GONAD DEVELOPMENT AND CONDITION INDEX OF GREEN MUSSEL, *PERNA VIRIDIS* (LINNAEUS, 1758) FROM YE ESTUARY, MON STATE

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Abstract

The study on the gonad development and condition index of green mussel, *Perna viridis* from Ye Estuary was conducted from January to December 2017. Sample collection was made by the help of local mussel collector during low tide. Ripe gonads were in good numbers from January and March and peak spawning ones were observed from April to July.The occurrence of spawning and partially spent specimens of both sexes recorded from April to October indicated that spawning took place during a prolonged period. *P. viridis* exhibited year-round gametogenesis and monsoon spawning. Surface water temperature at Ye estuary varied between 27.60°C in August and 32.00°C in March. CI_{shwt} and $CI_{commercial}$ values were high in June and low in July and December. High CI values in June may be due to ripe gonad states and food available from the river runoff. The low CI value in July coincides with the peak spawning stages of male and female mussels. The study indicated that there is obvious seasonal variation in the mussel condition and gonad maturation.

Keywords Condition index, Gonad Development, Mon State, *Perna viridis*, Ye estuary

Introduction

Green mussel, *Perna viridis* is a commercially important mussel species that distributes widely in the Indo-Pacific regions. It can grow in the optimum temperature ranging from 26 ºC to 32ºC and salinity ranging from 27‰ to 33 ‰ (Power *et al*., 2004). Sexes of this species are separate and gonad tissue of a sexually matured male shows creamy-white in colour, while that of the female appears reddish (Al-Barwani *et al*., 2013). *P. viridis* in tropical countries has been shown to spawn all year-round with two peaks which coincide with monsoon seasons (Soon & Ransangan, 2014).

Condition index (CI) is generally regarded as an indicator of the health status of mussels and the commercial quality of bivalve population (Crosby and Gale, 1990; Al-Barwani *et al*., 2011). The biotic and abiotic conditions of the environment such as food availability, temperature and salinity control the condition of mussel (Seed and Suchanek, 1992). Poor conditions in bivalves can be resulted by the limitations in food availability that is caused by unfavourable changes in environmental conditions (Bayne, 1976). Through the study of the condition index of *P. viridis* in Ye estuary, the spawning time of this mussel can also be estimated. The data observed from the mussels in Ye Estuary can also serve as baseline information for assessing the impacts of any future changes in the ecosystem.

Materials and Methods

Study area

Sample collection was conducted at Sitaw, Ye estuary (Lat. 15º 11' N, Long. 97º 48' E). Sitaw in Ye estuary is an intertidal rock shore area and rich mussel beds are located in subtidal rocky stretches up to the river (Fig. 1). This area is known for a daily level fishery of green mussel and oysters, locally called Be Won and Ka Mar. Mussels are removed from the natural beds, mainly for local consumption as food and for local fishermen's income. The mean values of salinity and temperature were monthly recorded by using a water monitor and refractometer.

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There were marked fluctuations in the salinity during monsoon seasons. The average salinity of the surface water varied from 6.38‰ in June to 30.5‰ in March. Surface water temperature ranged from 27.60°C in August to 32.00°C in March. Seasonal variations in the environmental parameters of Ye Estuary are primarily influenced by the prevailing monsoon regime. During the study period, the southwest monsoon commenced by the last week of May and the highest rainfall of 1628.14 mm was recorded in July 2017.

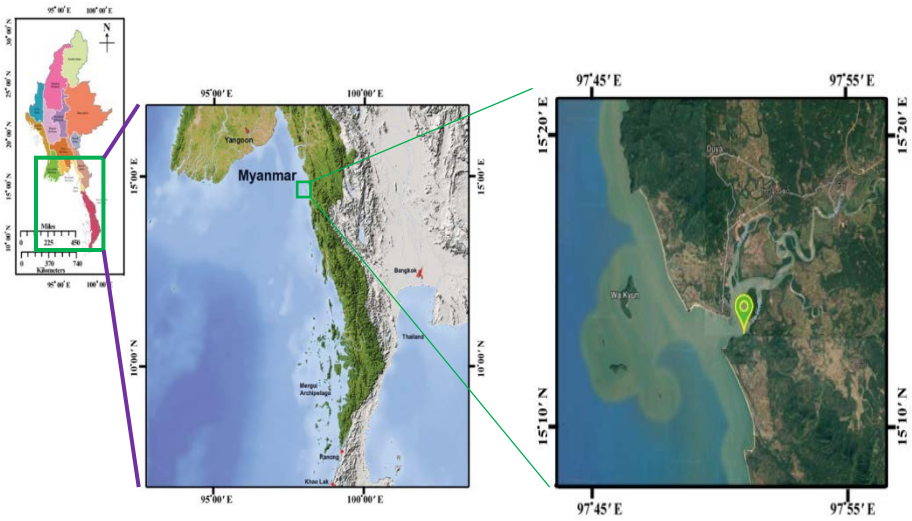

Figure 1 Map showing the sample collection site in Sitaw, Ye Estuary (Source: Google Maps).

Sample Collection

Samples were randomly collected from the subtidal rocky natural mussel beds under about 10 meters in Ye estuary on a monthly basis by the local mussel collector. The collection was made during low tide and samples were collected by using iron implements like chisels and kept in fishing net bags tied around their waist. The collected mussels were cleaned and extraneous water was wiped and transported to the laboratory using the insulated box.

Histological analysis of seasonal gonad development

For the study of gonad development, tissue sections were cut and processed according to standard histological techniques (Howard *et al.*, 2004). Sections of mantle, male and female gonads were immersed in Davidson's fixative (Shaw and Battle, 1957) for one week. The fixed tissues were washed in running tap water overnight and then dehydrated by the usual procedure in ascending grades of alcoholic series as 50%, 70% and 90% alcohol for 1 h, and 100% alcohol for 30 min. The tissues were first soaked in Alcohol-Acetone solution (1:1) for 30 min and then in xylem for 30 min to remove alcohol. After that tissues were infiltered in 2-3 changes of molten paraffin of melting point 58-62°C, and then embedded in wax at 58-60°C, made into blocks which were labeled. Paraffin blocks were trimmed to a suitable size and sections of tissue were cut using a microtome at 10 µm thickness. The resulting ribbons containing tissue sections were fixed on the glass slides using Mayer's egg albumin glycerol (1.1v/v) as an adhesive. Slides were placed in xylene to deparaffinise and were given brief dips in grades of alcohol (100%, 90%, 70% - 20 minutes each) to dehydrate. Hydrated slides were stained in Herris' hematoxylin (2-5 min). Sections were de-stained in acid alcohol, and counter stained in Eosin (1min). Slides were further dehydrated in alcohol and then transferred to xylene (15 min). Slides were mounted in DPX and a cover slip was applied and labeled. Thin layer of albumin was used as an adhesive. Sections were observed and photographed under a light microscope. For the interpretion of tissue structures and the stages of male and female gonads were followed

by the works of Narasimham (1980), Vasanthi *et al*., (2004), Hagger *et al*., (2008), Soumady (2012) and Mcfarland (2016).

Condition index

For the study of condition index (CI), the total weights of 50 mussels were first determined up to 0.01 g by using an electronic digital compact scale (SF-400A). Mussels were then opened; identified sex; tissue was removed from the shell and blotted to remove excess water to ensure accuracy before weighing the tissue. The individual weight of tissue and shell were then determined. CI_{shwt} recommended by Lucas and Beninger (1985) was used for calculating mussel condition index (CI).

$$CI_{shwt} = \frac{\text{Dry soft tissue weight (g)}}{\text{Dry shell weight (g)}} \times 100\,\%$$

According to the recommendation of Hickman and Illingworth (1980), $CI_{commercial}$ (meat yield) was used for studying the variations in wet meat percentage or the percentage edibility (% edibility).

$$CI_{commercial}\,(\text{Meat yield}) = \frac{\text{Wet tissue weight (g)}}{\text{Whole (live) weight (g)}} \times 100\,\%$$

To evaluate the variation of monthly CI values with reference to the mean CI during the study period, a CI ratio was calculated as:

$$\text{CI ratio} = \bar{x}\ CI_{month} / (\bar{x}\ CI_{all\ month}).$$

Based on the values obtained, the monthly CI which exceeded its annual mean was classified as "high (CI ratio >1)" and the remaining as "low (CI ratio <1)" (Hickman, 1991).

Result and Discussion

Gonad Development

The gonadal tissue of a sexually matured male mussel appears creamy-white in color, while that of the female is bright orange in color (Fig. 2 A & B). From the gonad examinations, it was observed that 50% of the mussel of both sexes were matured by about 40 mm. However, Sreenivasan *et al*., (1989) observed that the sexes were matured by about 20 mm and Power *et al*., (2004) reported that sexual maturity occurs at 15-30 mm shell length.

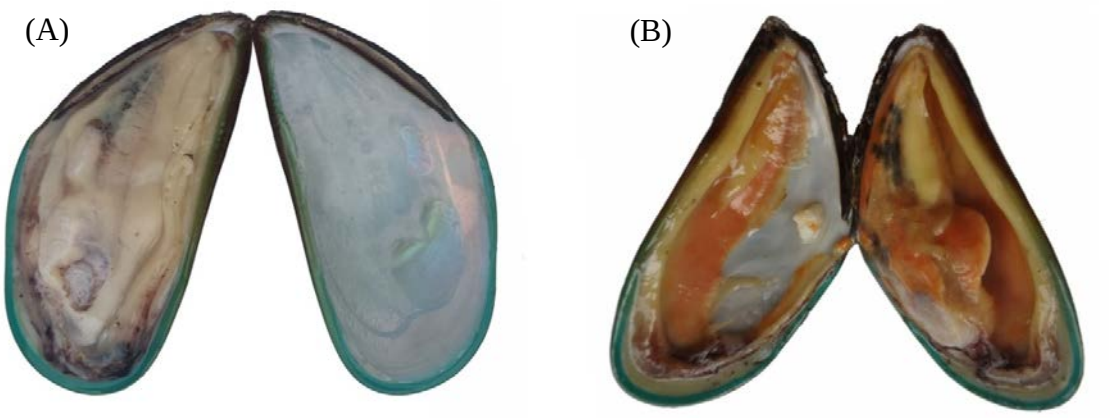

Figure 2 The internal morphology of male and female mussels, *Perna viridis* (A) ♂ Male with milky color, (B) ♀ Female with bright orange color.

Four main stages were distinguished in the reproductive cycle of *Perna viridis* from Ye estuary (Fig. 3 & 4). The percentage composition of different stages of maturity of males and females showed that developing gonads (Stage I) were encountered in November and December in male mussels and October to December in female ones. Ripe gonas (Stage II) were in good numbers from January and March, while partially spawned mussels (Stage III) were found in good numbers from April to July in both sexes. Spent and spent resorbtive individuals (Stage IV) were predominant from September to October in male mussles and August to September in female ones. However partially spawned mussels were observed in almost all months (Fig. 5 A & B).

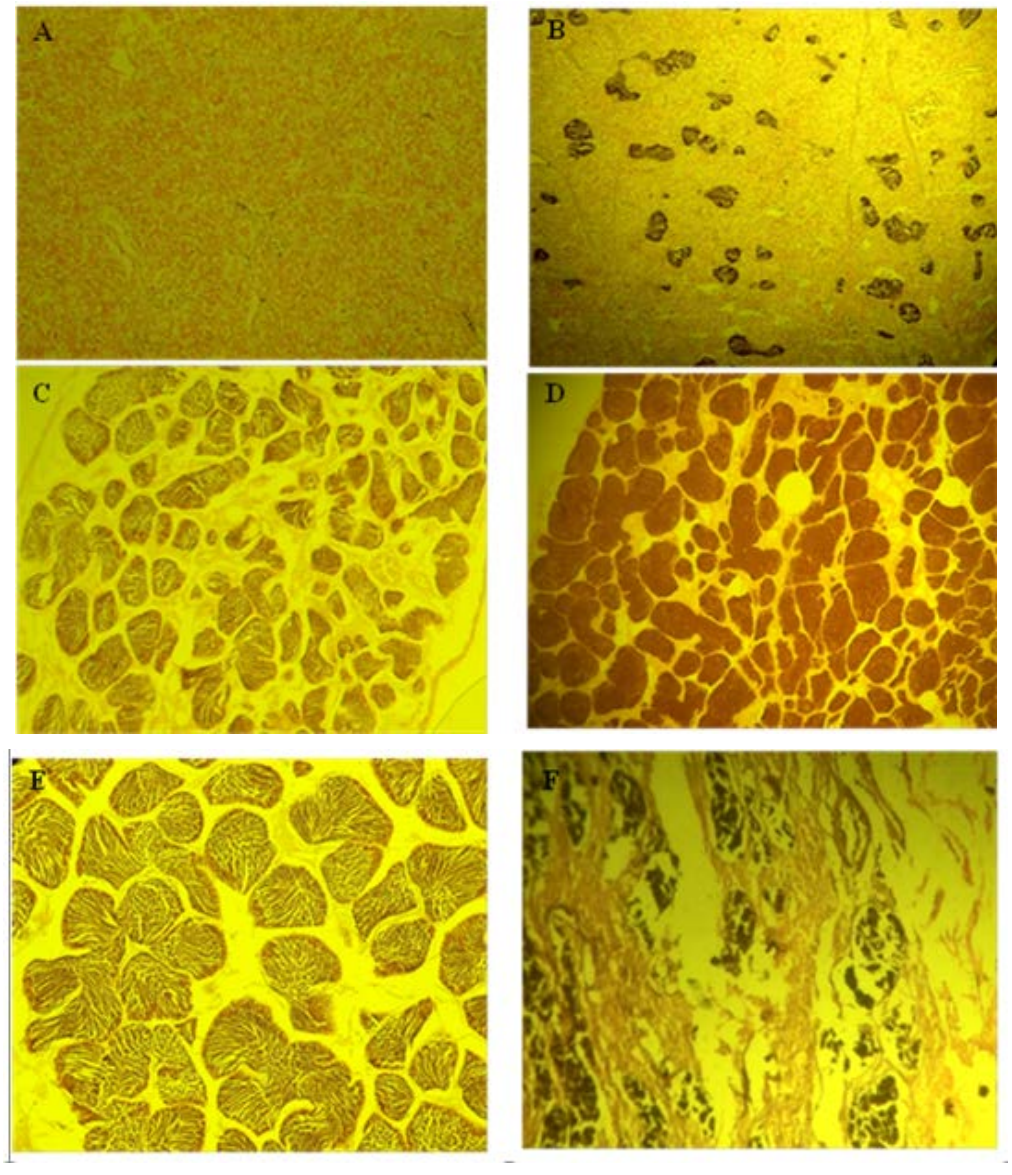

Figure 3 Photomicrographs of male gonads at different stages in sexual cycle of *Perna viridis* (10 x).

(A-C) developing/re-developing stages; (D) ripe gonads; (E) spawning in progress; (F) spent/resting gonads.

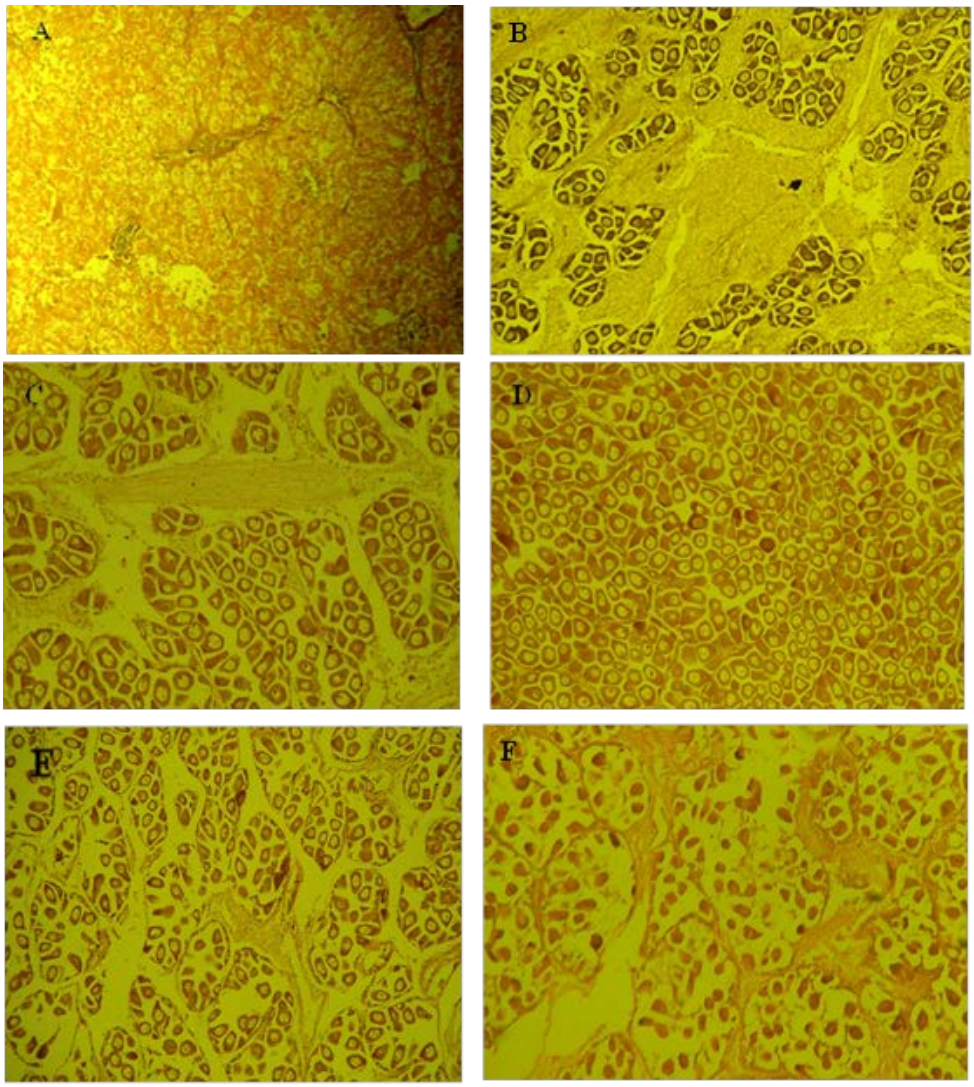

Figure 4 Photomicrographs of female gonads at different stages in sexual cycle of *Perna viridis* (10 x).(A-C) developing/re-developing stages; (D) ripe gonads; (E) spawning in progress; (F) spent/resting gonads.

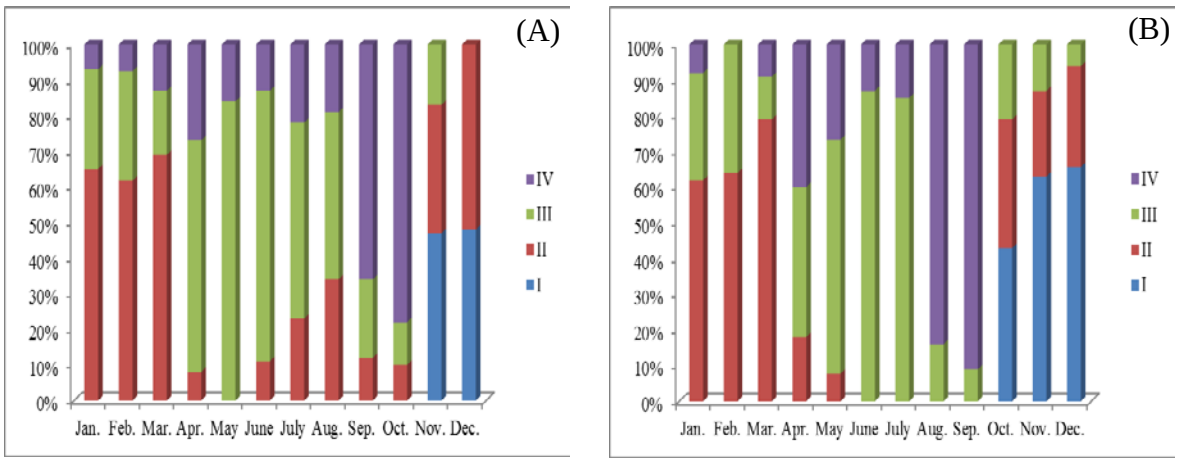

Figure 5 Stages of maturity among males and females *Perna viridis*.: A) Male; B) Female (I: developing/re-developing stage; II: ripe stage; III: spawning stage; IV: spent/resting stage)

The occurrence of spawning and partially spent specimens of both the sexes recorded from April to October indicates that spawning took place during a prolonged period. Though there was a drop in salinity and temperature during the monsoon season (May to October), ripe and spawning individuals continued to be present. This indicates that *Perna viridis* could spawn in salinities less than 30‰ also. Ripe individuals of both sexes were recorded with more numbers from January to March and peak spawning ones were observed from April to July. This indicates that there was peak spawning among green mussels during these months. Narasimham (1980) reported two main spawning periods for *P. viridis* from the east coast of India and stated that the spawning periods were seemingly associated with the seasonal distribution of temperature. Lee (1985) reported a single breeding period that extended from June to September for *P. viridis* population in the Victoria Harbour, Hong Kong. However, Cheung (1993) reported two breeding periods (July-September & November-March) per year for the population dynamics of *P. viridis* in the Tolo Harbour, Hong Kong. Yoshiyasu *et al*., (2004) reported that *P. viridis* in the Sagami Bay, Japan, reproduces successfully and spawns from summer to early autumn.

During the study period, the peak reproductive activity in April for male mussels and in May for female ones coincided with after rising in temperature in March (32.20˚C). Rajagopal (1998 a, b) stated that the temperature regulates the onset of reproductive activity of the mussels along the southeast coast of India. Chen *et al*., (1998) also reported that the spawning period, May-September, was observed when the temperature is 23-26°C. Rajagopal *et al*., (1998a) indicated that peak reproductive activity of *P. viridis* coincided with rising water temperature along the east coast of India. Temperature and food availability have been demonstrated as important decisive factors for somatic growth and gonadal development in bivalves (Seed and Suchanek, 1992; Ceballos *et al*., 2000). The annual reproductive cycle of *P. viridis* has a direct bearing on the degrees of fatness or condition, which has been related to environmental factors such as temperature, salinity and food availability (Rao *et al*., 1975; Nagabhushanam and Mane, 1975; Qasim *et al*., 1977; Ajithakumar, 1984; Parulekar *et al*, 1982; Rivonker *et al*., 1993 and Rajagopal *et al*., 1998a).

Condition Index (CI)

Variations in the value of condition index (CI) were studied by two methods; percentage of meat weight in shell weight of the mussel (CI_{shwt}) and meat weight in total weight of the mussel ($CI_{commercial}$). The highest CI_{shwt} (74.46 %) was recorded in June and the lowest (41.86 %) in December 2017 (Fig. 6A). Wet meat percentage or percentage edibility ($CI_{commercial}$) also followed similar trends as CI, with the highest meat percentage in March and June before spawning, which declined sharply after spawning in July. $CI_{commercial}$ values ranged from 25.61% in July to 37.32 % in June.The range of wet meat percentage observed in the study was comparable with the observations of Narasimham (1980) from natural beds and with the observations of Rivonker *et al*., (1993) and Rajagopal (1998b) from the suspended culture of *P. viridis* along the Indian coast. The comparison of CI values in their absolute terms between different periods was found irrelevant due to the differences in the methods of determination. Many workers applied different methods using wet tissue weight or volume in relation to shell cavity volume or weight.

Distinct peaks in CI with the values of 1.24 in June and 1.20 in October; a decrease was observed in May (0.88) and in July (0.82) which improved later in August. CI (CI_{shwt}) also displayed a continuous decline from March to May which later improved in August. Meat yield or percentage edibility ($CI_{commercial}$) also displayed similar patterns as CI_{shwt} in mussels from Ye estuary. Generally, the percentage edibility of mussels was low in December and July and high in June and August to September months (Fig. 6B). The index values were low when

spawning and spent resorbtive specimens occurred in considerable numbers in the population in July, too (Fig. 5). Mussels from Ye estuary generally showed low condition when compared to Surathkal and Someshwara mussel beds that yielded mussels with high condition (Sasikumar, 2007).

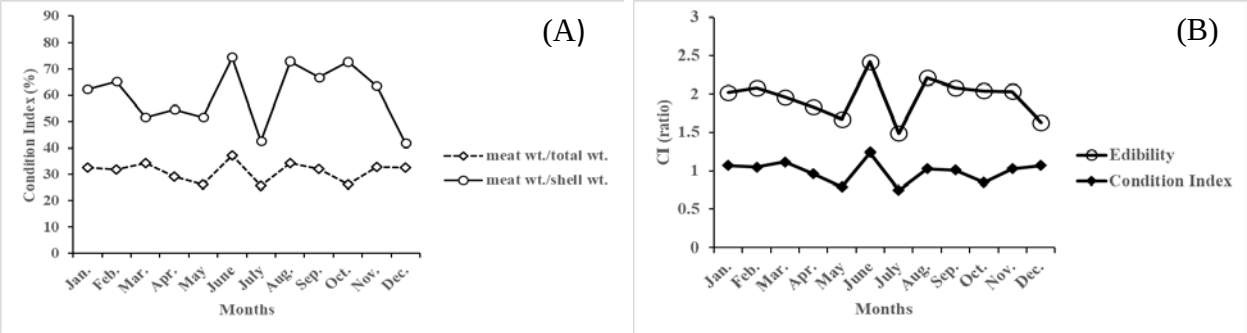

Figure 6 Condition Index of *Perna viridis* during the study period: A) Percentage of condition index; B) CI ratio = $\bar{x}$ CI_{month} / ($\bar{x}$ $CI_{all\ month}$).

The highest CI was observed in the monsoon season, followed by post moonsoon season and premonsoon seasons (Table 1). In the present study, relatively higher water temperature prevailed during March-May, June and December in the mussel bed with a difference of about 5°C between the maximum (32.00˚C) and minimum (27.60˚C) values. It was observed that the CI as well as the percentage edibility corresponded with these seasonal patterns in temperature, with poor mussel condition in pre monsoon and post monsoon. Temperature is a principal factor in controlling the broader aspects of the annual cycle of mussels (Seed, 1976). Chatterji *et al*., (1984) stated the changes in temperature can affect the growth rate of adult mussels in the tropical waters though they are exposed to thermally stable. Nair and Appukuttan (2003) also reported that the temperater changes can affect the development, growth, survival and settlement in spat. The optimum temperature for normal growth in green mussels is between 26 and 32°C (Sivalingam, 1977). The temperature (27.60˚C - 32.00˚C) recorded from the mussel beds in Ye estuary may be within the tolerable limits for this species.

Table 1 Seasonal variation in the condition index and edibility of green mussels from Sitaw

Season	Condition Index	Edibility
Premonsoon (February – May)	0.9800	0.945
Monsoon (June – September)	1.0225	1.028
Post monsoon (October – January)	0.9875	0.990

Conclusion

The study area covers the mussel bed off Sitaw, Ye estuary where green mussel, *Perna viridis* contributes to a significant fishery of commercial importance. Monthly monitoring of established *P. viridis* populations revealed year-round reproductive activity The observed year-round reproduction was fueled by an ability to maintain adequate energy reserves with minimal seasonal variation, which allowed for continuous gametogenesis. Peak spawning activity from April to July may be due to the trigger of rise in temperature during March and April. High CI values in June may be due to ripe gonad states and high food availability from the river and followed by low CI values in July may be due to peak spawning stages of male and female mussels.

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References

Ajithakumar, B.S. (1984) Reproductive physiology of Indian species of the genus Perna (fly. Mytilidae). Ph.D Thesis, Cochin University of Science and Technology (Unpublished).

Al-Barwani, S. M., Arshad, A., Amin, S. M. N., Japar, S. B., Siraj, S. S., and Yap, C. K. (2013) "Population dynamics of the green mussel Perna viridis from the high-spat-fall coastal water of Malacca, Peninsular Malaysia". Fish. Res, vol. 84, pp. 147-152.

Al-Barwani, S. M., Arshad, A., and Amin, S. M. N. (2011) Reproductive biology of green mussel P. viridis (Linnaeus 1758). LAP LAMBERT Academic Publishing GmbH & Co. K. G.

Bayne, B. L. (1976) The biology of mussel larvae. In: Marine Mussels: their Ecology and Physiology, Bayne, B. L. (Ed.). Cambridge University Press.

Ceballos, V. B. P., Martinez, M. A., Dominguez, F. G., and Fuerte, M. V. (2000) "Reproductive cycle of the rugosse pen shell, Pinna rugosa Sobewey, 1835 (Mollusca: Bivalvia) from Bahia Concepcion, Gulf of California and its relation to temperature and photoperiod". Shellfish. Res., vol. 19, no. 1, pp. 95-99.

Chatterji, A., Ansarl, Z. A., Imcole, S. S., and Parulekar, A. H. (1984) “Growth of the green mussel, Perna viridis L, In sea water circulating system”. Aquaculture, vol. 40, pp. 47-55.

Cheung, S.G. (1993) "Population dynamics and energy budgets of green-lipped mussel Perna viridis (Linnaeus) in a polluted harbor". Journal of Experimental Marine Biology and Ecology, vol 168, pp. 1-24.

Crosby, M. P., and Gale, L. D. (1990) "A review and evolution of bivalve condition index methodologies with a suggested standard method". J. Shell. Res, vol. 9, no. 1, pp. 233-237.

Hagger, J., Lowe, D., Galloway, T., and Jones, M. (2008) Ecosystem management bioindicators. Science Report-SCO 50062. Environmental Agency, Rio House, Bristol.

Hickman, R.W. (1991) " The relationship between farmed mussels, Perna canaliculus, and available food in Pelorus-Kenepuru Sound, New Zealand". Aquaculture, vol. 99, no. 1 & 2, pp. 49-68.

Hickman, R.W. and Illingworth, J. (1980) “Condition cycle of the green lipped mussel, Perna canaliculus, in New Zealand”. Mar. Biol, vol. 60, pp. 27-38.

Howard, D. W., Lewis, E. J., Keller, B. J., and Smith, C. S. (2004) Histological techniques for marine bivalve mollusks and crustaceans. NOAA Technical Memorandum. MOSNCCOS, Oxford, MD, USA.

Lee, S.Y. (1985) "Population dynamics of the green mussel Perna viridis (L.) (Bivalvia: Mytilacea) in Victoria Harbour, Hong Kong: dominance in a polluted environment". Asian Mar. Biol, vol. 2, pp. 107-118.

Lucas, A., and Beninger, P. G. (1985) "The use of physiological condition indices in marine bivalve aquaculture". Aquaculture, vol. 44, pp. 187-200.

Mcfarland, K. (2016) Ecology, environment. Seasonal variation in gametogenesis and proximal biochemical composition of the invasive green mussel, Perna viridis, in southwest Florida. In: Population dynamics of the invasive green mussel Perna viridis and their response to the toxic dinoflagellate Karenia brevis: apllication of Dynamic Energy Budget theory to determine population trends. Florida Gulf Coast University.

Nagabhushanam, R., and Mane, U. H. (1975) "Reproduction in the mussel, Mytilus viridis at Ratnagiri. Bull. Dept. Mar. Sci. Univ. Cochin. vol. 2, pp. 377-387.

Nair, M., and Appukuttan, K. K. (2003) "Effect of temperature on the development, growth, survival and settlement of green mussel Perna viridis (Linnaeus, 1758)". Aquaculture Research, vol. 34, pp. 1037-1045.

Narasimham, K. A. (1980) "Fishery and biology of green mussel, Perna viridis (Linnaeus). Mussel farming progress and prospects". CMFRI Bulletin, vol. 29, pp. 10-16

Parulekar, A. H., Dalal, S. G., Ansari Z. A., and Harkantra, S. N. (1982). "Environmental physiology of raft-grown mussels on Goa, India". Aquaculture vol. 29, pp. 83-93.

Power, A. J., Walker, R. L., Payne, K. and Hurley, D. (2004) "First Occurrence of the Nonindigenous Green Mussel, Perna viridis (Linnaeus, 1758) in Coastal Georgia, United States". Journal of Shellfish Research, vol. 23, no. 3, pp. 741-744.

Qasim, S. Z., Parulekar, A. H., Ilarkantra, S. N., Ansari, Z. A. and Nair, A. (1977) "Aquaculture of green mussel Mytilus viridis L., Cultivation on ropes from floating rafts". Indian Journal of Marine Science, vol. 4, pp. 189-197.

Rajagopal, S., Venugopalan, V. P., Nair, K. V. K., Velde, G. V. D., and Jenner, H. A. (1998a) "Reproduction, growth rate and culture potential of the green mussel, Perna viridis (L). in Edaiyur Back waters, east coast of India". Aquaculture, vol. 162, pp. 187-202.

Rajagopal, S., Venugopalan, V. P., Nair, K. V. K., Velde, G. V. D., and Jenner, H. A. (1998b) "Settlement and growth of the green mussel Perna viridis (L.) in coastal waters, influence of water velocity". Aquatic Ecology, vol. 32, pp. 313-322.

Rao, K.V., Kumari, L. K., and Dwevedi, S. N. (1975) "Biology of the green mussel, Mytilus viridis". Indian J Mar Sci, vol. 4, pp. 189-197.

Rivonker, C.U., Ansari Z. A., and Parulekar, A. H. (1993) "Cultivation of green mussel, Perna viridis L., on a floating raft in an estuary along the west coast of India". Aquaculture, vol. 112, pp. 47 –56.

Sasikumar, G. (2007) Studies on the coastal water quality in relation to the health of green mussel, Perna viridis (Linnaeus). PhD Dissertation. Department of Post-Graduate Studies and Research in Biosciences Mangalore University, Mangalagangothri-574 199, Karnataka, India (Unpublished).

Seed, R. (1976) Ecology. In: Marine mussels; their ecology and physiology, Bayne, B. L. (Ed). IBP 10. Cambridge University Press, Cambridge.

Seed, R., and Suchanek, T.H. (1992) Population and community ecology of Mytilus. In: The mussel Mytilus: ecology, physiology, genetic and culture, Gosling, E. M. (Ed). Elsevier Sci. Pub., Amsterdam, Netherland.

Shaw, B., and Battle, H. (1957) "The gross and microscopic anatomy of the digestive tract of the oyster Crassostrea virginica (Gmelin)". Canadian Journal of Fisheries and Aquatic Sciences, vol. 35, pp. 325-347.

Sivalingam, P. M. (1977) "Aquaculture of green mussel Mytilus viridis Linnaeus, in Malaysia". Aquaculture, vol. 11, pp. 297-312.

Soon, T. K., and Ransangan, J. (2014) "A review of feeding behavior, growth, reproduction and aquaculture site selection for green-lipped mussel, Perna viridis". Advances in Bioscience and Biotechnology, vol. 5, pp. 462-469.

Soumady, D. 2012. Ecological of Perna viridis in a portion of coastal waters of Nagapattinam District, Tamil Nandu, India. PhD Dissertation. Department of Zoology. Bharathidasan University, India (Unpublished).

Sreenivasan, P. V., Thangavelu, R., and Poovannan, P. (1989) "Biology of the green mussel, Perna viridis (Linnaeus) Cultured in Muttukadu Lagoon, Madras". Indian J. Fish, vol. 36, no. 2, pp. 149-155.

Vasanthi, L. A., Muruganandam, A., Revathi, P., Baskar, B., Jayapriyan, K., Baburajendran, R., and Munusamy, N. (2004) "The application of histo-cytopathological biomarkers in the mud crab Scylla serrata (Forskal) to assess heavy metal toxicity in Pulicat Lake, Chennai". Marine Pollution Bulletin, vol. 81, pp. 85-93.

Yoshiyasu, H., Ueda, I., and Kiyoshi, A. (2004) "Annual reproductive cycle of the green mussel Perna viridis (L.) at Enoshima Island, Sagami Bay, Japan". Sessile Organisms, vol. 21, pp. 19–26.