Fishery Technology 61 (2024) : 21 - 27

Shell Nacre Colour is Predictable from Shell Morphometric Measurements in Indian Pearl Mussel *Lamellidens corrianus* (Lea, 1834)

Rekha Das*[1#], Sankar Prasad Das[1#], Gopal Krishna[2], Annam Pavan Kumar[2], Chandan Debnath[1], Sadokpam Gojendro Singh[3], Hamari Debbarma[1] and Sourav Debnath[1]

[1] *ICAR Research Complex for North Eastern Hill Region, Tripura Centre, Lembucherra, Tripura - 799 210*

[2] *ICAR-Central Institute of Fisheries Education, Mumbai - 400 061*

[3] *ICAR Research Complex for North Eastern Hill Region, Umiam, Meghalaya - 793 103*

Abstract

Identification of nacre properties in live mussels could revolutionize pearl culture industry by aiding selective breeding and cutting down on losses. Indian freshwater pearl mussel *Lamellidens corrianus* was collected from fifteen different water bodies (375 mussels) spread across the Ganga-Brahmaputra-Meghna river basin for the study. We report for the first time the presence of two nacre colour morphotypes (pink and white, occurring in 1:3 ratio) in the species. Pink shells and white shells had hue angle values between 0º/360º & 180º and 180º & 270º respectively. The mantle tissue of the pink nacred mussels also sported a prominent black "lip" at its anterior edge. Deeper shells with elongated and more acute profiles and sharper shoulders are more likely to have white nacre phenotype with 73% accuracy ($P<0.05$). Lightness had a strong positive correlation to anterior length and moderate positive and negative correlation respectively to shell width and ligament length. Chroma did not show high correlation to any of the LMI, except for a weak negative ($P<0.05$) correlation to ventral posterior margin length. However, neither of these continuous colour parameters could be reliably predicted from linear shell morphometric measurements. Our results point to the presence of quantifiable shell shape differences between the two morphotypes. Further studies involving high throughput shell shape phenotyping can potentially enhance the reliability of prediction and can pave the way for phenotypic marker development for nacre quality traits in live mussels.

Keywords: Morphotypes, colourimetry, phenotypic marker, GBM basin

Introduction

Freshwater pearl production in the Indian subcontinent is focused on three major species- *Lamellidens marginalis, L. corrianus* and *Parreysia corrugata*. These bivalve species are found across the country in freshwater environments with gentle water flow (Rao, 1989). The species can also coexist and thrive in polyculture with most cultivated freshwater fish species with minimal additional inputs (Mishra & Mukhaadhyay, 2008). With these advantages, freshwater pearl culture with these species can be a lucrative business, either alone or in combination with conventional aquaculture.

The drainage basin of the Ganga-Brahmaputra-Meghna river system (GBM basin) is one of the largest river basins in the world with an area of 1.7 million square kilometers. Even though the confluence of these three major rivers occurs only a few hundred kilometers prior to draining into the Bay of Bengal, it is considered to be a single river basin. Exhibiting excellent diversity in the geo-climatic conditions, this transboundary river basin is also one of the most dynamic freshwater ecosystems known. Consequently, high levels of phenotypic diversity across populations in the region can also be expected for any species inhabiting the GBM basin area, as a result of

Received 25 July 2023; Revised 13 January 2024; Accepted 28 January 2024

**E-mail: rekhakalidas84@gmail.com*

 # (These authors contributed equally)

accumulated adaptations to suit their respective environments.

A major reason for the low adoption of freshwater pearl cultivation among farmers is the fact that more than 90% of cultured pearls are of suboptimal quality for jewellery and of little value (Zhu et al., 2019). Considering that a typical pearl farming cycle is around 1.5-2 years, during which the pearl quality remains unknown, this scenario is a serious gamble for the farmer for every cycle. A methodology to identify the nacre quality characteristics in an intact live mussel would be of high significance to the farmers.

Since the bio-mineralization process involved in shell development and pearl growth in bivalves has the same basic outline, several shell shape and growth parameters are highly correlated to pearl production potential (McGinty et al., 2010; Bai et al., 2016; Blay et al., 2017; Ky et al., 2017; Li et al., 2017). With this background, we attempted to study potential shell morphometry- nacre trait associations in an Indian freshwater pearl mussel, *Lamellidens corrianus* (Lea, 1834).

Materials and Methods

The samples for the study were collected from Ganga-Brahmaputra-Meghna river basin. The sampling sites were located at the uppermost, middle and lowermost reaches of the GBM confluence separated by more than 200km (Fig. 1). All the sampling sites were sheltered natural lentic water

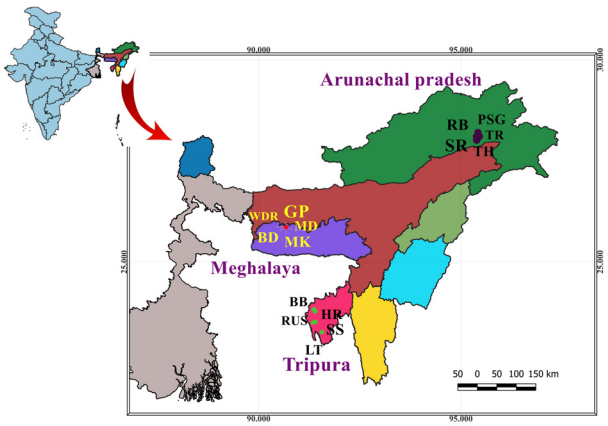

Fig. 1. Map showing location of sampling sites in the study falling within the upper, middle and lower stretches of the basin of the Ganga-Brahmaputra-Meghna confluence

bodies with soft sandy loam soil substrates close to the major tributaries of the GBM basin and not directly affected by the flow rates of the rivers. To include maximum genetic diversity in the study samples, we conducted samplings from 15 locations spread across the geo-climatically diverse GBM river basin. Twenty five live individuals of *L. corrianus* with shell length 8.0 cm or above were collected from each of the fifteen different wetlands (375 in total) within the river basin.

The sampled mussels were depurated in freshwater for a day and anaesthetized in a bath containing MS-222 to a final concentration of 50mg/L for 35-40 minutes prior to dissection. The taxonomic identity of the collected mussels was confirmed using conventional taxonomic keys (Rao, 1989). Soft tissues were dissected aseptically from completely relaxed mussels and preserved in appropriate media for future analyses. The shells were cleaned in diluted bleach (1:10 v:v), air dried and preserved in a moisture-free environment in labelled zip-lock pouches away from light.

The shell nacre colouration was quantified using the Hunterlab colourimeter (Hunter Colour labs, Germany). An approximate square of 5mm X 5mm was cut out using a diamond saw cutter from three different positions of the shell *viz.,* the pallial ventral margin of the shells directly beneath the umbo, the region of anterior adductor scar and the posterior adductor scar. The pieces were mounted and readings taken following the manufacturer's instructions. Five readings each were taken for the three shell positions for each of the shells.

Shell colour was expressed in terms of three parameters hue (the qualitative aspect of colour), chroma (intensity of colour) and lightness (level of brightness) calculated from the Hunterlab colour space variables L, a* & b* according to Pathare et al. (2013). The three colour variables mark aspects of lightness/ darkness, redness/blueness and greenness/yellowness of the measured object in the spherical colour space. The hue angle readings were scored into hue group categories based on their position on the hue angle quadrant (McLellan et al., 1995) and the modal hue score from the total readings of the shell was considered as the average hue of the shell. The average chroma and lightness values over the total reading per shell were used as the chroma and lightness values for individual shells.

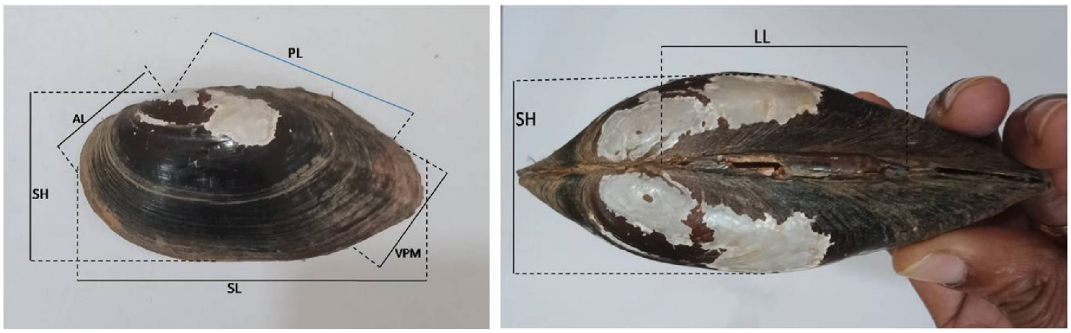

Fig. 2. Positions of measurement of the seven Landmark based in the study on an intact shell of *L. corrianus*. Legend: AL: anterior length; LL: ligament length; PL: posterior length; SH: shell height; SW: shell width; SL: Shell Length; VPM: ventral posterior margin length

Details of landmark based morphometric measurements used in the study:

Anterior length (AL): Distance between umbo and widest point at the anterior edge of the shell

Ligament length (LL): Length of the ligament

Posterior Length (PL): Distance between umbo and the end of the posterior shoulder

Shell height (SH): Distance between umbo and the ventral-most point directly perpendicular to the umbo on the shell

Shell Width (SW): the distance between the widest bilateral points of the intact shell

Shell Length (SL): Widest antero-posterior distance of the shell

Ventral posterior margin length (VPM): Distance between the the end of the posterior shoulder and beginning of the postero-ventral margin of the shell

Seven landmark based morphometric readings that are measurable on intact mussels were recorded from the left valves of the shells following Hamli et al. (2015) (Fig. 2). Three readings were taken for each of the parameter in every individual using calibrated Digital Vernier caliper (size range 0-200mm; Mitutoyo, Japan) and an average of the three readings were used.

For nullifying the effect of shell size on various measurements the parameters were converted into Landmark based relative indices (LMI) obtained as the quotient of the parameter and shell length; i.e. Relative index of parameter = value of the parameter/total shell length (Table 1).

All statistical analyses were performed on SPSS V 16 and PAST V 4.03. The test statistic was considered significant at $P<0.05$ for all statistical tests performed in our study. To reduce errors, shell whiteness index (WI) values returned by the equipment were used to eliminate samples discoloured during storage by excluding shells with WI values less than the 25^{th} percentile value from the analysis. Similarly, physically damaged shells that could lead to erroneous morphometric measurements were also removed from the analysis (71 in total).

To identify potential relationship between the LMI and the shell nacre hue score (which is a categorical variable), Binary Logistic regression was applied on the data. For shell nacre colour variables with a continuous distribution (chroma and lightness), Pearson's correlation coefficient between the colour parameters and LMI was estimated.

Results and Discussion

The quality of cultivated pearl is heavily dependent on the quality of the host and donor molluscs (McGinty et al., 2010; McDougall et al., 2016; Blay et al., 2017; Ky et al., 2017). Given the typical length of culture duration in freshwater pearl culture, assessment of the donor and host mussel morphology for drawing insights into the potential quality of the cultured pearl could alleviate a lot of uncertainty in the process.

Pearl colour, directly influenced by the shell nacre colour of the donor mussel (McGinty et al., 2010; Li et al., 2015; Ky et al., 2017), is one of the most important economic traits in pearl culture. While colour is a qualitative variable in its entirety, visual perception of colour quantitatively includes the factors of hue, chroma and lightness simultaneously

Table 1. Descriptive Statistics of the Landmark Based Morphometric Indices (LMI) expressed relative to the total shell length (SL) (n=304)*

	SH	AL	PL	VPM	LL	SW
Min	0.4363	0.2097	0.6396	0.1368	0.0608	0.2479
Max	0.5779	0.4088	1.2912	0.3427	0.3942	0.5744
Sum	150.37	85.234	228.43	69.280	52.629	100.87
Mean	0.4946	0.2803	0.7514	0.2278	0.1731	0.3318
Std. error	0.0012	0.0019	0.0028	0.0018	0.0040	0.0031
Variance	0.0005	0.0012	0.0024	0.0010	0.0050	0.0030
Stand. dev	0.0224	0.0347	0.0497	0.0323	0.0711	0.0555
Median	0.4963	0.2733	0.7489	0.2248	0.1537	0.3206
25 prcntil	0.4785	0.2527	0.7244	0.2074	0.1148	0.3006
75 prcntil	0.5102	0.3048	0.7750	0.2456	0.2282	0.3434
Skewness	-0.0020	0.7184	4.1337	0.4942	0.6504	2.2130
Kurtosis	0.0813	0.4809	44.740	0.9566	0.3877	5.1854
Coeff. var	4.53236	12.3935	6.62068	14.2062	41.1229	16.7378

(SH) shell height; (SW) shell width; (AL) anterior length; (PL) posterior length; (LL) ligament length; (VPM) ventral posterior margin length. Umbo length could not be measured consistently in most samples due to wear and tear. *After removal of 71 defective shells with discolourations or physical damages that could interfere with the analyses

(Delgado-Gonzalez et al., 2018). Quantification of colour variables in these terms also facilitates the objective comparison of multiple samples. Therefore, shell nacre colour was recorded in terms of hue, chroma and lightness in our study. The qualitative aspect of colour was recorded in terms of hue angles and visual perception of hue corroborated with hue angle quadrant (McLellan et al., 1995).

We observed two visually distinct nacre colour phenotypes in *L. corrianus* in our study - pink and white (Fig. 3).

This difference in colour could also be quantified and corroborated as hue angles McLellan et al. (1995). The white and pink nacred shells had hue angle values between 180° & 270° and 0°/360° & 180° respectively. The chroma and lightness values for white shells ranged from 0.628 to 9.416 and 45.93 to 78.22 respectively. In pink nacred shells, chroma and lightness values ranged from 0.628 to 4.324 and 55.43 to 78.22 respectively. The white phenotype was more abundant (73.76%) than the pink phenotype (26.23%). This percentage abundance of the two shell colour phenotypes did not differ significantly between the sampling sites (P>0.05; Fisher Exact Test). This indicates that the observed colour phenotypes are highly unlikely to be the result of an environmental factor or a population specific trait. Moreover, the mantle tissue of the pink phenotype sported a prominent black "lip" at its anterior edge (Fig. 4) which was absent in the white phenotype.

Fig. 3. Morphotypes in *L. corrianus* with distinct shell nacre colour phenotypes white (left) and pink (right)

Colour variability in the soft tissue of most molluscs is known to be under genetic control (Brake et al., 2004; Ky et al., 2016). Additionally, the *Lamellidens* spp from various natural environments are reported to have a sex ratio of 1:1 (Gaikwad & Kamble, 2013; Mondol et al., 2016; Niogee et al., 2019). Therefore, with the 3:1 ratio of white to pink morphotypes in our samples, we infer this to be a non-sex dependent trait as well. Read together, the two colour

Fig. 4. Differences in the mantle characteristics of the two morphotypes. Note the black 'lip' towards the anterior edge (bold arrow) of the soft mantle tissue in the pink nacred shell.

phenotypes observed in our study appear to be genetically distinct morphotypes, rather than environmentally induced colour variations. This is the first report of the presence of distinct nacre colour phenotypes in the Indian pearl mussel species till date.

With the intent of mining for shell morphology based markers for the identification of shell nacre colour in intact mussels, we estimated correlations between nacre color traits and shell morphology. Co-existence of non-causative markers and desirable traits in specific populations leading to false positive marker-trait association results are often a major problem in studies based on natural populations (Platt et al., 2010). Considering this, our sampling arrangement was done to ensure the inclusion of maximum diversity in the sampling populations that formed the base of our analyses. Encouragingly, none of the nacre colour parameters (hue, chroma or lightness) exhibited significant population specificity in our study (P>0.05).

Since hue was a categorical variable, binary logistic regression was used to identify LMI-hue correlations. Among the LMI eight parameters are observable on an intact mussel Shell height (SH), shell width (SW), anterior length (AL), posterior length (PL), ligament length (LL) & ventral posterior margin (VPM) and were included in a binary logistic regression analysis to enable prediction of nacre colour in a live mussel. SH, AL, PL and VPM were identified as significant unique predictors of hue score. Our logistic regression model (1) was significant ($\div^2(8)$= 6.132; P<0.05) and could predict the hue score from the morphometric indices data with 73% overall accuracy (Fig. 5)

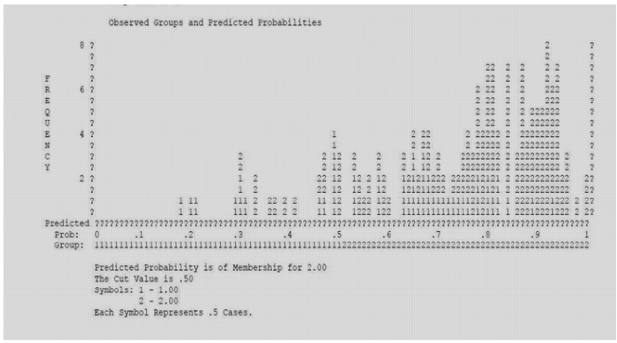

Fig. 5. Probability Plot for nacre hue based on shell morphometry from the binary regression model. The leaf diagram indicates that the logistic regression model has a very high probability of accurate prediction of white phenotype from the set of predictor variables. (Symbol "1" stands for pink hue and "2" for white hue)

L_i=11.75*SH+5.295*PL-16.278*AL-16.391*VPM-0.176……..(1)

where L_i refers to is the Log_e (odds of a shell to have white nacre)

Accordingly, deeper shells with elongated and more acute profiles and sharper shoulders are more likely to have white nacre. However, our model explains only 13.8% of variance in the data as estimated from the Nagelkerke's R^2 indicating there are other potential shape estimators that could contribute to the observed variability.

The continuous colour parameters lightness and chroma are known to indicate the luminosity of a surface (Granato & Masson, 2010) and intensity difference of a hue from gray with the same lightness (Pathare et al., 2013) respectively. Since the presentation of the nacre on the inner surface of the shell must have a direct bearing on both these parameters, strong correlation of these colour parameters to LMI was expected. Lightness had a strong positive correlation to anterior length and moderate positive and negative correlation respectively to shell width and ligament length. Chroma did not show high correlation to any of the LMI, except for a weak negative (P<0.05) correlation to ventral posterior margin length (Fig. 6). However, neither lightness nor chroma could be predicted reliably from the LMI parameters in our study.

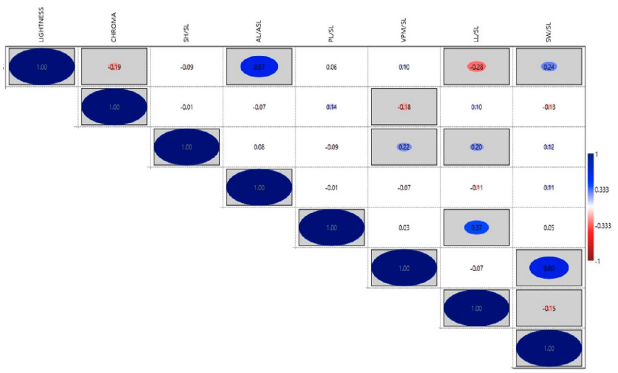

Fig. 6. Plot showing correlation between continuous colour variables (lightness and chroma) and shell morphometric indices. The statistically significant correlation coefficients are boxed

Our study reports the presence of two nacre colour phenotypes in Indian pearl mussel *Lamellidens corrianus* for the first time. Our results also indicate existence of quantifiable shell shape differences between the two morphotypes. However linear morphometric indices alone could not fully explain the nacre colour variability in our study. Further efforts on high throughput shell shape phenotyping that can enhance the nacre colour prediction accuracy in live mussels are warranted.

Acknowledgements

The authors are grateful to Department of Biotechnology, Government of India for funding this study under the Twinning Project Scheme (Project ID: BT/PR16977/NER/95/377/2015; PIMS code OXX04066). The authors gratefully acknowledge the Director, ICAR Research Complex for NEH Region, Umiam for kind support and providing the facilities for the conduct of this study.

References

Bai, Z.H., Han, X.K., Liu, X.J., Li, Q.Q. and Li, J.L. (2016) Construction of a high-density genetic map and QTL mapping for pearl quality-related traits in *Hyriopsis cumingii.* Sci. Rep. 6: 32608

Blay, C., Planes, S. and KY, C.L. (2017) Donor and recipient contribution to phenotypic traits and the expression of biomineralisation genes in the pearl oyster model *Pinctada margaritifera*. Sci. Rep. 7(1): p.2696

Brake, J., Evans, F. and Langdon, C. (2004) Evidence for genetic control of pigmentation of shell and mantle edge in selected families of Pacific oysters. *Crassostrea gigas.* Aquac. 229(1-4): 89-98

Delgado-Gonzalez, M.J., Carmona-Jiminez, Y., Rodriguez-Dodero, M.C. and Garcia-Moreno, M.V. (2018) Colour space mathematical modelling using microsoft excel. J. Chem. Educ. 95: 1885-1889

Gaikwad, S.S. and Kamble, N.A. (2013) Gametogenic phenology in freshwater molluscan species; *Lamellidens marginalis* and *Parreysia corrugata*. Asian J. Biol. Sci. 2 (1): 1-5

Granato, D. and Masson, M.L. (2010) Instrumental color and sensory acceptance of soy-based emulsions: a response surface approach. Food Sci. Technol. 30(4): 1090-1096

Hamli, H., Rahim, A.A., Idris, M.H., Kamal, A.H.M. and King, W.S. (2015) Morphometric variation among three local mangrove clam species of Corbiculidae. Songklanakarin J. Sci. Technol. 37(1): 15-20

Ky, C.L., Lo, C. and Planes, S. (2017) Mono- and polychromatic inner shell phenotype diversity in *Pinctada margaritifera* donor pearl oysters and its relation with cultured pearl colour. Aquac. 468: 199-205

Ky, C.L., Nakasai, S., Pommier, S., Sham Koua, M. and Devaux, D. (2016) The Mendelian inheritance of rare flesh and shell colour variants in the black-lipped pearl oyster (*Pinctada margaritifera*). Anim. Genet. 47(5): 610-614

Li, Q., Bai, Z., Han, X. and Li, J. (2017) Freshwater nucleated pearl quality is influenced by host mussel growth traits in *Hyriopsis cumingii*. Aquac. Res. 48(9): 4656-4665

Li, Q., Bai, Z., Liu, X., Han, X., Luo, H., Dong, S. and Li, J. (2015) Correlation analysis of non-nucleated pearl quality parameters with growth traits and inner shell color of *Hyriopsis cumingii*. J. Fish. Sci. China. 39(11): 1754-1762

McDougall, C., Moase, P. and Degnan, B.M. (2016) Host and donor influence on pearls produced by the silver-lip pearl oyster, *Pinctada maxima*. Aquac. 450: 313-320

McGinty, E.L., Evans, B.S., Taylor, J.U.U. and Jerry, D.R. (2010) Xenografts and pearl production in two pearl oyster species, *P. maxima* and *P. margaritifera*: Effect on pearl quality and a key to understanding genetic contribution. Aquac. 302(3-4): 175-181

McLellan, M.R., Lind, L.R. and Kime, R.W. (1995) Hue angle determinations and statistical analysis for multiquadrant Hunter L, a, b data. J. Food. Qual. 18(3): 235-240

Mishra, G. and Mukhapadhyay, P.K. (2008) Mussel farming: Alternative water monitoring practice. Aquac. Asia. 13(3): 32-34

Mondol, M.R., Nasrin, F. and Nahar, D.A. (2016) Length-weight relationships, condition index and sex ratio of

mussel *Lamellidens corrianus* (Lea, 1834) in a freshwater lake, Northwest Bangladesh. Ribar. Croat. J. Fish. 74(4): 172-178

Niogee, S.R., Tonii, K.F., Barman, A.C., Tanu, M.B., Sku, S. and Uddin, M.J. (2019) Ovarian cycle of freshwater pearl mussel, *Lamellidens marginalis* (Lamarck, 1819) collected from a culture pond in Bangladesh. Asian. Fish. Sci. 32(3): 117-123

Pathare, P.B., Opara, U.L. and Al Said, F.A. (2013) Colour measurement and analysis in fresh and processed foods: A review. Food. Bioproc. Tech. 6: 36-60

Platt, A., Vilhjálmsson, B.J. and Nordborg, M. (2010) Conditions under which genome-wide association studies will be positively misleading. Genetics. 186(3): 1045-1052

Rao, S.N.V. (1989) Handbook on Freshwater Molluscs of India. 289 p, Zoological Survey of India, Calcutta, India

Zhu, C., Southgate, P.C. and Li, T. (2019) Production of pearls. In: Goods and Services of Marine Bivalves (Smaal, A.C., Ferreira, J.G., Grant, J., Petersen, J.K. and Strand, O., Eds), pp 73-93, Springer, Switzerland