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Effect of temperature and body size on food utilization in the marine pearl oyster *Pinctada fucata* (Bivalvia: Pteridae)

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Physiological parameters such as clearance rates, absorption efficiency, oxygen consumption and ammonia excretion were estimated for four size groups ranging from 16 to 60 mm in Dorso Ventral Measurements (DVM) of the marine pearl oyster *Pinctada fucata* at different water temperatures and the results were integrated by means of two physiological indices, namely Scope For Growth (SFG) and Net Growth Efficiency (K_2). The rates of clearance, oxygen consumption and ammonia excretion were found strongly correlated ($p = 0.01$) with size groups (as tissue dry weight) at water temperature from 18° to 31°C. Absorption efficiency ranged from 43.2 to 56.9 % and was not related to body size in the tested temperature range. Oxygen consumption and ammonia excretion increased with temperature within the same size group from 18° to 31°C. Clearance rate increased with temperature from 18° to 28°C, but declined with further increase of temperature to 31°C. Excreted energy contributed 2.4 to 4.0% to the total absorbed energy for different size groups and water temperatures. The SFG and K_2 were higher at 26° and 28°C and were minimum at 18°C for all the size groups. The result showed that the optimum physiological conditions for survival and growth of *P. fucata* were in the temperature range of 26° to 28°C.

[**Key words**: Pearl oyster, *Pinctada fucata*, temperature effect, food utilization]

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Introduction

Temperature is considered as a major environmental factor influencing most physiological rates, including those involved in the energy balance, such as feeding, excretion and respiration among bivalves[1]. The effects of temperature on physiological processes studied for several species of pearl oysters[2-5] suggest that high temperature within tolerance limits does not always seem to have any direct effect on mortality, but appears to be associated with some sub-lethal effects such as reduced feeding, poor growth, high metabolic expenditure, etc. Among the Indian marine pearl oysters, *Pinctada fucata* (Bivalvia: Pteridae) is extensively utilized for the production of quality pearls. It is desirable to determine the physiological response of *P. fucata* to changes in temperature in order to standardize the optimum condition for their growth, survival and reproductive conditioning in the onshore culture system of marine pearls. There is no comprehensive study of the effects of temperature on the physiological rates involved in the energy balance in this species. Therefore, experiments were conducted on the pearl oyster, *P. fucata* to investigate the physiological rates involved in the energy balance in different size groups on exposure to temperature changes and to quantify the net energy gain under different thermal regimes. Hence, information on the physiological conditions and growth of the pearl oysters *P. fucata* is important to standardize the techniques of the culture of marine pearls in the onshore tanks.

Materials and Methods

The experiments were conducted at Kovalam (near Chennai, India) as a part of onshore culture of marine pearls. The average minimum and maximum water temperature recorded over a period of 10 years from 1985 to 1995 were 18°C (January) and 31°C (June). Therefore, in the present experimental set up the temperatures selected (within this range) are 18°, 23°, 26°, 28° and 31°C. Samples of *P. fucata* were collected off Mandapam coast in Gulf of Mannar and were transported to the Kovalam Field Laboratory.

The oysters were cleaned for the epibiota from the shell surface and Dorso Ventral Measurement (DVM) and weight were noted. The oysters were divided into four size groups consisting of 50 individuals each, Group A [16.0-18.0 mm (DVM); 0.054 g (average dry tissue weight)]; Group B [29.0-31.0 mm; 0.159 g]; Group C [38.0-40.0 mm; 0.294 g] and Group D [58.0-60.0 mm; 0.430 g]. Dry weight of the tissue was considered for the analysis so as to avoid possible variations in the water content of the tissues and different size groups were used for the purpose of measuring the effect of body size on the physiological rates.

Experimental test temperatures were kept constant by using either a thermostat heater or plastic bags containing ice-bricks. Constant aeration was provided to maintain oxygen level above 6 mg l^{-1} and the natural *p*H value was 7.8 ± 0.2 throughout the experiment. At the end of the experiment, the oysters were sacrificed and dry tissue weight was determined by drying the soft tissue at 105°C for 72 h.

Microalgae, *Isochrysis galbana* and *Chaetoceros calcitrans* (1:1) were used as feed since these species have been shown to support good growth and survival of pearl oysters[6]. Stock cultures of the microalgae were maintained in 3 liters Haufkin's flask by using Walne's medium with a 12:12 hr, L/D photoperiod. The algae were harvested after 4-7 days of inoculation in early stationary phase at densities in the range $10^6 - 15^6$ cells ml^{-1}. The algal cells were counted at regular intervals by using haemocytometer to determine the quantity of algae to be fed to the experimental oysters and were diluted with 0.2 µm filtered seawater to get the desired concentrations. The clearance rates, ingestion rates, absorption efficiency, oxygen consumption and ammonia excretion of the oysters acclimatised to different temperature regimes were determined and the scope for growth and net growth efficiency were calculated as detailed below.

Clearance rate of *P. fucata* in relation to temperature was determined in a flowing water system by measuring the algal cell concentration of the in-flowing and out-flowing water. The experiment consisted of six plastic tanks (volume: 4 liters) for each test temperature, five with 3 individual animals of each size group and the sixth was the control without animal. The experiment was performed in a flow-through system having a flow rate of 80 ml min^{-1} with 30,000 algal cells ml^{-1}. The experiment was performed for 3 hours and the measurements were recorded every hour. The concentration of the suspension was determined by measuring the absorbance at 450 nm in a spectrophotometer (Bausch & Lomb Spectronic 20) using a standard curve and corrected for any absorbance changes in a control chamber and counter checked by direct counting with haemocytometer[7]. The clearance rates were calculated following the formula given by Bayne *et al.*[8]:

$$CR = \frac{C_1 - C_2}{C_1} \times F$$

where, CR= Clearance rate (l h^{-1}), C_1=Inflow particle concentration (particles ml^{-1}), C_2=Outflow particle concentration (particles ml^{-1}), F = Flow rate through the experimental chamber (l h^{-1})

The ingestion rate per unit time was estimated as the product of clearance rate (l h^{-1}) and the concentration of the micro algae in the experimental chamber (mg l^{-1}).[9] Pseudofaeces were collected at the end of the clearance rate experiment and their weights were deducted from the total ingested food. Organic ingestion rate (OIR) in mg l^{-1} was calculated using the formula:

$$OIR = (IR. \% OS) / 100$$

where, IR= total ingestion rate (mg l^{-1}), % OS= percentage of organic seston. The ingestion ration (mg l^{-1}) was converted to energy equivalents[10] using the conversion factors, 1 mg protein equivalent to 24.0 J; 1 mg carbohydrate equivalent to 17.5 J and 1 mg lipid equivalent to 39.5 J.[11]

The percentage of consumed food that was absorbed by the oyster was determined[12] by comparing the fraction of faeces lost on ashing with the fraction of samples of food suspensions lost on ashing.

$$\text{Absorption efficiency} = (F - E) / [(1- E). F]$$

where, F= ash free dry weight: dry weight ratio of food (seston), E= ash free dry weight: dry weight ratio of faeces, F and E stand for the proportion of organic matter in the food and faeces.[13]

Fifty millilitre of seawater containing microalgae was filtered on to pre weighed (W_o) 40 mm GF/C grade filter. The filters with microalgae were then kept in a hot air oven for drying at 60° C for 48 hours and weighed again (W_d) in order to calculate the total dry weight of suspended microalgae per liter of seawater. These filters were then ashed in a muffle furnace at 450°C for 6 hours and weighed (W_{450}) to calculate the weight of organic material in the food combusted. The

component ($W_d - W_{450}$) represented the weight of organic matter in the food. During the experiments, the faeces accumulated were collected by pipette from the experimental chamber for subsequent analysis. The total weight and organic portion of faeces were determined in the same manner.

Oysters of different size groups (A, B, C and D) were acclimated to each experimental temperature for 10 days keeping all other environmental parameters constant in order to study the oxygen consumption among these organisms. Rates of oxygen consumption of 3 oysters of each size group were measured by maintaining them in aquaria containing 5 liter seawater, having stirring rod running at a speed of 5-10 r.p.s. In the aquaria, the surface of the water was covered with 1.5 to 2.0 cm layer of liquid paraffin to stop gaseous exchange between the seawater and atmosphere. To ensure steady oxygen consumption, the oysters were allowed 1 h acclimation in the oxygen chamber. Three replicates were measured each time for each size group. Control chambers (containers with no animals) were analyzed similarly to correct for changes in O_2 concentration. Dissolved oxygen was estimated in each chamber by the azide-modified Winkler method[14] and each time the water was siphoned out into a glass stoppered bottle of 150 ml capacity. Metabolic energy expenditure was calculated from the oxygen consumption using the conversion factor 1 ml O_2 equivalent to 19.9 joules[15].

Individual oysters were placed in glass beakers containing 3 liters of freshly filtered seawater after the experiment of clearance rates. One additional beaker without animals served as control. The temperatures were maintained by keeping them in a thermostatic water bath. After two hours, water samples of each experimental beaker were analyzed for ammonia nitrogen.[16]. Ammonia excretion values were expressed in µg NH_4-N h^{-1} and transformed to joules[15] using the conversion factor 1 µg NH_4-N equivalent to 0.025 J. Scope for growth (SFG) was calculated[17] by subtracting the energy lost in respiration (R) and excretion (U) from the energy absorbed from the food (A) after converting all values to joules, SFG = A – (R+U). The SFG was calculated after providing the pearl oysters a feed dose of 30,000 algal cells ml^{-1} of water, composed equal mixture of microalgae *I. galbana* and *C. calcitrans*, corresponding to total dry weight 0.96 mg l^{-1} with an organic content of 0.85 mg l^{1} (88.1%). Net growth efficiency (K_2) is the efficiency by which food is converted into body tissue and was calculated by the following formula:

$$K_2 = [A - (R+U)] / A,$$

where A is the absorbed energy, R is the energy lost in respiration and U, the energy lost in excretion.

The standard error and regression analysis were carried out followed by the procedure of Snedecor & Cochran [18].

Results

Clearance rates of *P. fucata* were inversely related with size groups at all the experimental temperatures (Fig. 1A) and there was a significant regression ($p < 0.01$) between clearance rate and dry tissue weight of the oyster (Table 1). In all the size groups, clearance rate (l. g dry tissue $weight^{-1}$. h^{-1}) was minimum at 18° and maximum at 28°C but declined at further higher temperature of 31°C.

The mean absorption efficiency of four size groups ranged from 43.2 to 57.1% within the range of temperature tested. It increased with water temperature from 18° to 31°C (Fig. 1B). Absorption efficiency was found independent of body size.

The oxygen consumption rate increased from 18° to 31°C in all the size groups (Fig. 1C). There was a significant regression between oxygen consumption and dry tissue weight of *P. fucata* at all the experimental temperatures (Table 1). The rate of oxygen consumption increased markedly with increase in temperature from 18° to 23°C.

The rate of ammonia excretion increased with temperature in all the size groups and decreased in each temperature with increasing size (Fig. 1D). There was a significant relationship between ammonia excretion rate and body size for all the experimental water temperatures. Highly significant regression ($p<0.01$) was observed between ammonia excretion (µg NH_4-N h^{-1}) and body size in all the water temperature (Table 1).

The effect of temperature on the scope for growth (SFG) of the pearl oyster, *P. fucata* is summarized in Table 2 after transformations of all the physiological rates into energy equivalents for each temperature and size group. The SFG of all the size groups showed the lowest value at 18° C. They were 1.56, 4.03, 6.27 and 8.46 J $individual^{1}$ h^{-1}, for the size groups A, B, C and D, respectively. It gave higher values at 26° and 28° C for all the size groups. There was considerable increase in the SFG for all the size classes with increase of water temperature from 18° to 23° and 23° to 26°C (Fig. 1E).

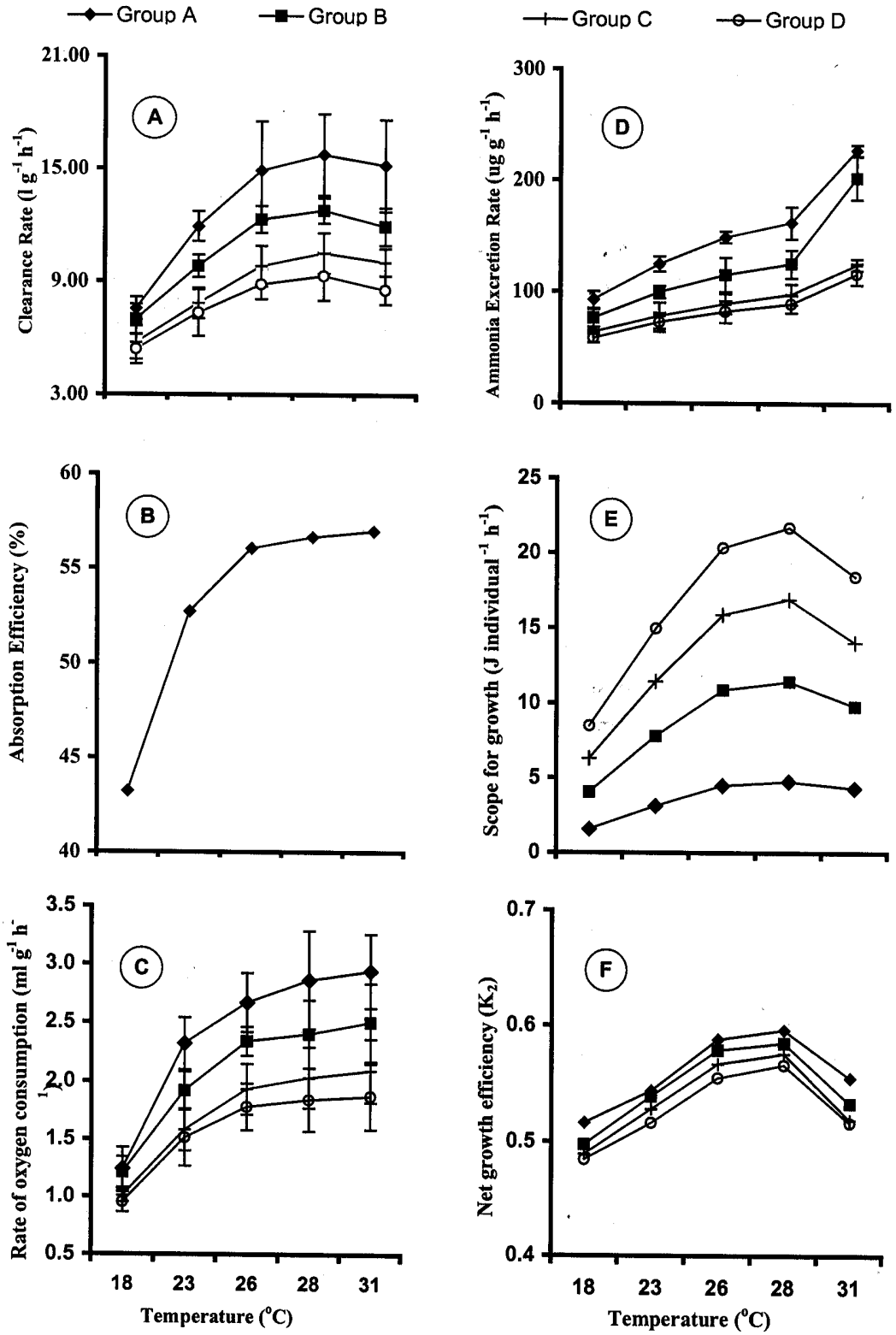

Fig 1— A) Clearance rates by different size groups of *P. fucata* exposed to different water temperatures, B) Absorption efficiency at different temperatures of *P. fucata* (values are means of different size groups), C) Relationship between water temperature and rate of oxygen consumption in different size groups in *P. fucata*, D) Ammonia excretion rates of different size groups as a unction of acclimatization temperature in *P. fucata*, E) Scope for growth for different size groups of *P. fucata* as a function of water temperature, F) Net growth efficiency for four body sizes of *P. fucata* at different temperatures.

Table 1—Regressions of physiological parameters [e.g. clearance rate (l h^{-1}); oxygen consumption (ml h^{-1}) and ammonia excretion (µg h^{-1})] against dry tissue weight (g) for different temperatures, in *P. fucata*. Regression equations are in the form $y = aX^b$, where y = oxygen consumption, X = dry tissue weight. The statistic F is measured as the significance of the difference between b and zero. All 'F' values is considered significant at $p < 0.01$.

Physiological parameters	Temp. (°C)	n	b	a	r
Clearance rate	18	12	0.83	4.74	0.97
(l h^{-1})	23	12	0.75	5.96	0.96
	26	12	0.74	7.24	0.97
	28	12	0.74	7.53	0.96
	31	12	0.73	7.00	0.95
Oxygen consumption(ml h^{-1})	18	12	0.90	0.90	0.95
	23	12	0.79	1.25	0.96
	26	12	0.82	1.55	0.97
	28	12	0.79	1.54	0.96
	31	12	0.79	1.59	0.97
Ammonia excretion	18	12	0.76	46.77	0.96
(µg h^{-1})	23	12	0.72	56.23	0.96
	26	12	0.70	61.66	0.95
	28	12	0.71	69.18	0.97
	31	12	0.65	87.10	0.94

Table 2 — Scope for growth and net growth efficiency (K_2) with relation to water temperature and size groups in *P. fucata*

Temp. (°C)	Size groups	Clearance rate (CR) (l $indl^{-1}$ h^{-1})	Organic ingestion (mg)	Acquired energy (J h^{-1})	Absorbed energy (J h^{-1})	Respired energy (RE) (J h^{-1})	Excreted energy (J h^{-1})	RE + EE (EE)	SFG J h^{-1}	Net growth efficiency K_2
18	A	0.41	0.347	6.978	3.014	1.340	0.119	1.459	1.555	0.516
	B	1.10	0.934	18.783	8.114	3.781	0.300	4.081	4.033	0.497
	C	1.74	1.471	29.582	12.809	6.070	0.469	6.539	6.270	0.489
	D	2.38	2.013	40.481	17.488	8.391	0.634	9.025	8.463	0.484
23	A	0.64	0.541	10.880	5.744	2.454	0.163	2.617	3.127	0.544
	B	1.61	1.362	27.390	14.407	6.235	0.400	6.635	7.772	0.539
	C	2.42	2.047	41.165	21.694	9.652	0.596	10.248	11.446	0.528
	D	3.23	2.732	54.941	29.009	13.247	0.796	14.043	14.966	0.516
26	A	0.80	0.677	13.614	7.624	2.952	0.188	3.140	4.484	0.588
	B	1.97	1.666	33.503	18.795	7.463	0.454	7.917	10.878	0.579
	C	2.94	2.486	49.993	28.046	11.476	0.667	12.143	15.903	0.567
	D	3.85	3.256	65.478	36.668	15.423	0.889	16.312	20.356	0.555
28	A	0.83	0.702	14.117	7.990	3.018	0.208	3.226	4.764	0.596
	B	2.03	1.717	34.529	19.578	7.628	0.490	8.118	11.460	0.585
	C	3.05	2.580	51.884	29.366	11.741	0.713	12.454	16.912	0.576
	D	3.98	3.366	67.690	38.380	15.721	0.950	16.671	21.709	0.566
31	A	0.80	0.677	13.614	7.760	3.151	0.300	3.451	4.309	0.555
	B	1.99	1.683	32.317	18.388	7.894	0.700	8.594	9.794	0.533
	C	3.01	2.546	47.623	27.097	12.040	1.000	13.040	14.057	0.519
	D	3.96	3.350	62.924	35.741	16.053	1.250	17.303	18.438	0.516

Net growth efficiencies (K_2) for *P. fucata* fluctuated widely with the change of water temperatures (Fig. 1F). They were minimum at 18°C ranging from 0.516, 0.497, 0.489 and 0.484 for the size classes A, B, C and D, respectively. The net growth efficiencies gave higher values at 26° and 28°C in all the size groups.

Discussion

In this experiment all the physiological parameters investigated within the range of tested temperatures showed to some extent a direct dependence on water temperature. In this study, the clearance rate (CR) of the pearl oysters, *Pinctada fucata* reduced considerably at water temperature above 28°C. Thus, temperature above 28°C might be considered a stressful condition for *P. fucata* to gain metabolic energy by clearance activity. Yukihira *et al.*[4] reported the CR of other species of pearl oysters, *Pinctada margaritifera* declined over 28° to 32°C but under the same conditions, the CR of *Pinctada maxima* remained steady. In the present study, the CR increased exponentially with body size (dry tissue weight). The value of the exponent in the relationship was 0.83, 0.75, 0.74, 0.74 and 0.73 for the temperatures 18°, 23°, 26°, 28° and 31°C respectively. There is a wide variation in the value of the exponent in the relationship between CR and dry tissue weight in different species of bivalves.[19, 20]

In the present experiment the mean absorption efficiency was minimum in 18°C for all the size groups and it increased with the increase of water temperature from 18° to 31°C. Yukihira *et al.*[4] found that *P. maxima* showed a significant increase in absorption efficiency from 19° to 32°C, but in contrast, the absorption efficiency of *P. margaritifera* was not influenced by temperature. A number of studies on bivalves show that the absorption efficiency is size independent[20,21] and the present investigation on *P. fucata* is in agreement with the previous studies, indicating that there were no significant differences between individuals of different sizes in all the experimental temperatures.

The temperature dependence of oxygen consumption observed in the present study is similar to that of the previous studies[4, 22]. It increased with the temperature from 18° to 31°C in all the size groups. Saucedo *et al.*,[5] reported in the Calafia mother-of-pearl oyster, *Pinctada mazatlanica* the metabolic activity was affected at higher level of temperature (33°C) and as well as at lower level (18°C). The allometric exponent for oxygen consumption reported in the literature is very variable e.g. 0.44 and 0.56 for *P. margaritifera* and *P. maxima*[20] and 0.94 for *P. martensii*[3]. In the present experiment, the values were in the range of 0.79 to 0.82 for 23 to 31°C and 0.90 for 18°C.

Ammonia excretion of molluscs is affected by an interaction between size, temperature and food availability[9, 23]. In the present study, the excretory energy formed 2.4 to 2.8% of the absorbed energy for the temperatures 23° to 28°C in different size groups, whereas, it was slightly higher (3.50 to 3.95 % of absorbed energy) at 18° and 31°C for the various size groups. Bayne *et al.*,[1] reported that excreted energy represent only a small proportion of the energy budget of a species. Excreted energy was estimated only 2-5% of total absorbed energy of another species of pearl oysters, *Pinctada margaritifera* and *Pinctada maxima* of different sizes.[20] The allometric exponent for excretion is variable and with body size it varied between 0.48 and 1.48 in the case of *M. edulis*[23], 0.91 in *P. martensii*[3] and 0.64 and 0.79 in *P. margaritifera* and *P. maxima*, respectively[20]. Comparing well with the values of *P. margaritifera* and *P. maxima*, the values observed for *P. fucata* in this study were, 0.76, 0.72, 0.70, 0.71 and 0.65 for 18, 23, 26, 28 and 31° C, respectively.

The scope for growth (SFG), which provides the index of the whole organism with the change of the environmental temperature, is derived from the integrations of the physiological parameters involved in the energy budget. Net growth efficiency (K_2) is the efficiency with which the assimilated ration is utilized. Widdows & Bayne[24] used the SFG to describe the initial and adaptive responses of *M. edulis* to change in temperature. When subjected to a rise in temperature, the SFG was reduced. Results of the present study on the acclimatization response of *P. fucata* over the range of temperatures from 18° to 31°C are similar to that of *M. edulis*.

In this experiment, the scope for growth was associated with the feeding activity by clearance, ingestion and absorption rates and the energy lost by excretion and respiration. Here the CR increased with temperature from 18° to 28°C, but it decreased with further increase of temperature in all the size groups. On the other hand, oxygen consumption and ammonia excretion increased with temperature within the same size group. These studies also indicate that respiratory

energy accounts for a major portion of the absorbed energy (e.g. 39.4 to 41.8% for different size classes at 28°C) and the energy allocated for excretion was minimum (2.5 to 2.6% of absorbed energy at 28°C). The thermal dependence of the metabolic rates found in this work is consistent with previous studies on different species of oysters[4]. Considering the energy acquisition and metabolic expenditure, it is evident from the present investigation that the SFG and K_2 were maximum at 26° and 28°C, reflecting optimum physiological conditions for survival and growth in the temperature range of 26 to 28°C. Further, in all the test temperatures and among the experimental animals the size groups with smaller oysters have greater growth efficiency than the larger ones.

The findings of the present study can be considered as useful information to predict and explain the physiological conditions and the growth of *P. fucata* exposed to known environmental temperatures. The data is useful for the improvement and perfection of onshore culture of marine pearls using *P. fucata* as a candidate species.

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