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Intraspecific Population Variability in Goldstripe Ponyfish, *Karalla daura* Sampled along the Pakistan Coast Based on Geo-Morphometric Approach

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ABSTRACT

The use of geo-morphometric variables to discriminate fish populations is one of the appropriate, effective and widely adopted approaches to study population dynamics. To determine intrinsic population variability in goldstripe ponyfish, *Karalla daura* sampled from Keti Bunder, Karachi Fish Harbour, Hawks Bay and Pasni along the coastal belt of Pakistan. Size frequency distribution based on normal distribution demonstrates that individuals of 13 cm total length were common in all groups. Some fifteen morphometric and six geometric variables were used to develop Principal Component Analysis (PCA) that revealed (76%, 13.9% and 9.8%) variation deduced from first three components. The population sampled from Karachi fish harbors showed significant taxonomic variability. An eigenvalue 7.251 and canonical correlation 0.937 among four groups was appraised from PCI whereas Wilks' Lambda test of function $\lambda = 0.27$ was significant at $P < 0.05$. The highest variance percentage was the most correlated among the groups defined by total length, girth, 2nd dorsal fin, pectoral fin rays, eye dia, snout to extended gill cover and head length parameters. PC-II accounted for 13.9% of the variance that are more correlated with standard length, snout to eye tip, eye tip to first dorsal fin, base of 1st dorsal fin, last fin ray to tip of caudal, anal fin ray to pelvic fin ray, chin to snout and pelvic fin to pectoral fin. PC-III showed 9.8% variance with fork length, total weight, anal fin rays, last anal fin ray to 1st dorsal fin, tip of caudal fin to last anal fin ray, last anal fin ray to 1st anal fin, first pelvic fin to chin, eye to chin, 1st dorsal fin to last anal fin ray and last dorsal ray to last anal fin ray. among the groups. Whereas PC-IV has 0.3 % variance denoted by total weight, tip caudal fin to last anal fin ray. The PCA displayed a clear discrimination by sampling site, especially specimen from Karachi fish harbor were distinct from other location. Keti Bandar specimen was not dispersing as much as by geographical discrimination. Overall, results revealed that different topographies and environmental conditions causing significant variation in populations of *Karalla daura*. Therefore, it is assumed that this species has ability to adopt different ecosystem for survival despite acquiring adverse impact on phenotypic characteristics.

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Key words
Truss network, Morphometric, Population variability, Goldstripe ponyfish

INTRODUCTION

Ponyfishes of the family Leiognathidae inhabiting in the pelagic regimes are widely distributed in tropical and subtropical waters (McFall-Ngai and Dunlap, 1983; Bianchi, 1985; Qamar *et al*., 2017). These shiny, tiny fishes display tremendous fashion of bio-luminance that may help them for communication and predation (Paxton and Esch, 1998; McFall-Ngai and Dunlap, 1983). One of the striking phenomena in the advanced biology is to understand behaviour, taxonomical and environmental adaptation of aquatic organism they encounter that ultimately alters their phenotypic characteristics. Such variations can thoroughly be tactful in species hence the auxiliary morphological changes display divergent evolution (Mallarino *et al*., 2012). For better understanding of such changes we need to recognize periodic changes, perform counting, absence record, meristic, length-weight measurements, phenotypic data, geometric morphometrics and statistical data of intended species. Starting from common techniques of counting and comparing of body parts e.g., vertebrae, pectoral and pelvic fin, mouth position, shape and size of head, length, width, weight, scales and otolith structure

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Abbreviations
SET, snout to eye tip; ETF, eye tip to 1st D-fin; FDR, 1st dorsal fin to base; LRC, base of dorsal fin to tip of caudal; LDA, base of 1st dorsal fin; VRC, anal fin ray to 1st dorsal fin; TAR, caudal fin tip to last anal ray; LRA, anal fin base anal fin base to pelvic fin; PFC, 1st pelvic fin to chin; CHS, chin to snout; EYD, eye diameter; SEG, snout to gill cover tip; EYC, eye to chin; DFA, 1st D-fin to 1st anal fin; DFP, 1st D-fin to pectoral fin; DRA, dorsal fin base to anal fin base; PFP, pelvic fin to pectoral fin; SHE, head length; SDF, snout to 1st D-fin.

(Ethin *et al*., 2019). Different ecosystems produce distinct stress level which reflects on the capability of an individual species to encounter such environmental pressures and continue to exist. In this context, different species produce diverge phenotypes in heterogeneous environment which consequence between environmental and developmental changes (Ernande and Dieckmann, 2004; Benitez *et al*., 2021). In the aquatic ecosystem, organism body size, population diversity and water dept with different latitude continuously studied, biotope characteristics, anthropogenic activities and physical parameters remarkably influenced body size and shape (Sukhodolskaya, 2013; Benitez *et al*., 2020, 2021).

The basic morphological measurements sometime may not match although species belong to same genus or intra-species, such as pigmentation, spots or blotches, spines, rays, lateral line, mouth parts and pelvic fin. Earlier multiple authors contributed toward evolutionary modification through morphological analysis (Prud'homme *et al*., 2011). The modification of pelvic fin in sticklebacks, *Gasterosteus aculeatus* is an evolutionary change (Mallarino and Abzhanov, 2012). Further they measured pelvic fin from tip to the point of articulation with simple meristic and length-weight hence proved evolutionary changes in stickleback between two different water bodies.

Traditional morphometrics deal with absolute measurements while landmark-based geometric morphometrics applied for multi dimension data that coordinate with landmarks. For different species some particular landmarks are used for geometric morphometric analysis including upper and lower mandible, pelvic fin and pre-pectoral length etc. The sequence of landmarks and semi-landmarks helped researchers to assess better perception among species and discovered the enumerative contribution of relative size, position and curvature of different body parts (Foster *et al*., 2008). In Pakistan Qamar *et al*. (2017) recorded eight species sampled from different fish landing sites along Pakistan coast. However, this family remained under uncertainties owing to close taxonomic resemblances in some of the family members.

Thus, the objective of this study was to develop discrimination model using geo-morphometric data to find out intraspecific variability of goldstripe ponyfish, *Karalla daura* populations inhabiting along the Pakistan coastline.

MATERIALS AND METHODS

Sampling and location description

Seventy individuals of goldstripe ponyfish, *Karalla daura* were collected from Keti Bandar, Karachi Fish coast. The specimens were collected using medium sized fishing boat at an hour hauling time with gill nets of three different mesh sizes (1.5, 2.0 and 2.4 inches). (Fig. 1). Specimens were immediately stored in ice boxes. In the laboratory frozen specimens were thawed for an hour under running water and open room temperature (26~30℃) before analysis of morphometrics, meristic and geometric measurements.

Morphometrics measurement and truss network

Geometric and morphometric measurement of 70 individual of goldstripe ponyfish were used to establish truss network on dorsal view of each specimen considered for land-marking. Some fourteen landmarks with seven truss networking were applied. The landmarks were digitized by tpsDig2 v 2.31. software and truss distances were measured by a divider at 0.01mm precision (Fig. 2). The measurements and truss networking were also tested with digital images following practice adopted by (Cardrin and Friedland, 1999; Pazhayamadom *et al*., 2015). In addition, we performed an exercise using digital images in MorphoJ software (V 1.07a) for accuracy and corroboration.

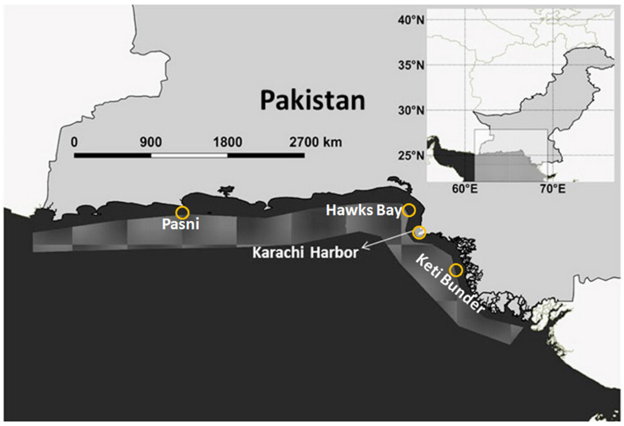

Fig. 1. Sampling locations and coastal fishing grounds of Pakistan are highlighted with shaded area.

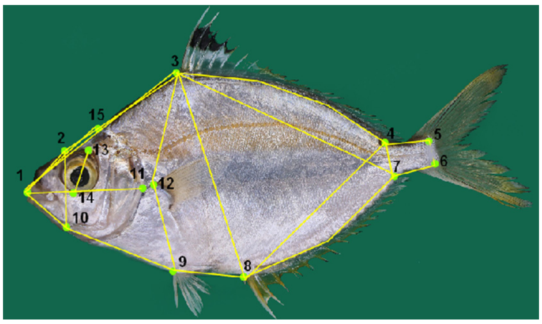

Fig. 2. Locations of fifteen landmarks and outline of truss network established for *Karalla daura* collected from four sampling sites.

Table I. Descriptive form of *Karalla daura* sampled from four locations along Pakistan coast.

Locations	N	Lengths (cm) Mean±S.E	Body weight (g) Mean±S.E	Standard length (cm) Mean±S.E	Girth (cm) Mean±S.E
Hawks bay	29	11.74 ± 0.22	25.07 ± 1.62	9.48 ± 0.18	10.01 ± 0.25
Fish harbor	18	11.37 ± 0.99	23.39 ± 1.53	9.25 ± 0.19	9.64 ± 0.27
Keti bunder	16	11.86 ± 0.13	23.07 ± 0.7	9.63 ± 0.1	9.86 ± 0.12
Pasni	7	11.45 ± 0.34	22.99 ± 2.3	9.35 ± 0.26	9.5 ± 0.4

Statistical analysis

Using SPSS ver. 24 all statistical analysis including Principal Component Analysis (PCA), Canonical Discriminant Analysis (CDA) and ANOVA were conducted.

Moreover, PCA based on covariance matrix of individual shape was applied to test the dimensions of shape, space and levels of variations. In order to discriminate intraspecific variation among four locations a canonical variation analysis was performed.

RESULTS

The lengths and weights of the specimens collected was more or less similar ranging from 9~14 cm (11.0cm mean), 22~25 g (23.63 g mean) (Table I, Fig. 3). Significance of the data was statistically tested using ($P<0.05$). The canonical discriminant variations showed a highly significant differences among the groups (=0.027, $p<0.00$) among four locations, population sampled from Karachi harbour revealed significant difference while rest of the locations Keti Bunder, Hawks Bay and Pasni showed 36:25:1.0 ratio of variation respectively. The highest variance percentage was the most correlated among the groups of measurements were TL, Girth, DII, PECFR, EYD, SEG, SHE, and SDF. Group II accounted for 13.9% of the variance that are more correlated with SL, SET, ETF, FDR, LRC, TEC, AFP, CHS, and PFP. Group III showed 9.8% variance with FL, TW, AFR, VRC, TAR, LRA, PFC, EYC, DFA, and DRA among the groups. Group IV defines trivial 0.3 % variance by correlation of TW and TAR (Table II). The canonical discrimination function reveals that specimens from Karachi Fish Harbour are distinctly different from rest of the locations. Keti Bandar specimens were not dispersing as much as by geographical discrimination.

A cumulative variance of PCI and PCII showed 90.2% divergence in the population sampled from four locations which were remarkably differs from each other particularly in body shape and head region. PC~I was basically deviation between the fish head and body measurements except snout length, nuchal crest, LRC, AFP, CHS, PFP, LRA, PFC and DFP which are predominant in PC~II, PC~III and PC~IV. *K. daura* from Keti Bunder have maximum body depth along with vast width of lateral line and head height than other population, on the other hand, shortest body depth, head length, and snout from Karachi Fish Harbor obviously discriminant the two distinct population (Fig. 4). Population sampled from Hawks Bay comparatively differs with upper body parts (snout, nape, dorsal fin to extend end of caudal fin). Whereas minimum deviation found in Pasni population along with width and body weight were higher from rest of the groups.

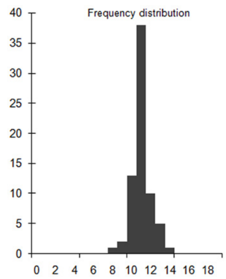

Fig. 3. Normal distribution of the size frequencies, central line falls at 13 TL_{cm} in the population of *Karalla daura* sampled form four locations along Pakistan coast.

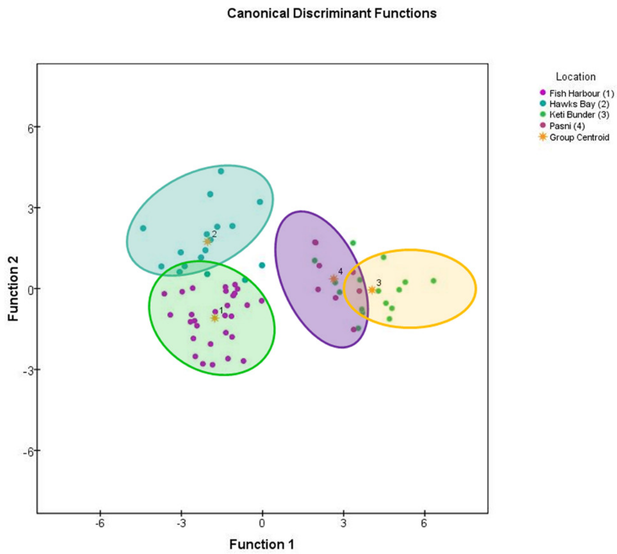

Fig. 4. Canonical discriminant function analysis plotted for goldstripe ponyfish collected from four locations along Pakistani coast using Geo-morphomteric variables.

Table II. Abbreviations and full forms of each taxonomic parameter used in PCA analysis.

S. No	Abbre-viations	Full form of the parameter	Landmarks
1	SET	Snout to eye	1-2
2	ETF	Eye to dorsal fin	2-3
3	FDR	1st Dorsal fin to 1st dorsal fin base	3-4
4	LRC	Last D-fin to base to tip of caudal fin	4-5
5	LDA	Last D-fin rays to 1st anal fin	4-8
6	VRC	Last anal fin base to dorsal fin base	3-7
7	TAR	Tip caudal fin tip to anal fin	6-7
8	LRA	Last anal ray to anal fin	7-8
9	AFP	Anal fin to pelvic fin	8-9
10	PFC	Pelvic fin to chin	9-10
11	CHS	Chin to snout	10-1
12	EYD	Eye diameter	13-14
13	SEG	Snout tip of gill cover	1-11
14	EYC	Eye to chin	2-10
15	DFA	1st D-fin to anal fin	3-8
16	DFP	1st D-fin to pectoral fin	3-12
17	DRA	Dorsal fin base to anal fin base	4-7
18	PFP	Pelvic fin to pectoral fin	12-9
19	SHE	Head length	1-15
20	SDF	Snout to 1st D-fin	1-3

The truss measurements in Fish harbor have maximum variation which covers the whole body of fish. Function 1 and function 2 alone make 90.2% of variance in total population and exceptionally showed important morphological variation in body and head region (Table III). The discriminant function analysis results indicated the dandrogram from location I was a strong differentiate whereas high degree of overlapping was interrelated between location II and location III demonstrates that the variables were appropriately discriminant at location I (Fig. 5).

Table III. Summary of first three canonical discriminant functions were used in the analysis.

Function	Eigenvalue	% of variance	Canonical correlation
1	7.251[a]	76.3	0.937
2	1.324[a]	13.9	0.755
3	.930[a]	9.8	0.694

The estimates of Wilk λ have showed significant variation among morphometric traits of total population except the function III because it is non-significant ($p > 0.05$) (Table IV).

Table IV. Wilks' λ test function of first three components.

Component	Wilks' Lambda	Chi-square	Df	P value
1/ 3	0.027	189.58	87	0
2/ 3	0.223	78.782	56	0.024
3	0.518	34.513	27	0.152

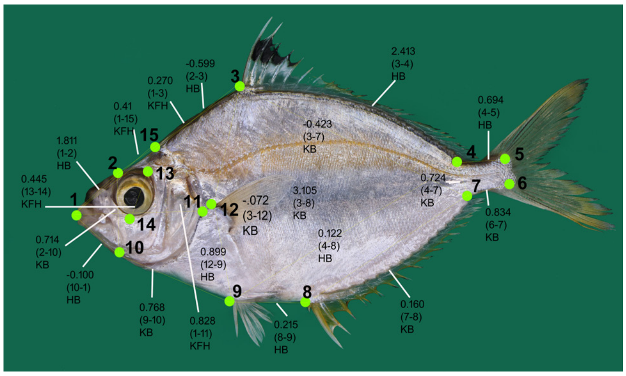

Fig. 5. Significantly diverging taxonomic variables, PCA values along with landmarks are closed in parenthesis (KB refers Keti Bunder, HB Hawks Bay, KFH Karachi Fish Harbour).

DISCUSSION

This study has established a stance that morphometric measurements and truss network analysis mutually expressing population variability among four locations along Pakistan coast. However, taxonomical characters in fishes are often unchanged condition through generation to generation but manmade changes affected aquatic ecosystem significantly that ultimately cause morphometrics deviation.

Despite their enormous ecological significance ponyfishes remain untargeted group of fishes in Pakistan. Four sampling locations Keti Bunder (a muddy creeks area), Karachi (mangroves with massive polluted area), Hawks Bay (sandy cum rocky area) and Pasni (muddy pollution free area) are comparable to evaluate fish health and taxonomic variations influenced by the topographies. However, population inhabiting in pollution free area for instance Pasni grew well and remained untapped from topographic influences. Adding to it, it is common that intraspecific morphological changes are basically triggered by environmental influences (Staunton-Smith *et al.*, 1999).

Use of truss network in fish taxonomical studies play an important role to underline physical and morphological changes that cannot be detected through traditional form of fish body measurements (Park *et al.*, 2015). Different physical parameters effects fish population, in this context, we found their significant taxonomic variation among four locations. Since samples obtained from commercial catches, we may assume that they used bigger mesh size and catch small numbers as bycatch. This may result bigger impact on weight and size variation. Nevertheless, the variations between head and nuchal crest may be caused of water density, wave speed and topography of the location.

Though there are limited anthropogenic activities and uniform quality of water around Pasni (Balochistan) did not show much influence on taxonomic characters of goldstripe ponyfish. The four locations are not only differed from anthropogenic activities but also diverse by planktons and nutrients density. We noticed that population dwelling around Karachi coastal waters encounter significant pollution pressure, almost all type of pollution.

Based on fifteen measurements our results suggest that there is a strong variation among the groups. There is a vast geographic division between these locations. All these locations from southern Keti Bunder to western Pasni is vary in terms of productivity, diversity, topography density, pressure and depth. Nevertheless, pressure and depth have more impact in growth rate or morphological variation, diversity and population. Although we do not know spawning nature of this species, we may assume it migrate to deep water for spawning or vice versa return into shoreline which can lead for morphological changes because of adaptation of diverse ecology as it is reported by several researcher (Jorgensen *et al*., 2008). Moreover, organisms living in Open Ocean their morphometrics changes caused by biological origins, particular water body, or other levels could be sort outduel to trait homogenization (Cronin-Fine *et al*., 2015).

Although we found better weight gain in Keti Bunder however the snout length, nuchal crest, dorsal fin, LCR (last dorsal fin ray to tip of caudal) AFP (1st anal fin to pelvic fin), CHS (chin to snout) and PFP (pectoral fin to pelvic fin) is bigger in Hawk's Bay. Whereas eye diameter (EYD), snout to gill covers (SEG), head length (SHE) and snout to dorsal fin (SDF) is lager in Karachi fish harbor. This pattern of body shape variation shows in opposite in group 3 (Keti Bunder) where body depth tends to be higher. Additionally, tip of caudal to last anal fin ray (TAR), anal fin (LRA), pelvic to chin (PFC), width of mandible (eye tip to chin), body depth (dorsal fin to anal fin DFA), dorsal fin to pectoral fin (DFP) and last dorsal fin ray to last anal fin ray (DRA) is prominent in last two groups. The major deviation between two location is in the anterior region and these data suggest variations in head can be influenced by pressure, density, turbidity, currents and temperature because the fluctuation of water parameters in both locations are higher and most fluctuation found in Karachi Fish Harbor. The morphological development in other parts of body including body depth or girth, pectoral to pelvic region and pelvic to anal fin are due to fullness of abdomen by planktons and nutrients abundance in ecosystem. We also found uniform development of body parts in Pasni location because it is the most undisturbed location in terms of anthropogenic activities and pollution and environmental changes. Topography can change over long-time scales consequence evolutionary and developmental alteration in fishes (Zhu *et al*. 2016; Chin *et al*., 2019). Besides, seasonal variations in tide, current, depth, water movement and upwelling are also adversely acting on fish and habitat. The topography also effects in vertical mixing and stratification substance transport.

We obtained the relative importance of morphological variations in the shape variation in the species. Shape modification seems to befall large and small spatial scales. The most important differences in body shape are whether body elongated or shorten, this may progress to high deviation in high and length among the groups. Overall, six canonical groups are identified based on shorten anterior region and larger body depth vice versa. Our results suggest that the 15 landmarks are valid to determine the origins of goldstripe ponyfish. Therefore, it seems that morphometric analysis can deliver easy accessible, fast, inexpensive and effective method for for fish stock identification.

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Data availability statement

Data will be made available on request.

Statement of conflict of interest

The authors have declared no conflict of interest.

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