21. Truss Networking: A Powerful Tool for Stock Structure Analysis

Dr. Rekha Devi Chakraborty

Shellfish Fisheries Division

ICAR-Central Marine Fisheries Research Institute, Kochi

A fish stock refers to a population or subpopulation of a species that is at least partially genetically or reproductively isolated. Stock identification is crucial for fisheries management, as different stocks may have distinct growth, mortality, and reproductive rates, influencing conservation and resource assessment (Reiss et al., 2009).

Various methods, including meristic, morphometric, otolith analysis, and molecular markers, are used for stock identification. Among these, morphometric analysis—particularly the truss network system—has gained prominence due to its accuracy and cost-effectiveness (Strauss & Bookstein, 1982). Traditional morphometric approaches rely on direct distance measurements, whereas truss networks use interconnected landmark points to create a geometric framework that captures shape differences more effectively (Cavalcanti et al., 1999).

The truss network system has proven valuable for distinguishing phenotypic stocks by quantifying shape variations within and between populations (Bookstein, 1991). It has been successfully applied to various fish and crustacean species, offering insights into stock structure and aiding sustainable fisheries management (Pazhayamadom et al., 2015).

Methods for Truss Network System

Sample Preparation and Imaging

Shrimp samples were first cleaned under running water, dried with tissue paper, and placed on graph paper. Each specimen was positioned on a flat platform with its rostrum facing left and telson on the right to ensure symmetry. Appendages were carefully arranged, and each specimen was assigned a unique ID for identification and reference.

High-resolution digital images were captured using a Canon G-15 camera mounted on a tripod. The camera was aligned so that the graph paper's margins matched the viewfinder's X-Y axes, and a scale was included in each image for standardization. The images were then processed in tpsDig2 v2.1 to mark anatomical landmarks and extract truss variables, ensuring accuracy and repeatability (Rohlf, 2006).

Data Extraction and Statistical Analysis

Truss measurements were obtained using tpsDig2 v2.1 for landmark digitization and Paleontological Statistics (PAST) for distance calculations (Hammer et al., 2001). Since shape differences may arise due to sex (Sajina et al., 2011; Reiss & Grothues, 2015; Pazhayamadom et al., 2015), both male and female specimens were included in the analysis.

To ensure data reliability, normality and variance homogeneity were tested using SAS PROC UNIVARIATE (SAS, 2014). Outliers (7-10%) were removed before further analysis. Log-transformed data were used in a Multivariate Analysis of Covariance (MANCOVA), incorporating carapace length (CL) as a covariate for size correction. Size-independent shape variables were calculated using the allometric equation (Reist, 1985):

$M_{trans} = \log M - \beta (\log CL - \log CL \text{ mean})$ ……..Equation 1,

Where M_{trans} is the truss measurement after transformation, M is the original truss measurement, CL is the carapace length of the shrimp which is reported to be more reliable than using total length (TL) in the case of crustaceans (FAO 1974), CL mean is the overall mean carapace length, and β is the slope regressions of the log M against log CL.

Correlation coefficients between variables were examined before and after size correction to ensure reduced dependency (Murta, 2000). Descriptive statistics, including mean ($\bar{x}$), standard deviation (SD), standard error (SE), maximum, minimum, and coefficient of variation (CV%), were calculated for each population.

Multivariate Analysis

The study employed Principal Component Analysis (PCA), Discriminant Function Analysis (DFA), and Hierarchical Cluster Analysis (HCA) for stock differentiation:

PCA identified morphometric variables contributing significantly to variation.

DFA tested the efficiency of variables in distinguishing different population groups (Tomović & Džukić, 2003; Loy et al., 2008).

Stepwise inclusion procedures reduced redundant variables, ensuring optimal separation of stocks (Hair et al., 1996; Jain et al., 2000; Poulet et al., 2005).

HCA, based on Mahalanobis distance matrices from DFA, evaluated population relationships (Slabová & Frynta, 2007; Ferrito et al., 2007).

All statistical analyses were performed using SAS (2014).

Truss Network Construction

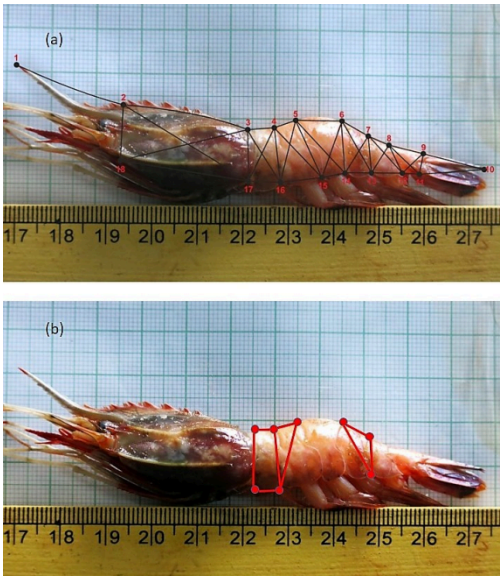
The truss network method (Strauss & Bookstein, 1982) involves overlaying a geometric framework on the specimen for shape analysis. Fish specimens were placed on water-resistant paper, and anatomical landmarks were marked using a needle. Digital images were then taken for precise landmark identification.

Morphometric landmarks were selected based on homology, ensuring consistency across specimens. The truss distances were extracted using a combination of tps Util, tps Dig2 v2.1, and PAST for accurate shape analysis. The truss network system enhances stock differentiation, providing a reliable and efficient approach for fisheries research.

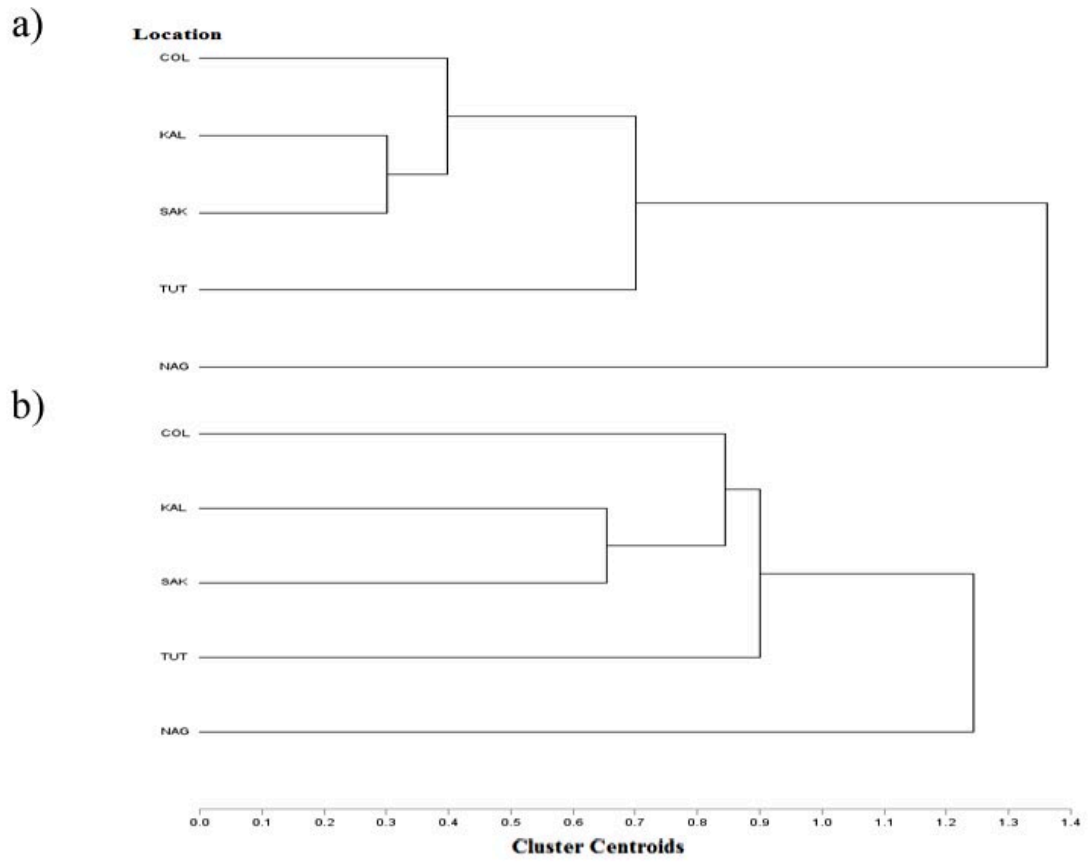
Case Study –I: *Heterocarpus chani*: A caridean deepsea shrimp

The samples of *H. chani* were collected from deepsea trawl shrimp catches obtained from five major fishing harbours along the southern coast of India. The sampling sites are Kalamuku (KAL), Sakthikulangara (SAK), Colachel (COL) on the southwest coast and Tuticorin (TUT), and Nagapattinam (NAG) on the southeast coast. Information on study sites, geographical coordinates, shrimp sex and the sample size from each location. A total of 1879 specimens of *H. chani* including 984 males and 895 female individuals were used in this study.

Digitisation of samples



The results of HCA showed three clear clusters from five populations of both sexes as shown below figure. The group-I included populations from NAG, group-II consisted of the TUT and group-III with SAK, KAL, and COL populations. Theinterpretation of resultsindicated that the samples obtained from the locations NAG and TUT represented a phenotypically distinct population while the morphometric resemblance between SAK, KAL, and COL stocks were observed to be high.



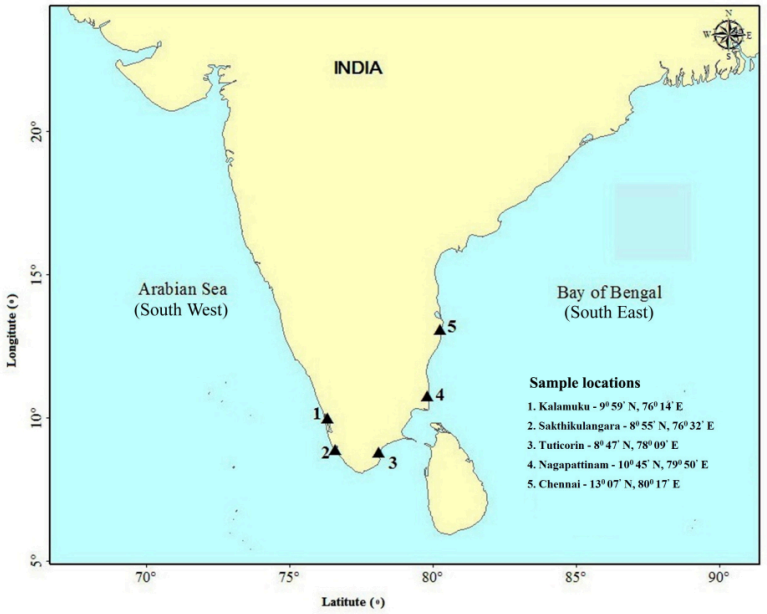
A Case Study: Deep-sea Shrimp: *Aristeus alcocki*- Penaeid shrimp

A. alcocki Ramadan, 1938 (Decapoda, Aristeidae), commonly known as Red Ring or Arabian red shrimp is distributed along the southern Indian coast at a depth range of 200-1000 m (Silas 1969; Suseelan 1989; Madhusoodana 2008; CMFRI 2015). It forms a commercial fishery confining only along the southeast and southwest coast, and it's not recorded along the northern coast of India (Mohamed and Suseelan 1973). The catch landed between 2008 and 2015 indicate that the *A. alcocki* is the prime species in order of biomass among the deep sea penaeid catch accounting to about 36% from the whole Indian coast and the trend in catch rates indicates a decline of these deep-sea shrimps (CMFRI 2008-2015). In this study we aim to investigate the effectiveness of the truss variables in differentiating the populations of *A.alcocki* along the Indian coast using truss morphometry, to provide management advisory for fisheries sustainability.

Sampling

Samples of *A. alcocki* were collected from five different fishing harbors i.e., Tuticorin (SEN), Chennai (SEC), Nagapattianam (SEN) on the southeast, and Sakthikulangara (SWS), Kalamuku (SWK) on the southwest Indian coast (shown in figure below). The sampling sites were chosen such that they are distantly apart in latitudinal aspect to reduce the chances of mixing specimens from the same population. In total, 1842 specimens were collected from the selected sampling sites i.e., from commercial fishing harbors where the catch is landed by

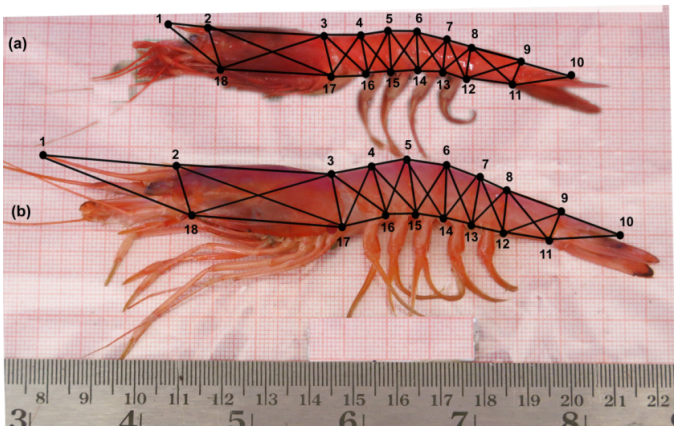
multiday trawlers along the southern coast during December 2014 and January 2015. The samples were collected during peak breeding season (November to January) to ensure that they represent to their parent population. The matured specimens (carapace length: female>3.5 cm; male: >2.0 cm) were sorted from the samples collected from each fishing location and used for truss morphometric analysis. The species exhibit a high degree of sexual dimorphism where males were identified by the presence of petasma and females were sorted based on the presence of thelycum. Specimens showing physical damage *viz*., broken rostrum or any other body parts may distort the shape characteristics and hence they were not included in the samples for the study.

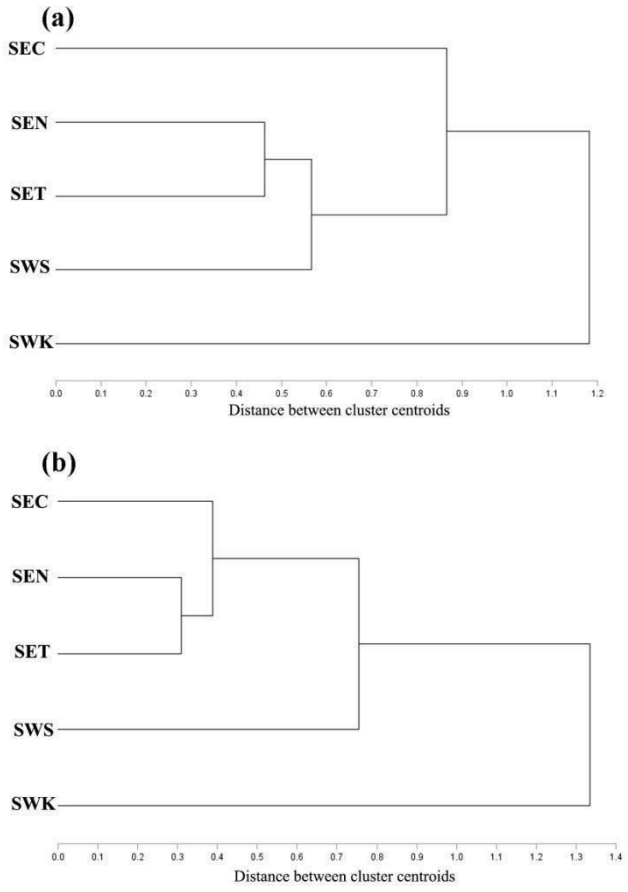


Digitization of specimens and fixing anatomical landmarks

Shrimp samples were first cleaned with running water, allowed the water to drain, wiped with tissue paper and finally placed on a graph paper (shown in figure below). Each specimen was placed on a flat platform with a graph paper over a thermofoam, appendages (pereiopods and pleopods) and telson were erected by positioning the rostrum portion towards the left side, telson on the right by assuming symmetry between left and right side of the shrimps and was labeled with a specific ID code. This helps us in identifying specimens if more landmarks are required to be fixed or if the morphometric measurements are to be repeated. Digital images of the specimens were captured using a camera (Canon G-15) which was fixed on a tripod stand directly above the specimen and the lens was adjusted so the margins of viewfinder align with margins of the graph paper in X-Y directions and each image included a scale to standardize the individual sizes and further scaling was applied in tpsdig utilizing the millimeter grid in graph paper. These images were used further in fixing the anatomical landmarks and measuring linear distances between them i.e., truss variables. In many previous studies, it has been found that differences in sex are likely to contribute to shape differences affecting total variance in morphometric distances (Reiss and Grothues 2015; Sajina et al. 2011; Pazhayamadom et al. 2015). In the present analysis, both males and females were included to accommodate the effect of sex on their morphometry. The extraction of numeric truss distances from the digital images of specimens were carried out

by using two software platforms, 1) tps Dig2 V2.1 for marking the landmark coordinates on the digital images (Rohlf 2006) and 2) paleontological statistics (PAST) for extracting the values pertaining to the marked distances (Hammer et al. 2001). The data extracted by this method ensures stability, accuracy, and repeatability.





Genetic Characterisation of the species

Genetic variation is considered to be an important feature of the population to reveal not only the short term fitness of individuals but also the long term survival of the population, through allowing adaptation to the changing environmental conditions. Information deduced from molecular markers can provide insight into genetic structure and geographical boundaries

(*i.e*. breeding stock) and vulnerability (*i.e*. genetic diversity) of the species (Buchholz-Sørensen & Vella, 2016).

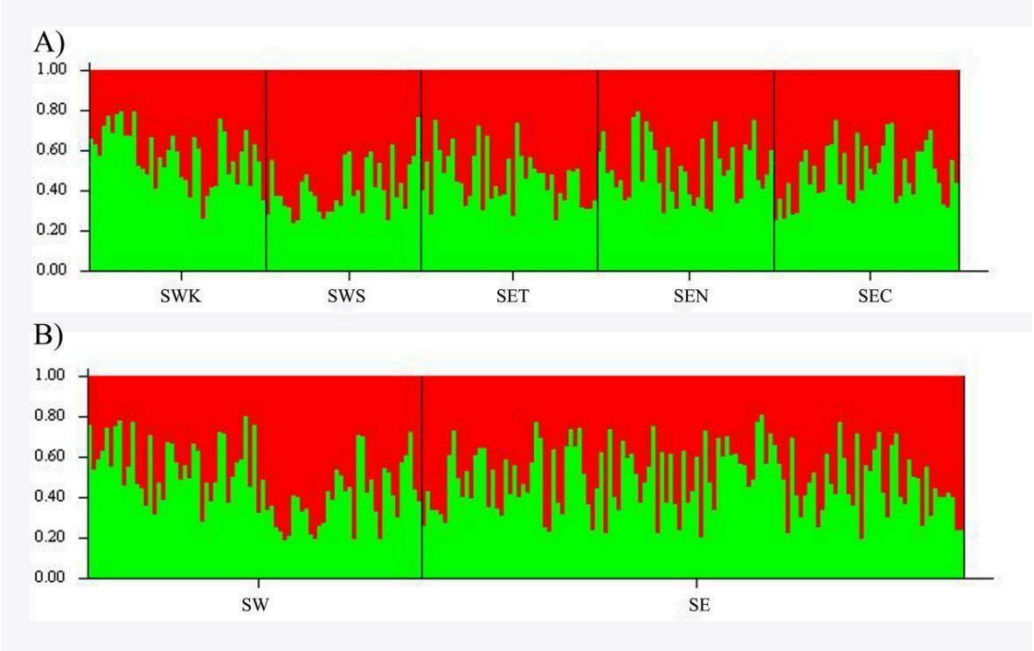
Molecular markers have been proved to be an effective indicator of genetic variation within and between fishery populations of shrimp species; *Aristeus antennatus* (Maggio et al., 2009; Cannas et al., 2012; Fernández et al., 2011b; Brutto et al., 2012), *Aristaeomorpha foliacea* (Fernández et al., 2011a), *Penaeus monodon* (Mandal et al., 2012; Sekar et al., 2014) and *Fenneropenaeus indicus* (Sajeela et al., 2015). Microsatellite markers are characterized as co-dominant and highly polymorphic in nature and addition to their abundance, even genomic distribution, small locus size, have quickly become useful molecular markers with great discriminatory power for the evaluation of genetic diversity in various species (Powell et al., 1996). Analyses of microsatellite nuclear markers were used to describe the differences and distribution patterns of natural populations of this species.

DNA extraction, amplification and genotyping of microsatellite loci

The total genomic DNA was extracted from the pleopod of the each individuals using DNeasy® Blood & Tissue Kit (Qiagen Inc.) according to the manufacturer's protocol. The cells were lysed by incubating at 56°C for 2 hrs and all other steps were followed as per the protocol. The primers for nine nuclear microsatellite loci were taken from Cannas et al., (2008), were originally designed for the *Aristeus antennatus*. The microsatellite loci were optimised for genotyping by following the general protocols of Palumbi (1996), and Cannas et al., (2008). The amplification of microsatellite markers were performed in 25 µl reaction cocktails containing genomic DNA (0.5 µg μl^{-1}), *Taq* DNA polymerase (0.05 U μl^{-1}), 1X buffer, MgCl2 (1.5 mM), 10 pM μl^{-1} of each primer and dNTPs (200 µM). The PCR thermal profile used was 94°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, annealing at 52–54°C for 1 min, extension at 72°C for 1.5 min, and a final extension at 72°C for 5 min (Table 1). Amplification of PCR products were confirmed by electrophoresis on a 1.5% agarose gel containing ethidium bromide and visualized under UV transilluminator (Lark, India). Analysis of fragment size was carried out by ABI prism genetic analyser (Applied Biosystems, USA) at AgriGenome Labs, Scigenom, Cochin, India.

Molecular Results

The pairwise F_{ST}, Nei, and AMOVA values calculated from microsatellites indicated the absence of significant variation among the samples of *A. Alcocki* collected from the South west (Arabian Sea) and South east (Bay of Bengal) coast of India. Moreover the results of AMOVA also indicated the proportion of genetic variation was mainly associated to differences among the individuals (99.2%) with F_{st}=0.0078 which is further confirmed by the cluster analysis performed using STRUCTURE (shown in figure below) directed towards the presence of homogeneous groups due to the absence of specific allelic variation in the sampled localities. The present study was in agreement with the results reported in *A. Antennatus* (among individual difference 99.3%; Fst=0.0067) using same markers in the Mediterranean Sea (Cannas et al., 2012) where no genetic differentiation was noticed between the localities.



Conclusion

The truss morphometric characters in *A. alcocki* and *H.chani* can be efficiently used in the discrimination of populations as studied in other species of freshwater and marine environments. The major discriminating variable to differentiate the populations into two groups was attributed to the abdominal measurements, suggesting a need to adopt separate management strategies for the resource sustainability and policy regulations. Further, studies based on the genetic markers in *A. alcocki* indicated the presence of single population. However, in *H.chani* molecular studies can be used to validate the findings of this study.

References

1. A.O.Anyanwu and O.A. Ugwumba, *The Zoologist* 2: 70-77 (2003). 1997).

2. Aini NK, Mashar A, Madduppa HH, Wardiatno Y. 2020. Genetic diversity of horseshoe crabs (*Carcinoscorpiusrotundicauda*and *Tachypleus gigas*) in Demak, Madura and Balikpapan waters based on Random Amplified Polymorphic DNA marker. *Journal of Natural Resources and Environmental Management.* 10(1): 124-137. doi: 10.29244/jpsl.10.1.124-137

3. Bhosale MM, Pawar RA, Bhendarkar MP, Sawant MS, Pawase AS. 2018. Truss based morphometric approach for the analysis of body shape in portunid crabs (*Charybdis feriatus, P. pelagicus*and *P. sanguinolentus*) along Ratnagiri coast, India. *Journal Entomology Zoology Studies*. 6(2): 2641-2648.

4. Booke, H.E., 1981. The conundrum of the stock concept—are nature and nurture definable in fishery science? Can. J. Fish. Aquat. Sci. 38, 1479–1480.

5. Bookstein, F.L., Chernoff, B., Elder, R.L., Humpheries, J.M., Smith, G.R., Strauss, S.E., 1985. Morphometrics in Evolutionary Biology, vol. 15. Acad. Natl. Sci. Philadelphia Spec. Pub, p. 277.

6. C. Turan, S. Yalcin, F. Turan, F. Okur and I. Akyurt, *Folia.Zool.* 54: 165-172 (2005).

7. Cadrin, S. X., Friedland, K. D. & Waldman, J. R. (2005). *Stock Identification Methods: Applications in Fishery Science*. Amsterdam: Elsevier Academic Press.

8. Cavalcanti, M.J., Monteiro, L.R., Lopez, P.R.D., 1999. Landmark based morphometric analysis in selected species of Serranid fishes (Perciformes: Teleostei). Zool. Stud. 38 (3), 287–294.

9. Clayton, J. W. (1981). The stock concept and the uncoupling of organismal and molecular evolution. *Canadian Journal of Fisheries and Aquatic Sciences* 38, 1515–1522. doi: 10.1139/f81-204

10. FAO (Food and Agriculture Organization). 1995. *Code of Conduct for Responsible Fisheries*. Rome (IT): FAO Technical Guidelines for Responsible Fisheries.

11. H.J. Meng and M. Stocker, *Can.J.Fish.aquat.Sci.* 41: 414-422 (1984).

12. Hart PJB, Reynolds JD. 2002. *Handbook of Fish Biology and Fisheries: Fish Biology.* Oxford (UK): Blackwell Publishing.

13. Hollander J, Butlin RK. 2010. The adaptive value of phenotypic plasticity in two ecotypes of a marine gastropod. *BMC Evol Biol.* 10(1): 333-340. doi: 10.1186/1471-2148-10-333.

14. J. E. Eyo, *The Zoologist* 2: 1-17 (2003).

15. J.A. Fowler, *Quart. Res. Bio.* 45, 148-167 (1970).

16. L.A. Krumhoitz and S.H. Cavanah, *Trans.Am.Fish.Soc.* 97: 429-441 (1968).

17. Ganesan, Kuberan& Chakraborty, Rekha &Paramasivam, Purushothaman. (2020). Phenotypic variation using truss network system in the deep-sea shrimp Heterocarpuschani Li, 2006 (Caridea: Pandalidae) off Arabian Sea and Bay of Bengal. 1839-1847.

18. M. J. Cavalcanti, L.R. Monteiro and P.R.D. Lopez, *Zool Stud* 38(3):287–294(1999)

19. M.C. Fabrizio, *Trans. Am. Fish. Soc.* 116: 728-736 (1987).

20. Marini M, Suman A, Farajallah A, Wardiatno Y. 2017. Identifying *Penaeus merguiensis*de Man, 1888 stocks in Indonesian Fisheries Management Area 573: a truss network analysis approach. *AACL Bioflux*. 10(4): 922-935.

21. Mojekwu TO, Anumudu CI. 2015. Advanced techniques for morphometric analysis in fish. *J Aquac Res Develop*. 6(8): 1-6. doi: 10.4172/2155-9546.1000354.

22. of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries* 10, 361–395. doi: 10.1111/j.1467-2979.2008.00324.x

23. P. Pepin and S.M. Carr, *Can.j.Fish.Aquatic Sci.* 50: 1924-1933 (1993).

24. Pawson, M. G. & Jennings, S. (1996). A critique of methods for stock dentification in marine capture fisheries. *Fisheries Research* 25, 3–4.

25. Pazhayamadom DG, Chakraborty SK, Jaiswar AK, Sudheesan D, Sajina AM, Jahageerdar S. 2015. Stock structure analysis of "Bombay duck" (*Harpadonnehereus*Hamilton, 1822) along the Indian coast using truss network morphometrics. *J ApplIchthyolo.* 31: 37-44. doi: 10.1111/jai.12629.

26. Rawat S, Benakappa S, Kumar J, Naik K, Pandey G, Pema CW. 2017. Identification of fish stock based on truss morphometric: a review. *J Fish Life Sci.* 2(1): 9-14.

27. Rebello VT, George MK, Paulton MP, Sathianandan TV. 2013. Morphometric structure of the jumbo tiger prawn, *Penaeus monodon* Fabricius, 1798 from southeast and southwest coasts of India. *J Mar Biol Assoc India*. 55(2): 11-15. doi: 10.6024/jmbai.2013.55.2.01784-02.

28. Reiss, H., Hoarau, G., Dickey-Collas, M. & Wolff, W. J. (2009). Genetic population structure

29. Sajina AM, Chakraborty SK, Jaiswar AK, Pazhayamadam DG, Sudheesan D. 2011. Stock structure analysis of *Megalaspiscordyla*(Linnaeus, 1758) along the Indian coast based on truss network analysis. *Fish Res*. 108: 100-105. doi: 10.1016/j.fishres.2010.12.006.

30. Secor DH. 2014. *The Unit Stock Concept: Bounded fish and fisheries. In: Stock Identification Methods.* San Diego (US): Academic Press.

31. Sen S, Jahageerdar S, Jaiswar AK, Chakraborty SK, Sajina AM, Dash GR. 2011. Stock structure analysis of *Decapterusrusselli*(Ruppell, 1830) from east and west coast of India using truss network analysis. *Fish Res*. 112: 38-43. doi: 10.1016/j.fishres.2011.08.008.

32. Smith, P. J., Francis, R. I. C. C. &McVeagh, M. (1991). Loss of genetic diversity due to fishing pressure. *Fisheries Research* 10, 309–316. doi: 10.1016/0165-7836(91)90082-Q

33. Strauss, R.E., Bookstein, F.L., 1982. The truss: body from reconstructions in morphometrics. Syst. Zool. 31, 113–135.

34. Turan, C., 1999. A note on the examination of morphometric differentiation among fish populations: the Truss System. Turk. J. Zool. 23, 259–264.