ml<sup>-1</sup>, although pumping activity took place, there was little assimilation of algal cells. In the smaller size group, the lowest salinity of 15 ppt profoundly depressed the FR,

while the larger size group mussels could cope with lower salinity better.

The IR of smaller size *P. viridis* also showed a similar pattern to that of FR (Fig.1b). However, the tested salinities did not affect the IR of larger size mussels significantly and at 25 ppt the IR continued to increase even after 10<sup>5</sup> cells.ml<sup>-1</sup>. Up to 250 million cells were filtered in an hour by each large size mussel.

According to Schulte<sup>15</sup> in the European blue mussel, *Mytilus edulis*, the FR generally decreased as the algal cell concentration increased from 3×10<sup>5</sup> to 1.5×10<sup>8</sup> cells.l<sup>-1</sup>. The differences in FR trend noted in the present study maybe due to differences in methodology (algal cell concentrations) and the faster growth exhibited by the tropical green mussel as compared to the temperate blue mussel. Hawkins *et al.*<sup>16</sup> reported that the clearance (filtration) rate of *P. viridis* in Malaysian mussels decreased with

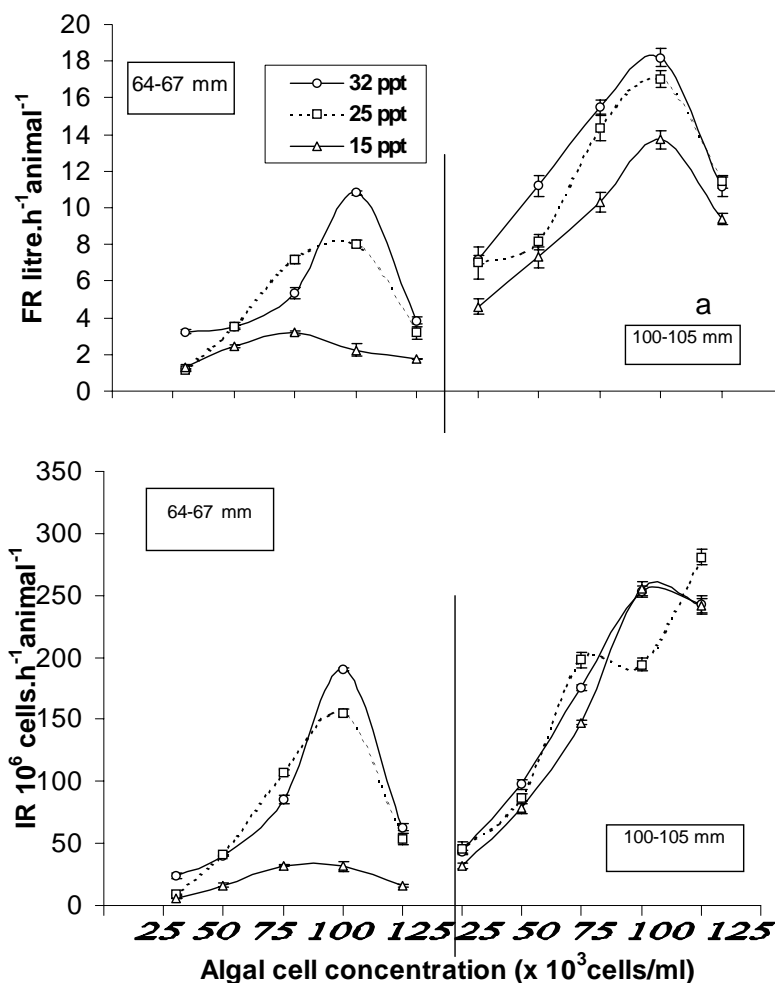


Fig. 1 —The filtration rate (a) and ingestion rate (b) of *Perna viridis* in varying algal concentration, salinity and body size. Error bars indicate ± 2 SE.

increasing particulate organic matter (POM). Specifically, feeding rate decreased in exponential relation with increasing concentration of POM, presumably because the gut becomes saturated with organics<sup>17,18</sup>.

The FR and IR of *C. madrasensis* in different salinities and body sizes are shown in Fig. 2. As in *P. viridis*, the maximum FR in both size groups of oysters was recorded at  $10^5$  cells.ml<sup>-1</sup>, except at 32 ppt salinity where it was observed at  $7.5 \times 10^4$  cells.ml<sup>-1</sup>. Pseudofaeces production was noticed at  $10^5$  cells.ml<sup>-1</sup> algal concentration and after. Both large and small oysters showed maximum FR at 20 ppt salinity and the FR of larger animals was significantly ( $P < 0.05$ ) higher. The FR at 10 ppt salinity was higher than that at 32 ppt indicating that this brackishwater oyster is more attuned and physiologically adapted to lower salinities prevailing in such environments.

The IR of the oysters also peaked at  $10^5$  cells.ml<sup>-1</sup> in both the size groups at 20 ppt. The effect of change

in salinity was not so marked in the large size group as in the case of smaller size group although all differences were statistically significant at 5% level.

Similar to the results obtained in the present study, Strychar & Macdonald<sup>19</sup> reported that in *Crassostrea virginica* ingestion was regulated as the concentration of particles increased both by producing pseudofaeces and reducing clearance rates even at low particle concentrations. Pseudofaeces production is an important mechanism to regulate ingestion and has typically been shown to increase with elevated seston concentrations in most of the bivalves studied<sup>19</sup>.

The FR and IR of *P. malabarica* was relatively lesser than in the other tested species (Fig. 3). The FR increased with increasing algal concentration until  $0.75 \times 10^5$  cells.ml<sup>-1</sup>. At this concentration pseudofaeces production was also observed. There was no significant ( $P > 0.05$ ) difference in the FR of larger clams as compared to smaller ones. In smaller clams

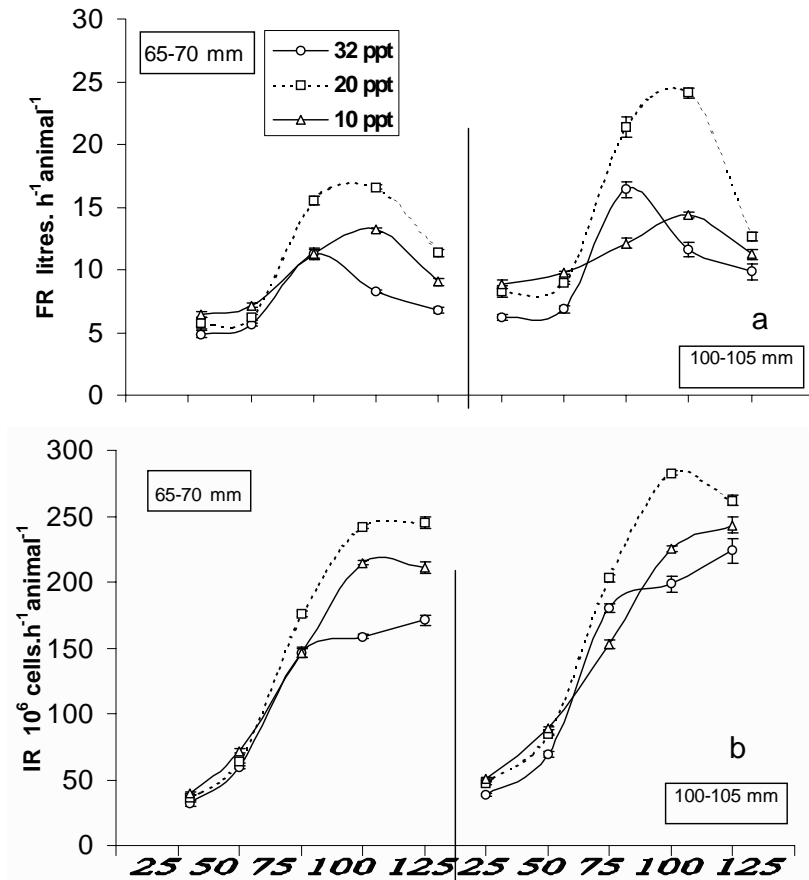


Fig. 2 —The filtration rate (a) and ingestion rate (b) of *Crassostrea madrasensis* in varying algal concentration, salinity and body size. Error bars indicate  $\pm 2$  SE.

the FR was maximum in 25 ppt salinity and in larger clams it was maximum at 32 ppt salinity. This indicates that larger clams are more adapted to the marine zones (near bar-mouth) of the estuarine habitat.

Durve<sup>9</sup> noted in the clam *Meretrix casta* that the FR falls in low and high salinities. Khalil<sup>5</sup> observed that in the clam *Tapes decussatus* the FR generally decreased with increased algal concentration, in contrast, the IR generally increased with increased algal concentration as observed in the present study. The FR and IR also increased with increased body size in this clam.

A comparison of the observed threshold (maximum) and critical (without pseudofaeces production) cell density for the tested bivalves together with the

corresponding FR and IR is given in Table 2. Maximum filtration and ingestion capacity was observed for the oyster *C. madrasensis* closely followed by the green mussel *P. viridis*. The clam *P. malabarica* had comparatively low FR and IR. This could be because both *P. viridis* and *C. madrasensis* are epifaunal species while *P. malabarica* is an infaunal species and hence has a lower capacity to ingest organic matter. Hawkins *et al.*<sup>16</sup> also arrived at similar conclusions when comparing the ingestion rates of epifaunal species *C. gigas* and *M. edulis* and the infaunal cockle *Cerastoderma edule*.

There are increasing requirements to predict the carrying capacity for culture of filter-feeding shellfish

Table 2—Species-wise threshold and critical cell densities and the peak FR and IR

Species	Threshold cell density cells.ml <sup>-1</sup>	Critical cell density cells.ml <sup>-1</sup>	Salinity at which maximum FR & IR	Peak FR l.h <sup>-1</sup> .animal <sup>-1</sup> ±2 SE	Peak IR cells (×10 <sup>6</sup> ) l.h <sup>-1</sup> .animal <sup>-1</sup> ±2 SE
<i>Perna viridis</i>	100,000	75,000	32 ppt	18.2±0.47	280.8±5.94
<i>Crassostrea madrasensis</i>	100,000	75,000	20 ppt	24.1±0.45	282.7±1.76
<i>Paphia malabarica</i>	75,000	50,000	32 ppt	5.61±0.28	116.0±0.95

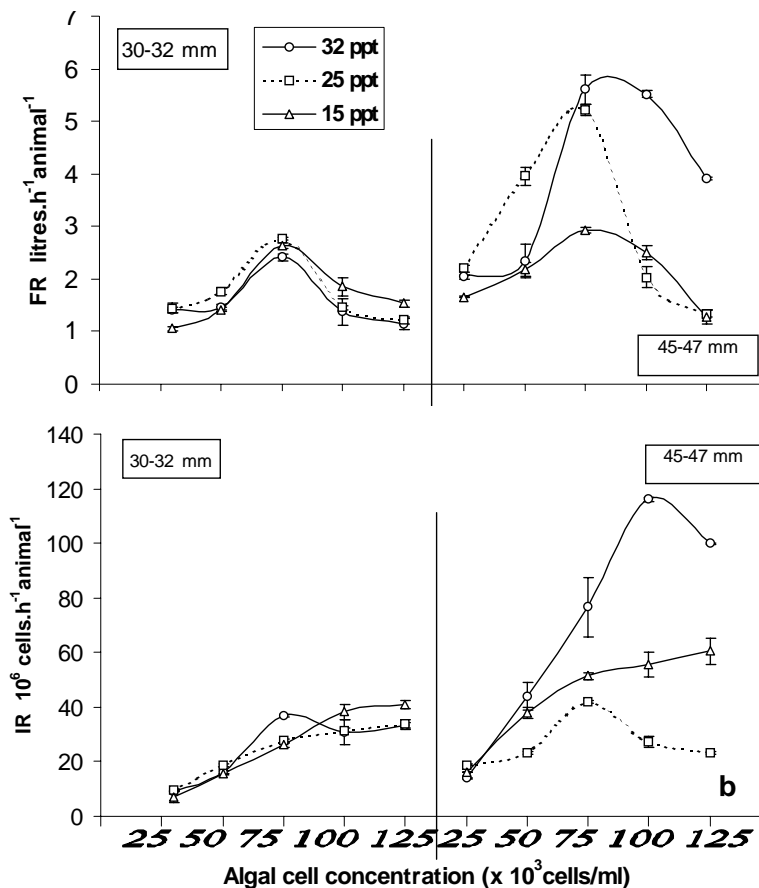


Fig. 3 —The filtration rate (a) and ingestion rate (b) of *Paphia malabarica* in varying algal concentration, salinity and body size. Error bars indicate ± 2 SE.

within nearshore environments, and to understand the impact of filter-feeding shellfish on ecosystem dynamics<sup>20</sup>. The present results could form the baseline for further studies of the FR and IR of these bivalves over complete ranges of seston availability and composition in their natural environment. Furthermore, for broodstock maintenance and conditioning of these bivalves in hatcheries the results from this study would be useful to calculate the daily ration.

### Acknowledgement

The authors are grateful to the Director, CMFRI, the Head, Molluscan Fisheries Division and the Officer Incharge, Fisheries Harbour Laboratory for facilities and encouragement. They are also thankful to the Indian Council of Agricultural Research, New Delhi for the award of a Research Fellowship to one of the authors.

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