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Fish communities in high-latitude mangrove in north-western Mexico

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Abstract

Studying fish communities in extreme habitats is important to better understand the variation in their ranges under climatic scenarios or anthropogenic pressure. In particular, the mangroves in Baja California Sur occupy the northernmost distribution range under two extreme conditions (temperate waters and arid weather). In this context, the aim of the presently reported study was to analyze the functional characteristics of ichthyofauna in two localities, La Paz Bay (BP) and Almejas Bay (BA), which are also located in different ecoregions. For both bays, the composition and frequency values were compiled from monthly samples and the literature. Eleven functional traits were assessed from the morphology of every fish species. Functional indices (Richness, Evenness, Divergence, and Originality) were used to evaluate different aspects of the community structure. A total of 83 species were found at both sites, with 54 in BP and 50 in BA. In BP, six species were residents, eight were temporal visitors, and 36 were occasional visitors. In BA, six species were residents, 15 species were temporal visitors, and 33 were occasional visitors. At both sites, 12% of the species were permanent residents; BA had a higher percentage of temporal residents (27%), while BP had a higher percentage of occasional visitors (72%). The functional analysis detected communities with specialized traits, but in comparison to BP, BA had higher evenness in the community structure. Although greater structural complexity was expected in the community during the warm months, because of the increase in richness and attributes, BA had higher values during the cold months, which was probably because the area is a transition zone and the fish communities are adapted to colder climates. In comparison to BA, BP had higher originality values, and colder months presented higher values than warmer months. Although the mangrove sites had the same northernmost latitudinal limits and both had extreme conditions compared to those in mangroves in tropical environments, the fish communities differed in their composition, frequency, and functionality with more extreme functional traits in colder weather than in warmer weather.

Keywords

arid, frequency, functional attributes, functional diversity, temperate

Introduction

Mangrove fish communities are distributed worldwide (30°N–30°S) on sheltered tropical and subtropical coastlines (Ellison 2002). Due to the importance of these communities from economic, ecological, and social perspectives (Costanza et al. 1997), the number of studies

has increased, especially in tropical distribution zones (Faunce and Serafy 2006). However, only a few studies have focused on higher latitudes around circumtropical boundaries (Quisthoudt et al. 2013). An analysis of habitats at latitudinal boundaries (which usually have extreme conditions) requires special attention (Ong et al. 2018), because environmental characteristics influence

the distribution, physiology, and survival of marine populations. In addition, the migration of fishes from the tropics to higher latitudes has started to become more frequent (Tedesco et al. 2017; Ford and Roberts 2018), which indicates that the changes in the community dynamics of the newly invaded habitats need to be understood.

Two kinds of mangrove environments have been defined at higher latitudes according to habitat characteristics: (1) temperate mangroves, which tolerate low water temperatures, with an average of 20°C in the coldest month (water and weather temperature combined; Cavanaugh et al. 2014), and (2) arid mangroves, which are in areas with low rainfall ($<300 \text{ mm} \cdot \text{year}^{-1}$), no riverine input, and high evaporation rates (Schaeffer-Novelli and Cintron-Molero 1993; Arreola-Lizárraga et al. 2004). Under both conditions, the trees are dwarfed with average heights of less than 3 m (Flores-Verdugo et al. 1993; Millán-Aguilar et al. 2019). In Baja California Sur (BCS), north-western Mexico, there are examples of mangroves that experience a combination of both conditions.

Although mangrove fish communities in BCS live under extreme weather conditions, few studies have been performed in this area. In this context, it is important to use the information available, which includes sampling performed at different time periods, and to implement the appropriate analytical tools to describe the characteristics of the community. In particular, functional ecological analyses have proven to be useful because they use species traits that are linked to ecosystem processes. These analyses focus on the difference between unique species characteristics (e.g., physiological, feeding, locomotion, and reproduction) and explain the functions performed by species in the environment from another perspective (Niche, Costa-Azevedo et al. 2017).

In the presently reported study, the main aim was to explore the arid and temperate mangrove fish communities and describe the functional aspects that may provide insights on broader ecological processes in high-latitude regions. We expected that the functional traits at the selected sites would be more specialized than those reported in tropical mangrove fish communities. However, based on the extreme weather conditions in BCS, the functional composition of the fish communities would likely be affected in a similar way (loss/gain of species) despite the original condition of the mangrove (arid/temperate) during the seasonal changes, as the temperature has been reported to guide local migration of fish to other near environments.

Methods

Study area

Two areas in the Baja California Peninsula, México, were selected: Bahía de La Paz (BP) and Bahía Almejas (BA). Both are strictly marine, without riverine inputs, and present arid and temperate environments (rainfall of $180 \text{ mm} \cdot \text{yr}^{-1}$ in BP and approximately $80 \text{ mm} \cdot \text{yr}^{-1}$ in BA with an evaporation rate of $1800 \text{ mm} \cdot \text{yr}^{-1}$), as-

pects that create close to hypersaline suboptimal conditions. As a consequence, dwarf mangrove forest trees are common, with a maximum height of approximately 3 m (González-Zamorano et al. 2013). In contrast, the sites belong to two separate ecoregions (sensu Spalding et al. 2007), which implies different oceanographic characteristics. BP is the largest protected bay on the eastern coast of the peninsula inside the Cortezian ecoregion ($24^{\circ}07'39''\text{N}$ and $24^{\circ}21'41''\text{N}$, and $110^{\circ}17'23''\text{W}$ and $110^{\circ}40'23''\text{W}$), as it includes the Gulf of California. It is characterized by warm climates, upwelling systems, and high levels of primary productivity. BA is located in the southern part of Bahía Magdalena on the western coast of Baja California Sur inside the Magdalena Transition ecoregion ($24^{\circ}17'18''\text{N}$ and $24^{\circ}20'51''\text{N}$, and $111^{\circ}20'30''\text{W}$ and $111^{\circ}27'47''\text{W}$), which is a transitional zone for cold and warm waters located below San Ignacio to the southernmost tip of Cabo San Lucas. This ecoregion has moderate productivity with higher peaks in upwelling systems (Spalding et al. 2007; Wilkinson et al. 2010; Fig. 1).

Sampling and database

In BP, monthly trawling occurred at four sites (with a repetition on each, for a total of eight sampling events) from July 2010 to June 2011, and all events occurred at the same hour, with the highest possible reproducibility. The fishing device was an experimental seine net (a length of 50 m, depth of 2 m, and mesh size of 1 cm). The BA data were published by Rodríguez-Romero et al. (2011) from monthly sampling at ten sites (one sampling event per site) from October 1993 to September 1994 using an experimental shrimp seine net (6 m length, 4 m depth, and mesh size of 1 cm at the codend). BP identification

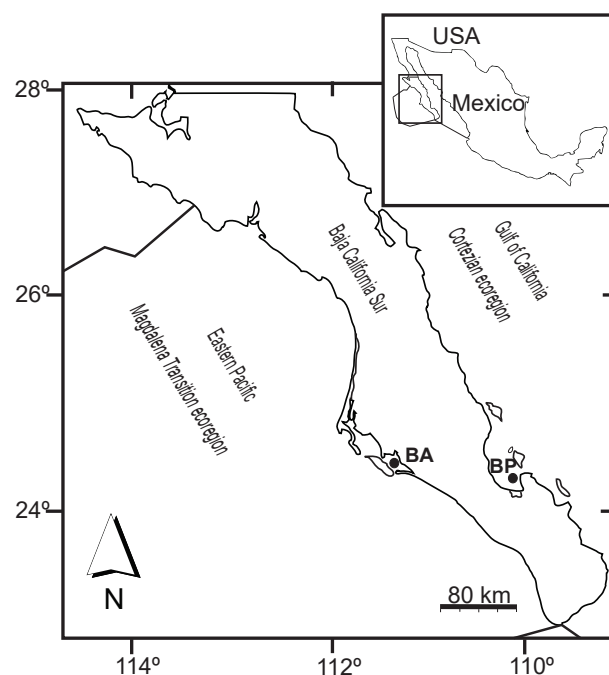


Figure 1. Sample sites location in the Baja California Peninsula. The two ecoregions are also represented in the map.

was carried out to the species level following the criteria of Fischer et al. (1995). All voucher specimens collected in BP were deposited in the Ichthyological Collection at CICIMAR, Instituto Politécnico Nacional, La Paz, BCS, Mexico. The list of species from Rodríguez-Romero et al. (2011) was reviewed using the FishBase *match name* tool (Froese and Pauly 2019). One species reported by Rodríguez-Romero et al. (2011) was detected out of its distribution range for *Sphoeroides angusticeps* (Jenyns, 1842); as a result, it was excluded from the analysis.

Frequency of the species











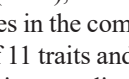
To identify the affinity and permanence time of the species in the environment, the percentage frequency throughout the year for each species was considered. The classification was modified from the proposal of Amezcua-Linares and Yáñez-Arancibia (1980): Resident species (frequency > 75%), Temporal visitors (75% > frequency > 25%), and Occasional visitor (frequency < 25%).

Functional diversity

One adult organism was selected to describe the morphology of the species, the photograph was taken following standard ichthyological guidelines, and the mouth of the organism was pointing to the left side with its fins extended. In the case where organisms were too damaged to follow this procedure, scientific photographs were gathered from available public web repositories such as FishBase (www.fishbase.org), Naturalista (www.naturalista.mx), and scientific fish collections (Ichthyological Collection of CICIMAR). Eleven body lengths were measured from the photographs using ImageJ software ver. 1.51j8 (Wayne Rasband, National Institutes of Health, Bethesda, MD, USA), and using these measures, proportions of the body were calculated describing nine functional traits (Table 1, modified from Toussaint et al. 2016). These functional traits were selected after a rigorous screening process from several potential descriptors (defining feeding and locomotion processes), and the correlation between the variables was considered to identify collinearity (cutoff variation inflation factor > 3; Zuur et al. 2010) as well as the explained variance in a PCA (which is not included here) to determine the traits that had relatively high explanatory power to describe the morpho-functional variation in the species.

Functional indices that could be calculated with the presence/absence of the species were selected, to avoid the introduction of bias in the community structure interpretation due to dominance aspects: Species abundance values could be related to external conditions (biotic and abiotic) present during the different years when the studies were carried out. Because abundance was not considered in the analysis, the interpretation of the functional indices was adjusted; instead of weighing the species by the number of individuals, each species was considered to have 1/S (Species) abundance (Villéger et al. 2008).

Table 1. Formulas and description for calculating functional attributes.

Attribute	Formula	Measures
Body elongation	$\frac{\text{Maximum body height}}{\text{Standard length}}$	
Body form	$\frac{\text{Head length}}{\text{Standard length}}$	
Body height	$\frac{\text{Head height}}{\text{Maximum body height}}$	
Oral gape position	$\frac{\text{Mouth position}}{\text{Head height}}$	
Mouth length	$\frac{\text{Jaw length}}{\text{Head length}}$	
Eye size	$\frac{\text{Eye diameter}}{\text{Head height}}$	
Eye orientation	$\frac{\text{Eye position}}{\text{Head height}}$	
Pectoral fin size	$\frac{\text{Pectoral fin length}}{\text{Standard length}}$	
Pectoral fin position	$\frac{\text{Pectoral fin insertion}}{\text{Maximum body height}}$	
Caudal peduncle throttling	$\frac{\text{Caudal peduncle height}}{\text{Caudal fin height}}$	
Caudal peduncle aspect	$\frac{\text{Caudal peduncle height}}{\text{Caudal peduncle length}}$	

The selected functional indices focused on four facets of functional diversity: functional richness (FRic), which is the functional space occupied by the species in the community represented by the multivariate set of 11 traits and, was calculated using a convex hull volume in an ordination space; functional evenness (FEve), which describes the regularity of the distribution of species in the functional space using a minimum spanning tree to be measured; functional divergence (FDiv), which describes how far highly frequent species are from the center (the median focus point that is at the same distance from each species) of the functional space (Villéger et al. 2008; Laliberté and Legendre 2010); and finally, functional originality (FOri), which describes how species modify redundancy between the species, where the lower the value, the closer species are together, and the larger the value, the more separated species tend to be from one another in the functional space (Mouillot et al. 2013). The indices were calculated with the multidimFD package (Villéger 2017). Due to low sample sizes for some months (extreme cold and warm water temperature), the indices FEve and FDiv could not be calculated in BP in December, January, February, and July.

Results

Fish community

A total of 83 species were recorded in this study: 54 were in Bahía de La Paz and 50 were in Bahía Almejas (Appendix 1–3). In both localities, Haemulidae was the most diverse family, with 10 species at each site, followed by Paralichthyidae and Gerreidae, with seven and five species in BP and seven and eight species in BA, respectively.

For BA, two species (*Orthopristis reddingi* Jordan et Richardson, 1895 and *Prionotus ruscarius* Gilbert et Starks, 1904) were found with a relatively small distribution range (from Bahía Magdalena to the Gulf of California), six species had a cosmopolitan range, whereas 42 species had a Tropical Eastern Pacific (TEP) distribution. For BP, we found one – *Exerpes asper* (Jenkins et Evermann, 1889) – with a restricted distribution range inside the Gulf of California, nine species with a cosmopolitan range and 43 species with a TEP distribution.

For BP, six species were defined as residents – *Diapterus brevirostris* (Sauvage, 1879); *Eucinostomus currani* Zahuranec, 1980; *Eucinostomus dowii* (Gill, 1863); *Eucinostomus entomelas* Zahuranec, 1980; *Mugil curema* Valenciennes, 1836; and *Paralabrax maculatofasciatus* (Steindachner, 1868); eight species as temporal visitors, and 36 as occasional visitors. For BA, six species were recognized as residents – *Achirus mazatlanus* (Steindachner, 1869); *Etropus crossotus* Jordan et Gilbert, 1882; *Paralabrax maculatofasciatus*; *Paralichthys cali-*

formicus (Ayres, 1859); *Sphoeroides annulatus* (Jenyns, 1842); and *Synodus lucioceps* (Ayres, 1855); 15 species as temporal visitors and 33 as occasional visitors. According to the proportions in each residence category, at both sites, 12% of the species were permanent residents, BA had more temporal residents (27%), and BP had more occasional visitors (72%).

Functional indices

Three similar morphologies were identified in the functional space: flatfish were on the right, tubular/elongated fish were on the left, and the remaining morphologies, mainly perch-like, were around the center (Fig. 2). Higher values of functional richness occurred during the months with more species, which were June, July, September, and October (warm season months), than during the other months, and these months had wider coverage areas in the convex hull (Appendix 4). The FEve did not show a general pattern among the seasons, but Bahía Almejas presented higher values than Bahía de la Paz and the posteriori test showed a significant difference ($t = 4.82$, $P = 0.0005$).

The FDiv plots for BP showed high value results (more than 70 to a total of 100), indicating specialized functional community composition. In comparison to other months, months with higher temperatures had species with extreme morphologies (i.e., *Mugil curema* and *Etropus crossotus*) that occur more frequently, each

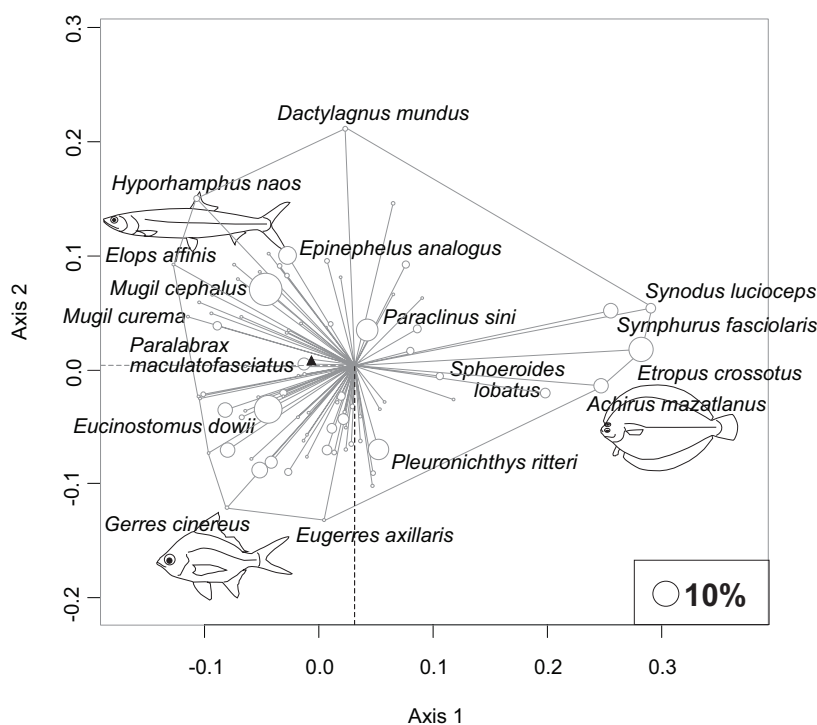


Figure 2. Functional space of the Baja California peninsula. Names describe the species position that is found to be of importance in the community, *Paralabrax maculatofasciatus* (black triangle) represent the point where most common morphological traits converge in the community.

month extending the border of the total functional space. On the other hand, in comparison to BP, BA presented a higher frequency of species presence with extreme morphologies (as indicated by the FEve high values), but the monthly functional space did not cover the whole functional space.

The species that seemed to have the highest frequency throughout the year in terms of functional divergence were those located farthest from the functional center of gravity, such as *Etropus crossotus*, *Mugil curema*, and *Symphurus fasciolaris* Gilbert, 1892, with the exception of *Paralabrax maculatofasciatus*, which was close to the center of gravity and represented a good example of the predominant fish morphotype (perc shaped) around this area. Statistical differences were found between sites ($t = 98.179$, $P < 2.2e-16$) for the months that were available. In comparison to BA, BP presented higher FOr values throughout the year, while BA showed wider variability in high and low values during different months. In general, months with lower temperatures presented higher FOr values, and warmer months presented lower FOr values.

Discussion

As expected from our first hypothesis, fish communities in strictly marine arid mangroves present highly functional specialized traits, as they are present throughout the year in both localities. Some studies in estuaries have identified communities with short functional spatial coverage in temporal analyses (months and seasons, Villeger et al. 2010; Pease et al. 2012), probably due to the physiological constraints required to overcome more dynamic and adverse environments than those of marine arid mangroves (Payán-Alcacio et al. 2020). Because functional analyses must be uniform in the selection of attributes (Violle et al. 2007) and although maximum biodiversity (1/S) was used instead of abundance data (both provides different results, but they reached the same conclusion; Villéger et al. 2008), we carefully examined our comparison.

Functional analyses confirmed the previously proposed regionalization (Spalding et al. 2007). It seemed that individual participation in the structuring and dynamic processing of the community in terms of its biological, morphological, and physiological traits was more related to oceanographic and biogeographic factors at a larger spatial scale than to similar habitat characteristics and the expected functional redundancy at a more local scale (Stuart-Smith et al. 2013; Frainer et al. 2017). According to the richness and frequency, we identified the families Haemulidae, Paralichthyidae, Gerreidae, Mugilidae, Tetraodontidae, and Serranidae as the most representative in the two mangrove localities on the Baja California Peninsula. Our finding agreed with the results of Castellanos-Galindo et al. (2013) in terms of the most important fish families in mangroves in the Eastern Tropical Pacific.

Although both localities had six resident species, only one (*Paralabrax maculatofasciatus*) occurred at both

sites. Based on the permanent resident species, BP seems more centrally structured with benthopelagic fishes (especially members of the Gerreidae family), while BA had a higher number of resident flatfish. Both groups are strong representatives of mangrove fish species in the Pacific (Yáñez-Arancibia et al. 1983; Castellanos-Galindo et al. 2013).

Regarding resident proportion, in comparison to BA, BP had a higher proportion of occasional visitors, and the results suggested a more open and dynamic environment. This scenario could be related to the geomorphology of the site; BP opens into two large channels connected to the Gulf of California, while BA is an enclosed cove with only one opening (Fig. 1).

Another similarity in the structural composition of these high-latitude mangroves with those in other areas of the Americas was the presence of the family Carangidae. Such fish have a preference for warmer temperatures (Sivakami 1996), and we found carangids at both sites from August to November. The temporal use of mangroves by this fish family could be related to the high availability of prey items, such as juveniles (mean total length <10 cm) of *E. dowii* and *M. curema*, as well as some species of Engraulidae and Clupeidae found during the same months at both sites. Although we found a low number of carangid species, as is usually reported for carangids in the mangroves of the Americas (Castellanos-Galindo et al. 2013), the low species diversity in this study could be related to the higher nocturnal feeding activity of the family (Sivakami 1996) and the lack of sampling during that time.

In general, season appeared to be the most important aspect determining the structural and functional characterization of the fish community at both locations. Species richness and organism abundance increased as the temperature increased at both sites, and the opposite effect occurred during the cold months. Similar results were obtained in other studies on the region (González-Acosta et al. 2005; López-Rasgado et al. 2012). The higher species richness during the warm months than during the cold months could be associated with higher temperature values, longer days, maximum litterfall rates, and the presence of nutrients that promote primary production (Félix-Pico et al. 2006; Bizzaro 2008). These characteristics result in food availability for the species inhabiting mangroves (López-Rasgado et al. 2012) and by default increase the range of available functional traits.

For our second hypothesis, the results showed a difference in the functional composition at both sites. Consequently, the results are consistent with the fish biogeography proposal for the Baja California Peninsula that separates BA within the transitional zone (considering the clash the sea currents temperatures, cold and warm from the northern and southern part of the peninsula respectively) and BP within the warmer zone (due to the degree of endemism and isotherms coming from the tropics preventing migration of genetic flow; Briggs and Bowen 2012).

For both localities, the FDiv results indicated specialized community composition. In BP, all extreme-mor-

phology species were present throughout the year, meaning that those specialized species were residents at the site. On the other hand, BA presented changes in the different extreme morphologies according to seasonality (i.e., from benthic flatfish to pelagic species such as *Hyporhamphus naos* Banford et Collette, 2001 to benthopelagic species such as *E. dowii*), indicating strong fragmentation in the community.

Although the community was more specialized, the most common morphological features indicated that the community contained *generalists*, as perch-shaped fish accounted for approximately 50% of the composition. Generalist traits are related to a greater swimming maneuvering capacity (due to the proportion of fins to the body and caudal peduncle; Villéger et al. 2010), allowing them to be efficient at competing for resources under a range of different circumstances (Bridge et al. 2016). In addition, generalists are less sensitive than specialists to alterations in the environment because they occupy a broader niche space (Villéger et al. 2010; da Silva et al. 2018).

The greater functional complexity of the fish community (higher presence of different traits) was related to the months with warm temperatures (August to November), mainly because higher FRic values were positively correlated with species richness (Schleuter et al. 2010). However, if the trade-off between species presence and the number of traits present are considered, greater complexity occurred in BA than in BP, regardless of the different mean temperatures present in the bays (Colder in BA and warmer in BP). This result could reflect a high degree of niche differentiation (Mason et al. 2013) rather than a high number of fish species. As BA is a transition zone between temperate and warm temperatures (Bizarro 2008), the functional traits could be more regularly distributed among species, as shown by the FEve results, and this scenario generates high differentiation of resources

and promotes distinct mechanisms for using the available resources per species (Dolbeth et al. 2016).

Functional originality differed between localities during warmer months (July to November). Although at both sites, an increase in the species occurred, and in comparison to BA, BP presented higher values of FOr_i due to the occurrence of species with extreme traits (e.g., opportunistic predators). In contrast, the FOr_i values in BA decreased because more generalist species entered the bay in the warmer months, which lowered community uniqueness, and the resident fishes had the extreme traits. The results suggest that mangrove fish communities in colder waters present a more complex set of traits than those in warmer waters, and this complex set of traits is also found in the functional life strategies of river fish in temperate water (Keck et al. 2014).

Both hypotheses were accepted. The first hypothesis indicated that due to extreme weather conditions (arid and temperate climate) mangrove fish communities at high latitudes present more specialized traits, and the second hypothesis indicated that biogeographic barriers and weather conditions alter the functional composition of the communities despite being found at similar latitudes in the same landmass. Future studies should focus on changes in the functional composition of fish communities in the same ecoregion with different environmental conditions at mangrove sites to validate our findings.

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Appendices

Appendix 1. List of fish species (Actinopterygii) in Baja California Peninsula and their geographic distribution.

Class	Family	Species	Author	Distribution
Pleuronectiformes	Achiridae	<i>Achirus mazatlanus</i>	(Steindachner, 1869)	Eastern Pacific
Perciformes	Scombridae	<i>Auxis thazard</i>	(Lacepède, 1800)	Eastern Pacific
Tetraodontiformes	Balistidae	<i>Balistes polylepis</i>	Steindachner, 1876	Eastern Pacific
Pleuronectiformes	Bothidae	<i>Bothus constellatus</i>	(Jordan, 1889)	Eastern Pacific and Gulf of California
Perciformes	Sparidae	<i>Calamus brachysomus</i>	(Lockington, 1880)	Eastern Pacific
Perciformes	Carangidae	<i>Caranx caninus</i>	Günther, 1867	Eastern Pacific and Gulf of California
Perciformes	Centropomidae	<i>Centropomus medius</i>	Günther, 1864	Eastern Pacific and Gulf of California
Perciformes	Ephippidae	<i>Chaetodipterus zonatus</i>	(Girard, 1858)	Eastern Pacific
Perciformes	Chaetodontidae	<i>Chaetodon humeralis</i>	Günther, 1860	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Citharichthys gilberti</i>	Jenkins et Evermann, 1889	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Citharichthys platophrys</i>	Gilbert, 1891	Eastern Pacific
Perciformes	Gobiidae	<i>Ctenogobius mangicola</i>	(Jordan et Starks, 1985)	Eastern Pacific
Perciformes	Gobiidae	<i>Ctenogobius sagittula</i>	(Günther, 1862)	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Cyclopsetta panamensis</i>	(Steindachner, 1876)	Eastern Pacific
Perciformes	Sciaenidae	<i>Cynoscion parvipinnis</i>	Ayres, 1861	Eastern Pacific
Perciformes	Sciaenidae	<i>Cynoscion stollmanni</i>	(Steindachner, 1879)	Eastern Pacific
Perciformes	Sciaenidae	<i>Cynoscion xanthulus</i>	Jordan et Gilbert, 1882	Eastern Pacific
Perciformes	Dactyloscopidae	<i>Dactylagnus mundus</i>	Gill, 1863	Eastern Central Pacific
Perciformes	Gerreidae	<i>Diapterus brevirostris</i>	(Sauvage, 1879)	Eastern Pacific
Tetraodontiformes	Diodontidae	<i>Diodon holocanthus</i>	Linnaeus, 1758	Circumtropical distribution
Tetraodontiformes	Diodontidae	<i>Diodon hystrix</i>	Linnaeus, 1758	Circumtropical distribution
Perciformes	Serranidae	<i>Diplectrum pacificum</i>	Meek et Hildebrand, 1925	Eastern Pacific
Elopiformes	Elopidae	<i>Elops affinis</i>	Regan, 1909	Eastern Pacific
Perciformes	Serranidae	<i>Epinephelus analogus</i>	Gill, 1863	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Etropus crossotus</i>	Jordan et Gilbert, 1882	Eastern Pacific
Perciformes	Gerreidae	<i>Eucinostomus currani</i>	Zahuranec, 1980	Eastern Pacific
Perciformes	Gerreidae	<i>Gerres simillimus</i>	Regan, 1907	Eastern Pacific
Perciformes	Gerreidae	<i>Eucinostomus entomelas</i>	Zahuranec, 1980	Eastern Pacific
Perciformes	Gerreidae	<i>Eucinostomus gracilis</i>	(Gill, 1862)	Eastern Pacific
Perciformes	Gerreidae	<i>Eugerres axillaris</i>	(Günther, 1864)	Eastern Central Pacific
Perciformes	Gerreidae	<i>Eugerres lineatus</i>	(Humboldt, 1821)	Eastern Pacific
Perciformes	Labrisomidae	<i>Exerpes asper</i>	(Jenkins et Evermann, 1889)	Eastern Pacific
Syngnathiformes	Fistulariidae	<i>Fistularia commersonii</i>	Rüppell, 1838	Circumtropical distribution
Perciformes	Gerreidae	<i>Gerres cinereus</i>	(Walbaum, 1792)	American distribution
Perciformes	Haemulidae	<i>Haemulopsis elongatus</i>	(Steindachner, 1879)	Eastern Pacific
Perciformes	Haemulidae	<i>Haemulon sexfasciatum</i>	Gill, 1862	Eastern Central Pacific
Perciformes	Haemulidae	<i>Haemulon steindachneri</i>	(Jordan et Gilbert, 1882)	Eastern Pacific
Perciformes	Haemulidae	<i>Haemulopsis leuciscus</i>	(Günther, 1864)	Eastern Pacific

Appendix 1 continues on next page.

Appendix 1. cont.

Class	Family	Species	Author	Distribution
Perciformes	Haemulidae	<i>Haemulopsis nitidus</i>	(Steindachner, 1869)	Eastern Pacific
Clupeiformes	Clupeidae	<i>Harengula thrissina</i>	(Jordan et Gilbert, 1882)	Eastern Pacific
Perciformes	Carangidae	<i>Hemicaranx zelotes</i>	Gilbert, 1898	Eastern Central Pacific
Perciformes	Lutjanidae	<i>Hoplopagrus guentherii</i>	Gill, 1862	Eastern Pacific
Beloniformes	Hemiramphidae	<i>Hyporhamphus naos</i>	Banford et Collette, 2001	Eastern Pacific
Perciformes	Blenniidae	<i>Hypsoblennius gentilis</i>	(Girard, 1854)	Eastern Central Pacific
Pleuronectiformes	Pleuronectidae	<i>Hypsopsetta guttulata</i>	(Girard, 1856)	Eastern Pacific
Perciformes	Lutjanidae	<i>Lutjanus aratus</i>	(Günther, 1864)	Eastern Pacific
Perciformes	Lutjanidae	<i>Lutjanus argentiventris</i>	(Peters, 1869)	Eastern Pacific
Perciformes	Lutjanidae	<i>Lutjanus colorado</i>	Jordan et Gilbert, 1882	Eastern Pacific
Perciformes	Lutjanidae	<i>Lutjanus novemfasciatus</i>	Gill, 1862	Eastern Pacific
Perciformes	Sciaenidae	<i>Menticirrhus undulatus</i>	(Girard, 1854)	Eastern Pacific
Perciformes	Haemulidae	<i>Microlepidotus inornatus</i>	Gill, 1862	Eastern Central Pacific
Perciformes	Sciaenidae	<i>Micropogonias ectenes</i>	(Jordan et Gilbert, 1882)	Eastern Central Pacific
Mugiliformes	Mugilidae	<i>Mugil cephalus</i>	Linnaeus, 1758	Circumtropical distribution
Mugiliformes	Mugilidae	<i>Mugil curema</i>	Valenciennes, 1836	American distribution
Siluriformes	Ariidae	<i>Occidentarius platypogon</i>	(Günther, 1864)	Eastern Pacific
Perciformes	Carangidae	<i>Oligoplites altus</i>	(Günther, 1868)	Eastern Pacific
Perciformes	Carangidae	<i>Oligoplites saurus</i>	(Bloch et Schneider, 1801)	American distribution
Clupeiformes	Clupeidae	<i>Opisthonema libertate</i>	(Günther, 1867)	Eastern Pacific
Perciformes	Haemulidae	<i>Orthopristis reddingi</i>	Jordan et Richardson, 1895	Eastern Central Pacific
Perciformes	Labrisomidae	<i>Paraclinus mexicanus</i>	(Gilbert, 1904)	Punta Concepción BCS
Perciformes	Labrisomidae	<i>Paraclinus sini</i>	Hubbs, 1952	Eastern Central Pacific
Perciformes	Serranidae	<i>Paralabrax maculatofasciatus</i>	(Steindachner, 1868)	Eastern Central Pacific
Perciformes	Serranidae	<i>Paralabrax nebulifer</i>	(Girard, 1854)	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Paralichthys californicus</i>	(Ayres, 1859)	Eastern Pacific and Gulf of California
Pleuronectiformes	Paralichthyidae	<i>Paralichthys woolmani</i>	Jordan et Williams, 1897	Eastern Pacific
Pleuronectiformes	Pleuronectidae	<i>Pleuronichthys ritteri</i>	Starks et Morris, 1907	Eastern Pacific
Perciformes	Haemulidae	<i>Pomadasyx branickii</i>	(Steindachner, 1879)	Eastern Pacific
Perciformes	Haemulidae	<i>Pomadasyx panamensis</i>	(Steindachner, 1876)	Eastern Pacific
Scorpaeniformes	Triglidae	<i>Prionotus ruscarius</i>	Gilbert et Starks, 1904	Gulf of California and Magdalena Bay
Perciformes	Mullidae	<i>Pseudupeneus grandisquamis</i>	(Gill, 1863)	Eastern Pacific
Perciformes	Gobiidae	<i>Quietula y-cauda</i>	(Jenkins et Evermann, 1889)	Eastern Pacific and Gulf of California
Perciformes	Haemulidae	<i>Rhonciscus bayanus</i>	(Jordan et Evermann, 1898)	Eastern Pacific
Clupeiformes	Clupeidae	<i>Sardinops sagax</i>	(Jenyns, 1842)	Circumtropical distribution
Perciformes	Scaridae	<i>Scarus perrico</i>	Jordan et Gilbert, 1882	Eastern Pacific
Scorpaeniformes	Scorpaenidae	<i>Scorpaena russula</i>	Jordan et Bollman, 1890	Eastern Pacific
Tetraodontiformes	Tetraodontidae	<i>Sphoeroides annulatus</i>	(Jenyns, 1842)	Eastern Pacific
Tetraodontiformes	Tetraodontidae	<i>Sphoeroides lobatus</i>	(Steindachner, 1870)	Eastern Pacific
Pleuronectiformes	Paralichthyidae	<i>Syacium ovale</i>	(Günther, 1864)	Eastern Pacific
Pleuronectiformes	Cynoglossidae	<i>Symphurus atramentatus</i>	Jordan et Bollman, 1890	Gulf of California and Magdalena Bay
Pleuronectiformes	Cynoglossidae	<i>Symphurus fasciolaris</i>	Gilbert, 1892	Gulf of California and Magdalena Bay
Aulopiformes	Synodontidae	<i>Synodus lucioceps</i>	(Ayres, 1855)	Eastern Pacific
Perciformes	Haemulidae	<i>Haemulon californiensis</i>	(Steindachner, 1876)	Eastern Pacific

Appendix 2. Presence of the species in Bahía Almejas.

Species	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
<i>A. mazatlanus</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>B. polylepis</i>	1	1	1	1	0	0	1	0	0	1	1	1
<i>B. constellatus</i>	0	1	1	1	0	1	0	0	1	1	1	0
<i>B. californiensis</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>C. brachysomus</i>	1	1	0	0	0	0	0	0	0	1	1	1
<i>C. caninus</i>	1	0	0	0	0	0	0	0	0	0	0	1
<i>C. medius</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>C. zonatus</i>	1	0	0	1	0	0	0	0	0	0	1	0
<i>C. humeralis</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>C. panamensis</i>	1	0	0	1	1	0	0	0	0	0	1	1
<i>C. parvipinnis</i>	0	0	0	0	0	0	0	0	0	0	1	1
<i>D. mundus</i>	0	0	0	0	0	0	0	1	0	1	1	0
<i>D. brevirostris</i>	1	1	0	0	0	1	0	0	0	1	1	1
<i>D. holocanthus</i>	0	0	0	1	0	0	1	0	0	0	1	0
<i>D. hystrix</i>	1	0	0	1	0	0	0	0	1	0	0	0
<i>D. pacificum</i>	1	0	0	0	0	0	0	0	0	0	1	0
<i>E. analogus</i>	0	0	1	0	0	0	0	0	0	0	1	1
<i>E. crossotus</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. dowii</i>	0	1	1	1	1	1	1	0	0	1	1	1

Appendix 2 continues on next page.

Appendix 2. cont.

Species	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
<i>E. gracilis</i>	1	1	0	0	0	0	0	1	1	1	1	1
<i>E. axillaris</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>F. commersonii</i>	0	0	0	0	0	0	0	1	0	0	0	0
<i>G. cinereus</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>H. steindachneri</i>	0	0	0	0	0	0	1	0	1	1	0	0
<i>H. leuciscus</i>	1	0	0	0	1	1	1	0	0	1	0	1
<i>H. nitidus</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>H. guentherii</i>	0	0	0	0	0	0	0	1	0	1	0	1
<i>H. gentilis</i>	0	0	0	0	0	0	1	1	1	0	1	0
<i>H. guttulata</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>L. aratus</i>	0	0	0	0	0	1	0	0	1	1	0	0
<i>L. argentiventris</i>	1	1	0	0	0	1	0	1	0	1	1	1
<i>L. colorado</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>L. novemfasciatus</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>M. undulatus</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>M. inornatus</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>M. ectenes</i>	0	0	0	1	0	0	1	0	0	0	0	0
<i>O. reddingi</i>	1	1	0	1	0	0	0	0	0	1	1	1
<i>O. platypogon</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>P. maculatofasciatus</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>P. nebulifer</i>	0	1	0	0	0	0	0	0	0	0	0	0
<i>P. californicus</i>	1	1	1	1	1	1	1	1	1	0	1	1
<i>P. woolmani</i>	0	0	1	0	0	0	0	1	1	0	1	0
<i>P. ritteri</i>	0	0	0	1	0	1	0	0	0	0	0	0
<i>P. panamensis</i>	1	0	0	0	0	0	0	0	0	1	0	0
<i>P. ruscarius</i>	0	1	0	1	1	1	1	0	0	0	1	1
<i>P. grandisquamis</i>	1	1	0	1	1	0	1	0	0	0	0	1
<i>R. bayanus</i>	0	1	0	0	0	0	0	0	0	1	1	0
<i>S. perrico</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>S. russula</i>	1	0	0	0	1	0	0	0	0	1	0	0
<i>S. annulatus</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>S. ovale</i>	0	1	0	1	0	0	0	0	0	0	1	0
<i>S. atramentatus</i>	0	0	1	1	1	1	1	1	1	0	1	0
<i>S. fasciolaris</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>S. lucioceps</i>	1	1	1	1	1	1	1	1	0	1	0	1

Appendix 3. Presence of the species in Bahía de La Paz.

Species	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
<i>A. mazatlanus</i>	0	0	1	0	0	0	0	1	0	0	1	0
<i>A. thazard</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>C. zonatus</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>C. gilberti</i>	0	0	0	1	0	0	0	0	0	0	0	0
<i>C. platophrys</i>	0	0	0	0	0	0	0	0	0	0	1	1
<i>C. mangicola</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>C. sagittula</i>	0	0	1	1	1	1	0	0	0	1	1	1
<i>C. stolzmanni</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>C. xanthulus</i>	0	0	0	0	0	0	0	0	0	0	1	1
<i>D. brevirostris</i>	1	1	0	1	1	1	1	1	1	1	1	1
<i>D. hystrix</i>	1	0	0	1	1	0	1	0	1	1	1	0
<i>E. affinis</i>	0	0	1	0	0	0	0	0	0	0	0	0
<i>E. crossotus</i>	0	0	0	0	1	1	0	0	1	1	1	1
<i>E. currani</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. dowii</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. entomelas</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>E. gracilis</i>	0	0	0	0	1	0	1	1	0	0	0	1
<i>E. lineatus</i>	0	0	0	0	0	0	1	0	0	1	0	0
<i>E. asper</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>F. commersonii</i>	0	0	0	0	0	1	0	1	0	0	0	0
<i>G. cinereus</i>	0	0	0	0	0	0	0	0	0	1	0	1
<i>H. sexfasciatum</i>	1	0	0	0	0	0	0	0	0	0	0	0
<i>H. steindachneri</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>H. elongatus</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>H. leuciscus</i>	0	0	0	0	0	0	1	0	1	0	0	0
<i>H. thrissina</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>H. zelotes</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>H. guentherii</i>	0	0	0	0	1	0	0	0	0	0	1	0
<i>H. naos</i>	0	0	1	0	0	1	1	1	1	1	1	1
<i>H. gentilis</i>	0	0	0	0	1	0	0	0	0	0	0	0

Appendix 3 continues on next page.

Appendix 3. cont.

Species	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov
<i>L. aratus</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>L. argentiventris</i>	0	0	0	0	1	0	1	1	1	1	1	1
<i>L. colorado</i>	0	0	0	0	0	0	0	0	1	0	1	0
<i>L. novemfasciatus</i>	0	0	0	0	1	0	0	0	0	1	0	1
<i>M. cephalus</i>	0	0	0	0	0	0	0	0	1	0	0	0
<i>M. curema</i>	1	1	1	1	1	1	1	1	1	1	1	1
<i>O. altus</i>	0	0	0	0	0	0	0	0	0	1	0	1
<i>O. saurus</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>O. libertate</i>	0	0	0	0	0	0	1	0	0	0	0	0
<i>O. reddingi</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>P. mexicanus</i>	0	0	0	0	1	1	0	0	0	0	0	0
<i>P. sini</i>	0	0	0	0	0	0	0	0	0	0	1	0
<i>P. maculatofasciatus</i>	1	1	1	1	1	1	1	0	1	1	1	1
<i>P. branickii</i>	0	0	0	0	0	0	0	0	0	0	0	1
<i>P. macracanthus</i>	0	0	0	0	0	1	0	0	0	1	0	0
<i>R. bayanus</i>	0	0	0	1	0	1	1	0	0	1	1	0
<i>Q. ycauda</i>	0	0	0	1	1	1	0	0	0	0	0	0
<i>S. sagax</i>	0	0	0	0	0	0	0	0	0	1	0	0
<i>S. annulatus</i>	1	0	1	1	1	1	0	0	0	1	0	1
<i>S. lobatus</i>	0	0	0	0	0	0	0	0	0	0	0	1

Appendix 4. Values of the different functional indexes in the Baja California Peninsula and between the two bays (Bahía de la Paz and Bahía Almejas); bold numbers represent higher values and different trends between sites.

	Peninsula				Magdalena Transition				Cortezian			
	FRic	FDiv	FEve	FOri	FRic	FDiv	FEve	FOri	FRic	FDiv	FEve	FOri
Jan	0.0011	0.7755	0.7345	0.0982	0.0007	0.7455	0.8203	0.0990	0.0088	—	—	0.1416
Feb	0.0007	0.7082	0.5709	0.1030	0.00001	0.7945	0.8872	0.1231	0.0140	—	—	0.1807
Mar	0.0087	0.8789	0.6951	0.1082	0.0040	0.7856	0.8844	0.1159	0.0059	0.000001	0.7660	0.1574
Apr	0.0019	0.7989	0.5890	0.1055	0.00004	0.7533	0.8488	0.1116	0.0078	0.0004	0.7559	0.1617
May	0.0038	0.7776	0.7178	0.1044	0.00004	0.7440	0.8121	0.1068	0.0014	0.0001	0.7402	0.1813
Jun	0.0207	0.8030	0.6197	0.1104	0.0027	0.7662	0.8567	0.1091	0.0025	0.0001	0.7477	0.1497
Jul	0.0059	0.7804	0.7153	0.1095	0.0006	0.7463	0.8014	0.1231	0.0124	—	—	0.1928
Aug	0.0033	0.8320	0.6905	0.1072	0.00003	0.7756	0.8241	0.1113	0.0022	0.00001	0.7393	0.1674
Sep	0.0321	0.7787	0.5059	0.1063	0.0006	0.6992	0.7485	0.0848	0.0042	0.0024	0.7573	0.1630
Oct	0.1136	0.7653	0.4876	0.1007	0.0573	0.7733	0.8580	0.1086	0.0034	0.0004	0.7330	0.1709
Nov	0.0096	0.7926	0.6739	0.0964	0.0007	0.7358	0.8150	0.0908	0.0005	0.0054	0.7512	0.1530
Dec	0.0022	0.8026	0.6347	0.0963	0.0003	0.7423	0.8154	0.0873	0.0130	—	—	0.1487

Population dynamics of an emergent invasive fish, striped piggy, *Pomadasys stridens* (Actinopterygii, Perciformes, Haemulidae) in the Gulf of İskenderun, north-eastern Mediterranean

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Abstract

The striped piggy, *Pomadasys stridens* (Forsskal, 1775), is a recent invasive fish species in the eastern Mediterranean. Although its population dynamics was investigated in detail in its natural distribution area, the western Indian Ocean, an important data gap still exists in the Mediterranean. This study was carried out to determine the growth, mortality, and the length–weight and length–length relations of invasive striped piggy right after its establishment in the area. Samples were collected seasonally between April 2014 and December 2015 by using bottom trawl from 10 and 20 m depth contours off the north-western coast of the Gulf of İskenderun. After the length, weight, and sex of fish were recorded, sagittal otoliths were extracted and examined for age determination. Then length–length, length–weight, and length at age relations, growth performance, and mortality rates were calculated for both sexes and pooled data. In the study, a total of 1131 individuals were investigated, the total length of which ranged between 7.3 and 18.9 cm. The mean length was 12.32 ± 0.11 cm. There was no significant difference between the length–weight relation (LWR) parameters of sexes and pooled data. The overall LWR was found to be $TW = 0.0113 * TL^{3.096}$ in all specimens. The von Bertalanffy growth parameters were not significantly different between males and females. The model parameters for both sexes were $L_{\infty} = 22.01$ cm, $K = 0.22$ years⁻¹, $t_0 = -1.30$ years. The growth performance index (ϕ') was calculated as $\phi' = 2.03$. For combined sexes, the total, natural and fishery mortality rates were $Z = 1.14$ years⁻¹, $M = 0.66$ years⁻¹, and $F = 0.48$ years⁻¹, respectively. The exploitation rate of the stock was found to be $E = 0.42$. The growth performance of the invasive striped piggy population was determined to be similar to the ones reported from the Gulf of Suez where was the closest natural distribution area of the species. Although striped piggy is proper for human consumption and commercially caught in its native range, its fishery has not yet been established in the Eastern Mediterranean and we found that there was insufficient fishery pressure on the invasive population.

Keywords

Exploitation, growth, Lessepsian fish, Levant Basin, mortality

Introduction

Striped piggy, *Pomadasys stridens* (Forsskal, 1775), naturally distributed in the western Indian Ocean extending from the Pakistani coasts to the east African coasts including the Red Sea (Froese and Pauly 2018), the Gulf of Suez, and the Suez Canal (El-Azim et al. 2017). It was introduced to the Mediterranean via the Suez Canal. According to Bodilis et al. (2013), its first occurrence in the Eastern Mediterranean was reported from Port-Said, Egypt. After a while, its first substantiated record was documented by Ben-Tuvia (1976), from the Israeli and by Mouneimne (1977) from Lebanese coasts. In Turkey, its occurrence was first reported by Bilecenoğlu et al. (2009) from Yumurtalık coast of the Gulf of Iskenderun and it spread westward quickly (Ergüden et al. 2010; Akyol and Çoker 2018). After 2013, striped piggy population significantly increased in a short while invading the coastal soft bottom habitats of the Gulf of Iskenderun and it became one of the most dominant species (Mavruk et al. 2017; Ozyurt et al. 2018).

The Gulf of Iskenderun is an important fishery ground (Gücü and Bingel 1994) along with being an invasion hotspot in the eastern Mediterranean. Lessepsian fishes unquestionably dominate the gulf in where their rate frequently exceeds 99% of total fish abundance and 95% of total fish biomass in the shallow soft bottoms (Mavruk et al. 2017). This is because the prevailing environmental condition of the gulf is quite similar to the Gulf of Suez (Avşar 1999). The Gulf of Iskenderun is characteristic with high temperature, salinity, and primary production as well as shallow bathymetry and sandy-muddy bottom structure, which are well pre-adapted by Lessepsian fishes (Golani 2010). As a consequence, Lessepsian fishes form an important part of the commercial fishery (Yemisken et al. 2014). Although striped piggy has economic importance in its native range (Osman et al. 2019; El-Azim et al. 2017), it is discarded in the Turkish fishery (unpublished data).

Striped piggy is a small sized demersal fish species inhabiting in shallow waters with sandy and muddy bottom structure feeding on crustaceans and small fishes (Froese and Pauly 2018). Its spawning period is in spring and summer in the Gulf of Iskenderun (Özbek 2017). Although the population dynamics of the striped piggy has been comprehensively studied in the Indo-Pacific region (Safi et al. 2013, 2014a; Osman et al. 2019; El-Azim et al. 2017), there is still insufficient information on its age, growth, mortality, and morphometry in the Eastern Mediterranean. Therefore, the aim of this study was to determine the population dynamical parameters in the Gulf of Iskenderun, five years after the establishment of the population. In this context, the von Bertalanffy growth parameters, mortality, length-weight and length-length relations of striped piggy, were calculated and growth performance of the Gulf of Iskenderun population was compared with those of the native range.

Materials and methods

Sample collection

Samplings were seasonally performed in April, July, October, and December 2014 and 2015 off Yumurtalık, Gulf of Iskenderun (Fig. 1) using commercial bottom trawl nets. Trawling transects were located at 10 and 20 m depth contours. Each tow lasted one hour using Mediterranean type trawl nets with 44 mm mesh size. The details of samplings were given in Mavruk et al. (2017). In the samplings, all striped piggy specimens were collected onboard and carried to the laboratory. When the sampling amount is high, a subsampling procedure proposed by Holden and Raitt (1974) was used.

Sample processing

In total, 1134 specimens were investigated in the context of the study. To determine the morphometric characteristics; total length (TL), fork length, standard length, body width, head width, body depth, head depth, and total weight were measured. Lengths and weights were measured to the nearest millimeter using a digital caliper, and to the nearest 0.01 g using a digital scale, respectively. The sex of specimens was determined by a visual examination when the gonads are ripe or with a stereomicroscope (Olympus SZ 60), otherwise.

The age of fish was determined by otolith investigations following the methods suggested by Holden and Raitt (1974). For this purpose, sagittal otoliths were removed from the fish, cleaned in alcohol to wipe off all tissue remnants, and kept in glycerin until examination. Age determination was performed under a stereomicroscope. The zones which form a complete circle around the otolith were considered as true annual rings whereas the rings which were not continuous around the otolith were excluded from the counting. This was a necessary step to correctly describe the seasonal increments, which is crucial so that a reliable length at age data can be provided and overestimation of growth coefficients can be prevented.

Statistical analyses and modelling

The sex-based changes of length frequency distribution were tested using Kolmogorov–Smirnov test. The season and sex-based variations of the mean TL were analyzed using two ways ANOVA. TL–length and TL–girth relations were fitted using ordinary linear regressions. TL–weight (W) relations were fitted using the allometric equation given by Ricker (1975);

$$W = a \cdot L^b$$

The von Bertalanffy (von Bertalanffy 1938) growth functions (VBGF) were fitted using a non-linear least

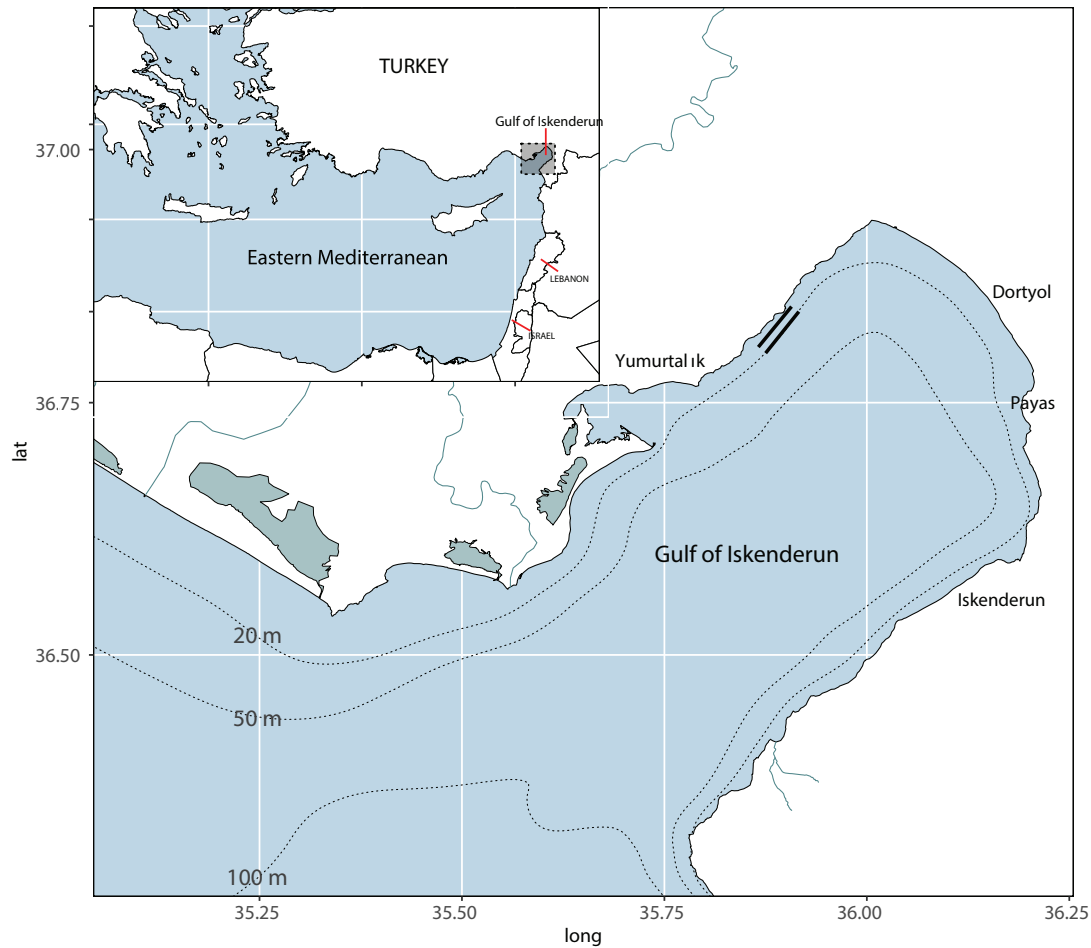


Figure 1. Sampling area and bottom trawl transects (10 m: 35.87°E, 36.82°N to 35.91°E, 36.86°N; 20 m: 35.89°E, 36.80°N to 35.93°E, 36.84°N).

square method with TropFishR package (Mildenberger et al. 2017). Then the lengths at age values were predicted using the parameters of VBGF. The 95% confidence intervals of predictions were calculated using R library propagate (Spiess 2018). VBGF was as follows

$$L_t = L_\infty \left(1 - e^{(-K(t-t_0))} \right)$$

where; L_t is the total length at age t , L_∞ is asymptotic length, K is growth coefficient and t_0 is theoretical age at zero length. Then the generation time was calculated using $\text{LN}[3]/K$ equation given by Froese and Pauly (2018).

In order to determine the growth performance, Pauly and Munro (1984)'s growth performance index (ϕ') was calculated using the following equation

$$\phi' = \text{Ln } K + 2 \cdot \text{Ln } L_\infty$$

The total mortality rate was calculated from the linearized catch curve method based on Ursin (1967). Natural mortality was calculated from the empirical equation given by Pauly (1980) using TropFishR library

$$\text{Ln } M = -0.0152 - 0.279 \cdot \text{Ln } L_\infty + 0.6543 \cdot \text{Ln } K + 0.463 \cdot \text{Ln } T$$

where T [°C] shows the mean annual water temperature of the study area which is assumed as 23°C. After the total and natural mortality were determined, fishing mortality was calculated from equation

$$Z = F + M$$

Then the exploitation level (E) of the stock was calculated using $E = F / Z$ equation given by Sparre and Venema (1992). All calculations were performed using R 4.0 Language and Environment for Statistical Computing (R Core Team 2020).

Results

In the study, a total of 1131 individuals were examined, 445 of which were males and 471 of which were females. In 215 specimens, sex could not be determined. The total length of females ranged between 7.8 and 18.9 cm, and of males between 9.1 and 18.6 cm. The overall mean length and weight values were 12.32 ± 0.11 (95% confidence interval) cm and 29.19 ± 0.85 g, respectively. The overall mean length and weight of females were found to be 12.79

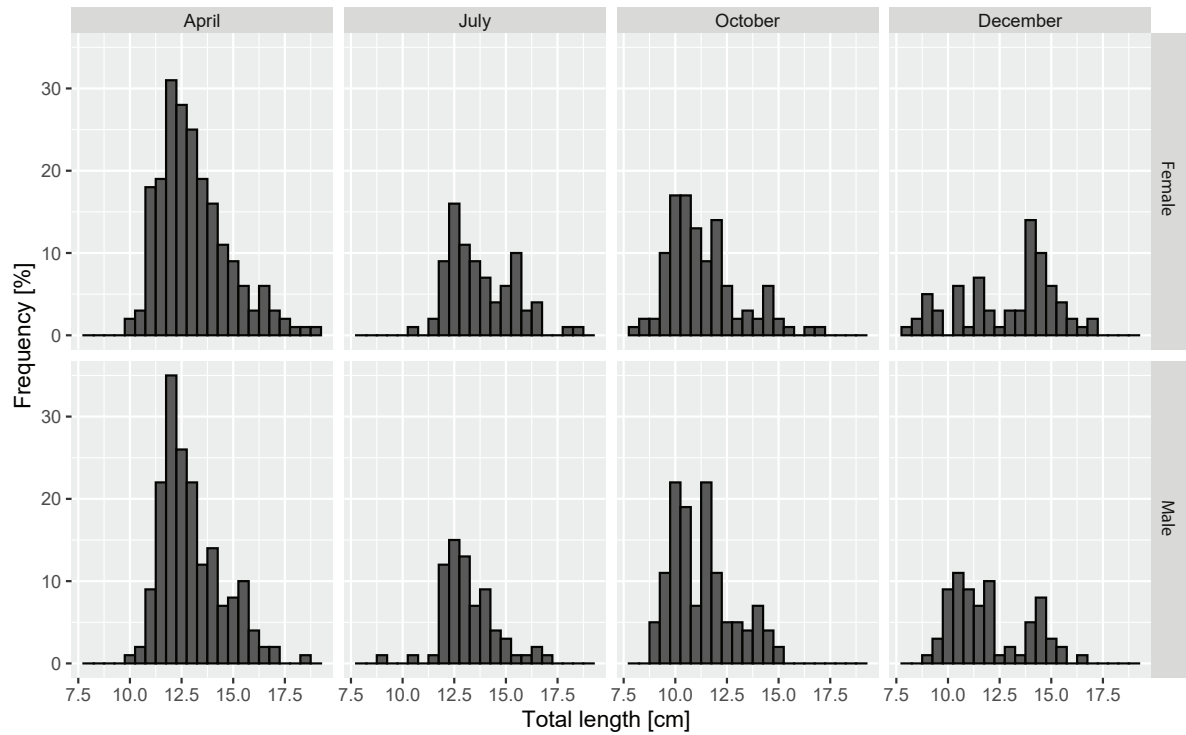


Figure 2. Length-frequency distributions of striped piggy, *Pomadasys stridens*, from the Gulf of Iskenderun, by sexes and seasons.

Table 1. Regression parameters of morphometric relations calculated for striped piggy, *Pomadasys stridens* from the Gulf of Iskenderun, by sexes and pooled data.

Total length–fork length						Total length–head width					
		Par.	Ste.	<i>t</i>	sig.			Par.	Ste.	<i>t</i>	sig.
Male	<i>a</i>	0.254	0.071	3.581	<0.001***	Male	<i>a</i>	0.172	0.067	2.551	0.011*
	<i>b</i>	0.921	0.006	162.513	<0.001***		<i>b</i>	0.835	0.005	154.901	<0.001***
	<i>r</i> ²	0.984					<i>r</i> ²	0.982			
Female	<i>a</i>	0.411	0.092	4.472	<0.001***	Female	<i>a</i>	−0.040	0.124	−0.320	0.749 ^{ns}
	<i>b</i>	0.909	0.007	128.037	<0.001***		<i>b</i>	0.852	0.010	89.239	<0.001***
	<i>r</i> ²	0.972					<i>r</i> ²	0.944			
Overall	<i>a</i>	0.267	0.056	4.801	<0.001***	Overall	<i>a</i>	0.065	0.060	1.083	0.279 ^{ns}
	<i>b</i>	0.920	0.004	206.002	<0.001***		<i>b</i>	0.844	0.005	174.770	<0.001***
	<i>r</i> ²	0.974					<i>r</i> ²	0.964			
Total length–standard length						Total length–girth at opercle					
		Par.	Ste.	<i>t</i>	sig.			Par.	Ste.	<i>t</i>	sig.
Male	<i>a</i>	−0.736	0.099	−7.451	<0.001***	Male	<i>a</i>	−0.397	0.085	−4.677	<0.001***
	<i>b</i>	0.286	0.008	35.365	<0.001***		<i>b</i>	0.199	0.007	28.720	<0.001***
	<i>r</i> ²	0.769					<i>r</i> ²	0.672			
Female	<i>a</i>	−0.601	0.084	−7.121	<0.001***	Female	<i>a</i>	−0.152	0.077	−1.972	0.049*
	<i>b</i>	0.275	0.007	40.890	<0.001***		<i>b</i>	0.180	0.006	29.257	<0.001***
	<i>r</i> ²	0.825					<i>r</i> ²	0.673			
Overall	<i>a</i>	−0.691	0.053	−13.138	<0.001***	Overall	<i>a</i>	−0.271	0.047	−5.799	<0.001***
	<i>b</i>	0.280	0.004	64.760	<0.001***		<i>b</i>	0.188	0.004	48.986	<0.001***
	<i>r</i> ²	0.816					<i>r</i> ²	0.706			
Total length–body width						Total length–maximum girth					
		Par.	Ste.	<i>t</i>	sig.			Par.	Ste.	<i>t</i>	sig.
Male	<i>a</i>	−1.434	0.169	−8.474	<0.001***	Male	<i>a</i>	−0.798	0.146	−5.450	<0.001***
	<i>b</i>	0.773	0.014	57.049	<0.001***		<i>b</i>	0.612	0.012	52.190	<0.001***
	<i>r</i> ²	0.881					<i>r</i> ²	0.861			
Female	<i>a</i>	−1.095	0.152	−7.185	<0.001***	Female	<i>a</i>	−0.612	0.140	−4.386	<0.001***
	<i>b</i>	0.745	0.012	63.270	<0.001***		<i>b</i>	0.600	0.011	55.597	<0.001***
	<i>r</i> ²	0.896					<i>r</i> ²	0.868			
Overall	<i>a</i>	−1.265	0.094	−13.508	<0.001***	Overall	<i>a</i>	−0.691	0.085	−8.150	<0.001***
	<i>b</i>	0.757	0.008	100.746	<0.001***		<i>b</i>	0.604	0.007	88.740	<0.001***
	<i>r</i> ²	0.900					<i>r</i> ²	0.875			

Par.: parameter, Ste.: standard error of parameter, ns: not significant.

± 0.18 cm and 32.85 ± 1.46 g, and of males were found to be 12.38 ± 0.16 and 29.18 ± 1.21 g. Length-frequency distributions of males and females were significantly different than each other ($D = 0.12$, $P < 0.01$). Females were slightly larger than males ($P < 0.01$). In addition, seasonal variations of the mean length were found to be significant ($P < 0.001$). Length frequency distributions by seasons and sexes are given in Fig. 2.

Linear relations between the TL and fork length, standard length, head and body width, as well as maximum girth and girth at opercle are given in Table 1. In general, determination coefficients TL–length and TL–width relations were higher than TL–girth regressions.

Table 2. Parameters of length–weight relations calculated for striped piggy, *Pomadasys stridens*, from the Gulf of Iskenderun, by sexes and pooled data.

Sex	N. of samples	a	b	r^2
Male	445	0.0116 ± 0.09	3.087 ± 0.053	0.967
Female	471	0.0123 ± 0.13	3.064 ± 0.069	0.941
Overall	1131	0.0113 ± 0.18	3.096 ± 0.038	0.958

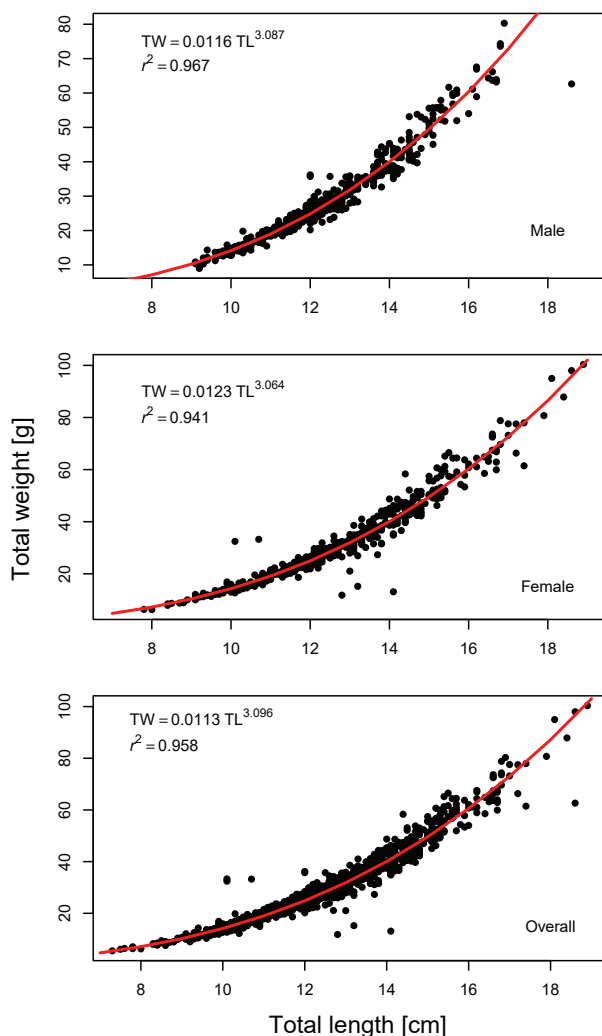


Figure 3. Length–weight relations by sexes and overall data in striped piggy, *Pomadasys stridens*, from the Gulf of Iskenderun.

The parameters of length–weight relations calculated for males, females and pooled data are given in Table 2 and Fig. 3. The b values of allometric equations revealed that males and pooled data showed a slightly positive allometric growth ($P < 0.05$); however, females showed isometric growth. There were not statistically significant differences among the intercept and slope parameters of regressions.

The maximum age determined for both sexes was found to be seven years. It was determined that the first age group was dominant for both sexes and pooled data constituting 62.5% of males and 52.7% of females. The von Bertalanffy growth parameters calculated for male, female, and pooled data were given in Table 3 and the length at age plots were given in Fig. 4. Based on 95% confidence intervals, no significant difference was found among L_{∞} , K , and t_0 values of sexes and pooled data. Using the growth coefficient for combined sexes ($K = 0.22 \text{ year}^{-1}$) the generation length of striped piggy was calculated as 5 years in the Gulf of Iskenderun. Growth performance index values were calculated as 2.01 for males and 2.03

Table 3. Von Bertalanffy growth parameters with confidence intervals (95%) and growth performance indices calculated for males, females and combined data in this study and previous studies.

Ref.	Location	Sex	L_{∞} [cm]	K [year ⁻¹]	t_0 [year]	θ'
ps	Mediterranean	M	20.99 (± 2.56)	0.24 (± 0.07)	-1.31 (± 0.46)	2.03
ps	Mediterranean	F	21.60 (± 2.04)	0.23 (± 0.06)	-1.25 (± 0.37)	2.01
ps	Mediterranean	C	22.01 (± 1.63)	0.22 (± 0.04)	-1.30 (± 0.25)	2.03
1	Mediterranean	M	17.96	0.34	-1.54	2.04
1	Mediterranean	F	32.70	0.10	-1.14	2.01
1	Mediterranean	C	22.52	0.19	-2.05	1.98
2	Suez Canal	C	16.64	0.51	-0.65	2.15
3	Suez Canal	C	23.15	0.51	-0.29	2.44
4	Gulf of Suez	C	20.37	0.28	-1.33	2.07
5	Gulf of Suez	C	20.60	0.19	-2.40	1.91
6	Persian Gulf	C	26.00	0.70	-0.65	2.68
7	Persian Gulf	C	24.54	0.14	-4.41	1.93

ps: presently reported study, 1: Uyan et al. (2018), 2: Al-Ganainy and Sabra (2008), 3: El-Azim et al. (2017), 4: El Sayed (1990) taken from Al-Ganainy and Sabra (2008), 5: Osman et al. (2019), 6: Hashemi and Taghavi-motlagh (2012), 7: Karimi et al. (2015), M: male, F: female, C: combined.

Table 4. Mean length values, lower (CIL), and upper (CIU) limits of 95% confidence intervals calculated from von Bertalanffy growth equation.

Age	Male			Female		
	CIL TL [cm]	Mean TL [cm]	CIU TL [cm]	CIL TL [cm]	Mean TL [cm]	CIU TL [cm]
I	8.50	8.75	8.92	8.39	8.82	9.08
II	11.18	11.38	11.48	11.14	11.48	11.64
III	13.26	13.49	13.60	13.21	13.58	13.76
IV	14.92	15.17	15.30	14.86	15.25	15.45
V	16.23	16.53	16.70	16.13	16.57	16.81
VI	17.23	17.61	17.87	17.09	17.62	17.95
VII	17.99	18.48	18.85	17.79	18.45	18.90

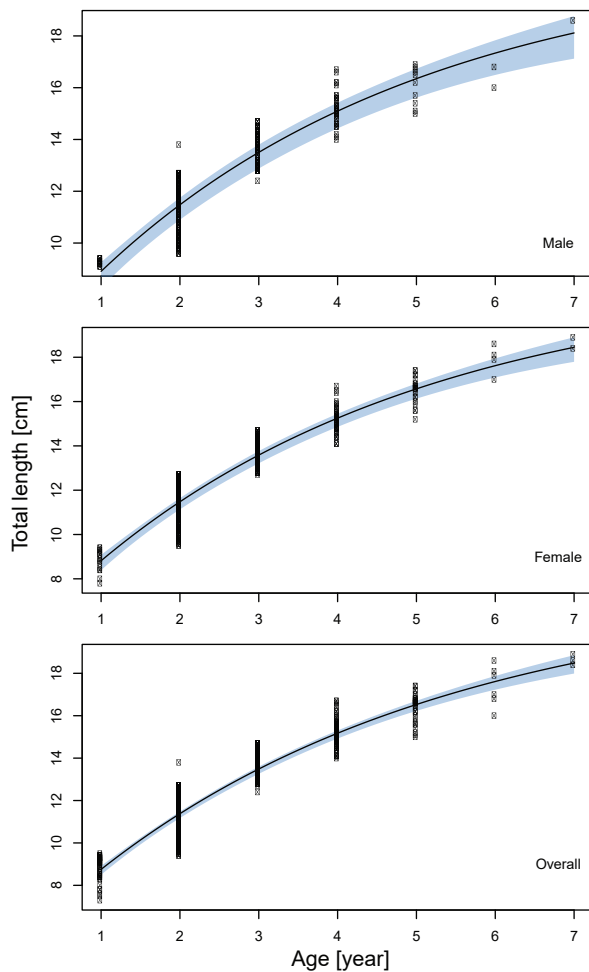


Figure 4. Observed and fitted length at age values and von Bertalanffy growth curves with 95% confidence intervals for both sexes and overall specimens of striped piggy, *Pomadasys stridens*, from the Gulf of Iskenderun.

Table 5. Total mortality (Z), natural mortality (M), fishing mortality (F), and exploitation rate (E).

	Z [year ⁻¹]	M [year ⁻¹]	F [year ⁻¹]	E
Male	1.19	0.71	0.48	0.40
Female	1.02	0.68	0.34	0.33
Overall	1.14	0.66	0.48	0.42

for females and all individuals. ϕ' values calculated using the growth parameters reported in the previous studies were between 1.91 (Gulf of Suez) and 2.44 (Suez Canal) (Table 3). There was no difference between the lengths at age values of sexes calculated from VBGF (Table 4).

The total mortality (Z), natural mortality (M), fishing mortality (F), and exploitation rate (E) calculated for each sex of the *P. stridens* are given in Table 5. The deaths caused by fisheries on both sex groups were less than those of natural causes. Therefore, the exploitation rates were calculated less than 0.5 showing the population was underexploited in the Gulf of Iskenderun (Table 5).

Discussion

Here, we investigated the population dynamical parameters of an invasive fish species striped piggy (*Pomadasys stridens*) soon after its establishment in the study area. In the context of the study, the mean length of investigated individuals was 12.32 ± 0.11 cm ranging from 7.3 to 18.9 cm. In previous studies, the maximum length was reported as 23.5 cm by Hashemi and Taghavimotlagh (2012), 19.3 cm by Wright (1989) in the Persian Gulf, 21.9 cm in Pakistan coasts (Safi et al. 2013 and 2014a, 2014b). In comparison with the studies performed in the natural distribution area; various parts of the western Indian Ocean, the maximum length values were apparently smaller in our study. In accordance with this, Edelist (2014) reported the maximum length of striped piggy as 14.5 cm from Israeli coasts and Ergüden et al. (2015) as 17.7 cm from the Gulf of Iskenderun in the Mediterranean where is out of the native range. Along with the differences among the ecological properties of different study areas, this is probably because of that the striped piggy do not have a long history in our study area. Although its first record in the Gulf of Iskenderun was given in 2009 (Bilecenoğlu et al. 2009), its population abundance dramatically increased after 2013 (Mavruk et al. 2017). The samples investigated in this study were collected in 2014 and 2015, right after striped piggy has established a dominant population in the area.

Contrary to the above-mentioned hypothesis, the studies performed in the Gulf of Suez and the Suez Canal reported maximum length values close to our study which were 19 cm (Osman et al. 2019) and 15.6 cm (El-Ganainy and Sabra 2008) even though they are in the native range of the species. Based on exploitation rates reported in these studies, striped piggy populations are overfished in the Suez Canal ($E = 0.64$; El-Ganainy and Sabra 2008 and $E = 0.69$; El-Azim et al. 2017) and in the Gulf of Suez ($E = 0.72$; Osman et al. 2019). In comparison, we determined that the population inhabit the Gulf of Iskenderun was clearly under fished with an exploitation rate of 0.42.

Although, the first specimens of the striped piggy were collected in 2009 in our study area (Bilecenoğlu et al. 2009), our oldest specimens were seven years old. Based on this, several individuals may be existing in the area at least one year before the first observation. On the other hand, age determination increasingly harder in older age groups since zone formation gradually tightens (Matta and Kimura 2012). Therefore, this may also be a methodological fault.

Although the observed the mean length of females was found to be larger than males, no statistically significant difference was observed between the von Bertalanffy growth parameters and length at age values of sexes. Therefore, the presence of larger females in the sampling area may indicate differences between the distribution patterns of sexes. This should be investigated in further studies.

In the context of our study, b values of length–weight relation were calculated between 3.064 and 3.096. A slightly positive allometric growth profile was observed

for males and pooled data; however, isometric growth was detected in females of the striped piggy population in the Gulf of Iskenderun. In general, negative allometry is prevalent for striped piggy in the studies performed in both native and introduced range of the species. From the northern Persian Gulf, Safi et al. (2013) and (2014a) reported b values between 2.73 and 2.86. Within the introduced range, in the Gulf of Iskenderun, Özbek (2017) reported isometric growth for males and pooled data, however, females revealed negative allometric growth. From the same area, Uyan et al. (2018) also reported isometric growth, although their b values were far below 3 ranging between 2.03 and 2.84. In accordance with our study, Edelist (2014) reported positive allometric growth from the Israeli coast of the Mediterranean ($b = 3.07$). Additionally, Ergüden et al. (2015) also reported a remarkably positive allometric growth ($b = 3.406$) from the Gulf of Iskenderun. Although the differences between the Indian Ocean and the Mediterranean can be explained with the different ecological properties of study areas, the studies performed in the Mediterranean, even in the same gulf, revealed different growth profiles for the same striped piggy population. In addition, these differences cannot be attributed to the different sampling methodologies since all of the studies performed in the Mediterranean used bottom trawls to collect the striped piggy samples. Possible explanations of this discrepancy may be the different sampling periods or differences in the sample processing procedures of the studies.

Although the asymptotic length (L_{∞}) is a theoretical size of the infinitely old fish, its value is influenced by the number of large sized fishes in the sample. Therefore, it does not represent a reliable measure of the maximum growth potential of an average fish in a population if the population is not sampled representatively, including all available size classes. Moreover, its value directly influences the growth parameter (K), e.g., an overestimated L_{∞} value causes underestimation of K . Accordingly, the asymptotic length (L_{∞}), growth coefficient (K), and theoretical age at zero length (t_0) of striped piggy varied in a wide range in the both native and introduced distribution area. For example, in the Suez Canal, El-Ganainy and Sabra (2008) reported L_{∞} value as 16.64 cm and t_0 value as -0.653 year^{-1} , whereas El-Azim et al. (2017) reported L_{∞} value as 23.15 cm and t_0 value as -0.29 year^{-1} in the same area. The same discrepancy also exists in the Gulf of Iskenderun. Uyan et al. (2018) reported that the L_{∞} was 32.7 cm and K was 0.096 year^{-1} in females of the striped piggy population in the Gulf of Iskenderun. These values look quite unrealistic and significantly different than that calculated in our study, in where L_{∞} was 21.6 cm and K was 0.23 year^{-1} in females. Although females' parameters were statistically significantly different from males, the parameters calculated for males and pooled data were closer in this study and Uyan et al. (2018).

In spite of the above-mentioned methodological obstacles in making direct comparisons among the growth parameters, the growth determined in different studies can

be compared using the growth performance index (Φ') of Pauly and Munro (1984). However, this index is apparently calculated using different logarithmic bases in the previous studies. In addition, only a few studies reported Φ' values. Therefore, we recalculated Φ' values for different striped piggy populations employing the same base with this study and using L_{∞} and K values reported in the previous studies. Growth performance values calculated in this study and Uyan et al. (2018) were in a narrow range between 2.01 and 2.03 in accordance with each other. Since the both studies performed in the same area, the Gulf of Iskenderun, this was an expected outcome. Moreover, the growth performance values calculated from the Gulf of Suez were also close to the values given in our study. From the growth parameters given by El-Sayed (1990), Φ' value was calculated as 2.07 and by Osman et al. (2019), Φ' value was calculated as 1.91. This conformity may be explained with the similar ecological conditions, particularly temperature of the Gulf of Iskenderun and the Gulf of Suez (Ben-Tuvia 1966, Avşar 1999). The Gulf of Iskenderun is believed to be providing an ultimate habitat for Lessepsian fish species because of the mentioned ecological similarity (Mavruk and Avşar 2008). In addition, the Gulf of Suez is the last point before the Suez Canal, the biota of which constitutes the source for founder populations of Lessepsian organisms. Therefore, the populations that inhabit the Gulf of Suez can be expected to be more similar to Lessepsian ones genetically, however, this hypothesis should be investigated with further studies.

The striped piggy populations inhabit the Suez Canal seem to have better growth performance with 2.15 (El-Ganainy and Sabra 2008) and 2.44 (El-Azim et al. 2017) Φ' values than in the Mediterranean and the Gulf of Suez. As mentioned above, the populations are overfished here, and they are smaller in size. Therefore, the higher growth rates can be attributed to the younger population structure along with specific environmental conditions in the canal.

The establishment and dispersal of an invader is a complicated process depending on a lot of intrinsic and extrinsic factors from the biological traits of the species to climate change (Bianchi and Morri 2003; Arndt and Schembri 2015). Koutsidi et al. (2020) showed that the opportunist life history strategy can be associated with high establishment success in Lessepsian fishes. According to our results, striped piggy can be considered an opportunistic species with a short generation time, small body size, and fast growth.

Conclusions

The striped piggy is an invasive species, the first observation of which has been reported in 2009 in the study area (Bilecenoğlu et al. 2009). After its first record, its population remarkably increased spreading throughout the Mediterranean coasts of Turkey within a few years (Mavruk et al. 2017; de Meo et al. 2018). Recently, it

constitutes one of the most dominant fish in the shallow coastal waters (Ozyurt et al. 2018) completely invading the soft bottoms. This study assessed the growth patterns of a founder population in the Gulf of Iskenderun, the northeastern Mediterranean. In addition, no other study has been found that investigated the population dynamics of striped piggy in other places of the Mediterranean. Therefore, the results presented here will constitute an important baseline for future studies.

Although striped piggy is suitable for human consumption and has economic importance in its native range (Osman et al. 2019), it is discarded in the Turkish fishery. Consequently, we found that the fishery pressure

on striped piggy is insufficient in the study area. Therefore, creating a market for striped piggy can increase economic importance and this may prevent further invasion and spread as well as reduce fishery pressure on the native species, the majority of which are already overfished (Demirel et al. 2020).

Acknowledgments

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Distributional range extension of a rare scorpionfish, *Hipposcorpaena filamentosa* (Actinopterygii, Scorpaeniformes, Scorpaenidae)

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Abstract

The distributional range of *Hipposcorpaena filamentosa* Fowler, 1938, previously recorded only from the Philippines, Indonesia, and Papua New Guinea is extended to include South Africa and Australia, on the basis of two specimens (17.2–29.5 mm standard length) which are described in detail. In addition, the first underwater photograph of *H. filamentosa*, taken at Kashiwa-jima Island, Kochi, Japan, is included. The species is apparently widely distributed in the Indo-West Pacific.

Keywords

Australia, description, Japan, morphology, new records, South Africa

Introduction

The monotypic genus *Hipposcorpaena* (Scorpaenidae), represented by *Hipposcorpaena filamentosa* Fowler, 1938, was originally described based on a single specimen collected from the Philippines (Fowler 1938). Although the status of the genus has been questioned, with its relation to *Rhinopias* Gill, 1905 (Eschmeyer et al. 1973; Poss 1999), a review of *Hipposcorpaena* based on the holotype and two non-type specimens of *H. filamentosa* by Motomura and Senou (2005) reassured its validity. Since then, no additional specimens have been reported. However, two small scorpionfish specimens from the southwestern Indian Ocean and north-western Australia, found recently by HM in the collections of the South African Institute for Aquatic Biodiversity, Grahamstown (SAIAB) and Museums Victoria, Mel-

bourne (NMV), respectively, are herein identified as *H. filamentosa*, as is an underwater photograph of a scorpionfish from Kashiwa-jima Island, Kochi, Japan. The specimens are described in detail and distributional records of *H. filamentosa*, previously known only from the type locality, Indonesia and Papua New Guinea, are reviewed.

Methods

Counts and measurements followed Motomura and Senou (2005). The last two soft rays of the dorsal and anal fins were counted as single rays, each pair being associated with a single pterygiophore. Standard length is abbreviated as SL. The terminology of the head spines follows Randall and Eschmeyer (2001: fig. 1).

Results

Family Scorpaenidae Risso, 1827

Hipposcorpaena Fowler, 1938

Hipposcorpaena filamentosa Fowler, 1938

[Standard English name (Australia): Filamentous Scorpionfish; new standard Japanese name: Itohiki-kasago]

Figs 1–3; Table 1

Material examined. 2 specimens. NMV A 29729-041, 29.5 mm SL, Ashmore Islands, Western Australia, 12°26'42"–58"S, 123°36'03"–35"E, 95 m, beam trawl, RV *Southern Surveyor*, 7 July 2007; SAIAB 57321, 17.2 mm SL, Aliwal Shoal off Scottburgh, KwaZulu-Natal, South Africa, 30°18'S, 30°48'E, 34–36 m, A. Bentley et al., 9 February 1998.

Description. Meristics and morphometrics of the specimens are shown in Table 1. Dorsal fin with 11 or 12 spines and 9 or 10 soft rays; all rays, except posteriormost, divided into 2 at base, unbranched; 1st spine length less than half that of 2nd spine; 2nd or 3rd spine longest, length much greater than upper-jaw length; 2nd to 10th (3rd to 10th in small specimen) spines progressively shorter; interspinous membranes of adjacent dorsal-fin spines reaching tips of posterior spines, except 2nd, 11th, and 12th (2nd and 11th in small specimen) spines; 5th ray longest (broken in small specimen); 5th to last soft rays progressively shortening; intersoft-rayed membrane reaching tips of posterior rays; posterior branch of last soft ray joined by membrane to caudal peduncle for less than half its length. Anal fin with 2 spines and 6 soft rays; all rays, except posteriormost, divided into 2 at base, unbranched; 1st spine 2.6 in 2nd spine; 1st to 4th soft rays progressively lengthening, 4th longer than longest dorsal-fin soft ray; 4th to 6th soft rays progressively shortening; posterior branch of last soft ray joined by membrane to caudal peduncle for less than one-sixth its length; membranes between rays weakly notched. Pectoral fin with 14 rays, all rays unbranched; 2nd to 7th rays progressively shortening (about equal length in small specimen); 9th ray longest, length slightly greater than head length; 9th to lowermost rays progressively shortening; membranes between 2nd and 7th rays reaching tip of each ray; membranes between 8th and lowermost rays strongly notched, membranes between 9th and lowermost rays extending to one-third to half (half to two-thirds in small specimen) length of each upper adjacent ray; lower 7 rays filamentous. Pelvic fin with 1 spine and 5 soft rays, 1st to 3rd soft rays branched, remaining rays unbranched; 2nd soft ray longest, longer than upper jaw length. Caudal fin with 14 rays, all unbranched; 8th (counted from above) ray longest, slightly greater (shorter in small specimen) than body depth.

Longitudinal scale rows 53 (not obvious in small specimen); pored lateral-line scales 22 (counted from right side in large specimen); scale rows between origin of last dorsal-fin spine and lateral line 8 (not obvious in small

Table 1. Morphometrics (expressed as percentages of standard length) of *Hipposcorpaena filamentosa*.

	This study		Motomura and Senou (2005)	
	South Africa	Australia	Philippines	
	SAIAB	NMV A	KPM-NI	USNM
	57321	29729-041	13005	168183
Standard length [mm]	17.2	29.5	35.2	31.1
Body depth	40.6	39.7	39.5	41.2
Body width	9.0	11.5	16.8	13.5
Head length	46.0	49.5	48.0	46.9
Snout length	20.0	23.9	24.1	21.2
Orbit diameter	9.2	9.8	9.9	10.9
Interorbital width ^a	7.5	7.0	6.3	6.4
Interorbital width ^b	9.3	9.9	9.1	8.7
Upper-jaw length	18.0	18.5	19.0	18.0
Postorbital length	19.1	16.4	17.0	17.4
Pre-dorsal-fin length	41.0	43.7	41.5	39.9
Pre-anal-fin length	67.0	70.4	71.6	68.8
Pre-pelvic-fin length	43.5	38.7	40.3	38.6
1 st dorsal-fin spine length	13.7	12.9	11.4	—
2 nd dorsal-fin spine length	33.8	26.7	22.7	—
3 rd dorsal-fin spine length	32.7	27.6	21.9	—
4 th dorsal-fin spine length	29.3	23.9	18.5	22.5
5 th dorsal-fin spine length	27.0	19.9	15.1	19.6
6 th dorsal-fin spine length	22.5	17.1	13.6	—
7 th dorsal-fin spine length	20.2	15.4	12.8	—
8 th dorsal-fin spine length	16.8	12.9	11.1	12.9
9 th dorsal-fin spine length	10.6	9.7	8.5	9.3
10 th dorsal-fin spine length	7.8	4.9	5.4	6.1
11 th dorsal-fin spine length	17.5	5.6	5.1	5.5
12 th dorsal-fin spine length	absent	15.6	13.9	14.1
1 st dorsal-fin soft ray length	17.8	18.1	16.2	—
2 nd dorsal-fin soft ray length	—	21.0	17.6	—
3 rd dorsal-fin soft ray length	—	23.0	17.9	—
4 th dorsal-fin soft ray length	—	24.0	19.3	—
5 th dorsal-fin soft ray length	—	25.1	19.3	—
6 th dorsal-fin soft ray length	19.8	21.3	19.0	—
7 th dorsal-fin soft ray length	19.1	20.7	15.1	—
8 th dorsal-fin soft ray length	18.1	17.2	12.8	—
9 th dorsal-fin soft ray length ^c	15.5	14.1	10.2	—
9 th dorsal-fin soft ray length ^d	absent	12.7	8.0	—
10 th dorsal-fin soft ray length ^c	12.3	absent	absent	absent
10 th dorsal-fin soft ray length ^d	9.9	absent	absent	absent
1 st anal-fin spine length	6.7	4.4	4.3	—
2 nd anal-fin spine length	16.9	10.7	10.2	—
1 st anal-fin soft ray length	18.9	18.6	16.2	—
2 nd anal-fin soft ray length	20.9	22.4	20.2	—
3 rd anal-fin soft ray length	21.0	27.5	23.6	—
4 th anal-fin soft ray length	21.3	28.7	24.1	—
5 th anal-fin soft ray length	18.2	26.1	23.0	—
6 th anal-fin soft ray length ^c	16.6	20.9	19.3	—
6 th anal-fin soft ray length ^d	14.8	18.1	15.3	—
Pectoral-fin ray length	49.5	51.1	49.4	47.9
Pelvic-fin spine length	25.2	14.6	14.5	—
1 st pelvic-fin soft ray length	25.4	21.9	19.3	—
2 nd pelvic-fin soft ray length	27.4	26.5	21.9	—
3 rd pelvic-fin soft ray length	24.6	26.4	21.6	—
4 th pelvic-fin soft ray length	19.1	20.9	18.5	—
5 th pelvic-fin soft ray length	17.0	17.9	14.2	—
Caudal-fin length	34.8	43.4	40.9	—
Caudal-peduncle length	14.9	14.6	14.5	14.1
Caudal-peduncle depth	9.4	9.9	9.7	10.3

^a at posterior end of preocular spine base, ^b at vertical midline of eye, ^c anterior element, ^d posterior element.

specimen). Gill rakers on upper limb 5 or 6, on lower limb 14–16, including 5 or 6 rakers on hypobranchial; gill rakers short, spinous, longest raker on 1st gill arch less than one-fourth length of longest gill filament; 4th gill slit closed by membrane.

Body and head strongly compressed, head width less than greatest body width. Body moderately deep, deepest at origin of 2nd dorsal-fin spine. Head large, length greater than body depth. Posterior lacrimal spine with rounded tentacle, length approximately equal to orbit diameter (slightly greater than pupil diameter in small specimen). Large tentacle on supraocular spine, its length greater than orbit diameter. Pair of tentacles projecting from anterior surface of lip at symphysis of upper jaw. Tentacle on posterior margin of anterior nostril, length less than posterior lacrimal tentacle. Small tentacle on cheek (absent in small specimen). Three to four tentacles along preopercular margin; lowermost largest, elliptical, length less than pupil diameter; uppermost slender, on uppermost preopercular spine (absent in large specimen). Two obvious tentacles on each side of ventral surface of mandible, 1st simple, long, slender, located between 1st and 2nd dentary pores; 2nd larger, on posterior margin of dentary. Few tentacles on lateral-line scales. Small elliptical fleshy tentacles on outer part of eye membrane. Few tiny tentacles on spinous portion of dorsal fin and 7 uppermost rays of pectoral fin (absent in large specimen).

Numerous small papillae covering head and body. Posterior nostril opening upward, diameter about equal to anterior nostril, not visible laterally. Two distinct sensory pores, 1st anterior to anterior nostril, 2nd between anterior and posterior nostrils (not obvious in small specimen). Underside of dentary with 3 small sensory pores on each side, small pore behind nodular portion of lower jaw on each side. No scales on surface of head, including opercle, interorbital space, and occipital pit. Body covered with small cycloid scales, not extending onto fins. Embedded scales covering pectoral-fin base (not obvious in small specimen). No scales on ventral body surface.

Mouth oblique, forming an angle of ca. 30 degrees to horizontal axis of head and body. Posterior margin of maxilla not reaching (just reaching in small specimen) vertical level of anterior margin of orbit. Lateral surface of maxilla smooth, without ridges. Lower jaw with thickened symphyseal portion fitting into shallow median depression of upper jaw when mouth completely closed. Width of symphyseal gap separating premaxillary teeth bands broader than width of each band. Upper and lower jaws with approximately equal-width bands of villiform teeth; majority of upper- and lower-jaw teeth of similar length. Vomer with narrow patch of villiform teeth. No teeth on palatine.

Dorsal profile of snout strongly curved, initially convex, thereafter deeply concave (steep anterior to orbit, but slightly concave behind ascending process of premaxilla in small specimen). Nasal spine simple, small, directed dorsoposteriorly (indistinct in small specimen). Posterior margin of ascending process of premaxilla not reaching vertical level of posterior nostril. Interorbital ridges present, low, beginning level with supraocular spine and ending at base of postocular spine. Interorbital space relatively narrow, deep (broader and shallower in small specimen), forming V-shape in anterior view. Preocular spine

simple (not obvious in small specimen), directed upward, tip extending slightly beyond horizontal line through upper margin of pupil in lateral view. Supraocular spine simple, located considerably anterior to vertical midline of eye. Postocular spines simple, triangular in lateral view. Tympanic spine simple, small, directed upward. No coronal spines. Occipital pit shallow, behind distinct transverse ridge, curved posteriorly in dorsal view. Parietal and nuchal spines well developed, joined at base. Sphenotic with 1 small spine. Postorbital with 4 short, small, pointed spines (smooth without ridge or spines in small specimen). Pterotic spine simple, small, pointed, with narrow base, located just above uppermost end of preopercular ridge. Posttemporal spine simple, pointed, small, directed dorsoposteriorly; no upper posttemporal spine. Supracleithral spine simple, pointed.

Lateral surface of lacrimal with 5 low ridges radiating from center, but lacking spines; uppermost end of upwardly directed ridge with bump directly below posterior nostril; anterior end of forward ridge and lower end of downward ridge not projecting over upper lip. Suborbital ridge very low, with 4 small spines in large specimen (1st spine located above posterior margin of maxilla, 2nd just below anterior margin of orbit, 3rd and 4th adjoining, below posterior part of orbit); 3 spines in small specimen (1st spine just above tip of posterior lacrimal spine; 2nd and 3rd spines adjoining, below posterior part of orbit). Broad space between ventral margin of orbit and suborbital ridge. Suborbital pit shallow, front rimmed by an oblique low lacrimal ridge. Preopercle with 4 blunt spines; uppermost spine largest with low median ridge; 2nd with low median ridge; 3rd and 4th spines without median ridge. No supplemental preopercular spine. Upper and lower opercular spines simple, each with median ridge; lower spine almost horizontal.

Origin of 1st dorsal-fin spine above posttemporal spine base. Posterior margin of opercular membrane and upper end of pectoral-fin base extending slightly beyond vertical from base of 3rd dorsal-fin spine. Posterior tip of longest pectoral-fin ray extending beyond vertical from posterior end of anal-fin base. Origin of pelvic fin slightly anterior to base of pectoral fin; posterior tip of depressed pelvic fin extending slightly beyond anus (extending to 2nd anal-fin spine base in small specimen). Origin of 1st anal-fin spine slightly posterior to origin of last dorsal-fin spine.

Color of fresh specimens (Fig. 1A, C). Body red-brown with whitish blotches surrounding pectoral-fin base. Snout whitish, posterior half of head red with distinct small black or purplish spots below orbit. Dorsal fin red, posterior of spinous and soft ray portions with broad white band or whitish irregular markings or spots, basal area with 2–4 whitish spots (width slightly larger than pupil diameter). Pelvic fin blackish or reddish. Anal fin reddish basally, blackish distally. Caudal fin reddish posteriorly, whitish anteriorly, with black spots on lower rays (3 spots between 10th and 12th rays in large specimen, 4 on four lowermost rays in small specimen).

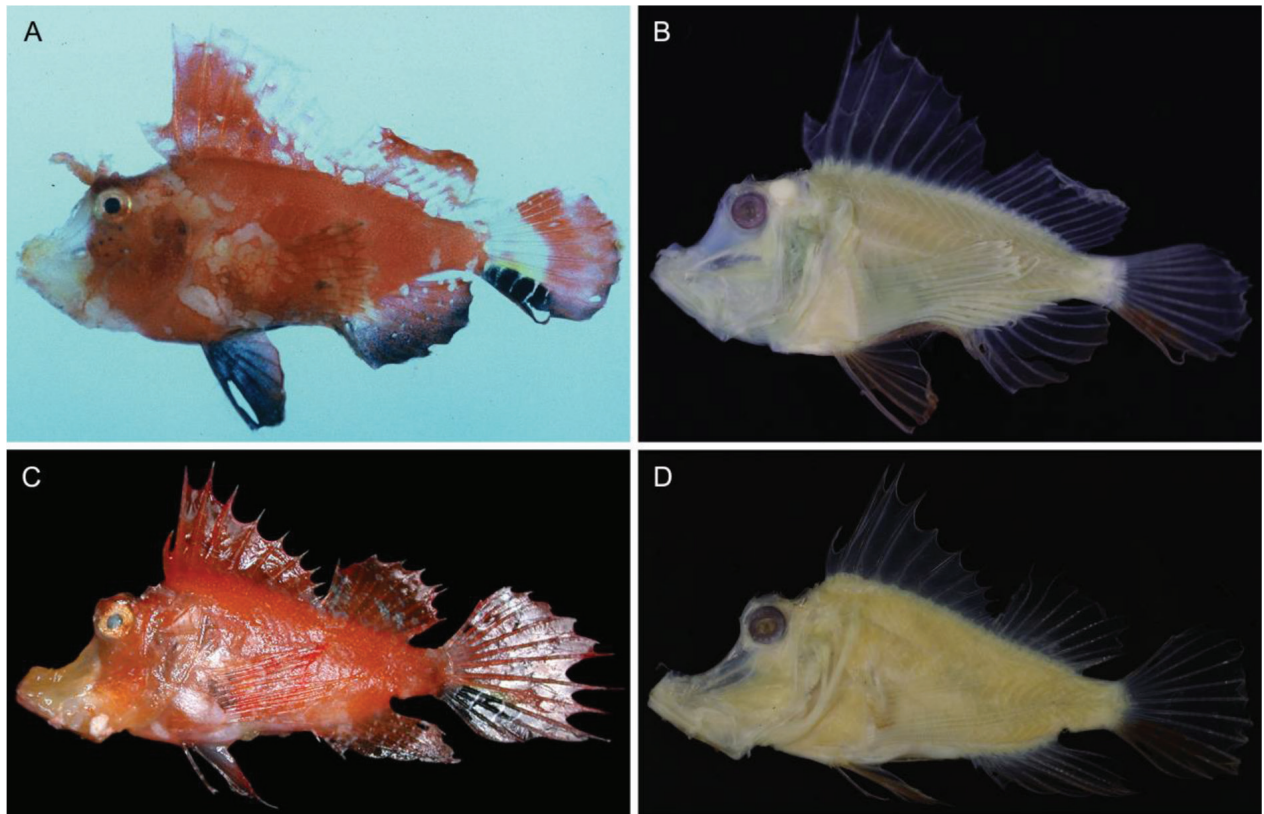


Figure 1. Photographs of *Hipposcorpaena filamentosa*. (A) fresh and (B) preserved specimens of SAIAB 57321, 17.2 mm SL, South Africa, and (C) fresh and (D) preserved specimens of NMV A 29729-041, 29.5 mm SL, Australia. Fresh South African and Australian specimen photos by P. Heemstra and M. Gomon, respectively and also available on the websites of http://fishwatch.tri-pod.com/REPORTS/FW_final_2006_files/page0002.htm and <https://fishesofaustralia.net.au/home/species/2673>, respectively.



Figure 2. Underwater photograph of *Hipposcorpaena filamentosa*, taken at Kashiwa-jima Island, Kochi, Japan, at a depth of 45 m on 10 May 2007 (Photo by K. Matsuno).

Color of preserved specimens (Fig. 1B, D). Head and body uniformly pale yellowish. Dorsal fin translucent. Pelvic fin translucent brownish. Anal fin translucent with brownish margin. Caudal fin translucent, with obvious dark brown spots on lower basal region.

Color in life. In general, similar to color when fresh (see Allen and Erdmann 2012: 224, unnumbered fig.; this study: Fig. 2).

Distribution. *Hipposcorpaena filamentosa* is widely distributed in the Indo-West Pacific (Fig. 3), being recorded from South Africa (Aliwal Shoal off Scottburgh), Japan (Kashiwa-jima Island), and Australia (Ashmore Islands), as well as previously from the Philippines (Luzon, Mindanao, and Sulu islands) (Fowler 1938; Motomura and Senou 2005), Indonesia (Lembah Strait and Bali), and Papua New Guinea (Milne Bay Province) (Allen and Erd-



Figure 3. Distributional records of *Hipposcorpaena filamentosa*, based on previous studies (circles) and this study (stars). Closed and open symbols indicate specimen- and underwater photograph-based records, respectively.

mann 2012). Previously reported from depths of 10–51 m (Fowler 1938; Motomura and Senou 2005; Allen and Erdmann 2012), the South African and Australian specimens were collected in depths of 34–36 m and 95 m, respectively, and the Japanese underwater photograph was taken at 45 m.

Discussion

The specimens collected from South Africa and Australia (Fig. 1) were identified as *Hipposcorpaena filamentosa* on the basis of the following combination of characters, which agreed well (except for dorsal-fin ray number of the South African specimen) with the diagnostic features given for the species by Motomura and Senou (2005): dorsal fin with 12 spines and 9 soft rays; anal fin with 2 spines and 6 soft rays; all dorsal- and anal-fin soft rays (except the last ray of each fin which was divided into 2 at the base) and all pectoral- and caudal-fin rays unbranched; 14 pectoral-fin rays, the longest ray extending well beyond vertical through the posterior end of the anal-fin base, lower ca. 7 rays filamentous; head and body strongly compressed; body deep, depth 39.5%–41.2% of SL; no palatine teeth; body covered with cycloid scales; interorbital ridges and tympanic spine present; posttemporal spine simple, upper posttemporal spine absent; and distinct dark brown markings on the lower part of the caudal fin. In addition, an underwater photograph from Kashiwa-jima Island, Japan (Fig. 2) was also identified as *H. filamentosa*, the body appearance, including filamentous pectoral-fin rays and black markings on the lower part of the caudal fin of the individual, clearly matching the species description (see Motomura and Senou 2005: fig. 1; Allen and Erdmann 2012: 224, unnumbered fig.).

The South African specimen differed slightly from all other known specimens of *H. filamentosa* in having XI, 10 dorsal-fin rays (vs. XII, 9 in the latter). However, Allen and Erdmann (2012) reported a similar dorsal-fin ray configuration in underwater photographs of *H. filamentosa*

from Indonesia and Papua New Guinea. Accordingly, a variable number of dorsal-fin rays in *H. filamentosa* is considered to represent intraspecific variation only.

The South African specimen was a small subadult or juvenile (17.2 mm SL), with the occipital pit surface, teeth, maxilla, and most head spines relatively weak. In addition, the nasal and opercular spines were indistinct and difficult to observe. The snout profile (length and angle) differed between the former (Fig. 1A, B) and specimens from other localities (29.5–35.2 mm SL; Motomura and Senou 2005: fig. 1; this study: Fig. 1C, D), suggesting an ontogenetic change.

Although the snout colors of *H. filamentosa* (whitish with small blackish or purplish spots below the orbit) had been lost in the preserved specimens, such colors are consistent in living and fresh specimens (see Motomura and Senou 2005: fig. 1; Allen and Erdmann 2012: 224, unnumbered fig.; this study: Figs 1A, C, 2), and, in addition to black spots on the lower basal caudal fin, are strongly diagnostic of the species.

Hipposcorpaena filamentosa is very similar to species of *Rhinopias* in having the head and body strongly compressed, body depth 38%–54% of SL, body covered with cycloid scales, and no palatine teeth (Eschmeyer et al. 1973; Poss 1999). However, *Hipposcorpaena* is generically distinct from the latter in several aspects, including some meristics, head spination, and coloration. Detailed generic characters of *Hipposcorpaena* and comparisons with related genera were given by Motomura and Senou (2005).

The new standard Japanese name “Itohiki-kasago” is herein proposed for *H. filamentosa*, “itohiki” meaning “filament” in reference to the filamentous pectoral fin rays and “kasago” being the common Japanese name for scorpionfish.

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Evaluating the size at sexual maturity for 20 fish species (Actinopterygii) in wetland (Gajner Beel) ecosystem, north-western Bangladesh through multi-model approach: A key for sound management

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Abstract

Effective fisheries management depend on having an exact assessment of biological parameters, including growth parameters, reproduction, size at sexual maturity (L_m), and stock assessment. The purpose of this research was to estimate the size at sexual maturity (L_m) for 20 fish species belongs to 14 families from a wetland (Gajner Beel) ecosystem in the north-western (NW) Bangladesh through multi-models such as length (L_{max}) based empirical model, gonadosomatic index (GSI)-based model, and logistic model using commercial catches from January to December 2018. Also, we assessed the L_m in other water-bodies worldwide. Specimens' total length (TL) was noted up to 0.1 cm using measuring board body weight (BW) and gonad weight (GW) weighed by digital electronic balance with 0.01 g accuracy. To assess the L_m , maximum body length (L_{max}) based empirical model; the relation between TL (total length in cm) vs. GSI (gonadosomatic index in %); and a logistic model were considered. The minimum L_m was 4.64, 3.90, and 4.15 cm for *Chanda nama* Hamilton, 1822 and the maximum was 25.33, 24.50, and 24.70 cm for *Channa striata* (Bloch, 1793) through L_{max} , GSI, and logistic-based models, respectively. From these three models, the minimum mean L_m was 4.23 cm for *C. nama* and the maximum was 24.84 cm for *C. striata*. The L_m with 50.0% species was in 8.80 cm TL. We also calculated the L_m from different bodies of water based on L_{max} . This study was generated data of 17 new L_m among 20 species, which are globally absent. Therefore, the study will help develop sustainable management strategies, conservation through the implementation of mesh size based on the size at sexual maturity (L_m).

Keywords

Bangladesh, logistic models, fish species, size at first sexual maturity, Gajner Beel

Introduction

In Bangladesh, fishes are the most affluent organisms, which secure livelihood, contribute food, generate employment, and are used to develop the nation's economy

(Hamilton 1971; Godfray et al. 2010; Costello et al. 2012; FAO 2012). The fisheries sector plays a key role in the national economy, contributing 3.50% to the GDP (Gross Domestic Product) of the country and 25.71% in agricultural GDP (DoF 2019). A large variety of aquatic animals

is found in the inland, estuarine, and marine waters of Bangladesh (Rahman 1989).

Bangladesh is fortunate to have vast aquatic resources and rich fish genetic diversity. It has a lot of inland water bodies that host 267 freshwater fish species. Biodiversity of fishes is very essential for nutrition and livelihoods for the rural people in Bangladesh (Thilsted 2013). Fishes, which spend their life in freshwater, (rivers and lakes), where the salinity is below 1.05‰ are considered freshwater fishes. Fishes require a range of physiological modification to live in the freshwater environment (Rohalin et al. 2019).

A land, which is inundated by water, annually or seasonally, permanently or temporarily that is called a wetland (Keddy 2010). Water purification, processing of carbon and other nutrients, maintenance of shorelines, water storage, and assistance of animals and plants are the important functions of a wetland (Butler 2010). Marsh, swamp, pen, and bog are the main types of wetlands (Keddy 2010). The wetlands can be freshwater, brackish, or saltwater (Ramsar Conservation 1971). The Pantanal in South America, the Amazon River basin, the West Siberian Plain (Fraser and Keddy 2005), and the Sundarbans in the Ganges–Brahmaputra delta (Giri et al. 2007) are the largest wetlands on the Earth. Rivers and streams, *Hoars*, *Baors*, Beels, lakes and marshes, reservoirs, ponds, cultivated fields flooded by water, and estuarine systems are considered wetlands in Bangladesh (See Table 1). The freshwater wetlands are Haors, Baors, Beels, and Jheels. The man-made wetlands are dighis, lakes, ponds, and borrow pits (Banglapedia 2004).

Gajner Beel is situated at Sujanagar, Pabna in north-western (NW) Bangladesh. This Beel is used as an imperative feeding and spawning ground by many freshwater fish species. Near about 0.5 million people of sur-

Table 1. Types and area of wetlands in Bangladesh.

Types	Wetland	Area [km ²]
Open waters	Rivers	7497
	Estuaries and mangrove swamps	6102
	Beels and haors	1142
	Inundable floodplains	54 866
	Kaptai Lake	688
Closed water	Ponds	1469
	Baors (Oxbow Lakes)	55
	Brackish-water farms	1080
	Total	72 899

rounding villages of this Beel are directly or indirectly reliant on this wetland for their livelihood (Mazid et al. 2005; Hasan et al. 2020).

Effective fisheries management depends on having an exact assessment of biological parameters, including growth parameters, reproduction, size at sexual maturity (L_m), and stock assessment (Tracey et al. 2007). The L_m in fish species is a fundamental requirement to find out the reasons on behalf of modifications of the length of maturity (Templeman 1987). Subsequently, it is habitually castoff as a sign of least-acceptable capture dimensions (Lucifora et al. 1999).

Scanning of the literature shows non-availability of species-specific data on size at sexual maturity (L_m) of these 20 species except *Channa striata* (Bloch, 1793) (see Herre 1924; Makmur et al. 2003), *Gudusia chapra* (Hamilton, 1822) (see Hossain et al. 2010), and *Puntius sophore* (Hamilton, 1822) (see Hossain et al. 2012a) from the Gajner Beel, Bangladesh. The objective of our research is to estimate the L_m for 20 species from the Gajner Beel in Bangladesh that will be helpful for the management strategies of these species in Gajner Beel in Bangladesh and adjacent aquatic ecosystems.

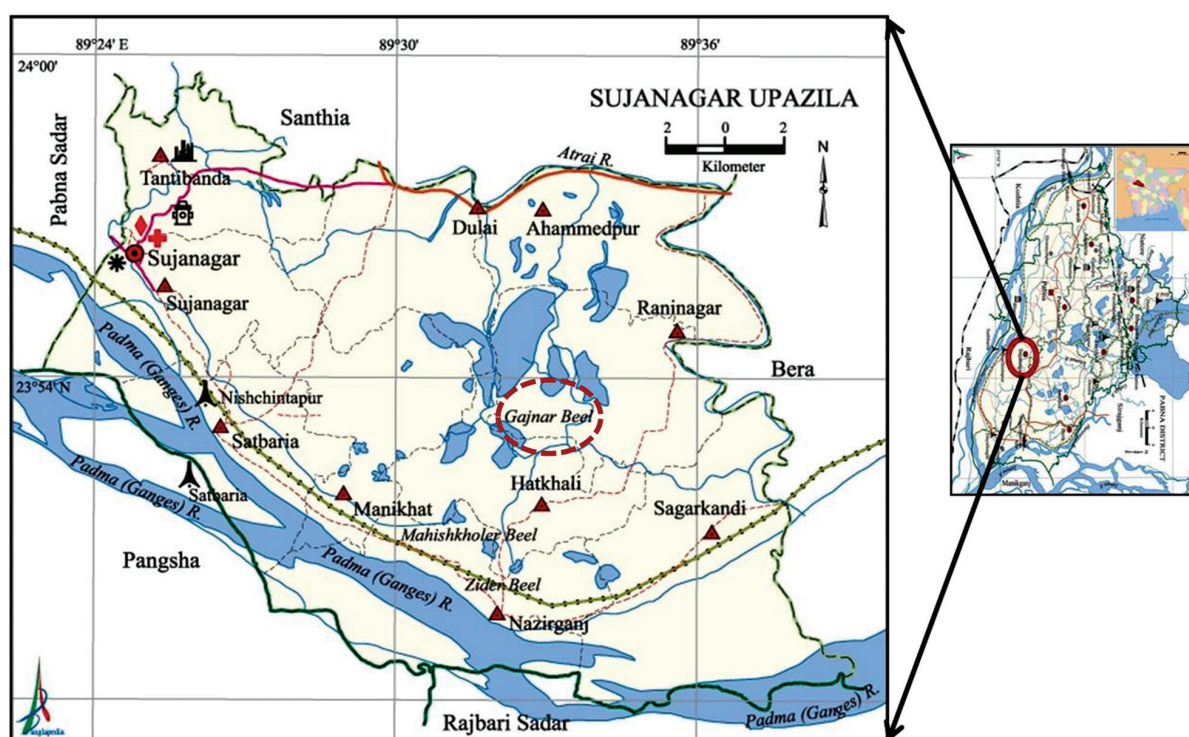


Figure 1. Sampling sites in a wetland ecosystem (Gajner Beel) (indicated by red circle), northwestern Bangladesh.

Materials and methods

The presently reported study was conducted in Gajner Beel (23°55'N, 89°33'E), which is located at Sujanagar, Pabna, NW Bangladesh (Fig. 1). Sampling was done from January to December 2018. Fishes were caught by several types of net (gill nets, long seine) and then preserved in 10% formalin for the further process. Species identification was done by observation of morphometric characters and reviews the various pieces of literature. Each individual was measured by measuring board (0.1 cm) and weighed by digital weight balance (0.01 g). After dissection the fishes, gonads have been removed and weighed. Sexing was determined under the microscopic view, and then only female specimens were used for this analysis. The gonadosomatic index was estimated based on Nikolsky (1963)

$$\text{GSI (\%)} = \text{GW/BW} \times 100$$

where, GW referred to the gonad weight (g) and BW were body weight (g). The length of 50% maturity (50% L_m) of the 20 fish species was estimated using three models, which were shown in Table 2.

Table 2. Size at sexual maturity of 20 fish species was calculated by these three models.

Model name	Equations	Reference
Empirical model	$\log(L_m) = -0.1189 + 0.9157 * \log(L_{\max})$	Binohlan and Froese 2009
GSI based model	$L_m = \text{TL vs. GSI}$	Hossain et al. 2010
Logistic model	$\text{PMI} = 100 / [1 + \exp\{-f(\text{TL}_m - \text{TL}_{50})\}]$	King 2007

Analysis of L_{50} , a logistic curve following King (2007) was applied for the data by plotting the percentage of mature individuals (PMI) against TL class. TL = total length (cm), GSI = Gonadosomatic index (%) and L_m = Size at sexual maturity.

Results

Altogether 3040 specimens of 20 fish species were considered in the presently reported study and a list of fish species is given in Table 3. The minimum length was 2.40 cm in TL for *C. nama* and the maximum length was 46.00 cm for *C. striata*. The estimated minimum L_m was 4.64, 3.90, and 4.15 cm for *C. nama* and the maximum was 25.33, 24.50, and 24.70 cm for *C. striata* through L_{\max} , TL vs. GSI, and logistic-based models, respectively, and the mean value was 10.04 cm for the 20 species of Gajner Beel, Bangladesh. The maximum length, minimum length, and L_m with 95% CL are given in Table 4. We also calculated the L_m from the different water bodies (Table 5) based on L_{\max} which are collected from the previous works on these species by previous workers. As an example of TL vs. GSI and logistic models and figures are presented in Fig. 2. L_m shows that 50% of mature fishes are below 8.80 cm, so the selection of this net-mesh size would protect half the adults in the Gajner Beel ecosystem. On the other hand, 80% of mature fishes are below 12.10 cm, so such a larger, more-conservative mesh size might play a vital role for sustainable fish production in wetland ecosystems (Fig. 3).

Table 3. List of total 20 fish species in a wetland ecosystem (Gajner Beel), NW Bangladesh.

Sl. No	Family	Scientific name	Common name
01	Ambassidae	<i>Chanda nama</i>	Chanda
02	Anabantidae	<i>Anabas testudineus</i>	Koi
03	Bagridae	<i>Mystus cavasius</i>	Gulsa
04		<i>Mystus tengra</i>	Tengra
05	Belontiidae	<i>Xenotodon cancila</i>	Kakila
06	Channidae	<i>Channa orientalis</i>	Cheng
07		<i>Channa punctata</i>	Taki
08		<i>Channa striata</i>	Shol
09	Clupeidae	<i>Gudusia chapra</i>	Chapila
10	Cobitidae	<i>Lepidocephalichthys guntea</i>	Gutum
11	Cyprinidae	<i>Amblypharyngodon mola</i>	Moa
12		<i>Puntius sophore</i>	Jat punti
13		<i>Salmostoma bacaila</i>	Chela
14	Gobiidae	<i>Glossogobius giuris</i>	Bele
15	Heteropneustidae	<i>Heteropneustes fossilis</i>	Shingi
16	Mastacembelidae	<i>Macrognathus aculeatus</i>	Shal baim
17		<i>Macrognathus pancalus</i>	Guchi
18	Nandidae	<i>Nandus nandus</i>	Bheda
19	Osphronemidae	<i>Trichogaster fasciata</i>	Kholisa
20	Siluridae	<i>Ompok pabo</i>	Pabda

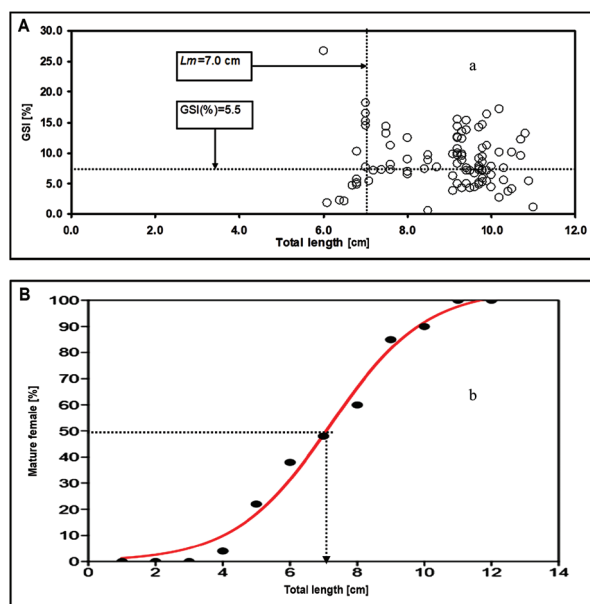


Figure 2. An example figure of size at sexual maturity which produced by TL vs. GSI (A) and logistic model (B) for the 20 species in wetland ecosystem (Gajner Beel) northwestern Bangladesh.

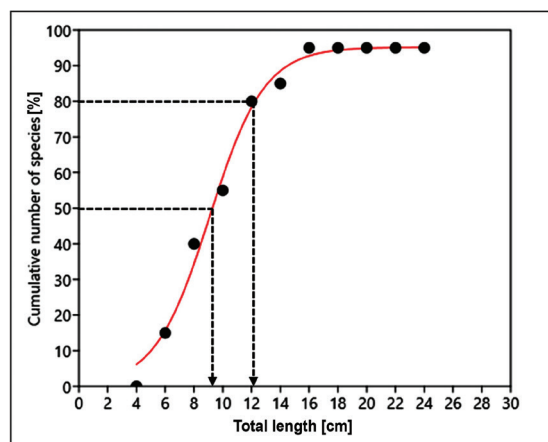


Figure 3. Relation between the maximum total length attained by a species and the number of species attaining that length in a wetland ecosystem (Gajner Beel) northwestern Bangladesh.

Table 4. Size at first sexual maturity (L_m) of 20 fish species in a wetland ecosystem (Gajner *Beel*), NW Bangladesh.

Scientific name	n	Minimum length [cm]	Maximum length [cm]	Size at sexual maturity (L_m)			
				Maximum length based	GSI based	Logistic-based models	Mean
<i>Chanda nama</i>	196	2.40	7.20	4.64 (3.81–5.68)	3.90	4.15	4.23
<i>Anabas testudineus</i>	130	7.50	16.40	9.85 (7.84–12.39)	10.20	9.10	9.72
<i>Mystus cavasius</i>	124	5.30	16.90	10.13 (8.05–12.75)	9.80	9.70	9.88
<i>Mystus tengara</i>	139	4.80	12.60	7.74 (6.23–9.65)	7.00	7.10	7.28
<i>Xenentodon cancila</i>	118	8.50	24.00	13.96 (10.95–17.77)	12.98	13.45	13.46
<i>Channa orientalis</i>	152	8.10	19.00	11.27 (8.92–14.24)	12.48	11.00	11.58
<i>Channa punctata</i>	178	5.30	19.40	11.49 (9.09–14.53)	12.20	11.25	11.65
<i>Channa striata</i>	128	9.50	46.00	25.33 (19.35–32.89)	24.50	24.70	24.84
<i>Gudusia chapra</i>	126	4.40	14.60	7.18 (5.79–8.93)	6.90	6.95	7.00
<i>Lepidocephalichthys guntea</i>	117	5.00	10.30	6.44 (5.22–7.98)	6.50	6.20	6.38
<i>Amblypharyngodon mola</i>	193	3.90	7.80	4.99 (4.09–6.13)	4.90	4.85	4.91
<i>Puntius sophore</i>	191	4.20	11.00	6.83 (5.53–8.49)	7.00	6.50	6.78
<i>Salmostoma bacaila</i>	114	4.20	10.00	6.26 (5.09–7.76)	6.50	5.90	6.22
<i>Glossogobius giuris</i>	189	3.90	14.70	8.91 (7.13–11.17)	8.10	8.45	8.49
<i>Heteropneustes fossilis</i>	180	6.30	24.10	14.02 (10.99–17.84)	12.20	13.96	13.39
<i>Macrognathus aculeatus</i>	115	8.70	27.00	15.55 (12.14–19.86)	16.18	15.00	15.58
<i>Macrognathus pancalus</i>	190	6.90	15.70	9.47 (7.55–11.89)	9.80	9.00	9.42
<i>Nandus nandus</i>	168	6.50	17.20	10.29 (8.18–12.96)	10.40	9.95	10.21
<i>Trichogaster fasciata</i>	170	3.30	9.30	5.86 (4.77–7.24)	6.00	5.00	5.62
<i>Ompok pabo</i>	122	4.80	17.80	10.62 (8.43–13.39)	9.85	10.20	10.22

Table 5. Calculate the size at sexual maturity based on maximum length from the different water bodies in world wide.

Species name	Sex	Habitat	L_{max} [cm]	L_m (95% CL)
<i>Chanda nama</i>	C	Brahmaputra River tributary, Bangladesh	6.40	4.16 (3.44–5.08)
	C	Deepor <i>beel</i> , Assam, India	7.00	4.52 (3.72–5.53)
	C	Hirakud Reservoir, India	10.10	6.32 (5.13–7.83)
	C	Brahmaputra River, Bangladesh	7.40	4.75 (3.91–5.83)
	C	Ganges River, Rajshahi, Bangladesh	7.20	4.64 (3.81–5.68)
<i>Anabas testudineus</i>	C	India	11.00	6.83 (5.53–8.49)
	C	Chi River, Thailand	16.50	9.91 (7.89–12.46)
	C	Pampanga River, Candaba, Philippines	11.70 (SL)	7.23 (5.84–9.00)
	C	Agusan Marsh, Philippines	17.00	10.18 (8.09–12.82)
	C	Tetulia River, Bangladesh	16.10	9.69 (7.72–12.18)
<i>Mystus cavasius</i>	C	India	25.00	14.49 (11.35–18.47)
	C	Betwa River, India	27.40	15.76 (12.30–20.14)
	C	Ganges River, Bangladesh	15.00	9.08 (7.25–11.39)
	C	Brahmaputra River tributary, Bangladesh	11.30	7.00 (5.66–7.71)
	C	Qadirabad barrage, Chenab River, Pakistan	17.80	10.62 (8.43–13.39)
<i>Mystus tengara</i>	C	India	40.0	22.29 (17.12–28.81)
	C	Brahmaputra River, Bangladesh	11.20	6.95 (5.62–8.64)
	C	Ganges River, Bangladesh	11.60	7.18 (5.79–8.93)
	C	India	18.00	10.73 (8.51–13.53)
	C	Atrai River, Bangladesh	18.10	10.78 (8.55–13.60)
<i>Xenentodon cancila</i>	C	Hirakud reservoir, India	18.60	11.06 (8.76–13.96)
	C	Chi River, Thailand	23.00	13.43 (10.55–17.07)
	C	India	40.00	22.29 (17.12–28.81)
	C	Basantar River, India	19.60	11.60 (9.17–14.67)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	18.40	10.95 (8.68–13.82)
<i>Channa orientalis</i>	C	India	33.00	18.69 (14.47–24.02)
	F	Siruvani River, Tamil Nadu, India	24.40	14.18 (11.11–18.05)
	M	India	25.00	14.49 (11.35–18.47)
	F	Vellar River, Tamil Nadu, India	24.50	14.23 (11.15–18.12)
	M	India	27.90	16.03 (12.49–20.49)
<i>Channa punctata</i>	F	Cauvery River, Tamil Nadu, India	25.90	14.97 (11.70–19.10)
	M	India	25.40	14.71 (11.51–18.75)
	F	Tamirabrani River, Tamil Nadu, India	27.40	15.76 (12.30–20.14)
	M	India	26.80	15.45 (12.06–19.72)
	C	Hirakud Reservoir, India	19.20	11.38 (9.01–14.38)
<i>Channa striata</i>	C	Mathabhanga River, Bangladesh	18.90	11.22 (8.88–14.17)
	C	India	31.00	17.65 (13.70–22.64)
	F	North Kerian rice agroecosystem, Malaysia	54.00	29.34 (22.27–38.28)
	M	India	45.20	24.93 (19.06–32.35)
	C	Agusan Marsh, Philippines	61.0	32.80 (24.78–42.96)
	C	Chi River, Thailand	51.00	277.84 (21.18–36.26)
	C	Pampanga River, Candaba, Philippines	41.40 (SL)	23.0 (17.65–29.77)

Table 5 continues on next page.

Table 5. cont.

Species name	Sex	Habitat	L_{\max} [cm]	L_m (95% CL)
<i>Channa striata</i>	C	Pearl River, China	39.30	21.93 (16.86–28.34)
	C		100.00	51.58 (38.20–68.60)
<i>Gudusia chapra</i>	F	Lake, Mymensingh, Bangladesh	13.70 (SL)	8.36 (6.70–10.45)
	M		12.60 (SL)	7.74 (6.23–9.65)
	C	Lower Brahmaputra, India	13.80	8.41 (6.74–10.52)
	C	Betwa River, India	15.00	9.08 (7.25–11.39)
	C	Hirakud Reservoir, India	11.60	7.18 (5.79–8.93)
	C	Ganges Lower region, Bangladesh	13.40	8.19 (6.57–10.23)
	C		20.00	11.82 (9.33–14.95)
<i>Lepidocephalichthys guntea</i>	C	Atrai River, Bangladesh	8.70	5.51 (4.50–6.80)
	C	Ganges Lower region, Bangladesh	9.60 (SL)	6.03 (4.91–7.46)
	C		15.00	9.08 (7.25–11.39)
<i>Amblypharyngodon mola</i>	F	Wetlands of Dishoi and Neamatighat, Assam, India	9.00	5.69 (4.64–7.02)
	M		6.60	4.28 (3.53–5.23)
	C	Hirakud Reservoir, India	7.20	4.64 (3.81–5.68)
	F	Payra River, Bangladesh	5.80 (SL)	3.80 (3.16–4.63)
	M		5.40 (SL)	3.56 (2.97–4.33)
	C	Atrai River, Bangladesh	6.20	4.04 (3.35–4.93)
	C	Ganges River, Bangladesh	8.10	5.16 (4.23–6.35)
	F	Garjan <i>beel</i> , India	8.30	5.28 (4.32–6.50)
	M		7.60	4.73 (4.00–5.98)
	C	Ganges lower region, Bangladesh	5.9 (SL)	3.86 (3.20–4.71)
	C	Mathabhanga River, Bangladesh	7.00	4.52 (3.72–5.53)
	U	South 24 Parganas, India	8.70	5.51 (4.50–6.80)
	C	India	20.00	11.82 (9.33–14.95)
<i>Puntius sophore</i>	C	Ganga basin tributaries, India	18.50	11.00 (8.72–13.89)
	C	Mathabhanga River, Bangladesh	10.20	6.38 (5.18–7.90)
	C	Hirakud Reservoir, India	10.80	6.72 (5.44–8.34)
	C	Brahmaputra River basin, India	7.40 (SL)	4.75 (3.91–5.83)
	C		20.00	11.82 (9.33–14.95)
<i>Salmostoma bacaila</i>	C	Atrai River, Bangladesh	10.50	6.55 (5.31–8.12)
	C	Hirakud Reservoir, India	14.70	8.91 (7.13–11.17)
	C		18.00	10.73 (8.51–13.53)
<i>Glossogobius giuris</i>	C	Brahmaputra River, Bangladesh	9.70	6.09 (4.95–7.54)
	C	Hirakud Reservoir, India	22.50	13.16 (10.35–16.71)
	C	Hongshui River, China	17.50	10.46 (8.30–13.18)
	C	Agusan Marsh, Philippines	19.50	11.54 (9.13–14.60)
	C	Ganges lower region, Bangladesh	23.60	13.75 (14.79–17.49)
	F		22.80	13.32 (10.47–16.92)
	M		23.60	13.75 (14.79–17.49)
	C		17.90 (SL)	10.67 (8.47–13.46)
	C	Estuaries, South Africa	11.90 (SL)	7.34 (5.92–9.15)
	C		50.00 (SL)	27.34 (20.82–35.59)
<i>Heteropneustes fossilis</i>	C	Atrai River, Bangladesh	13.70	8.36 (6.70–10.45)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	16.50	9.91 (7.89–12.46)
	C	Ganga River, India	31.00	17.65 (13.70–22.64)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	26.80	15.45 (12.06–19.72)
	C		24.10	14.02 (10.99–17.84)
<i>Macrognathus aculeatus</i>	C	Ganges River, NW Bangladesh	23.40	13.64 (10.71–17.35)
	C	Thailand	38.00	21.27 (16.37–27.45)
<i>Macrognathus pancalus</i>	C	Atrai River, Bangladesh	12.60	7.74 (6.23–9.65)
	C	Mathabhanga River, Bangladesh	16.20	9.74 (7.76–12.25)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	14.40	8.75 (7.00–10.95)
	C	Hirakud Reservoir, India	16.60	9.96 (7.93–12.53)
	C		18.00	10.73 (8.51–13.53)
<i>Nandus nandus</i>	F	Ganges River, NW Bangladesh	13.60	8.30 (6.66–10.38)
	M		12.60	7.74 (6.23–9.65)
	C	Brahmaputra River, Bangladesh	14.00	8.52 (6.83–10.67)
	C	Mathabhanga River, Bangladesh	14.20	8.63 (6.91–10.81)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	14.10	8.58 (6.87–10.74)
<i>Trichogaster fasciata</i>	C		20.00	11.82 (9.33–14.95)
	C	Deepor <i>beel</i> , Assam, India	8.10	5.16 (4.23–6.35)
	C	Gajner <i>beel</i> floodplain, Pabna, Bangladesh	9.40	5.92 (4.82–7.32)
<i>Ompok pabo</i>	C		12.50	7.68 (6.18–9.58)
	C		25.00	14.49 (11.35–18.47)
	F	Feni and Gomati River, Tripura, India	19.00	11.27 (8.92–14.24)
	M		20.70	12.19 (9.62–15.45)
	C	Payra River, southern Bangladesh	22.30	13.05 (10.27–16.57)

Discussion

This study referred to the first strive to evaluate the size at sexual maturity of 20 fishes through multiple models in the Gajner Beel wetland ecosystem. The selection of permissible capture size at first maturity is broadly used and it is also used as an important tool in fisheries management (Lucifora et al. 1999; Hossain et al. 2012b) in open waters. Available information on size at sexual maturity of fishes from plots of percentage occurrence of mature females against length class can be obtained from the resulting logistic equation (King 2007). Some studies have narrated low exactness in the estimation of L_m of fishes using this logistic equation (Hossain and Ohtomi 2008; Hossain et al. 2013) but its accuracy for short life cycle organisms is addressed. Garcia (1985) also reported that using the proportion of mature females as an index of population reproduction was highly biased.

Nevertheless, the L_m was estimated by several models including brooding of eggs over time (especially for crustaceans), the appearance of the ovary and maturation stages over time (King 2007), the relative weight of gonad (TL vs. gonadosomatic index, modified gonadosomatic index, and Dobriyal index) over time (Hossain et al. 2017; Ahamed et al. 2018; Khatun et al. 2019), and histological studies (Chelemal et al. 2009; Jan and Ahmed 2019; Lucano-Ramirez et al. 2019). These methods differ with processing time, precision, accuracy, or suitability when we used these singly (De Martini and Lau 1999). To prevent this problem, we used three models (L_{max} , TL vs. GSI, and logistic-based models) and their mean value was used to calculate their size at sexual maturity.

Among the 20 fishes, *C. nama* was the smallest and *C. striata* the largest in TL. Information on L_m was available only for three species (*Channa striata*, *Gudusia chapra*, and *Puntius sophore*) in FishBase (Froese and Pauly 2020). In our study L_m (mean L_m) was 24.84 cm for *C. striata* whereas Makmur et al. (2003) recorded 15.40 and 18.00 cm in the Musi River, south Sumatera, and 25.00 cm was found in Indonesia (Herre 1924). For the *G. chapra* L_m was 7.00 cm in this study. Hossain et al. (2010) narrated 8.00 cm in the Ganges River. We found 6.78 cm L_m for *P. sophore*. Halls et al. (1999), Halls (2005), and Hossain et al. (2012a) reported that L_m was 6.10, 4.50, and 5.00 for the *P. sophore* in the Talimnagar sluiceway, Lohajang River, and Padma River, respectively. The L_m of fish specimens might differ due to several factors like feeding rate, sex and gonadal development, behavior, season, the flow of water, populations density, water temperature, and food (Hossain et al. 2006, 2012a, b; Tarkan et al. 2006; Muchlisin et al. 2010). Most importantly it was the first attempt on L_m for 20 species in Gajner Beel wetland ecosystem so it can be used as baseline information for the future studies and essential for the selection of the permissible mesh size of nets which will be helpful for the sustainable management strategies of these 20 fish species from Gajner Beel in Bangladesh and contiguous

ecosystems. Optimum catchable length (L_{opt}) is the length where the biomass of an unexploited cohort would be maximum (Froese et al. 2016). We also observed the L_{opt} which is essential for the management of these 20 fish species (Table 6).

Table 6. Optimum catchable length (L_{opt}) of 20 fish species in a wetland ecosystem (Gajner Beel), NW Bangladesh.

Scientific name	n	Minimum length [cm]	Maximum length [cm]	Optimum catchable length of individuals (L_{opt})
<i>Chanda nama</i>	196	2.40	7.20	4.80
<i>Anabas testudineus</i>	130	7.50	16.40	10.93
<i>Mystus cavasius</i>	124	5.30	16.90	11.27
<i>Mystus tengara</i>	139	4.80	12.60	8.40
<i>Xenentodon cancila</i>	118	8.50	24.00	16.00
<i>Channa orientalis</i>	152	8.10	19.00	12.67
<i>Channa punctata</i>	178	5.30	19.40	12.93
<i>Channa striata</i>	128	9.50	46.00	30.67
<i>Gudusia chapra</i>	126	4.40	14.60	9.73
<i>Lepidocephalichthys guntea</i>	117	5.00	10.30	6.87
<i>Amblypharyngodon mola</i>	193	3.90	7.80	5.20
<i>Puntius sophore</i>	191	4.20	11.00	7.33
<i>Salmostoma bacaila</i>	114	4.20	10.00	6.67
<i>Glossogobius giuris</i>	189	3.90	14.70	9.80
<i>Heteropneustes fossilis</i>	180	6.30	24.10	16.07
<i>Macrornathus aculeatus</i>	115	8.70	27.00	18.00
<i>Macrornathus pancalus</i>	190	6.90	15.70	10.47
<i>Nandus nandus</i>	168	6.50	17.20	11.47
<i>Trichogaster fasciata</i>	170	3.30	9.30	6.20
<i>Ompok pabo</i>	122	4.80	17.80	11.87

Fish diversity of Gajner Beel wetland ecosystem is declining at a faster rate because of many factors; damage of habitat, aquatic pollution, fishing pressure, natural disaster, extreme floodplain siltation, and reclamation of wetland (Dudgeon 1992; Hossain et al. 2014; Rahman et al. 2016). Therefore, to conserve the wild stock of wetlands, more population surveys and stock assessments are urgently needed. Identification of the causative factors for declining of the species, the establishment of suitable sanctuaries, conservation of habitats, and protection of adult species during the spawning – and/ or peak spawning season is highly recommended. Besides this, the mesh size of harvesting nets based on size at sexual maturity should be confirmed throughout the year for sustainable conservation and management. Furthermore, public awareness is most important for the conservation of this species.

The presently reported study concludes that around 50.0% of species were sexually matured in 8.80 cm TL. So, we strongly suggest that ≤ 8.80 cm TL fishes cannot be recommended for harvesting. As a result, at least 50% of species survive in the wetland ecosystem.

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First data on the structure of the vertebral column in *Gobio* and *Romanogobio* species (Actinopterygii, Cypriniformes, Gobionidae) from Ohrid Lake and the Vardar River basin

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Abstract

The validity of four gudgeon species known for the ichthyofauna of North Macedonia has been a matter of different taxonomic disputes (except for *Gobio ohridanus* Karaman, 1924). Recently, a restoration of the species status was proposed for *Gobio balcanicus* Dimovski et Grupče, 1977 and *Romanogobio banarescui* (Dimovski et Grupče, 1974). So far, *Romanogobio stankoi* (Karaman, 1974), has not been a part of any recent investigation and it is still considered as a junior synonym of *Romanogobio elimeius* (Kattoulas, Stephanidis et Economidis, 1973) or *R. banarescui* due to the lack of distinctive morphological characters. The osteological characters in the structure of the vertebral column, have significance to the systematics of the gudgeons, especially in elevation of *Romanogobio* at the level of genus. To contribute to resolving the taxonomic status in these gudgeon species, the goal of this paper is to identify distinctive characters by presenting and analyzing the structure of their vertebral column. Samples were inspected from the fish collections in the Institute of Biology (*G. ohridanus*), the Institute of Animal Science (*G. balcanicus*), and the Macedonian Museum of Natural History (both *Romanogobio* species). Fish samples were X-rayed as well as cleared and double-stained. Obtained data on the number of vertebrae were presented through the vertebral formula following Naseka (1996). Multiple Correspondence Analysis (MCA) was used to check the interrelation between taxa and the number of vertebrae in all units of the vertebral column. *G. balcanicus* is distinguished from *G. ohridanus* mainly in the predorsal and preanal subregion (10 vs. 11 vertebrae and 1 vs. 0 vertebrae, respectively), while *R. stankoi* distinguishes from *R. banarescui* by the number of vertebrae in the preanal and postanal subregion (2 vs. 4 vertebrae and 19 vs. 17 vertebrae, respectively). The structure of vertebral column offers distinctive morphological characters for the four gudgeon species from Ohrid Lake and the Vardar River basin.

Keywords

Gudgeons, MCA analysis, vertebrae, vertebral column, vertebral formula

Introduction

Although the monophyly of the family Gobionidae (formerly in the family Cyprinidae) is well supported (Yang

et al. 2006; Tang et al. 2011), its systematics still contains unresolved issues that deem taxonomic challenge due to ongoing speciation (Takács et al. 2014) and the application of different species concepts (Kullander 1999; Lusk

and Šlechta 2005; Nowak et al. 2008b, 2009). New data to make an advance in the taxonomy, systematics, and phylogeny of Gobionidae is still required, Naseka (1996) analyzed the structure of the Gobionine vertebral column, proposing a vertebral formula for each genus and species. Genus *Gobio* Cuvier, 1816 (type species *G. gobio*) formerly included subgenera *Romanogobio* Bănărescu, 196—type species *R. kessleri*—and *Rheogobio* Bănărescu, 1961—type species *Romanogobio uranoscopus* (Agassiz, 1828). The structure of the vertebral column, among other morphological characters, gave recognition to *Romanogobio* as a distinct genus (Naseka 1996) based on analysis of *Romanogobio kesslerii* (Dybowski, 1862), *Romanogobio al-bipinnatus* (Lukasch, 1933), *Romanogobio ciscaucasicus* (Berg, 1932), and *Romanogobio persus* (Günther, 1899). Later, Naseka and Freyhof (2004) synonymized subgenus *Rheogobio* with the accepted *Romanogobio* reevaluating the status of 15 species (see Naseka 1996; Kottelat 1997) in *Romanogobio* based on the external morphology, as well as vertebral column's features. Then, the presence of two native genera in Europe, *Gobio* and *Romanogobio*, became widely accepted (Lusk and Šlechta 2005; Kottelat and Freyhof 2007; Naseka and Bogutskaya 2009; Nowak et al. 2008a, 2011). On the other hand, some authors, based on osteological analyses including the vertebral column, proposed restoration of *Rheogobio* and required revision of all gudgeon subspecies that were previously under the species name *uranoscopus* (Talabishka 2014).

Four native gudgeon species that currently belong to genera *Gobio* and *Romanogobio* are known for the ichthyofauna of North Macedonia. Extensively studied in the past, one gudgeon species—*Gobio ohridanus* Karaman, 1924—was described for the old tectonic Ohrid Lake, while the other three were described for the Vardar River drainage. *Gobio balcanicus* Dimovski et Grupče, 1977 is widely distributed throughout the Vardar River and its tributaries. *Romanogobio banarescui* (Dimovski et Grupče, 1974) was described for the middle and lower course of the Vardar River, and *Romanogobio stankoi* (Karaman, 1974) (as interpreted by Dimovski and Grupče 1976a) for fast-flowing upper courses of the Vardar River, and its tributaries Lepenec, Bregalnica, Crna, and Treska rivers.

The validity of these species (except that of the *G. ohridanus*) has been a matter of different taxonomic disputes in which the need for contemporary morphological description was often emphasized. Dimovski and Grupče (1976b) showed differences between *R. stankoi* and *Romanogobio elimeius* (Kattoulas, Stephanidis et Economidis, 1973) from the Aliakmon River. Later, both *R. stankoi* and *R. banarescui* were suggested to be junior synonyms to *R. elimeius* (see Economidis et al. 1981; Kottelat and Freyhof 2007). Owing to the lack of comparative morphological data, Kottelat and Freyhof (2007) suggested that *G. balcanicus* should be a synonym of *Gobio bulgaricus* Drensky, 1926. Conversely, recent studies based on molecular data (Geiger et al. 2014; Jelič et al. 2018; Friedrich et al. 2018) proposed restoring the taxonomic status of *G. balcanicus* and *R. banarescui* as clearly distant from *G. bulgaricus* and *R. elimeius*, re-

spectively. So far, *R. stankoi* from the type locality has not been subject to recent studies and is still considered either a synonym of *R. banarescui*, or of *R. elimeius* (see Banarescu 1992; Kottelat and Freyhof 2007). In this paper, the name *R. stankoi* is used for material from the type locality aiming to contribute to the clarification of the taxonomic status of this taxon.

Having in mind that ambiguities in species delimitations seriously hamper the conservation measures (Kottelat 1998, 2013; Economou et al. 2007; Kottelat and Freyhof 2009) and that gudgeon species are very important to both conservation and ecology (Lusk and Šlechta 2005; Telcean and Cupșa 2012; Curtean-Bănăduc et al. 2019), it is of urgent matter to clarify the taxonomic status of all gudgeon taxa from the Balkan Peninsula. In this context, the goal of this paper is to analyze and present in detail, for the first time, the vertebral formula in gudgeons from North Macedonia following Naseka's (1996) methodology to shed light on the taxonomic potential of the structure of the vertebral column.

Material and methods

For this study, the material from the collections of the Macedonian Museum of Natural History, the Institute of Biology, and the Institute of Animal Science from Skopje was used. *Romanogobio banarescui* and *R. stankoi* were analyzed from the type material, collected by Dimovski and Grupče from 1961 to 1975 and deposited at the Macedonian Museum of Natural History. The material of *G. balcanicus* was collected in 2007 from the Vardar River and deposited at the Institute of Animal Science, while the material of *G. ohridanus* was collected in 2015 from Ohrid Lake and stored at the Institute of Biology. The specimens of *G. ohridanus* ($n = 22$), *G. balcanicus* ($n = 16$), *R. banarescui* ($n = 36$), and *R. stankoi* ($n = 36$) were X-rayed as well as cleared and double-stained according to the protocol of Hanken and Wassersug (1981).

The structure of the vertebral column was analyzed following Naseka (1996). Namely, to present the vertebral formula, seven characters of the vertebral column were analyzed: total number of vertebrae (T), abdominal number of vertebrae (A), caudal number of vertebrae (C), predorsal number of vertebrae (a_1), intermediate number of vertebrae (i), preanal number of vertebrae (c_1) and postanal number of vertebrae (c_2). The vertebral formula is composed of the mean values of these characters. Additionally, the number of vertebrae was counted in the distance between the origin of the dorsal fin and the origin of the anal fin (D–A distance), as well as in the distance between the origin of the dorsal fin and first caudal vertebrae (D–C1 distance). The last D–A and D–C1 distances are not part of the vertebral formula, but were counted as additional explorative characters.

The Multiple Correspondence Analysis (MCA) is a multivariate analysis that is used to examine the relations between more than two categorical variables (Sourial et al. 2010; Di Franco 2016). In this paper, MCA was ap-

plied to explore the interrelations between taxa and a certain number of vertebrae in different units of the vertebral column. As each unit of the vertebral column was considered as a character, the number of vertebrae observed in it was considered as a character state. The characters of the vertebral column (T , A , a_1 , i , C , c_1 , and c_2) and taxa (genera *Gobio* and *Romanogobio* and species *G. balcanicus*, *G. ohridanus*, *R. banareseui*, and *R. stankoi*) were analyzed as active variables. The distances D–A and D–C1 were plotted as supplementary variables (they do not contribute to forming of the dimensions in MCA) to enhance the interpretation of the analysis. MCA was performed using Statistical Software for Excel–XLSTAT 2014 5.03.

Results

Vertebral formula

The total number of vertebrae in *G. ohridanus* ranges from 37 to 39 (Table 1). The abdominal vertebrae (20 or 21) dominate over the caudal vertebrae (17 or 18) in all specimens. There was no variation in the predorsal vertebrae number (11) in all examined specimens. The number of intermediate vertebrae ranged from 3 to 5. An absence of preanal caudal vertebrae was noted in 18% of individuals in the sample. The number of postanal caudal vertebrae ranged from 16 to 18, with a mode of 17 in 59% of the specimens. The D–A distance had a mode of 10 vertebrae, the same as the mode of the A–C1 distance. The vertebral formula of *G. ohridanus* was: 38.23: (11) 20.45 (4.36) + (0.95) 17.73 (16.77).

The total number of vertebrae in *G. balcanicus* ranged from 37 to 39 (Table 1). All specimens had a greater number of abdominal vertebrae (20 or 21) compared to the number of caudal vertebrae (17–19). The predorsal subregion most often included 10 vertebrae. The range of intermediate vertebrae was from 3 to 5. The number of preanal vertebrae was 0–2, and the absence was observed in only 5% of the specimens. The number of postanal vertebrae ranged from 15 to 18 vertebrae. The D–A distance had a mode of 11 vertebrae, while D–C1 distance had a mode of 10 vertebrae. The vertebral formula of *G. balcanicus* was: 38.67: (10.33) 20.27 (4.40) + (1.33) 18.40 (17.07).

The examined specimens of *R. stankoi* had a total number of 39 or 40 vertebrae (Table 1). The abdominal and caudal vertebrae had the identical range (19 or 20), but the mean value of the specimens showed a dominance of the caudal vertebrae. A higher number of caudal than abdominal vertebrae (19:20 and 19:21) was observed in the majority of the examined specimens. The number of abdominal vertebrae was higher in 7 specimens (20:19) while an equal number between the abdominal and the caudal vertebrae (20:20) was observed in 11 specimens. The number of predorsal vertebrae most often was 11, while the intermediate vertebrae most often were 4. There is a mode of 2 preanal caudal vertebrae, while the number of postanal caudal vertebrae ranged from 17 to 19. The D–A distance had a mode of 10 vertebrae, while the D–C1 distance had a mode of 8 vertebrae. The vertebral formula of *R. stankoi* was: 39.42: (11.08) 19.50 (4.08) + (1.69) 19.92 (18.22).

The total number of vertebrae in *R. banareseui* ranged from 38 to 40 (Table 1). The number of abdominal vertebrae ranged from 18 to 20, while that of the caudal vertebrae ranged from 19 to 21. A higher number of caudal vertebrae, over the abdominal ones, was recorded in the majority of the examined specimens, 18:21 (2), 19:20 (20), and 19:21 (11). The number of abdominal vertebrae prevailed (20:19) in one specimen, while an equal number of the abdominal and the caudal vertebrae (19:19 and 20:20) was observed in 2 specimens. There were 10–12 predorsal vertebrae, while the number of intermediate vertebrae ranged from 3 to 5. There were 2–4 preanal caudal vertebrae, while postanal caudal vertebrae were most often 17. The most common value of D–A distance was 11 vertebrae and a mode of 8 vertebrae was counted for the D–C1 distance. The vertebral formula of *R. banareseui* was: 39.31: (10.97) 19.00 (4.06) + (2.92) 20.31 (17.42).

Multiple Correspondence Analysis

MCA was used to investigate the taxa as a function of the vertebral column structure. The overall inertia of the sample was 2.8889. The first two dimensions accounted for the largest part of the total variability, explaining 70.93% of the inertia (Fig. 1). The first dimension (F_1) accounted 62.75% of the total inertia and grouped specimens according to their

Table 1. Frequency table of the vertebral column of *Gobio* and *Romanogobio* species.

Species	T					A				a_1			i		
	37	38	39	40	18	19	20	21	10	11	12	3	4	5	
<i>G. ohridanus</i>	1	15	6				12	10		22		2	10	10	
<i>G. balcanicus</i>	2	6	12			1	15	4	12	8		2	9	9	
<i>R. stankoi</i>			21	15		18	18			33	3	5	23	8	
<i>R. banareseui</i>		1	23	12	2	32	2		3	31	2	4	26	6	
Species	C					c_1				c_2					
	17	18	19	20	21	0	1	2	3	4	15	16	17	18	19
<i>G. ohridanus</i>	6	16				4	15	3				7	13	2	
<i>G. balcanicus</i>	1	11	8			1	11	8			1	3	11	5	
<i>R. stankoi</i>			7	25	4		12	22	2				3	22	11
<i>R. banareseui</i>			2	21	13			7	25	4		1	19	16	

Abbreviations are given in Material and methods.

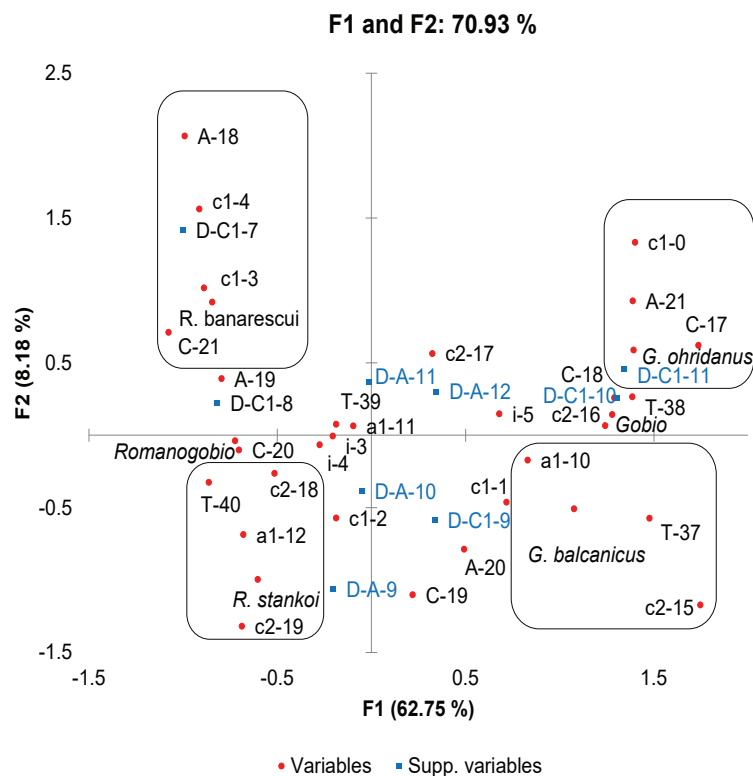


Figure 1. Results of Multiple Correspondence Analysis (MCA). Squares represent closely associated character states of the vertebral column with the analyzed species. The numeric suffixes in the active variables represent the actual number of vertebrae (the character state). Abbreviations: T – total vertebrae, A – abdominal vertebrae, C – caudal vertebrae, a1 – predorsal vertebrae, i – intermediate vertebrae, c1 – preanal vertebrae, c2 – postanal vertebrae.

genera. Thus, it can be called the generic dimension. Positive loadings on the F_1 featured genus *Gobio* with a lower total number of vertebrae, a higher number of abdominal vertebrae, a lower number of caudal vertebrae, a lower number of preanal vertebrae, a lower number of postanal vertebrae, and a lower number of predorsal vertebrae (Table 2, Fig. 1). Negative loadings of the first dimension (F_1) represented genus *Romanogobio* with a higher total number of vertebrae, a lower number of abdominal vertebrae, a higher number of caudal vertebrae, a higher number of preanal vertebrae, and a higher number of postanal vertebrae (Table 2, Fig. 1).

The second dimension (F_2) accounted 8.18% of the total inertia and grouped each species with certain character states of the vertebral column. Thus, this dimension can be called the species dimension (Fig. 1). Positive loadings associated *G. ohridanus* with 21 vertebrae in abdominal region and with the absence of preanal vertebrae; and *R. banarescui* with 18 abdominal vertebrae, 3 and 4 preanal vertebrae, as well as 21 caudal vertebrae. Negative loadings of F_2 associated *R. stankoi* with the highest number (19) of postanal vertebrae observed in all taxa (Fig. 1). *Gobio balcanicus* was associated with the lowest number of total vertebrae (37), the lowest number of postanal vertebrae (15), and the presence of 10 predorsal vertebrae. These character states did not contribute significantly to the forming of the second dimension, but to the third dimension (F_3) (Table 2).

The D–A distance was not closely associated with any of the genera, since it significantly contributed to the creation of the second, species dimension (Fig. 1; Table 2). This was not the case with D–C1 distance, where lower values were associated with *Romanogobio* and higher values with *Gobio* (Fig. 1; Table 1).

Discussion

The great variability in European gudgeon species that makes their identification difficult perpetuates the scientific interest for resolving of their taxonomy, morphological and genetic diversity, and phylogenetic relations between them (Freyhof et al. 2000; Kottelat and Persat 2005; Lusk et al. 2005; Mendel et al. 2008, 2012; Takács 2012; Nowak et al. 2013, 2014; Szlachciak and Nowak 2015; Zangl et al. 2020). The structure of the vertebral column offers a powerful tool in resolving taxonomic distinction between gudgeon species (Naseka 1996, 2001; Talabishka 2014). The total number of vertebrae in gudgeon species is usually provided as a meristic count, merely a character used in either description or diagnosis of the species (Karaman 1924; Drensky 1926; Kattoulas et al. 1973; Grupče and Dimovski 1975; Dimovski and Grupče 1974, 1976a, 1977). Species within *Gobio* can have both low (e.g., 38–39 in *Gobio maeandricus* Naseka, Erk'akan

Table 2. MCA Test values of active and supplementary variables of the first three dimensions (F_1 – F_3) of *Gobio* and *Romanogobio* species.

Variables/dimensions	F_1	F_2	F_3
<i>G. balcanicus</i>	5.2747	-2.4917	-6.0344
<i>G. ohridanus</i>	7.2362	3.0602	5.0598
<i>R. banarescui</i>	-6.1045	6.6366	-2.1616
<i>R. stankoi</i>	-4.3550	-7.1959	2.8035
<i>Gobio</i>	10.0791	0.5389	-0.6185
<i>Romanogobio</i>	-10.0791	-0.5389	0.6185
T-37	2.5814	-1.0021	-1.9610
T-38	7.2024	1.3744	3.3163
T-39	-2.1713	0.8810	-6.6659
T-40	-5.1138	-1.9306	5.4694
A-18	-1.4083	2.9341	0.0652
A-19	-7.6140	3.7337	-1.5859
A-20	4.3842	-7.0118	0.6810
A-21	5.5227	3.6861	1.3550
a_1 -10	3.4333	-0.7142	-6.1559
a_1 -11	-2.2183	1.4762	5.4949
a_1 -12	-1.5473	-1.5629	-0.0443
i-3	-0.7498	-0.0191	1.2853
i-4	-3.6924	-0.8966	0.5179
i-5	4.5125	0.9844	-1.4389
C-17	4.7232	1.6875	2.2329
C-18	7.6394	1.5278	0.9230
C-19	0.9732	-4.9035	-4.6068
C-20	-6.1563	-0.8856	-0.6382
C-21	-4.7953	3.1625	2.8794
c_1 -0	3.1875	3.0305	1.3755
c_1 -1	5.4977	-3.5517	2.2954
c_1 -2	-1.4358	-4.3843	-1.2417
c_1 -3	-5.2658	6.0253	-2.2559
c_1 -4	-1.8533	3.1669	0.9649
c_2 -15	1.7473	-1.1717	-2.8202
c_2 -16	4.4425	0.4983	2.2279
c_2 -17	2.8343	4.9311	-2.6849
c_2 -18	-4.4188	-2.2599	-1.5547
c_2 -19	-2.3886	-4.5805	5.6978
D-A-9	-0.3529	-1.8554	-0.8431
D-A-10	-0.4792	-3.5798	3.7303
D-A-11	-0.1206	3.5399	-1.0774
D-A-12	1.1978	1.0468	-3.9830
D-C1-7	-2.0285	2.8691	0.2018
D-C1-8	-7.4095	2.0236	-0.7995
D-C1-9	2.5828	-4.4943	2.1404
D-C1-10	6.3905	1.2563	-0.7728
D-C1-11	2.3469	0.7953	-2.2733

Abbreviations and grouping are given in Material and methods. Values displayed in bold are significant at the level $\alpha = 0.05$.

et Küçük, 2006, 38–40 in *G. bulgaricus*) and a high total number of vertebrae (e.g., 39–41 in *Gobio battalgilae* Naseka, Erk'akan et Küçük, 2006 and *G. gobio*, 40–42 in *Gobio microlepidotus* Battalgil, 1942) (see Naseka et al. 2006; Szlachciak and Ząbkiewicz 2008). The ancestral vertebral structure of Gobionidae has a low total number of vertebrae (Naseka, 1996). *Romanogobio* has undergone a specialization by increasing the total number of vertebrae, however, this count (36–39) is low within Gobionidae, yet higher than *Gobio* (Naseka, 1996). The results in this study showed that the total number of ver-

tebrae in *R. stankoi* (39–40) and *R. banarescui* (38–40) is higher than in *G. balcanicus* (37–39) and *G. ohridanus* (37–39, thus confirming their intergeneric difference suggested by Naseka (1996) (Fig. 1).

As the total number of vertebrae does not reflect the internal structure of the vertebral column (Ford 1937), only recently it has been analyzed for systematic purposes in Gobionidae, revealing that three states of abdominal–caudal ratio (A:C) can be noticed within *Romanogobio* and *Gobio*: an equal number of abdominal and caudal vertebrae ($A = C$), the prevalence of the abdominal over the caudal vertebrae ($A > C$) and prevalence of the caudal over the abdominal vertebrae ($C > A$) (Naseka 1996). The latter is the synapomorphy of *Romanogobio* that supported its re-evaluation as a distinct genus (Naseka 1996). The literature data from other studies mainly follow this distribution pattern of A: C ratio with few exceptions (see Table 3). *G. balcanicus* and *G. ohridanus* in this study show only prevalence of the abdominal vertebrae ($A > C$) (Table 3), with the abdominal region of *G. ohridanus* being longer than in *G. balcanicus* (Fig. 1, Table 4). This is due to the high number of abdominal vertebrae (21) in *G. ohridanus* (Fig. 1), as well as the lower number of predorsal vertebrae (10) in *G. balcanicus* (Tables 2, 4). On the other hand, even though both *R. stankoi* and *R. banarescui* have all three states of abdominal–caudal ratio, the caudal region is relatively longer than the abdominal in both taxa (Fig. 1, Tables 3, 4), but longest, up to 21 vertebrae, in *R. banarescui* (Fig. 1, Tables 3, 4). The results of these studies, once again confirmed the usefulness of the A:C ratio in determining the intergeneric difference and should be further pursued to unlock its taxonomic potential within *Gobio* and especially *Romanogobio* species where different A:C ratios are present within one sample of the population (Table 3).

Table 3. Relative frequency of abdominal–caudal ratio in different species of *Romanogobio* and *Gobio* including results of this study.

Species	A = C	A > C	C > A
<i>G. gobio</i> ^[1]	7%	81.4%	1.6%
<i>G. soldatovi</i> ^[1]		100%	
<i>G. delyamurei</i> ^[3]		100%	
<i>R. uranoscopus</i> ^[1]	45%	30%	25%
<i>R. kesslerii</i> ^[1]	46%	4%	50%
<i>R. ciscaucasicus</i> ^[1]	31%	22%	47%
<i>R. pentatrichus</i> ^[4]	39%	28%	33%
<i>R. albipinnatus</i> ^[2]		2%	98%
<i>R. belingi</i> ^[2]	10%	3%	87%
<i>R. vladikovii</i> ^[2]	55%	9%	36%
<i>R. tanaiticus</i> ^[2]			98%
<i>R. parvus</i> ^[5]	28%		72%
<i>R. benacensis</i> ^[6]		100%	
<i>G. ohridanus</i> (this study)		100%	
<i>G. balcanicus</i> (this study)		100%	
<i>R. banarescui</i> (this study)	5.5%	2.7%	91.6%
<i>R. stankoi</i> (this study)	30.6%	19.4%	50%

Abbreviations are given in Material and methods. Superscript explanation: [1] Naseka (1996); [2] Naseka (2001); [3] Freyhof and Naseka (2005); [4] Naseka et al. (2002); [5] Naseka and Freyhof (2004); [6] Jelič et al. (2018).

Table 4. Relative frequency of modal numbers in vertebral column in different species of *Gobio* and *Romanogobio* including results from this study.

Characters/ Species	A% of T	a ₁ % of T	a ₁ % of A	i% of T	i% of A	C% of T	c ₁ % of T	c ₁ % of C	c ₂ % of T	c ₂ % of C
<i>G. gobio</i> ^[1]	52	28	53	12	22	48	5	10	43	90
<i>G. soldatovi</i> ^[1]	53	28	53	11	21	47	3	6	44	94
<i>G. coriparoides</i> ^[1]	54	28	52	13	24	46	3	6	44	94
<i>R. uranoscopus</i> ^[1]	50	30	59	10	20	50	5	9	45	91
<i>R. kessleri</i> ^[1]	49	28	56	12	25	51	8	15	44	85
<i>R. ciscaucasicus</i> ^[1]	50	26	53	12	24	50	5	11	45	89
<i>R. persus</i> ^[1]	49	27	55	11	23	51	5	9	47	91
<i>R. albipinnatus</i> ^[2]	47	—	—	—	—	53	11	21	42	79
<i>R. tanaiticus</i> ^[2]	46	—	—	—	—	54	12	22	42	78
<i>R. belingi</i> ^[2]	48	—	—	—	—	52	10	19	42	81
<i>R. vladykovi</i> ^[2]	50	—	—	—	—	50	7	14	43	86
<i>G. bulgaricus</i> ^[3]	54	26	48	—	—	46	4	8	—	—
<i>G. carpathicus</i> ^[4]	51	25	49	8	16	49	5	11	44	89
<i>R. uranoscopus</i> ^[4]	49	25	52	9	18	51	4	7	47	93
<i>R. kessleri</i> ^[4]	44	25	57	9	21	54	9	16	46	84
<i>R. vladykovi</i> ^[4]	48	25	52	9	19	52	7	13	45	86
<i>G. ohridanus</i> ^[5]	54	29	54	11	21	46	2	5	44	95
<i>G. balcanicus</i> ^[5]	52	27	52	11	22	48	4	7	44	93
<i>R. stankoi</i> ^[5]	49	28	57	10	21	51	4	9	46	91
<i>R. banarescui</i> ^[5]	48	28	58	10	21	52	7	14	44	86

Abbreviations are given in Material and Methods. Superscript explanation: [1] Naseka (1996); [2] Naseka (2001); [3] Naseka et al. (2006); [4] Talabishka (2014); [5] this study.

In addition to the abdominal and caudal regions, the number of vertebrae in their respective subunits plays an important role in the taxonomic distinction between *Gobio* and *Romanogobio* species (Naseka 2001; Naseka et al. 2006; Bogutskaya et al. 2013). The increased number of preanal vertebrae (with consequent increase of the caudal vertebrae) is another synapomorphic character that distinguishes *Romanogobio* from *Gobio* (see: Naseka 1996). The preanal subregion has its external morphological reflection in the position of the anus, being closer to the anal fin in *Gobio* and distant from the anal fin in *Romanogobio*, favoring it as an important taxonomic key character (Naseka 1996; Naseka and Freyhof 2004; Kottelat and Freyhof 2007; Bogutskaya et al. 2013; Friedrich et al. 2018). Preanal vertebrae in some *Romanogobio* species (e.g., *R. kessleri*) comprise 8%–9% of the total number of vertebrae, while in others (e.g., *Romanogobio tanaiticus* Naseka 2001, *Romanogobio belingi* (Slastenenko, 1934), and *R. albipinnatus*) they are high in number up to 10%–12% of the total number of vertebrae (Table 4). It is hypothesized that the low value of preanal vertebrae (plesiomorphic characteristic) in *R. uranoscopus* (4%–5% of T) (Table 4) is due to its specialization towards rapid waters that moves the anal fin forward, elongating the caudal peduncle at the expense of preanal vertebrae (Talabishka 2014). The elongation of the postanal subregion (up to 47% of the total number of vertebrae) (Table 4), which was seen as ecological adaptation, is one of the osteological peculiarities that Talabishka (2014) used to propose a revision of all gudgeon species previously assigned under the species name *uranoscopus*.

The presently reported study of *Romanogobio* species showed that *R. banarescui* has up to 4 preanal vertebrae

that comprise 7% of the total number of vertebrae. On the other side, *R. stankoi* has a lower number of preanal vertebrae (4% of T), contributing to a significantly higher number of postanal vertebrae (19) that comprise 46% of the total number of vertebrae (Tables 1, 4; Fig. 1). Concerning *Gobio* species examined in this study, the preanal subregion in *G. ohridanus* is one of the shortest (2% of the total number of vertebrae) (Table 4) due to the frequent character state of absence of preanal vertebrae (Fig. 1). Therefore, the preanal subregion is shorter in *G. ohridanus* than in *G. balcanicus*, even though both species have identical values of range (0–2 vertebrae) and mode (1 vertebra) (Table 1). Moreover, this study showed that the D–C1 distance in *R. banarescui* was the shortest (7 vertebrae), while in *G. ohridanus* it was the longest (10–11 vertebrae) (Fig. 1), which is in line with the high number of preanal vertebrae in the first species and their absence in the second one (Fig. 1; Table 1), strongly confirming again the importance of the number of preanal caudal vertebrae as a key taxonomic character in Gobionidae.

Conclusions

In this publication, for the first time, the vertebral formula of 4 gudgeon species is presented, and also for the first time, the structure of the vertebral column is analyzed through MCA analysis, which enables defining the closely associated character states with the analyzed taxa. The genera herein fall in line with the already known conditions of low total vertebrae and dominance of the abdominal region in *Gobio*, and the high total number of vertebrae with the dominance of the caudal region in *Romanogobio*.

Within genus *Gobio*, the character state of 10 predorsal vertebrae in *G. balcanicus* contributes to this subregion being shorter than in *G. ohridanus*, where 11 vertebrae without variation are present. The abdominal region of *G. ohridanus*, closely associated with 21 vertebrae, is longer than that of *G. balcanicus* (20 vertebrae). Due to the character state of 0 preanal vertebrae, more frequently noted in *G. ohridanus*, the preanal subregion is shorter than in *G. balcanicus*. Within *Romanogobio* the preanal subregion is significantly longer in *R. banarescui* (closely associated with 3 and 4 vertebrae) than in *R. stankoi*. And finally, *R. stankoi* is associated with 19 postanal vertebrae displaying elongation of the postanal subregion compared to *R. banarescui*. So, based on the structure of the vertebral

column, *R. stankoi* and *R. banarescui* considerably differ and we offer a hypothesis that they are not conspecific—an opinion that should be further investigated using morphological, osteological, and molecular markers.

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Information source preferences of small-scale fishers in the Aegean Sea coast of Turkey

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Abstract

Information source preferences of small-scale fishers can play a role in decision-making processes and affect the sustainability of small-scale fisheries. In this respect, determining useful communication tools to eliminate the information gaps and lack of information of fishers is important for sustainable and effective fisheries management. The purpose of this study was the determination of the preferred source of information and priorities of the small-scale fishers who operate along the Aegean Sea coast of Turkey. Data were collected from a random sample of 278 small-scale Turkish fishers located along the Aegean coastline via face-to-face interviews. Information source preferences of fishers were determined by Repertory Grid Technique. In the analysis, obtaining information about fisheries focused on three criteria such as marine ecology, fisheries technology, and fisheries policies. The level of importance given by fishers for each criterion was determined. Fishers prefer to get information from other fishers, followed by fishery cooperatives, and their own experiences (χ^2 (11, $n = 278$) = 1305.920, $P < 0.001$). Fishery cooperatives are the closest organizations to fishers. The use of cooperatives as a source of information can be interpreted as an element that can facilitate access to information when evaluated through the “availability” of behavioral economics. Fishery cooperatives, which stand out in fishers’ information source preferences, have the potential to be a valuable source of information in all aspects. The results of the research are thought to benefit researchers from non-governmental organizations, research institutes, and universities that carry out national and international projects with fishers.

Keywords

Availability, fishery cooperatives, information behavior, repertory grid technique, behavioral economics

Introduction

The correct and proper use of information sources is important for sustainable fisheries management to train, raise the awareness of fishers, or meet their information needs. Any individual needs an information (Hisyam Selamat and Choudrie 2004; Wilson 2006). The term “information” is an abstract concept used to denote any amount of data, code or text stored, sent, received, or manipulated in any medium (Capurro and Hjørland 2003).

Information is a tool used to reduce uncertainties in in-active action selection and is the necessary input in decision-making processes (Solano et al. 2003). There is a direct relation between the quality of the information used by a decision-maker and decision-making performance, and between the availability of information and the sharpness of the decision (O’Reilly 1982). The individuals’ perception of “useful” knowledge leads him/her directly to the “useful” decision-making process (Streufert 1973). Therefore, the right decision does not guarantee a good outcome; such

pragmatism has paid off (Buchanan and O'Connell 2006). The limited knowledge of the individual reveals the need for information in the decision-making process (Simon 1955).

The information gap appears in the case of semantic inconsistency or systematic deficiency in the individuals' social environment. Information, assistance, or a link is needed to eliminate this gap (Case 2002). Lack of knowledge, skills, or abilities required for adequate access, interpretation, and application of information reveals the lack of information (Britz 2004). The information gap is one of the factors affecting political participation (Cho and McLeod 2007). The success of the Ecosystem Approach to Fisheries (Anonymous 2003, 2005, 2012; Dimech et al. 2014; Ünal et al. 2018) which considers co-management approach and stakeholder participation into management process (Berkes 2009; Claudet and Guidetti 2010) for the sustainable management of natural resources, particularly the management of marine living resources is getting more important and remarkable. In this respect, the information gap may negatively affect stakeholder engagement, thus may prevent the achievement of sustainable fisheries management targets.

In the literature, information source preferences are evaluated within the framework of information behavior (Wilson 2000; Case 2002; Dawes and Sampson 2003). Information behavior is the interaction (information seeking and access to information) of several potential sources that can respond to individuals' interests and information needs (Wilson 2000). Some researchers (Julien and Michels 2004; Remenova and Jankelova 2019) have pointed out that information behavior and decision-making processes are intertwined. It is stated that information behavior is also associated with externality (Swinton and Lowenberg-DeBoer 1998; Osei et al. 2017).

On the other hand, there are few studies on the choices and preferences of information sources (Fourie 2009). There are also very few studies, especially on the information behavior of fishers (Ikoja-Odongo and Ocholla 2003; Dutta 2009; Okwu et al. 2011). There may be a relation between fishers' information source preferences and sustainable small-scale fisheries. This situation arises from the potential information gap and lack of information on fishers and thus, from the information behavior of fishers. Reviewing the literature, we haven't found any direct research which considers information source preferences of small-scale fishers neither in Turkey nor in the Mediterranean.

The purpose of this research was the determination of the preferred source of information and priorities of the small-scale fishers operating at the Aegean Sea, Turkey. Because it is important to identify the sources of information for fishers located in the coastal-rural areas, to use accurate and sufficient communication resources, knowledge generated by the research can provide insight into policymakers, non-governmental organizations, and researchers to improve acceptable, less costly, and applicable policy interventions for supporting sustainable fisheries.

Materials and methods

Study area and data collection

The primary material of the research is the data obtained from small-scale fishers operating at the Aegean Sea, Turkey. It has been determined that the number of registered small-scale fishing vessels (<12 m) in the Aegean Sea is 4355 (Anonymous 2014). Proportional sample volume formula was utilized in determining the number of small-scale fishers to be interviewed (Newbold 1995). To reach the maximum sample volume, p : 0.50 and $(1-p)$: 0.50 are accepted. The number of fishers interviewed was calcu-

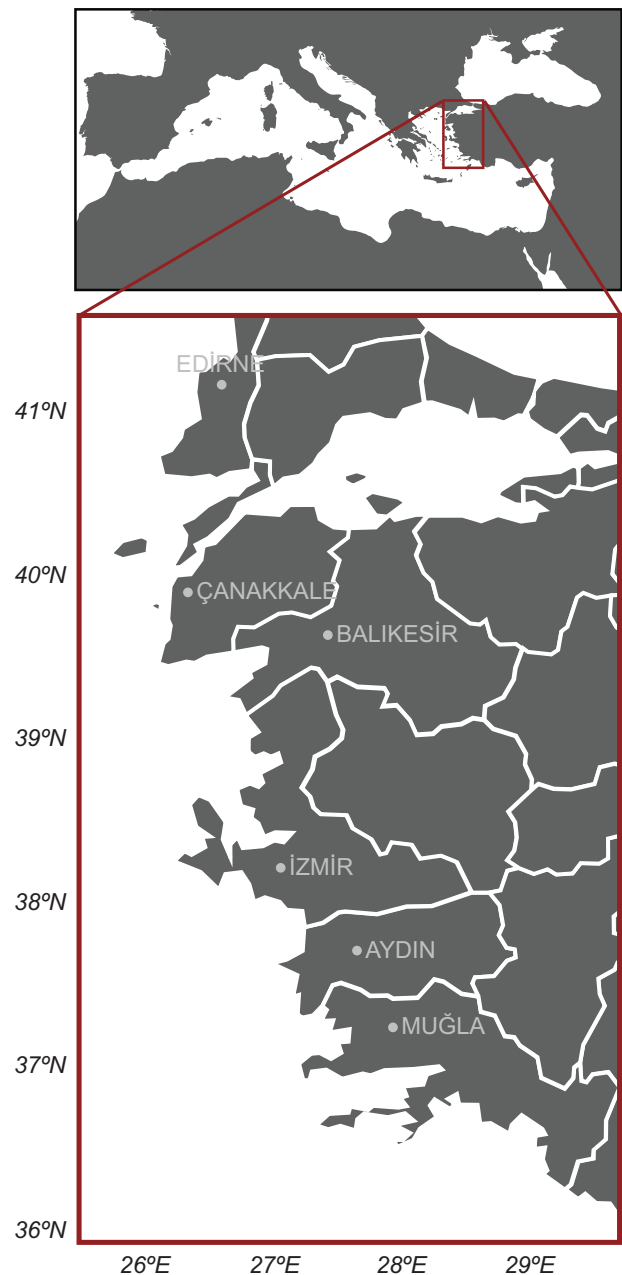


Figure 1. Study area. Ocean Data View.

lated as 278, taking into account the 99% confidence interval and a 7.5% margin of error.

The sample volume was distributed proportionally according to the provinces Edirne ($n = 9$), Çanakkale ($n = 9$), Balıkesir ($n = 8$), İzmir ($n = 143$), Aydın ($n = 13$), and Muğla ($n = 96$), respectively (Fig. 1). All interviews were carried out at the 62 fishing ports along the Aegean sea coast of Turkey in 2016, face-to-face, by an experienced interviewer. In order not to be affected by other fishers, face-to-face interviews were conducted in an isolated environment.

Information source framework

As a result of uncertainty, experienced individuals need to use personal and non-personal or internal and external sources of information (Kuhlthau 1993). Accordingly, the findings of Kuhlthau (1993) have been used to frame information source alternatives in the presently reported study. Criteria and information source alternatives are given in Table 1. Information sources (alternatives) are; Coast Guard, scientists (universities, institutes), non-governmental organizations (NGOs), television, and radio broadcasting, Other fishers (other small-scale fishers at the same port and/or colleagues), ministry, print media (newspaper, magazine), fishery cooperative, family members, fishing gear vendors, social media (Facebook, Twitter, Instagram, etc.) and fisher's own experience. The three criteria considered by fisheries experts as important components of the fisheries are; marine ecosystem, fisheries technology, and fisheries policy.

Table 1. Information sources (alternatives) and criteria for information preference.

Criteria	Information source (Alternatives)			
Marine Ecosystem	Fishery cooperative	Print media	Ministry	Coast guard
Fisheries Technology	Fishing gear vendors	Television and radio	Social media	NGOs
Fisheries Policy	Family members	Fisher's own experience	Other fishers	Scientists

The following questions were asked the fishers in the presently reported study: 1) To what extent would you prefer the information sources (alternatives) for the marine ecosystem? 2) To what extent would you prefer the information sources (alternatives) for the fisheries technology? 3) To what extent would you prefer the information sources (alternatives) for the fisheries policy? 4) Please rate the importance level for each criterion.

Fishers evaluated the importance levels (1: not important, 5: very important) that they gave to each three criterion and preference levels (1: strongly disagree, 5: strongly agree) for twelve alternatives on the five-point Likert (1932) type scale.

Data analysis

Information sources preferences of small-scale fishers were determined by the Repertory Grid Technique (RGT). In the technique, obtaining information about fisheries is discussed from three criteria. The level of importance given by fishers to 12 information sources for each criterion was determined. RGT is a useful cognitive mapping approach to assess how individuals and groups derive meaning from the people and objects around them (Walsh 1995). RGT, which is based on Kelly (1955) personal construct theory, is a method used to reveal personal fiction about his/her thoughts on a subject presented to people. Personal fiction theory is based on expectations for future events that were based on past experiences or based on similar events (Kelly 1955).

According to this theory, there is no absolute truth or objective reality (Ilbery and Hornby 1983). Each fiction has bi-polar construction (Curtis et al. 2008). The matrix used in RGT helps individuals to express themselves more quickly and better (Fransella et al. 2004). In short, RGT reveals what people think in their world (Tan and Hunter 2002). Although RGT is generally used by psychologists (Saúl et al. 2012), it is a frequently used method in agricultural economics, sociology, and marketing studies (Ilbery and Hornby 1983; Neimeyer 1993; Coakes et al. 1999; Bourne and Özbilgin 2008).

In the RGT, scores are assigned to each alternative/criterion combination. The scores obtained in the next stage were weighted according to the relative importance of the criterion. Finally, the aggregated scores were summed, and the total score for each alternative was obtained. The magnitude of the scores obtained gave the priorities of the alternatives.

In the meantime, the Friedman (1937) test was used to compare the priorities of information sources and information sources preferences in the current study.

Results

Table 2 presents the criteria for evaluating information source preferences of fishers. It is seen that the Friedman test was statistically significant. This result indicates that the importance of some information criteria is higher than others. Accordingly, it is determined that the most important information criterion for fishers is fishing technology, followed by marine ecosystem, and fisheries policies ($\chi^2(2, n = 278) = 67.329, P < 0.001$).

Table 2. Information source evaluation for criteria.

Criteria	Mean*	Std Dev
Fisheries Technology	4.14	1.16
Marine Ecosystem	3.37	1.38
Fisheries Policy	3.31	1.39

* indicates significance level is 0.05; 1: Not all-important, 5: Very important

When the priorities of information sources are evaluated according to the above criteria, the Friedman test was statistically significant ($\chi^2 (11, n = 278) = 1305.920, P < 0.001$). This result indicates that some information sources are more prominent than others in terms of fisher preferences. In the light of such information, it is possible to say that fishers prefer to get information from other fishers. Other primary sources of information for fishers were fishery cooperative and their own experience (Table 3). As a source of information about fisheries, non-governmental organizations, family members, and fishing gear vendors were in the last place.

Table 3. Information source preferences.

Information source	Mean*	Std. Dev.	Ranking
Other fishers	45.71	13.60	1
Fishery cooperative	44.19	15.91	2
Fisher's own experiences	41.43	12.53	3
Television and radio	40.45	16.02	4
Scientists	38.62	16.07	5
Ministry	36.54	15.79	6
Print media	34.67	16.44	7
Social media	26.97	18.55	8
Coast Guard	26.22	13.64	9
NGOs	24.59	14.57	10
Family members	22.82	15.20	11
Fishing gear vendors	19.86	11.05	12

* indicates significance level is 0.05 according to the Friedman test.

Discussion

According to our findings, other fishers were found as the primary information source in the study area. This result supports the findings of Smith and Hanna (1993). Because, according to these researchers, face-to-face information transfer in fisheries is one of the most important reasons for the continuation of the fishing community.

Fishing technology has been determined by fishers as the most important information criterion. This may be due to the fishers' unlimited interest in increasing the Catch Per Unit Effort (CPUE) and the eagerness to receive such information. Another important finding obtained from the survey is that fishers considered the importance of knowledge criterion about marine ecosystem moderately important. However, during the fieldwork, it was observed that fishers have knowledge gaps, especially in subjects such as gonadal sex differentiation, seagrass beds, taxonomic identification. Therefore, future studies that focus on local ecological knowledge (Farr et al. 2018) in the study area should consider fishers' knowledge gaps on the marine ecology. On the other hand, the criterion of information about fisheries policies was found to be low, with no difference with the marine ecosystem criterion, in the study. Information on fisheries policies includes practices that can indirectly raise fishers' CPUE. At the same time, neglecting information about fisheries policies may prevent the development and implementation of the co-management approach. In fact, one outcome of the current fisheries management shows that fishers care about fish-

ing policies only moderately. However, in order to play a role in fisheries management, fishers are also expected to show more interest in relevant policies. Here the question arises: do current management practices hinder fishers' eagerness to learn relevant policies? Considering the results of the study, we can claim that the present fisheries management system does not support fishers in this regard in the study area.

The fishery cooperative is among the primary information choices. Cooperatives are organizations established and managed by themselves. Therefore, it can be said that fishers see cooperatives as entities that are close to them. The concept of availability in behavioral economics (Thaler and Sunstein 2008) can be seen as a phenomenon that can facilitate access to information. Using fishery cooperatives as an information source can be interpreted as a factor that can facilitate access to information. Hence, it is an expected result that cooperatives, which are a cumulative source of information, come to the fore in fishers' preferences. Differently, it is necessary to clarify that the academy and ministry were in the middle of the preferences list. Factors such as the fishers' inadequate relations with the academy and the ministry, choosing to avoid these two institutions or having problems with these institutions may have played a role in this preference. Still, at this stage, they are only assumptions and have no scientific basis. For this reason, future research should focus on the information sources of fishers as well as reasons or factors affecting these preferences.

Curtis et al. (2008) pointed out some drawbacks of the RGT such as being cognitively demanding, being limited to the specified scope, monotony. In RGT rating, ranking, and dichotomizing commonly are used to link alternative and criterion (Tan and Hunter 2002). The reason we use a rating scale in this study is that it is relatively less cognitively demanding compared to the other two methods. It would not be wrong to say that a situation that is less cognitively demand can trigger System 1 (Frankish 2010) and offer clues on the irrationality of the behavior.

Individuals consider resource preferences in the early stages of the information-seeking process. Decisions for questioning the suitability of information are made in the process of using the next stage of information (Savolainen 2008). However, the information behavior on decision making has been a controversial issue because of cognitive bias (Allen 2011). When a reasoned choice is made by a reasonable individual using relevant information about the advantages and disadvantages of all the possible courses of action, in accord with the individuals' beliefs, there is an informed decision (Bekker et al. 1999). Therefore, it is not possible to talk about a pattern that will suit every segment of society in information preferences. Hence, another issue that should be emphasized in future studies on determining fishers' information source preferences should be taking into account the cognitive biases (Thaler and Sunstein 2008) and other psychographic features such as personal values and attitudes of fishers.

Conclusion

Consequently, while developing policy interventions for fishers' information behavior, it may be advisable to do this through other fishers (leader fishers, reputable fishers) among fishing cooperatives. The peer effect among fishers is observed as a significant phenomenon (Felthoven et al. 2014). Institutional stakeholders who work in the study area can initiate information campaigns to close information gaps of fishers by focusing on those leaders and reputable fishers in cooperatives. In this way, access to accurate, correct and usable information of the fishers' community in the region can be provided. It is thought that the results obtained from the research will benefit NGOs, policymakers, and researchers to improve acceptable, less costly, and applicable policy interventions for supporting sustainable fisheries management.

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agement. Besides, the Ministry of Agriculture and Forestry, which is officially responsible for fisheries management, and the Coast Guard Command, which carries out protection-control-surveillance services, are among the leading institutions that can benefit from the outputs of the presently reported study. Besides, as required by terms of references, both institutions should continuously inform fishers.

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First records of the bandfin scorpionfish, *Scorpaenopsis vittapinna* (Actinopterygii, Scorpaeniformes, Scorpaenidae), from Australia

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Abstract

Seven specimens (15.6–43.5 mm standard length) of *Scorpaenopsis vittapinna* Randall et Eschmeyer, 2002 (Scorpaenidae), a widely distributed Indo-West Pacific species, are recorded from Australian waters for the first time. A full description of the specimens is given, and intraspecific variations in comparison with the type specimens are noted.

Keywords

Australia, description, distribution, morphology, new records, variations

Introduction

The Indo-Pacific scorpionfish genus *Scorpaenopsis* Heckel, 1837 (Scorpaenidae), currently including 28 valid species (Fricke et al. 2013), is characterized by 12 dorsal-fin spines, three or more suborbital spines, and absence of palatine teeth (Randall and Eschmeyer 2001).

Seven small scorpionfish specimens collected from northwestern and northeastern Australia, found recently by HM in the collections of several Australian museums, are herein identified as *Scorpaenopsis vittapinna* Randall et Eschmeyer, 2002, a widely distributed Indo-West Pacific species, although not previously recorded from Australian waters. The first recorded specimens from the Australian region are described herein.

Methods

Counts and measurements followed Randall and Eschmeyer (2001). The last two soft rays of the dorsal and anal fins were counted as single ray, respectively, each pair being associated with a single pterygiophore. Standard length and head length are expressed as SL and HL, respectively. Descriptive characters are based on the Australian specimens. Two specimens (AMS I. 33728-021, 35.9 mm SL and QM I. 15552, 24.7 mm SL) were not measured because of their widely opened mouths. Institutional codes follow Sabaj (2019).

Results

Family Scorpaenidae Risso, 1827 *Scorpaenopsis* Heckel, 1837

Scorpaenopsis vittapinna Randall et Eschmeyer, 2002

[English name: bandfin scorpionfish]

Figs 1–3; Table 1

Material examined. (7 specimens, 15.6–43.5 mm SL). AMS I. 18740-026, 2 specimens, 42.5–43.5 mm SL, Yonge Reef, Lizard Island, Coral Sea, Queensland (Qld), 14°36'S, 145°36'E, 10–12 m, D. Hoese et al., 8 Nov. 1975; AMS I. 33728-021, 35.9 mm SL, outer slope on west side of Ashmore Reef, Coral Sea, Qld, 10°16'11"S, 144°24'07"E, 11–15 m, FNQ Team, 21 Jan. 1993; NTM S. 12319-029, 15.6 mm SL, east channel in Ashmore Reef, Timor Sea, Western Australia (WA), 12°08'S, 123°06'E, 11–12 m, H. Larson, 20 Sep. 1987; NTM S. 13585-027, 20.7 mm SL, outer reef slope of Great Detached Reef, Coral Sea, Qld, 11°42'36"S, 144°04'00"E, 21–23 m, H. Larson et al., 12 Jan. 1993; QM I. 15552, 24.7 mm SL, west of Raine Island, Coral Sea, Qld, 11°36'S, 144°01'E, team of AIMS, AM, and QM, 13 Feb. 1979; WAM P. 28022-012, 42.3 mm SL, Clerke Reef, Rowley Shoals, WA, 17°18'S, 119°22'E, 7–12 m, G. Allen and R. Steene, 4 Aug. 1983.

Description. Body moderately elongate, depth 2.8–3.0 in SL; width 1.4–1.7 in body depth. Head length 2.1–2.2 in

SL; snout length 3.3 in HL. Orbit diameter 4.3–4.6 in HL. Interorbital width 5.8–6.9 in HL. Dorsal profile of head not arched (Fig. 1). Dorsal fin with 12 spines and 9 soft rays; fifth or sixth spine longest; all soft rays branched, second longest; posterior branch of last soft ray joined by membrane to caudal peduncle for about three-fourths its length. Origin of dorsal fin above supracleithral spine. Anal fin with 3 spines and 5 soft rays; first spine shortest, second longest; all soft rays branched, second longest; origin of first anal-fin spine below base of eleventh dorsal-fin spine. Pectoral fin with 1 uppermost unbranched ray, 4 or 5 branched rays, 12 or 13 lower unbranched rays (all rays unbranched in two smallest specimens 15.6–20.7 mm SL), total 18 rays (17 in one specimen 24.7 mm SL), eighth or ninth rays longest. Pelvic fin with 1 spine and 5 branched soft rays; second soft ray longest; last soft ray joined by membrane to abdomen for more than half its length. Caudal fin slightly rounded, with 13 principal rays. Lateral-line scales 20–22. Longitudinal scale series 41 or 42. Scale rows above lateral line in middle of body 5, below lateral line to base of first anal-fin spine 13 or 14. Gill rakers on upper limb of first gill arch 3–5 (mode 4), lower limb 8–10 (8) on ceratohyal and absent on hypobranchial, total 11–15 (12). Rakers short with small spinules, longest at angle about two-thirds length of longest gill filament. Branchiostegal rays 7.

Mouth large, slightly oblique, forming an angle of 20–25 degrees to horizontal axis of head and body. Posterior margin of maxilla slightly beyond posterior margin of or-

Table 1. Morphometrics (expressed as percentages of standard length) of *Scorpaenopsis vittapinna*.

	AMS I. 18740-026	AMS I. 18740-026	NTM S. 12319-029	NTM S. 13585-027	WAM P. 28022-012	Mean value
Standard length [mm]	42.5	43.5	15.6	20.7	42.3	
Body depth	36.0	35.2	35.3	33.3	35.2	35.0
Body width	24.0	23.4	20.5	21.7	24.8	22.9
Head length	46.4	45.5	48.7	48.3	45.9	47.0
Snout length	14.1	13.8	14.7	14.5	13.9	14.2
Orbit diameter	10.1	10.6	10.9	11.1	10.4	10.6
Interorbital width	7.1	6.7	8.3	8.2	6.6	7.4
Upper-jaw length	24.5	23.0	23.7	24.2	23.4	23.7
Postorbital length	23.1	23.7	23.7	23.2	23.6	23.5
Pre-dorsal-fin length	43.3	43.4	46.2	46.9	43.5	44.7
Pre-anal-fin length	66.1	69.0	66.7	69.6	67.1	67.7
Pre-pelvic-fin length	36.2	37.2	39.1	36.7	37.8	37.4
1 st dorsal-fin spine length	4.7	5.3	3.8	5.3	4.7	4.8
2 nd dorsal-fin spine length	7.8	8.5	6.4	8.2	7.3	7.6
Longest dorsal-fin spine length	15.3	15.9	16.0	15.9	15.1	15.7
11 th dorsal-fin spine length	9.4	10.3	6.4	8.2	8.7	8.6
12 th dorsal-fin spine length	13.9	—	13.5	13.0	12.5	13.2
Longest dorsal-fin soft ray length	20.5	20.0	16.0	17.9	22.5	19.4
1 st anal-fin spine length	10.4	11.7	—	11.1	9.0	10.5
2 nd anal-fin spine length	21.4	20.9	—	17.4	18.0	19.4
3 rd anal-fin spine length	18.4	18.4	16.0	15.0	16.1	16.8
Longest anal-fin soft ray length	22.8	21.6	—	17.9	22.0	21.1
Pectoral-fin length	36.0	34.0	—	30.9	31.0	32.9
Pelvic-fin spine length	16.9	17.0	17.3	17.9	16.5	17.1
Longest pelvic-fin soft ray length	26.4	26.0	25.0	24.6	25.5	25.5
Caudal-fin length	28.2	28.5	29.5	28.5	27.0	28.3
Caudal-peduncle length	17.9	17.5	16.0	15.9	18.2	17.1
Caudal-peduncle depth	11.8	11.7	12.2	11.6	11.3	11.7



Figure 1. Preserved specimen of *Scorpaenopsis vittapinna*, AMS I. 18740-026, 42.5 mm SL, Lizard Island, Queensland, Australia.

bit (not reaching the posterior orbit margin in two smallest specimens). Jaws with a band of slender, incurved, conical teeth; about 7 tooth rows at anterior of upper jaw and 6 in lower; band narrowing to 1 or 2 teeth posteriorly; teeth progressively longer inwardly. Vomer with short conical teeth, longer posteriorly, forming a V-shaped patch. Palatine teeth absent. Tongue thick, rounded, with fleshy tip and a median skeletal rim.

Interorbital ridges low, rounded, beginning with indistinct ridge from anterior interorbital space, conjoined level with tympanic spines posteriorly and forming a low ridge to anterior angular edge of occipital pit. Occipital pit very shallow. Nasal, preocular, supraocular, and postocular spines simple; postocular spine slightly canted laterally, base wide. Tympanic spine simple, pointed, slightly canted laterally; base joined to interorbital ridge or to parietal-spine base by low ridge. Parietal spine simple, base distinctly medial to tympanic spines. Nuchal spine simple, base continuous with parietal spine. A low transverse ridge posteriorly in occipital pit between bases of parietal and nuchal spines. Sphenotic with two small spines. Postorbital smooth, without pointed spines or with 1 or 2 tiny spines. Pterotic spine simple, located below parietal spine. Upper and lower posttemporal spines simple, upper shorter than lower. Supracleithral spine simple, with distinct ridge on dorsal margin. Cleithral spine flattened, strongly pointed. Upper opercular spine divided into 2 points (simple and divided into 3 points, respectively, in two specimens 15.6 and 35.9 mm SL), with low median ridges (Fig. 2A). Lower opercular spine simple, with distinct median ridge. Suborbital pit rimmed anteriorly by vertical lacrimal ridge, narrow, relatively deep, deepest anteriorly below anterior part of orbit. Suborbital ridge with four spines (five in one specimen 43.5 mm SL), first on lacrimal, small; first and second directed slightly ventrally. Preopercle with five spines, uppermost longest with a supplemental spine, second to fifth flat. Lacrimal

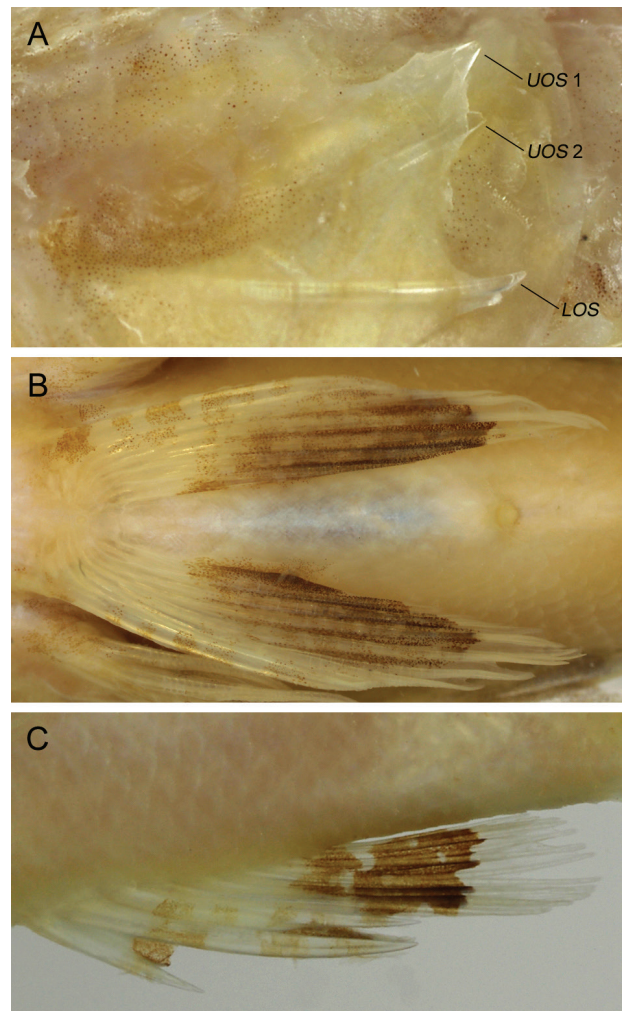


Figure 2. Preserved specimens of *Scorpaenopsis vittapinna*. (A) opercular spines of WAM P. 28022-012, 42.3 mm SL (UOS, upper opercular spine; LOS, lower opercular spine); (B) ventral view of pelvic fins of same individual as Fig. 1; (C) anal fin of same individual as Fig. 2A.

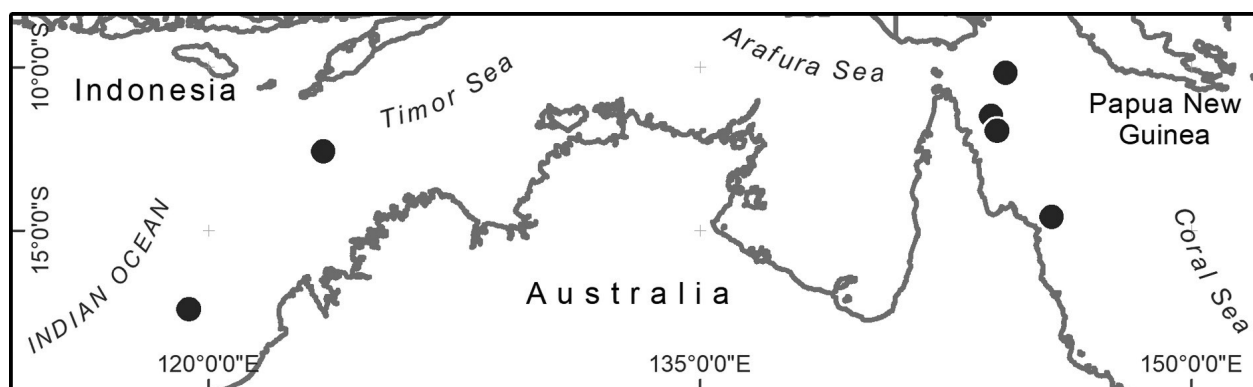


Figure 3. Distributional records of *Scorpaenopsis vittapinna* in Australian waters.

with two anterior parallel ridges. Anterior lacrimal spine simple, directed slightly anteroventrally. Posterior lacrimal spine simple (2 points in two specimens 35.9 and 42.5 mm SL), directed posteroventrally.

Lateral surface of body covered with ctenoid scales, becoming cycloid ventrally. Exposed cycloid scales covering pectoral-fin base and anteroventral surface of body. Cycloid or ctenoid scales embedded in thin skin covering cheek. Body scales not extending onto fin rays or membranes, except basally on pectoral and caudal fins. Ctenoid scales covering preopercle behind eye, below pterotic and lower posttemporal spines, and distal area between upper and lower opercular spines. Lateral line complete, first two scales with spine-like projection at end of sensory tube.

Underside of dentary with three sensory pores; single pores behind and on each side of lower-jaw symphyseal knob; a small pore behind nasal spine and on each of mid-interorbital ridge; some small pores associated with suborbital ridge and preopercular spine bases.

A short slender tentacle on posterior end of preocular spine base. A fleshy tentacle associated with supraocular spine, length about equal to orbit diameter. A slender tentacle posteriorly on parietal and nuchal spine bases. Many small papillae on outer margins of eye membrane. A tentacle, with several short branches along distal margin, on upper posterior edge of low membranous tube associated with anterior nostril, extending beyond posterior nostril when laid back. Anterior lacrimal spine associated with a short slender tentacle. Posterior lacrimal spine associated with a broad fleshy tentacle; its length greater than that of anterior nostril tentacle; posterior lacrimal spine tentacle linked posteriorly to head by fringed skin. A broad, thin skin flap associated with each of 3rd–5th preopercular spines. Many fleshy tentacles on ventral surface of head, anteriormost longest. A slender, fleshy tentacle centrally on cheek. Several slender tentacles associated with pored lateral scales, scattered on lateral surface of body.

Color of preserved specimens (based on all specimens). Head and body yellowish brown or whitish; faint brownish blotches below eye and dorsally on operculum;

ca. three faint broad brownish blotches dorsolaterally on body. Dorsal fin with small brownish spots scattered on basal membranes of spinous and soft-rayed portions in some specimens. Pectoral fin with some small brownish spots dorsally or dark brownish membranes between rays. Pelvic fin with broad dark brown medial band (Fig. 2B). Anal fin with broad dark brown medial band across soft rays (Fig. 2C).

Discussion

Scorpaenopsis vittapinna can be distinguished from all other congeners by the following combination of characters: pectoral-fin rays 17–19 (usually 18, rarely 19), longitudinal scale series 40–44; interorbital width 5.7–6.9 in HL; snout length 3.0–3.3 in HL; mouth slightly oblique; posterior margin of maxilla just reaching a vertical through posterior margin of orbit or slightly beyond (except in juveniles); occipital pit shallow; upper opercular spine usually with 2–4 points; lower opercular spine single; and a broad dark brown medial band on pelvic and anal fins (Randall and Eschmeyer 2001; this study).

The present specimens were identified as *S. vittapinna*, agreeing closely with the diagnostic features of the species given by Randall and Eschmeyer (2001). However, the Australian specimens differed slightly from the type specimens of *S. vittapinna* in the following characters (regarded here as intraspecific variations): head length 2.1–2.2 in SL (vs. 2.2–2.3 in SL in the latter); orbit diameter 4.3–4.6 in HL (vs. 4.5–5.0 in HL); and interorbital width 5.8–6.9 in HL (vs. 5.7–6.5 in HL). Although Randall and Eschmeyer (2001) and Motomura et al. (2011) indicated that *S. vittapinna* had the posterior maxilla margin just reaching a vertical through posterior margin of orbit or slightly beyond, and the upper opercular spine with two or more points, the two smallest of the present specimens (15.6–20.7 mm SL) had the posterior maxilla margin not reaching the posterior orbit margin (both specimens), and a simple upper opercular spine (the smallest specimen).

Randall and Eschmeyer (2001) indicated that the number of spinous points on the posterior lacrimal spine in *S. vittapinna* changes with growth [simple in juveniles, two points in adults (largest recorded size 65 mm SL)]. The three apparently juvenile Australian specimens (15.6–24.7 mm SL) had simple posterior lacrimal spines, the remaining (subadult) specimens having double (35.9 and 42.5 mm SL) and simple (42.3 and 43.5 mm SL) posterior lacrimal spines. In addition, Randall and Eschmeyer (2001) also indicated that the second to fifth pectoral-fin rays are branched. However, of the present specimens, the two smallest (15.6–20.7 mm SL) had all pectoral-fin rays unbranched, the largest (43.5 mm SL) had 5 rays branched, and the remaining specimens (24.7–42.5 mm SL) had 4 rays branched, suggesting that the number of branched pectoral-fin rays in *S. vittapinna* tends to increase with growth, a well-known feature of Scorpaenidae (e.g., genus *Scorpaena*) (Motomura et al. 2005; Wibowo et al. 2019).

Scorpaenopsis vittapinna is widely distributed in the Indo-West Pacific, having been recorded from the following localities: Red Sea, South Africa (type locality), Mauritius, Comoro Islands, Seychelles, Maldives Islands, Indonesia, Philippines, Papua New Guinea, Caroline Islands, Coral Sea (New Caledonia), Fiji, Wallis and Futuna Islands, Samoa, French Polynesia (Randall and Eschmeyer 2001); Ryukyu Islands, Japan (Motomura et al. 2004); Madagascar (Fricke et al. 2018); Taiwan (Moto-

mura et al. 2011); and Marquesas Islands (Delrieu-Trottin et al. 2015), occurring on coral reefs or rubble bottoms in depths 0–40 m (Randall and Eschmeyer 2001). The present specimens, being the first records from Australian waters, were distributed from Rowley Shoals, Western Australia to Lizard Island, Queensland in depths of 10–23 m (Fig. 3).

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Temporal stability in the otolith Sr:Ca ratio of the yellow croaker, *Larimichthys polyactis* (Actinopterygii, Perciformes, Sciaenidae), from the southern Yellow Sea

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Abstract

Otolith chemical signatures are sufficiently stable across time to allow for accurate stock classification. The classification of the southern Yellow Sea population for *Larimichthys polyactis* (Bleeker, 1877) and its connectivity with others from 1962 is controversial. The study aimed to study the inter-annual variation in otolith strontium:calcium (Sr:Ca) ratios of *L. polyactis* to determine whether otolith natural tags are representative over long periods and can then be used for population structure classification. Spawning *L. polyactis* individuals were captured by stow nets in the same site of the southern Yellow Sea coastal waters during April–May in 2003, 2012, and 2013. EPMA (electron probe microanalysis) was used to determine the Sr:Ca ratios of a total of 25 otolith samples. Mann–Whitney *U*-test was used to test the differences of otolith Sr:Ca ratios from the core to edge for each otolith. One-way ANOVA was performed to compare the mean otolith Sr:Ca values among 2003, 2012, and 2013. Otoliths from 2003, 2012, and 2013 showed similar patterns of Sr:Ca ratios and Sr:Ca ratios could be divided into higher and lower phases in the core and remaining regions, respectively. Inter-annual significant differences for each high or low Sr:Ca phase of otoliths were not observed over short- (between 2012 and 2013) or long-time (between 2003 and 2012, and between 2003 and 2013) scales. Univariate contrasts across the adjacent year and decade classes were statistically similar. The Sr:Ca ratio signatures in the otolith were relatively stable across years and can be used as a reliable natural tag for connectivity assessments and stock identification with little or no genetic differentiation among *L. polyactis* populations. The short- and long-term temporal stability of otolith Sr:Ca ratios also revealed, the existence of stable *L. polyactis* stocks in the southern Yellow Sea, consistent with a previous finding of capture survey.

Keywords

Larimichthys polyactis, southern Yellow Sea, Sr:Ca ratio, temporal stability

Introduction

Otoliths are used as natural tags in fish, because of their capacity to record time-resolved lifetime environmental histories, which provides an opportunity for geolocating individual fish in time and space (Campana et al. 2000;

Gillanders 2002). Retrospective determination of the natal source of fish stocks depends on the premise that the otolith chemical signatures are sufficiently stable across time to allow for accurate classification (Rooker et al. 2003; D’Avignon and Rose 2013). Therefore, understanding the temporal scale of otolith chemistry is essential to evaluate

the reliability of natural tags among years (Walther and Thorrold 2009). There has been considerable research on the geographical variation of natal geochemical signatures, but limited studies have examined the temporal stability of otolith chemistry. Studies that examined temporal variation in otolith chemical composition found substantial variations within (Thorrold and Shuttleworth 2000; Reis-Santos et al. 2012) and among years (Campana et al. 2000; Gillanders 2002; Walther and Thorrold 2009; Pruell et al. 2012), which could lead to misinterpretation of spatial variability in otolith signatures (Reis-Santos et al. 2012; Avigliano et al. 2018). For instance, in *Platichthys flesus* (Linnaeus, 1758) and *Dicentrarchus labrax* (Linnaeus, 1758), collected from several estuaries along the Portuguese coast in two years and three seasons within a year, significant differences were observed in the majority of otolith elements among estuaries and sampling times, which were likely to be a reflection of the observed variability in environmental conditions, incorporating seasonal variation, which resulted in an 11% increase in the correct classification of individual estuaries (Reis-Santos et al. 2012). There was some overlap in elemental fingerprints among years for different estuaries for juvenile snapper *Pagrus auratus* (Forster, 1801), which was likely to have consequences in the assignment of adult fish to recruitment estuaries, especially if juvenile fish were collected from different estuaries in different years and adult fish were not assigned to recruitment estuaries using elemental fingerprints from the year class of recruits in which they were juveniles (Gillanders 2002). Furthermore, the long-term stability of otolith chemical signatures has been less frequently investigated (Avigliano et al. 2018), suggesting that chemical signatures may serve only as short-term natural tags (1–3 y) (Rooker et al. 2001; Walther and Thorrold 2009; Pruell et al. 2012).

The yellow croaker, *Larimichthys polyactis* (Bleeker, 1877) (Perciformes: Sciaenidae), is an important fish species endemic to the Northwest Pacific, inhabiting coastal waters across the Yellow, Bohai, and East China seas (Wang et al. 2013; Xiong et al. 2015), and supporting demersal fisheries in China, Korea, and Japan (Lim et al. 2010; Xiong et al. 2016). Therefore, it is important to understand the population structure and connectivity patterns between stocks to conserve and manage this species in danger of overexploitation. Among *L. polyactis* populations in China's coastal waters, the classification of the southern Yellow Sea population and its connectivity dynamics from 1962 is controversial (Xiong et al. 2016). Previous fishery ecological studies have suggested four, three, or two groups of *L. polyactis* across the China coast. Some researchers (Liu 1990; Jin 2005) have pointed out the presence of the Bohai Sea and Northern Yellow Sea group, the Middle Yellow Sea group, the Southern Yellow Sea group, and the East China Sea group across the China coast. Others (Zhang et al. 2007; Yan et al. 2014) believe that there were three groups (the Bohai Sea, the Southern Yellow Sea, and the Central Yellow Sea groups) along the coast of China. However, Xu and Chen (2010) suggested that there were two *L. polyactis* groups: the Northern

Yellow Sea and Bohai Sea group and the Southern Yellow Sea and the East China Sea group. Therefore, the temporal variability in otolith elemental signatures is essential to determine whether classifications and retroactivity of unknown individuals can be made using baseline data of otolith chemical signatures of this fish species.

The otolith strontium:calcium (Sr:Ca) ratio of concentrations have been applied as a useful scalar to estimate habitat use, migration history, and distinguish population structures of fish (Secor and Rooker 2000; Zimmerman 2005; Yang et al. 2011; Khumbanyiwa et al. 2018), successfully used in marine fish, including the Sciaenidae species *Argyrosomus japonicus* (Temminck et Schlegel, 1843) (see Ferguson et al. 2011) and *Collichthys lucidus* (Richardson, 1844) (see Liu et al. 2015). Recently, the habitat reconstruction and early life history of *L. polyactis* from the southern Yellow Sea was determined, based on the otolith microchemical analysis of the Sr:Ca ratio (Xiong et al. 2014, 2017). The presently reported study aimed to investigate otolith Sr:Ca ratio variability across an adjacent year class and 10-year interval class of *L. polyactis* in the southern Yellow Sea. This is a key step to establishing baseline characteristics of *L. polyactis* otolith Sr:Ca tags, which can then be used for connectivity assessments in the southern Yellow Sea.

Materials and methods

Spawning *L. polyactis* individuals were captured by stow nets in the southern Yellow Sea coastal waters (Fig. 1), as described by Xiong et al. (2017). Sampling was conducted during the late spring spawning run (April–May) in 2003, 2012, and 2013, at the same sampling site (32°05'N, 121°50'E) to determine the otolith Sr:Ca ratio. Eight specimens of *L. polyactis* [standard length (L_s): range, 13.4–18.3 cm; mean \pm SD, 15.1 ± 1.7 cm; similarly hereafter] aged 1–2 years were captured in 2003, ten (10.7–15.6 cm, 11.9 ± 1.5 cm) aged 1–2 years in 2012, and seven (12.2–18.6 cm, 14.6 ± 2.0 cm) aged 1–2 years in 2013 (Table 1).

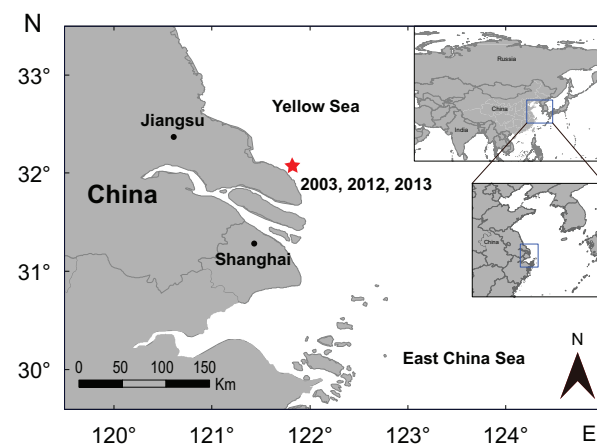


Figure 1. Sampling site for the yellow croaker *Larimichthys polyactis* used in the microchemistry analyses. The sampling site in coastal waters of the southern Yellow Sea in 2003, 2012, and 2013 is indicated by a red star.

Table 1. High and low phases of the strontium:calcium (Sr:Ca) ratio in *Larimichthys polyactis* otoliths. High and low phases of the Sr:Ca ratio in *Larimichthys polyactis* otoliths collected in 2003, 2012, and 2013.

Year	Sample code	Standard length [mm]	Estimated age	High Sr:Ca phase		Low Sr:Ca phase	
				Distance from the core [μm]	Sr:Ca (mean ± SD)	Distance from the core [μm]	Sr:Ca (mean ± SD)
2003	LSPP12	134	1+	0–240	7.57 ± 1.20 ^a	260–3,180	5.77 ± 0.80 ^b
	LSPP13	136	1+	0–140	6.97 ± 0.73 ^a	160–2,180	4.60 ± 0.74 ^b
	LSPP14	148	1+	0–140	7.16 ± 1.51 ^a	160–2,500	4.74 ± 0.63 ^b
	LSPP15	141	1+	0–180	7.10 ± 1.16 ^a	200–2,100	4.71 ± 0.78 ^b
	LSPP18	162	1+	0–40	7.68 ± 1.50 ^a	60–3,040	4.31 ± 0.80 ^b
	LSPP19	161	1+	0–200	6.85 ± 1.24 ^a	220–4,260	4.76 ± 0.99 ^b
	LSPP20	183	2+	0–160	6.90 ± 0.96 ^a	180–1,900	4.88 ± 0.78 ^b
	LSPP21	141	1+	0–300	7.64 ± 0.95 ^a	320–2,320	5.48 ± 0.92 ^b
2012	LSPP01	156	1+	0–200	7.67 ± 1.03 ^a	220–3,300	4.31 ± 0.88 ^b
	LSPP02	106	1+	0–280	7.31 ± 0.59 ^a	300–2,480	5.18 ± 0.92 ^b
	LSPP03	115	1+	0–80	7.02 ± 2.36 ^a	100–3,280	4.69 ± 1.01 ^b
	LSPP04	117	1+	0–160	6.63 ± 0.58 ^a	180–2,760	4.60 ± 0.96 ^b
	LSPP05	109	1+	0–180	7.04 ± 1.09 ^a	200–2,940	5.33 ± 0.92 ^b
	LSPP06	122	1+	0–60	6.68 ± 0.26 ^a	80–3,140	4.82 ± 0.95 ^b
	LSPP07	107	1+	0–60	7.74 ± 1.46 ^a	80–3,440	4.73 ± 0.86 ^b
	LSPP08	118	1+	0–300	7.58 ± 0.88 ^a	320–3,420	4.36 ± 0.98 ^b
	LSPP09	123	1+	0–240	7.14 ± 0.83 ^a	260–3,340	4.85 ± 1.00 ^b
	LSPP10	112	1+	0–380	6.40 ± 0.64 ^a	400–2,780	5.16 ± 1.09 ^b
2013	LSLP02	138	1+	0–80	7.62 ± 1.17 ^a	100–3,480	4.28 ± 0.81 ^b
	LSLP04	186	2+	0–220	7.18 ± 1.17 ^a	240–2,520	4.76 ± 0.81 ^b
	LSLP05	152	1+	0–80	7.55 ± 1.94 ^a	100–4,340	4.77 ± 0.90 ^b
	LSLP07	122	1+	0–160	6.79 ± 1.14 ^a	180–2,020	4.57 ± 0.85 ^b
	LSLP08	143	1+	0–80	7.75 ± 1.10 ^a	100–2,760	5.09 ± 0.92 ^b
	LSLP09	133	1+	0–120	7.61 ± 1.30 ^a	140–2,200	4.42 ± 1.02 ^b
	LSLP10	151	1+	0–80	7.34 ± 1.43 ^a	100–1,980	4.48 ± 0.86 ^b

The different superscript lowercase letters (a and b) indicate a significant difference at the 0.01 level for the Mann–Whitney *U*-test, which was used to compare the differences between the high and low Sr:Ca phases of each otolith sample.

Methods of preparing *L. polyactis* otoliths for use in electron probe microanalysis (EPMA) measurement have been described by Xiong et al. (2017). The sagittal otoliths were extracted, cleaned, and embedded in epoxy resin (Epofix; Struers, Copenhagen, Denmark), mounted on a glass slide, and then ground using a grinding machine (Discoplan-TS; Struers, Copenhagen, Denmark) equipped with a diamond cup-wheel. Each otolith was further polished on an automated polishing wheel (LaboPol-35; Struers, Copenhagen, Denmark), then cleaned in an ultrasonic bath, and rinsed with Milli-Q water. Finally, all otoliths were carbon-coated in a high vacuum evaporator (JEE-420, JEOL Ltd, Tokyo, Japan) for further EPMA measurements.

EPMA was used to study the Sr and Ca concentrations, based on the method described by Xiong et al. (2017) but with a slight modification. Each otolith sample was measured along the longest axis, from the core to the edge, using a wavelength dispersive X-ray electron probe micro-analyzer (JEOL JXA-8100; JEOL Ltd, Tokyo, Japan).

Two reference materials, CaCO_3 and SrTiO_3 , were used as standards. The accelerating voltage and electron beam current was set at 15 kV and 2×10^{-8} A, respectively. The electron beam was focused on a point 5 μm in diameter with measurements spaced at 20 μm intervals, and the counting time was 15 s. The Sr X-ray intensity maps were developed from the representative *L. polyactis* otoliths, using the aforementioned electron probe micro-analyzer with the same methods described in our previous study (Xiong et al. 2014; Fig. 2). The beam current was 5×10^{-7} A, counting time was 30 ms, and pixel size was 7×7 μm in diameter.

Based on our previous study in 2012, which was the first time *L. polyactis* otoliths from the southern Yellow Sea were analyzed (Xiong et al. 2014), we established the baseline of otolith Sr:Ca ratio (Table 2 and Fig. 2). Otolith Sr:Ca ratios from the core to edge were separated into high Sr:Ca (i.e., $(\text{Sr:Ca}) \times 1000 > 7$, reddish regions in X-ray intensity map) and low Sr:Ca ($7 \geq (\text{Sr:Ca}) \times 1000 \geq 3$, greenish-yellowish regions in X-ray intensity map) regions, which corresponded to a high salinity habitat in early developmental

Table 2. Comparison for high and low strontium:calcium (Sr:Ca) phases in *Larimichthys polyactis* otoliths. One-way ANOVA results for high Sr:Ca and low Sr:Ca phases in *Larimichthys polyactis* otoliths compared in 2003, 2012, and 2013.

		MS	df	F	P	Significance level
High Sr:Ca phase	Inter-groups	0.167	2	1.082	0.356	NS
	Within-groups	0.154	22			
Low Sr:Ca phase	Inter-groups	0.151	2	1.070	0.360	NS
	Within-groups	0.141	22			

NS: Non-significant.

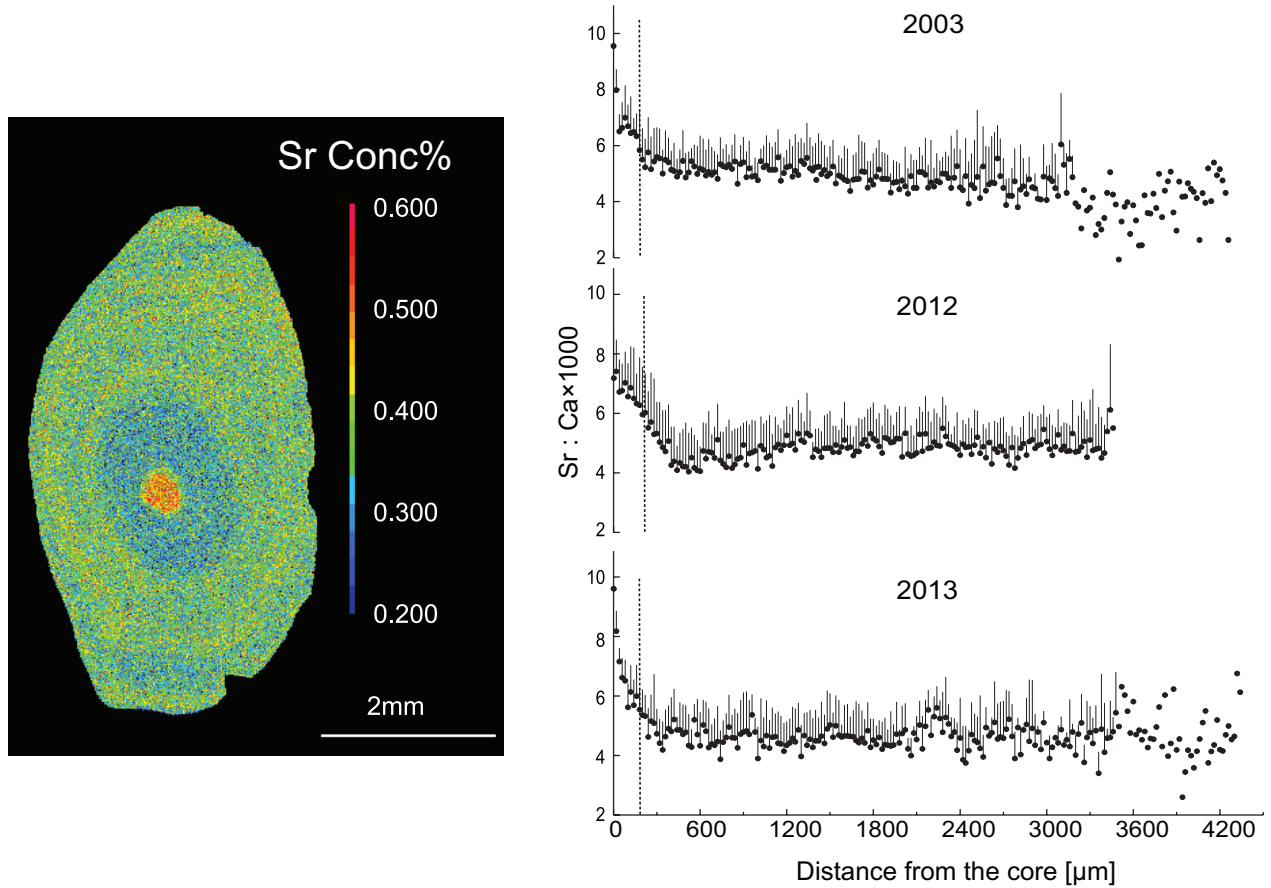


Figure 2. X-ray intensity map of strontium (Sr) content and strontium:calcium (Sr:Ca) ratios fluctuation in *Larimichthys polyactis* otolith. X-ray intensity map of the Sr content in *Larimichthys polyactis* otoliths collected in 2012 from the southern Yellow Sea (left). This pattern is representative of with overall low Sr levels (greenish and yellowish color), except for higher Sr contents in the core (reddish color). Mean Sr:Ca ratios fluctuated (with positive SD values) along the transect from the core (0 μm) to the edge of otoliths (right). The figure in 2012 has been referenced from Xiong et al. (2014). The dashed line represents the boundary between the high and low Sr phases; the high phases are on the left and the low phases are on the right.

stages, and middle salinity habitat in later developmental stages, respectively. The X-ray intensity map corresponds to 16 color map patterns of Sr content from blue (lowest) through green and yellow to red (highest).

According to *L. polyactis* baseline data from 2012 (Xiong et al. 2014), otolith Sr:Ca ratios in 2003 and 2013 from the core to edge were separated into high and low regions, respectively. The Mann–Whitney U -test is a non-parametric statistical technique, which is used to analyze differences between the medians of two data sets. This study presents the Mann–Whitney U -test as a statistical technique to examine differences between two independent groups on a continuous scale, namely, the differences in otolith Sr:Ca ratios between high and low phases of each otolith sample as described in previous studies (Yang et al. 2006; Liu et al. 2015; Jiang et al. 2019; Sokta et al. 2020). The Mann–Whitney U -test was defined as follows:

$$W_{XY} = \sum_{i=1}^m \sum_{j=1}^n U_{ij}$$

where X and Y represent two samples (high and low phases) in this study, and m and n are their sample sizes, respectively.

Unlike the Mann–Whitney U -test, which compares median values between two groups, one-way ANOVA compares values among multiple sets of groups. In this study, one-way ANOVA was used to determine whether the otolith Sr:Ca values varied with time for three comparisons across an adjacent year (2012 vs. 2013) and decade (2003 vs. 2012, and 2003 vs. 2013), calculated from the high and low Sr:Ca phases, respectively. The assumption of normality and homogeneity of variance for each variable was examined using Kolmogorov–Smirnov (KS) and Levene’s tests, and the variables were log-transformed if necessary. Variables that did not pass the normality and homogeneity tests were excluded from further analysis. Statistical analysis was performed using IBM SPSS (version 19.0; IBM Corp., Armonk, NY, USA).

One-way ANOVA was defined as:

$$SS_B = \sum_{i=1}^k n_i (\bar{x}_i - \bar{x})^2$$

$$MS_B = \frac{SS_B}{V_B}$$

$$SS_W = \sum_{i=1}^K \left\{ \sum_{j=1}^n (x_{ij} - \bar{x}_i)^2 \right\}$$

$$MS_W = \frac{SS_W}{V_W}$$

where SS_B represents the sum of squares between groups, SS_W represents the sum of squares within-group, MS_B represents the mean square between groups, MS_W represents the mean square within-group, V_B represents the degrees of freedom between-groups ($V_B = 2$), V_W represents the degrees of freedom within-group ($V_W = 22$), i represents three groups (2003, 2012, and 2013), and j represents the total number of samples ($n = 25$) minus K (3 groups), namely 22.

The age of each sample of the presently reported study was estimated with the body length-at-age growth equation

$$\left(L_t = 240.6 \times \left[1 - e^{-0.56 \times (t+0.25)} \right] \right)$$

reported by Zhang et al. (2010), because the growth rings were indistinguishable in both scales and otoliths (Table 1).

Results

Otoliths from 2003, 2012, and 2013 showed similar patterns of Sr:Ca ratios (Table 1, Fig. 2). All otolith samples had Sr-rich cores (regions < 380 μm radius from the core), whereas the remaining regions were characterized by relatively lower and more stable Sr patterns. Despite the individual variation, the corresponding results from the life history transects of the otolith Sr:Ca ratios could be divided into higher and lower phases in the core and remaining regions, respectively (Mann–Whitney U -test, $P < 0.01$) (Table 1).

The mean Sr:Ca ratios of the regions in the high Sr:Ca phase ranged from 6.79 to 7.68 in 2003, 2013, and 2012, whereas the corresponding ratios in the low Sr:Ca phase varied substantially in the remaining stages (Fig. 2).

Inter-annual differences in otolith chemistry were not observed over short- (between 2012 and 2013) or long-time (between 2003 and 2012, and between 2003 and 2013) scales (Table 2). Univariate contrasts for each high or low Sr:Ca phase across the adjacent year and decade classes were statistically similar (ANOVA, $P > 0.05$) (Table 2).

Discussion

In the presently reported study, the *L. polyactis* otolith Sr:Ca ratios were the highest and most variable near the otolith core in 2003, 2012, and 2013, which was possibly influenced by ontogenetic physiology and ambient chemistry. High Sr in the adult stage is more likely to reflect physiological state, particularly reproduction, than to reflect waterborne sources (Sturrock et al. 2015). However,

the edge of high Sr region in the otolith core of larval *L. polyactis* is corresponding to the age of around 5–7 days and the first feeding ring is formed on the second day after hatching, which is demonstrated in Xiong et al. (2017). Moreover, for other sciaenid species *C. lucidus*, the Sr-rich regions (radius < 90 μm regions from the core) were attributed to the brachyhaline sea water brought by large flood tides (Liu et al. 2015). So the high Sr otolith core cannot be explained as the influence of the maternal yolk. Coastal areas are characterized by greater chemical heterogeneity owing to upwelling and fluvial (Sturrock et al. 2012). Furthermore, at the sampling site of the presently reported study, upwelling enriched trace elements (e.g., Sr) occurs in the coastal waters of the Lüsi near the Yangtze River estuary (Zhu et al. 2004; Lü et al. 2006). In March, the mixed water masses of the East China Sea and the Yellow Sea with low temperature and high salinity appeared in the coastal waters of the southern Yellow Sea (Su et al. 1983). Therefore, high Sr:Ca values and fluctuations during the early development of *L. polyactis*, which more likely correlated with waterborne sources in coastal areas of the southern Yellow Sea. These spawning fish were almost 1-year-old during this study, and fish of this age would probably have experienced drastic environmental changes in the western seawater of Jeju Island in which *L. polyactis* migrate for both overwintering and spawning (Xiong et al. 2016) because temperatures there have been observed to range from 26.1°C in November to 12.2°C in March (Hu 2013). However, such remarkable variations in water temperature did not appear to influence the otolith Sr:Ca ratios of *L. polyactis*, because the remaining development showed stable Sr:Ca ratios. Therefore, the otolith Sr contents or Sr:Ca ratios of *L. polyactis* in the presently reported study appeared to correspond to the salinity habitat. Although Sr uptake in otoliths of diadromous fish may be influenced to some degree by temperature, growth rate, age, diet, and stress (Yang et al. 2011), temperature and other factors did not seem to be the major factors (Howland et al. 2001; Yang et al. 2011). Additionally, the salinity of the ambient water is the most consistent and prominent factor influencing Sr uptake and may mask the effects of other factors (Yang et al. 2006).

In *L. polyactis* later developmental stages, the otolith Sr:Ca ratios remained stable and showed no significant differences between short- and long-time, suggesting that this ontogenetic stage experienced a relatively uniform physicochemical environment, which is supported by the fact that Ca and Sr exhibit quasi-conservative distributions resulting in comparatively stable Sr:Ca levels (Brown and Severin 2009) or near-constant Sr:Ca ratios in marine ecosystems (Secor et al. 1995; Steele et al. 2009).

The Sr:Ca ratio was selected to infer migration through habitats with different salinities in otolith microchemistry studies. Otolith Sr:Ca from *Pangasius krempfi* Fang et Chaux, 1949 varied between 1999 and 2017, possibly owing to changes in water environmental conditions with the development of hydropower dams along the Mekong

River (Tran et al. 2019). Otherwise, the inter-annual variability of elemental composition (including Sr:Ca) in the otolith nuclei of *Coilia nasus* Temminck et Schlegel, 1846 at each site was minimal, indicating that the elemental signatures at a given site might be maintained at comparable levels over a period of 3 years, resulting in a characteristic marker for that stock (Dou et al. 2012). In the presently reported study, the *L. polyactis* otolith Sr:Ca results in adjacent year and decade intervals may be indicative of consistent environmental conditions along the migration route across years. The tendency of temporal variation across years in Sr:Ca for *L. polyactis* was similar to that reported by Liu et al. (2015) for the Sciaenidae species *C. lucidus* from 2003 and 2010, although the prior study did not compare interannual variation.

Furthermore, we documented directly, for the first time, the uniform migratory history demonstrated by stable Sr:Ca over short- and long-term scales, which could suggest the existence of a stable *L. polyactis* stock in the southern Yellow Sea. This corroborates the findings of a previous fishery investigation during 2003 and 2013 that showed a similar migration distribution for *L. polyactis* in the southern Yellow Sea (Xiong et al. 2016).

Up to now, genetic studies have failed to provide compelling evidence for the existence of a single stock of *L. polyactis* in the southern Yellow Sea, despite the development of numerous discriminatory tools and genetic markers (Li et al. 2013; Zhang et al. 2020). Compared to molecular approaches, the Sr:Ca ratio of otoliths, especially the inter-annual stability of the Sr:Ca ratio, might provide an alternative method for stock identification when little or no genetic differentiation exists among *L. polyactis* populations.

In conclusion, the *L. polyactis* otolith Sr:Ca signatures were found to be stable across years in the southern Yellow Sea, and therefore we suggest that further studies on *L. polyactis* should focus on the connectivity of spawning groups and overwintering groups, and their population structure in China seawater by analyzing the otolith microchemistry.

Acknowledgments

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Comparative osteology of three endemic cichlids (*Iranocichla* spp.) (Actinopterygii, Perciformes, Cichlidae) from southern Iran

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Abstract

Iranian cichlids are isolated disjunct populations of the African cichlids group, restricted to the Hormuz Strait region in the Hormuz basin, and are a very important group from both zoogeographic and phylogenetic points of view. Thus, the osteological structures, as one of the most reliable structures, of the three nominal species of Iranian cichlid fishes, *Iranocichla hormuzensis* Coad, 1982, from the Mehran River, *Iranocichla persa* Esmaeili, Sayyadzadeh et Seehausen, 2016, from Khorgo hot spring, and *Iranocichla* sp., from the Kol River were described and compared. Ten specimens of each species were cleared and stained with Alcian blue and Alizarin red. A digital camera was used for taking pictures and CorelDraw X6 software for preparing drawings. The three species have some differences in the caudal fin skeleton, shape of the urostyle, neural spine of preural 2, hypural spines 2, and 3, anterior part of parhypural and hypurapophysis, the size of neural spines of preural 2 and 3, hypural 3 and 4, and epurals. Although there are some differences among the species, osteology of these species is very conservative and other traits like behavioral and molecular should be used.

Keywords

Biodiversity, bone, Cichlidae, *Iranocichla*, osteology

Introduction

The family Cichlidae contains about 202 genera and more than 2000 species. The geographical distribution of freshwater cichlids includes Jordan Valley, southern Iran, Sri Lanka and southern India, Cuba and Hispaniola, Madagascar, Africa, central, southern, and North America (Kullander 1998; Nelson et al. 2016). The Iranian cichlid, *Iranocichla* genus has three species, two described, *Iranocichla hormuzensis* Coad, 1982, *Iranocichla persa* Esmaeili, Sayyadzadeh et Seehausen, 2016, and one undescribed, *Iranocichla* sp. (see Schwarzer et al. 2016). *Iranocichla hormuzensis* distributed in the Mehran River, *I. persa* in Shur, Hasanlangi and Minab rivers and *Iranocichla* sp.,

in the Kol River drainages (Keivany et al. 2016; Esmaeili et al. 2017), flowing into the Persian Gulf at the Strait of Hormuz. Osteological studies help to understand processes such as feeding, respiration and swimming abilities through cognition jaw bones, branchial bones and fins, also osteology is necessary for understanding the phylogenetic relations among fishes and their classification (Helfman et al. 2009). Only a few osteological works on *I. hormuzensis* is available. Esmaeili and Teimory (2006) mentioned the morphology of the urohyal bone and its importance in the taxonomy of freshwater fishes of Iran, including *I. hormuzensis*. Stiassny et al. (2010) in studying a new species of *Danakilia* (Cichlidae) compared the lower pharyngeal, posterior neurocranium and anterior vertebral elements of

D. dinicolai with those of *I. hormuzensis*. Therefore, the aims of this study is to provide a detailed osteological description of *I. hormuzensis* and compare it to other Iranian cichlids, *I. persa* and *Iranocichla* sp.

Materials and methods

Thirty specimens of *Iranocichla* spp. with 3–5.5 cm total length collected from Mehran and Kol rivers and Khorgo hot spring in southern Iran, were examined. They were kept in ethanol (70%) and stored at Isfahan University of Technology Ichthyology Museum (IUT-IM). They were cleared and stained with Alizarin red and Alcian blue according to Taylor and Van Dyke (1985) protocol. A digital camera (Tucson) was used for taking pictures of them. Then, they were drawn using CorelDraw X6 software. The terminology of the bones is based on Rojo (1991).

Results

Cranium (Fig. 1). The posterior part of the skull is wider than its anterior part and its roof includes the ethmoid, nasal, frontal, parietal, sphenotic, epiotic, pterotic and supraoccipital and its crest (Fig. 1a). The ethmoid region consists of the paired nasals, lateral ethmoids and unpaired ethmoid, and prevomer. The nasal is short and attached to the frontal. The prevomer is horizontally triangular, elongated posteriorly and bears a strong connection to the parasphenoid. The lateral ethmoid is connected to the prevomer via an anterior process and, in dorsal view, is connected to the frontal (Fig. 1b).

The orbital region consists of the paired orbitosphenoids, circumorbital series, frontals and the unpaired parasphenoid. The frontal is a large element of skull roof with an elongated posterior edge which is linked to the sphenotic and parietal (Fig. 1a). In the ventral view, the

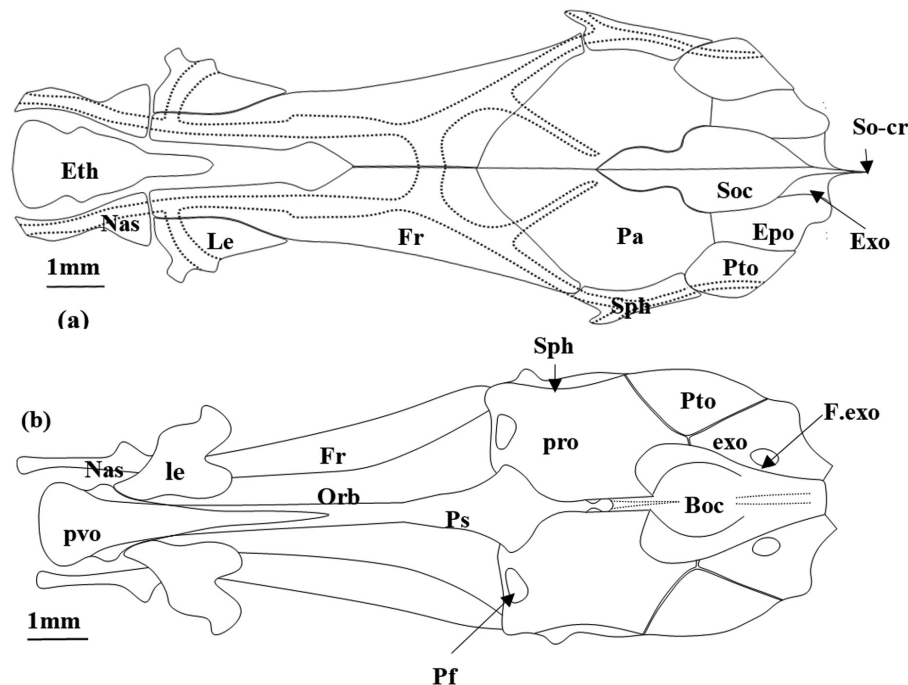


Figure 1. Dorsal (a) and ventral (b) views of the cranium in *Iranocichla hormuzensis*. Boc: Basioccipital; Epo: Epiotic; Eth: Ethmoid, attached to the surroundings by cartilage not shown in the figure; Exo: Exoccipital; Fr: Frontal; F.exo: foramen exoccipital; Le: Lateral ethmoid; Nas: Nasal; Os: Orbitosphenoid; Pa: Parietal; Pf: Prootic foramen; Pro: Prootic; Ps: Parasphenoid; Pto: Pterotic; Pvo: Prevomer; Sph: Sphenoid; Soc: Supraoccipital; So-cr: Supraoccipital crest.

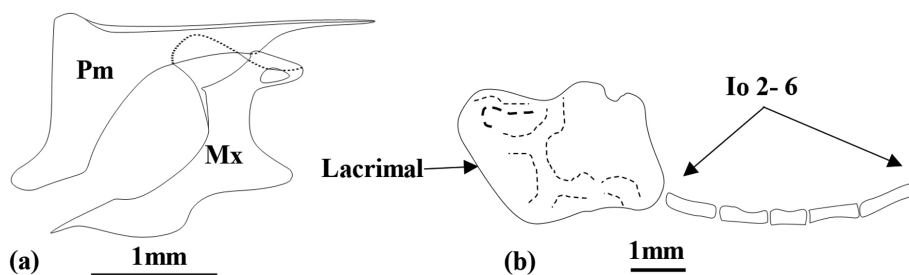


Figure 2. Internal view of the upper jaw (a) and circumorbital series (b) of *Iranocichla hormuzensis*. Abbreviations: Aap: Anterior ascending process; Io 2–6: Infraorbitals 2–6; Mx: Maxilla; Pmx: Premaxilla; Rc: Rostal cartilage.

parasphenoid connects to the prootic by its posteriolateral edges and is elongated posteriorly and bears two foramina in this part (Fig. 1b). The two orbitosphenoids are attached to the frontal laterally and to the parasphenoid dorsally. The infraorbital series includes the lachrymal and five abutting elements (Fig. 2b). The lachrymal is almost oval-shaped and the biggest element which is the anterior most part of infraorbital series. A sensory canal traverse amid the lachrymal. Infraorbitals 2–6 are short and narrow (Fig. 2b).

The parietals are situated between the supraoccipital and frontal which are linked to each other in the midline. Epiotic is located between parietal, supraoccipital, exoccipital, and pterotic. This bone has an enlarged process in the posteriodorsal edge. The pterotic is nearly triangular which is ventrally connected to the parietal and epiotic, and laterally to the prootic. The prootic ventrally contacts the orbitosphenoid and frontal, dorsally the sphenotic and posteriorly the basioccipital and pterotic. There is a foramen in the prootic for the passage of auditory nerves and blood vessels. The sphenotic bears a small process anterodorsally. The supraoccipital is wide in the middle and linked to the parietal by its anterior process and posteriolaterally to the exoccipitals and epiotic and bears a blade-shaped crest. The exoccipital is linked to the pterotic laterally and bears a foramen on its ventral part. The basioccipital is almost jug-shaped that is cheeky in its middle portion. This bone is located between the prootic and exoccipitals in the lateral view of the skull (Fig. 1b). The posterior part of the basioccipital is connected to the first centrum.

Jaws (Figs. 2, 3). Premaxilla bears teeth (not shown). The anterior part of premaxilla has a sharp ascending process which is longer than the premaxillary length. Having an ascending appendage with middle cartilage at its dorsal tip (rostral cartilage in Fig. 2a) helps the ethmoid to move forward and slide around as the mouth opens (Fujimura and Okada 2008). Both premaxillae are connected from the ascending to the middle part by a maxillary cartilage

linkage. The maxilla possesses a mid-lateral ascending process and a posterior descending process. This bone has a hole in the middle outer edge, connected to the middle long ascending process of the premaxilla. The lower jaw is triangle and connected to anteroventral part of the quadrate. The anguloarticular anterior shaft enters the dentary mid cavity. The retroarticular is a tiny bone linking to the posterior inner edge of the angular. The Meckel cartilage is elongated and located in the internal face of the angular (Fig. 3).

Suspensorium (Fig. 3). The palatine is small, with a round head, and is posteriorly flat. The ectopterygoid is thin and short, in part, anteriorly binds to the ventral surface of palatine and laterally to the quadrate. The endopterygoid is flat, small and located at the anterior part of the metapterygoid and above the posterior part of quadrate. The metapterygoid is broad and connected to the symplectic and hyomandibular. The quadrate is almost axe-shaped and the symplectic is bar-like. The upper part of hyomandibular is broad and inserted below the upper edge of the preopercle.

Opercular Series (Fig. 3). The opercle is nearly triangular and the largest component of the opercular series. The opercle slightly covers the upper rim of subopercle. The preopercle is L-shaped which is broader ventrally. The interopercle is broad and joined to the subopercle posteriorly. The posterior edge of hyomandibular covers the anterior border of preopercle and the ventral corner of the preopercle covers the ventral corner of the interopercle. The hyomandibular is connected to the pterotic through the hyomandibular fossa. The opercle is linked to the hyomandibular posteriorly. Subopercle is broad with a serrated edge and has a sharp and small ascending process that its inner face connects to posterior part of the opercle.

Hyoid arch (Fig. 4a). The basihyal is a short anteriorly flattened bar. The urohyal consists of a vertical and horizontal blade and has an anteriodorsal process. The hypohyals include the ventral and dorsal parts, the dorsal hypo-

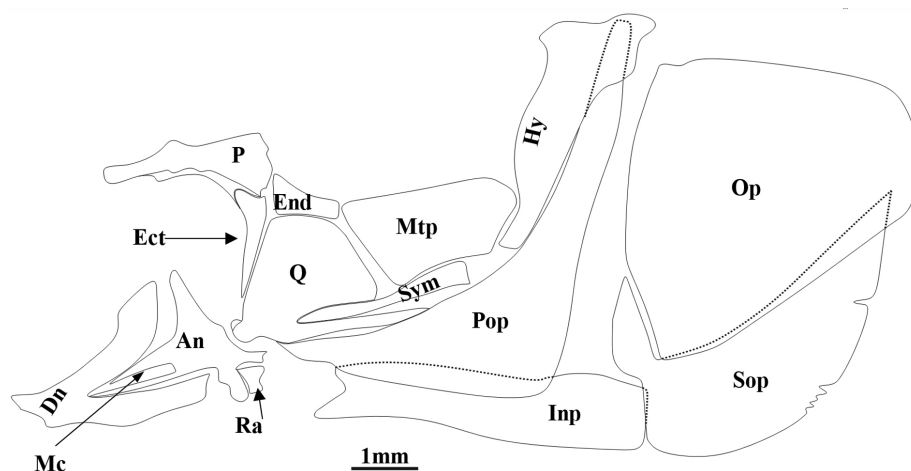


Figure 3. The suspensorium and opercular series and upper jaw in *Iranocichla hormuzensis*. Abbreviations: An: Angular; Dn: Dentary; Ect: Ectopterygoid; End: Endopterygoid; Hy: Hyomandibular; Inp: Interopercle; Mc: Meckel cartilage; Mtp: Metapterygoid; Op: Opercle; P: Palatine; Pop: Preopercle; Q: Quadrate; Ra: Retroarticular; Sop: Subopercle; Sym: Symplectic.

hyal bears a hole posteriorly. The (anterior) ceratohyal is posteriorly flatter than anteriorly and connected to the epihyal (posterior ceratohyal) with a small blade. The epihyal is almost triangular. The interhyal is cylindrical in shape. Five branchiostegal rays are present, two branchiostegals articulate with the epihyal and three with the ceratohyal.

Branchial arch (Fig. 4b). There are three basibranchials that have different shapes. The first basibranchial is almost crescent-shaped and smaller than the others. The second and third basibranchials are rod-shaped; the second is flattened at the end and the third at the middle. The hypobran-

chials are three pairs, the third pair have been surrounded by the third basibranchial. The last ceratobranchials are covered by dermal toothplates. and the third and fourth pairs of pharyngobranchials are fused and covered by a single toothplate. The four epibranchials are tripartite.

Vertebral columns (Fig. 5a). *Iranocichla hormuzensis* has 25–27 vertebrae. Parapophyses are located in the posterolateral part of the third and fourth vertebrates, this position was observed in three specimens from the Mehran River, in eight from Khorgo hot spring and in seven from the Kol River (in one specimen from the Kol River

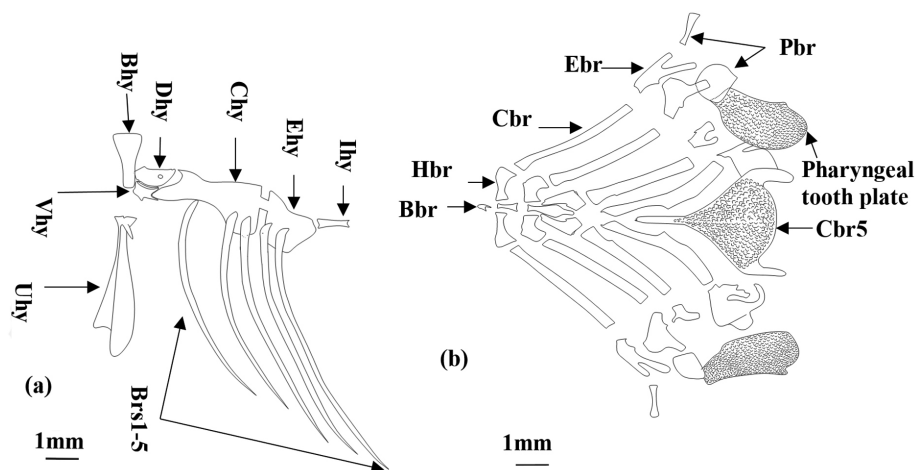


Figure 4. (a) Hyoid arch of *Iranocichla hormuzensis*. Bhy: Basihyal, Brs 1–5: Branchiostegal 1–5; Chy: Ceratohyal, Dhy and Vhy: Dorsal and Ventral hypohyal; Ehy: Epihyal; Ihy: Interhyal; Uhy: Urohyal. (b) Branchial apparatus of *I. hormuzensis*. Bbr: Basibranchial; Cbr: Ceratobranchial (Cbr5: Pharyngeal bones); Ebr: Epibranchial; Hbr: Hypobranchial; Pbr: Pharyngobranchials.

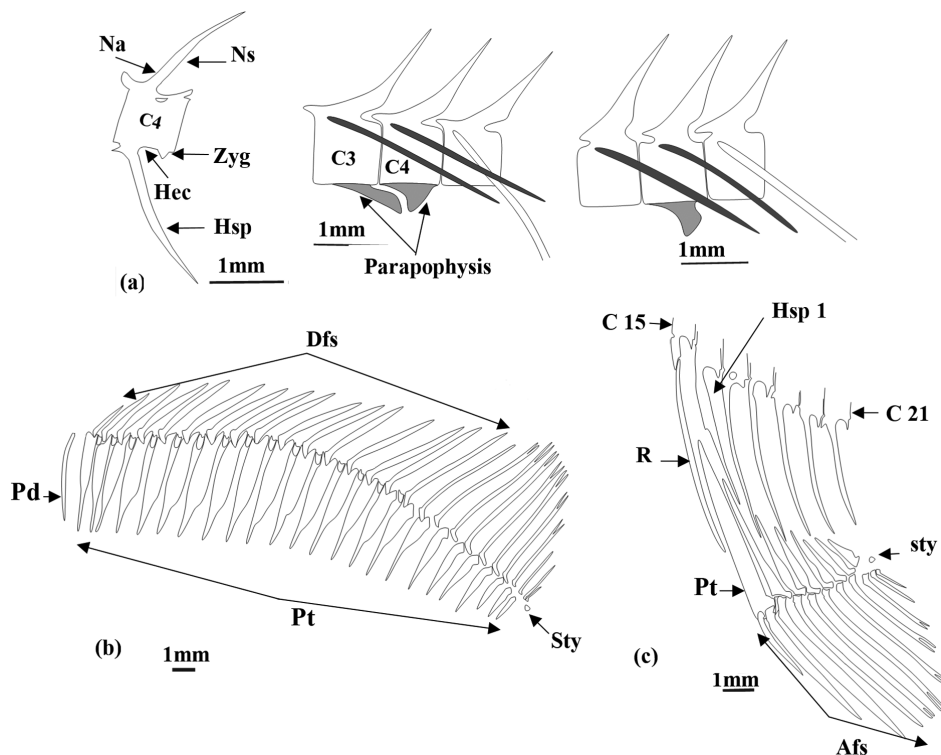


Figure 5. Lateral view of vertebrae (a), dorsal fin (b) and anal fin (c) of *Iranocichla hormuzensis*. Abbreviations: Afs: Anal fin spine; C3–C4, C15–C21: Centrum 3–4, Centrum 15–21; Dfs: Dorsal fin spine; Dpt: Distal part of the pterygiophore; Hec: Hemal canal; Hsp (1): Hemal spine (1); Na: Neural arch; Ns: Neural spine; Pd: Predorsal; Pt: Pterygiophore; R: Rib; Sty: Stay; Zyg: Zygopophysis.

and Khorgo hot spring, there was only the fourth centrum and in the Mehran River only the third or fourth or the centrum is absent).

Dorsal and anal fins (Fig. 5b, c). D XV–XVI 10–12. There are 24–25 pterygiophores and one stays in the dorsal fin (Fig. 6b). The first pterygiophore is between 1st and 2nd vertebrae. The distal pterygiophores are linked to the branched rays which decline in size anterioposteriorly. One pretarsal is located before the first pterygiophore. The last two branched rays are not connected to the pterygiophore and a tiny stay support them. A III7–9. The first anal pterygiophore is located between the 15th and 16th centrum. There are 8–9 pterygiophores and one tiny stay. The first pterygiophore is largest and supports two spines (Fig. 5a).

Caudal skeleton (Fig. 6). There are five hypurals (in some specimens, hypural 1 and 2 or 3 and 4 are fused) (Fig. 6a). Hypurals 1–5 and parhypural directly support the caudal fin rays. The first and fourth hypurals are the largest. Two long epurals are bowed to the posterior part of hypural

plate to support the procurent rays. Hypurapophysis is sharp and stretched toward the hypural 3. The uroneural is narrower than the hypural 5 and in the ventral part is bent to the urostyle. The Urostyle is thin and elongated at the end and part of the beginning is hooked and in general, this bone has insignificant differences in size and shape in different people of each population. The first neural spine of preural 2 is short and the second is elongated in *I. persa* and there is only a short neural spine in *Iranocichla* sp., but in *I. hormuzensis* in addition to this condition (short and elongated), there are also two other conditions; both short or both long. *Iranocichla* sp. and *I. persa* have a haemal spine in the preural 3 but in *I. hormuzensis* might be more than one. Epural 2 in *Iranocichla* sp. and *I. persa* is longer than epural 1 with a space between them, but in *I. hormuzensis* they are almost equal in size and attached all along. The differences in the caudal skeletons of all three species are shown in Fig. 6 and a comparison between them is summarized in Table 1.

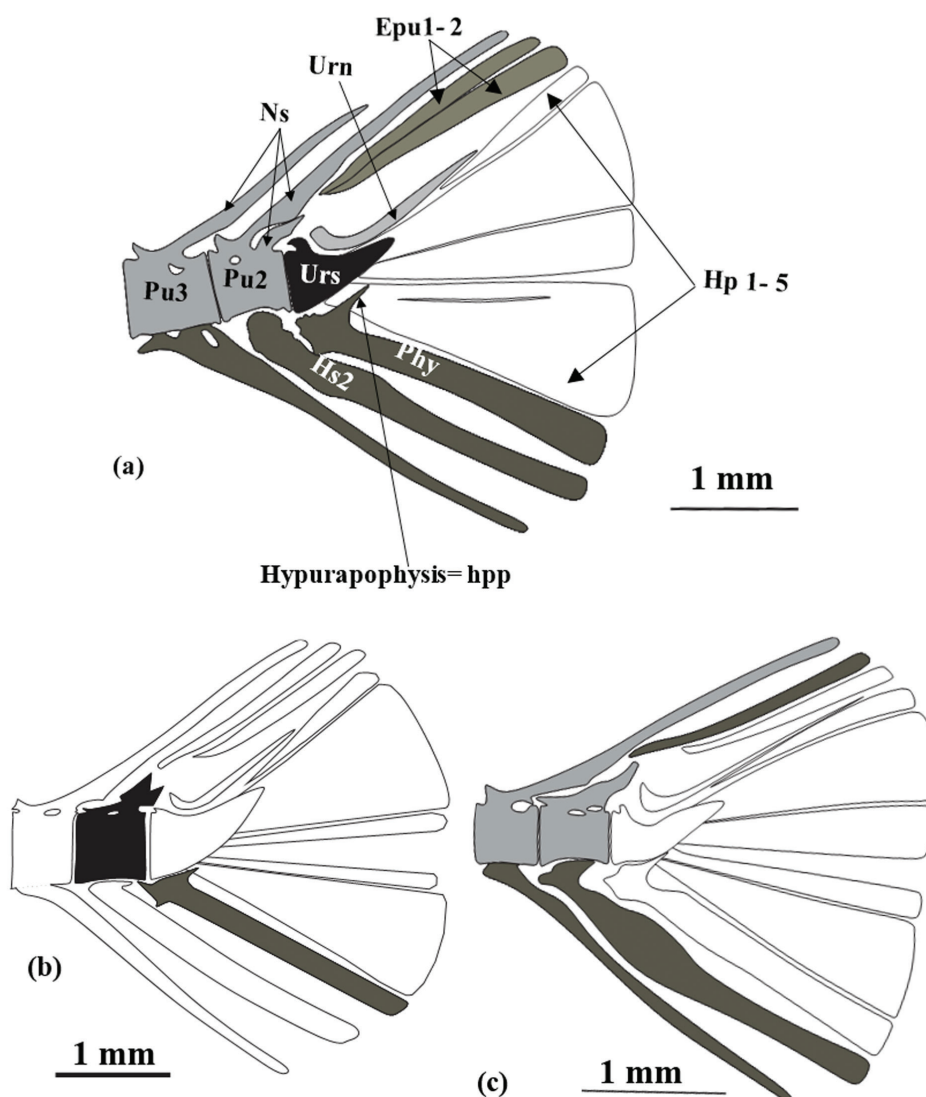


Figure 6. Lateral view of the caudal skeletons of *Iranocichla hormuzensis* (a), *Iranocichla* sp. (b) and *I. persa* (c). Abbreviations: Epu 1–2: Epural 1–2; Hp 1–5: Hypurals 1–5; Hs2: Haemal spine 2; hpp: Hypurapophysis; Ns: Neural spine; Phy: Parhypural; Pu2 (3): Preurals 2 (4); Urn: Uroneural; Urs: Urostyle.

Table1. Comparison of the caudal skeletons of *Iranocichla hormuzensis*, *Iranocichla persa* and *Iranocichla* sp.

PHY, URN, URS, HS2	HS3	NS3		NS2		Epu1-2		Hyp5		Hyp1-4			Character /Species																	
		No.	Length	No.	Length	No.	Status	No.	Status	No.	Status																			
											3,4	1,2																		
Slight difference in shape	2 elements (first: elongated second: short)	6	Elongated	1	Both elongated	2 elements	2	Both free, second longer	2	Wider posteriorly	5	No fusion	No fusion	<i>I. hormuzensis</i>																
							1	First: free second: fused with pu2				1	Complete fusion		Complete fusion															
							2	Attached to each other with curvature					1		Complete fusion	No fusion														
		1 element: elongated	4	Short	1	First: short, second: elongated (fused together)	1 element	2	Attached to each other without curvature	1	Narrow anteriorly	1	1		Complete fusion	No fusion														
								1	Fused to each other				1		No fusion	Incomplete fusion														
								5	Short				1		Incomplete fusion	No fusion														
	1 element: elongated	4	Short	1	Short (fused with epu2)	1 element	1	Without curvature	1 element	Anterior needle-shaped	All: free (wedge-shaped)	1	Incomplete fusion		Incomplete fusion															
							1	With curvature				1	Incomplete fusion		Incomplete fusion															
							Slight difference in shape	1 element: elongated				6	Elongated		1	both Short	2 elements	6	Both free, second longer	2 elements	9	All free (wedge- shaped of different sizes)	5	No fusion	No fusion	<i>Iranocichla</i> sp.				
																							3	First: short second: elongated	4		Attached to each other	1	Complete fusion	No fusion
																												6	Short	1 element
							Slight difference in shape	1 element: branched				3	Short		7	Short	1 element	5	Both free, second longer with hook-shaped end	2 elements	10	All free (wedge-shaped of different sizes)	3	No fusion	No fusion	<i>I. persa</i>				
1 element: elongated	7	3	Elongated	3	First: short, second: elongated	2 elements			5	Both free, second longer with flat end	2			Complete fusion									Complete fusion							
											1			Complete fusion									No fusion							
1 element: branched	3	Short	7	Short	1 element	5		Both free, second longer with hook-shaped end	2 elements	10	All free (wedge-shaped of different sizes)	1	No fusion	Complete fusion																
												3	No fusion	Incomplete fusion																
												3	No fusion	Incomplete fusion																

*The only exception in *Iranocichla* sp. population; haemal spine in preural 4 is short.

Pectoral girdle (Fig. 7a). The largest bony element of the pectoral girdle is the cleithrum. This bone is posteriorly attached to the coracoid, to the scapula anteriorly and to the supracleithrum ventrally. The coracoid is curved anteriorly making a large hole between itself and the cleithrum. The postcleithra are elongated and thin. The second postcleithrum ends into a sharp point. Two postcleithra are linked to each other behind the scapula. The first ray is slim and directly attached to the posteriodorsal part of the scapula. The scapula is almost square with a foramen in the middle. The elongated supracleithrum is located anteriodorsally on the cleithrum. The base of posttemporal is broadened to attach to the supracleithrum and has a pore on its wide part, but the anterior part of

the posttemporal is elongated and the sensory canal of the head passes through it. The first actinost is attached directly to the scapula, but other actinost are connected to the scapula by cartilage and the fourth actinost is the largest.

Pelvic girdle (Fig. 7b). The pelvic includes the paired distal processes, basipterygia, posterior processes, anteroventral processes, and fin rays. This fin is horizontally situated in the thoracic area and directly linked to pectoral fin and fixed to it by muscles and ligaments. Pelvic fins are attached together via the combined posterior processes. The anteroventral processes are rod like and thin that fused together between the posterior distal processes. The anterior part of the distal process is sharp but wide posteriorly. There is one spine that is forked at the junction with the distal process.

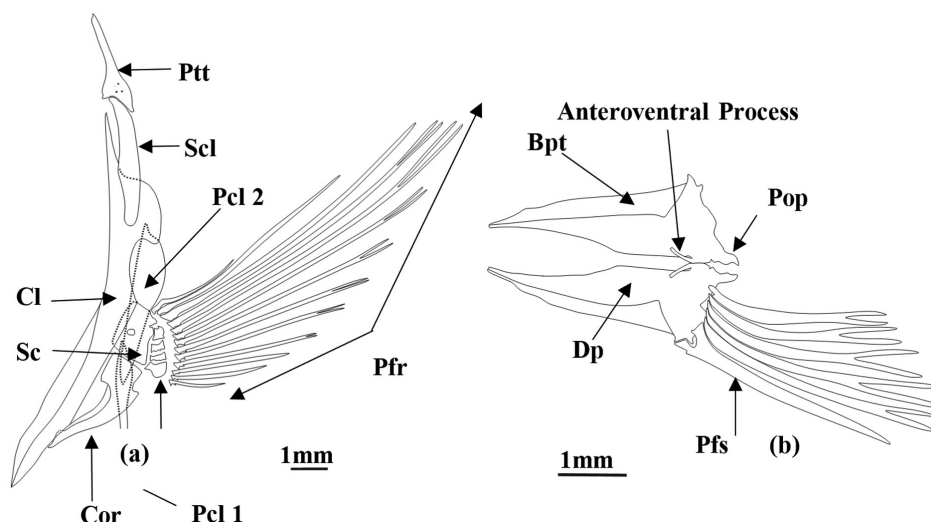


Figure 7. Lateral view of the pectoral girdle (a) and internal view of the pelvic (b) of *Iranocichla hormuzensis*. Abbreviations: Act: Actinost; Anp: Anteroventral Process; Bpt: Basipterygium; Cl: Cleithrum; Cor: Coracoid; Dp: Distal process; Pcl 1, 2: Postcleithrum 1, 2; Pfr: Pectoral fin ray; Pfs: Pelvic fin spine; Pop: Posterior process; Ptt: Posttemporal; Sc: Scapula; Scl: Supracleithrum.

Discussion

The osteology of three endemic species of Iranian cichlid, *Iranocichla hormuzensis*, *I. persa*, and *Iranocichla* sp. is described and compared for the first time. Coad (1982) introduced the Iranian cichlid as a new genus and species from the Mehran River. Schwarzer et al. (2016), studied phenotypic and genetic diversity of populations of *Iranocichla* from the Mehran, Kol, Shur, and Minab rivers and found genetic differences between populations of the western and the eastern branches of the Kol River system. They suggested that the genetically differentiated populations with different nuptial coloration represent distinct biological species. Esmacili et al. (2016) introduced a new cichlid species as *I. persa* based on mitochondrial DNA sequence and male nuptial coloration from the Shur, Hasanlangi, and Minab river drainages flowing into the Persian Gulf and pointed out that *Iranocichla* sp. was closer to *I. hormuzensis* in terms of mitochondrial sequence and to *I. persa* in male nuptial coloration.

The majority of the bony elements including the neurocranium and the branchiocranium were basically similar in all the three species and did not show any significant differences among the species. The mesethmoid is found in many species of tilapia but the genera *Sarotherodon*, *Oreochromis*, and *Iranocichla* are reported to lack this bone (Trewavas 1973, 1983), however, our findings indicate the presence of this bone in *Iranocichla* species. Like the genus *Gymnogeophagus*, it is assumed that there is no predorsal in the *Iranocichla* (see Reis and Malabarba 1988), but again, our observation indicates the presence of this bone in *Iranocichla* species, although it is limited to one. In the genus *Gymnogeophagus*, the outer part of the epiotic is connected to the posttemporal bone, but in *Iranocichla*, the outer part of the pterotic is connected to the posttemporal bone (Reis and Malabarba 1988). Some

little differences were found in the vertebral parapophyses of centrum 3 or 4. Stiassny et al. (2010) showed that there were two inferior vertebral apophysis on 3rd and 4th centurs of *Iranocichla hormuzensis*, as seen in our study. As Keivany (2014a) pointed out, the premaxillary ascending process although reduced in many eurypterygian taxa, is well developed in most of the higher percomorphs including Perciformes. The fourth pharyngobranchial is absent or reduced in most eurypterygian fishes (Keivany 2014c) but present in *Iranocichla* spp., however, in the majority of taxa, it bears a separate toothplate, but in *Iranocichla*, the third and fourth pharyngobranchials share a relatively large toothplate. The postcleithra could be present or absent in percomorphs (Keivany 2014d) including cichlids. Also, as in these species, the preopercle is L-shaped in most eurypterygians (Keivany 2014b). As Esmacili and Teimory (2006) denoted that the ventral surface of urohyal of *I. hormuzensis* is triangular, grooved and only a thorn in the dorsal surface can be seen. In the genus *Gymnogeophagus*, the interhyal is articulated with a separate cartilage to the symplectic and the hyomandibular, but in the genus *Iranocichla*, this bone connects to the interopercle (Reis and Malabarba 1988). Caudal skeleton probably is the most variable structure among the species. Generally, the elements of caudal fin of Eurypterygii (see Keivany 2017a) and the hypurals of some cichlids tend to fuse to each other (Vandewalle 1973; Keivany 2017a). In some cichlids of African lakes, this tendency to fuse with each other is seen first on a double plate and then on a single plate (Vandewalle 1973); this trend is apperceived in some individuals of the Mehran and Kol populations. The differences between caudal skeleton of populations were in the shape of the urostyle, neural spine of preural 2, hypural spine 2 and 3, anterior part of parhypural and hyporapophysis, the size and number of neural spines of preural 2 and 3, hypural 3 and 4 and epurals. Sebilia and

Andreata (1990) suggested that the shape of the parhypural, urostyle and haemal spine 2; the degree of ossification of the hyporapophysis and the number of caudal fin rays are suitable for taxonomy of cichlid fishes. As in the majority of eurypterygians (Keivany 2017b), the pelvic girdle is a simple structure in cichlids and featured by the presence of anterioventral process.

Sebilias and Andreata (1990) found that the characteristics of the total number of radius of the caudal fin and how they are located on the supporting bony elements, urostyle shape, parhypural, haemal spine 2, degree of ossification of the hypurapophysis, are valuable characteristics in the classification of cichlids. However, these characteristics do not seem to be suitable for the Iranian cichlid species due to the great variety in the structure of the caudal skeleton. Ottoni (2015) showed that the morphology of the anterior structure of the ceratohyal was not useful for the detection of *Laetacara* species as well as a key factor for the classification of Cichlasomatini members. Therefore, it is possible that in different species, only some structures be suitable for the separation of the species. In general, the osteology of these cichlids, like other cichlids, is quite stable and conservative and could not resolve the relations among the species. However, despite the findings of

Esmacili et al. (2016) (Based on mitochondrial DNA sequencing and male staining during the breeding season), it seems that *Iranocichla* sp. and *I. persa* are more similar to each other than to *Iranocichla hormuzensis*.

The presence of two types of oral and pharyngeal teeth and the parental care system in Cichlids has increased various feeding strategies and increased the survival of larvae and population (Yoder et al. 2010). In addition, the difference in the structure of the tail stem causes more efficiency of swimming, reduces the cost of energy and mechanism and increases the power of moving forward in the river. It seems that the Iranian cichlid, using the three factors of having two types of teeth, the type of parental care system and the diversity in the structure of the caudal fin, has created motor strategies for living in harsh environmental conditions, thereby improving its survival and reproduction.

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Redescription of a rare cusk eel, *Pycnocraspedum squamipinne* (Actinopterygii, Ophidiiformes, Ophidiidae), from Bay of Bengal

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Abstract

Pycnocraspedum squamipinne Alcock, 1889 is a rare species of pelagic cusk eel described from specimens collected off the Bay of Bengal. The descriptive literature on *P. squamipinne* is limited to the original description owing to the rarity in collections. The genus and the species need a thorough revision to identify specific characters of taxonomic importance as the descriptive information regarding the species in the genus *Pycnocraspedum* is limited to the holotypes and syntypes. Fifteen fresh specimens of *P. squamipinne* were collected from the Chennai coast, Tamil Nadu, Bay of Bengal region. The syntypes of *P. squamipinne* (ZSI F 11700 and ZSI F 11703) available at the Zoological Survey of India, Kolkata were examined. The morphometric and meristic characters of *P. squamipinne* were compared with those of syntypes and other species of the genus. Molecular analysis was carried out using partial mtDNA cytochrome oxidase subunit I gene (COI). The specimen is deposited in the national fish repository of the ICAR, National Bureau of Fish Genetic Resources, Lucknow, India. A detailed redescription of *P. squamipinne* based on the syntypes and fifteen recently collected specimens are provided. A detailed comparison with other species of the genus is also provided which helps in establishing the taxonomic identity of *P. squamipinne*. Molecular information was generated which would help in the species identification of this rare deep-sea species. This study provides morphological and molecular information of the rare cusk eel, *P. squamipinne*, which would help in the precise identification of the species. However, due to paucity, the majority of species of the genus are still only known from single specimens and comprehensive examination of holotypes and intensive sampling is needed to better understand the species identity and distribution.

Keywords

Bay of Bengal, COI, cusk eel, redescription

Introduction

The order Ophidiiformes consists of five families, Aphyonidae, Bythitidae, Carapidae, Ophidiidae, and Parabrotulidae and is one of the diverse groups of deep-sea demersal ichthyofauna inhabiting the continental slope to the abyssal plain (Haedrich and Merrett 1988; Merrett and Haedrich 1997). Alcock (1891) compiled the first

list of deep-sea fishes from the Indian waters in his book ‘Indian deep-sea fishes in the Indian museum’. Nielsen et al. (1999) published a world catalog of fishes of the order Ophidiiformes, which included 48 genera and 218 species under the family Ophidiidae.

The family Ophidiidae consists of 266 species in four subfamilies: Brotulinae (7 species), Brotulotaeniinae (4 species), Ophidiinae (65 species), and Neobythitinae

(190 species) (Nielsen et al. 1999; Fahay and Nielsen 2003; Okiyama and Yamaguchi 2004; Fricke et al. 2020). The diagnostic characters for the genus *Pycnocraspedum* are the large head, longer than half of the preanal length, four developed gill rakers, eight branchiostegal rays, small granular teeth on premaxillaries, vomer and palatines, 2 basibranchial tooth patches, caudal-fin rays 10, pelvic fins as bifid filaments united basally; opercular spine present, 2–3 spines at the lower angle of preopercle and 12 precaudal vertebrae (Alcock 1889; Cohen and Nielsen 1978; Nielsen 1997).

According to Nielsen et al. (1999), the genus *Pycnocraspedum* has five nominal species, *Pycnocraspedum armatum* Gosline, 1954, *Pycnocraspedum fulvum* Machida, 1984, *Pycnocraspedum microlepis* (Matsubara, 1943), *Pycnocraspedum phyllosoma* (Parr, 1933), and *Pycnocraspedum squamipinne* Alcock, 1889. *Pycnocraspedum squamipinne* was originally described from specimens collected off the Bay of Bengal (Alcock 1889). The descriptive literature on *P. squamipinne* is limited to the original description. It has been suggested by many authors that the genus needs a thorough revision to identify specific characters of taxonomic importance (Cohen and Nielsen 1978; Nielsen 1997). The present paper provides a detailed redescription of *P. squamipinne* based on the syntypes and fifteen recently collected specimens.

Material and methods

Fifteen fresh specimens of *Pycnocraspedum squamipinne* were collected from the Chennai coast, Tamil Nadu, Bay of Bengal region. All the measurements were made with the digital vernier caliper to the nearest 0.1 mm, following Hubbs and Lagler (1947) and Cohen and Nielsen (1978). The body proportions were expressed in terms of standard length (SL: length from the snout tip to the base of the caudal fin) and head length (HL: length from the snout tip to the posterior margin of the opercle). The vertebral counts were taken from radiographs. The syntypes of *P. squamipinne* (ZSI F 11700 and ZSI F 11703) available at Zoological Survey of India, Kolkata were examined. The morphometric characters of *P. squamipinne* were compared with syntypes and other species of the genera (Tables 1, 2). The specimen is deposited in the National Fish Repository of the ICAR, National Bureau of Fish Genetic Resources, Lucknow, India (Accession numbers NBFGR/OPHPSQU1, NBFGR/OPHPSQU2, NBFGR/OPHPSQU3, NBFGR/OPHPSQU4, NBFGR/OPHPSQU5).

The molecular analysis was carried out using partial mtDNA cytochrome oxidase subunit I gene (COI). The genomic DNA was extracted using Qiagen Kit as per the manufacturer's protocol. The universal primers COI F (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC - 3') and COI R (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') (Ward et al. 2005) were used to amplify the partial COI gene. The amplifications were performed in

25 µL reactions containing 10× assay buffer (100 mM Tris, 500 mM KCl, pH 9.0) with 20 mM MgCl₂, 10 pmoles of each primer, 200 µM of each dNTP, 0.25 U TaqDNA polymerase and 25 ng of template DNA. PCR products were sequenced bi-directionally. DNA sequences developed in the presently reported study were aligned and edited using BioEdit sequence alignment editor version 7.0.5.2 (Hall 1999). Phylogenetic and molecular evolutionary analysis using Kimura 2-parameter method (Kimura 1980) was conducted using MEGA version 7.0 (Kumar et al. 2016). The edited sequences were submitted to GenBank (Accession No: [MT231514](#), [MT231515](#), [MT231516](#), [MT231517](#), [MT231518](#), and [MT231519](#)).

Systematic account

Family Ophidiidae

Pycnocraspedum squamipinne Alcock, 1889

Figs 1–4, Tables 1, 2

Pycnocraspedum squamipinne Alcock, 1889 (Type locality: Bay of Bengal, 20°17'30"N, 88°50'E)

Materials examined. INDIA 1; Syntype of *P. squamipinne* (TL = 291 mm); Bay of Bengal; 20°17'30"N, 88°50'E R.I.M.S. Investigator coll. leg.; ZSI F11700 1; Syntype of *P. squamipinne* (TL = 299 mm) same collection data as preceding; ZSI F 11703 (1, 299 mm TL).

INDIA 5; Chennai coast, Tamil Nadu; March 2018; Teena Jayakumar T.K and T.T Ajith Kumar leg.; NBFGR / OPHPSQU1 to 5 • 10; same collection data as for preceding; NBFGR / CE1 to 10.

Diagnosis. Deep bodied cusk eel with head 3.0–3.9 times in SL; eye diameter 5.5–6.8 in HL; interorbital 4.8–6.3 in HL; snout 3.8–5.2 in HL; depth 4.3–6.8 in SL; predorsal length 3.3–4.7 in SL, distance between pelvic origin to anal origin 2.5–4.4 in SL; no pseudobranchiae and 12–13 pyloric caeca. Two median basibranchial tooth patches. Single opercular spine and 3 short blunt spines at lower angle of preopercle. *Pycnocraspedum squamipinne* differs from its congeners in origin of dorsal fin above preopercle, absence of pseudobranchial filaments, pectoral fins and pelvic fins short and not reaching anus.

Description. Body compressed, moderately deep and tapers caudally, its depth 4.3–6.8 in SL (Fig. 1). Body covered by small cycloid scales. Head large, compressed, fully scaled except lips, head length 3.0–3.9 in SL. Eye diameter 15.2%–18.4% HL; preorbital length 19.2%–23.6% HL and postorbital length 61.9%–72.7% HL. Head length in preanal distance 1.6–1.9. Anterior nostril tubular, behind snout rim with small skin flap, smaller than posterior nostril being simple pore in front of eye. Eyes moderately large, elliptical, snout length greater than diameter of eye, interorbital space wide equal to or slightly greater than eye diameter. Mouth large and oblique, snout blunt, maxilla extending beyond posterior margin of eye,

Table 1. Comparison of morphometric and meristic characters of *Pycnocraspedum squamipinne* ($n = 15$) and syntypes (ZSI F11700 and ZSI F 11703).

Measurements	<i>P. squamipinne</i> (ZSI F11700 and ZSI F 11703)	<i>P. squamipinne</i> (this study) $n = 15$		
		Min	Mean	Max
Total length [mm]	291–299	210	—	405
Standard length [mm]	270–277	190.4	—	380
As percentage of standard length				
Head length	29.6–30.4	25.6	29.0	33.0
Preorbital length	7.4–7.8	5.6	6.2	7.3
Eye diameter	4.3–4.8	4.1	4.8	5.8
Postorbital length	16.3–18.1	16.4	19.1	21
Interorbital width	4.8–5.9	4.8	5.5	6.4
Length of upper jaw	13.9–14.1	12.2	14.3	16.1
Predorsal length	25.1–25.6	21.2	25	30
Prepectoral length	33.5–33.5	26.7	31	35.7
Prepelvic length	24.8–27.1	19.8	21.6	24.5
Preanal length	50.0–50.5	46.2	50.6	59.9
Pelvic fin origin to anal fin origin	27.6–30.4	25.2	32.9	40.0
Length of longest pelvic filament	9.6–9.8	5.6	8.9	11.7
Length of pectoral fin	13.7–15.5	12.4	14.5	16.6
Body depth	16.7–17.3	14.7	18.3	23.1
Meristic counts				
Dorsal rays	63–68	69	—	92
Anal rays	56–58	54	—	79
Caudal rays	10	10	—	10
Pectoral rays	24	24	—	24
Pelvic filaments	2	2	—	2
Developed gill rakers	4	4	—	4
Branchiostegal rays	8	8	—	8
Transverse scale rows	58–60	49	—	86
Transverse scale rows ahead of anal origin	52–55	43	—	78
Scales above lateral line	11–15	10	—	24
Scales below lateral line	38–43	39	—	52

Table 2. Comparison of morphometric and meristic characters among *Pycnocraspedum* species.

Characters	<i>P. squamipinne</i>	<i>P. fulvum</i>	<i>P. armatum</i>	<i>P. microlepis</i>	<i>P. phyllosoma</i>
Proportional measurements in standard length					
Body depth at anal origin	4.3–6.8	5.1	4.4	—	—
Head length	3.0–3.9	3.7	3.9	4.2	4.4
Predorsal length	3.3–4.7	4.0	5.1	5.4	5.3
Preanal length	1.7–2.2	2.1	2.2	2.5	2.4
Prepelvic length	3.7–5.1	4.6	4.7	—	—
Proportional measurements in head length					
Eye diameter	5.5–6.8	6.4	5.8	5.9	5.0
Interorbital width	4.8–6.3	3.6	3.3	3.5	2.8
Upper jaw length	1.9–2.3	1.8	1.8	2.0	2.0
Snout length	3.8–5.2	4.3	3.9	4.2	3.5
Postorbital length	1.4–1.9	1.6	1.7	1.8	—
Pectoral fin length	1.7–2.2	1.7	1.6	1.4	1.4
Counts					
Dorsal fin rays	63–92	81	±90	81–98	97
Anal fin rays	54–79	63	±72	63–76	71
Pectoral fin rays	24	26/26	26	26	26
Pelvic fin rays	2	2	2	2	2
Caudal fin rays	10	10	10	10	10
Branchiostegal rays	8	8	8	8	8
Developed gill rakers	4	4	5–6	4	4
Pseudobranchiae	0	4	Rudimentary	0	6
Pyloric caeca	12–13	13	12	20	21
Preopercular spines	3	2	4	2	2
Scales above lateral line	10–24	~17	±20	25	21
Dorsal fin origin	About over preopercular margin	Slightly before opercular margin	Slightly before preopercular margin	About over preopercular margin	Before opercular margin
Vertebrae	47–49	52	—	52	—



Figure 1. (A) *Pycnocraspedum squamipinne*, 40.3 cm TL, off Chennai coast, Bay of Bengal; (B) *Pycnocraspedum squamipinne* syntype 29.9 cm TL, ZSI F11703, Bay of Bengal.

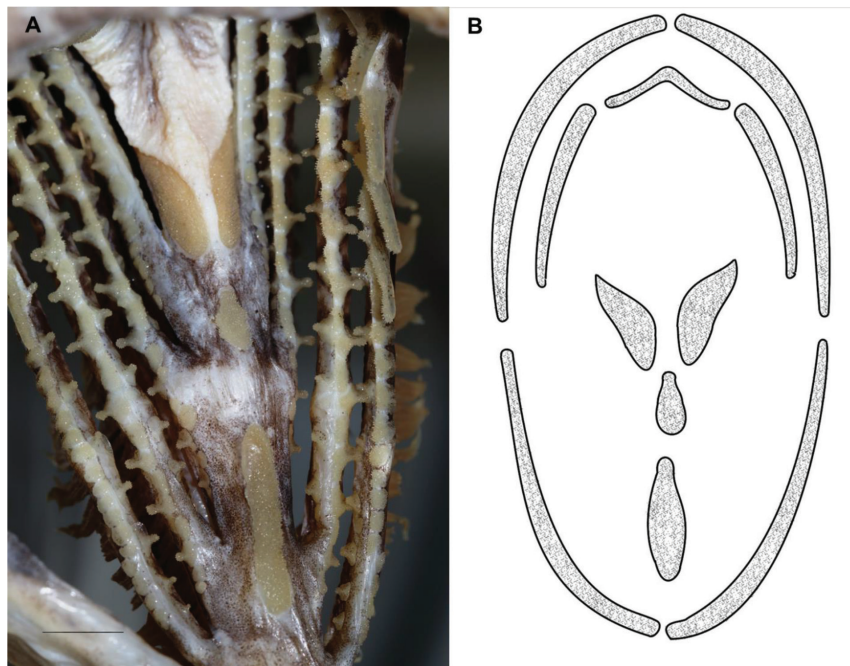


Figure 2. (A) Basibranchial tooth patches of *Pycnocraspedum squamipinne*, 29.8 cm SL, scale bar = 1 cm; (B) Dentition in jaws premaxillary, vomerine, palatine, dentary, and basibranchial tooth patches, scale bar = 1 cm.

wide posteriorly. Granular teeth in bands in jaws, vomer and palatines, vomerine tooth-patch narrow, v-shaped, tongue pointed, reaching below vomer. Median basibranchial with elongate tooth patch from first gill arch to third and second small tooth patch near fourth gill arch followed by pair of teardrop-shaped (rounded anteriorly and pointed posteriorly) lower pharyngeal tooth patches (Fig. 2). Sagittal otolith long and oval (Fig. 3A, B). Ratio

of length to height ranging from 2.4 to 2.9 times; length to thickness from 5.9 to 7.4 times. Pseudobranchial filaments absent. Developed gill rakers 4 on first gill arch, having small granular teeth (Fig. 3C). Branchiostegal rays 8; 6 in ceratohyal and 2 on epihyal. Opercle thin, soft with weak posterodorsal spine, three broad and blunt spines at lower angle of preopercle. Origin of dorsal fin above hind margin of preopercle. Pectoral fins elongate with

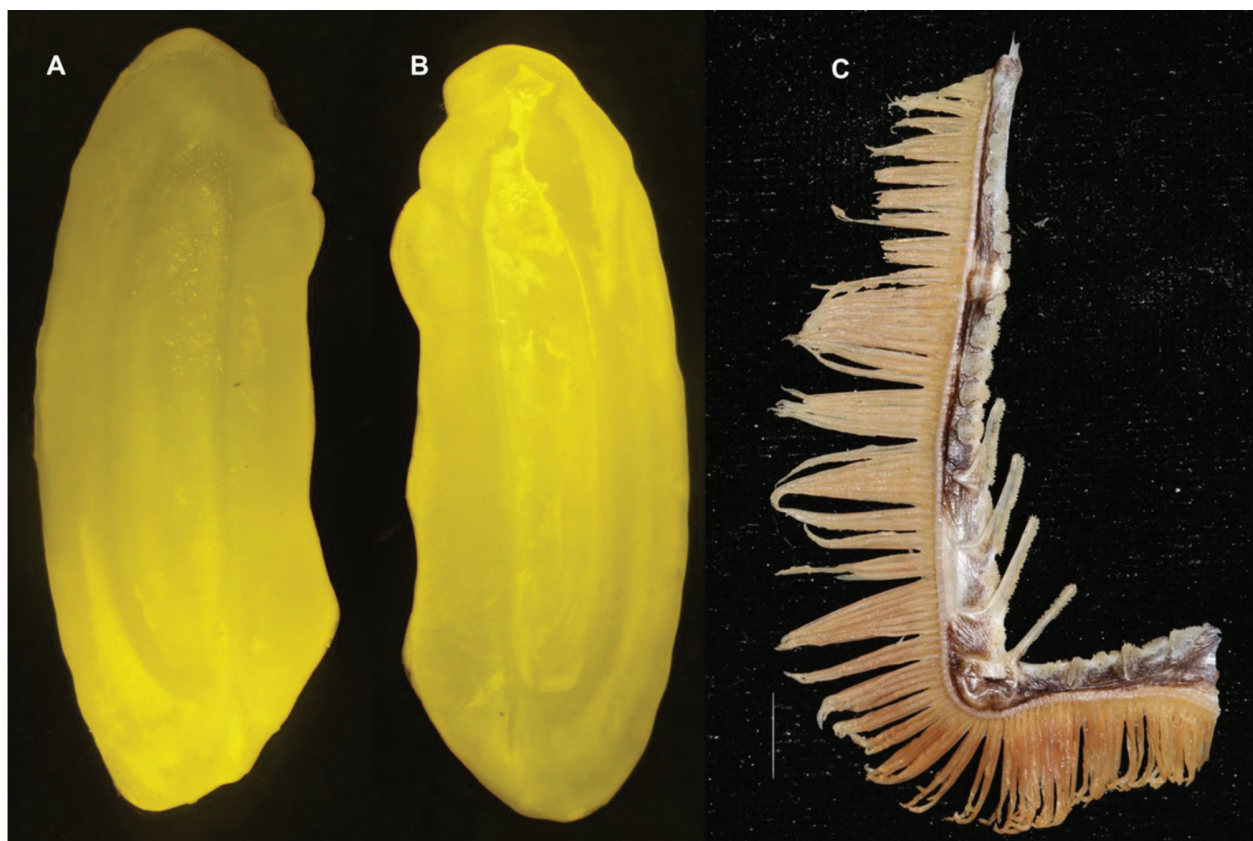


Figure 3. *Pycnocraspedum squamipinne*, 20 cm SL (A) left sagitta, (B) right sagitta; (C) First gill raker of *Pycnocraspedum squamipinne*, 29.8 cm SL, scale bar = 1 cm.

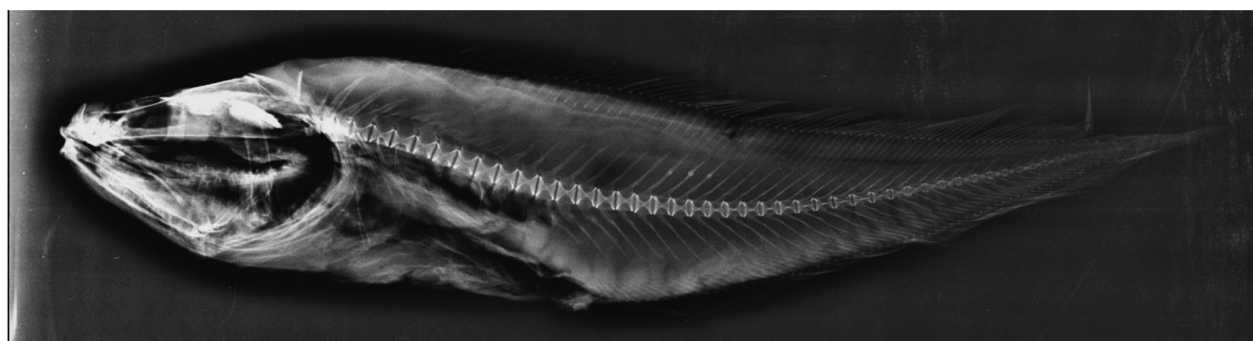


Figure 4. Radiograph of *Pycnocraspedum squamipinne*, 38 cm SL.

prominent skin flap above its base but not reaching anus. Pelvic fins united basally and separated distally. Caudal distinguishable from dorsal and anal by closer grouping of last 10 rays. Lateral line well developed, terminating at point about length of postorbit from caudal fin base. Stomach black with 13 long and finger-like pyloric caeca. Total of 12 precaudal vertebrae and 47–49 total vertebrae number (Fig. 4). Meristic and morphometric characters of the specimen are presented in Tables 1, 2.

Color. Body greyish brown but on ventral side dull silvery brown. Body without markings or spots. Distal margins of dorsal and anal fins dark black, ground color of dorsal and anal fin pale brown. Pectoral fins thick black but base of the pectoral light grey. Pelvic fin rays white with blackish tinge. Lateral line with blackish tinge. Orobranchial cavity greyish white, peritoneum black.

Discussion

Nielsen et al. (1999) reported five nominal species in the genus *Pycnocraspedum*, but noted that a thorough revision was required for confirming the validity of some species. *Pycnocraspedum fulvum*, *P. armatum*, *P. microlepis*, and *P. phyllosoma* have been described based on a single specimen and the descriptions are incomplete and need further elucidation (Gosline 1954; Machida 1984). The original description of *P. squamipinne* was lacking certain diagnostic characters such as the meristic counts and few measurements. The diagnostic features of *P. squamipinne* include short body with large head, eyes smaller than snout, single opercular spine, three preopercular spines, four developed gill rakers on first gill arch, two median basibranchial tooth patches, dorsal fin origin

above preopercular margin, and short pectoral and pelvic fins not reaching anus.

Norman (1939), reported *Pycnocraspedum squamipinne* from Zanzibar, Indian Ocean, however, has not provided a detailed description of the specimen. Nielsen (1997) described a species obtained from the waters of New Caledonia. The specimen was similar to *P. squamipinne*, but differed in the presence of 7 pseudobranchial filaments, dorsal fin origin, and also the length of pectoral fin which extends beyond the anus to the anal fin origin (fig. 20 in Nielsen 1997). Prokofiev (2005) described a species of *Pycnocraspedum*, collected off the east coast of Southern Africa, which closely resembled *P. squamipinne*. However, the specimen differed from *P. squamipinne* in the presence of 4 well developed pseudobranchial filaments (absent in *P. squamipinne*) and elongate pectoral and pelvic fins (short in *P. squamipinne*). The specimen shows similarity with *P. armatum* in having long pectoral fins, but distinguishable with the same in having weak spines in preopercle and the origin of the dorsal fin. The specimen had a single minute lateral basibranchial tooth-plate on the left side of the gill arch base which is not found in the rest of the species. The long pectoral fin, the presence of pseudobranchiae, and single lateral basibranchial tooth patch suggest that this may be a new species or a rare individual variation (Prokofiev 2005). Psomadakis et al. (2019), reported *Pycnocraspedum* cf. *squamipinne* from Myanmar, distinguished from *Pycnocraspedum squamipinne* in possessing a greater number of dorsal fin rays (90–91 vs. 63–92) and anal fin rays (57–62 vs. 54–79). Our data for *P. squamipinne*, encompasses a range that includes the Myanmar fish specimen, the only difference being the greater number of pectoral fin rays (27–30 vs. 24) for the specimen from Myanmar. Further analysis is required to verify the identity of the Myanmar fish.

Pycnocraspedum squamipinne resembles *P. armatum* with the pattern of basibranchial tooth patch but is distinguishable from the latter in the number of gill rakers (5–6 in the latter), absence of pseudobranchial filaments (rudimentary in latter), preopercular spines (4 in the latter), the origin of dorsal fin (originates forward of the preopercular border for latter), and shorter pectoral fin (long and reaches to the anus in the latter). The species differ from *P. fulvum* with the number of preopercular spines (2 in the latter), and the absence of pseudobranchial filaments, whereas *P. fulvum* has pseudobranchial filaments. Moreover, *Pycnocraspedum fulvum* has a pair of small tooth patches behind the anterior large tooth patch on the median basibranchial, whereas tooth patches on the lateral sides are absent in *P. squamipinne*. Furthermore, the dorsal fin origin of *P. fulvum* is located before the posterior margin of the opercle whereas, in *P. squamipinne* it is about over the preopercular margin. *Pycnocraspedum squamipinne* is clearly separable from *P. phyllosoma* in having a fewer number of pyloric caeca (12–13 vs. 20), the absence of pseudobranchiae (6 in the latter), a number of preopercular spines (3 vs. 2), and the differences in

proportions of the predorsal and preanal lengths, interorbital distance, eye diameter, snout, and pectoral length. Though *Pycnocraspedum microlepis* does not have pseudobranchiae, it differs from *P. squamipinne* with the number of preopercular spines, pyloric caeca, the proportion of head length, predorsal length, preanal length, interorbital length, and pectoral fin length. The counts of dorsal and anal fin rays are too variable to be used in discriminating related species in the genus.

Descriptive information regarding the species in the genus *Pycnocraspedum* is limited to the holotypes and syntypes mainly owing to the rarity in collections. A comprehensive study of their systematics is lacking. An in-depth study on the species of the genus *Pycnocraspedum* is required by examining holotypes and specimens to resolve their taxonomic ambiguity. As suggested by various authors, a revision of the genus is very much required to overcome the confusion in the species identification. Moreover, the molecular information generated for the species in the study would help in the species identification of this rare deep-sea species.

Recent studies have documented the diversity of deep-sea Ophidiiform fishes from the Indian ocean (Anderson 2005; Cubelio et al. 2009; Kurup et al. 2009; Nielsen and Møller 2011; Kannan et al. 2013a, 2013b; Kannan et al. 2014; Kannan 2017; Reethas et al. 2018; Uiblein and Nielsen 2018). More intensive deep-sea exploratory surveys and closer monitoring of deep-sea bycatch are required which may result in discoveries of more species in the Indian Ocean.

Conclusion

The morphological and molecular information generated for the cusk eel, *Pycnocraspedum squamipinne*, would help in the precise identification of the species. However, due to paucity, the majority of species of the genus are still only known from single specimens and comprehensive examination of holotypes and intensive sampling is needed to better understand the species identity and distribution.

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Assessment of morphological variation between stocks of bluefish, *Pomatomus saltatrix* (Actinopterygii, Perciformes, Pomatomidae), in the Aegean Sea, Black Sea, and Sea of Marmara

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Abstract

The population structure of the bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), in Turkish waters is scarcely described in the literature. To identify any distinct population units of bluefish, and reaffirm the findings of a previous study, four areas were selected: the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara. In this study, truss network morphometrics, meristics, and otolith shape analyses were successfully applied for different population identification of the bluefish. Multivariate analysis of variance (MANOVA) revealed no differences for truss network morphometrics, meristic, and otolith shape characters between males and females. Hence, both sexes were combined for the discriminant function (DFA) and the Principal Component Analysis (PCA). Using univariate ANOVA based on the stepwise method revealed a highly significant difference among different locations for each truss-morphometrics and otolith shape characters. Furthermore, six out of seven meristic characters also showed significant differences between different areas. Based on PCA, 25 out of 27 truss-morphometric characters had a loading value above 0.70, which was considered significant in this study. The results of DFA show clear patterns of truss-morphometric character variations, forming four distinct clusters that were well separated from each other, indicating the existence of four morphologically differentiated populations of the bluefish. The proportion of the correctly classified Aegean Sea, western Black Sea, and eastern Black Sea bluefish samples to their original groups were 100%, demonstrating clear separation of these stocks from each other. Whereas up to 5% of the total samples of the Sea of Marmara were incorrectly classified, assigning to the eastern Black Sea. These findings were supported by meristic and otolith shape characters that also indicated four morphologically differentiated populations of the bluefish. However, their overall proportion of correct classification was relatively lower than the truss-morphometric traits method. The findings suggest the requirement of strategic assessment and management of each bluefish stock separately to use them sustainably in the future.

Keywords

Climate change, factor analysis, Pomatomidae, stock structure, truss network system

Introduction

The bluefish, *Pomatomus saltatrix* (Linnaeus, 1766), is a highly migratory pelagic streamlined predatory species with a wide geographical distribution that occurs in the majority of major ocean basins throughout the world except for the eastern Pacific (Helfman et al. 2009; Carpenter et al. 2015). It comprises an integral part of billfishes, sharks, and tunas' diets, constituting up to 80% of their diets (Feldman 2013). It is also an economically important marine fish species in the temperate and subtropical waters (Shepherd 2010). In the Turkish territorial waters, bluefish begin their spawning migration in spring via the Aegean Sea northwards from the Mediterranean and return south in the early autumn (Ceyhan et al. 2007). Its spawning season is limited to the warmest months in the region at water temperatures of 20–26°C from July to September (Ceyhan et al. 2007; Sabatés et al. 2012).

Bluefish is subjected to over-exploitation threats and has been considered a globally vulnerable species (Carpenter et al. 2015). The overall global landings of bluefish have generally trended from a peak to down over the past 15–24 years, plummeted by 7 percentage points to 46% (Carpenter et al. 2015; MAF 2019). The maximum capture of bluefish was 25 000 tons in 2002 (MAF 2019), and since then, their population has been on a steady decline, hitting its lowest level in 2019 (TÜİK 2020). The total Turkish landings of bluefish from the Aegean Sea, Black Sea, Mediterranean Sea, and Sea of Marmara were 5767 and 1213 tons in 2018 and 2019, respectively (TÜİK 2020).

A previous study by Turan et al. (2006) reported the existence of a total of three morphologically isolated subpopulations of bluefish in the Turkish territorial waters. The first stock was made by the Aegean Sea, the Sea of Marmara, and the western Black Sea, while the two other

morphologically isolated subpopulations of bluefish were represented the Mediterranean Sea and the east Black Sea (Turan et al. 2006). However, no other comprehensive research has been undertaken to evaluate the bluefish population structures in the Turkish territorial waters after a study conducted by Turan et al. (2006). According to Rawat et al. (2017) the identification of stock with distinguished phenotypic and genetic differentiation among fish populations within a species may help to effectively: 1) manage the stock separately, 2), achieving biologically sustainable productivity, 3) determine stock-wise population abundance, 4) estimate how each stock respond to fisheries exploitation, and 5) accomplish the objectives of fishery stock assessment by modeling (Rawat et al. 2017). Thus, the presently reported study aimed to investigate the morphological population structure of bluefish for the second time after a decade to determine the possible existence of any new geographically isolated populations of bluefish. In this study, the inter-population morphometric variability of bluefish was investigated in the Aegean Sea, the western Black Sea, eastern Black Sea, and the Sea of Marmara by truss-morphometric traits, meristic characters, and otolith characters.

Materials and methods

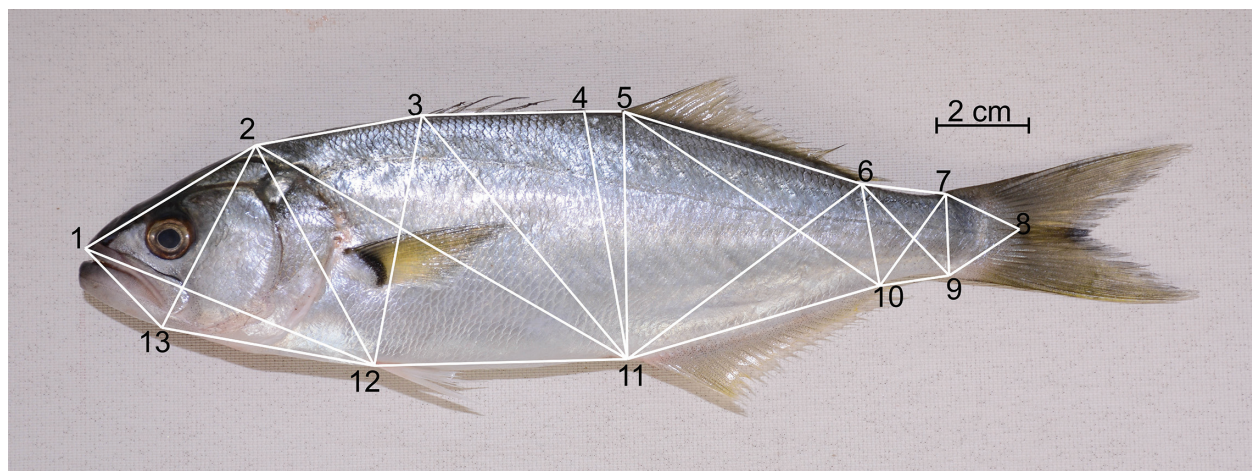
Samples of bluefish were collected from four commercial fish landing centers: Aegean Sea (Gulf of İzmir), western Black Sea (Şile İstanbul), eastern Black Sea (Trabzon: Akçaabat), and the Sea of Marmara (Erdek Balıkesir) (Fig. 1). The sampling details of the bluefish are provided in Table 1. Samples were carefully preserved in iceboxes (ca. –20°C) to transfer to the laboratory for further examination.



Figure 1. Map of the study area (Sources: ESRI World Ocean GDAL basemap layer).

Table 1. Descriptive data of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, Western Black Sea, Eastern Black Sea, and Sea of Marmara.

Sea	Location	Coordinates	n	Sex ratio (♀:♂)	Date of capture	Sampling gear
Aegean Sea	Gulf of İzmir	38°36'32.8"N, 26°38'53.9"E	31	1.0:1.1	05 Apr. 2014	Fishhook
Western Black Sea	Şile İstanbul	41°13'39.4"N, 29°43'09.1"E	36	1.0:1.1	29 Sep. 2014	Gillnet
Eastern Black Sea	Akçaabat Trabzon	41°02'45.3"N, 39°36'18.5"E	33	1.0:0.9	14 Nov. 2014	Fishhook
Sea of Marmara	Erdek Balıkesir	40°28'59.1"N, 27°33'37.9"E	31	1.0:0.8	29 Feb. 2014	Purse-seine

**Figure 2.** Truss-morphometric characters measured on bluefish, *Pomatomus saltatrix*.

Data acquisition of morphometric traits and meristic characters

Before taking the measurements, the frozen samples of bluefish were thawed for 1 hour under running water, placed on their right side on a water-resistant graph. Body posture and fins were forced into a natural position. Each fish was examined for physical damage, and a sample with any physical damage was removed from the analysis. Furthermore, their sexes were determined by reviewing their gonads under a dissecting microscope.

A total of 13 anatomical landmarks were chosen for the study, and by inter-connecting these landmarks, the box-truss network was produced, representing a truss network of 27 lines (Fig. 2, Table 2). Each landmark line was measured via manual methods by piercing the paper with a needle (Strauss and Bookstein 1982; Hanif et al. 2019).

Using a binocular microscope, the number of branched and un-branched rays in dorsal fin spines, dorsal fin rays, ventral fin rays, pectoral fin rays, and anal fin rays as well as right and left gill rakers were obtained (Turan et al. 2006).

Otolith extraction

The sagittal otoliths were removed from all individuals. Each otolith was carefully wiped, clean, and stored dry in U-plates (Bal et al. 2018a). A digitized image for each otolith was produced using a binocular microscope coupled with a digital camera. The digitized im-

Table 2. Description of morphometric measurements made for each sample of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara between February 2014 and November 2014.

Measurement No.	Distance code	Distance	Landmarks
1	HL1	Head length 1	1–2
2	BL1	Body length 1 (Pre-dorsal length)	2–3
3	DFBL1	First dorsal fin base Length	3–4
4	MDL	Mid dorsal length	4–5
5	DFBL2	Second dorsal fin base length	5–6
6	PDL	Post-dorsal length	6–7
7	CL1	Caudal length 1	7–8
8	CL2	Caudal length 2	8–9
9	BL2	Body length 2	9–10
10	AFBL	Anal fin base length	10–11
11	BL3	Body length 3	11–12
12	BL4	Body length 4	12–13
13	HL2	Head length 2	1–13
14	BD1	Head diagonal 1	2–13
15	BH1	Body height 1	3–12
16	BD2	Body diagonal 2	4–11
17	BH2	Body height 2	5–11
18	BD3	Body diagonal 3	6–11
19	BD4	Body diagonal 4	7–10
20	BD5	Head diagonal2	1–12
21	BD5	Body diagonal 5	2–12
22	BD6	Body diagonal 6	3–11
23	BD7	Body diagonal 7	5–10
24	BH2	Body height 2	6–10
25	BH3	Body height 3	7–9
26	BD8	Body diagonal 8	2–11
27	BD9	Body diagonal 9	6–9

ages were then used to measure the otolith dimensions using ImageJ2 software (Rueden et al. 2017). Each otolith was weighed individually to the nearest 0.01 g on a digital balance.

Statistical analysis

Truss-morphometric and otolith variables were standardized separately for each region to eliminate the effect of fish size on these variables. The meristic characters were not standardized as they did not show a significant correlation with the bluefish body size (Turan et al. 2006). The variables were standardized using the following allometric equation (Reist 1986)

$$V_{\text{trans}} = \log V - \hat{\alpha}(\log SL - \log SL_{\text{mean}})$$

where V_{trans} is the transformed morphometric variable, V is the non-transformed variable, SL is the standard length of each fish, SL_{mean} is the overall mean standard length of all the fish from each group (region), and β is the slope of the relation between $\log V$ and $\log SL$.

The modified morphometric variables were tested for normality check, and outliers, if any, were excluded before subsequent analysis. Multivariate analysis of variance (MANOVA) was performed to check significant variation between different sex groups as well as sampling locations based on morphometrics, meristic, and otolith characters. The univariate ANOVA for each variable was then used to test significant differences among different sampling areas. The differences were considered statistically significant at P -values below 0.05. Principal component analysis (PCA) was used to uncover the morphometric variables with a highly influential role in distinguishing between the four populations. Discriminant function analysis (DFA) was used to demonstrate the variations among different bluefish stocks by classifying

them to their respective groups based on morphometrics, meristic, or otolith characters. Dendrogram based Euclidean distance method was used to depict similarities between different locations. All statistical analyses were carried out with IBM SPSS Statistics software ver. 25.0.

Results

The size distribution of the bluefish based on total length is presented in Fig. 3. None of the sizes corrected truss measurements showed statistical significance with standard length by using correlation analysis, which indicates the allometric transformation method efficiently removed the effect of body size.

Truss-morphometric traits

There was no statistical difference observed between truss-morphometric characteristics for females and males (one-way MANOVA; $F_{(27, 32)} = 26.4$, Wilk's $\lambda = 0.456$, $P = 0.172$); hence, sexes were combined for further analysis. While there were highly significant differences among the stocks of bluefish from different locations using all data (one-way MANOVA; $F_{(81, 108)} = 26.4$, Wilk's $\lambda = 0.0001$, $P < 0.0001$). Also, the univariate ANOVA based on the stepwise method further revealed a highly significant difference among different locations for each truss-morphometric trait (Table 3). Furthermore, the PCA uncovered the truss-morphometric traits with a highly influential role in distinguishing

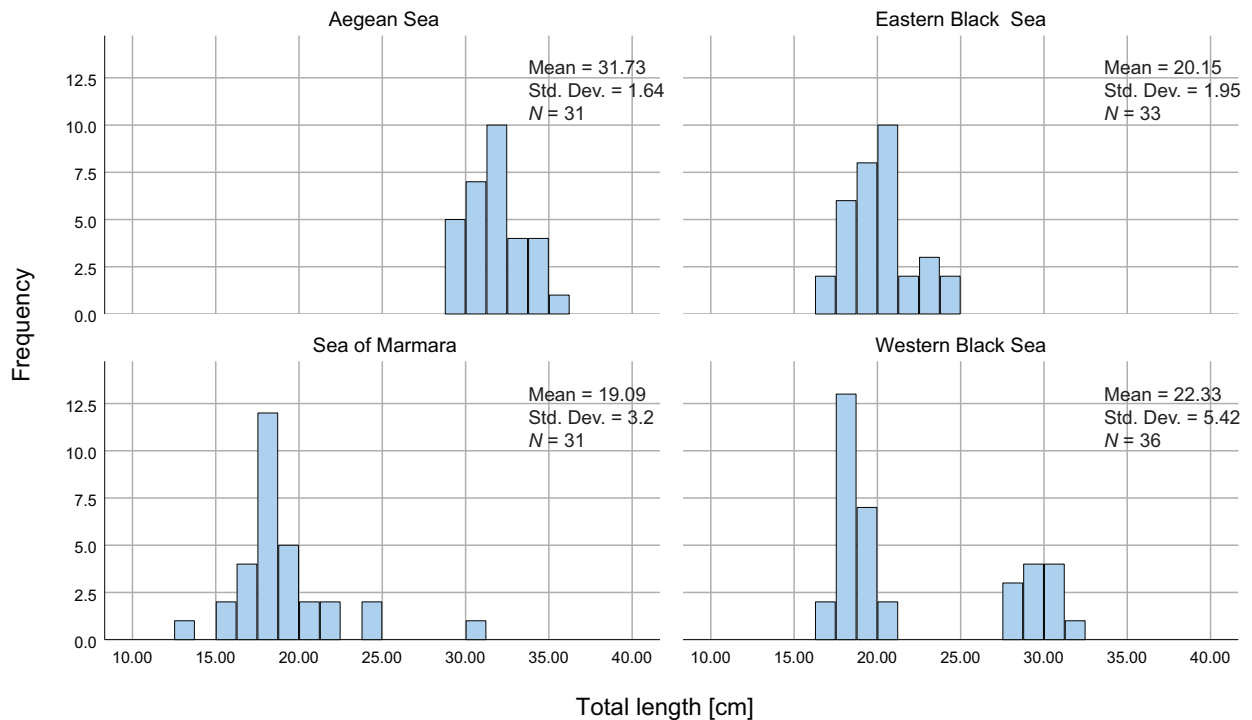
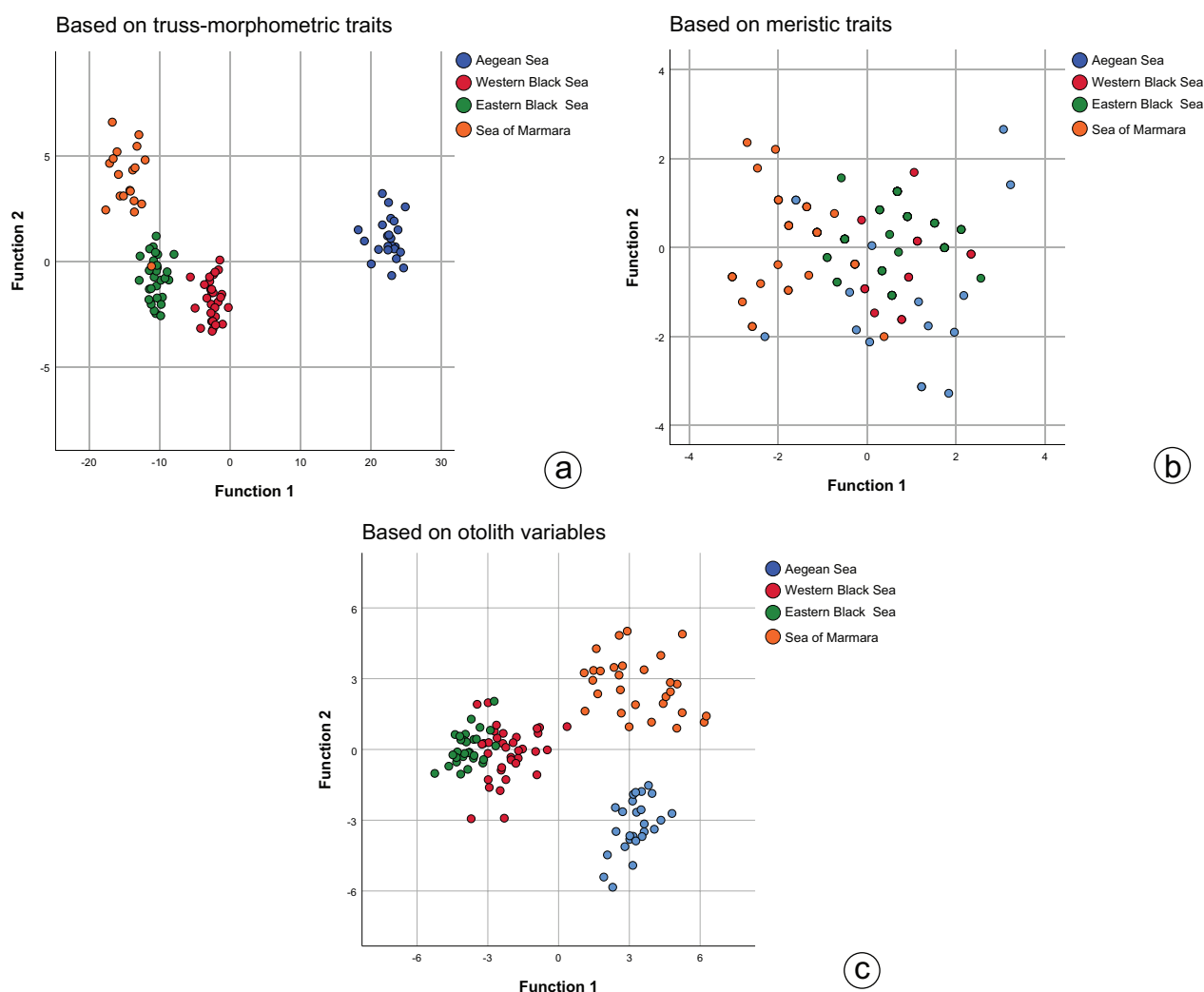


Figure 3. Frequency distribution of bluefish, *Pomatomus saltatrix*, according to their total length.

Table 3. Descriptive statistics of univariate ANOVA based on morphometric characters of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Morphometric characters	Univariate ANOVA			Morphometric characters	Univariate ANOVA		
	Wilks' λ	F	Sign.		Wilks' λ	F	Sign.
1–2	0.276	54.241	<0.0001	3–12	0.068	285.212	<0.0001
2–3	0.085	221.971	<0.0001	4–11	0.056	349.181	<0.0001
3–4	0.163	106.011	<0.0001	5–11	0.070	274.448	<0.0001
4–5	0.315	44.959	<0.0001	6–11	0.035	575.511	<0.0001
5–6	0.036	557.940	<0.0001	7–10	0.093	201.551	<0.0001
6–7	0.176	96.828	<0.0001	1–12	0.067	289.156	<0.0001
7–8	0.215	75.242	<0.0001	2–12	0.067	289.493	<0.0001
8–9	0.174	97.954	<0.0001	3–11	0.038	526.114	<0.0001
9–10	0.228	70.132	<0.0001	5–10	0.025	807.113	<0.0001
10–11	0.033	602.729	<0.0001	6–10	0.072	265.311	<0.0001
11–12	0.055	351.826	<0.0001	7–9	0.132	135.666	<0.0001
12–13	0.113	162.162	<0.0001	2–11	0.022	925.575	<0.0001
1–13	0.308	46.441	<0.0001	6–9	0.157	110.827	<0.0001
2–13	0.201	82.171	<0.0001				

Abbreviations of morphometric characters are given in Fig. 2.

**Figure 4.** Discriminant function analysis (DFA) of bluefish, *Pomatomus saltatrix*, populations based on the truss-morphometric traits, meristic characters, and otolith variables.

between the four populations. The estimated value of Kaiser–Meyer–Olkin (KMO) was 0.911, suggesting that the data was appropriate for factor analysis. The first two principal components accounted for 84.81% (PC1) and

5.27% (PC2) of the total variance, explaining 90.1% of the total variation. The truss-morphometric trait that had loadings > 0.70 was considered significant in this study. Except for 1–13 and 4–5, all truss-morphometric traits

Table 4. The first two component-loading scores of principal components based on morphometric characters of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Morphometric characters	Principal component				
	PC1 (84.81%)	PC2 (5.27%)	Morphometric characters	PC1 (84.81%)	PC2 (5.27%)
1-2	0.757	0.479	3-12	0.970	-0.092
2-3	0.936	-0.049	4-11	0.981	-0.035
3-4	0.859	-0.402	5-11	0.977	-0.093
4-5	0.615	0.616	6-11	0.983	-0.067
5-6	0.976	0.025	7-10	0.964	-0.104
6-7	0.917	0.077	1-12	0.969	0.028
7-8	0.894	0.007	2-12	0.967	-0.113
8-9	0.894	0.144	3-11	0.984	-0.079
9-10	0.888	-0.200	5-10	0.986	0.020
10-11	0.973	0.016	6-10	0.974	-0.052
11-12	0.968	-0.010	7-9	0.954	-0.021
12-13	0.863	-0.373	2-11	0.984	-0.041
1-13	0.676	0.570	6-9	0.943	-0.058
2-13	0.887	0.242			

Abbreviations of morphometric characters are given in Fig. 2. Bold values indicated significance loading at >0.70 .

Table 5. Summary output of stepwise canonical discriminant analysis based on morphometric characters bluefish, *Pomatomus saltatrix*, samples from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara collected between February 2014 to November 2014; Overall, 99.0% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	100%				100%
Western Black Sea		100%			100%
Eastern Black Sea			100%		100%
Sea of Marmara			5.26%	94.74%	100%

had a loading value above 0.70 on PC1 (Table 4). The second PC2 was strongly associated with 4-5 and 1-13 truss-morphometric traits, and their loading values were 0.616 and 0.570, respectively.

DFA results show clear patterns of truss-morphometric trait variations, forming four distinct clusters that are well separated from each other (Fig. 4). In DFA, the first DF accounted for 97.7%, and the second corresponded to 1.9% of the between-group variability. The proportion of correctly classified Aegean Sea, western Black Sea, and eastern Black Sea samples to their original groups were 100%, demonstrating clear separation of these stocks from each other. Up to 5% of the Sea of Marmara samples were incorrectly classified (Table 5).

Dendrogram, based on the Euclidean distance method, formed three main clusters (Fig. 5). The first cluster formed by the Aegean Sea was separated with maximum Euclidean distance evincing apparent isolation of the Aegean Sea population from others, which supports the result highlighted by DFA (Fig. 4). The minimum Euclidean distance was found between the western Black Sea and the eastern Black Sea, sharing a high similarity.

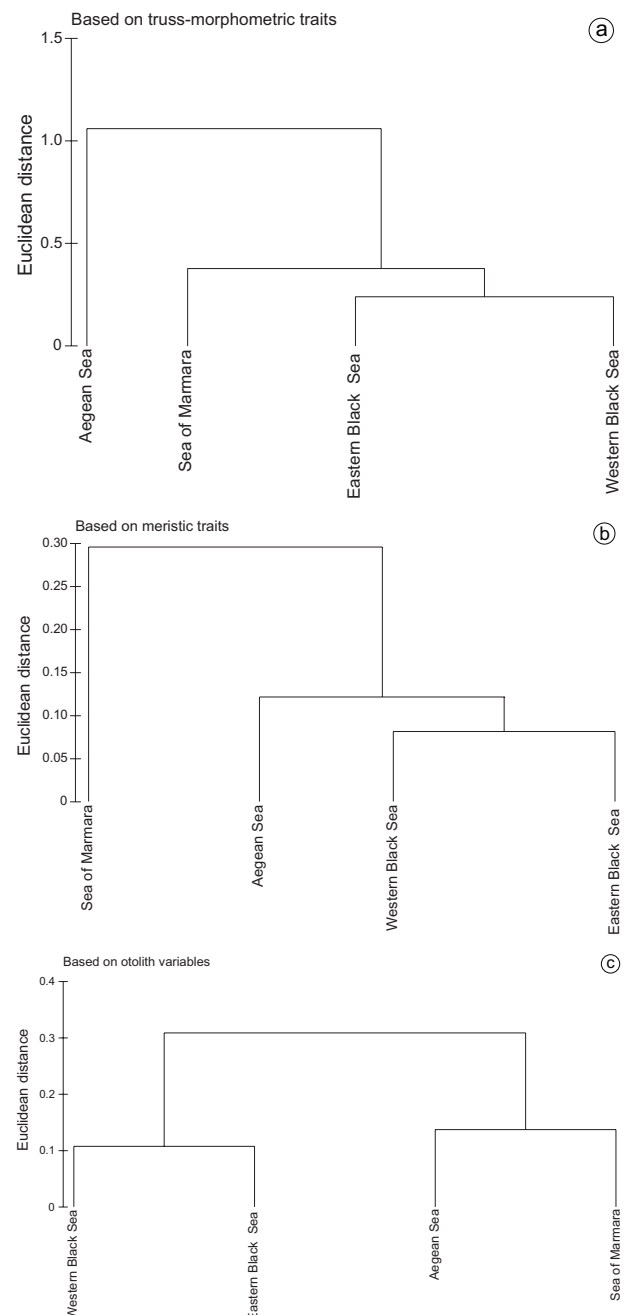


Figure 5. Dendrogram based on the Euclidean distance method depicting the dissimilarity of bluefish, *Pomatomus saltatrix*, populations based on the truss-morphometric traits, meristic characters, and otolith variables.

Meristic characters

The range of the bluefish meristic counts from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara are given in Table 6. The effect of sex on meristic characters were not significant (one-way MANOVA; $F_{(7.0, 123)} = 1.57$, Wilk's $\lambda = 0.918$, $P = 0.150$); therefore, further analysis was done disregarding the sex. The meristic characters showed significant variations for different stocks of the bluefish (one-

Table 6. Descriptive data of the meristic counts of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Meristic characters	Aegean Sea		Western Black Sea		Eastern Black Sea		Sea of Marmara	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Right gill rakers	10.97 \pm 0.84	9–12	11.17 \pm 0.97	9–13	11.00 \pm 0.00	11–11	10.39 \pm 1.15	9–12
Left gill rakers	10.87 \pm 0.81	9–12	11.25 \pm 1.00	9–13	10.91 \pm 0.52	10–12	10.39 \pm 1.05	9–12
Dorsal fin spines	7.19 \pm 0.70	6–10	7.72 \pm 0.45	7–8	7.55 \pm 0.51	7–8	6.65 \pm 0.49	6–7
Dorsal fin rays	24.39 \pm 1.17	22–27	24.44 \pm 0.88	23–26	24.15 \pm 0.94	22–26	22.68 \pm 0.79	22–24
Ventral fin rays	11.77 \pm 0.72	10–13	12.00 \pm 0.00	12–12	12.00 \pm 0.00	12–12	12.10 \pm 0.30	12–13
Pectoral fin rays	15.13 \pm 0.85	12–16	14.97 \pm 0.38	14–16	15.18 \pm 0.46	14–16	15.03 \pm 0.66	14–16
Anal fin rays	25.48 \pm 1.09	24–28	25.03 \pm 1.03	23–27	24.70 \pm 0.88	24–27	24.10 \pm 1.14	23–28

Table 7. Descriptive statistics of univariate ANOVA based on meristic characters of the bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara collected between February 2014 and November 2014.

Characters	Wilks' λ	F	Significance
Right gill rakers	0.889	5.311	0.002
Left gill rakers	0.884	5.562	0.001
Dorsal fin spines	0.618	26.214	0.000
Dorsal fin rays	0.630	24.873	0.000
Ventral fin rays	0.910	4.195	0.007
Pectoral fin rays	0.982	0.786	0.504
Anal fin rays	0.808	10.083	0.000

Table 8. The component-loading scores of principal components based on meristic characters of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Character	Principal component	
	PC1 (37.45%)	PC2 (20.94%)
Right gill rakers	0.769	0.466
Left gill rakers	0.786	0.440
Dorsal fin spines	0.626	–0.157
Dorsal fin rays	0.612	–0.574
Ventral fin rays	0.020	0.482
Anal fin rays	0.521	–0.508

Bold values indicated significance loading at >0.70 .

Table 9. The summary output of stepwise canonical discriminant analysis based on meristic characters of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014; overall, 64.1% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	67.74%	9.68%	9.68%	12.90%	100%
Western Black Sea	16.67%	50.00%	27.78%	5.56%	100%
Eastern Black Sea	15.15%	24.24%	51.52%	9.09%	100%
Sea of Marmara	6.45%		3.23%	90.32%	100%

Table 10. Descriptive data of otolith variables of bluefish, *Pomatomus saltatrix*, collected from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Otolith variables	Aegean Sea		Western Black Sea		Eastern Black Sea		*Sea of Marmara	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
Otolith length	7.07 \pm 0.32	6.28–7.55	5.38 \pm 1.09	4.34–7.39	5.02 \pm 0.38	4.37–5.95	6.36 \pm 0.87	4.65–8.98
Otolith width	2.58 \pm 0.14	2.23–2.81	2.16 \pm 0.23	1.8–2.58	2.09 \pm 0.1	1.91–2.26	2.50 \pm 0.26	1.99–3.16
Otolith area	13.03 \pm 0.99	10.79–14.94	8.46 \pm 2.86	5.53–13.63	7.56 \pm 0.97	5.82–9.37	12.63 \pm 3.05	7.25–21.63
Otolith perimeter	17.57 \pm 1.03	15.66–19.79	14.3 \pm 2.87	10.71–19.79	12.47 \pm 1.03	10.52–14.48	19.48 \pm 3.95	14.51–32.09
Form factor	0.53 \pm 0.04	0.46–0.6	0.52 \pm 0.06	0.39–0.64	0.61 \pm 0.04	0.52–0.72	0.43 \pm 0.08	0.18–0.55
Roundness	0.33 \pm 0.02	0.3–0.39	0.37 \pm 0.03	0.29–0.42	0.38 \pm 0.02	0.34–0.41	0.39 \pm 0.03	0.33–0.44
Aspect ratio	2.74 \pm 0.14	2.36–3.03	2.47 \pm 0.26	2.13–3	2.4 \pm 0.13	2.16–2.69	2.54 \pm 0.17	2.28–3.07

*Bal et al. (2018b).

way MANOVA; $F_{(21, 348)} = 7.259$, Wilk's $\lambda = 0.352$, $P < 0.0001$). Univariate ANOVA, based on the stepwise method, further revealed a highly significant difference among different locations for six out of seven meristic characters (Table 7). The pectoral fin rays were not considered in the PCA analysis as it was constant among different stocks of the bluefish. The estimated value of KMO was 0.596. The PC1 and PC2 accounted for 37.45% and 20.94% of the total variance, explaining 58.4% of the total variation. Only two meristic characters, viz. right and left gill rakers, had a loading value above 0.70 (Table 8).

In DFA, the first DF accounted for 83.0%, and the second corresponded to 16.8% of the between-group variability. Overall, 64.1% of original grouped cases were correctly classified, and the bluefish correct classification into their original population/location ranged from 50.0% to 90.3% by canonical discriminant analysis (Table 9). The remarkably high reclassification rate was recorded by the bluefish individuals from the Sea of Marmara (90.3%) clearly separated from the other stocks (Fig. 4). The dendrogram based on the Euclidean distance method also proved that the stock of the Sea of Marmara was the most clearly distinguished stock isolating it from the

other groups with the highest Euclidean distance (Fig. 5). The stocks of the eastern and western Black Sea shared high similarity having the lowest Euclidean distance.

Otolith characters

The mean, standard deviation, minimum and maximum values for each otolith variable of the bluefish (*Pomatomus saltatrix*) are given in Table 10. Similar to truss-morphometric variables, the otoliths variables were free from the influence of body size using the allometric transformation method. The effect of sex on otolith bluefish characters was also not significant (one-way MANOVA; $F_{(4, 110)} = 0.597$, Wilk's $\lambda = 0.979$, $P = 0.666$); therefore, both sexes were combined for further analysis. Similar to truss-morphometric and meristic characters, the otolith variables were showed significant differences among the stocks of bluefish from different locations (one-way MANOVA; $F_{(12, 286)} = 100.275$, Wilk's $\lambda = 0.013$, $P < 0.0001$). Also, univariate ANOVA, based on the stepwise method, further revealed a highly significant difference among different locations for otolith variables (Table 11). This matrix was not positive defi-

Table 11. Descriptive statistics of univariate ANOVA based on otolith variables of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014 from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Variable	Wilks' λ	F	Significance
Otolith length	0.102	324.325	0.000
Otolith width	0.257	107.157	0.000
Otolith area	0.114	288.710	0.000
Otolith circumference	0.121	269.433	0.000
Form factor	0.390	57.986	0.000
Roundness	0.295	88.584	0.000
Aspect ratio	0.425	50.075	0.000

Table 12. The component-loading scores of principal components based on otolith variables of bluefish, *Pomatomus saltatrix*, sampled from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara.

Variables	Principal component	
	PC1 (66.07%)	PC2 (21.33%)
Otolith length	0.981	-0.123
Otolith width	0.893	0.181
Otolith area	0.964	0.112
Otolith circumference	0.938	0.331
Form factor	-0.557	-0.572
Roundness	-0.445	0.832
Aspect ratio	0.741	-0.551

Bold values indicated significance loading at > 0.70 .

Table 13. Summary statistics of stepwise canonical discriminant analysis based on otolith variables of bluefish, *Pomatomus saltatrix*, collected between February 2014 and November 2014; overall, 96.6% of original grouped cases correctly classified.

Populations	Predicted group membership				Total
	Aegean Sea	Western Black Sea	Eastern Black Sea	Sea of Marmara	
Aegean Sea	100				100
Western Black Sea		91.43	8.57		100
Eastern Black Sea		3.45	96.55		100
Sea of Marmara				100	100

nite, and hence the KMO was not displayed for otolith variables. The PC1 and PC2 accounted for 66.07% and 21.32% of the total variance, explaining 91.95% of the total variation. Except for CI and RD, all had a loading value above 0.70 (Table 12).

In DFA, the first DF accounted for 69.1%, and the second corresponded to 28.9% of the between-group variability. Overall, 96.6% of original grouped cases were correctly classified, and the bluefish correct classification into their original population ranged from 91.4% to 100% by canonical discriminant analysis (Table 13). The bluefish from the Aegean Sea, as well as the Sea of Marmara, each formed a distinct cluster that was well separated from others (Fig. 4). Furthermore, the Aegean Sea and the Sea of Marmara samples' reclassification rate to their original group were 100%, and hence they both were the most clearly isolated groups. Dendrogram, based on the Euclidean distance method, formed two main clusters (Fig. 5). The first cluster formed by the Aegean Sea and Sea of Marmara were separated with maximum Euclidean distance evincing apparent isolation of these populations from others, which supported the result highlighted by DFA (Fig. 4). Similarly, in truss-morphometric traits and meristic characters methods, the minimum Euclidean distance was found between the western Black Sea and the eastern Black Sea, sharing a high similarity.

Discussion

The truss-morphometric characteristics analysis provided evidence of the existence of four morphologically differentiated populations of bluefish, with 95% to 100% correct allocation of bluefish individuals into their original stock. These results are in line with the findings of Turan et al. (2006), who also observed the existence of morphologically differentiated groups of bluefish in Turkish sea waters. Turan et al. (2006) observed three morphologically differentiated groups of bluefish: first included samples from the Aegean Sea, Sea of Marmara, and the western Black Sea and formed a stock, while the other two groups were made by the north-eastern Mediterranean Sea and east Black Sea, and each represented a separate stock. In contrast to Turan et al. (2006), this study evinced the populations of bluefish from the Aegean Sea, the Sea of Marmara, and the western Black Sea did not overlap in DFA analysis, and they are clearly distinct stocks based on truss-morphometric characteristics (Fig. 4a). According to

Turan et al. (2006), the existence of low phenotypic differentiation among the Aegean Sea, Sea of Marmara, and the western Black Sea was attributed to the extensive migration of bluefish in these waters (i.e., Pardiñas et al. 2010), resulted in a higher level of intermingled bluefish stocks.

Several studies suggest that the population structure of highly migratory marine species is strongly regulated by some behavioral traits such as spawning site fidelity, homing behavior (Danancher and Garcia-Vazquez 2011), but can also be promoted by oceanic barriers to gene flow (Machado-Schiaffino et al. 2010), temperature (Crow et al. 2007) or salinity (Nielsen et al. 2004). The Bosphorus is an important migration route for fishes between the Sea of Marmara and the Black Sea (Atilgan et al. 2017; Ceyhan et al. 2007; Kokos 2011). A recent increase in anthropogenic activities, such as an increase in the pollutant loads from industrial and domestic sources, together with high sea traffic and coastal erosion in the Bosphorus, might prevent fish migration (Özsoy and Mikaelyan 1997). They might also restrict the intermingling of bluefish stocks among the western Black Sea, Sea of Marmara, and the Aegean Sea, and consequently showed stock separation.

The use of more than one stock identification approach and comparison between them can enhance the likelihood of extracting differences between classifying for a comprehensive conclusion (Waldman et al. 1988; Begg and Waldman 1999; Cadrin et al. 2014). The truss-morphometric characteristics analysis with meristic characteristics or otolith characters has been used combined to investigate between subpopulations of a fish (Begg and Waldman 1999; Turan et al. 2006; Khan et al. 2012; Bose et al. 2020). The ability of each method to correctly allocate individuals into their original stock change from species to species (Turan et al. 2006; Khan et al. 2012; Hari et al. 2019). In this study, the truss morphometric approach demonstrated a higher success rate (99%) in individuals'

allocation to their original locations than the meristic characters method, which had a 64% success rate. The success rate of the otolith characters' approaches demonstrated was higher (97%) than the meristic characters approach. On the contrary, Turan et al. (2006) recorded a higher success rate for meristic characters (64%) than the truss morphometric approach (54%). Consequently, these differences indicate that the ability of a stock identification approach to correctly allocating individuals into their original stock might change over time.

Conclusions

Bluefish stock from the Aegean Sea, western Black Sea, eastern Black Sea, and the Sea of Marmara demonstrated considerable morphometric variations and hence they should be considered as four self-contained stocks that are geographically isolated from each other. Environmental differences between areas probably influence these inter-population morphometric distinctions. This might indicate new environmental consequences hindering the intermingling of bluefish stocks; since the stocks of the Aegean Sea, the Sea of Marmara, and the western Black Sea were observed as a single, morphometrically homogeneous stock by Turan et al. (2006). This study suggests the requirement of strategic assessment and management of each bluefish stock separately to use them sustainably in the future.

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Feeding habits of the spotted rose snapper, *Lutjanus guttatus*, (Actinopterygii, Perciformes, Lutjanidae), in the central Gulf of California, BCS, Mexico

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Abstract

The spotted rose snapper, *Lutjanus guttatus* (Steindachner, 1869), is an important resource for the coastal fisheries of the Gulf of California, mainly due to its high commercial value. Despite this, there are no management measures for this species, owing in part to a lack of information on its basic biology and its trophic ecology in the area. In this context, the presently reported study had as objective to describe the feeding habits of *L. guttatus* through stomach content analyses, as well as to describe possible changes linked to sex, size, and season. Specimens were caught monthly from June 2016 to September 2017 with gillnets in Santa Rosalía, BCS, Mexico. The abundance, weight, and frequency of occurrence of each prey were assessed, and these parameters were integrated into the index of relative importance (%IRI) to determine the importance of each prey item in the *L. guttatus* diet. The Levin's index was used to assess the trophic niche width of the species, the feeding strategy was evaluated using Costello's graphic method and the trophic level was calculated. Finally, to establish whether there were significant differences in the diet by sex, size, or season a PERMANOVA test was used with a 95% confidence level. A total of 202 *L. guttatus* stomachs were analyzed, 191 of which contained food. A total of 26 prey items were identified. According to the %IRI, the most important prey were the teleost fishes *Harengula thrissina* (Jordan et Gilbert, 1882) (45.7%) and *Sardinops sagax* (Jenyns, 1842) (34.8%), the euphausiid *Nyctiphanes simplex* (13.4%), and the crustacean *Penaeus* spp. (5.6%). The PERMANOVA analysis resulted in significant differences between the analyzed categories; however, there were no significant differences in the interactions among the categories. According to Levin's index, *L. guttatus* had a narrow trophic width, with changes in the main prey consumed by the different categories. According to our results, *L. guttatus* can be considered a benthopelagic opportunistic carnivorous predator with a narrow trophic niche, presenting mostly quantitative variations in its diet according to sex, size, and season. Its trophic plasticity allows it to take advantage of the most available and abundant food resources.

Keywords

Lutjanidae, diet, sardines, euphausiids, Gulf of California.

Introduction

Studies on the food habits of fish are fundamental to understand the structure and functioning of marine ecosystems (Díaz-Ruiz et al. 2004; Freitas et al. 2015), as they allow us to understand ecological aspects of species such as trophic interactions, their role in the food chain, and the energy flow through ecosystems (Brown et al. 2012). They are also extremely important when establishing management plans for species that are exploited due to their economic importance (Rojas-Herrera et al. 2004; Moreno-Sánchez et al. 2016). Within the family Lutjanidae, commonly known as snappers, the genus *Lutjanus* is the most diverse, as it includes 73 of the 113 species recorded in the family; among these, nine species are distributed in the eastern Pacific (Froese and Pauly 2019).

Snappers are commercially important components of artisanal fisheries worldwide. They are appreciated as a high-quality food resource, they are in high demand by the population, and their commercial value is higher than that of other fish species (Rojas 1997; Rojas-Herrera and Chiappa-Carrara 2002). This is reflected in the global catch numbers; according to the FAO (2020) over the past two decades, an average of 217 000 tons of snapper was caught annually.

Within this family, the spotted rose snapper, *Lutjanus guttatus* (Steindachner, 1869), is distributed from the Gulf of California, through Mexican Pacific coasts, to Peru. This is a demersal species that inhabits coastal reefs to a maximum depth of 30 m. Sexes are separate and those fish reach the size at first sexual maturity at 30 cm (Sarabia-Méndez et al. 2010).

Lutjanus guttatus is an important resource for fisheries in the coastal area of the Gulf of California, where one of the main economic activities is coastal fishing. Approximately 136 tons are captured annually in the area, representing an economic value of 4 million MXN (~ 207 590 USD) (CONAPESCA 2014).

Various studies have reported on the feeding habits of this species, although the majority of studies have been performed in the southernmost portion of its distribution, corresponding to tropical regions. These studies have reported that *L. guttatus* is a benthic carnivorous predator that feeds mainly on fish and small crustaceans (Rojas-Herrera and Chiappa-Carrara 2002; Rojas-Herrera et al. 2004; Tripp-Valdez and Arreguín-Sánchez 2009).

Previous studies have also shown latitudinal variations in the feeding habits of *L. guttatus*. The primary and secondary food items were, respectively, crustaceans and fishes at El Salvador (Rojas-Herrera et al. 2004), whereas they were, respectively, small-sized fish (Engraulidae and Clupeidae) and crustaceans, off the Guerrero coast, Mexico (Rojas-Herrera and Chiappa-Carrara 2002). Moreover, both crustaceans (Xanthidae) and fishes (Engraulidae) were the main prey items in the southern Gulf of California, Mexico (Tripp-Valdez and Arreguín-Sánchez 2009).

These data have led scientists to infer that the composition of the diet in *L. guttatus* depends mainly on variations in food availability, more than on resource selection by

the predator (Rojas-Herrera and Chiappa-Carrara 2002). Moreover, the previously mentioned studies showed that there were changes in the diet of *L. guttatus* according to intraspecific variations such as size, and that sex and season did not lead to significant differences in diet (Rojas-Herrera and Chiappa-Carrara 2002). It should be noted that *L. guttatus* plays a role as predator and as prey and that this species is considered important in recirculation and energy transfers from the epifauna and infauna to upper trophic levels (Rojas 2006; Navia et al. 2016).

Despite its economic and ecological importance, there are no studies on the feeding habits of *L. guttatus* in the subtropical portion of its distribution area. The objective of the presently reported study was to evaluate the trophic spectrum of *L. guttatus* in the central Gulf of California, analyzing variations in the diet by sex, size, and season, to generate information on its diet in the higher latitudes of its distribution and identify possible variations compared with lower latitudes.

Materials and methods

Sample collection, processing, and data analysis

Monthly sampling was undertaken from June 2016 to September 2017 in the mining town of Santa Rosalía, Baja California Sur, in the central Gulf of California (Fig. 1). Specimens were obtained from the coastal fishery, which employs 300-m long gillnets with 102 mm mesh size; nets are left approximately 10 h in the water, from sunset to sunrise. Specimens were frozen and transported to the Ecology Laboratory of the Interdisciplinary Centre of Marine Sciences of the National Polytechnic Institute (Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, CICIMAR-IPN). The total length (L_t , cm) and weight (W , g) of each organism were recorded. Sex was identified through the direct observation of the gonads and

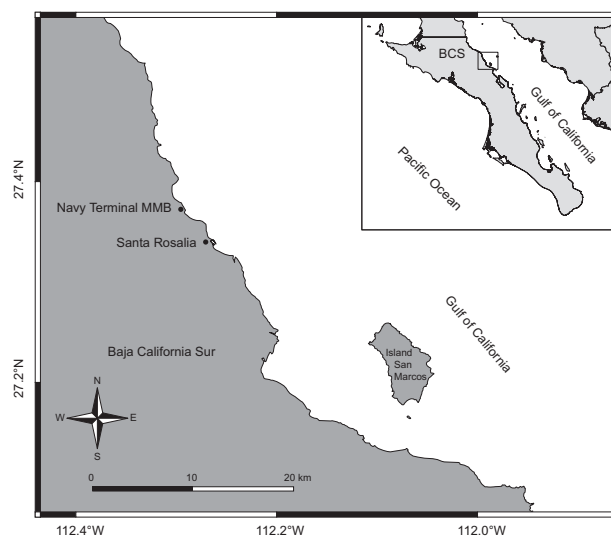


Figure 1. Map of the study area, Santa Rosalía, BCS, central Gulf of California.

confirmed through histological analyses following methods proposed by Arellano-Martínez et al. (2001). Because captured individuals were at or above the size at first maturity (i.e., 30 cm) (Sarabia-Méndez et al. 2010), the specimens were categorized into three groups following Sturges' rule (Daniel 1997) (group 1 = 28–38 cm, group 2 = 39–48 cm, and group 3 = 49–58 cm).

To identify the seasonal variation in sea surface temperature, the monthly and annual mean values of sea surface temperature were calculated based on MODIS-AQUA satellite images with 1.1 km resolution. Temperature data were obtained from the ERDDAP portal of the National Oceanic and Atmospheric Administration (NOAA). The general mean value of the time series was calculated and was used to define the seasons: cold-season months were below the surface temperature mean value (November–May) and warm-season months were above the surface temperature mean value (June–October) (Fig. 2).

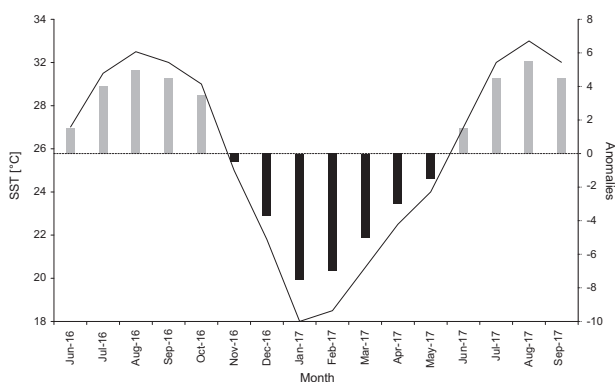


Figure 2. Monthly surface sea temperature (SST) records (black line) in the central Gulf of California during the sampling period. The dotted line indicates the general mean value of the time series. The gray bars indicate the warm months (months with SST above the mean value) and the black bars indicate the cold months (months with SST below the mean value). The primary y axis shows surface sea temperature values in degrees Celsius and the secondary y axis shows anomalies with respect to the general mean value of the time series during the sampled period.

Prey items were identified to the lowest possible taxonomic level using specialized identification keys. For fish, the keys by Whitehead (1985), Fischer et al. (1995), and Lowry (2011) were used. For crustaceans, the key by Morris et al. (1980) was used, and for other invertebrates, the keys by Iverson and Pinkas (1971), Brusca (1980), and Keen (2012) were used. For the trophic analysis of *L. guttatus*, specimens were categorized according to sex (male or female), size (group 1 = 28–38 cm, group 2 = 39–48 cm, and group 3 = 49–58 cm), and season (cold or warm).

Feeding habits

A species accumulation curve was graphed to assess whether the number of stomachs containing food was adequate to represent the diet of *L. guttatus*. The curve

was created using the program EstimateS Swins820 (Colwell 2009), using the numerical abundance of each prey item and Shannon–Wiener's diversity index (H') for each stomach. The coefficient of variation (CV) was calculated to assess the number of stomachs necessary to adequately represent the diet in general and by category (sex, size, and season). If the CV took on values equal to or below 5% (0.05), the number of stomachs was deemed sufficient to adequately represent the diet (Jiménez-Valverde and Hortal 2003; Moreno-Sánchez et al. 2019).

The quantitative importance of each prey item in the *L. guttatus* diet was described using the numerical (%N), gravimetric (%W), and frequency of occurrence (%FO) indices (Hyslop 1980). These indices were then integrated into the index of relative importance (IRI) proposed by Pinkas et al. (1971):

$$IRI = (\%N + \%W) \cdot \%FO$$

To contrast the results of the presently reported study with those found in previous studies, results are presented as a percentage (Cortés 1997):

$$\% IRI_i = \frac{100 IRI_i}{\sum_{i=1}^n IRI_i}$$

Levin's standardized index (B_i) was used (Krebs 1989) to assess the trophic width of *L. guttatus*. Values close to zero indicate that the species present a specialist feeding strategy, whereas values close to one indicate that the species has a generalist strategy (Labropoulou and Eleftheriou 1997):

$$B_i = \frac{1}{n-1} \left(\frac{1}{\sum_j P_{ij}^2} - 1 \right)$$

Where B_i is the niche width, $\sum_j P_{ij}^2$ is the proportion of the j^{th} item in the diet of the i^{th} predator, and n is the total number of prey items.

Data analysis

To interpret the feeding strategy of *L. guttatus* in the study area, we created a dispersion diagram based on Costello's graphic method (1990), modified by Amundsen et al. (1996). According to the authors, four strategies can be distinguished: 1) specialized on different trophic resources, 2) more generalist with little individual variation in trophic width, 3) specialist with one prey type, but occasional consumption of other species, and 4) mixed strategy where there are individuals with a specialized diet and other individuals with a more generalist diet. This technique was used complementarily to corroborate the trophic width niche of *L. guttatus*.

A permutational multivariate analysis of variance (PERMANOVA) with 1000 permutations was undertaken to evaluate possible differences in the *L. guttatus* diet with respect to sex (male or female), size (group 1 = 28–38 cm, group 2 = 39–48 cm, and group 3 = 49–58 cm), or season (warm or cold), and possible interactions between categories. For this analysis, a numerical matrix was constructed where columns were the prey species and rows were the analyzed stomachs. A Bray–Curtis dissimilarity matrix was used as a similarity measure for the PERMANOVA. This analysis was performed using the Adonis function in the Vegan 2.2-1 library (Oksanen et al. 2015) in the R platform version 3.0.1 (R Core Team 2016), with a 95% confidence interval.

The *L. guttatus* trophic level was calculated using the formula proposed by Cortés (1999). This equation took into account the type of prey found in stomach contents:

$$TL = 1 + \left(\sum_{j=1}^n P_j \cdot TL_j \right)$$

Where TL = trophic level of *L. guttatus*, TL_j = trophic level of each prey category consumed, P_j = proportion of each prey category in the diet of the predator, and n = number of prey items.

The trophic levels of prey were obtained from Fish-Base (Froese and Pauly 2019) and the Sea Around Us Project DataBase (Pauly et al. 2020).

Results

A total of 202 *L. guttatus* specimens were caught, ranging in size from 28 to 55 cm L_t and weighing from 290 to 1675 g. A total of 191 specimens (94.5%) had stomachs containing food and 11 (5.5%) were empty. The prey species accumulation curve reached an asymptote at 109 stomachs, which indicated that the number of analyzed stomachs was sufficient to characterize the diet ($CV \leq 0.05$). The minimum sample size was also achieved for the categories of sex, size, and season (Table 1).

Table 1. Minimum sample size for *Lutjanus guttatus* for all samples, by sex, size, and season.

Category	N_s	N_{sm}	CV
General	191	109	0.05
Female	125	82	0.05
Male	66	61	0.05
Group 1	94	79	0.05
Group 2	79	54	0.05
Group 3	18	16	0.05
Cold season	141	110	0.05
Warm season	50	43	0.05

N_s = number of analyzed stomachs, N_{sm} = minimum number of stomachs, CV = coefficient of variation for the respective sample size.

General diet

The trophic spectrum of *L. guttatus* comprised 26 categories of prey items; it included 15 fish species, 11 invertebrate species, and fish remains. A total of 502 prey items were counted; the most abundant were invertebrates (65%, $n = 327$), mainly the euphausiid *Nyctiphanes simplex* (43.6%, $n = 219$) and the shrimp *Penaeus* spp. (16.9%, $n = 85$), as well as fish (35%, $n = 175$), mainly the sardines *Sardinops sagax* (Jenyns, 1842) (16.3%, $n = 82$) and *Harengula thrissina* (Jordan et Gilbert, 1882) (15.1%, $n = 76$).

The total biomass of stomach contents was 978 g, most of which corresponded to fishes (94.2%, 921.3 g), mainly *H. thrissina* (49.7%, 486.8 g) and *S. sagax* (29.8%, 291.5 g), and invertebrates (5.8%, 56.7 g), mainly *Penaeus* spp. (2.8%, 27.7 g) and *N. simplex* (1.8%, 18 g).

The most frequent prey items were the fishes *S. sagax* (38.7%, $n = 82$) and *H. thrissina* (36.1%, $n = 76$), the euphausiid *N. simplex* (15.1%, $n = 219$), and the crustacean *Penaeus* spp. (14.6%, $n = 85$). According to the %IRI the most important prey species were the fishes *H. thrissina* (45.7%) and *S. sagax* (34.8%), the euphausiid *N. simplex* (13.4%), and the shrimp *Penaeus* spp. (5.6%) (Table 2, Fig. 3).

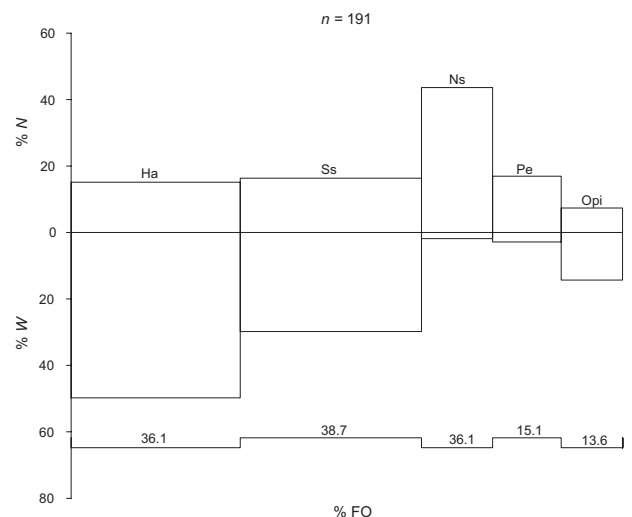


Figure 3. General trophic spectrum of *Lutjanus guttatus* in the central Gulf of California, measured with the index of relative importance (%IRI). %W = Prey-specific weight, %N = Prey-specific abundance, %FO = frequency of occurrence, Ss = *Sardinops sagax*, Ha = *Harengula thrissina*, Ns = *Nyctiphanes simplex*, Pe = *Penaeus*, Opi = Other prey items.

Diet by sex

Of 191 analyzed stomachs containing food, 66 were from males and 125 were from females. There were 10 prey items in male stomachs (5 fishes and 5 invertebrates), and the most important prey according to the %IRI were the fishes *S. sagax* (64.6%) and *H. thrissina* (15.4%), and the crustaceans *N. simplex* (15.2%) and *Penaeus* spp. (3.7%). There were 19 prey items in

Table 2. General diet of *Lutjanus guttatus* in the central Gulf of California, Mexico.

Tx	Prey	N	W	FO	%N	%W	%FO	IRI	%IRI	TL
Mo	<i>Chione</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	2.00
	<i>Loligo</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.05
	<i>Parvanachis</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	2.10
Cr	<i>Poecilostomatoida</i>	4	1	2	0.80	0.10	1.05	0.94	0.018	2.00
	<i>Penaeus</i> spp.	85	27.7	28	16.93	2.83	14.66	289.74	5.651	2.70
	<i>Callinectes</i> spp.	10	2.5	2	1.99	0.26	1.05	2.35	0.046	3.70
	<i>Sicyonia disedwardsi</i>	1	0.5	1	0.20	0.05	0.52	0.13	0.003	2.40
	<i>Nyctiphanes simplex</i>	219	18	29	43.63	1.84	15.18	690.32	13.464	2.25
	<i>Cymothoa exigua</i>	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.18
	<i>Squilla</i> spp.	2	4.5	2	0.40	0.46	1.05	0.90	0.018	2.40
	Salpidae	2	0.5	1	0.40	0.05	0.52	0.24	0.005	3.00
Ac	<i>Acanthurus</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	2.00
	<i>Achirus</i> spp.	1	38	1	0.20	3.89	0.52	2.14	0.042	3.00
	<i>Ophioblennius steindachneri</i>	1	9	1	0.20	0.92	0.52	0.59	0.011	2.50
	<i>Harengula thrissina</i>	76	486.8	69	15.14	49.78	36.13	2345.08	45.737	3.10
	<i>Opisthonema libertate</i>	1	19	1	0.20	1.94	0.52	1.12	0.022	2.89
	<i>Sardinops sagax</i>	82	291.5	74	16.33	29.81	38.74	1787.64	34.865	2.84
	<i>Engraulis mordax</i>	1	12	1	0.20	1.23	0.52	0.75	0.015	2.96
	<i>Mugil curema</i>	1	12	1	0.20	1.23	0.52	0.75	0.015	2.01
	<i>Benthoosema panamense</i>	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.00
	<i>Diaphus</i> spp.	1	3	1	0.20	0.31	0.52	0.26	0.005	3.30
	<i>Triphoturus</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.00
	<i>Ophichthus</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.40
	<i>Sebastes</i> spp.	2	0.5	1	0.40	0.05	0.52	0.24	0.005	3.50
	<i>Scomber japonicus</i>	1	33	1	0.20	3.37	0.52	1.87	0.036	3.38
	<i>Scorpaenodes</i> spp.	1	0.5	1	0.20	0.05	0.52	0.13	0.003	3.87
	Fish remains	3	14	1	0.60	1.43	0.52	1.06	0.021	
	Total	502	978	191	100	100		5127.29	100	

IRI = index of relative importance, %IRI = percent index of relative importance, Tx = highest taxon, Mo = Mollusca, Cr = Crustacea, Tu = Tunicata, Ac = Actinopterygii, N = number of individuals for each prey, W = total weight of the prey, FO = frequency of occurrence of each prey, %N = percent abundance of each prey, %W = percent weight of each prey, %FO = percent frequency of occurrence of each prey, TL = trophic level.

female stomachs (12 fishes and 7 invertebrates); the most important prey items were *H. thrissina* (56.8%), *S. sagax* (24.7%), *N. simplex* (11.7%), and *Penaeus* spp. (6.1%) (Fig. 4). The PERMANOVA showed significant differences in the diet between the two sexes ($F = 2.472$, $P < 0.05$) (Table 3).

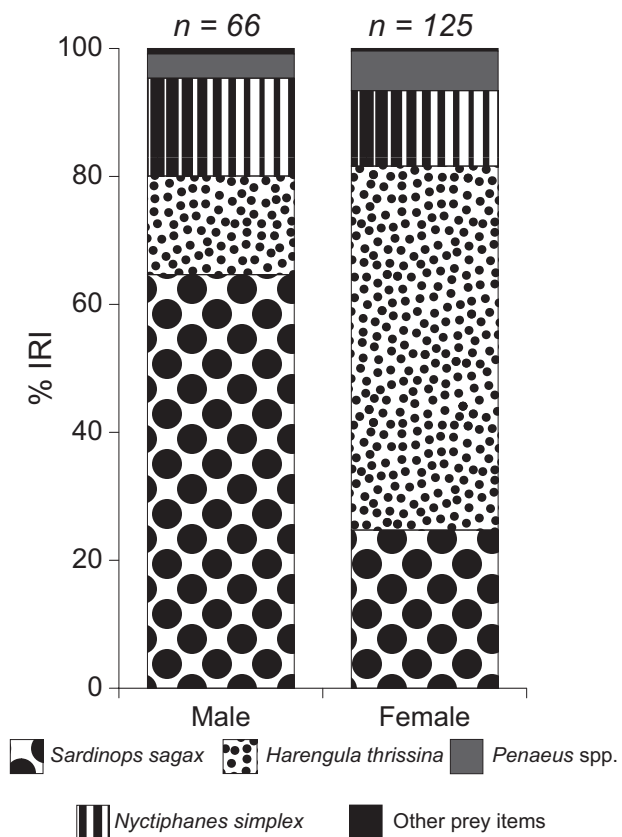
Diet by size

A total of 94 stomachs belonging to group 1 (28–38 cm Lt) were analyzed; 14 prey items were found in these stomachs (8 fishes and 6 invertebrates). According to the %IRI, the most important prey in this group were the fishes *S. sagax* (42.7%) and *H. thrissina* (29.5%),

Table 3. Results of the PERMANOVA (Permutational multivariate analysis of variance) analysis of the *Lutjanus guttatus* diet between sexes (male and female), sizes (G1, G2, and G3), and seasons (warm and cold) in the central Gulf of California, Mexico.

Factor	F	r	P(>F)	Significance
Sex	2.472	0.005	0.022	Yes
Size	45.440	0.101	0.002	Yes
Season	5.054	0.011	0.002	Yes
Sex:Size	1.223	0.003	0.248	No
Sex:Season	0.628	0.001	0.767	No
Size:Season	1.585	0.004	0.120	No
Sex:Size:Season	0.605	0.001	0.799	No

F = Fisher's F statistic, r = similarity among groups, P = probability values.

**Figure 4.** Inter-sexual variation (male or female) in prey items consumed by *Lutjanus guttatus* in the central Gulf of California, measured with the index of relative importance (%IRI).

the euphausiid *N. simplex* (23.4%), and the shrimp *Penaeus* spp. (3.9%). A total of 79 stomachs belonging to group 2 (39–48 cm L_t) were analyzed; 15 prey items were found in these stomachs (8 fishes and 7 invertebrates). The most important prey were *H. thrissina* (65.3%), *S. sagax* (21.7%), *Penaeus* spp. (7.9%), and *N. simplex* (4.2%). A total of 18 stomachs belonging to group 3 (49–58 cm L_t) were analyzed; 7 prey items were found in these stomachs (5 fishes and 2 invertebrates). The most important prey items were the fishes *S. sagax* (46.8%) and *H. thrissina* (44.4%), and the shrimp *Penaeus* spp. (3.2%) (Fig. 5). The PERMANOVA test showed significant differences in the diet between the three size groups ($F = 45.4$, $P < 0.05$) (Table 3).

Diet by season

A total of 141 stomachs from the cold season and 50 stomachs from the warm season were analyzed. During the cold season, the diet included 16 prey items (7 fishes and 9 invertebrates). According to the %IRI, the most important prey were *H. thrissina* (46.4%), *S. sagax* (29.4%), *N. simplex* (21.1%), and *Penaeus* spp. (2.6%). During the warm season, the diet included 14 prey items (11 fishes and 3 invertebrates). According to the %IRI, the most important prey were *S. sagax* (43.1%),

H. thrissina (34.1%), and *Penaeus* spp. (21.3%) (Fig. 6). The PERMANOVA test showed that there were significant differences in the diet between the two seasons ($F = 5$, $P < 0.05$) (Table 3).

According to the PERMANOVA test, there were no significant differences in the interaction between sex and size ($F = 1.2$, $P = 0.24$), between sex and season ($F = 0.62$, $P = 0.76$), between size and season ($F = 1.5$, $P = 0.12$), or between sex, size and season ($F = 0.6$, $P = 0.79$) (Table 3).

Trophic niche width and feeding strategy

According to Levin's standardized index (B_i), *L. guttatus* can be considered a specialist predator ($B_i = 0.13$). B_i values were consistent across the studied categories: by sex (males: $B_i = 0.12$; females: $B_i = 0.12$), size (G1: $B_i = 0.12$; G2: $B_i = 0.13$; G3: $B_i = 0.11$), and season (cold: $B_i = 0.11$; warm: $B_i = 0.15$). The feeding strategy confirmed that *L. guttatus* is a benthopelagic predator with a narrow trophic niche; it feeds on a reduced number of prey items that are abundant and frequent (*S. sagax*, *H. thrissina*, and *Penaeus* spp.). However, according to Costello's graph, the dominance of the main prey varies according to sex, size, and season (Fig. 7).

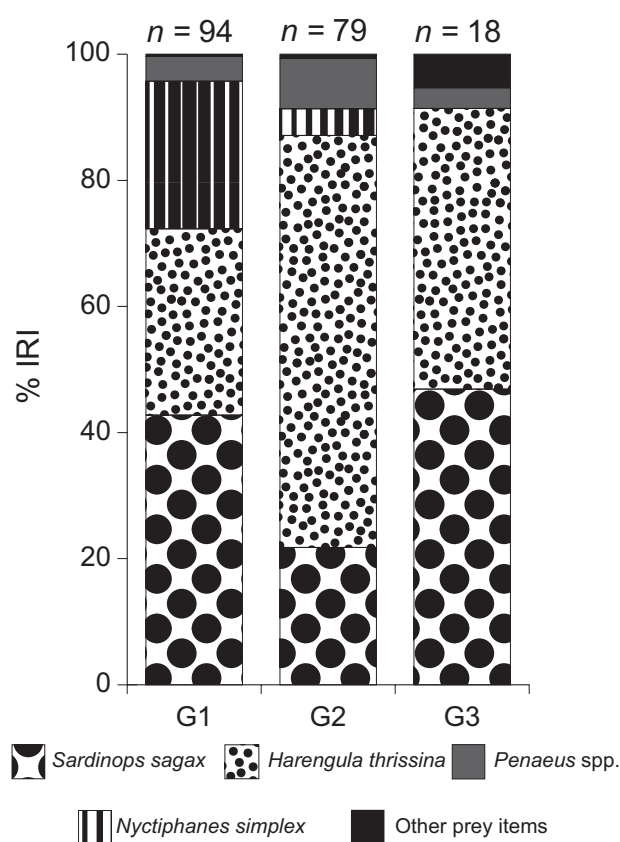


Figure 5. Size variation (G1, G2, or G3) in prey items consumed by *Lutjanus guttatus* in the central Gulf of California, measured with the index of relative importance (%IRI).

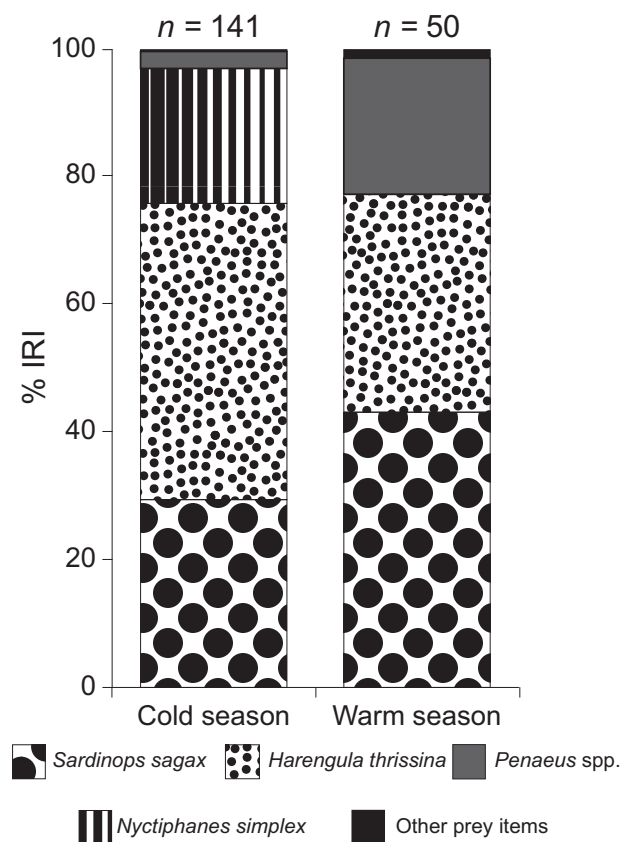


Figure 6. Seasonal variation (cold or warm) in prey items of *Lutjanus guttatus* in the central Gulf of California measured with the index of relative importance (%IRI).

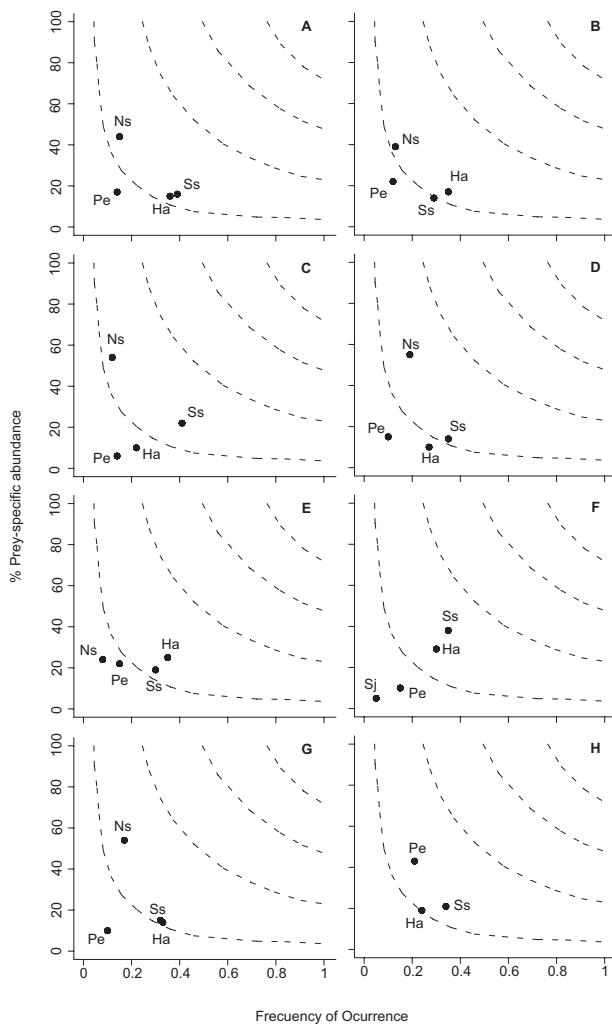


Figure 7. Costello graph. Prey-specific abundance (%N) vs. frequency of occurrence (%FO) in the general diet of *Lutjanus guttatus* in the central Gulf of California. (A) General, (B) female, (C) male, (D) size group 1, (E) size group 2, (F) size group 3, (G) cold season, (H) warm season. Ss = *Sardinops sagax*, Ha = *Harengula thrissina*, Ns = *Nyctiphanes simplex*, Pe = *Penaeus* spp., Sj = *Scomber japonicus*.

Trophic level

The trophic level calculated for *L. guttatus* was 3.9. The trophic levels for males and females were 3.8 and 3.9, respectively; for size group 1, 2, and 3 trophic levels were 3.8, 4.0, and 4.0, respectively; for cold and warm seasons trophic levels were 3.8 and 3.9, respectively.

Discussion

Several studies have reported on the feeding habits of lutjanid species at various locations. For example, studies on *Lutjanus analis* (Cuvier, 1828) (see Duarte and García 1999), *Lutjanus argentiventris* (Peters, 1869) (see Vázquez et al. 2008), *Lutjanus campechanus* (Poey, 1860) (see Wells et al. 2008), *Lutjanus decussatus* (Cuvier,

1828), *Lutjanus fulvivflamma* (Forsskål, 1775), *Lutjanus fulvus* (Forster, 1801), *Lutjanus gibbus* (Forsskål, 1775) (see Kamukuru and Mgaya 2004; Nanami and Shimose 2013), *Lutjanus griseus* (Linnaeus, 1758) (see Guevara et al. 2007), *Lutjanus malabaricus* (Bloch et Schneider, 1801) (see Takahashi et al. 2020), *Lutjanus peru* (Nichols et Murphy, 1922) (see Moreno-Sánchez et al. 2016), *Lutjanus sanguineus* (Cuvier, 1828), and *Lutjanus sebae* (Cuvier, 1816) (see Senta and Peng 1977) have found that snappers are active, mainly carnivorous predators that feed on a wide variety of pelagic and benthic prey, mainly fishes and crustaceans, as well as on bivalves, gastropods, cephalopods, and planktonic organisms such as urochordates. Several authors have also reported that the species within the genus feed on different prey according to the study area, and they have therefore been considered opportunistic predators, which could reflect the high trophic plasticity that allows them to take advantage of the most abundant resources.

In the presently reported study, the trophic spectrum of *L. guttatus* included 26 categories of prey items. The most important prey items in the diet were fish from the family Clupeidae and crustaceans of the families Euphausiidae and Penaeidae. This is similar to what was reported by Rojas (1997) for this species off the Costa Rica coast, where it fed on 22 categories of prey items, mainly crustaceans from the family Penaeidae. Rojas et al. (2004) reported that off the coast of El Salvador the *L. guttatus* trophic spectrum comprised 15 categories of prey items, mainly crustaceans of the families Squillidae, Portunidae, and Penaeus. Tripp-Valdez and Arreguín-Sánchez (2009) reported that off Nayarit, Mexico, *L. guttatus* fed on 26 categories of prey items, the most important of which were crustaceans of the family Xanthidae and fish of the family Engraulidae. However, what was found in the presently reported study differs from what was found by Rojas-Herrera and Chiappa-Carrara (2002) off Guerrero, Mexico, mainly regarding the number of prey item categories; these authors found that at that location the trophic spectrum of the species comprised 88 prey item categories, mainly fish of the families Engraulidae, Clupeidae, and Bregmacerotidae.

Differences among trophic spectra at different locations could be associated with the characteristics of each habitat. At locations where the number of prey items consumed by the species was lower, the ecosystems presented more homogeneous conditions; for example, *L. guttatus* individuals in Costa Rica are surrounded by mangroves, whereas in Nayarit, Mexico, the area has sandy bottoms and rocky substrates (Tripp-Valdez and Arreguín-Sánchez 2009). However, off Guerrero, Mexico (Rojas-Herrera and Chiappa-Carrara 2002) where the number of prey items consumed by the species was greatest, the species richness could be due to the heterogeneity of the ecosystem, which includes rocky substrates, reef patches, soft bottoms, and a wide continental platform with variable oceanography dynamics (Palacios-Salgado et al. 2014), which allows the species to feed on a greater number of

prey items. The study area in the presently reported investigation (Santa Rosalía, BCS) was characterized by sandy and rocky bottoms, where oceanographic processes such as the dominance of regional winds that favor upwelling led to large numbers of prey species such as *S. sagax*, with approximate abundances of 488 640 t (Martínez-Zavala et al. 2010), *Harengula thrissina*, with approximate abundances of 150.3 ind. 10 m⁻² (Franco-Gordo et al. 2008), and *N. simplex*, with approximate abundances of 889 ind. 1000 m⁻³ (Gómez-Gutiérrez et al. 2010).

Significant inter-sexual differences in the proportion of prey items were found; the main prey consumed by the two sexes were the same, but there were differences in the proportions of each prey type consumed. Females consumed a greater proportion of *Sardinops sagax*, whereas males ate a greater proportion of *Harengula thrissina*. This same behavior has been reported for other species in the study area (e.g., *Mycteroperca rosacea*; see Moreno-Sánchez et al. 2019), and could be the result of an ecological strategy by the species to optimize prey consumption and reduce or avoid intraspecific competition (Werner 1979).

Moreover, the difference in diet between the sexes could reflect the energy needs of males and females. For other species in the genus, such as *L. campechanus*, it was reported that females presented greater energy reserves in muscle as well as liver compared with males; these energy reserves were later used for the formation and maturation of gonads (Schwartzkopf and Cowan 2016). In the case of prey species consumed by *L. guttatus*, sardines are known for their high energy value, as was reported by Abitia-Cárdenas et al. (1997) and Navarro-García (unpublished*) for the striped marlin, *Kajikia audax* (Philippi, 1887), and the leopard grouper *Mycteroperca rosacea*, with values oscillating around 3.19–4.97 kcal·g⁻¹ dry weight.

This suggests that the diet differences observed are not due to the energetic demands of females and their different metabolic requirements, but to both sexes having a marked preference for seasonally abundant prey, providing thus an excellent example of the optimal foraging theory. Individuals are selecting prey based on the prey's vulnerability to capture and time spent to find and handle prey, maximizing thus their energy gains to maximize meeting their requirements (Gerking 1994).

Regarding the difference in the number of prey item categories between sexes, we found a greater number of prey categories ($n = 19$) in females than in males ($n = 10$). This type of result has been reported by Doncel and Paramo (2010) for the species *Lutjanus synagris* (Linnaeus, 1758) in the Colombian Caribbean, where females fed on a greater number of prey categories ($n = 23$) than males ($n = 16$). These authors attributed this result to differences in size between the sexes; females were smaller and consumed more crustaceans and mollusks compared with males, which were larger and consumed large amounts of crustaceans and fish. In the presently reported study,

the two sexes were of similar size (females = 38.4 ± 5 cm L_t ; males = 38.1 ± 4.9 cm L_t), so differences in the diet could be due to other factors. Differences could be due to variations in the distribution and habitat of the two sexes. Santamaría-Miranda et al. (2003) reported that off Guerrero, Mexico, *L. peru* females were more abundant in areas close to the coast compared with males. This would agree with what was found in the presently reported study because the proportion of males to females was 1:1.9 (M:F), resulting from their capture relatively close to the coast. It has been observed that *H. thrissina* forms large schools near the coast (Hobson 1968). In this study it was found that *L. guttatus* females fed on large amounts of *H. thrissina* compared with males, which could reflect differences in distribution between the two sexes.

There were changes in diet according to size with differences in the proportions of prey consumed, as well as in the variety of prey present in stomach contents. There was an increase in the proportion of fish in the diet compared with invertebrates with increasing *L. guttatus* size. This change in diet with predator ontogeny has been observed in other species of the genus such as *L. analis* (see Duarte and García 1999), *L. campechanus* (see Wells et al. 2008), and *L. peru* (see Moreno-Sánchez et al. 2016), and has also been observed in other locations where this species has been studied (e.g., Rojas-Herrera and Chiappa-Carrara 2002; Rojas-Herrera et al. 2004; Tripp-Valdez and Arreguín-Sánchez 2009). This has been attributed to morphological differences among the size groups. According to Allen (1985), prey selection in snappers is linked to mouth diameter, with smaller individuals having a smaller mouth aperture, which leads them to consume small-sized prey (e.g., crustaceans), compared with larger individuals with larger mouth apertures that can consume larger prey such as fish. Moreover, the ability to move, hunt, and capture prey could increase with increasing spotted rose snapper size (Rojas 1997; Moreno-Sánchez et al. 2019).

Seasonal variations in prey items were also detected. For example, there was a notable increase in the consumption of the euphausiid *N. simplex* during the cold season. It has been reported that euphausiids *N. simplex* carry out daily vertical migrations in the water column; they are found at greater depths during the day and move to the surface at night. It has also been reported that they undertake their migrations closer to the surface in the cold season when the water column homogenizes, reaching temperatures $\leq 17^\circ\text{C}$, whereas in the warm season euphausiids migrate upwards to waters over 50 m deep, avoiding warm surface waters (Gómez-Gutiérrez et al. 2010).

This could explain the increase in the importance of *N. simplex* in the diet of *L. guttatus* in the cold season and its lower importance in the diet in the warm season. According to this and the optimal foraging theory, *L. guttatus* individuals could obtain greater energy benefits by

* Navarro-García RA (2018) Bioenergética de la cabrilla sardinera *Mycteroperca rosacea* (Streets, 1877) en Santa Rosalía, Baja California Sur, México. Tesis de Licenciatura. Universidad Autónoma de Sinaloa, Facultad de Ciencias del Mar (UAS-FACI-MAR), 93 pp.

feeding on prey items that are abundant in winter, as they do not spend energy searching for less abundant organisms that are harder to catch (Gerking 1994). This could also be due to the reproductive season of the spotted rose snapper; there are two reproductive periods, one from March to April, which coincides with euphausiid consumption, and a longer period from August to November when *L. guttatus* consumed mainly sardines. These changes in the consumption of prey species could be due to the reproductive season having a high energetic cost for individuals (Arellano-Martínez et al. 2001).

In the presently reported study, according to Levin's standardized index values obtained, *L. guttatus* could be considered a predator with a narrow trophic width, as it used few trophic resources. Of 26 categories of prey items, only four (i.e., *Harengula* spp., *Sardinops sagax*, *Nyctiphanes simplex*, and *Penaeus* spp.) were found in great proportions in stomach contents, with high abundance and frequency of occurrence. However, it should be mentioned that according to Costello's graph, there was a change in the importance of the main prey according to sex, size, and season, which would allow us to classify this species as an opportunist predator that feeds on the most available and abundant prey in a given time and place (Gerking 1994).

This behavior has been observed in other species of the genus *Lutjanus* such as *L. argentiventris* (see Vázquez et al. 2008) and *L. synagris* (see Doncel and Paramo 2010). The strategy of reducing the trophic niche and alternating prey allows an efficient distribution of trophic resources and therefore a reduction in intra- and interspecific competition, as *L. guttatus* in the Gulf of California shares its habitat with similar predators (e.g., *L. peru*, *L. argentiventris*, *M. rosacea*, among others) (Gerking 1994; Moreno-Sánchez et al. 2016). It has been reported that differences in the diet with other sympatric predators can be a strategy to reduce interspecific competition. This could have an evolutionary component, with the shape of the body and head, the type of dentition, and the mandibular mechanism influencing the type of prey consumed (Rooker 1995; Rojas-Herrera et al. 2004; Nanami and Shimose 2013). Nanami and Shimose (2013) described differences in the type of prey consumed by four sympatric lutjanids based on the body type and dentition. *L. decussatus* and *L. fulviflamma* presented a compressed body, long teeth, and a mandibular mechanism that allowed them to open

and close the mouth rapidly, and they tended to consume a large number of fish. *L. fulvus* and *L. gibbus* had a wider body, short conical teeth, a mandibular mechanism with greater strength in the bite, and consumed a larger number of crustaceans. The species *L. peru* fed mainly on invertebrates such as the shrimp *Penaeus californiensis*, the crab *Pleuroncodes planipes*, and the ostracods *Myodocopida* gen. spp. in the Gulf of California (Moreno-Sánchez et al. 2016), whereas in the presently reported study *L. guttatus* fed mainly on fish such as *S. sagax* and *Harengula thrissina*, as well as on euphausiids *N. simplex* and shrimp *Penaeus* spp. These differences in the type of prey consumed could be due to morphometric differences in the dental and premaxillary bones, as was mentioned by Rojas-Herrera et al. (2004).

The trophic level calculated for *L. guttatus* was 3.9, which classifies it as a tertiary consumer, coinciding with what has been reported for other species in the genus, such as *L. campechanus* (TL = 4.2) (Tarnecki and Paterson 2015), *Lutjanus purpureus* (Poey, 1866) (TL = 3.8), and *L. synagris* (TL = 3.5) (García and Contreras 2011) and for the same species in Colima, Mexico (TL = 3.7) (Tripp-Valdez and Arreguín-Sánchez 2009). This reflects its feeding habits as a carnivorous predator that feeds mainly on intermediate trophic levels.

According to the results obtained, we conclude that *L. guttatus* in the central Gulf of California is an opportunistic carnivorous benthopelagic predator, presenting a narrow trophic niche and also displaying changes in feeding strategy according to sex, size, and season, which allows it to minimize intra- and interspecific competition.

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First record of two squirrelfishes, *Sargocentron spinosissimum* and *Sargocentron tiereoides* (Actinopterygii, Beryciformes, Holocentridae) from the Egyptian Mediterranean coast

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Abstract

Holocentrinae and Myripristinae (Holocentridae) are among the most apparent species in the nocturnal reef fish community. However, there is no clear assent regarding their phylogenetic relations, which is reported in their complicated taxonomic history. In this study, *Sargocentron spinosissimum* (Temminck et Schlegel, 1843) and *Sargocentron tiereoides* (Bleeker, 1853) were reported from the Mediterranean coast of Egypt (Damietta coast). This is the first record of these species which is greatly distributed across Indo-Pacific regions and eastern Africa showed the success of these species to migrate to the Mediterranean water with a good adaptation to the new habitats.

In the presently reported study, 26 morphometric measurements were recorded and cytochrome c oxidase subunit I (COI) barcodes were recovered for a total of 20 specimens (8 from *S. spinosissimum* and 12 from *S. tiereoides*).

The specimens from the Damietta coast, Egypt show character states diagnostic of *S. spinosissimum*: Head is curved. Spinous dorsal fin base straight. Soft dorsal fin base slightly raised. Spinous dorsal fin membranes red. Anterior margin of pelvic and anal fins white. Other fin rays are red. While character states diagnostic of *S. tiereoides*: Head is straight and pointed. Spinous dorsal fin base almost straight. Soft dorsal fin base not raised and spinous dorsal fin membranes vaguely red. Sequences of both species were submitted to the GenBank and Barcode of Life Database (BOLD) publication database which displayed 99%–100% similarity value *S. tiereoides* from GenBank and BOLD databases but, *S. spinosissimum* has not any deposited sequences from GenBank or BOLD.

DNA barcoding based on COI gene was demonstrated as a powerful and useful molecular marker in the identification and differentiation of *S. spinosissimum* and *S. tiereoides* fish species.

Keywords

COI gene, DNA barcoding, fish, Holocentrinae, phylogeny, taxon

Introduction

The squirrelfishes and soldierfishes (family Holocentridae Bonaparte, 1833) are widespread from tropical to warm temperate waters in shallow water on coral reefs or rocky

bottoms. The Holocentridae is divided into two subfamilies, Holocentrinae Bonaparte, 1833 and Myripristinae Nelson, 1955, based on the communication between the swim bladder and skull (Nelson 1955). Woods and Sonoda (1973) recognized these subfamilies in a recent review

of the western Atlantic holocentrids. These two stems are recognized in fossil beryciforms (see Dunkle and Olsen 1959) and fossil otoliths (Frizzell and Lamber 1961; Lamber unpublished¹). Woods (1955) classified Holocentrinae into four subgenera: *Holocentrus* Scopoli, 1777, *Flammeo* Jordan et Evermann, 1898, *Sargocentron* Fowler, 1904, and *Adioryx* Starks, 1908. *Flammeo* has been displaced to synonymy with *Neoniphon* Castelnau, 1875, due to the uncertain status of the type species for *Neoniphon* (see Woods 1955). Then, *Adioryx* was elevated to the generic level (Woods 1965). However, Randall and Heemstra (1985, 1986) recognized *Flammeo* as a junior synonym of the *Neoniphon* depending on the identification of the type species of *Neoniphon* as *Neoniphon sammara* (Forsskål, 1775).

Similarly, Matsuura and Shimizu (1982) reported that the species of *Adioryx* and *Sargocentron* could not be identified by features of the swim bladder and auditory bulla, and then they classified all *Adioryx* as *Sargocentron*. Hubert et al. (2010) took eight *Sargocentron* species and one species of *Neoniphon* for the mtDNA cytochrome c oxidase subunit I (COI) gene and postulated that *Sargocentron* is paraphyletic to *Neoniphon*. Moreover, phylogenetic analyses of rhodopsin amino acid sequences resolved a paraphyletic *Sargocentron* to *Neoniphon* (see Yokoyama and Takenaka 2004; Yokoyama et al. 2008).

Genus *Sargocentron* has a great diversity among its species, which belongs to the family Holocentridae known as squirrelfish. This genus includes about 33 species (Froese and Pauly 2019, eight of which were found in the Red Sea (Golani and Bogorodsky 2010). *Sargocentron rubrum* (Forsskål, 1775), is one of the oldest migratory Red Sea species that have entered the Mediterranean Sea via the Suez Canal (Golani and Ben-Tuvia 1985). Until recently, *Sargocentron rubrum* was considered to be the only representative of the squirrelfish family in this basin, with a single record from Libyan waters (Štirn 1970). *Sargocentron rubrum* has been recorded from Egyptian waters (Ibrahim and Soliman 1996; Alwany 2011; Bakhoun 2018; Farrag et al. 2018), and it was mentioned in the marine ichthyofauna of Egypt (Akel and Karachle 2018). *Sargocentron* was proved as a genus without comment (Fowler 1944). Starks (1908) realized differences in the formation of the swim bladder with respect to the back of the skull in several holocentrids and then separated *Holocentrus adscensionis* (Osbeck, 1765) from *Holocentrus suborbitalis* Gill, 1863, grouping the latter into a new genus, *Adioryx*. Whitley (1933) added two subgenera to *Holocentrus*: *Faremusca* Whitley, 1933 for the Indo-Pacific *Holocentrus punctatissimum* Cuvier, 1829 and *Cephalofarar* Whitley, 1933 for the western Atlantic species *Holocentrus vexillarium* Poey, 1860. In this study, *Sargocentron spinosissimum* (Temminck et Schlegel, 1843) and *Sargocentron tiereoides* (Bleeker, 1853) were reported for the first time from the Mediterranean coast of Egypt (Damietta coast). These species which are greatly distributed across Indo-Pacific regions and eastern Africa showed the successful of these species to migrate to the Mediterranean water with a good adaptation to the new habitats.

Methods

Study area and sample collection

Twenty specimens of the North Pacific squirrelfish, *Sargocentron spinosissimum*, and the pink squirrelfish *Sargocentron tiereoides*, (Fig. 1A, B) were collected from the Damietta coast of the north of Egypt, in the south-eastern part of the Mediterranean Sea at 31°46'48.0"N, 31°40'48.0"E.

Morphological data

Twenty-six morphometric measurements were recorded with vernier calipers to the nearest 0.05 mm. Body proportions were expressed in percentage of standard length (SL). All measurements are presented in Table 1 and abbreviations for measurements are as follows: Total length (TL); Fork length (FL); Standard length (SL); Prepectoral fin length (PPL); Predorsal fin length (PDL); Prepelvic fin length (PVL); Preanal fin length (PAL); Caudal peduncle length (CPL); Head length (HL); Body depth (BD); Eye diameter (ED); Preorbital length (POL); Pectoral fin base length (PBL); Dorsal fin base length (DBL); Pelvic fin base length (VBL); Anal fin base length (ABL); Caudal fin length (CL). Pectoral fin length (PL); Dorsal fin length (DL); Pelvic fin length (VL); Anal fin length (AL); Pectoral fin count (PC); Dorsal fin count (DC); Pelvic fin count (VC); Anal fin count (AC); Lateral line scale (LS).

DNA extraction PCR amplification and sequencing

Liver tissue was obtained from *Sargocentron* species, then preserved in 95% alcohol and stored in a deep freezer at -4°C, where the *Sargocentron* spp. DNA was extracted using a GeneJET kit Genomic DNA Kit#K0721 following the manufacturer's protocol.

Cytochrome c oxidase subunit I gene was amplified using primers FF-5' TTC TCC ACC AAC CAC AAR GAY ATY GG -3' and FR-5' CAC CTC AGG GTG TCC GAA RAA YCA RAA -3' (Ivanova et al. 2007). The polymerase chain reactions (PCR) consisting of approximately 50 ng of template DNA were carried out in volumes of 15 µL with 1 × PCR Buffer, 2 mM MgCl₂, 0.5 µM of each FF and FR, 0.2 mM of dNTP, and 0.6 U of Taq DNA Polymerase. The thermal program started with an initial denaturation at 94°C for 5 min, followed by 10 cycles of 1 min at 94°C, 30 s at 60°C, 1 min at 72°C, and a final extension of 5 min at 72°C.

PCR product was visualized in a 2.0% agarose gel stained with ethidium bromide and photographed under UV transillumination. PCR product was purified using a GeneJET kit (Thermo K0701) according to the manufacturer's recommendations. A purified PCR sample was sent for sequencing to a GATC Company in England that uses an ABI 3730xl DNA sequencer.

¹ Lamber CK (1963) Fossil and recent beryciform otoliths: an adjunct to ichthyological classification. M. S. Thesis, Univ. Rolla, 134.

Molecular analysis

The resulting sequences were confirmed as being derived from *Sargocentron* species DNA using the GenBank Blast algorithm. The DNADynamo software version 1.459 was used for editing the sequences and they were aligned using Clustal W. Finally, the phylogenetic analyses used were Maximum Evolution, Neighbor Joining, and Maximum Likelihood in MEGA 6.0 software (Tamura et al. 2013).

Results

Fish species belonging to the family Holocentridae were collected and identified based on traditional morphotaxonomy and further confirmed by molecular marker using DNA sequencing. In this study, it was recorded for the first time two different fish species such as; eight specimens of *Sargocentron spinosissimum* and twelve specimens of *Sargocentron tiereoides* of the order Beryciformes, from the Damietta coast. The morphometric as well meristic data of both species were presented in Table 1.

Table 1. Morphometric and meristic counts of *Sargocentron spinosissimum* ($n = 8$) and *Sargocentron tiereoides* ($n = 12$), collected from the Damietta coast, Egypt.

	<i>Sargocentron spinosissimum</i>	<i>Sargocentron tiereoides</i>
Morphometric variables	Measurement [mm]	
Total length (TL)	172	186
Fork length (FL)	149	152
Standard length (SL)	134	130
Head length (HL)	42	47
Body depth (BD)	50	53
Eye diameter (ED)	17.5	15
Pre orbital length (POL)	8	17
Pre dorsal length (PDL)	48	59
Pre pectoral length (PPL)	44	60
Pre pelvic length (PVL)	51	67
Pre anal length (PAL)	101	122
Dorsal fin base length (DBL)	76	87
Pectoral fin base length (PBL)	7.6	6
Pelvic fin base length (VBL)	7	10
Anal fin base length (ABL)	17	21
Dorsal fin length (DL)	95	100
Anal fin length (AL)	26	29
Pectoral fin length (PL)	19	35
Pelvic fin length (VL)	24	34
Caudal fin length (CL)	38	56
Caudal peduncle length (CPL)	11	14
Relative characters:		
SL/BD	2.86	2.45
SL/HL	3.19	2.76
SL/ED	7.65	8.66
SL/PDL	2.79	2.20
SL/PAL	1.32	1.06
HL/ED	2.4	3.1
Meristic counts		
Dorsal fin count (DC)	XI + 13	XI + 14
Anal fin count (AC)	IV + 9	IV + 9
Pelvic fin count (VC)	I + 7	I + 8
Pectoral fin count (PC)	14	14
Lateral line scale (LS)	36	40

Key to the subfamilies

Holocentrinae has anal fin rays 7–10 (soft rays). A stout long spine was found in the angle of preopercle which is longer than its width while, Myripristinae species have anal fin rays more than 10 (soft rays). Angle of preopercle without spine in adult (Atlantic species *Corniger spinosus* Agassiz, 1831 has one or two strong spines).

Key to the genera

Last dorsal fin spine located at middle between the first dorsal fin ray and the penultimate dorsal fin spine in *Sargocentron*. In *Neoniphon* last dorsal fin spine located nearer to the first dorsal fin ray than the penultimate dorsal fin spine.

Morphological data

The following morphometric characters of *Sargocentron spinosissimum* were registered: scales are very rough ctenoid and large; ridges and mucous channels dorsally on rounded red head and edges of external bones of head serrate or with spines. Opercle with two sharp spines, almost equal in length. Preopercular spine moderate, about a half of orbit; very large eyes; dorsal fin with 11 spines and 12 soft rays; 13 anal fin rays (4 spines and 9 soft); 14 pectoral fin rays; pelvic fin with 1 spine and 7 soft rays; standard length 134 mm; fork length 149 mm; head length 42 mm; eye diameter 17.5 mm. Body depth 50 mm; length of caudal peduncle length 11 mm; length from tip of snout to origin of dorsal fin 48 mm; length from tip of snout to origin of pectoral fin 44 mm; length from tip of snout to origin of anal fin 101 mm; length from tip of snout to origin of pelvic fin 51 mm. Base of dorsal fin 76 mm; base of pectoral fin 7.6 mm; base of pelvic fin 7 mm. Base of anal fin 17 mm. Caudal fin forked. Morphometric data are given as percentages of standard length in Table 1. Coloration of body is brilliant reddish. Body with 9 red stripes alternating with 9 narrower white stripes that pass along the middle of the longitudinal scale rows; head is red with a white bar along the posterior margin of preopercle (Fig. 1A).

The following morphometric characters of *Sargocentron tiereoides* was registered: scales are very rough ctenoid and large; ridges and mucous channels dorsally on rounded red head and edges of external bones of head serrate or with spines. Opercle with two spines, the upper slightly longer. Preopercular spine long, slightly longer than two-thirds of orbit; very large eyes; dorsal fin with 11 spines and 14 soft rays; 13 anal fin rays (4 spines and 9 soft); 14 pectoral fin rays; pelvic fin with 1 spines and 8 soft rays; standard length 130 mm; fork length 152 mm; head length 47 mm; eye diameter 15 mm. Body depth 53 mm; length of caudal peduncle length 14 mm; length from tip of snout to origin of dorsal fin 59 mm; length from tip of snout to origin of pectoral fin 60 mm; length from tip of



Figure 1. *Sargocentron* species, (A) *Sargocentron spinosissimum*, 134 mm SL and (B) *Sargocentron tieeoides*, 130 mm SL, specimens caught from Coast of Damietta, Egypt

snout to origin of anal fin 122 mm; length from tip of snout to origin of pelvic fin 67 mm. Base of dorsal fin 87 mm; base of pectoral fin 6 mm; base of pelvic fin 10 mm. Base of anal fin 21 mm. Caudal fin forked. Morphometric data are given as percentages of standard length in Table 1. Coloration of body is brilliant reddish orange. Body with 9 reddish orange stripes alternating with 9 narrower white stripes that pass along the middle of the longitudinal scale rows; head is reddish with a white bar along the posterior margin of preopercle (Fig. 1B).

Sequencing analysis

Cytochrome c oxidase subunit I (COI) barcodes were recovered for a total of twenty specimens (eight from *Sargocentron spinosissimum* and twelve from *Sargocentron tieeoides*) of the family Holocentridae. No insertions/deletions, heterozygous sites or stop codons were discovered, accepting the view that all of the amplified sequences form functional mitochondrial COI sequences. BLAST outcomes of all nucleotide sequences succeeded to identify sequence similarity of *Sargocentron tieeoides* species under study. While *Sargocentron spinosissimum* has not any similarity of sequence deposited in GenBank. The COI sequence analysis of *Sargocentron tieeoides* resulted the average nucleotide frequencies as 25.7% (A), 30.3% (T), 26.9% (C), and 17.1% (G). Similarly, in *Sargocentron spinosissimum* the nucleotide frequencies are 25.8% (A), 29.8% (T), 26.9% (C) and 17.6% (G).

Phylogenetic analysis revealed a well-determined hypothesis of relations at the species level. Overall Maximum Evolution, Neighbor Joining, and Maximum Likelihood trees analyses (Figs 2–4 respectively), new sequences from

the two species grouped in different clusters. Moreover, where applicable, the sequences from the same species (newly obtained in this study and deposited from Barcode of Life Database (BOLD) and GenBank) were grouped, resulting in homology and more or less conspecific distances between them. Furthermore, sequences from *Sargocentron tieeoides* species retrieved from National Center for Biotechnology Information (NCBI) grouped in the same cluster.

The phylogenetic trees generated through Maximum Evolution, Neighbor Joining, and Maximum Likelihood analyses shows same topology (Figs 2, 3, 4). Sequences from above specimens of *Sargocentron tieeoides* were submitted to the NCBI GenBank Barcode database and BOLD with accession and BIN numbers HM034176, HM034280, HM034281, BOLD: BIFZB182, FPFL040, MBFA391 and MBFA553 respectively.

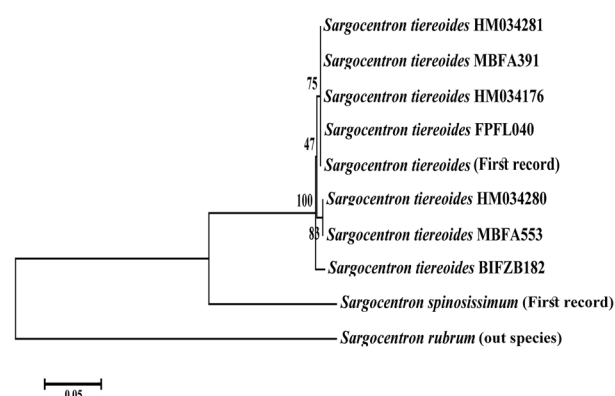


Figure 2. Maximum Evolution phylogenetic of COI variation for reconstruction for *Sargocentron spinosissimum* and *Sargocentron tieeoides* using Kimura 2-Parameter distances and values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.

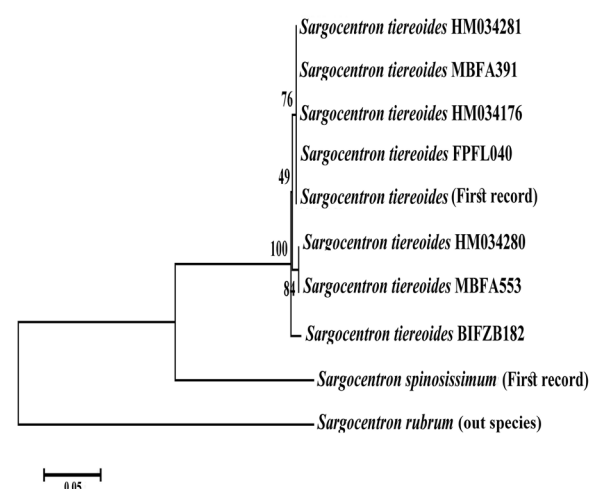


Figure 3. Kimura 2-Parameter distances Neighbor Joining tree of COI variation for *Sargocentron spinosissimum* and *Sargocentron tieeoides*. Values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.

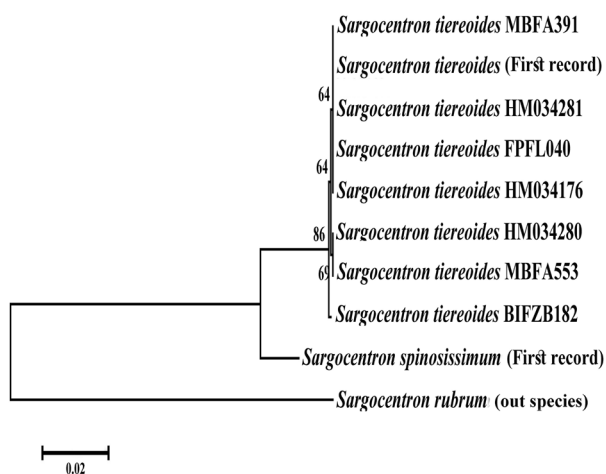


Figure 4. Phylogenetic tree of Maximum Likelihood analyses based on COI gene from *Sargocentron spinosissimum* and *Sargocentron tiereoides* using Kimura 2-Parameter distances and values at nodes represent bootstrap confidence level (1000 replicates). Specimen's number denotes the accession number of NCBI and BOLD database, rooted with *Sargocentron rubrum*.

Discussion

Species of the genus *Sargocentron* have compressed and an elongated body with thin caudal peduncle; ridges and mucous channels dorsally on head and have very large eyes. They have a single spine in pelvic fin with soft rays 5–8. Although dorsal fin is long with 10–13 spines but, a notched soft-rayed part is found with 11–17 rays. Anal fin has four spines and 7–16 soft rays. Forked caudal fin has 18 or 19 rays. Scales are very rough ctenoid and large. The majority of them are brilliant reddish in color. Usually are nocturnal. Usually cryptic during the day in beneath ledges of reefs or crevices. *Sargocentrons* feed on worms and small fishes (Randall 1998).

The specimens from the Damietta coast, Egypt show character states diagnostic of *Sargocentron spinosissimum*: Head is curved. Spinous dorsal fin base straight. Opercle with two sharp spines, almost equal in length. Preopercular spine moderate, about a half of orbit. Soft dorsal fin base slightly raised. Spinous dorsal fin membranes red. Anterior margin of pelvic and anal fins white. Other fin rays are red.

On the contrary, opercle with two sharp spines, the upper longer. Preopercular spine long, about two-thirds of orbit in *Sargocentron rubrum* and its ground color of head and body crimson to violet. Each longitudinal body scale row with a wide white stripe. Dorsal fin spines red. Spinous dorsal fin membranes white. Anterior margin of soft dorsal fin red, the rest of fin yellow. The doubt on the distinction between *Sargocentron spinosissimum* and *Sargocentron rubrum* was the reason for Re-examination of *Sargocentron spinosissimum* indicates that this species has spinules on the posterior portion of the nasal bone. On the other hand, the posterior portion of the nasal bone in *Sargocentron rubrum* is quite smooth without any supination. Therefore, both are regarded as distinct species.

While character states diagnostic of *Sargocentron tiereoides*: Head is straight and pointed. Spinous dorsal fin base almost straight. Opercle with two spines, the upper slightly longer. Preopercular spine long, slightly longer than two-thirds of orbit. Soft dorsal fin base not raised and spinous dorsal fin membranes vaguely red. Besides morphology, morphological measurements are agreed to Shimizu and Yamakawa (1979) which aid in identifying these *Sargocentron* species.

DNA barcoding is a recent and greatly used molecular-based recognition system that aims to recognize biological specimens. Studies on the biodiversity of coral reefs inevitably require taxonomic coverage. DNA barcoding is a method for taxonomic identification of organisms that is entirely based on the 5' portion of the mitochondrial gene cytochrome oxidase subunit I (COI-5). It can be a beneficial gene for identification of fish species (Neigel et al. 2007). Under any form of anthropogenic change, species will have to move, adapt or die. Progress in genetic studies of adaptation until recently had been constrained by the lack of resolution and absence of genomic perspective. Genetic tools can give us crucial insights into these processes. At the largest scale, molecular tools can identify cryptic species and their introductions, as in this study.

The presently reported study is based on the first occurrence of two fish species *Sargocentron spinosissimum* and *Sargocentron tiereoides* from the Damietta coast, Egypt. DNA barcoding uses a genetic marker (often a single gene) to assign an individual to a particular known species. It has also been suggested that barcoding can be used to identify unknown species based on the expectation that interspecific genetic divergence considerably exceeds intraspecific variation to form a clear “barcode gap”. In addition to that, these fish species have never been reported earlier, hence this happens to be the first record from the Damietta coast, Egypt.

Ward et al. (2009) revealed that the simplest method of seeking the recognition of an unknown specimen is to place its cytochrome b sequence into the BOLD identification engine. In the presently reported study, COI gene sequence was corresponding with *Sargocentron* species base which displayed 99%–100% similarity value *Sargocentron tiereoides* from GenBank database but, *Sargocentron spinosissimum* has not any deposited sequences from GenBank or BOLD. Overall COI constructed Maximum Evolution, Neighbor Joining, and Maximum Likelihood trees placed *Sargocentron spinosissimum*, *Sargocentron tiereoides* and *Sargocentron rubrum* (as out species of this study) in three different clades due to these are three distinct species. While *Sargocentron tiereoides* and all deposited COI sequences from GenBank and BOLD formed high bootstrap-supported clusters without any overlap between species.

Conclusion

This study contains novel findings of *Sargocentron spinosissimum* species that is distributed Northwest Pacific:

southern Japan to Taiwan; also reported from Hawaii and *Sargocentron tiereoides* is distributed in Indo-Pacific regions and East Africa showed the success of the migration of these species to the Mediterranean water with a

good adaptation to the new habitats. In this work, DNA barcoding based on the COI gene was demonstrated as a powerful and useful molecular marker in identifying fish species.

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The first record of the pharaoh cardinal fish, *Apogonichthyoides pharaonis* (Actinopterygii: Perciformes: Apogonidae), from Libyan waters

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Abstract

The occurrence of the pharaoh cardinalfish, *Apogonichthyoides pharaonis* (Bellotti, 1874), is documented for the first time from the Libyan waters, after two subsequent findings reported in September and November 2020 in the far eastern region of the country. The location of these findings represents the westernmost area of distribution in the southern Mediterranean for this species, which has the western Indian Ocean and Red Sea origin and which entered into the Mediterranean via the Suez Canal.

Keywords

Non-Indigenous Species (NIS), Lessepsian migration, Suez Canal, citizen science, southern Mediterranean, spear fishing

Introduction

There is only one member of the cardinal fish family (Apogonidae) native to the Mediterranean, the Mediterranean cardinal fish, *Apogon imberbis* (Linnaeus, 1758), while at least five non-indigenous members of the same family have been recorded to date from the basin. These include the spotfin cardinal, *Jaydia queketti* (Gilchrist, 1903), the Smith's cardinalfish *Jaydia smithi* Kotthaus, 1970, the broadband cardinalfish, *Ostorhinchus fasciatus* (White, 1790), the Indian Ocean twospot cardinalfish, *Cheilodipterus novemstriatus* (Rüppell, 1838), and the pharaoh cardinalfish, *Apogonichthyoides pharaonis* (Bellotti, 1874), all introduced via the Suez Canal

(Lessepsian migrant fishes) (Rothman et al. 2013; Irmak and Engin 2015).

Apogonichthyoides pharaonis is native to the Red Sea, the Persian Gulf, and the western Indian Ocean (Gon 2000; Gon and Randall 2003). The species was first recorded in the basin at Port Said, Egypt, in 1924 (Norman 1927) and later off the Palestinian coasts before 1946, misidentified as *Apogonichthyoides taeniatus* (Cuvier, 1828) (see Haas and Steinitz 1947; Golani 2010). The species was subsequently recorded from Cyprus in 1961 (Demetropoulos and Neocleous 1969), Lebanon in 1962 (George et al. 1963), from south-eastern Turkey in 1984 (Mater and Kaya 1987), from Syria in 1992 (Sbaihi and Saad 1995), from the south-eastern Aegean Sea, at Symi

Island, Greece, in 1982 (Zachariou-Mamalinga 1990), and at Rhodes Island, Greece, and the Datça Peninsula region, Turkey, in 2002 (Corsini-Foka et al. 2004; Öz et al. 2007) (Fig. 1). The species is currently classified as an established non-indigenous species in the Levantine Sea, being regularly caught as bycatch in the artisanal fishery (Carpentieri et al. 2009; Anonymous 2010) in relatively shallow waters of Egypt (Ragheb et al. 2019), Turkey (Bilge et al. 2019), Cyprus (Iglésias and Frotté 2015), Lebanon (Carpentieri et al. 2009), and Greece (Corsini-Foka et al. 2014; Corsini-Foka and Kondylatos 2015).

In this study, the record of *A. pharaonis* is documented for the first time in the Libyan waters, being the species not yet reported in recent literature on marine non-indigenous species of the country (Shakman et al. 2019 and references therein; Abdelghani et al. 2020; Bariche et al. 2020; Osca et al. 2020). The locations of the two subsequent findings of *A. pharaonis* described in this paper represent the westernmost Mediterranean area of colonization of this non-indigenous fish along the southern shores of the basin.

Material and methods

On 19 September 2020, a spear fisher submitted photos to the social media citizen science platform for Libyan waters called 'Marine Biology in Libya' (<https://www.facebook.com/MarineBiologyinLibya>) of an unknown fish he had just caught. The catch was made on rocky bottom

interrupted by sand at a depth of approximately 1.5 m, along the coast of the Al Burdi (Bardia) region in the far eastern extremity of Libya, not far from the Egyptian border (31°46'45"N, 25°04'40"E) (Fig. 1). The same sports fisher preserved in a freezer the specimen (specimen A), which was retrieved by one of us (SAM), to enable the assessment and measurement of its main morphometric and meristic attributes. Measurements were taken with a caliper to the nearest 0.01 mm, following Bauchot (1987). The sample is stored in formaldehyde solution at the fish collection of the Omar Al-Moukhtar University, El Bayda, Libya. The specimen was identified following Gon (1986, 2000), Gon and Randall (2003), and Golani et al. (2013).

On 17 November 2020, a recreational fisherman posted pictures of the second specimen of *A. pharaonis* (specimen B) on a Facebook group of spear fishing lovers (<https://www.facebook.com/groups/377703789082199/?ref=share>). The fish was captured with a fishing rod at 2 m of depth, on a mixed rocky and sandy bottom, off Tobruk city (32°04'20"N, 23°58'09"E), western to the previous site (Fig. 1). The sample was returned to the sea. The specimen was identified through examination of photos taken immediately after its capture.

Results

The specimen A, slightly damaged, featured the following meristic characters: D1: VII; D2: I + 9 (the fourth

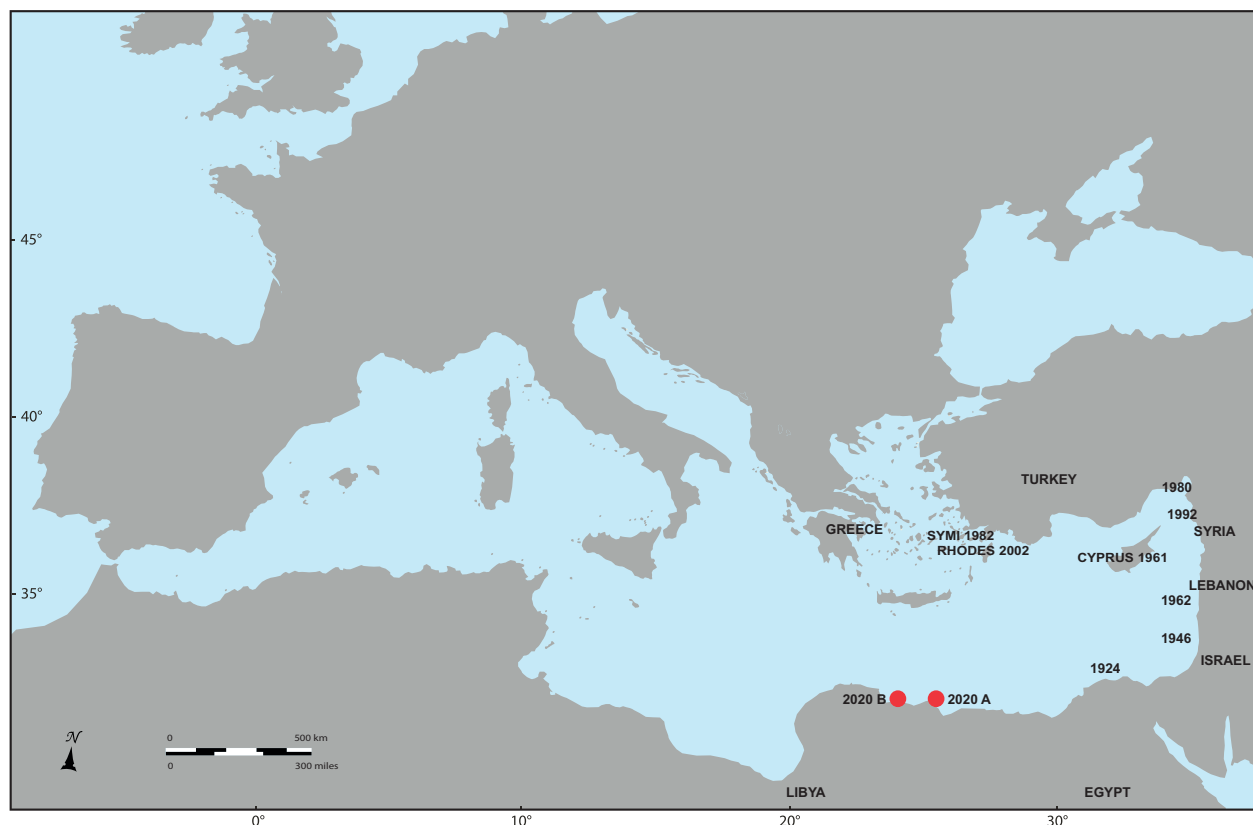


Figure 1. Locations and dates of first records of *Apogonichthyoides pharaonis* in the Mediterranean Sea, updated with the two findings in Libyan waters in 2020 (red bullets; **A.** specimen from Al Burdi; **B.** specimen from Tobruk).

soft ray broken); P: 15; A: II + 8; V: I + 5; LL: 24; Gill rakers: 17. The fish measured 52.92 mm in total length (TL), 40.81 mm in standard length (SL), and weighed 2.6 g. The main proportions were: body depth 2.2 and head length 2.4 in SL; snout length 4.7, eye diameter 3.3 and upper-jaw length 1.9, all in head length; caudal peduncle depth 1.2 in its length; peduncle length 5.1 in SL. The fish presented a body laterally compressed, pale brown with two distinct dorsal fins, pelvic fin origin under the base of the pectoral fin, truncate tail, and large eye size. Color in fresh specimens: four dark vertical bars, the first running under the first dorsal fin, the second under posterior half of the second dorsal fin and extending to the tip of the spine of the second dorsal fin and to the tip of second spine in anal fin, the third, faint, on the caudal peduncle and the fourth on caudal fin base (Fig. 2A, B); a dark caudal spot visible (Fig. 2A, B); a black and silvery ‘eye-spot’ (ocellus) on the right flank of specimen A (left side damaged) and on both sides of specimen B, within the first black vertical bar under the lateral line (Fig. 2A detail, B); anterior part of first dorsal fin dark, second dorsal and anal fins dusky (except the dark extension of the second bar), pectoral and caudal fin pale, ventral fin dusky. The gross morphology and livery of the two specimens, along with the morphometric and meristic attributes of the specimen A were generally consistent with *A. pharaonis*, as described in Gon (2000), Gon and Randall (2003), Goren et al. (2009a) and Golani et al. (2013). The common TL of the species ranges between 5 cm and 8 cm, with a maximum of 12 cm (Golani et al. 2013). Citing Gon and Randall (2003), the smallest mature female ever recorded for the species had a TL of 3.4 cm. The *A. pharaonis* specimen A recorded in the presently reported study can be thus considered to represent a young adult stage of the species.

Discussion

The gross morphology and livery of *A. pharaonis* is similar to that of the congeneric and sympatric *Apogonichthyoides pseudotaeniatus* (Gon, 1986), *A. taeniatus*, and *Apogonichthyoides timorensis* (Bleeker, 1854), following

Gon and Randall (2003). *Apogonichthyoides pseudotaeniatus* differs in lacking a bar on the caudal peduncle. In *A. taeniatus*, the second bar originates at the rear part of the second dorsal-fin base, usually fades out on the middle of the body, and does not extend onto the second dorsal fin. In addition, *A. taeniatus* has a caudal spot, but no bar on the caudal-fin base and 5–6 narrow dusky stripes along the body, which are never present in *A. pharaonis*, and usually 3 median predorsal scales. The color pattern of *A. timorensis* is similar to that of *A. pharaonis*, but the first never has an eye-spot between the lateral line and pectoral fin, and its bars are less distinct. Furthermore, according to the same authors, *A. pharaonis* is similar to *Apogonichthyoides nigripinnis* (Cuvier, 1828), whose distribution is restricted to the eastern Indian and western Pacific oceans, while *A. pharaonis* occurs in the western Indian Ocean. Also, both species have the ocellus below the lateral line, but *A. nigripinnis* lacks the two wide dark bars below the dorsal fins. They differ furthermore in some morphometric and meristic characteristics.

The Apogonidae species known from the Red Sea and the Arabian Sea live mainly in shallow waters and inhabit coral reefs and rocky areas; some species can be found on soft bottoms, seagrass beds, on silty reefs and on mangroves (Smith 1961; Gon and Randall 2003; Debelius 2011). Cardinal fish species of the Red Sea are nocturnal planktivorous predators, hidden by day (Goren et al. 2009b), but some species are active during sunlight (Debelius 2011). Among the apogonid migrants, *A. pharaonis* occurs on mangroves, seagrass beds, and on silty reefs in its native range (Gon 2000) and in the Mediterranean occupies prevalently seagrass meadows as well as soft bottom habitats (sandy and muddy) (Goren et al. 2009a; Corsini-Foka et al. 2014; Corsini-Foka and Kondylatos 2015), although it may be observed in front of rocks, in sandy or weedy shallow areas (MCF pers. comm.). The specimens of *A. pharaonis* under study were found on very shallow rocky bottom interrupted by sand. The preferred depth range of *A. pharaonis*, which extends up to 50 m, partly overlaps with the depth range of the native Mediterranean *A. imberbis*, which occupies rocky reefs and seagrass habitats at 2–70 m and more (Goren et al. 2009a; Froese and Pauly 2019).

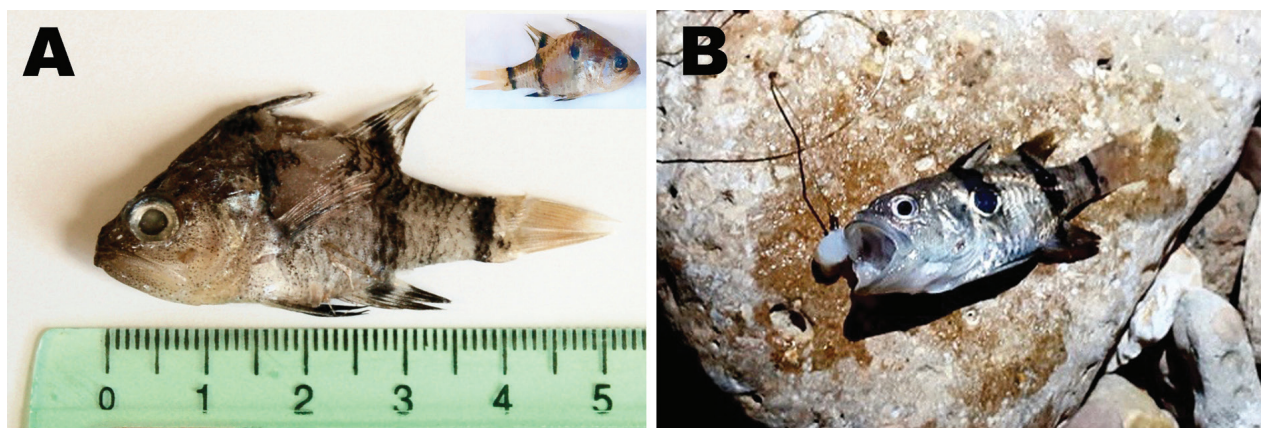


Figure 2. *Apogonichthyoides pharaonis* from Libya (A. specimen from Al Burdi; B. specimen from off Tobruk).

In their native range, *O. fasciatus* inhabits coastal reefs, in sandy or algal-dominated areas, *J. smithi* occurs on silty bottom, and *J. queketti* is a benthopelagic fish (Froese and Pauly 2019). In the Mediterranean, all three above-mentioned Lessepsian migrant species have been collected on bare muddy-silt bottoms (Golani et al. 2008; Goren et al. 2009a, 2009b) and a possible reversion of feeding habits from zooplankton to zoobenthos needs yet to be determined (Goren et al. 2009b). The last recorded *C. novemstriatus* lives in front of holes and under ledges of coral or rocky reefs; frequently it finds refuge among the spines of sea-urchins of the genus *Diadema* in groups of up to about 30 individuals (Gon and Randall 2003). In the Mediterranean, *C. novemstriatus* lives on various types of hard bottoms and near crevices (Rothman et al. 2013), and it has been observed among the spines of the already established non-indigenous sea urchin *Diadema setosum* in Rhodes Island (Ragkousis et al. 2020). The present species richness of Lessepsian apogonids, their rapid spread and abundance within the eastern Mediterranean are potentially of marine conservation and management concern for native communities, due to their establishment in diversified habitats.

It is interesting to note that the pharaoh cardinalfish is the earliest colonizer of Mediterranean waters among the Lessepsian apogonids, and it apparently expanded slowly its distribution in Levantine waters. The other four apogonid species mentioned above have been all recorded from the Mediterranean basin many decades later than the initial *A. pharaonis* record, between 2004 and 2010, and they appear to be extending rapidly their distribution range in the Levant (Rothman et al. 2013 and references therein), easily adapting to and establishing in the new environment. In particular, *J. queketti*, first recorded in the southern Turkey in 2004, has been observed in the south-eastern Aegean waters of Turkey in 2009 and again in 2014 (Akyol and Ünal 2015), while *C. novemstriatus*, first recorded along the Mediterranean coasts of Israel in 2010, has been detected at Rhodes, Greece, in 2020 (Ragkousis et al. 2020).

Nevertheless, it should be emphasized that the successive enlargements of the Suez Canal could be associated with the increase of new introductions of tropical and subtropical species from the Red Sea (Galil et al. 2017) and that the warming of Mediterranean waters due to climate change facilitates their expansion (Zenetos and

Galanidi 2020). Furthermore, the intensification all over the basin of research efforts and of underwater observations performed by citizen scientists during recent years has greatly contributed to the early detection of new or overlooked non-indigenous biota in many countries at a much faster pace than in the pre-citizen science era (Roy et al. 2018; Katsanevakis et al. 2020).

In this work, the occurrence of the Lessepsian migrant fish *A. pharaonis*, probably overlooked for a long time, is documented herewith for the first time in Libyan waters, due to the input of citizen science information. The Mediterranean Sea is a hotspot of biological invasions and introduced species are one of the major threats to Mediterranean biodiversity (Coll et al. 2010), resulting in changes of composition, function, and service of marine biocommunities, mainly in the eastern part of the basin (Katsanevakis et al. 2014, 2020). More than 100 alien fish species of the Red Sea/Indo-Pacific Ocean origin have been introduced via the Suez Canal into the Mediterranean basin (Golani and Fricke 2018), the majority established and integrated within the native food webs; many of these species do not have a commercial value and they are discarded in fishery activities, while other species dominate the fish catches, often with socio-economic and ecological impacts (Galil et al. 2017; Katsanevakis et al. 2020). Monitoring the expansion and abundance of non-indigenous species in the Mediterranean waters is of huge importance at the national and international level in order to contribute to the assessment of the quality of the waters of the basin and to manage its resources and sustainability, under the pressure of biological invasions and other factors (Anonymous 2020). The record of *A. pharaonis* in Libya not only adds knowledge on the poorly known ichthyofauna diversity of the whole country but contributes to fill gaps concerning the range distribution of this alien species in the Mediterranean basin.

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