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Microalgal Size, density and salinity gradients influence filter feeding of *Pinctada margaritifera* (Linnaeus 1758) spat

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Present study has revealed the feeding performance of pearl oyster *P. margaritifera* spat was comparatively better in salinities ranging from 28 to 37 ppt among the tested salinities. But a perfect feeding performance was noticed with a salinity between 31 to 34 ppt. Clearance rate, ingestion rate and retention efficiency of different sized algae showed that in these salinities spat can able to do a normal feeding activities in all the tested seston concentrations. these parameters were better in the optimal algal concentration of 50 x 10^3 cells.ml^{-1}. Clearance rate and ingestion rate lower with diatoms than flagellates. Salinity, size of the food particle and its concentrations are also important factors influence the ingestion rate. The ingestion rate was proportionally increased with food concentration but the retention efficiency was inversely proportional. The smaller sized *Chlorella marina* and *Nanochloropsis oculata* showed a less retention than that of the other larger algal species, *Pavlova salina, Isochrysis galbana* and *Chaetoceros calcitrans*. The study has revealed that the best live feed for the blacklip pearl oyster spat should have a size of above 3 µm with cell concentration of 25 to 50 x10^3 cells.ml^{-1} required for nursery rearing.

[**Key words:** pearl oyster, spat, nursery rearing, microalgae, filter feeding, Salinity gradients]

Introduction

The blacklip pearl oyster, *Pinctada margaritifera* is one of the economically important tropical bivalves, which is extensively used for cultured black pearl production. They are suspension feeders and the unicellular microalgae are widely used as food for rearing their larvae, spat and adults[1]. These food particles, not only provide the energy needs but also the nutritional requirements of the pearl oyster. Food concentration and type of microalgal diet are the major factors influencing its growth and reproduction. The nutritive values and growth rates of pearl oysters on various microalgae diets is based on the difference in algal species and influence of its feeding processes such as filtration, ingestion, digestion and absorption. There may be other factors affecting feeding depending upon different types of microalgae such as the chemical stimulants on the surface of food particles. It is reported the presence of chemoreceptors, sensitive to different tastes, on the labial palps of the American edible oyster *Crassostrea virginica*[2].

In nursery culture of bivalve spat, the energy balances obtained for various microalgal diets and its concentrations are fundamentally important in spat growth and development. An increase in food concentration promote growth rate only up to a level and beyond which there is a decline in growth rate[3]. Particle retention efficiency is a crucial factor in determination of clearance rate for many bivalves and it has about 100% efficiency in filtering particles greater than 4 µm diameter[4]. Clearance rate is the rate at which particles are captured by the gill filament cilia from the flow of water through the mantle cavity. Therefore, clearance rate is related to the food concentration.

Filtration rates and clearance rates of bivalves are not constant within a species or a population and even for an individual, are variable dependent

on the balance between endogenous (size, reproductive state) and exogenous factors (temperature, salinity, food density, food quality)[5]. Marine bivalves, especially blacklip pearl oysters are found in the intertidal to shallow subtidal habitats and are adapted to withstand varying levels of salinity. Mostly they exhibit a sudden reduction in filtration rate initially and then acclimation to different salinities will restore it to previous levels[6]. Such adaptive mechanisms were noticed in the blue mussel, *Mytilus edulis*[7]. We have observed the blacklip oysters hung in chaplets in our raft farm closing their valves or limiting the gaping during the sudden inflow of freshwater with high level of suspended solids during the monsoon season.

There are intra-specific and inter-specific differences in the particle capture efficiency in camparison to particle diameter. It was opined that comparatively *P. margaritifera* in Tahiti Islands have highest retention of the finest particles (1.5 µm)[4]. While in the silty conditions like Merbok mangrove system of Malaysia, *P. margaritifera* showed lower retention efficiencies (6 - 40 $mg.l^{-1}$)[8]. Values ranged from 30 % in the 3 - 10 µm particles size range down to about 15 % for 16 µm particles. At high algal concentrations, suspension feeding bivalves generally decrease clearance rates[3,9] and absorption efficiency[9,10] and increase pseudofaeces production[6]. This has also been reported in many Indian species of cultivable bivalves such as clams, oysters and mussels[11]. There were no differences in clearance rate at low food concentration (0.5 $mg.l^{-1}$), although *P. margaritifera* had lower absorption efficiency than *P. maxima*[12]. Whereas, in higher food concentrations, *P. maxima* had significantly higher absorption efficiency and clearance rate than *P. margaritifera* regardless of algal diet[13]. When all or almost all the particles in the incurrent water are retained on the gill filaments, clearance rate (CR) is the same as pumping rate (PR), the rate of water flow through the mantle cavity[12]. CR increases exponentially with size[12]. In French Polynesia, the high CR values for *P. margaritifera* resulted from a combination of large gill surface area and high pumping rate[14].

The present study focuses on effects of salinity gradients and different sized algae in varying concentrations on the feeding physiology of the blacklip pearl oyster, *Pinctada margaritifera* spat in the Andaman and Nicobar Islands. To investigate these, experiments were set up to study the clearance rate (CR) of spat using five species of phytoplankton having different sizes from 1 to 9 µm. The particle retention efficiency (RE) of these microalgae and the differences in the ingestion rate (IR) in the various algal concentrations were also studied.

Materials and Methods

P. margaritifera spat (10 ± 5 mm DVM) were raised in the hatchery as per methods outlined[15]. Spat from a single brood were stocked with a density of 5 spat in 500 mL in 1000 mL acrylic containers (effective density 1 spat.100 ml^{-1}) and acclimatized in different salinities for 20 days, before initiating the experiments. The nine salinity gradients set up for the experiment were 19, 22, 25, 28, 31, 34, 37, 40, and 43 ppt and 3 replicates were made for each treatment. The higher salinities were manipulated by adding natural sea salt to seawater and lower salinities made up by adding freshwater to the normal seawater. Complete (100 %) water exchange was done daily using filtered seawater.

The microalgal species used for the experiments were *Chlorella marina* (2 ± 1 µm, Chl), *Nannochloropsis oculata* (2.5 ± 1.5 µm, Nan), *Pavlova salina* (6.5 ± 1.5 µm, Pav), *Isochrysis galbana* (7 ± 2 µm, Iso) and *Chaetoceros calcitrans* (7 ± 2 µm, Cha). Axenic cultures of these microalgae were developed and maintained indoor by using Walne's medium[16] with a photoperiod of 12: 12 (light / dark) following the methods[1]. Cultures were harvested during exponential growth phase for feeding. The density of algal culture (cells.ml^{-1}) was calculated by counting with help of a haemocytometer.

The experiment was designed with minimum disturbance to filter feeding mechanisms of spat based on the indirect method of filtration and ingestion rates estimated by the measurement of concentration of algal cell suspension in uniform time intervals. The spat were raised on a mixed algal diet comprising of *P. salina, I. galbana* and *C. calcitrans.* Each set of spat was experimented with algal species one by one in various food concentrations (25 x 10^3, 50 x 10^3, 75 x10^3 and 100 x 10^3 cells.ml^{-1}) with a starvation period of one day between the experiments with each seston concentration as well as algal species. Assessment of remaining algal cell concentration was done at a time interval of 30 minutes for 6 hours.

Clearance rate (CR) is defined as the theoretical

water volume cleared of all particles per unit time. The clearance rate was calculated in a closed system based on algal concentration in experimental suspensions, by monitoring decrease in cells[17].

$$CR\ (l.h^{-1}) = [(\ln C_0 - \ln C_t) / (t - t_0)] \times V$$

Where, C_0 = initial algal concentration of suspension; C_t = algal concentration at time't';
V = volume of the suspension; $(t - t_0)$ = time interval (h)
Ingestion rate (IR) was determined by the formula

$$IR\ (cells.\ h^{-1}.\ animal^{-1}) = [(C_1 - C_2) / nt] \times V \times 60$$

Where, C_1 = initial algal concentration; C_2 = final algal cell concentration after time't'; V = volume of water; n = number of oysters per replicate.
The Relative retention efficiency (RE) of food particles was determined by using five algal species having differences in size. It is defined as the number of specific cell type retained per unit time, relative to the initial available number of the same cell type at the beginning of the experiment[18].

$$Re\ (\%) = 100 \times [(C_0 - C_t) / C_0]$$

Where, C_0 = initial algal concentration; C_t = algal concentration at time 't'
Variations in clearance rate, ingestion rate and retention efficiency in relation to different salinities were tested for significant differences by means of 2 way ANOVA using SPSS version 16.

Results

In different feed concentrations, all the 5 species of algae showed an increase in clearance rates with increasing salinity from 25 to 34 ppt (Fig. 1). Low clearance rates were observed for all the algal species in lower and higher salinities tested irrespective of the feed concentrations. The clearance rate of different algal species was depending on size and cell concentration. In lower food concentrations (25 and 50 x 10^3 cells.ml^{-1}) clearance rate was more and it decreases with increase of food concentrations (75 and 100 x 10^3 cells.ml^{-1}). ANOVA showed that the CRs were significantly different ($p < 0.01$) among different salanities and between species of algae. However, at 100 x 10^3 cells.ml^{-1} concentration the CR was not significantly different ($p > 0.05$) between algal species (Table 1).

Spat showed variations in ingestion rate for different algal species tested according to their mean cell size as well as the cell concentrations. The larger algal species such as *C. calcitrans* and *I. galbana* generally had higher ingestion rate in almost all the food concentrations tested. But in higher concentrations, smaller species (*N. Oculata* and *C. marina*), also showed a high ingestion rate (Fig. 1). In different salinities, the ingestion rates were always more in salinities between 28 and 37 ppt irrespective of the food concentration. In lower and higher salinities, ingestion rates were low in any food concentrations compared with the above mentioned salinity range. However, the ingestion rates were significantly ($p < 0.001$) high for all algal species in different food concentrations and among the range of salinities tested (Table 1).

As the size of algae reduces a progressive decrease in retention efficiency was observed. Retention efficiency was directly linked with the size and concentrations of food particles present in the medium. Bigger sized phytoplankton such as *C. calcitrans* and *I. galbana* showed more retention than smaller sized *P. salina*, *N. oculata* and *C. marina* (Fig. 1). The size range and class of maximum percentage of cells of each algal species used in the experiment is shown in Figure 2. In the case of *I. galbana* and *C. calcitrans*, cell size ranged between 5 – 9 µm with a maximum frequency percentage between 7 µm (35 %) and 8 µm (45 %) respectively. whereas it was 6 µm (55 %) for *P. salina* having a size ranged between 5 - 8 µm. Smaller sized algae, *N. oculata* and *C. marina* ranged between 1 - 4 µm and their maximum frequency percentage of cells (60 %) were in 2 and 3 µm respectively. The smaller sized *N. oculata* and *C. marina* had comparatively low retention efficiency.

In lower food concentrations (25, 50, 75 x 10^3 cells.ml^{-1}) the mean retention efficiency curves were well raised and showed high percentage retention efficiency in the salinities between 25 and 37 ppt. But in higher food concentration (100 x 10^3 cells.ml^{-1}), the retention efficiency curves were low in all the salinities tested. ANOVA showed that in all the salinities the retention efficiency was significant different ($p < 0.001$) (Table 1).

Table 1-ANOVA table of variations in clearance rate (CR), ingestion rate (IR) and retention efficency (RE) of pearl oyster *P. margaritifera* spat of algal species in different food concentrations

Food Concentration	Source of Variation	F value of CR	P value	F value of IR	P value	F value of RE	P value
100 x 10^3	Salinities	6.31	0.000	8.206	0.000	6.626	0.000
	Algal species	2.045	0.112*	5.831	0.001	3.645	0.015
75 x 10^3	Salinities	10.679	0.000	10.011	0.000	7.300	0.000
	Algal species	5.326	0.002	10.960	0.000	2.270	0.083*
50 x 10^3	Salinities	4.402	0.001	5.361	0.000	5.321	0.000
	Algal species	7.344	0.000	4.697	0.004	3.106	0.029
25 x 10^3	Salinities	5.955	0.000	9.706	0.000	7.819	0.000
	Algal species	11.129	0.000	6.100	0.001	3.762	0.013

* Not significant at 5 %

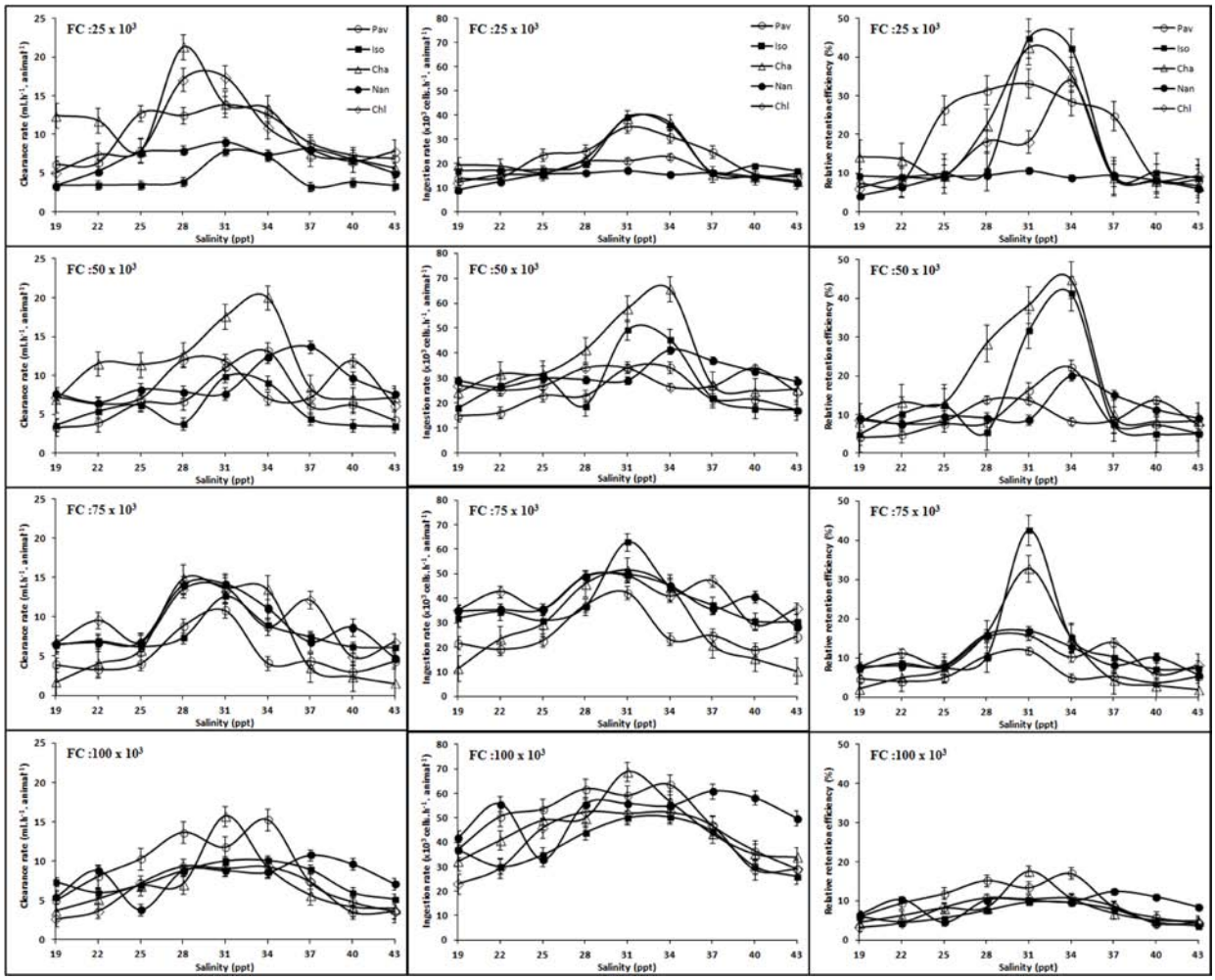

Fig. 1-Clearance rate (CR), Ingestion rate (IR) and retention efficiency (RE) of *P. margaritifera* spat for 5 species of microalgae in different salinities and food concentrations (FC)

Discussion

The present study has revealed that the feeding performance of pearl oyster *P. margaritifera* spat was comparatively better in salinities ranging from 28 to 37 ppt among the tested salinities. But a perfect feeding performance was noticed with a narrow range of salinity from 31 to 34 ppt among the wide range of salinities tested. Clearance rate, ingestion rate and retention efficiency of different sized algae showed that in these salinities spat were able to do normal feeding activities in all the tested seston concentrations. However, the CR and IR were better in the algal concentration of 50 x 10^3 cells.ml^{-1}. This is because of the under optimal salinity and seston concentration the filter pumping processes is at its full capacity. Whereas, higher concentrations of phytoplankton, cause reduction of the valve gape and retraction of mantle edges and siphons, correlated with reduced water pumping[6]. It was observed that clearance rate of Akoya pearl oyster *P. fucata* was increased by seawater salinity increases and peaked at 34 ppt and then declined with further increase in salinity[19]. It was also reported that the filtration rates are highest at medium particle concentrations and may respond to both the volume and the chlorophyll *a* concentration of particles[20]. In addition, higher concentrations of suspended particles in the ambient water elicit secretion of mucus and particles become entangled in mucus, get converted into pseudofaeces before ejection[6]. Particle sorting for ingestion or rejection is the physical process that is correlated with secretion of mucus for the production of pseudofaeces[6]. This process is depended upon size, shape and other physical characteristics of the food particles.

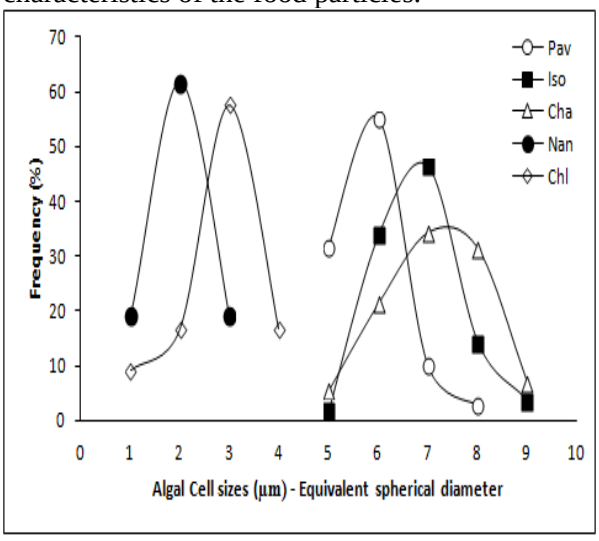

Fig. 2-Sizes and percentage frequency of different species of microalgae used in the study

Seston load not only influences the clearance activity, but also other physiological functions. However, seston particles < 2 µm were too small to be captured by adult Akoya oysters *P. fucata martensii*[21] and *P. margaritifera*[18] therefore not considered of nutritious value. This was explained well that the distance between the branching cilia of gill cirrus is about 1µm, so only particles about 4 µm and above is completely retained in suspension feeding bivalves[22]. But, It was stated that picoplankton (0.4 – 2 µm) and nanophytoplankton (2 – 20 µm) are very important components of the seston for pearl oyster culture[23]. However, the present study has concluded that the feeding selectivity is size specific and microalgae having a particle size > 3 µm are required for *P. margaritifera* spat rearing.

According to the energy balance, faster spat growth is achieved at environmental conditions supporting higher rates of energy acquisition[12]. Such energy needs met through food ration relay on the ingestion capacity and the maximum ingestion ration of an algal species which considered as a parameter for nutritional studies in bivalves. Clearance and ingestion rates are lower with diatoms than flagellates[24]. Size of the food particle and its concentrations are an important factors influence the ingestion rate along with morphology of pearl oyster[13]. In *P. margaritifera* larvae, clearance rate and ingestion rate of *P. salina* was about five times higher than those for *Chaetoceros* spp.[24]. Whereas in the present study, *P. margaritifera* spat showed more clearance and ingestion rate in the order of *C. calcitrans, I. galbana, P. salina, C. marina* and *N. oculata.* It is clear that the size of algae is also an important factor influencing the feeding performance. The ingestion rate increased proportionally with food concentration but the retention efficiency was inversely proportional.

Food uptake in bivalve molluscs mainly depends on the retention efficiency and clearance rate. Retention of particles was determined by the pumping capacity of bivalves and concentration of food in the ambient water. In the present study the relative retention efficiency of different microalgae in *P. margaritifrea* spat was more in lower concentrations of algae. The smaller sized *C. marina* and *N. oculata* showed less retention than that of the other larger algal species, *P. salina, I. galbana* and *C. calcitrans*. Poor retention efficiency was noticed with smallest alga tested *N. oculata* even in the lowest food concentration 25 x 10^3 cells.ml^{-1}. This is well

supported by the earlier findings that in *P. margaritifera* in Great Barrier Reef showed maximum capture efficiency (> 90 %) when the seston particle diameter was between 3 - 10 µm[25]. However, it was 8 - 35 µm in the case of Akoya oysters[26] and 4 - 10 µm in *P. maxima*[4,25].

Conclusion

This study revealed that the best live feed for the spat of blacklip pearl oyster, *P. margaritifera* should have a size above 3 µm with cell concentration of 25 to 50 x 10^3 cells.ml^{-1} during nursery rearing. This was evident from the clearance and ingestion rate and the mean retention efficiency of the five species of algae tested during the experiment. The algal food particles not only provide the energy needs but also the nutritional requirements of the pearl oyster. The complete feeding performance of the spat reared in different salinities proved that the blacklip pearl oyster, can survive in a salinity ranging between 28 and 37 ppt and the ideal salinity is from 31 to 34 ppt. Thus the outcome of the present study will lead to better nursery rearing practices for the blacklip pearl oyster spat.

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References

1 Lavens P & Sorgeloos P, Manual on the production and use of live food for aquaculture. *FAO Fisheries Technical Paper*, 361, (FAO, Rome) 1996, pp. 295.

2 Dwivedy R C, A study of chemoreceptors on labial palps of the American oyster using miroelectrodes, *Proc. Nat. Shellfish Ass.*, 63 (1973) 20-26.

3 Winter J E, A review on the knowledge of suspension feeding in Lamellibranchiate bivalves with special reference to artificial aquaculture systems, *Aquaculture*, 13 (1978) 1-33.

4 Pouvreau S, Jonquieres G & Buestel D, Filtration by the pearl oyster, *Pinctada margaritfera* under conditions of low seston load and small practice size in tropical lagoon habitat, *Aquaculture*, 176 (1999) 295-314.

5 Barnes P, Shellfish culture and particulate matter production and cycling: *A literature review* (BC Aquaculture research and development committee) 2006, pp. 8-13.

6 Jorgensen C B, Bivalve filter feeding revisited, *Mar. Ecol. Prog. Ser.*, 142 (1996) 287-302.

7 Bohle B, Effects of adaptation to reduced salinity on filteration activity and growth of mussels (*Mytilus edulis*), *J. Exp. Mar. Biol. Ecol*., 10 (1972) 41-49.

8 Hawkins A J S, Smith R F M, Tan S H & Yasin Z B, Suspension feeding behaviour in tropical bivalve molluscs: *Perna viridis, Crassostrea belcheri, Crassostrea iradelei, Saccostrea cucculata* and *Pinctada margaritifera*, *Mar. Ecol. Prog. Ser.*, 166 (1998) 173-185.

9 Griffiths R J, Filtration, respiration and assimilation in the black mussel *Choromytilus meridionalis, Mar. Ecol. Prog. Ser*., 3 (1980) 63-70.

10 Bayne B L, Hawkins A J S, Navarro E & Iglesias I P, Effects of seston concentration on feeding digestion and growth in the mussel *Mytilus edulis*, *Mar. Ecol. Prog. Ser*., 55 (1989) 57-54.

11 Rajesh K V, Mohamed K S & Kripa V, Influence of algal cell concentration, salinity and body size on the filtration and ingestion rates of cultivable Indian bivalves, *Indian J. Mar. Sci*., 30 (2001) 87-92.

12 Yukihira H, Klumpp D W & Lucas J S, Effects of body size on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*, *Mar. Ecol. Prog. Ser.*, 170 (1998) 119-130.

13 Yukihira H, Klumpp D W & Lucas J S, Comparative effects of microalgal species and food concentration on suspension feeding and energy budgets of the pearl oysters *Pinctada margaritifera* and *P. maxima*, *Mar. Ecol. Prog. Ser.*, 171 (1998) 71-84.

14 Pouvreau S, Bodoy A, & Buestel D, *In situ* suspension feeding behaviour of the pearl oyster, *Pinctada margaritifera*: combined effects of body size and weather-related seston composition, *Aquaculture*, 181 (2000) 91-113.

15 Mohamed K S, Kripa V, Libini C L, Shanmugam K, Rao K P & Velayudhan T S, Hatchery production of blacklip pearl oyster spat in the Andaman and Nicobar Islands – A success story, *Fish. Chimes*, 30 (2010) 40-41.

16 Walne P R, The influence of current speed, body size and water temperature on the filtration rate of five species of bivalves, *J. Mar. Biol. Ass. U K*, 52 (1972) 245-374.

17 Coughlan J, The estimation of filtering rate from the clearance of suspensions, *Mar. Biol.*, 2 (1969) 356-358.

18 Loret P, Le Gall S, Dupuy C, Blanchot J, Pastoreaud A, Delesalle B, Caisey X & Jonquieres G, Heterotrophic protists as a trophic link between picocyanobacteria and the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia), *Aquat. Microb. Ecol.*, 22 (2000) 215-226.

19 Algarswami K & Victor A C C, Salinity tolerance and rate of filtration of the pearl oyster *Pinctada fucata*, *J. Mar. Biol. Ass. India*, 18 (1976) 149-158.

20 Loret P, Pastoreaud A, Bacher C & Delesalle B, Phytoplankton composition and selective feeding of the pearl oyster *Pinctada margaritifera* in the Takapoto lagoon (Tuamotu Archipelago, French Polynesia): in situ study using optical microscopy and HPLC pigment analysis, *Mar. Ecol. Prog. Ser.*, 199 (2000) 55-67.

21 Tomaru Y, Udaka N, Kawabata Z & Nakano S, Seasonal change of seston size distribution and phytoplankton composition in bivalve pearl oyster *Pinctada fucata martensii* culture farm, *Hydrobiologia*, 481 (2002) 181-185.

22 Mohlenberg F & Riisgard H U, Efficiency of particle retention in 13 species of suspension feeding bivalves, *Ophelia*, 17 (1978) 239-246.

23 Numaguchi K, Growth and physiological condition of the Japanese pearl oyster, *Pinctada fucata martensii* in Ohmura Bay, Japan, *J. Shellfish Res*., 13 (1994) 93-99.

24 Doroudi M, Southgate P C & Lucas J S, Variation in clearance and ingestion rates by larvae of blacklip pearl oyster (*Pinctada margaritifera*) feeding on various microalgae, *Aquac. Nutr.*, 9 (2003) 11-16.

25 Yukihira H, Klumpp D W & Lucas J S, Feeding adaptations of the pearl oysters *Pinctada margaritifera* and *P. maxima* to variations in natural particulates, *Mar. Ecol. Prog. Ser.*, 182 (1999) 161-173.

26 Ward J E & Mc Donald B A, Preingestive feeding behaviours of two subtropical bivalves (*Pinctada imbricata* and *Arca zebra*) responses to an acute increase in suspended sediment concentration, *Bull. Mar. Sci*., 59 (1996) 417-432.