

## Spatio-temporal variations in biochemical composition, condition index and percentage edibility of the clam, *Paphia malabarica* (Chemnitz) from estuarine regions of Goa

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Biochemical compositions, condition index (CI) and percentage edibility (PE) of an estuarine clam, *Paphia malabarica*, were studied from two estuarine regions of Goa during March 2009 to March 2010. Relationships between nutritive components and ambient environmental parameters were also established. Significant ( $P < 0.001$ ) spatio-temporal changes in biochemical composition, CI and PE were noticed. Protein content was higher (15.64-86.79%) round the year, followed by carbohydrate (4.29-72.73%) and lipid (2.25-5.12%) contents. Maximum nutritive values i.e. protein (64.37-86.78%), lipid (2.85-5.21%), calorific values (4.61-6.52Kcal/g), CI (31.75-62.06%) and PE (17.27-21.00%) during premonsoon (February-May) period are suggestive of suitable harvesting period for *P. malabarica*.

[**Keywords:** Estuarine, Condition index, Biochemical composition, Percentage edibility, *Paphia malabarica*]

### Introduction

Clam fisheries forms secondary source of livelihood for the small fishermen community. Of the several species edible clams, the short-neck clam *Paphia malabarica* (Chemnitz) forms one of the nutritious and inexpensive sources of food and are widely distributed round the world<sup>1</sup>. They are commercially exploited for locals and external market for Japan and other European countries. Generally, biochemical compositions indicate quality of animal and thus determine its nutritional value in comparison to other organisms<sup>2</sup>. International trade industries have adopted condition index (CI) as a standard criterion to select the best product and also serves as a useful biomarker reflecting the ability of bivalves to withstand natural and anthropogenic stress<sup>3</sup>. Seasonal variations in biochemical composition, physiological condition and PE (meat content/yield), of organism occur in relation to energy metabolism necessary for growth and reproduction and vary seasonally with latitude and strongly related to water temperature, food availability, and the gametogenic cycle<sup>4</sup>.

There is a great amount of literature on biochemical composition of other bivalves<sup>5-7</sup>. However, very limited studies have been attempted to understand the

biochemical constituents and their seasonal variations in *P. malabarica*<sup>8</sup> though it is common fishery resource. This species occurs abundantly in the estuaries of Goa and harvested round the year. Mandovi and Zuari are the two major estuaries, characterized by mixed tides with semidiurnal dominance with heavy fresh water influx during the southwest monsoon (June to September). These estuaries forms major navigation route for ore transport and harbor relatively rich mangrove vegetation, particularly, in the polyhaline and oligohaline zones<sup>9</sup>. As a result the selected bivalve beds (Nerul and Chicalim) towards confluence regions of Mandovi and Zuari, respectively are relatively more influenced by mangroves and mining activities. Since clams form rich protein food, and are regularly consumed by local people, the understanding of the biochemical constituents in different seasons would be valuable for sustainable utilization and management of such natural resource. In the present study efforts have been made to investigate the nutritional quality of *P. malabarica* with respect to its ambient environment.

### Materials and Methods

Specimens of *P. malabarica* were collected monthly from Zuari and Mandovi estuaries at

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Chicalim (Lat-15° 24' 35"N, Lon-73° 52' 59"E) and Nerul (Lat-15° 30' 55"N, Lon-73° 46' 59"E), respectively, from March 2009 to March 2010 (Fig. 1). Organisms (n=25) were handpicked at monthly intervals at ebb and were transported immediately to the laboratory in ice box and were cleaned carefully to remove adhered or fouling organisms/materials on the shells. Their meat was excised and blotted and weighed. Valves too were blotted dry and weighed. Meat and valves thus weighed were dried in an oven at 60°C ± 2 until constant weights were obtained. Water (Moisture) content was estimated by calculating the difference between the wet and dry weight of meat. Dried meat samples were pulverized using mortar and pestle and analyzed for its biochemical composition and energy value (caloric value).

Protein was estimated by the method of Lowery *et al.*,<sup>10</sup> using Bovine Serum Albumin as standard. While carbohydrates were analyzed using phenol sulphuric acid method<sup>11</sup> using glucose as standard. For lipid extraction and estimation method of Folch *et al.*,<sup>12</sup> was followed using chloroform-methanol mixture (2:1, v/v). Ash contents were obtained by calcinations in a muffle furnace at 550°C for 4 h. All determinations were performed in triplicate and values have been expressed as percentages. Calorific

values were calculated using the conversion factors 5.56 Kcal/g, 4.20 Kcal/g, and 9.45 Kcal/g dry weights for proteins, carbohydrates and lipids, respectively<sup>13</sup>.

Condition Index of *P. malabarica* was calculated using equation<sup>14</sup>:

$$CI = \frac{\text{Weight of dry meat (g)}}{\text{Weight of dry shell (g)}} \times 1000$$

Percentage Edibility value was determined by calculating the percentage ratio of the wet weight to the whole weight of meat of the individual specimen<sup>15</sup>:

$$PE = \frac{\text{Weight of dry meat (g)}}{\text{Weight of dry shell (g)}} \times 100$$

A study of ecological parameters viz water temperature, salinity and chlorophyll *a*, were conducted as described by Parsons *et al.*<sup>16</sup>. Data were expressed in terms of mean ± standard deviation and range. ANOVA (Analyses of variance) and Turkey's (honestly significant difference) post hoc analysis was carried out to find out the significance of spatial and temporal variations on biological parameters. Pearson correlation coefficients (r) were computed between biochemical composition, CI, PE, and environmental parameters. Data was subjected to statistical analyses using Statistica 6.

## Results

Monthly values of water temperature, salinity and chlorophyll *a* from Chicalim and Nerul stations are depicted in Fig. 2. Water temperature varied from a minimum of 27°C (at Nerul) to a maximum of 34°C (at Chicalim). Salinity ranged between 1 psu in the month of July (monsoon period) at both stations to 36 psu during premonsoon period (February and May) at Nerul. Chlorophyll *a* was highest in January (4.93 µg/l) at Chicalim while it was least in August (0.71 µg/l) at Nerul (Fig. 2). Biochemical composition, CI and PE showed significant differences amongst the station ( $P < 0.001$ ) and months ( $P < 0.001$ ). Water content in *P. malabarica* was significantly ( $P < 0.001$ ) higher from Chicalim (ranging from 77.90 ± 6.19 to 87.49 ± 1.74%) compared to those from Nerul (ranging from 78.46 ± 2.97 to 85.79 ± 2.94%) (Table 1, Fig. 3). Tukey's post hoc test confirmed significantly high water content in the month of September in organisms collected from Chicalim.

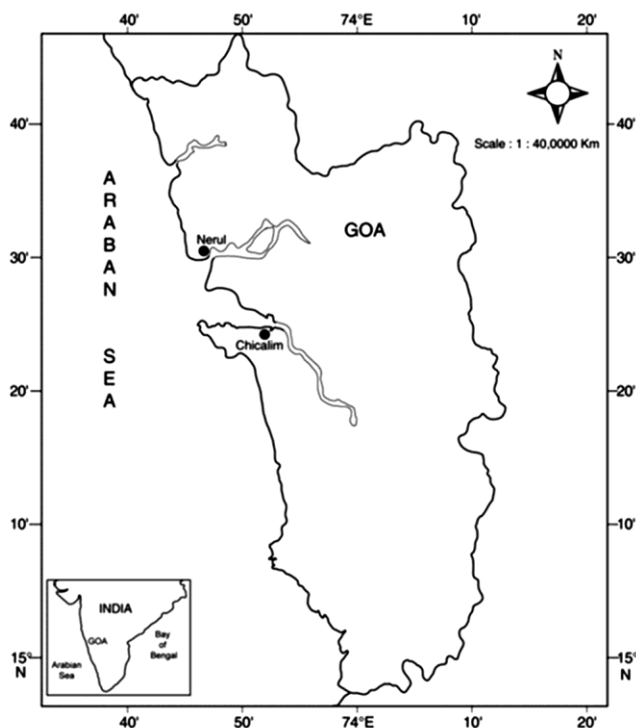


Fig. 1 — Map showing the study area

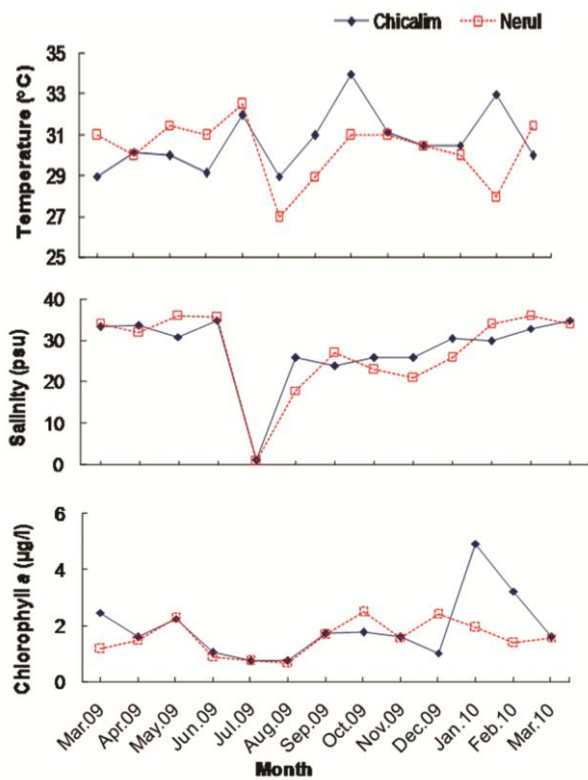


Fig. 2—Monthly variation in environmental physico-chemical parameters

The protein content varied significantly ( $P < 0.001$ ) in the range of  $15.64 \pm 3.48\%$  to  $86.78 \pm 4.16\%$  (Fig. 3). Tukey's post hoc test showed significantly low value of protein during September 2009 in organism from Chicalim while it was highest during March 2010, in organisms from Nerul (Table 1). Total carbohydrates ranged between  $4.29 \pm 0.08\%$  to  $72.73 \pm 0.51\%$  with highest during September 2009 in organism of Chicalim and the lowest ( $4.29\%$ ) during March 2010 in organism from Nerul (Table 1, Fig. 3). A steady increase in carbohydrate was recorded from March till September 2009 and declined thereafter (Fig. 3). Post hoc test showed significantly greater values of carbohydrate for organisms from Chicalim with annual average of  $34.34 \pm 20.05\%$  while in clams from Nerul average of  $22.85 \pm 10.82\%$  was reported (Table 1). Lipid contents of *P. malabarica* from Nerul (range  $2.59 \pm 0.18$  to  $4.65 \pm 0.14\%$ ) was found to be significantly ( $P < 0.001$ ) greater than in the organism of Chicalim (range  $2.25 \pm 0.13$  to  $5.21 \pm 0.00\%$ ) with annual mean value of  $3.28 \pm 0.68\%$  and  $3.12 \pm 0.98\%$ , respectively (Table 1, Fig. 3). Tukey's post hoc test showed significantly high mean value of lipids in organisms from Chicalim during May 2009.

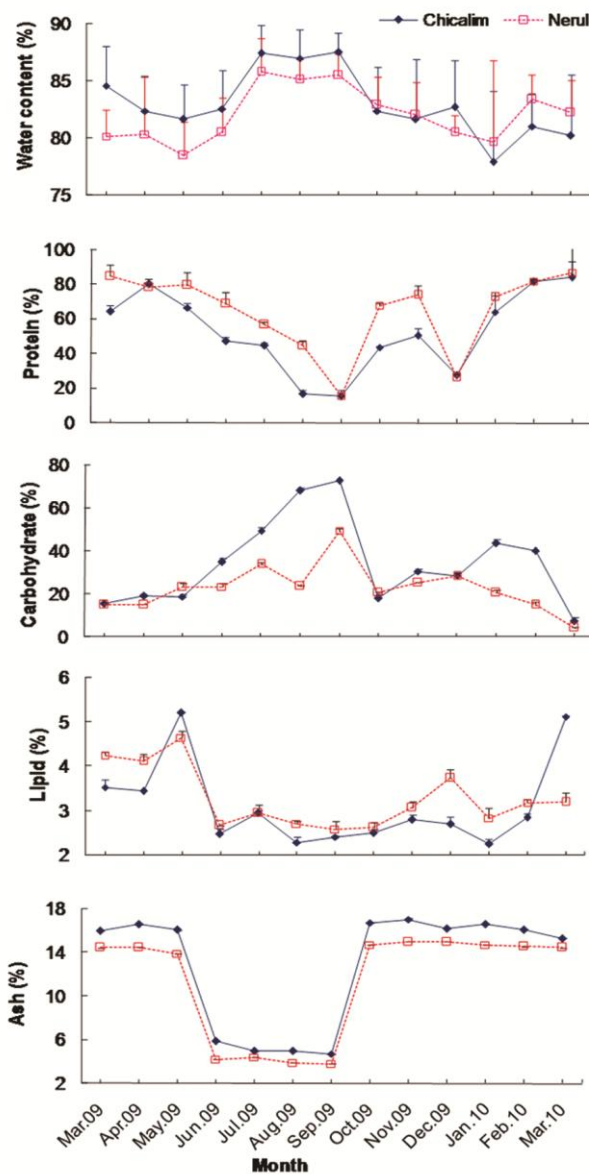


Fig. 3—Monthly variation in percentage (mean±standard deviation) biochemical composition of *P. malabarica*

Ash content in *P. malabarica* from Chicalim was maximum ( $17.05 \pm 0.07\%$ ) during November 2009, minimum value ( $3.8 \pm 0.01\%$ ) was noted during September 2009 in organism of Nerul (Fig. 3). A rapid increase in ash contents of organisms were noticed from October 2009 to March 2010 from both the station. Calorific value ranged from 2.99 to 6.52 Kcal/g with significantly ( $P < 0.001$ ) highest value in February 2010 and lowest in December 2009 at Chicalim (Table 1, Fig. 4). At Nerul higher value was recorded in May 2009 and lower in December 2009 (Fig. 4).

Table 1—Results of ANOVA per parameters studied in *P. malabarica* from study area

Parameters	Factor	df	MS	F value	P value	Post hoc test
Water (%)	Station	1	544.89	33.45	<10 <sup>-6</sup>	
	Month	12	86473.00	53.08	<10 <sup>-6</sup>	
	Station-Month	12	269.68	16.55	<10 <sup>-6</sup>	Chicalim-Sep
Protein (%)	Station	1	2639.46	30.81	<10 <sup>-6</sup>	
	Month	12	2954.82	34.56	<10 <sup>-6</sup>	
	Station-Month	12	179.93	2.10	0.032	Nerul-Mar
Carbohydrate (%)	Station	1	2572.63	1859.02	<10 <sup>-6</sup>	
	Month	12	1221.92	882.98	<10 <sup>-6</sup>	
	Station-Month	12	335.59	242.50	<10 <sup>-6</sup>	Chicalim-Sep
Lipid (%)	Station	1	0.47	29.29	<10 <sup>-7</sup>	
	Month	12	3.54	218.13	<10 <sup>-7</sup>	
	Station-Month	12	0.81	49.84	<10 <sup>-7</sup>	Chicalim-May
Calorific (Kcal/g)	Station	1	0.74	2.68	0.10	
	Month	12	5.48	19.80	<10 <sup>-7</sup>	
	Station-Month	12	1.25	4.52	5.9×10 <sup>-5</sup>	Chicalim-Feb
Condition index	Station	1	1059.04	5.82	0.01	
	Month	12	17072.53	93.96	<10 <sup>-7</sup>	
	Station-Month	12	6358.61	34.99	<10 <sup>-7</sup>	Chicalim-Apr
Percentage edibility	Station	1	171.68	17.44	3.04×10 <sup>-5</sup>	
	Month	12	1348.75	137.01	<10 <sup>-7</sup>	
	Station-Month	12	82.79	8.41	6.85×10 <sup>-16</sup>	Chicalim-May

df: degree of freedom, MS: mean squares

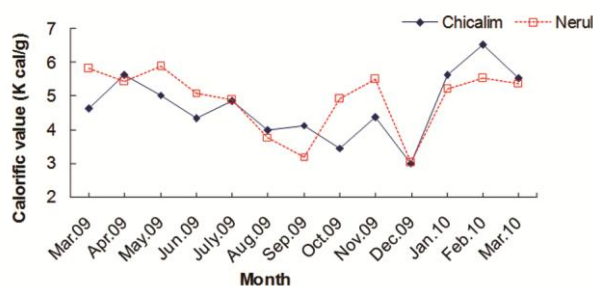


Fig. 4—Monthly variation in calorific value (mean±standard deviation) of *P. malabarica*

The average CI of *P. malabarica* varied from  $20.00 \pm 5.37$  to  $62.05 \pm 7.82$  (Fig. 5). Organism from Chicalim showed high CI (annual mean  $38.25 \pm 12.68$ ) compared to organism from Nerul (annual mean  $36.91 \pm 8.71$ ). Post hoc test confirmed these values significantly ( $P < 0.001$ ) higher in organism from Chicalim during April 2009 whereas, significantly ( $P < 0.001$ ) lower in organisms collected from Nerul in November 2009 (Table 1). Significantly ( $P < 0.001$ ) greater values of PE were reported in *P. malabarica* from Chicalim with annual average of  $16.47 \pm 2.93$  while the same in organisms

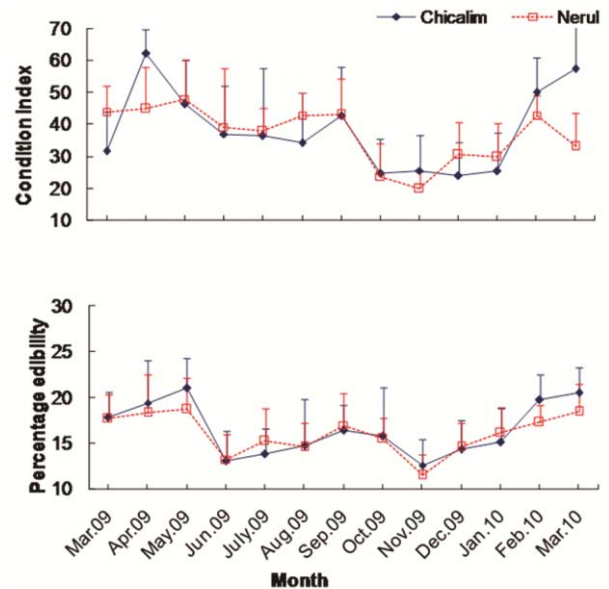


Fig. 5—Monthly variation in CI and PE values (mean±standard deviation) of *P. malabarica*

from Nerul showed average value of  $16.01 \pm 2.17$  (Table 1, Figure 5). Tukey's post hoc test showed significantly low mean value of PE in organisms

Table 2—Correlation coefficients (r values) between environmental parameters and biochemical composition, CI, PE of *P. malabarica*

	Temp	Salinity	Chl <i>a</i>	Protein	Carbohydrate	Lipid	Ash	Calorific value	PE	CI	Water content
<b>Chicalim</b>											
Protein	0.05	0.40*	0.47*	1							
Carbohydrate	0.00	-0.45**	-0.08	-0.70***	1						
Lipid	-0.24	0.25	-0.03	0.60***	-0.64***	1					
Ash	0.21	0.51**	0.51*	0.64***	-0.76***	0.36*	1				
Calorific value	0.04	0.18	0.56***	0.81***	-0.14	0.35*	0.24	1			
PE	-0.02	0.43*	0.29	0.67***	-0.44	0.75***	0.42*	0.59***	1		
CI	-0.14	0.25	-0.07	0.56***	-0.19	0.58***	0	0.65***	0.76***	1	
Water content	-0.09	-0.59***	-0.65***	-0.68***	0.59*	-0.26	-0.76***	-0.43*	-0.28	-0.05	1
<b>Nerul</b>											
Protein	0.31	0.40*	-0.08	1							
Carbohydrate	-0.09	-0.46**	0.00	-0.83***	1						
Lipid	0.27	0.37*	0.25	0.36*	-0.33	1					
Ash	0.21	0.46**	0.64***	0.54**	-0.62***	0.52**	1				
Calorific value	0.39*	0.34	-0.09	0.96***	-0.65***	0.40*	0.46*	1			
PE	-0.01	0.44*	0.19	0.29	-0.31	0.54**	0.27	0.27	1		
CI	-0.29	0.26	-0.38	0.00	0.06	0.44*	-0.35*	0.06	0.62***	1	
Water content	-0.33	-0.69***	-0.45**	-0.48**	0.46**	-0.66***	-0.63***	-0.46**	-0.21	-0.01	1

\*Significance ( $P < 0.05$ ) \*\*Significance ( $P < 0.01$ ) \*\*\*Significance ( $P < 0.001$ )

collected from Nerul during November 2009 and highest value during May 2009 in organisms from Chicalim.

Salinity showed significant ( $P < 0.001$ ) negative correlation with water content of *P. malabarica* whereas, it was positively correlated with ash content and PE. Chlorophyll *a* was positively correlated with protein in organisms from Chicalim whereas positively correlated with ash content in organism from both stations (Table 2). Significant ( $P < 0.001$ ) negative correlation was recorded between protein and carbohydrate, while protein was positively correlated with ash content and calorific content. Lipid contents too were found to be significantly ( $P < 0.001$ ) correlated with ash and calorific content as well as with CI and PE values (Table 2).

## Discussion

The data revealed significant spatio and temporal variations in biochemical composition, CI and PE of *P. malabarica* which could be attributed to various life stages and influence of ambient environment<sup>4,17</sup>.

High percentage of water content during monsoon (September 2009) (Fig. 3) might be due to the replacement for salt in tissue<sup>6,8,18</sup>. Reduced salinity during monsoon leads to more absorption of water which resulted in increased water content in tissue<sup>7</sup>. Significant negative correlation between water content in tissue and water salinity (Table 2) further strengthen the present findings.

Maximum protein content of *P. malabarica* (Fig. 3) during pre-monsoon season (March 2010) could be a mechanism of storage of reserves during gametogenesis to meet energy requirement during spawning season<sup>8</sup>. Further it may also be attributed to better metabolic conditions with increasing accumulation in gametes, proteins constitute the major organic component<sup>19</sup>. Other possibility for elevated proteins content could be the increased feeding efficiency with increased chlorophyll *a*, resulting in proper assimilation of food and better metabolic condition<sup>19</sup>. Significant positive correlation between protein and chlorophyll *a* (Table 2) justifies the above views. Low proteins in an organism during

September 2009 may be due to the spawning activity and decreased chlorophyll *a* value as reported earlier<sup>20</sup>. Reduced protein contents during post-spawning period (Fig. 3) suggest that much of the energy spent during active spawning period was contributed by protein<sup>6</sup>. Spawning period of *P. malabarica* was reported to be from September to January<sup>21</sup>, hence the lower values of protein could be attributed to spawning activity.

Lipids are more influenced by the clam's annual reproductive cycle because of their relationship with gonad maturation<sup>17</sup>. Protein and lipid contents followed almost the similar pattern with highest concentration during premonsoon (May 2009). Previous studies have also suggested the importance of lipids in the economy of adult marine bivalves, for maturation of gametes and as an energy reserve in conditions of either imposed or natural nutritional stress. However, the lower amounts were mainly due to utilization of accumulated lipid for building up of tissue materials<sup>6</sup>.

Carbohydrate values showed a reverse trend with respect to protein and lipid, with carbohydrates (and water content) reaching minima during pre-monsoon (March 2010), when protein and lipid content peaked (Fig. 3). Carbohydrate patterns are strongly related to those of glycogen, which represents about 50% of total carbohydrates<sup>22</sup>. Link between the lipid and carbohydrate contents may thus be rooted in the assumption that lipid accumulations by clams are sourced from glycogen reserves<sup>23</sup> thus resulting in lower value of carbohydrate during premonsoon period (March 2010). During spawning season, energy requirements are met by proteins and lipid to a greater extent as compared to carbohydrates<sup>24</sup>. This is because gonads consist mainly of protein and fat in bivalves.

The increased ash content during pre and post monsoon period (Fig. 3) might be due to an increased inorganic content in the body constituents<sup>18</sup>, which could be confirmed by highly significant correlation of ash content with the protein and lipid contents (Table 2). Further, the lower value of ash content during monsoon period was correlated with low content of chlorophyll *a* and lower salinity. Calorific value of *P. malabarica* is comparable with other edible bivalves<sup>6,8,18</sup>. In general, energy stored prior to gametogenesis, and utilised in the production of gametes, when metabolic demand remains high<sup>8</sup>. *Paphia malabarica* too showed the high energy storage during pre-monsoon (Fig. 4) as noticed by

high protein and lipid values. Higher and lower calorific values were related to proteins and lipids contents (Table 2), which directly influenced the energy content<sup>6</sup>.

Seasonal changes in biochemical composition influenced the changes in PE and CI, which indirectly reflect reproductive phases in *P. malabarica*<sup>5,26</sup>. Values of CI and PE during premonsoon commenced with the maturation stages<sup>18</sup> while low values during post monsoon (Fig. 5) could be attributed to spawning season<sup>8</sup>. These values of clam are considered as very important criteria for bivalve producers, and the CI in particular, gives an indication of the general physiological status of the animals<sup>23</sup>. Condition index values observed were >148, indicative of high meat quality of *P. malabarica*. Significantly higher values of CI and PE in organism from Chicalim compared to organisms collected from Nerul suggests better health of *Paphia* beds in Zuari estuary. Low CI and PE in organism collected from Nerul could be attributed to relatively lower concentration of chlorophyll *a* at Nerul compared to the same from Chicalim. Appukuttan and Aravindan<sup>8</sup> have observed the similar pattern in CI and PE values on the same species from Ashtamudi estuary. However, these values were slightly higher than those reported in *P. malabarica* from Mulki estuary and Ratnagiri coast<sup>15,27</sup>. Phytoplankton concentrations tend to be positively correlated with salinity, hence higher PE values during premonsoon period (May 2009) thus governed by phytoplankton abundance in ambience<sup>28</sup>. Ansell *et al.*<sup>29</sup> reported that, the differences in CI are mainly due to the differences in the seasonal availability of phytoplankton. The correlations of CI and PE with lipid contents (Table 2) are suggestive of the energetic variations in CI and PE in *P. malabarica* could principally be related to the lipid content variations produced by gamete emission and gamete maturation<sup>30</sup>. In other words, an increase in chlorophyll *a*, protein, and lipid results in an increase in CI and PE. The present findings compare well with studies on the seasonality of various edible bivalves<sup>6-8,15</sup>.

## Conclusion

Although the seasonal variation was similar in both stations, annual mean values for protein ( $64.36 \pm 22.48\%$ ), lipid ( $3.27 \pm 0.68\%$ ) and calorific value ( $4.89 \pm 0.95 \text{Kcal/g}$ ) of *P. malabarica* from Nerul were significantly higher than those from

Chicalim, whereas, carbohydrate ( $34.33 \pm 20.05\%$ ), water ( $82.99 \pm 2.89\%$ ), ash content ( $12.84 \pm 5.39\%$ ), CI ( $38.24 \pm 12.68$ ) and PE ( $16.47 \pm 2.92$ ) were found to be significantly higher from Chicalim. Among the three components, the protein showed the highest values round the year followed by carbohydrate and lipid as the smallest component. It seems therefore, that in *P. malabarica*, proteins may be the major energy reserve. Higher values of protein, lipid, calorific value, CI and PE during premonsoon period are suggestive of suitable harvesting period of *P. malabarica*.

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