

Original Article

Evolution of ecophenotypic plasticity in Indian Oyster, *Crassostrea madrasensis* (Preston) population from Ashtamudi Lake, Kerala, India

Vineetha Vijayan Santhi, Mano Mohan Antony*, Leeanda Lopez, Lekshmi Vasanthi, Joelin Joseph

Post Graduate and Research Department of Zoology, (Research Centre, University of Kerala) University College, Palayam, Thiruvananthapuram, Kerala, India.

Abstract: The Indian Oyster, *Crassostrea madrasensis* are abundant in the coastal waters of Tamil Nadu and Kerala. Since, they are benthic filter feeders, the external environmental conditions impose ceaseless effects on their shell affecting one or more of size, sculpture, anatomy pattern, shape and colour resulting in ecophenotypic plasticity. However, the identification of Oyster species is still based on phenotypic characters that are highly plastic. Therefore, this study aimed to investigate the phenotypic plasticity of the Indian Oyster, *C. madrasensis* population of Ashtamudi Lake with respect to certain ecological parameters. Individuals were collected from the barmouth and upper reaches of Ashtamudi Lake, Kerala and apparent variations were measured. Significant variations were found in the Shell colour and shell pattern. Accordingly, two morphotypes of *C. madrasensis* were recorded. Since, the two population exhibit remarkable differences in morphology, species confirmation was made possible using mitochondrial 16S rRNA gene. Even though 2.7% genetic distance observed between the two morphotypes of *C. madrasensis* is not enough to consider them as different species, it calls attention to the possibility of evolutionary divergence in the near future.

Article history:

Received 3 March 2021

Accepted 12 October 2021

Available online 25 October 2021

Keywords:

Crassostrea madrasensis

Ashtamudi

Phenotypic plasticity

Gene sequencing

Introduction

Many speciation events and indeed whole adaptive radiations, result from the colonization of a new environment, and this entry into a new environment results in selection pressures favouring divergence from the ancestor, induce changes in an individual's physiology, behaviour and morphology. Such changes are collectively called as phenotypic plasticity. The Indian Oyster, *Crassostrea madrasensis* are abundant in the coastal waters of Tamil Nadu and Kerala. Since, they are sessile and benthic, and the external environmental conditions such as salinity, temperature, substrate, diet, moisture, predators and pollution rate impose interminable effects on their shell traits resulting in ecophenotypic plasticity.

Ecophenotypic plasticity is referred to as the ability of a genotype to produce multiple phenotypes when exposed to different environmental conditions. The morphological variations which is an outcome of phenotypic plasticity had led to the description of at least 4000 freshwater mussel species, among which

only ~840 species have been recognised (Haas, 1969; Graf and Cummings, 2007). This has been an interminable and a controversial issue that give rise to exaggerating the extant number of freshwater bivalves, as the species with high morphological plasticity variability on its shells tends to inflate the number of species, which is in reality, the ecophenotypes of the same species (Klishko et al., 2018).

Ecophenotypic plasticity and cryptic morphology are far prevalent among relatively sessile or immobile animals as they have to persistently adapt themselves to cope up with the surrounding conditions (Schlichting, 1986). Moreover, the theory of genetic isolation by geographic distance predicts that increasing distance between populations results in the decreasing gene flow that will lead to the increasing phenotypic divergence (Slatkin, 1987; Hendry et al., 2001). This morphological variation has been the subject of many evolutionary studies. These have increased greatly due to increasing availability of DNA sequences (Bickford et al., 2007).

*Correspondence: Mano Mohan Antony
E-mail: manomohanantony@universitycollege.ac.in

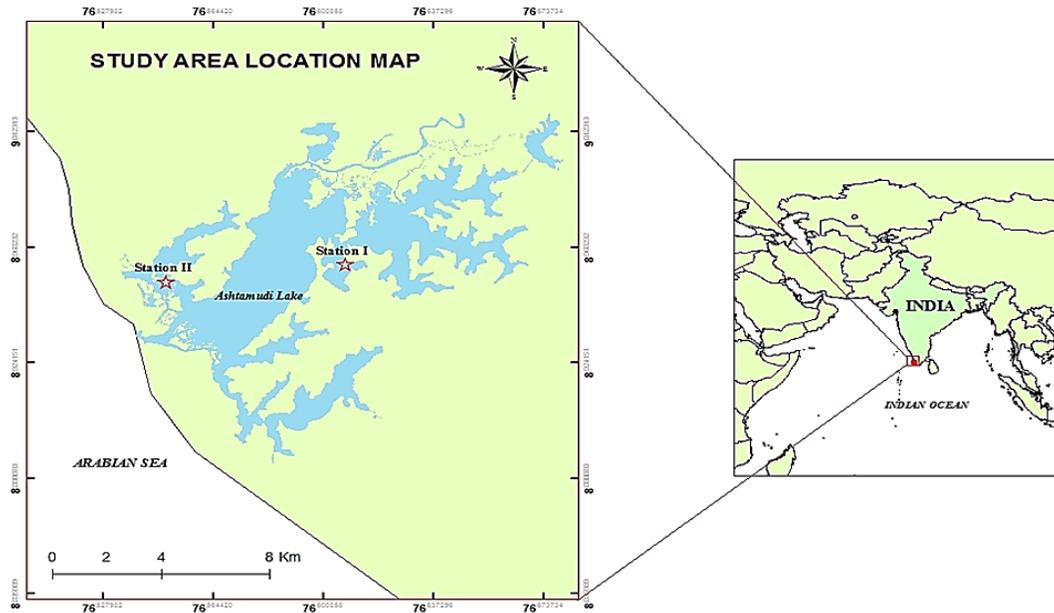


Figure 1. Location map of Ashtamudi Lake showing the study sites (Station I-Perumon Lake, Station II-Thekkumbhagam Lake).

As bivalve molluscs are closely related to their external environment, the changes in the allied habitat can be meticulously monitored using their shell morphology. Therefore, this is an attempt to look over the phenotypic plasticity of the Indian Oyster, *C. madrasensis* population of Ashtamudi Lake with respect to certain ecological parameters.

Materials and Methods

Live samples of *C. madrasensis* was collected randomly from the oyster beds of two ecologically different sites of Ashtamudi Lake, viz. Site I, Perumon (upper reach, Lat-8.961508, Long-76.607392) and Site II, Thekkumbhagam (estuarine barmouth region, Lat- 8.955707, Long-76.549566) for a period of six months, from March 2019 to August 2019 (Fig. 1). The study sites were surveyed every month at low tides and samples were collected by hand picking method from intertidal regions and shallow coastal waters. After collection, the live samples were brought to the laboratory for further investigations. Samples for molecular analyses were immediately transferred to absolute alcohol. Fifty individuals from each site was used for the morphological and morphometrical analysis. Water samples were collected from the sampling sites during morning hours between 8 a.m.

to 11 a.m. and used for the analyses.

The pH of water samples was recorded using a calibrated digital pH pen. The surface water temperature was recorded at the sites using an accurate mercury thermometer. Dissolved oxygen which was fixed in situ was estimated by Winkler's method (APHA, 1989). Salinity of the water sample was estimated by silver nitrate method (Trivedy et al., 1987). Total hardness and TDS was estimated using standard procedures of APHA (1989).

Samples were photographed and morphological characters were examined according to Durve (1974). Classification were referred to the World Register of Marine Species (Preston, 1916). Thirty individuals were randomly selected and used for morphometric analysis. According to Moore et al. (1971) and Dillon and Manzi (1989), 12 morphometric characters were selected and abbreviated as TW (Total Weight - gm), SWW (Shell Wet weight - gm), SDW (Shell Dry Weight - gm), MW (Meat weight - gm), MC (Meat Content - %), SCV (Shell Cavity Volume - ml), SV (Shell Volume - gm), SD (Shell Density - SDW to SV), SH (Shell Height - mm), SL (Shell Length - mm), SD (Shell Width - mm) and SH/SL (Ratio of Shell Height to Shell Length). Data analysis of the morphometric parameters were done by ANOVA. The

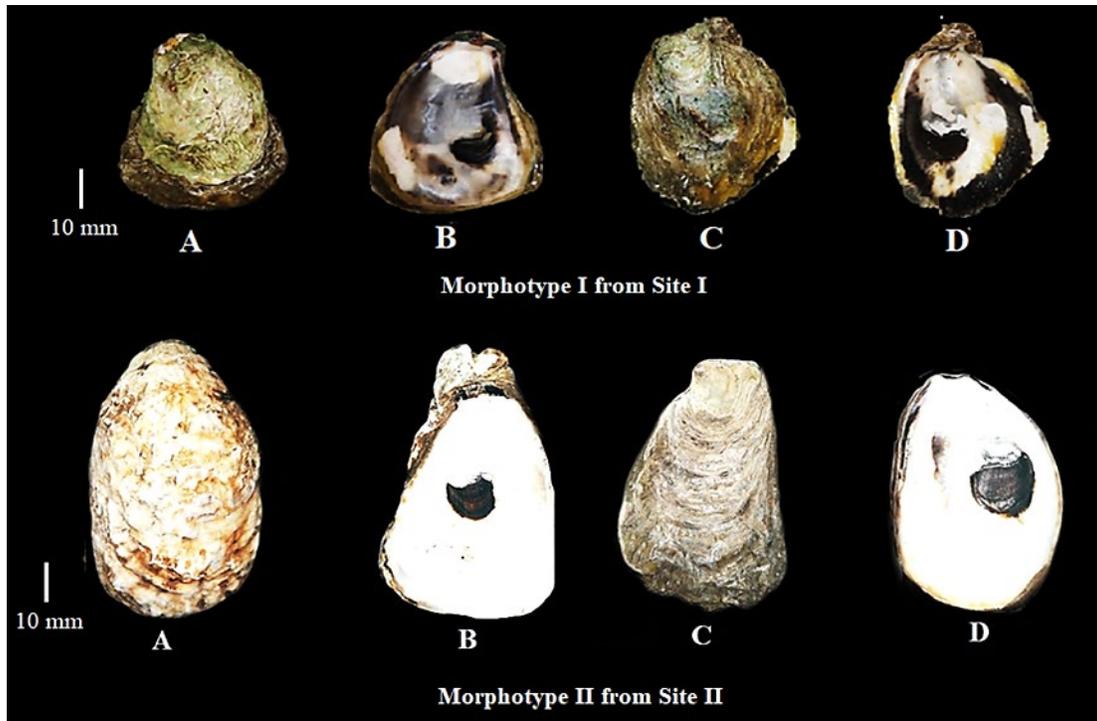


Figure 2. Morphotypes of *Crassostrea madrasensis*. (A) right valve dorsal (B) right valve ventral (C) left valve dorsal (D) left valve ventral.

differences in mean were tested using Duncon analysis. Significant level used was $P \leq 0.05$. All the statistical analysis was performed using the software SPSS 22.0 for windows.

The uncertainty in species identification due to phenotypic plasticity was addressed by performing 16S rRNA gene sequencing preceded by BLAST analysis. The genomic DNA was isolated from the muscle tissue using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instruction. DNA amplification of partial mitochondrial 16S rRNA was obtained using the primers 16Sar 5' CGCCTGTTTATCAAAAACAT 3' and 16Sbr 5'CCGGTCTGAACTCAGATCAC GT3' (Palumbi et al., 1991). The PCR cycles were carried out in a Mastercycler PCR System (Eppendorf) under the following conditions: an initial denaturation for 3 min at 94°C, followed by 35 cycles of 40 s at 94°C, 40 s at 48°C annealing temperature, 40 s at 72°C, and with a final 5-min extension at 72°C. PCR products were purified using ExoSAP-IT (GE Healthcare). Sequencing reaction was done in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Bio systems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Bio systems,

USA) following manufactures protocol. Genetic distance between two morphotypes ('p' distance) was calculated, the estimates of evolutionary divergence between the sequences were inferred using MEGA 7.

Results

Morphological and morphometrical analysis: Two different morphotypes of *C. madrasensis* were recorded from Ashtamudi Lake with respect to the two different ecological sites. The two morphotypes were clearly distinguishable from each other in terms of the shell type, shape, size, internal shell colour, colour and position of muscle scars (Fig. 2):

Morphotype I from Site I: Greenish yellow shells with brittle lamellae, umbos not prominent with shallow cavity, smooth inner surface and having purple around whole or part with dark purple to black shell outline. Adductor muscle scars reniform, deep purplish in colour and posterior in position.

Morphotype II from Site II: Whitish yellowish shells with solid lamellae, left valve (lower valve) thick, heavy, concave and deeply cupped, umbos prominent with deep and chalky cavity, inner surface white, outlines absent, adductor muscle scars dark purplish to black, D-shaped and sub-central in position.

Table 1. Morphometric analysis of Morphotype I and Morphotype II of *Crassostrea madrasensis*.

	Morphotype I (from Site I)			Morphotype II (from Site II)		
	range	mean	SD	range	mean	SD
TW	51.94-72.14	60.38	6.95	149.14-268.1	183.5	29.9
SWW	34.82-81	48.35	11.17	60.28-165.43	133.7	34.3
SDW	12.1-52.38	32.92	9.1	59.8-206.4	130.54	35.92
MW	4.9-8.7	6.3	1.32	7.76-19.07	11.33	2.9
MC	8.23-13.4	10.5	2.26	4.49-7.72	6.17	1.05
SCV	7.00-31	16.93	7.2	21-48	34.2	8.17
SV	10.54-46.37	29.82	12.1	52.6-112.26	80.51	15.55
SDN	0.38-3.33	1.3	0.8	1.14-2.59	1.62	0.38
SH	48.2-107	69.2	13.43	79-125	103.21	12.9
SL	38.2-69	52.6	8.05	52-95	73.61	13.93
SD	21-46	31	7.71	22-61	37.62	10.91
SH/SL	1.02-1.55	1.32	0.14	1.04-1.75	1.43	0.22

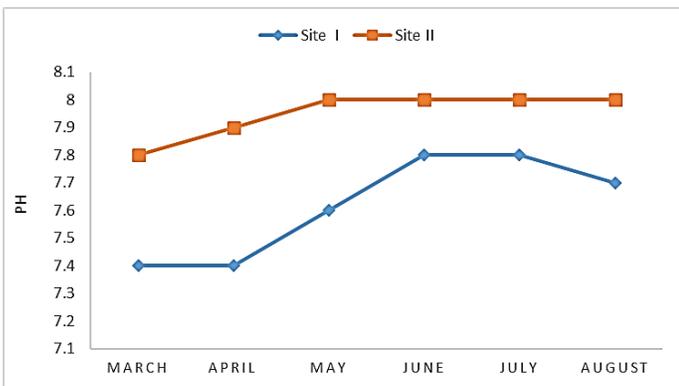


Figure 3. Monthly variation of pH from the two sites of Ashtamudi Lake.

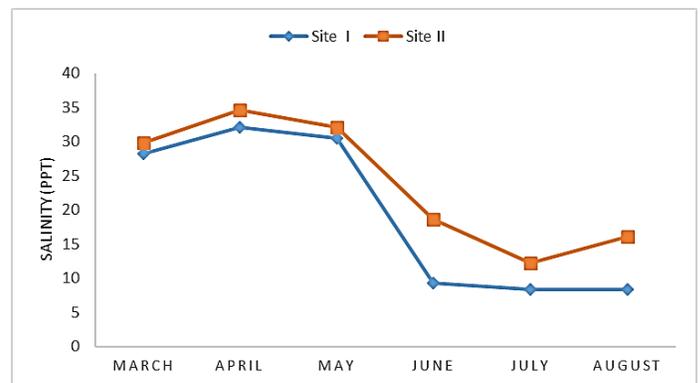


Figure 5. Monthly variation of salinity from two sites of Ashtamudi Lake.

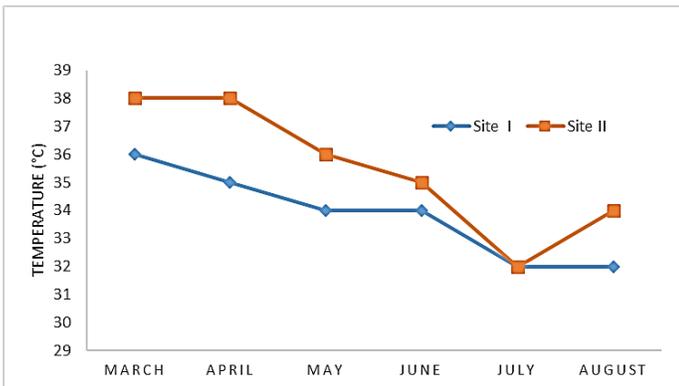


Figure 4. Monthly variation of temperature from the two sites of Ashtamudi Lake.

Remarkable variations were also observed in the overall morphometry of two morphotypes. Apart from MC, all the parameters show higher values for Morphotype II. The morphological parameters such as TW, SWW, SDW and SV were two times greater for Morphotype II, whereas MW, SCV and SH were doubled in Morphotype II. (Table 1).

Molecular analysis: Since the two morphotypes exhibited phenotypic plasticity, species confirmation was done through mitochondrial 16S rRNA gene sequencing method. FASTA sequences with 558 and 590 bp were interpreted from the Chromatogram. The BLAST analysis of the 16S rRNA gene sequences obtained from morphotypes 1 and II showed 99.79 and 99.78% similarity with *C. madrasensis* (Accession no. JF915515) in the NCBI GenBank, respectively. These sequences were submitted in NCBI GenBank with accession number of MN508435 (Morphotype I) and MN508481 (Morphotype II). Evolutionary divergence between the sequences were estimated. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 487 positions in the final dataset with 13 base differences contributed to 2.7% genetic distance between both the morphotypes.

Water quality parameters: The water quality of Perumon Lake and Thekkumbhagam Lake exhibited a

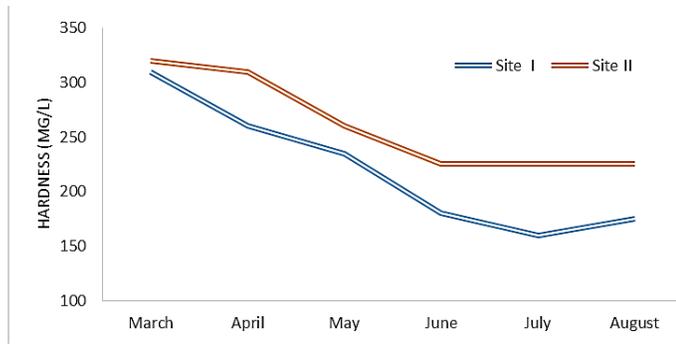


Figure 6. Monthly variation of hardness from two sites of Ashtamudi Lake.

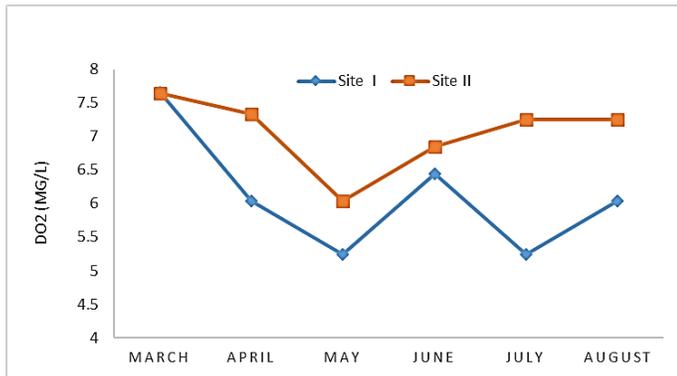


Figure 7. Monthly variation of dissolved oxygen from the two sites of Ashtamudi Lake.

continuous change with respect to the environmental conditions. A seasonal pattern was observed in both the sites, where highest values were recorded from Thekkumbhagam region (Figs. 3-8).

Discussions

The observed ecological parameters determine the morphological and physiological properties of *C. madrasensis*, so the variation in the external environment creates differences in the individuals of the same species between the different ecological sites. Being ectosomatic organ, shell faster than the internal organs reacts to fluctuations in the external environment, which allows them to adapt to a wide range of environmental conditions (Severtsov, 1939).

Several studies have showed morphological variations of the shell among populations in different habitats (Mackie and Topping, 1988; Hornbach et al., 2010). A study reported that a single species could be light and laterally compressed in form in headwaters, but heavier and more laterally inflated in downstream

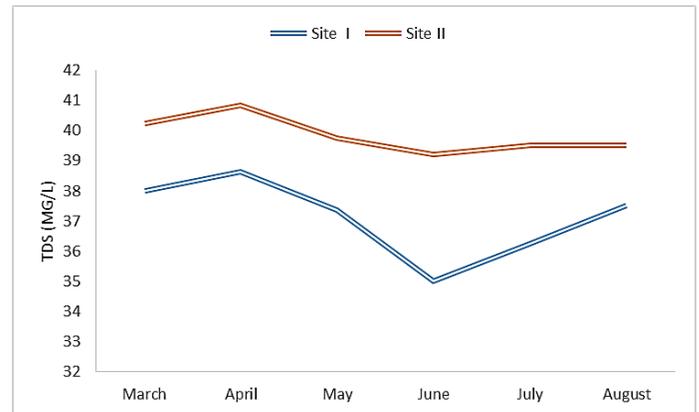


Figure 8. Monthly variation of Total Dissolved Solids (TDS) from the two sites of Ashtamudi Lake.

waters (Utterback, 1917). This agrees with the findings of the present study where the populations of *C. madrasensis*, exhibited remarkable variation in the external shell traits between the barmouth region of Thekkumbhagam Lake and upper stream portions of Perumon Lake. As a result, two morphotypes of *C. madrasensis* were observed in the present study with respect to the changes in the morphology and morphometrical parameters.

The uneven shell thickness and shorter forms of *C. madrasensis* from Site I, was the result of crowding due to population density and increased depth gradient. Therefore, the morphotype I showed an allometric growth of the shell and also resulted in the crooked forms of shells in Site I (Rao and Nayar, 1956). Therefore, crowding strongly affects shell shape. The shell size was found to be doubled, deeper and elongated in barmouth population (Site II), in order to counter involuntary dislodgement by turbulence and currents in the barmouth region and the protection offered by rocks or hard substrates in the Site II influences the lateral growth of Morphotype II (Denny et al., 1985).

The shell weight of *C. madrasensis* from sites I and II varied greatly (48.348 ± 2.883 gm and 133.662 ± 8.854 gm, respectively). The comparative drop in salinity in the Perumon region, cause the decreased CaCO_3 supply thereby shell thickness and shell density in Site I population, whereas a reciprocal reaction was observed in Site II with increased salinity ranges upto 32.08 ppt due to the influx of sea water.

This is in accordance with the findings of Aguirre et al. (2006) and Krapivka et al. (2007) in the mytilids, *Mytilus chilensis* and *Brachidontes* species, respectively, where the influence of salinity was observed on the shell shape along the clines. The parameters such as total weight, meat weight, shell weight, shell length, shell height, shell width etc. were also found to be doubled in the samples collected from Thekkumbhagam region. Being an estuarine portion, Thekkumbhagam region possesses more nutrient sources than Perumon region and the buffering activity of the estuary in turn have an influence on the fluctuations in water quality.

The conventional identification thus failed to address the species identity due to phenotypic plasticity, where morphological and morphometrical data supports two distinct taxa, while it is the genetic data that provided information of phenotypic plasticity. Therefore, combinations of genetic, biological, and morphological information are recommended for developing conservation strategies of these highly imperiled animals (Inoue et al., 2013). Ecophenotypic plasticity is controlled by one or two environmental factors that are subjected to the evolutionary change (Schneider et al., 2010). Even though 2.7% genetic distance observed between the two morphotypes of *C. madrasensis* is not enough to consider them as different species, in turn, it calls attention towards the emergence of evolutionary divergence of these two populations in the near future. Stanley (1970) showed that much of this diversity is the result of evolutionary adaptation to the hydrology and sedimentology of a particular niche. Therefore, the pattern of phenotypic integration can be modified by natural selection to enhance adaptation to the external environment or to maintain the coherence of the internal developmental system (Hansen and Houle, 2004), therefore 'constraints' are actually forms necessary substrate for natural selection to work (Matthen and Ariew, 2002).

In this context, it is expected that if gene flow between distant localities is low, adaptation to local environments generates shell-shape variation via selection. Therefore, variation between adjacent

populations could be due to phenotypic plasticity, while between distant populations the restricted gene flow caused by physical and biological barriers which could promote adaptive divergence (Marquez and Molen, 2011). Since bivalves are sessile, the habitat fragmentation and the restricted gene flow can give rise to reproductive isolation and genetic divergence. Moreover, the given anthropogenic disturbance of habitats and human driven climate change accelerates this process, therefore, it is crucial to understand evolutionary responses to this vicariant phenomena and to develop proper conservation practices for taxa in peril.

References

- Aguirre M.L., Perez S.I., Sirch Y.N. (2006). Morphological variability of *Brachidontes swainson* (Bivalvia, Mytilidae) in the marine Quaternary of Argentina (SW Atlantic). *Palaeogeography, Palaeoclimatology, Palaeoecology*, 239(1-2): 100-125.
- APHA. American Public Health Association, Water Pollution Control Federation. (1989). Standard methods for the examination of water and wastewater, 17.
- Bickford D., Lohman D.J., Sodhi N.S., Ng P.K., Meier R., Winker K., Ingram K.K., Das I. (2007). Cryptic species as a window on diversity and conservation. *Trends in ecology and Evolution*, 22(3): 148-155.
- Denny M.W., Daniel T.L., Koehl M.A.R. (1985). Mechanical limits to size in wave-swept organisms. *Ecological monographs*, 55(1): 69-102.
- Dillon R.T., Manzi J.J. (1989). Genetics and shell morphology in a hybrid zone between the hard clams *Mercenaria mercenaria* and *M. campechiensis*. *Marine Biology*, 100(2): 217-222.
- Durve V.S. (1974). On the specificity of Madras backwater oyster *Crassostrea madrasensis* Preston and the American oyster *Crassostrea virginica* Gmelin. 142 p.
- Graf D.L., Cummings K.S. (2007). Review of the systematics and global diversity of freshwater mussel species (Bivalvia: Unionoida). *Journal of Molluscan Studies*, 73(4): 291-314.
- Haas F. (1969). Superfamilia Unionacea. *Das Tierreich* 88. Gruvter. Berlin. 663 p.
- Hansen T.F., Houle D. (2004). Evolvability, stabilizing selection, and the problem of stasis. Phenotypic integration: Studying the ecology and evolution of complex phenotypes. pp.130-150.

- Hendry A.P., Day T., Taylor E.B. (2001). Population mixing and the adaptive divergence of quantitative traits in discrete populations: a theoretical framework for empirical tests. *Evolution*, 55(3): 459-466.
- Hornbach D.J., Kurth V.J., Hove M.C. (2010). Variation in freshwater mussel shell sculpture and shape along a river gradient. *The American Midland Naturalist*, 164(1): 22-36.
- Inoue K., Hayes D.M., Harris J.L., Christian A.D. (2013). Phylogenetic and morphometric analyses reveal ecophenotypic plasticity in freshwater mussels *Obovaria jacksoniana* and *Villosa arkansasensis* (Bivalvia: Unionidae). *Ecology and Evolution*, 3(8): 2670-2683.
- Klishko O.K., Lopes-Lima M., Bogan A.E., Matafonov D.V., Froufe E. (2018). Morphological and molecular analyses of *Anodontinae* species (Bivalvia, Unionidae) of Lake Baikal and Transbaikalia. *PloS One*, 13(4): e0194944.
- Krapivka S., Toro J.E., Alcapán A.C., Astorga M., Presa P., Pérez M., Guíñez R. (2007). Shell-shape variation along the latitudinal range of the Chilean blue mussel *Mytilus chilensis* (Hupe 1854). *Aquaculture Research*, 38(16): 1770-1777.
- Mackie G.L., Topping J.M. (1988). Historical changes in the unionid fauna of the Sydenham river watershed and downstream changes in shell morphometrics of three common species. *Canadian Field-Naturalist*, 102(4): 617-626.
- Márquez F., Van Der Molen S. (2011). Intraspecific shell-shape variation in the razor clam *Ensis macha* along the Patagonian coast. *Journal of Molluscan Studies*, 77(2): 123-128.
- Matthen M., Ariew A. (2002). Two ways of thinking about fitness and natural selection. *The Journal of Philosophy*, 99(2): 55-83.
- Moore R.C., Teichert C., McCormick L., Williams R.B. (1971). *Treatise on Invertebrate Paleontology, Part N*, 3(3). The Geological Society of America. Inc and the University of Kansas, 19(1): 131-133
- Palumbi S.R., Martin A., Romano S., McMillan W.O., Stice L., Grabowski G. (1991). *The simple fool's guide to PCR, version 2.0*. University of Hawaii, Honolulu. 45 p.
- Preston H.B. (1916). Report on a collection of Mollusca from the Cochin and Ennur backwaters. *Records of the Indian Museum*, 12(1): 27-39.
- Rao K.V., Nayar K.N. (1956). Rate of growth in spat and yearlings of the Indian backwater oyster *Ostrea madrasensis* Preston. *Indian Journal of Fisheries*, 3(2): 231-260.
- Schlichting C.D. (1986). The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, 17(1): 667-693.
- Schneider S., Fürsich F.T., Schulz-Mirbach T., Werner W. (2010). Ecophenotypic plasticity versus evolutionary trends—morphological variability in Upper Jurassic bivalve shells from Portugal. *Acta Palaeontologica Polonica*, 55(4): 701-732.
- Severtsov A. (1939). *Morphological patterns of evolution*. Izd. Moscow, Leningrad: Publ. Acad. Sci. USSR.
- Slatkin, M. (1987). Gene flow and the geographic structure of natural populations. *Science*, 236(4803): 787-792.
- Stanley S.M. (1970). Relation of shell form to life habits of the Bivalvia (Mollusca) (Vol. 125). Geological Society of America. 296 p.
- Trivedy R.K., Goel P.K., Trisal C.L. (1987). *Aquatic ecosystem. Practical Methods in Ecology and Environmental Sciences*. Enviro Media Publications, Karad, India. pp: 57-113.
- Utterback W.I. (1917). Naiadogeography of Missouri. *American Midland Naturalist*, 5(1): 25-30.