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Age, growth, and population structure of endemic *Telestes karsticus* (Actinopterygii: Cyprinidae: Leuciscinae) from Sušik Creek, Croatia

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Abstract

This study provides the first description of the age, growth, and population structure of the endemic karstic dace, *Telestes karsticus* Marčić et Mrakovčić, 2011, from Sušik Creek in Croatia. The oldest individual in the sample was a female of age 5[+] years and the oldest males were 4[+]. Females of the karstic dace have a higher maximum standard length than males (122.0 vs. 95.1 mm, respectively). In both sexes, the most numerous length category was 41–50 mm. The most numerous age group of karstic dace was 1[+], with a gradual decline in the abundance of older age groups. In larger length categories, males were fewer, while females showed a bimodal distribution with a second maximum in the length category of 81–90 mm. The total ratio of males to females in the sample was 2.8:1, in favor of males.

Keywords

age composition, endemic species, life history, population traits, size composition

Introduction

The karstic dace, *Telestes karsticus* Marčić et Mrakovčić, 2011, is a relatively recently described species (Marčić et al. 2011), and the information on its ecology and population structure is scarce. Despite being a very old species, most likely originating at the Miocene–Pliocene boundary (5.3 million years ago) following separation from the common ancestor this species shared with *Telestes polylepis* Steindachner, 1866 (see Buj et al. 2017), it was not recognized as a separate species. Marčić et al. (2011) revealed cryptic diversity within *T. polylepis* at both the molecular and morphological levels, leading to description of *Telestes karsticus* as a new species. This

species is endemic to streams at only four locations on the north-western side of the Velika Kapela and Mala Kapela mountains in Croatia (Marčić 2013; Marčić et al. 2013; Ćaleta et al. 2019). Such a small distribution range is very unusual for fish inhabiting the Black Sea basin, where rivers are large, with many tributaries and mostly interconnected. Its distribution is the consequence of the karst landscape of these watersheds, which lack surface connections with other rivers, draining instead towards the Danube River via underground connections.

The karstic dace is listed as endangered (EN) at the European level, i.e., in the Red List of Freshwater Fishes of Europe (Freyhof 2018), and also at the national level in the Red List of Croatian Freshwater Fishes with an

assessment of B1ab(i,ii,iii)+2ab(i,ii,iii) (ver. 12; Marčić et al. unpublished data). Though this species is perfectly adapted to karst environments, its extremely limited distribution range makes it particularly vulnerable. Any modifications to its small habitat could have irreversible effects on the population structure and viability. Furthermore, options for reintroduction or population augmentation are lacking, given the existence of only four populations. The distribution range of this species was somewhat larger in the past, but it has since vanished from several streams and has been severely reduced in the remaining localities. In the Stajničko Polje field, the karstic dace was last recorded in 2007 (with 12 individuals) in the Holjevačko Vrilo spring (Marčić 2013), despite visits to the spring at least once per year, while the population in the Drežničko Polje field is very small and includes just a few individuals within the spring itself.

Endemic species with very restricted distribution ranges, such as *T. karsticus*, are particularly vulnerable and typically have a reduced capacity to adapt to environmental change. The lack of knowledge about this species has also undermined its protection, and therefore, without an understanding of the biology of this species, it is not possible to ensure its protection and survival. To date, published studies on the biology of this species have examined its reproductive biology and seasonal diet profile (Marčić et al. 2017a, 2017b). However, an understanding of the population structure is crucial for adequate conservation plans, since biases from the normal age pyramid or population structure are indicative of pressures acting on the population. A balanced age structure provides a buffering capacity for the population, minimizing the effects of short-term environmental fluctuations on recruitment (Planque et al. 2010). In contrast, truncation of the age structure increases variability in the stock and its sensitivity to climate variation and other environmental modifications (Hidalgo et al. 2011; Rouyer et al. 2011). Moreover, since large, old, and experienced individuals have a higher reproductive value than young, small, and inexperienced ones, the age structure of the spawning population may be just as important as its biomass (Trippel et al. 1997; Berkeley et al. 2004). A skewed sex ratio is usually indicative of population disturbance. Thereafter, only populations with a normal age and sex distribution can be considered stable and viable. The aim of this study was to describe the age, growth, and population structure of this endangered endemic fish.

Methods

Specimens of *T. karsticus* were collected at regular monthly intervals from July 2007 to July 2008 in Sušik Creek (45°44.13′N, 15°41.17′E). Sušik Creek is a sinking karst river that flows over about 5 km through a karst field at an elevation of 463 m. The creek width ranges from 2 to 10 m. Water levels are not constant through the year, with strong flows after rains or snowmelt raising depths to over 2 m in places, while in summer the

creek partially dries out and its depth elsewhere is less than 20 cm. Sampling was conducted using a backpack electrofishing device (Hans-Grassl, model IG-200-1) and captured specimens were anesthetized with an overdose of MS-222 and subsequently frozen. Fish were captured in a 200-m long transect in all habitats with the same effort during each sampling session. The use of a block net was impossible due to terrain configuration and the water depth and velocity. A total of 630 individuals (68 juveniles, 414 males, and 148 females) were analyzed. Standard length (SL) was measured using digital calipers with a precision of 0.1 mm and individuals were placed in standard length categories (each of 10 mm). Mass was determined with a digital scale with a precision of 0.1 g. Sex was determined by visual examination of the gonads. Population from Sušik Creek was assessed as the only location with a population sufficiently stable and large to ensure that sampling would not be detrimental for the species. Furthermore, the population was monitored in subsequent years and on last monitoring on June 2021, 800 adult individuals were recorded on the same locality in a 200 meter long transect making the current population at least 20000 strong (Marčić unpublished data).

The analysis of age and growth was performed on a subsample of 87 individuals. In the subsample, there were 47 males, 32 females, and 8 juveniles and the fish were selected to cover all standard length categories present in each monthly sample. Fish age was determined by direct readings of the scale rings, with independent readings performed by two different researchers (Busacker et al. 1990). Scales were removed from the dorsal part of the fish, under the dorsal fin, using histological needles, and cleaned of organic impurities in 3% KOH. Cleaned scales were placed on slides and observed under the microscope (Olympus BX51). Scales were photographed using the microscope Zeiss Axiovert 200 and measurements for back calculation taken using the program AxioVision Rel. 4.8. Each scale clearly showed the focus and concentric rings formed during growth. The scale radius and the radius of all rings on the scale were measured. The scale radius was measured as the largest length from the scale focus to the edge of the scale. Radii were measured along the same line. For the remainder of the sample, age was assessed using the length–weight relations for each month of sampling. Statistical significance of the number of males and females was determined with the χ^2 test. All mathematical calculations were performed in the Excel 2010 and Statistica 10.0 packages.

Results

The range of standard length was examined for the entire sample of 630 individuals of the karstic dace from Sušik Creek. The length composition of the populations, particularly for males, females, and juveniles, is shown in Table 1 and expressed as the minimum, maximum, mean, and standard deviation.

Table 1. Length composition of total population, males, females, and juveniles of *Telestes karsticus* from Sušik Creek, Croatia, in period from July 2007 to July 2008.

Standard length [mm]	n	Min	Max	Mean ± SD
Total	630	15.0	122.0	49.7 ± 18.5
Males	414	18.5	95.1	48.5 ± 14.3
Females	148	20.2	122.0	63.0 ± 21.6
Juveniles	68	15.0	44.7	27.9 ± 6.7

n = number of specimens, SD = standard deviation.

The smallest male with developed testes had a standard length of only 18.5 mm, while the smallest female with developed ovaries had a standard length of 20.2 mm. The difference in the mean value of standard lengths between males and females was statistically significant ($P < 0.0001$); females were larger on average. The largest reported individual was a female with a standard length of 122.0 mm. The largest individual without developed gonads had a standard length of 44.7 mm, which overlaps with mature individuals. The number of individuals by sex and length category is shown in Fig. 1.

Juvenile individuals were divided into four length categories, males in nine and females in ten length categories. The majority of juveniles (51.5%) fell within the 21–30 mm category. For both males and females, the highest abundance was found for the 41–50 mm category (M: 38.2%; F: 20.9%). Juveniles and males showed a unimodal distribution, while females showed a bimodal distribution, with a second peak in the 81–90 mm category.

Scales were cycloid, normally developed, and small, covering the entire body. They overlapped on the entire body, except on the ventral side of the head, and in some individuals along the flank under the lateral line. Scales were round to oval in shape and varied in size, with a radius of 160.55 μm, and the largest 1084.62 μm, depending on their position on the body. Circuli were clearly visible and the annuli were relatively easy to view. The mean radius with standard deviation of sampled scales for each individual age group were: $R_{0+} = 234 ± 57$ μm, $R_{1+} = 372 ± 75.6$ μm, $R_{2+} = 574 ± 42.8$ μm, $R_{3+} = 683 ± 79.4$ μm, $R_{4+} = 868 ± 107.3$ μm, and $R_{5+} = 940$ μm.

Six age groups were observed (0^+ through 5^+). All juvenile individuals fell within the two age groups (0^+ and 1^+). Males were found in five age groups (from 0^+ to 4^+), while females were found in all six (Fig. 2). Table 2 shows the number of the karstic dace individuals from the subsample in individual age groups, separated by sex, with mean values and standard deviations for standard length and mass.

Table 2. Individuals of *Telestes karsticus* from Sušik Creek, Croatia, in individual age categories with mean values and standard deviations of standard length (SL) and mass (m) by gender.

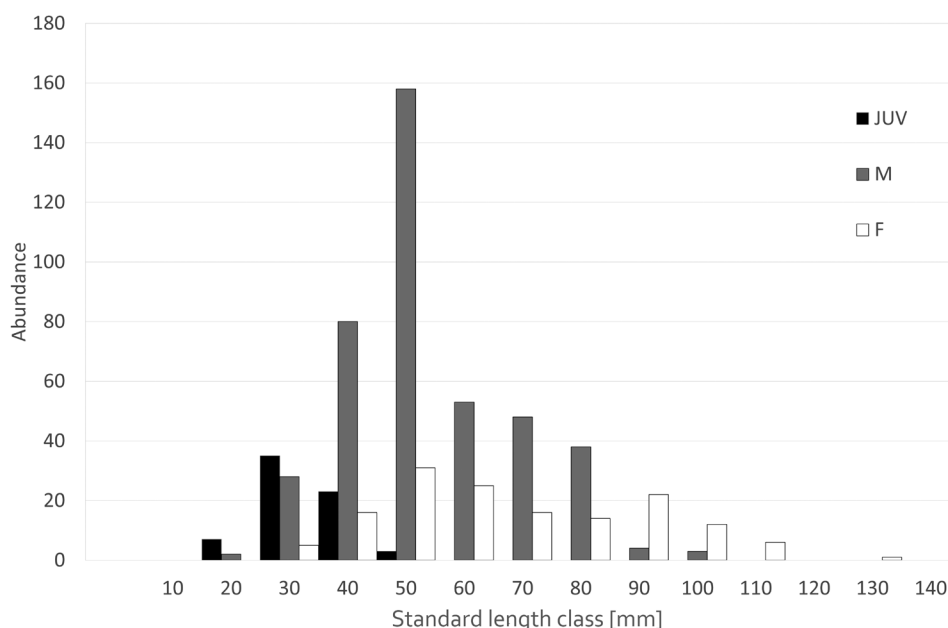| Sex	Juveniles			Males			Females		
Age	n	SL [mm]	m [g]	n	SL [mm]	m [g]	n	SL [mm]	m [g]
0^+	3	26.5 ± 5.7	0.3 ± 0.2	15	35.2 ± 9.4	0.9 ± 0.7	2	22.9 ± 3.8	0.3 ± 0.1
1^+	—	—	—	21	48.4 ± 11.0	2.6 ± 1.6	4	57.0 ± 11.2	3.7 ± 1.9
2^+	—	—	—	6	68.7 ± 6.0	5.9 ± 2.4	3	62.7 ± 6.3	4.5 ± 0.6
3^+	—	—	—	7	78.6 ± 8.6	11.1 ± 4.8	12	84.1 ± 5.7	10.7 ± 2.3
4^+	—	—	—	2	89.9 ± 4.3	11.7 ± 2.3	10	95.0 ± 6.3	16.7 ± 2.7
5^+	—	—	—	—	—	—	1	122.0	36.7

Figure 1. Abundance of *Telestes karsticus* individuals in Sušik Creek, Croatia, by sex (F = female; M = male; JUV = juvenile) and standard length (SL) categories (each 10 mm).
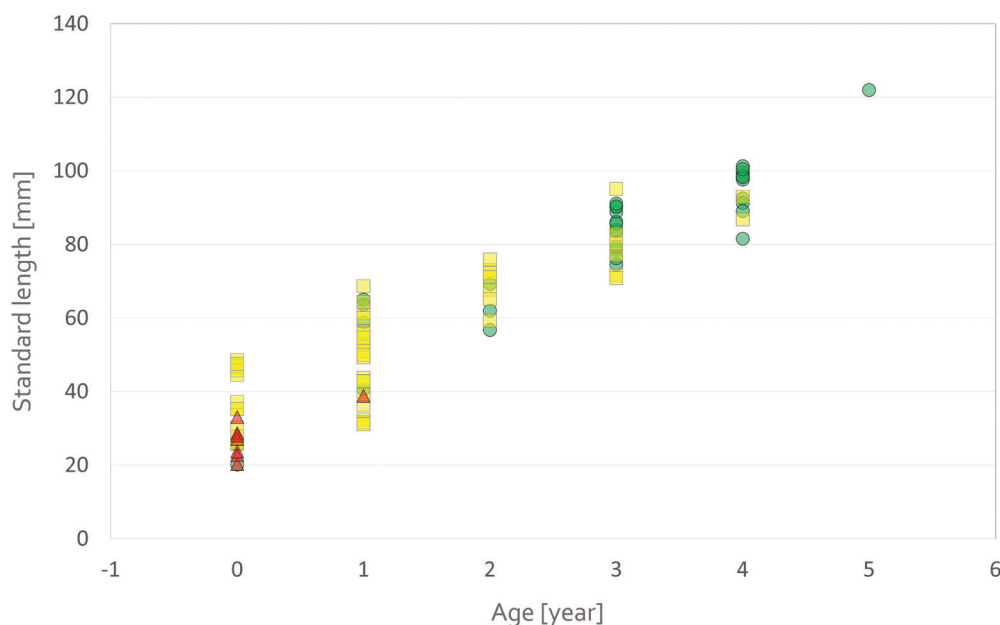
Figure 2. Age categories of *Telestes karsticus* from Sušik Creek, Croatia, in relation to standard length (SL) for males (yellow squares), females (green dots), and juveniles (red triangles).

The Mann–Whitney U test showed that there were no statistically significant differences in the mean SL values between males and females in any age group ($P > 0.05$), with the exception of the age group 0+ ($P < 0.05$). The mean SL of juvenile individuals did not differ statistically from males or females in the age group 0+. The Mann–Whitney U test showed that there were no statistically significant differences in the mass of males and females in any age group ($P > 0.05$). Table 3 shows the mass and length increments between age groups for males and females.

Table 3. Mass and length increments between consecutive age groups for males, females, and the total sample of *Telestes karsticus* from Sušik Creek, Croatia.

Sex	Age group	Mean mass increment [g]		Mean length increment [%]	
Females	0+–1+	3.4	1133.3	34.1	148.9
	1+–2+	0.8	22.4	5.7	9.9
	2+–3+	6.1	135.3	21.4	34.1
	3+–4+	6.0	56.7	11.0	13.1
	4+–5+	20.0	119.8	27.0	28.4
Males	0+–1+	1.7	180.9	13.3	37.7
	1+–2+	3.2	121.6	20.3	41.9
	2+–3+	5.3	90.1	9.9	14.4
	3+–4+	0.6	5.2	11.3	14.4

The highest mass increment (1133.3%) and standard length increment (148.9%) for females were recorded between the 0+ and 1+ age groups. Males also showed the highest increase in mass (180.9%) in that time period, though the highest length increase (41.9%) was achieved between the age groups 1+ and 2+. Females had the lowest mass increase (22.4%) and standard length increase (9.9%) between the age groups 1+ and 2+, while males had

the lowest mass increase (5.2%) between the age groups 3+ and 4+, and standard length increase (9.9%) between the age groups 2+ and 3+.

The mean SL values obtained through back calculations are shown in Table 4. Though these back-calculated SL values of standard length differed less than 7 mm from the measured mean standard length used in the determination of age for all age groups, the Mann–Whitney U test showed that those differences differed significantly ($P < 0.05$), with the exception of the age groups 4+ ($P = 0.077$) and 5+ for which there were too few individuals to determine statistical significance.

Table 4. Standard length of karstic dace, *Telestes karsticus*, from Sušik Creek, Croatia, obtained using back calculations by age group.

Age	*n*	Mean SL	Back calculated mean SL at age [mm]				
			SL 1+	SL 2+	SL 3+	SL 4+	SL 5+
0+	20	32.6					
1+	25	49.8	45.4				
2+	9	66.7	42.0	59.0			
3+	19	82.0	42.9	61.7	76.3		
4+	12	94.2	39.7	59.3	74.5	87.3	
5+	1	122.0	47.4	63.0	86.2	101.8	115.8
Total back calculated mean SL at age			43.5	60.8	79.0	94.6	115.8

According to the Kruskal–Wallis test, standard length obtained using back calculations did not differ significantly between individuals of different age groups ($P > 0.05$).

The population composition by sex and age is shown on the entire sample of 630 karstic dace from Sušik Creek (Fig. 3). Table 5 shows the calculated sex ratios by age category and the P values of the χ^2 test.
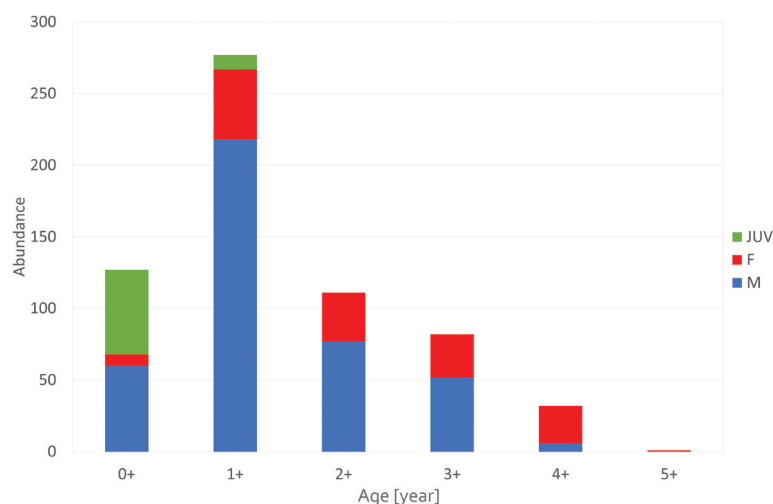
Figure 3. Age composition of the total sample of *Telestes karsticus* from Sušik Creek, Croatia (F = female, M = male, JUV = juvenile).

Table 5. Sex ratio of males (M) and females (F) of *Telestes karsticus* from Sušik Creek, Croatia, by age categories, with *P* values of the χ^2 test. Significant values ($P < 0.05$) are shown in bold.

Age	Sex ratio M:F	P
0^+	7.5:1	**2.9E-10**
1^+	4.4:1	**4.5E-25**
2^+	2.3:1	**4.5E-05**
3^+	1.7:1	**0.0151**
4^+	0.2:1	**0.0004**
5^+	0:1	0.3173

The most abundant age category of the karstic dace was the 1^+ category (43.9%), with declining abundance in the older age classes. The oldest age category was 5^+ (0.1%), and consisted of a single female.

The total ratio of males to females in the sample was 2.8:1 in favor of males, which was statistically significant ($P < 0.05$). Age categories from 0^+ to 3^+ were dominated by males, while females dominated in the 4^+ and 5^+ categories. The χ^2 test indicated significant differences in all age categories with the exception of the 5^+ category which included only a single female. Table 6 shows the calculated sex ratios by sampling date and *P* values of the χ^2 test.

Table 6. Sex ratio of males (M) and females (F) of *Telestes karsticus* from Sušik Creek, Croatia, by sampling date, with *P* values of the χ^2 test. Significant values ($P < 0.05$) are shown in bold.

Sampling date	Sex ratio M:F	P
24.7.2007	1.5:1.0	0.1036
29.8.2007	2.6:1.0	**0.0004**
27.9.2007	0.9:1.0	0.8474
31.10.2007	4.0:1.0	**0.0000**
27.11.2007	15.0:1.0	**0.0000**
20.12.2007	6.8:1.0	**0.0000**
4.2.2008	4.3:1.0	**0.0046**
3.3.2008	4.3:1.0	**0.0000**
3.4.2008	1.9:1.0	0.0947
29.4.2008	1.0:0.0	**0.0000**
4.6.2008	6.8:1.0	**0.0000**
2.7.2008	0.9:1.0	0.7963
29.7.2008	2.3:1.0	0.2059

On the majority of sampling dates, the sex ratio was dominated by males, with the exception of 27 September 2007 and 2 July 2008 which were dominated by females. However, the χ^2 test showed that the majority of sampling dates had a statistically significant difference in the sex ratio.

Discussion

The population structure of the karstic dace, *Telestes karsticus*, has hitherto been completely unknown. There are literature reports for the population composition of the related species *Telestes ukliva* (Heckel, 1843) (see Zanella, unpublished thesis; Zanella et al. 2009), *Telestes montenigrinus* (Vuković, 1963) (see Krivokapić 1992), and *Telestes souffia* (Risso, 1827) (see Vuković, unpublished thesis), and those results are compared with the results presented here. The maximum standard length of the karstic dace in Sušik Creek was 122 mm (total length 146 mm), confirming the assumption that it is a small bodied fish, and even smaller than other species in the genus *Telestes*. Zanella (unpublished thesis) reported the largest total length of *T. ukliva* at 150 mm, and Krivokapić (1992) reported the largest length category to 140 mm for *T. montenigrinus*. For *T. souffia*, Vuković (unpublished thesis) reported the largest length category to 180 mm from the Drina River and Vuković and Ivanović (1971) reported a maximum length of 250 mm for the same species. Kottelat and Freyhof (2007) listed maximum standard lengths of 120 mm for *Telestes beoticus* (Stephanidis, 1939), 165 mm for *Telestes croaticus* (Steindachner, 1866), 80 mm for *Telestes fontinalis* (Karaman, 1972), 100 mm for *Telestes metohiensis* (Steindachner, 1901), 160 mm for *T. montenigrinus*, 170 mm for *Telestes muticellus* (Bonaparte, 1837), 140 mm for *Telestes pleurobipunctatus* (Stephanidis, 1939), 150 mm for *T. polylepis*, 200 mm for *T. souffia*, 160 mm for *Telestes turskyi* (Heckel, 1843), and 100 mm for *T. ukliva*.

Females of the karstic dace from Sušik Creek, were generally larger than males, as was also found for *T. ukliva*

(see Zanella, unpublished thesis; Zanella et al. 2009) and *T. montenigrinus* (see Krivokapić 2002). Larger females are capable of producing greater numbers of eggs, therefore increasing reproductive success (Andersson 1994). The lower abundance within the age category 0^+ (20.1%) is likely a consequence of the sampling method, as electrofishing is known to underrepresent the 0^+ age category (Reynolds 1996). Such a population composition was also determined for *T. montenigrinus* (see Krivokapić 1992), while Zanella (unpublished thesis) found the highest abundance of *T. ukliva* in the 2^+ age category, and Vuković (unpublished thesis) found the highest abundance for *T. souffia* in the 3^+ and 4^+ age categories, depending on the season of sampling.

In the colder part of the year (November through March), when the water temperature is below 10°C, samples were comprised of individuals with an upper quartile of standard length less than 50 mm, and a median standard length of about 40 mm, suggesting that larger individuals overwintered at another, unknown location. One assumption is that larger individuals retract into subterranean areas where water temperatures are higher than in the surface stream, as suggested by Zanella (unpublished thesis) and Zanella et al. (2009), though such an assumption requires further study for the karstic dace.

The scale morphology of the karstic dace is similar to the scale morphology in other species in the genus *Telestes* (see Trgovčević 1905; Vuković, unpublished thesis). Clearly visible annuli on the scales enabled the assessment of individual age, as according to Busacker et al. (1990) the proportionality between most hard structures and fish size enables the determination of age, even for previous years of life. For the karstic dace, six age groups were found, with the oldest age group of 5^+. This is less than the 7^+ that determined for *T. souffia* (see Vuković, unpublished thesis; Schwarz 1998) and *T. ukliva* (see Zanella, unpublished thesis), and 6+ determined for *T. muticellus* (see Zerunian 2004) and *T. montenigrinus* (see Krivokapić 1998). However, this was higher than the 3^+ determined for *T. pleurobipunctatus* (see Barbieri et al. 2002). Both Zerunian (2004) and Vuković (unpublished thesis) also recorded only females in the oldest age group, which corresponds to the results presented here. The karstic dace and *T. pleurobipunctatus* are species that live in inconstant conditions, which is likely the reason for their lifespan being shorter than other members of this genus. Individuals of karstic dace in the aquarium survived longer than those ages recorded in nature (Marčić, unpublished data). This should be examined with further research on the remaining species of the genus *Telestes*.

The mass and length of males and females of the karstic dace have increased at a different pace. Males had a relatively high increase in mass between the age groups 0^+ and 1^+ (180%) which then declined with increasing age, to the minimum increase recorded between the age groups 3^+ and 4^+, which was also the oldest recorded male in nature. Such a growth model indicates that males grow faster and achieve their maximum increase in the age group 3+, after which time growth stagnates and the majority of individuals die. The length increase in males was similar to the mass increase. In females, the highest mass increase was also recorded between the age groups 0^+ and 1^+, though this is likely the consequence of the small number (2) of females in the 0+ category and the immaturity of their gonads. These results suggest that females stagnate in growth between the age categories 1^+ and 2^+ and then increase again between the age categories 2^+ and 3^+. Such growth could be interpreted with the development of the reproduction potential in subsequent age groups, when energy is invested in gonad development, thereby increasing the mass of the individual. As expected, the mass increase dropped between the age groups 3^+ and 4^+, and the large increase in mass increase between the age groups 4^+ and 5^+ should be viewed in light of the fact that there was only one female in the 5^+ age category, which was also the largest (SL 122 mm) and heaviest (36.7 g) individual of the entire sample. The length increases in females were similar to the mass increases. Vuković (unpublished thesis) determined for *T. souffia* from the Drina River that the mass increase rises while the length increase gradually decreases with advancing fish age. For *T. montenigrinus*, Krivokapić (1998) reported the highest mass increase between the age groups 2^+ and 3^+, though she did not examine specimens separately by sex. The reasons for the variations in mass increases between the species may be explained as the result of the different ecological conditions in the distribution area, or the result of biological or genetic isolation.

Using back calculations, the standard lengths were determined for earlier years of the life of individuals. The standard length obtained using this method should correspond to the standard lengths of individuals of that age group, which was confirmed here. Though the mean values of SL obtained using back calculations differed from the mean SL values for the measured individuals by age groups, the differences were not statistically significant, with the exception of age group 4^+. The back calculated SL values were generally smaller than the actual SL values measured by age groups, which corresponds to Lee's phenomenon (Lee 1912). The values obtained by back calculations are the values that the individual had at the time of formation of the annulus, i.e., in late February or early March. After this time, the individual continues its normal growth and a higher SL will be measured if the individual is caught in summer or later.

Sex ratio is an important demographic parameter crucial for population viability (Ospina-Alvarez and Piferrer 2008). According to the Fisher (1930) model, the sex ratio in animals should create offspring with an even sex ratio. In principle, populations with skewed sex ratios caused by environmental conditions can be considered disturbed or poorly adapted for the given conditions. As the reproductive potential of many fish species is determined by the number of females capable of producing eggs, a strong shift in sex ratio in favor of males can re-

duce the viability of vulnerable populations. The sex ratio of the entire sample of the karstic dace was statistically significant in favor of males, which was also reported for *T. ukliva* which had a ratio between 1.6:1 and 1.82:1 in favor of males (Zanella, unpublished thesis; Zanella et al. 2009). Male domination in the smaller length categories and females in larger categories were also seen for *T. montenigrinus* (see Krivokapić 2002). Zerunian (2004) also reported that the 7[+] age category for *T. muticellus* was comprised exclusively of females. However, the presently reported results differed from the even sex ratio for the species *T. montenigrinus* (see Krivokapić 1992) and *T. souffia* (see Vuković, unpublished thesis). Furthermore, the sex ratio of karstic dace was dominated by males in the majority of months, particularly in the colder months when only smaller specimens were captured. A stable sex ratio was confirmed during the spawning period (3 April 2008), when the sex ratio was also in favor of males, though this was not statistically significantly different from the theoretical ratio of 1:1. The sex ratio was also even and not significantly different from 1:1 in July 2007, September 2007, and July 2008, when larger individuals were captured, and in July 2008 when very few individuals were caught (Table 6). The uneven sex ratio in karstic dace is unlikely to be due to a segregation of the sexes due to their varying microhabitat use, as sampling included all habitats and sampling effort was uniform. The dominance of females in the older age categories might be explained by a higher mortality rate among males; however, such an occurrence would not support the prominent dominance of males in younger categories.

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PENSOFT.

ACTA
ICHTHYOLOGICA
ET PISCATORIA

First record of slender red scad, *Decapterus smithvanizi* (Actinopterygii: Perciformes: Carangidae), from the Philippines

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Abstract

Ten specimens (187.3–226.9 mm standard length) of slender red scad, *Decapterus smithvanizi* Kimura, Katahira et Kuriiwa, 2013, previously reported from the Andaman Sea, South China Sea, Taiwan, Japan, Thailand, Indonesia, Myanmar, and Pakistan, were collected off Iloilo (Panay Island), the Philippines. The presently reported specimens represent the first record of the species from the Philippines. A detailed description of the specimens is provided, with a comparison to other commonly-caught species of red-fin *Decapterus* in the area.

Keywords

Introduction

The carangid genus *Decapterus* Bleeker, 1851, currently including 11 valid species (Kimura et al. 2013; Fricke et al. 2021), is characterized by having a single finlet behind both the second dorsal and anal fins, absence of scutes on the anterior curved part of the lateral line, two low papillae on the shoulder girdle, and well-developed adipose eyelid (Gushiken 1983; Smith-Vaniz 1999). In 2013, Kimura et al. grouped the *Decapterus* species with red fins and identified *Decapterus smithvanizi* as a new species. Four species have been included in the group:

Decapterus akaadsi Abe, 1958; *Decapterus kurroides* Bleeker, 1855; *Decapterus tabl* Berry, 1968, and *Decapterus smithvanizi* Kimura, Katahira et Kuriiwa, 2013.

Specimens of red-fin *Decapterus* were collected by researchers from the University of the Philippines Visayas during an ichthyofaunal survey in Miagao Fish Market in Panay Island in November 2016. The specimens were identified with the help of a taxonomist from the Kagoshima University Museum and included *Decapterus smithvanizi* amongst the specimens collected. However, the collected specimens were poorly preserved and not accessioned. Subsequently, the same fish market

was visited in February–May 2020 and additional specimens of *D. smithvanizi* were obtained. The specimens of *D. smithvanizi*, used in this study, represent the first records of the species from the Philippines with complete examination and description. This report completes the presence of all red-fin *Decapterus* in the country.

Materials and methods

Counts and measurements followed Hubbs and Lagler (1947) and Kimura et al. (2013) with additional measurement, from the snout to the central posterior tip of the sideways "W-shaped" margin at the dorsal head. Measurements were made to the nearest 0.1 mm with a digital caliper (≤180 mm) and a manual caliper (>180 mm). Standard and head lengths are abbreviated as SL and HL, respectively. Curatorial procedures followed Motomura and Ishikawa (2013). Counts of lateral-line scales and scutes followed Kimura et al. (2013) and are defined according to Smith-Vaniz and Carpenter (2007). Characters, such as gill raker, lateral line scale, and scutes, were counted under a dissecting microscope. Identification of specimens followed Kimura et al. (2013). The specimens examined in this study are deposited at the University of the Philippines Visayas Museum of Natural Sciences, Iloilo, Philippines (**UPVMI**).

Results

Decapterus smithvanizi Kimura, Katahira et Kuriiwa, 2013

Material examined. *Decapterus smithvanizi*: UPVMI 3059 through 3068, 10 specimens, 187.3–226.9 mm SL, Miagao Fish Market, Iloilo, Feb.–May 2020, E. Delloro and R. Babaran. *Decapterus tabl*: UPVMI 3069 through 3077, 10 specimens, 185.4–208.96 mm SL, Miagao Fish Market, Iloilo, May 2020, E. Delloro. *Decapterus kurroides*: 9 specimens, UPVMI 3078 through 3086, 166.7–191.3 mm SL, Miagao Fish Market, Iloilo, Feb.–May 2020, E. Delloro and R. Babaran.

Description. Body elongate, compressed, and comparatively slender, deepest between first dorsal and second dorsal fin. Dorsal and ventral profile body slightly convex from tip of snout to caudal-fin base. Mouth terminal, large; posterior tip of maxilla reaching vertical through anterior margin of eye; posterior tip of upper jaw not hooked. Interorbital space slightly convex, with scales. Predorsal scaly area extending to anterior margin of eye. Upper end of pectoral-fin base anterior to vertical through opercular margin; lower end of pectoral-fin base vertical through pelvic-fin origin. Pectoral fin asymmetrical, its posterior tip pointed, reaching beyond vertical through second dorsal-fin origin. Pelvic-fin origin anterior to vertical through dorsal-fin origin. First dorsal fin higher than second; single finlet present both dorsally and ventrally on caudal peduncle. Caudal fin forked; covered with small scales. Body scales small and ctenoid. Lateral line extends downwards from origin of second dorsal fin; running straight from middle of second dorsal fin to caudal-fin base; curved part longer than straight part, covered with ctenoid scales and scutes at posteriormost area; straight lateral line covered with scutes reaching beyond hypural bone. Head covered with scales, except snout area, mandible and anteroventral region of head; posteriormost end of scaly head region anterior to vertical through opercular margin; adipose eyelid developed. Teeth on jaws minute, two rows on upper jaw and single row on lower jaw. Lower jaw slightly protruding. Gill rakers slender, covered with spinules at inner surface.

Color when fresh. Head and body bluish to pale black dorsally, pale white to silvery ventrolaterally; black blotch present on upper edge of opercle; both margins of dorsal, caudal, and pectoral fins and finlet red with fine melanophores; anal fin, pelvic fins and ventral finlet pinkish to white with fine melanophores (Fig. 1A).

Color when preserved. Head and body greyish to brownish dorsolaterally, pale white ventrolaterally; black blotch present on upper edge of opercle; both margins of dorsal, caudal, and pectoral fins and finlet brownish to dirty white with fine melanophores; anal fin, pelvic fins, and ventral finlet brownish to white with fine melanophores (Fig. 2A–C).

Distribution. *Decapterus smithvanizi* is distributed in the Andaman Sea, South China Sea, Indonesia, and the western coast of Thailand (Kimura et al. 2013), Taiwan (Smith-Vaniz et al. 2018b), Japan (Iwatsubo et al. 2016; Hata and Motomura 2017), Myanmar (Psomadakis et al. 2020), and Pakistan (Psomadakis et al. 2015). Specimens were collected off Iloilo Province (Panay Island), Philippines. The specimens, used in this study, represent the first record from the Philippines.

Comparison. *Decapterus smithvanizi* can be easily distinguished from the other members of red-fin *Decapterus* group in having fewer cycloid scales along the curved part of the lateral line (54–62), long pectoral fin (25.5%–29.6% SL) reaching beyond a vertical through the origin of second dorsal fin and fewer lower gill rakers (26–28) (Table 1). Melanophores scattered on the pectoral, pelvic and anal fin rays of *D. smithvanizi* (Fig. 2A–C) are more distinct than those of *D. tabl* (Fig. 2D–F) and *D. kurroides* (Fig. 2G–I). Two other species in red-fin *Decapterus* group, *D. kurroides* and *D. tabl*, are commonly caught together with *D. smithvanizi* in Panay Island. Similarities in morphological characters and coloration may lead to confusion and misidentification. However, they can be distinguished from each other with proper examination. In specimens of *D. kurroides*, the body depth is deeper (23.4%–26.6% SL) compared to *D. tabl* (18.4%–21.2% SL) and *D. smithvanizi* (19.7%–22.5% SL) (Fig. 3A; Table 1). The head length of *D. kurroides* (30.8%–31.7% SL) is longer than *D. smithvanizi* (29.3%– 31.0% SL) and *D. tabl* (29.0%–30.0% SL) (Fig. 3B; Table 1). The

Figure 1. Species of red-fin *Decapterus* commonly caught in Panay Island. **A:** *Decapterus smithvanizi*, UPVMI-3075, 201.91 mm SL; **B:** *Decapterus tabl*, UPVMI-3061, 205.47 mm SL; **C:** *Decapterus kurroides*, UPVMI-3085, 191.31 mm SL.

pectoral fins of *D. smithvanizi* (25.5%–29.6% SL) and *D. kurroides* (27.6%–32.8% SL) are longer than *D. tabl* (17.6%–20.5% SL) (Fig. 3C; Table 1). Additionally, it was observed that body scales of the specimens extend on to the head dorsally and formed a sideways "W-shaped" margin (Fig. 4). The distance from the snout to the central posterior tip of the margin was measured. *D. kurroides* is longer in proportion to head length (91.0%–97.1% HL) compared to *D. smithvanizi* (85.0%–87.2% HL) and *D. tabl* (80.0%–87.4% HL) (Fig. 3D; Fig. 4; Table 1).
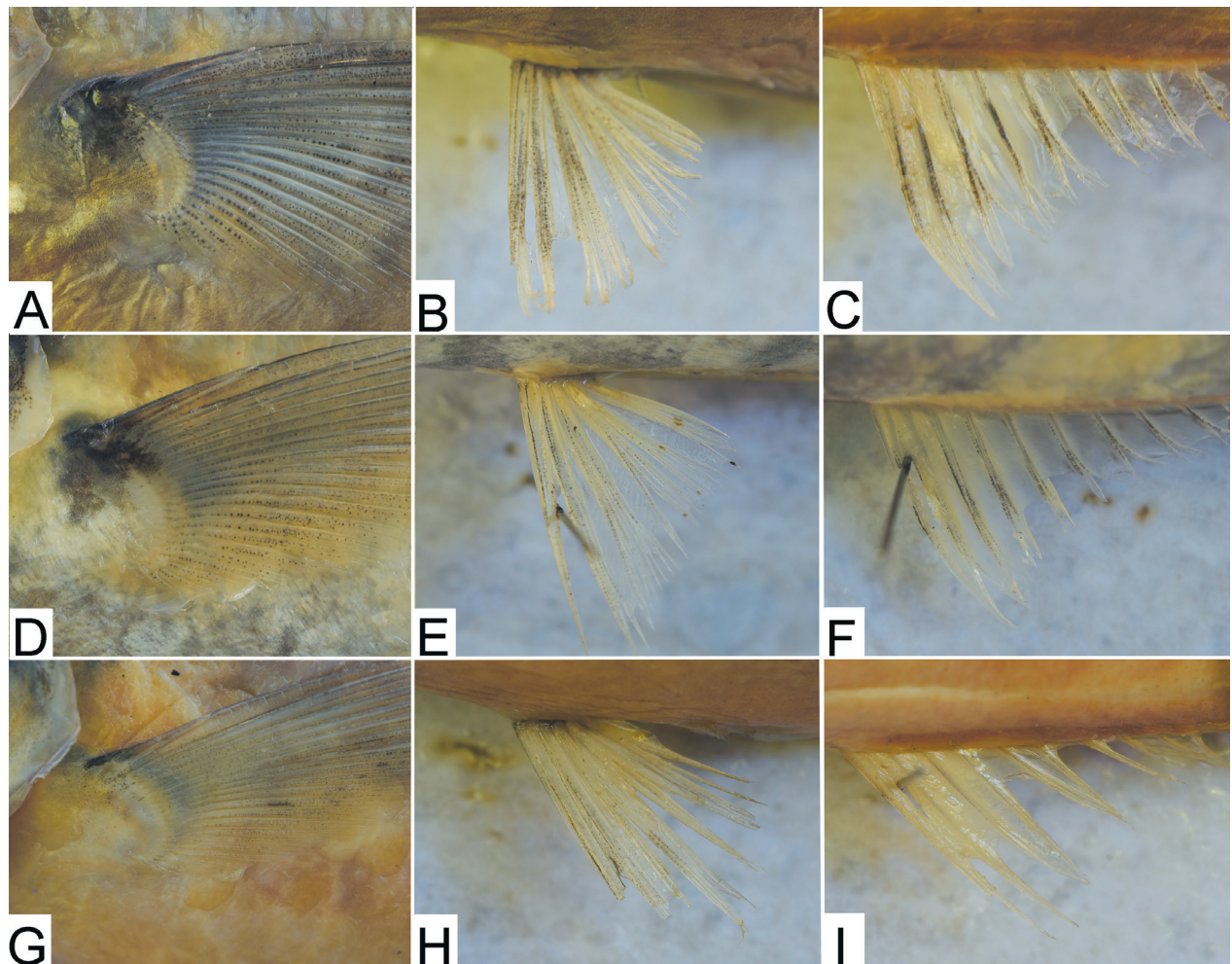
Figure 2. Pectoral, pelvic and anal fins of preserved specimens of *Decapterus smithvanizi* (**A–C**), UPVMI-3075, 201.91 mm SL, *Decapterus tabl* (**D–F**), UPVMI-3061, 205.47 mm SL and *Decapterus kurroides* (**G–I**), UPVMI-3085, 191.31 mm SL.

Figure 3. Relation of body depth (**A**), head length (**B**), and pectoral length (**C**) to standard length (SL) and of central posterior tip of dorsal head margin (**D**) to head length (HL) of *Decapterus smithvanizi* (▪), *Decapterus tabl* (▲) and *Decapterus kurroides* (o).

Table 1. Counts and measurements of *Decapterus smithvanizi*, *Decapterus tabl*, and *Decapterus kurroides*.

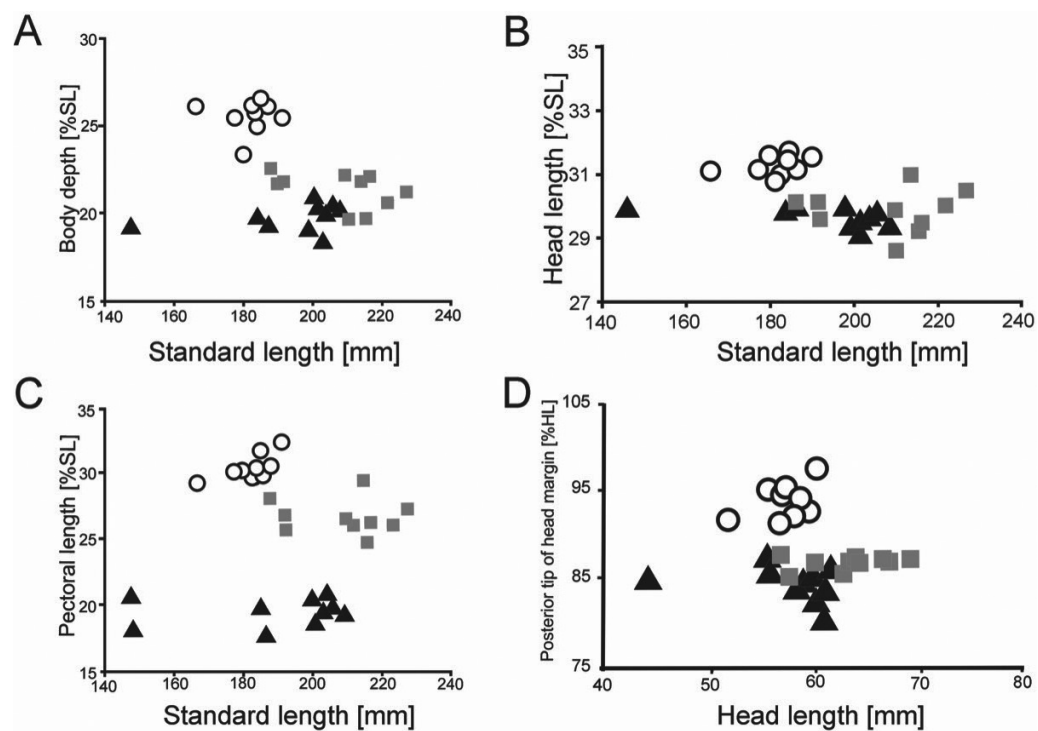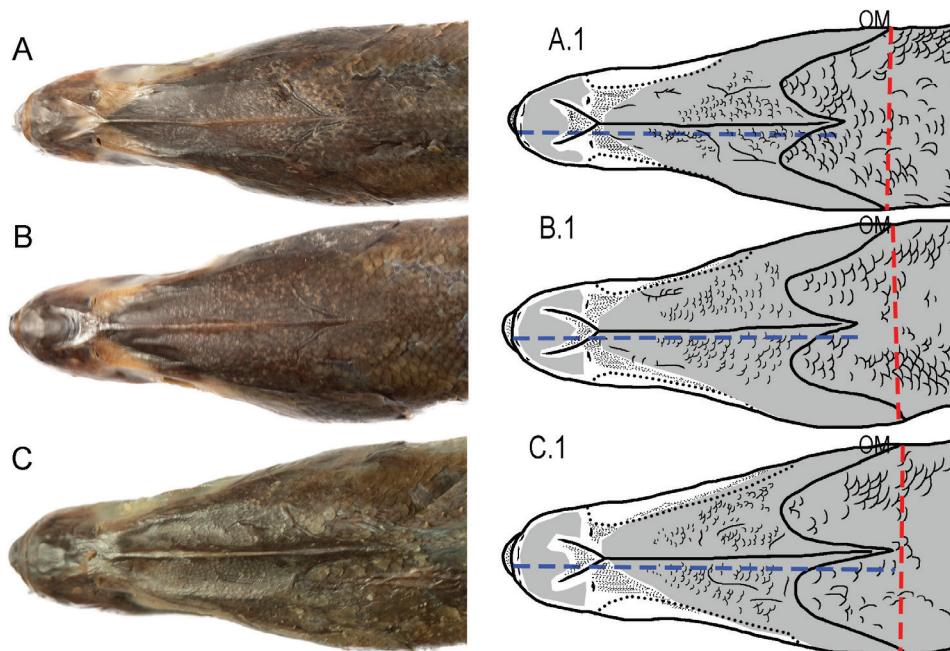| Character	*Decapterus smithvanizi*			*Decapterus tabl*			*Decapterus kurroides*		
	n	Range	Mean	*n*	Range	Mean	*n*	Range	Mean
Standard length [mm]	10	187.3–226.9	208.6	10	147.9–209.0	194.4	9	166.7–191.3	182.3
Measurements									
As % of standard length									
Head length	10	29.3–31.0	30.0	10	29.0–30	29.6	9	30.8–31.7	31.3
Predorsal length	10	34.6–36.8	35.9	10	36.0–37.3	36.4	9	35.9–37.2	36.4
First dorsal-fin base length	10	13.8–15.5	14.4	10	12.8–14.6	13.7	9	15.1–16.0	15.6
Second dorsal-fin base length	10	35.5–36.4	36.1	10	35.1–36.6	35.8	9	34.6–36.6	35.3
Anal-fin base length	10	26.9–28.2	27.5	10	25.9–27.6	26.6	9	26.2–27.9	26.9
Snout to pectoral-fin insertion	10	28.9–31.1	29.9	10	28.7–29.9	29.5	9	30.9–32.4	31.3
Snout to pelvic-fin insertion	10	27.4–32.7	30.1	10	30.2–33.0	31.3	9	31.7–33.7	32.8
Snout to anal-fin origin	10	56.8–60.3	58.6	10	57.4–59.5	58.7	9	55.4–60.2	59.0
Pelvic-fin insertion to anal-fin origin	10	26.4–29.1	27.8	10	26.7–29.3	28.0	9	24.7–27.0	31.9
Snout to anus	10	53.6–57.2	55.8	10	51.4–57.7	55.1	9	53.7–56.6	55.2
Caudal-peduncle length	10	9.0–10.4	9.6	10	9.5–11.2	10.1	9	9.4–11.7	10.4
Body depth	10	19.7–22.5	21.4	10	18.4–21.2	19.7	9	23.4–26.6	25.6
Caudal-peduncle depth	10	2.8–3.4	3.2	10	2.9–3.5	3.1	9	3.5–3.9	3.7
Pectoral-fin length	10	25.5–29.6	27.1	10	17.6–20.5	19.4	9	27.6–32.8	30.4
Pelvic-fin length	10	10.5–12.5	11.8	10	9.9–11.2	10.7	9	12.5–13.7	13.1
Length of second spine of first dorsal fin	10	11.7–14.4	13.4	10	10.7–14.5	12.7	8	14.0–15.3	14.8
First anal-fin spine length	10	3.9–5.5	4.8	10	4.3–5.8	5.2	9	5.1–6.1	5.6
Head length [mm]	10	56.6–69.1	61.8	10	44.2–61.4	57.6	9	51.9–60.3	57.0
As % of head length									
Snout length	10	27.7–31.0	29.3	10	29.3–32.2	30.6	9	27.9–31.0	28.9
Upper jaw length	10	31.7–35.1	33.2	10	31.5–33.0	32.4	9	34.5–36.5	35.5
Eye diameter	10	25.6–31.2	29.0	10	27.4–30.7	28.9	9	29.1–33.9	30.5
Postorbital head length	10	41.5–44.8	44.6	10	41.1–45.9	44.0	9	41.9–44.3	43.2
Interorbital width	10	18.5–29.6	21.3	10	20.1–22.8	21.4	9	20.8–24.2	22.4
Posterior tip of dorsal head margin	10	84.0–87.2	86.1	10	80.0–87.4	84.3	9	91.0–97.1	93.5
Counts									
Dorsal-fin rays	10	VIII + I, 29–31	30.0	10	VIII + I, 29–32	30.8	9	VIII + I, 27–29	28.1
Anal-fin rays	10	II + I, 22–26	24.5	10	II + I, 24–26	24.6	9	II + I, 22–23	22.3
Pectoral-fin rays	10	21–22	21.1	10	22–22	22	9	20–22	21
Pelvic-fin rays	10	I, 5	5	10	I, 5	5	9	I, 5	5
Gill rakers on upper arch	10	9–11	10	10	10–13	11.9	9	10–12	11
Gill rakers on lower arch	10	26–28	27.5	10	31–33	31.9	9	27–31	29.2
Cycloid scales on curved part of lateral line	10	54–62	58.7	10	60–67	62.5	9	45–51	48.7
Scutes on posterior curved part of lateral line	10	0–3	1.4	10	0	0	9	3–4	3.1
Cycloid scales on anterior straight part of lateral line	10	0–4	1.6	10	5–8	6.2	9	0	0
Scutes on straight part of lateral line	10	30–33	32.5	10	34–39	37.6	9	31–33	32.0

n = number of specimens studied.

Figure 4. Dorsal views of the head of red-fin *Decapterus* with their respective illustration. **A:** and **A.1:** *Decapterus smithvanizi*, UPVMI-3075, 201.91 mm SL; **B:** and **B.1:** *Decapterus tabl*, UPVMI-3061, 205.47 mm SL; **C:** and **C.1:** *Decapterus kurroides*, UPVMI-3085, 191.31 mm SL. The red broken line traced the posterior margin of the operculum. The blue broken line traced the distance from the snout to the central posterior tip of the sideways "W-shaped" margin. OM = operculum margin.

Discussion

Decapterus smithvanizi can be distinguished from other red-fin *Decapterus* by the following combination of characters; lower gill rakers 25–31, curved part of lateral line with 54–62 cycloid scales, body depth 19.4%–22.5% SL, pectoral-fin beyond the level of second dorsal-fin (Kimura et al. 2013). The presently reported specimens were identified as *D. smithvanizi* agreeing closely with the description of the species given by Kimura et al. (2013).

Four species of *Decapterus* with red caudal fins were grouped by Kimura et al. (2013) as red-fin *Decapterus*, *Decapterus kurroides*, *Decapterus akaadsi*, *Decapterus tabl* and *Decapterus smithvanizi*. Three species of red-fin *Decapterus* were reported in the Philippines, *D. akaadsi* (Smith-Vaniz et al. 2018a), *D. kurroides* (Smith-Vaniz 1999; Kimura et al. 2013) and *D. tabl* (Narido et al. 2016; Kimura 2017; Motomura et al. 2017). *Decapterus smithvanizi*, a newly-described species, is previously known from Thailand, Indonesia, Andaman Sea (Kimura et al. 2013), South China Sea, Taiwan (Smith-Vaniz et al. 2018b), Myanmar (Psomadakis et al. 2020) and Pakistan (Psomadakis et al. 2015). The report of *D. smithvanizi* in Iloilo Province confirmed its presence in the Philippines and completed all red-fin *Decapterus* in the country.

Red-fin *Decapterus* species are similar to each other. It was observed that *D. smithvanizi* are commonly caught with other species of red-fin *Decapterus* in Iloilo. Other than the description given by Kimura et al. (2013), it was observed that this species has more melanophores at its dorsal, anal, and pelvic fins compared to other species. Further examination of the specimens showed that their body scales extend on to the head dorsally, forming a sideways "W-shaped" margin in dorsal view. The distance was taken from the snout to the central posterior tip of the margin and showed that *D. kurroides* has a longer tip compared to *D. tabl* and *D. smithvanizi*. These diagnostics characters can help to identify and differentiate the species from each other, which is necessary for effective conservation and management of this group.

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ACTA
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A candiru, *Paracanthopoma* sp. (Siluriformes: Trichomycteridae), associated with a thorny catfish, *Doras phlyzakion* (Siluriformes: Doradidae), in a tributary of the middle Rio Negro, Brazilian Amazon

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Abstract

Amazonian vampire catfish, known regionally as "candiru", are recognized as hematophagous fishes, but information on their ecology remains limited. We provide the first report of *Paracanthopoma* sp. (Vandelliinae) found attached to the body surface of a thorny catfish, *Doras phlyzakion* Sabaj Pérez et Birindelli, 2008, bellow the lateral bony plates, rather than at the gills where they have usually been found. The specimens had not recently ingested blood or other identifiable fish remains (flesh, skin, or mucus), which could be an indication they have been using this host for protection or as a phoretic association, rather than for feeding. Thus, the interaction of vampire fish with the host catfish may be more complex than previously understood.

Keywords

Introduction

Although predation has often been considered the main interspecific relation structuring ecological communities (Menge and Sutherland 1987; Rodríguez and Lewis 1997; Okada et al. 2003; Piana et al. 2006; Petry et al. 2010), there are other biotic interactions that can be important to regulate the abundance of some species, such as parasitism, mutualism, and commensalism.

Candiru is the common name of Vandelliinae catfishes (Trichomycteridae) that are known to be parasites of other fish (de Pinna and Wosiacki 2003; Zuanon and Sazima 2004a, 2005) and sometimes humans (Spotte 2002). However, only species in the subfamily Vandelliinae are considered truly hematophagous parasites, feeding on blood extracted from the gill arteries of host fish (de Pinna and Wosiacki 2003; Zuanon and Sazima 2004a).

Some species of parasitic catfishes, such as those of the genus *Paracanthopoma* (Vandelliinae), seem to exhibit behavior of long-lasting attachment to their host fish, which may relieve them of the time and energy
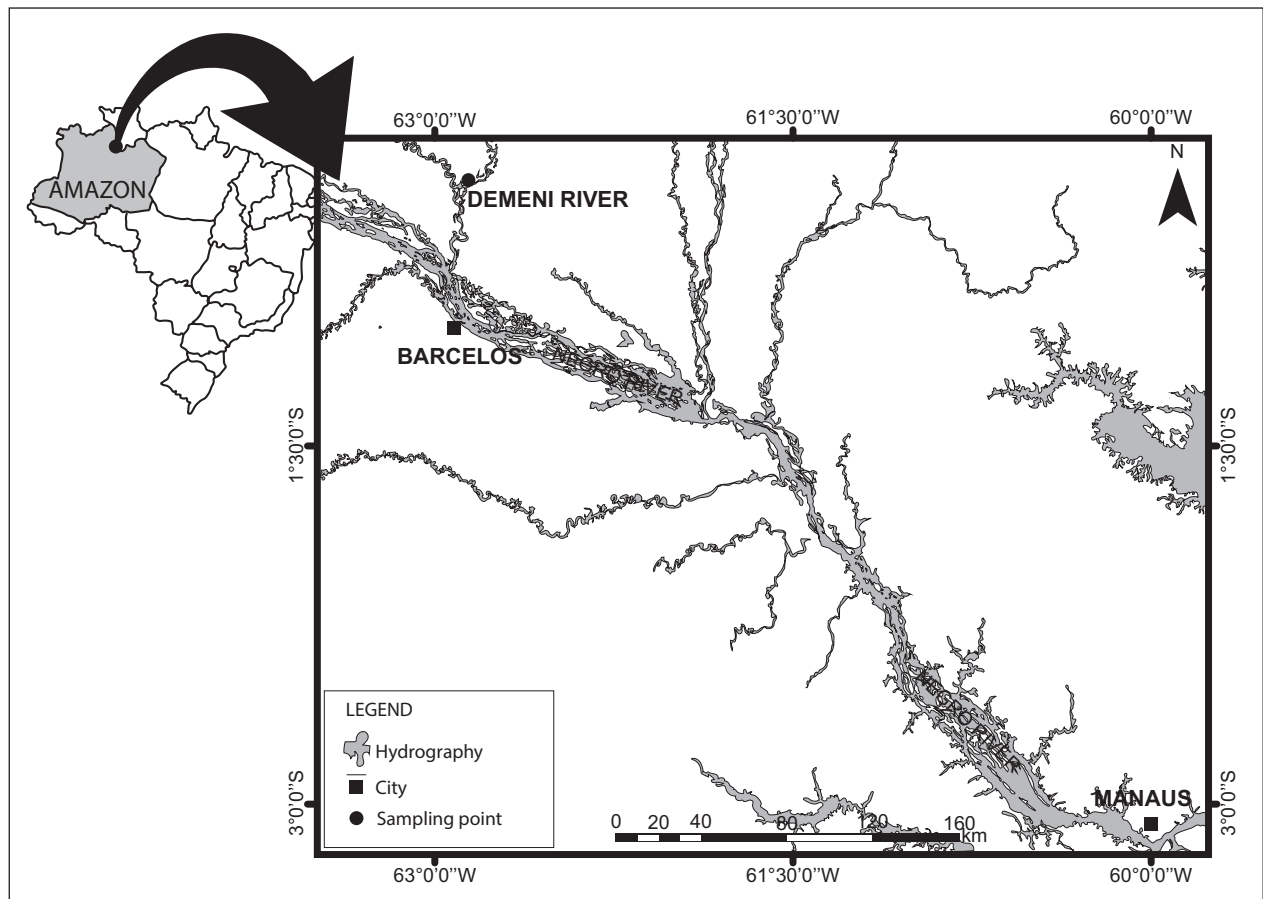
Figure 1. Location of the new occurrence of vampire fish (*Paracanthopoma* sp.) in the Demeni River, left bank tributary of the Negro River, Amazonas State, Brazil.

required to search for prey (de Pinna 2013). Attachment to a larger fish host may also offer a certain amount of protection against predation, especially since candirus are tiny and transparent, and therefore can avoid being noticed by visual predators while attached to their hosts (de Pinna 2013).

Zuanon and Sazima (2005) already reported an occurrence of individuals of *Paracanthopoma* sp. found attached to a large pimelodid catfish—*Zungaro zungaro* (Humboldt, 1821)—landed by a small-scale commercial fisherman. Here we present the first report of the occurrence of *Paracanthopoma* sp. attached to the body surface of a doradid thorny catfish, *Doras phlyzakion* Sabaj Pérez et Birindelli, 2008, a non-migratory species that was caught in a blackwater floodplain lake of the Negro River in Brazilian central Amazon.

Material and methods

The catch sample we examined was part of a study on the fish assemblages done on 7 April 2019, at a lake of the Demeni River basin (0°23′36.2″S, 62°51′43.4″W), a left-bank tributary of the Negro River (Fig. 1). We used gillnets of 30, 40, and 60 mm mesh sizes, which remained in the water from 1700 h to 2100 h. All the

fish caught were identified, weighed [g], and measured (standard length, SL) [mm]. The collected fish were euthanized with benzocaine hydrochloride and fixed in 10% formalin. Subsequently, the samples were transferred to 70% ethanol and voucher specimens were deposited in the Fish Collection of the Instituto Nacional de Pesquisas da Amazônia (INPA, Manaus, Amazonas State; deposit number #59058). Fish samples were collected under the license SISBIO 22121.

A regression was performed to evaluate the host length vs. the number of parasites. The regression was performed using the software R version 4.0.2 (R Core Team 2020).

Results

Twelve specimens of the vampire catfish, *Paracanthopoma* sp. (Trichomycteridae, Vandelliinae), measuring 10.1–18.0 mm SL (15.7 ± 2.5 mm) were found attached to the body of nine adult specimens of *Doras phlyzakion* weighing 30–106 g (69.11 ± 25.40 g) and measuring 110–160 mm (141.9 ± 17.6 cm). Each specimen of *D. phlyzakion* hosted one or two specimens of *Paracanthopoma* sp. attached to the outside of the body near the lateral bone plates (Fig. 2), where several reddish wounds
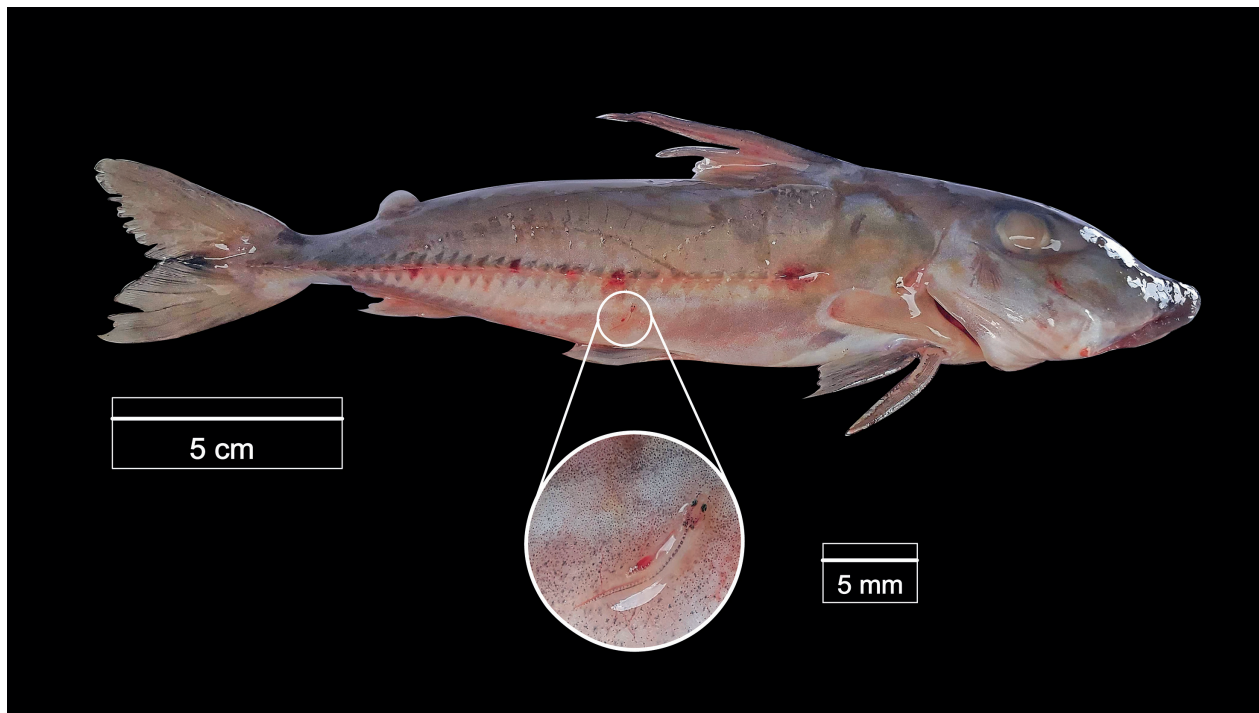
Figure 2. *Doras phlyzakion* with vampire fish (*Paracanthopoma* sp.) fixed into its epidermis close to the bony plates of the lateral line. Collected from the Demeni River, left bank tributary of the Negro River, Amazonas State, Brazil.

(small holes) were also observed. According to the values obtained from the regression, the length of the hosts explains approximately 50% the variation in the number of parasites (residual standard error = 0.6582, r^2 = 0.4969, F = 8.901, P-value = 0.02042). Therefore, larger fishes hosted more parasites.

Discussion

Paracanthopoma is a monophyletic genus of vampire catfish (Ochoa et al. 2020) that contain one described (*Paracanthopoma parva* Giltay, 1935) and several undescribed species (e.g., Pedroza et al. 2012). Fishes of the genus *Paracanthopoma* have long and robust snouts, with strong dentary teeth (Spotte 2002) that help them to attach to the epidermis of their hosts. However, a macroscopic analysis of the stomach contents of the preserved specimens of *Paracanthopoma* failed to detect coagulated blood or other fish remains such as flesh, skin, or mucus. Machado and Sazima (1983) hypothesize that the presence of vampire catfish attached to a host may be related to its inability to swim long distances by itself, which could indicate a phoretic relation. In that study, an individual of *Pseudoplatystoma fasciatum* (Linnaeus, 1766) (in fact, *Pseudoplatystoma reticulatum* Eigenmann et Eigenmann, 1889; see Buitrago Suárez and Burr 2007) was trapped on the river bank, and when removed from the water, individuals of a vampire catfish (*Paravandellia oxyptera* Miranda Ribeiro, 1912; Siluriformes: Trichomycteridae) left the body of the host. Vandelliinae and Stegophilinae species may perceive occasions when

a host is injured or at a disadvantageous situation, and take advantage to attach and feed on them (Machado and Sazima 1983).

Previously it has been reported that Vandelliinae species attach to the gills of their hosts for feeding (Machado and Sazima 1983; Spotte 2002; Zuanon and Sazima 2004b), which allows them to passively intake of blood pumped by the host's heart through gills arteries. During an ichthyological survey on the Orinoco River, Lasso et al. (2015) found two individuals of *Vandellia beccarii* Di Caporiacco, 1935 in the branchial chamber of two mature females of *Potamotrygon orbignyi* (Castelnau, 1855), as well as one individual of *Paracanthopoma* sp. on an adult male of *Potamotrygon scobina* Garman, 1913. Judging by the location of the vampire catfish, they would possibly be feeding on the hosts' blood, since species of Vandelliinae can be considered truly hematophagous parasites, feeding on blood extracted from the gill arteries of host fish (de Pinna and Wosiacki 2003; Zuanon and Sazima 2004a).

The absence of blood or other food remains in the stomach of the *Paracanthopoma* specimens apparently weakens the hypothesis of a parasitic relation with *D. phlyzakion*. However, such kind of relation cannot be discarded, since empty stomachs are a common fact among carnivorous fish and may reflect a momentaneous condition related to the time of collection of the candiru catfishes, as well as to a fast digestion rate of the ingested food. Despite this uncertainty, our finding of several *Paracanthopoma* individuals attached to the 9 collected individuals of *D. phlyzakion* suggests the existence of some strong interspecific association. For instance, the

association could provide some form of protection from predators to the candiru catfish (de Pinna and Wosiacki 2003; Zuanon and Sazima 2005). The candirus are tiny and nearly transparent, so they might pass unnoticed by predators while attached to the host catfish (de Pinna 2013). Moreover, the attachment near the sharp hooks of the lateral bony plates of the catfish host could strengthen the protective effect to the small candirus, which remains to be tested.

In addition to feeding and protection, riding on the host can greatly extend the movement capabilities of small candirus. Zuanon and Sazima (2005) found specimens of an undescribed species of *Paracanthopoma* attached to the body surface of *Zungaro zungaro*, a giant pimelodid catfish, and hypothesized that this could facilitate the dispersal of the candiru over the long migrations across the Amazon basin of their host. Enhanced dispersal does not seem to be the case of the association described here, since *D. phlyzakion* is not a migratory catfish. However, the candirus may still benefit from short-distance movements by the host catfish by saving energy, while moving unnoticed to predators.

Conclusion

In conclusion, the observed interspecific association may combine elements of parasitism, protection from predators and a phoretic association between *Paracanthopoma* sp. and *D. phlyzakion*.

Acknowledgments

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ACTA ICHTHYOLOGICA ET PISCATORIA

After four decades—Occurrence of the daggertooth pike conger, *Muraenesox cinereus* (Actinopterygii: Anguilliformes: Muraenesocidae), in the Mediterranean Sea

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Abstract

In October 2020, a single specimen of the daggertooth pike conger, *Muraenesox cinereus* (Forsskål, 1775), was caught by longline at a depth of 120 m, from the Mediterranean coast of Turkey. This species has been recorded from the Mediterranean for the second time after approximately four decades. This new finding shows that the distribution of the species has expanded.

Keywords

Introduction

The Mediterranean Sea is the sea most affected by alien species, in terms of the high rate of introduction and the number of established populations (Bonanno and Bonaca-Orlando 2019). In particular, the Levantine Sea is intensively targeted by the fishes passing through the Suez Canal. Until now, more than one hundred Lessepsian fish species have crossed into the Mediterranean (Seyhan et al. 2017). While some species, such as *Siganus rivulatus* Forsskål et Niebuhr, 1775 and *Lagocephalus sceleratus* (Gmelin, 1789), have established in the Mediterranean ecosystem which is evident from their reproductive success (Sala et al. 2011; Khalaf et al 2014), some species have been recorded only once—*Chanos chanos* (Forsskål, 1775), *Diplogrammus randalli* Fricke, 1983 (see Ozvarol and Gokoğlu 2012; Seyhan et al. 2017).

The daggertooth pike conger, *Muraenesox cinereus* (Forsskål, 1775), which represents the order Anguilliformes and the family Muraenesocidae, is widely distributed in the Indo-west Pacific Ocean and the Red Sea. Globally, the family Muraenesocidae is represented by 6 genera and 15 species. In the Mediterranean there are two species: *Cynoponticus ferox* Costa, 1846 and *Muraenesox cinereus* (see Froese and Pauly 2020). *Muraenesox cinereus* inhabits the bathypelagic-benthic region at depths down to 800 m. Its feeds mainly on small fishes and crustaceans and reaches a maximum length of 220 cm (Masuda et al. 1984; An et al. 2012). The daggertooth pike conger has importance as a fishing resource of the Far East, and it is also usually caught by small trawlers and longline fishing (Watari et al. 2014).

Muraenesox cinereus was recorded for the first time in the Mediterranean Sea in 1982 (Golani and Ben-Tuvia 1982) and had never been observed ever since. This study reports that *M. cinereus* was recorded from the Mediterranean Sea after a long time, and its distribution in the Levantine Sea has expanded.

Materials and methods

On 8 October 2020, a specimen (Fig. 1) of *M. cinereus* was caught by longline at a depth of 120 m, from the southern coast of Turkey (Gazipaşa; 36°22′30″N, 32°08′95″E) (Fig. 2). The specimen was identified according to Golani and Ben-Tuvia (1982) and Bauchot and Saldanha (1986). All morphometric measurements and meristic counts were following Golani and Ben-Tuvia (1982) together with the dental formula. After photographing, the specimen was fixed in 4% formaldehyde and deposited in the Fish Collection Centre of İzmir Kâtip Çelebi University where it was received the catalog number (IKC. PIS.1261).

Results and discussion

The obtained specimen of *M. cinereus* was 124.7 cm long (TL) (Fig. 1A) and weighed 2923 g. Its morphometric measurements and meristic counts are given in Table 1. The fresh coloration of the body was grey, the ventral side lighter, the margin of fins black, body elongated and laterally compressed, dorsal-fin origin in front of pectoral-fin base, the anal fin length is almost 70% of the dorsal fin length, the upper jaw extending downwards covering the tip of the lower jaw, large and elliptic eyes, scales were absent, visible lateral line pores, sharp and well-developed teeth with a row of enlarged teeth on the vomer, maxillary teeth with several rows extend inward on the
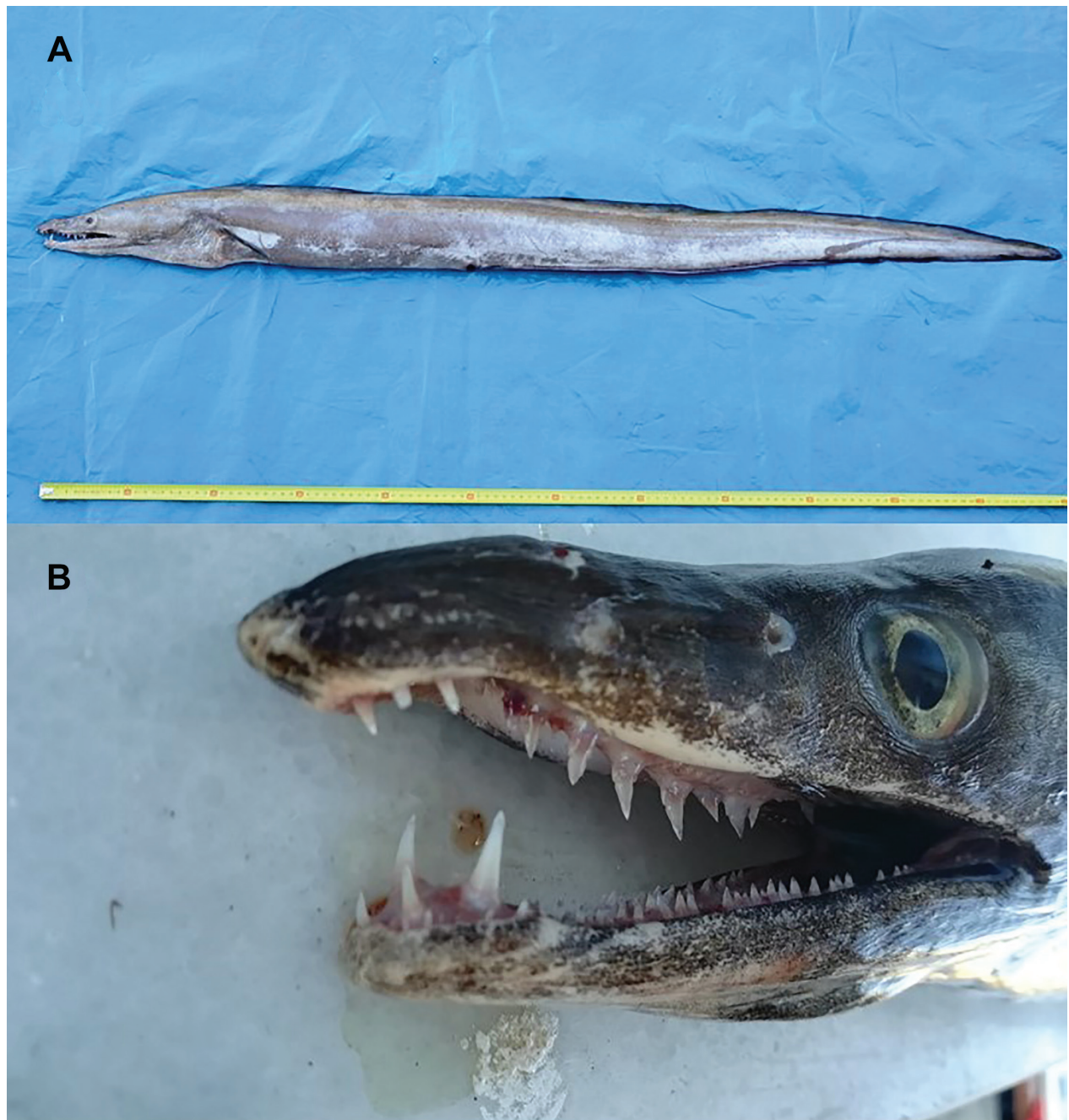

Figure 1. *Muraenesox cinereus* (124.7 cm TL) whole view (**A**); A closeup of the head with teeth (**B**).
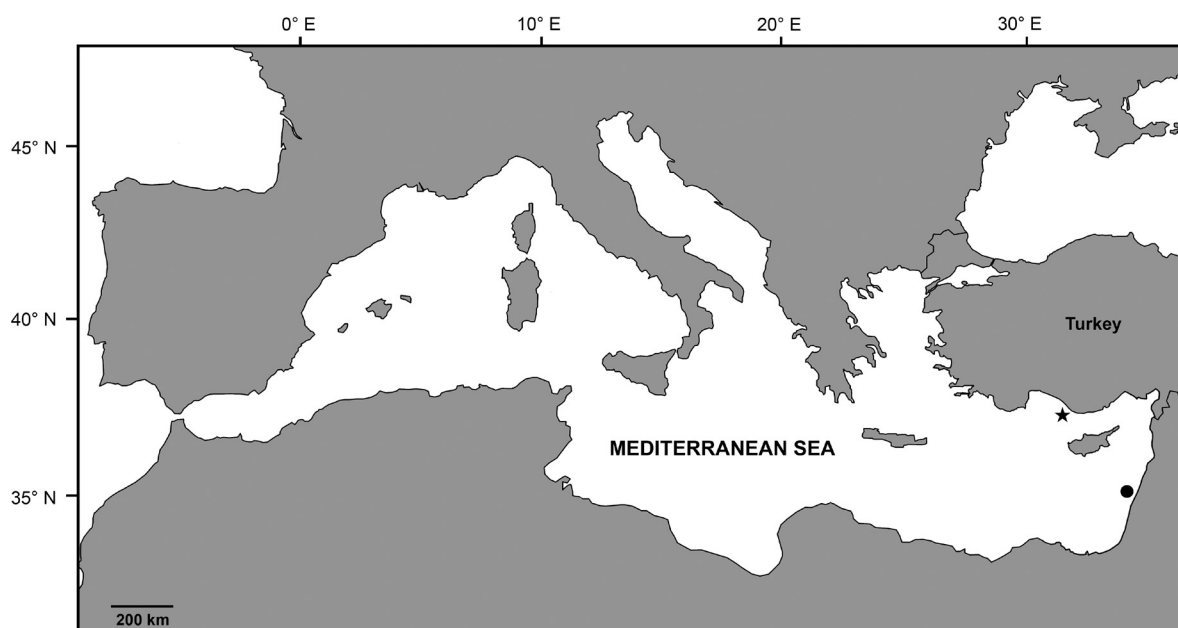
Figure 2. Sampling area of *Muraenesox cinereus* in this study (asterisk) off Gazipaşa/Turkey and the first record of this species in Mediterranean in 1982 in Jaffa/Israel (circle).

Table 1. Morphometric measurements and meristic counts of *Muraenesox cinereus* caught of eastern Mediterranean Sea.

Metrics	[cm]	[%HL]
Total length	124.7	
Preorbital length	6.3	28.3
Eye diameter	2.1	9.4
Prenostril lenght	4.7	21.1
Interorbital length	3	13.5
Gill height	3.5	15.7
Mouth length	9.5	42.6
	[cm]	[%TL]
Head length	22.3	17.9
Predorsal length	19.6	15.7
Prepectoral length	22.9	18.4
Preanal length	52.3	41.9
Pectoral fin length	7.3	5.9
Meristics	Count	
Dorsal fin rays	269	
Pectoral fin rays	14	
Anal fin rays	190	
Lateral line pores	146	
Before anus lateral line pores	40	
Before anus dorsal fin rays	62	
Premaxillary teeth (right)	2	
Premaxillary teeth (left)	4	
Vomerine teeth	12	
Dentary teeth (right)	44 + 3	
Dentary teeth (left)	39 + 2	

eye level. Blache and Tortonese (1968) stated that *C. ferox* and *M. cinereus* could be differentiated by the shape of vomer teeth. The examined specimen had a dagger-like vomerine tooth shape (Fig. 1B).

Until now, this fish had been seen only once in the Mediterranean (Golani and Ben-Tuvia 1982). It had not been observed in almost four-decades. Similarly, *Rhynchoconger trewavasae* Ben-Tuvia, 1993 and *Gymnothorax reticularis* Bloch, 1795 were recorded only once in the Mediterranean in 1993 and 2012, respectively (Ben-Tuvia 1993; Stern and Goren 2013). The reasons for the rarity of these species in the Mediterranean may be listed as having cryptobenthic lifestyle, preferring deep-water habitats, and being released back to the sea by fishermen even if they were caught.

It is clear that alien species negatively change the structure and function of the Mediterranean ecosystem by affecting native species and critical habitats. Among the Mediterranean countries, Turkey is one of the most affected by Lessepsian bioinvasion (Galil et al. 2018; Irmak and Özden 2020).

Acknowledgments

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Filling in knowledge gaps: Length–weight relations of 46 uncommon sharks and rays (Elasmobranchii) in the Mediterranean Sea

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Abstract

Large sharks and rays are generally understudied in the Mediterranean Sea, thus leading to a knowledge gap of basic biological characteristics that are important in fisheries management and ecosystem modeling. Out of the 76 sharks and rays inhabiting the Mediterranean Sea, the length–weight relations (LWR) are available for 28 (37%) of them, usually for common small-sized species that are not protected and may be marketed. The aim of the presently reported study was to fill in the knowledge gap through the estimation of LWR of rare and uncommon sharks and rays in the Mediterranean Sea using the information from single records or few individuals. The analysis was based on a Bayesian hierarchical method for estimating length–weight relations in fishes that has been recently proposed for data-deficient species or museum collections and uses the prior knowledge and existing LWR studies to derive species-specific LWR parameters by body form. The use of this method was applied to single records of rare and uncommon species and here we report the LWR of 46 uncommon sharks and ray species, 14 of which are first reported LWR at a global scale and 21 are the first reported LWR for the Mediterranean Sea; the remaining 11 species are first time records for the western or eastern Mediterranean regions. Museum collections and sporadic catch records of rare emblematic species may provide useful biological information with the use of appropriate Bayesian methods.

Keywords

gap analysis, fisheries management, ecosystems, Mediterranean Sea

Introduction

According to a recent gap analysis on the biology of Mediterranean fishes, sharks and rays (Class Elasmobranchii) are among the least studied species in the Mediterranean Sea and the lack of knowledge is higher for the less abundant large sharks and rays that are protected and rarely collected in a commercial catch or even scientific surveys (Dimarchopoulou et al. 2017). Despite their conservation status, many sharks are still illegally targeted for their fins

and these records are not officially reported (Clarke et al. 2006). Due to the scarcity of samples, the records on the biology of sharks and rays in the Mediterranean are sporadic (Dulvy et al. 2016; Dimarchopoulou et al. 2017), however, because of the importance of large sharks and rays in marine ecosystems and their conservation status (Dulvy et al. 2014), there are several single-catch records available of emblematic species such as the great white shark *Carcharodon carcharias* (Linnaeus, 1758) (see Kabasakal 2020). The majority of Mediterranean records

originate from Tunisia and Turkey owing to the lifetime commitment of a few scientists (e.g., Capapé et al. 2006; Kabasakal 2020 among others) and also, in the case of Tunisia, to the higher species richness of sharks and rays in the western Mediterranean Sea (Dulvy et al. 2016). The majority of biological characteristics of small and common sharks—e.g., lesser spotted dogfish, *Scyliorhinus canicula* (Linnaeus, 1758)—and rays—e.g., thornback ray, *Raja clavata* Linnaeus, 1758—that are marketed and not protected in the Mediterranean Sea are better studied (Froese and Pauly 2020).

Length–weight relations (**LWR**) of fishes (Froese 2006) are important in stock assessments for converting length measurements to weight and estimation of stock biomass (Froese et al. 2011). The LWR is the most studied biological characteristic and studies are available for a large proportion of fish stocks and the majority of finfish in the Mediterranean Sea (Dimarchopoulou et al. 2017). However, rare sharks and rays are underrepresented (Froese and Pauly 2020) and some of the existing LWR records deviate from the cube law dynamics (Froese 2006) because of the small sample size and narrow length and weight distributions (Froese et al. 2011).

A Bayesian hierarchical method for estimating length–weight relations in fishes has been proposed for data-deficient species that uses the prior knowledge and existing LWR studies to derive species-specific LWR parameters by body form (Froese et al. 2014). According to this method, the approximate values of the slope (*b*) and the intercept (*a*) of LWR have been estimated for all fish species and are available in FishBase (Froese and Pauly 2020). Recently, a new approach for estimating LWR from single the length and weight records has been proposed and applied to preserved museum specimens for species for which LWR are not available in the literature (Hay et al. 2020). Based on this approach the LWR of several uncommon fish species, for which museum records are available but their LWR were not, were generated (Hay et al. 2020).

Overall, out of the 43 species of sharks and 33 species of rays that are present in the Mediterranean Sea (Dulvy et al. 2016), LWR are available for 28 species (37%) based on FishBase records (Froese and Pauly 2020) and the literature (updated dataset of Dimarchopoulou et al. 2017). The aim of the presently reported study was to combine these two approaches and generate LWR for 46 rare shark and ray species in the Mediterranean Sea based on single records (or records of a few individuals) that were available in the literature and for which LWR are lacking at a global or regional scale. Thus, the gap between available and desired knowledge on the LWR of Mediterranean marine fishes will be minimized to only a handful of species.

Methods

We collected the single specimen records (or few specimens) of total length (*L*) [cm] and total weight (*W*) [g] data for 46 uncommon shark and ray species in the Mediterranean Sea that belong to 10 orders and 16 families from the literature. Then, we estimated their LWR (Table 1) using the Bayesian estimate available for the species based on LWR of taxonomically related species (Froese and Pauly 2020). The number of specimens ranged from a single (17 cases) and double specimens (10 cases) to 27 specimens of the great white shark that are available in a recently published book (Kabasakal 2020). The mean number of specimens was 3.65. All length data were converted to cm of total length and all weight data to g of total weight.

In species for which a range of sizes was not available to estimate the parameters of LWR, such as with museum specimens, Hay et al. (2020) assumed that the LWR were isometric, i.e., that the parameter $b = 3$, which is a good approximation in the majority of cases (Froese 2006). A similar approach was followed in the presently reported study with *b* approximated based on related species or species with similar body form (Froese and Pauly 2020) following a Bayesian hierarchical method (Froese et al. 2014).

When total length (*L*) and total weight (*W*) measurements from single specimen were available, the parameter *a* was estimated as (Hay et al. 2020):

$$a = \frac{W}{L^b}$$

where *b* is the approximation based on the Bayesian estimate available for the species based on LWR of related species (Froese and Pauly 2020) or body form when a Bayesian estimate was not available (Froese et al. 2014).

When several specimens (*n*) were available, but not enough to support a valid LWR, because of a small sample size or narrow length range, the mean value of parameter *a* was estimated as (Hay et al. 2020):

$$a = \frac{\sum\limits_{i=1}^{n} \times \frac{W}{L^b}}{n}$$

where *b* is the approximation based on the Bayesian estimate available for the species based on LWR of related species (Froese and Pauly 2020) or body form when a Bayesian estimate was not available (Froese et al. 2014).

Results

The LWR of 46 uncommon sharks and ray species are reported for the Mediterranean Sea based on published sources of stranded or incidentally caught animals; none of the specimens was preserved or in a museum collection. For 14 species this is the first reported LWR at a global scale and for 21 species this is the first reported LWR for the Mediterranean Sea (Table 1). The remaining 11 species are first-time LWR records for the western (nine species), eastern Mediterranean (one species) regions, and Aegean Sea (one species).

Table 1. Selected biometric data for 46 species of sharks and rays in the Mediterranean Sea.

Scientific name	N	Total length [cm]	Total weight [g]	b	b source	a	a range	First LWR	Country	Reference
CARCHARHINIFORMES										
Carcharhinidae										
Prionace glauca (Linnaeus, 1758)	1	288.0	174000	3.11	BFB	0.0039	—	Mediterranean	–	Kohler et al. 2002
Carcharhinus altimus (Springer, 1950)	2	65.2–68.0	2200–3100	3.12	BFB	0.0054	0.0048–0.0059	Mediterranean	Turkey	Ayas et al. 2020, Turan et al. 2020
Carcharhinus brachyurus (Günther, 1870)	1	253.0	200000	3.09	BFB	0.0075	—	Mediterranean	Italy	Storai et al. 2007
Carcharhinus brevipinna (Müller et Henle, 1839)	1	115.0	11500	3.07	BFB	0.0054	—	Mediterranean	Turkey	Ayas et al. 2019
Carcharhinus falciformis (Müller et Henle, 1839)	1	209.0	48000	3.09	BFB	0.0033	—	Mediterranean	Italy	Garibaldi and Orsi-Relini 2012
Carcharhinus plumbeus (Nardo, 1827)	6	89.0–300.0	3500–70000	3.17	BFB	0.0019	0.0010–0.0023	Mediterranean	Tunisia	Capape et al. 2018, Soufi et al. 2018
Galeocerdo cuvier (Péron et Lesueur, 1822)	2	95.8–97.4	2750–2840	3.15	BFB	0.0016	0.0015–0.0016	Mediterranean	Libya	Tobuni et al. 2016
ECHINORHINIFORMES										
Echinorhinidae										
Echinorhinus brucus (Bonnaterre, 1788)	6	170.0–254.0	45000–300000	3.12	BFB	0.0074	0.0031–0.0147	Global	Algeria, Turkey	Hemida and Capape 2002, Kabasakal and Bilecenoglu 2014
HEXANCHIFORMES										
Hexanchidae										
Heptranchias perlo (Bonnaterre, 1788)	5	70.0–110.0	1000–5000	3.11	BFB	0.0022	0.0016–0.0035	western Med	Tunisia, Spain	El Kamel-Moutalibi et al. 2014, Guallart et al. 2019
Hexanchus griseus (Bonnaterre, 1788)	17	250.0–600.0	200000–1000000	3.04	BF	0.0062	0.0034–0.0123	Global	Turkey	Kabasakal 2006
Hexanchus nakamurai Teng, 1962	1	230.0	85000	3.11	BFB	0.0038	—	Mediterranean	Albania	Bakiu et al. 2018
LAMNIFORMES										
Alopiidae										
Alopias vulpinus (Bonnaterre, 1788)	1	395.0	180000	2.86	BFB	0.0067	—	Mediterranean	Turkey	Erguden et al. 2015
Alopias superciliosus Lowe, 1841	7	151.0–450.0	10000–180000	2.91	BFB	0.0051	0.0027–0.0068	Mediterranean	Many countries	Kabasakal and Karhan 2007, Clo et al. 2008, Damalas and Megalofonou 2012, Kabasakal et al. 2011, Lanteri et al. 2017
Cetorhinidae										
Cetorhinus maximus (Gunnerus, 1765)	1	236.0	70000	3.04	BF	0.0043	—	Mediterranean	Turkey	Bilecenoglu et al. 2013
Lamnidae										
Isurus oxyrinchus Rafinesque, 1810	1	69.8	2285	3.03	BFB	0.0059	—	Mediterranean	Turkey	Bilecenoglu et al. 2013
Lamna nasus (Bonnaterre, 1788)	5	91.0–236.0	35000–120000	3.03	BFB	0.0240	0.0077–0.0753	Mediterranean	Italy	Storai et al. 2005
Carcharodon carcharias (Linnaeus, 1758)	27	132.0–642.0	27650–2500000	3.05	BFB	0.0074	0.0043–0.0108	Mediterranean	Tunisia	Saidi et al. 2005, Morey et al. 2003
Odontaspididae										
Carcharias taurus Rafinesque, 1810	1	99.7	3318	3.03	BFB	0.0029	—	Mediterranean	Turkey	Ismen et al. 2009
Odontaspis ferox (Risso, 1810)	2	190.0–250.0	34000–180000	3.04	BF	0.0066	0.0040–0.0092	Mediterranean	Turkey	Kabasakal and Bayri 2019
MYLIOBATIFORMES										
Dasyatidae										
Bathytoshia centroura (Mitchill, 1815)	1	60.1	5200	3.07	BFB	0.0180	—	western Med	Croatia	Dulcic et al. 2003
Dasyatis chrysonota (Smith, 1828)	1	36.9–44.5	149–445	3.07	BFB	0.0030	0.0023–0.0039	Global	Israel	Golani and Capape 2004
Dasyatis marmorata (Steindachner, 1892)	1	33.0	171.8	3.07	BFB	0.0037	—	Global	Greece	Chatzispyrou et al. 2020
Himantura leoparda Manjaji-Matsumoto et Last, 2008	2	26.0–135.2	722–55000	3.17	BFB	0.0166	0.0097–0.0236	Global	Turkey	Yucel et al. 2017
Himantura uarnak (Gmelin, 1789)	3	140.0–148.6	40000–150000	3.17	BFB	0.0117	0.0047–0.0195	Mediterranean	Turkey	Basusta et al. 1998, Ali et al. 2010
Taeniura grabata (Geoffroy Saint-Hilaire, 1817)	4	60.0–117.8	1681–16600	3.17	BFB	0.0040	0.0033–0.0045	Global	Turkey	Basusta et al. 1998, Ali et al. 2013
Mobulidae										
Mobula japanica (Müller et Henle, 1841)	2	97.5–120.0	67000–100000	3.04	BFB	0.0540	0.0478–0.0602	Global	Tunisia	Capape et al. 2015
Mobula mobular (Bonnaterre, 1788)	3	200.0–291.5	100000–105000	3.04	BFB	0.0052	0.0022–0.0101	Global	Italy, Turkey	Scacco et al. 2009, Basusta and Ozbek 2017
Myliobatidae										
Aetomylaeus bovinus (Geoffroy Saint-Hilaire, 1817)	2	152.7–160.0	14800–16200	3.04	BF	0.0033	0.0032–0.0034	Western Med	Tunisia	El Kamel et al. 2010
Myliobatis aquila (Linnaeus, 1758)	4	50.0–114.0	2000–29400	3.09	BFB	0.0111	0.0096–0.0130	Western Med	France	Capape et al. 2006
RAJIFORMES										
Rajidae										
Leucoraja melitensis (Clark, 1926)	1	27.0	80.2	3.13	BFB	0.0027	—	Global	Tunisia	Ben Amor et al. 2018
Dipturus nidarosiensis (Storm, 1881)	4	24.0–148.2	20.12–13783	3.24	BFB	0.0010	0.0009–0.0013	Global	Italy	Follesa et al. 2012
Dipturus oxyrinchus (Linnaeus, 1758)	1	48.0	443	3.25	BFB	0.0015	—	Western Med	France	Capape et al. 2006
Leucoraja circularis (Couch, 1838)	6	61.0–101.0	1250–5650	3.08	BFB	0.0052	0.0038–0.0087	Western Med	Tunisia	Mnasri et al. 2009
Leucoraja fullonica (Linnaeus, 1758)	11	19.0–76.0	191–2300	3.13	BFB	0.0065	0.0019–0.0240	Global	Italy	Zupa et al. 2010
Leucoraja naevus (Müller et Henle, 1841)	2	22.0–52.0	59–864	3.10	BFB	0.0041	0.0041–0.0041	Mediterranean	Spain	Valls et al. 2011
Raja brachyura Lafont, 1871	1	91.5	5450	3.27	BFB	0.0021	—	Eastern Med	France	Capape et al. 2006
Raja undulata Lacepède, 1802	2	48.4–58.0	765–1356	3.20	BFB	0.0031	0.0031–0.0031	Mediterranean	France	Capape et al. 2006
RHINOPRISTIFORMES										
Glaucostegidae										
Glaucostegus halavi (Forsskål, 1775)	1	102.3	3005	2.99	BFB	0.0029	—	Global	Tunisia	Ben Souissi et al. 2007

Table 1 continues on next page.

Table 1. cont.

Scientific name	N	Total length [cm]	Total weight [g]	b	b source	a	a range	First LWR	Country	Reference
Rhinopteridae										
Rhinoptera marginata (Geoffroy Saint-Hilaire, 1817)	5	38.5–87.4	104–9980	3.10	BFB	0.0044	0.0010–0.0096	Western Med	Turkey	Basusta et al. 2012
SQUALIFORMES										
Oxynotidae										
Oxynotus centrina (Linnaeus, 1758)	4	53.3–79.0	1649–5020	3.04	BF	0.0087	0.0080–0.0093	Aegean Sea	Greece	Kousteni and Megalofonou 2016
SQUATINIFORMES										
Squatinidae										
Squatina aculeata Cuvier, 1829	1	79.9	3690	3.04	BFB	0.0061	—	Global	Turkey	Basusta 2002
Squatina oculata Bonaparte, 1840	6	29.1–79.5	173–3750	3.04	BFB	0.0067	0.0061–0.0076	Global	Greece, Italy	Corsini and Zava 2007, Zava et al. 2016, Erguden et al. 2019
Squatina squatina (Linnaeus, 1758)	2	38.0–156.0	1900–32600	3.02	BFB	0.0200	0.0078–0.0322	Mediterranean	Turkey	Akyol et al. 2015, Cavallaro et al. 2015
TORPEDINIFORMES										
Torpedinidae										
Tetronarce nobiliana (Bonaparte, 1835)	4	17.0–102.0	150–20000	2.96	BFB	0.0256	0.0133–0.0342	Western Med	France	Capape et al. 2006
Torpedo marmorata Risso, 1810	4	16.0–50.0	40–3500	2.94	BFB	0.0241	0.0115–0.0354	Western Med	France	Capape et al. 2006
Torpedo torpedo (Linnaeus, 1758)	2	25.0–39.0	300–1012	2.90	BFB	0.0255	0.0246–0.0265	Mediterranean	France	Capape et al. 2006

N = sample size, *b* and *a* are LWR parameters of information; BFB = Bayesian FB, BF = Body form.

A Bayesian estimate of parameter *b* based on LWR of related species was available for 41 species (Froese and Pauly 2020), while for the remaining 5 ones—*Hexanchus griseus* (Bonnaterre, 1788), *Cetorhinus maximus* (Gunnerus, 1765), *Odontaspis ferox* (Risso, 1810), *Aetomylaeus bovinus* (Geoffroy Saint-Hilaire, 1817), *Oxynotus centrina* (Linnaeus, 1758)—an estimate based on their body form was used (Froese et al. 2014). In cases where more than one specimen was available, the range of length, weight, and parameter *a* is provided (Table 1).

Discussion

Although isometric growth (*b* = 3) is the most common type of growth in the majority of families (Froese 2006), strong deviations from isometry have been observed due to a narrow range of sizes and/or low sample size (Froese et al. 2011; Hay et al. 2020). In addition, as the seasonality in *b* values is strong along with the corresponding estimates of *a* (Froese 2006), a limited sampling period, even with a large sample size of the entire somatic range of the species may lead to varying LWR (Hay et al. 2020). This effect of seasonality is partly related to the spawning period of fishes and it is especially strong for the female viviparous sharks that deviate a lot in somatic weight during their gestation period (Castro 2000).

Length data for a few specimens of some other very rare shark species exist in the literature—pigeye shark, *Carcharhinus amboinensis* (Müller et Henle, 1839) (see Da Maddalena and Della Rovere 2005); common sawfish, *Pristis pristis* (Linnaeus, 1758) (see Capape et al. 2006);

dusky shark, *Carcharhinus obscurus* (Lesueur, 1818) (see Bilecenoglu et al. 2013)—but weight data are lacking as those specimens were spotted at fish markets, where only part of the body was "available" usually the head, or are based on photos or anecdotal records from newspapers and magazines. Anecdotal evidence and historical records may be really valuable in reconstructing the history of these iconic predators (Ferretti et al. 2016) and gaining insight into the previous status of marine ecosystems and ecosystem effects of fishing (Pauly 1995; Pauly et al. 1998).

This work extends the approach of gaining valuable information from museum collections (Hay et al. 2020) to rare and sporadic catch records of emblematic species such as large sharks and rays for which biological information is lacking, at least in the Mediterranean Sea. The importance of the Bayesian approach to data-deficient areas and species is highlighted along with the need for recording the basic biological information (length, weight, and sex) even from single specimens of rare and uncommon sharks and rays. As the majority of these species are rare and protected (although illegally landed in many areas of the Mediterranean Sea), sometimes such data are only available in fish markets and newspapers/magazines; nowadays also through social media (Kabasakal and Bilecenoglu 2020).

Acknowledgments

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Length–weight analysis of ten species (Actinopterygii) supporting subsistence fishery in Lakshadweep waters, southern Arabian Sea

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Abstract

The length–weight relations of ten fish species representing eight genera and four families and that formed the backbone of the subsistence fishery in the Lakshadweep islands were estimated. These fishes which included four species of tuna [*Katsuwonus pelamis* (Linnaeus, 1758); *Thunnus albacares* (Bonnaterre, 1788); *Auxis thazard* (Lacepède, 1800); *Euthynnus affinis* (Cantor, 1849)], three species of needlefishes[*Ablennes hians* (Valenciennes, 1846); *Tylosurus crocodilus* (Péron et Leseur, 1821); *Tylosurus acus melanotus* (Bleeker, 1850)], two species of bait fishes [*Spratelloides delicatulus* (Bennett, 1832); *Spratelloides gracilis* (Temminck et Schlegel, 1846)], and one species of halfbeak (*Hemiramphus archipelagicus* Collette et Parin, 1978) contributed to 96% of the total fish landings. The samples were collected from fish landing centers of ten inhabited islands of Lakshadweep from 2015 to 2017. *Katsuwonus pelamis* showed isometric growth, *S. delicatulus* and *S. gracilis* exhibited positive allometry, while negative allometric growth was seen in other species. The coefficient *a* of the LWR ranged from 0.001 (*A. hians*) to 0.035 (*T. albacares*), while *b* ranged from 2.7 (*T. acus melanotus*) to 3.4 (*S. delicatulus*). The results of the presently reported study provide useful biological information on the stock of ten commercially important pelagic fish species supporting the traditional fisheries in Lakshadweep waters.

Keywords

length–weight relation, pelagic stock, traditional fishery, Lakshadweep, India

Introduction

The relation between body weight and length is important for fishery biology, especially in understanding the state of fish stock and assessing the population structure based on the age- and length-structured models (Pope 1972; Sparre et al. 1989). Length–weight relations (**LWRs**) are also important tools for the morphological comparisons of different species within the same taxon and popula-

tions from different geographical area (Panda et al. 2016; Karna et al. 2020). This is significantly important in developing any policy frameworks for fisheries management and conservation pertaining to a particular species or locality (Gonçalves et al. 1997; Froese et al. 2011). LWRs are usually calculated through linear regression on the log-transformed length and weight data, however, in recent years, the use of nonlinear procedures for the estimation of LWR, as well as other population parameters,

has been increasing among researchers (De Giosa and Czerniejewski 2016).

The Lakshadweep archipelago, which includes a group of 36 islands lies in the southern Arabian Sea and is the only coral reef complex of India. The fishery in the Lakshadweep islands has traditionally been sustainable and for subsistence. In all the ten of its inhabited islands, fishing is the major source of livelihood. Tuna and needlefishes contribute to about 95% of the total commercial fishery in the Lakshadweep islands and have been historically harvested using pole and line, handline, troll line, and drift gillnet. The bait fishes available in the lagoon are used for chumming of the tuna. Although there have been studies in the past to assess the stock of these species individually (Appukuttan et al. 1977; Mohan and Kunhikoya 1985; Koya et al. 2013; Shahul Hameed et al. 2018), comprehensive documentation of LWR of all the major pelagic commercially exploited fishes from all the ten inhabited has been found lacking. The presently reported study is a compilation of LWRs of eight highly landed pelagic fish species and two live-baits that support traditional pelagic fisheries in Lakshadweep waters.

Methods

The Lakshadweep Archipelago includes a group of ten inhabited and 17 uninhabited islands, under the jurisdiction of the Government of India, scattered between 08°16′–13°58′N and 071°44′–074°24′E in the southern Arabian Sea. The samples were collected on a monthly basis between June 2015 to May 2017 from fish landing center of ten inhabited Lakshadweep islands: Androth (10°49.11′N, 073°41.05′E), Kavaratti (10°33.25′N, 072°38.52′E), Minicoy (08°17.41′N, 073°04.53′E), Agatti (10°15.17′N, 072°11.32′E), Kiltan (11°29.17′N, 073°04.12′E), Chetlat (11°41.21′N, 072°43.05′E), Bithra (11°66.11′N, 072°10.42′E), Amini (11°07.29′N, 072°44.45′E), Kadmath (11°13.19′N, 072°47.05′E), and Kalpeni (10°05.51′N, 073°39.02′E) (Fig. 1). Round the year sampling was done for ten species that represented eight genera and four families. The species included the flat needlefish, *Ablennes hians* (Valenciennes, 1846); the frigate tuna, *Auxis thazard* (Lacepède, 1800); the kawakawa, *Euthynnus affinis* (Cantor, 1849); the jumping halfbeak, *Hemiramphus archipelagicus* Collette et Parin, 1978; the skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758); the delicate round herring, *Spratelloides delicatulus* (Bennett, 1832); the silver-stripe round herring, *Spratelloides gracilis* (Temminck et Schlegel, 1846); the yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788); the keel-jawed needlefish, *Tylosurus acus melanotus* (Bleeker, 1850); and the hound needlefish, *Tylosurus crocodilus* (Péron et Leseur, 1821).

Tuna were collected using a diverse type of gears viz., pole and line, hook and line, handline, and drift
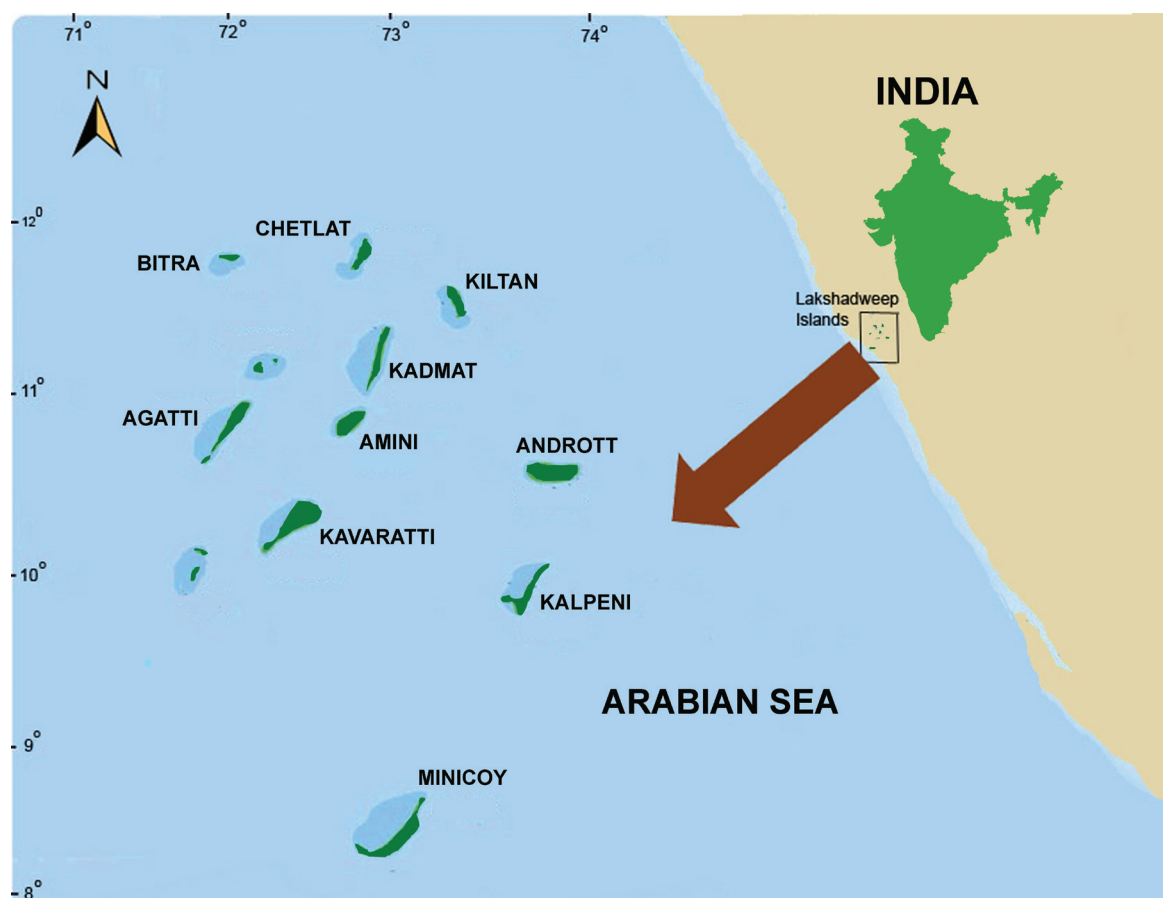

Figure 1. Map of Lakshadweep showing the ten inhabited islands from where fish landing data was collected.

gillnets (55–80 mm mesh size), while the needlefishes and halfbeaks were harvested using gillnet (22–55 mm mesh size) and bait fishes using encircling nets (4–6 mm mesh size). Specimens without physical damage were carefully transferred to the laboratory in iced condition and identified following Day (1878), Fischer and Bianchi (1984), and Collette (1984, 2003). The total length (TL) of all fishes was measured to the nearest 0.1 cm using a measuring board and scale (0.1 cm accuracy), and the individual weight (*W*) was recorded using an electronic balance (0.1 g accuracy). The length–weight relation described by the equation:

$$W = a\text{TL}^b$$

where *W* is the total weight [g], TL is the total length [cm], *a* is the intercept related to body form, and *b* is the regression coefficient (Froese 2006) was estimated, together with the parameters *a*, *b*, and *r²* (coefficient of determination) using least-square regression analysis of the logarithm-transformed LWR expression (Garcia 2010):

$$\log W = \log a + b \log \text{TL}$$

Normalization of the data was carried out using log-log plot of the length–weight pairs, and the 95% confidence limits (Cl) of *a*, *b*, and *r²* were estimated (Froese 2006; Roul et al. 2017). The null hypothesis that *b* = 3 (i.e., individuals show isometric growth pattern; Froese 2006) was tested using two-tailed t-tests. The statistical analysis was performed in PAST 3.20. All the statistical analyses were considered at a significance level of 5% ($P < 0.05$).

Results

During the presently reported study, 2474 specimens were measured. The length–weight relation parameters including the number of specimens (*N*), length range, weight range, length–weight parameters (both *a* and *b* values), and coefficient of determination (*r²* value) derived from regression analysis for each species are presented in Table 1. The highest number of specimens was measured for *Katsuwonus pelamis* (765), while the lowest was for

Hemiramphus archipelagicus (70). The linear regression was highly significant ($P < 0.05$) for all species with *r²* values ranging from 0.854 (*Tylosurus acus melanotus*) to 0.979 (*Spratelloides delicatulus*). The estimated allometric coefficient *a* of the LWR ranged from 0.001 (*Ablennes hians*) to 0.035 (*Thunnus albacares*), while *b* ranged from 2.745 (*T. acus melanotus*) to 3.404 (*S. delicatulus*).

Discussion

The LWR of fishes is important in fisheries biology because it allows the estimation of the mean weight of fish in a given length group (Beyer 1987) and is particularly important in parameterizing yield equations and estimations of stock size (Abdurahiman et al. 2004). The exact relation between length and weight differs among species of fish according to their inherited body shape, and within a species according to the condition (robustness) of individual fish (Froese et al. 2011). In the presently reported study, the calculated allometric coefficient *b* values were well within the expected range of 2.5–3.5 (Froese 2006). Similarly, the confidence intervals (95%) in this study were also found within the range and overlapped with the Bayesian confidence limits (Froese et al. 2014). Of the ten species, only one species (*Katsuwonus pelamis*) showed isometric growth, while *Spratelloides delicatulus* and *S. gracilis* exhibited significantly higher *b* value, while for the rest of the species the growth recorded was negatively allometric. The LWR *b* values calculated for all the tuna species in the presently reported study were slightly lower than those from the earlier reports (Stequert et al. 1996; Khan 2004; Ghosh et al. 2010, 2012; Koya et al. 2012; Rohit et al. 2012; Mariasingarayan et al. 2018). A similar trend was observed for all the needlefishes; wherein, the *b* values were significantly lower compared to earlier reports of Roul et al. (2017) and Shahul Hameed et al. (2018). For the remaining fish species, the *b* value was significantly higher than those already reported (Milton et al. 1991; Nasser 1999; Tabassum et al. 2015). The differences in *b* values for some of the species caught from the Lakshadweep islands could be attributed to the differences in the fishing gear employed, variation in sex ratio, size of the fish, stages of growth, temporal and spatial distribution, gastro-somatic index, stages of

Table 1. Descriptive statistics and estimated parameters of length–weight relation for the major pelagic species from Lakshadweep waters, southern Arabian Sea.

Family	Species	*N*	TL [cm]	W [g]	*a*	95% CI of *a*	*b*	95% CI of *b*	*r²*
Scombridae	*Thunnus albacares*	309	28.0–136.6	350–40 600	0.03533	0.02672–0.04472	2.846	2.778–2.913	0.957
	Katsuwonus pelamis	765	23.9–70.6	200–6400	0.01779	0.01501–0.02108	3.018	2.973–3.063	0.957
	Euthynnus affinis	271	23.0–64.0	300–3300	0.03283	0.02482–0.04343	2.848	2.773–2.922	0.954
	Auxis thazard	224	26.0–61.1	380–3200	0.02936	0.01992–0.04328	2.871	2.767–2.975	0.929
Belonidae	*Tylosurus acus melanotus*	288	45.0–82.3	180–1176	0.00492	0.00284–0.00854	2.745	2.614–2.877	0.854
	Tylosurus crocodilus	103	42.0–111.0	148–2389	0.00208	0.00093–0.00465	2.963	2.778–3.149	0.908
	Ablennes hians	77	40.0–112.0	85–1310	0.00132	0.00067–0.00256	2.972	2.818–3.126	0.951
Hemiramphidae	*Hemiramphus archipelagicus*	70	16.0–34.0	22–176	0.00636	0.00455–0.00888	2.903	2.798–3.007	0.978
Clupeidae	*Spratelloides delicatulus*	106	1.6–5.3	0.013–1.102	0.00377	0.00338–0.00421	3.404	3.307–3.501	0.979
	Spratelloides gracilis	261	3.0–7.8	0.137–2.953	0.00455	0.00399–0.00517	3.130	3.055–3.205	0.963

gonad maturity, ontogeny, climatic variability, general condition or number of species examined (Froese 2006; Percin and Akyol 2009).

Conclusion

The presently reported study is a comprehensive analysis encompassing landings from all the inhabited islands of Lakshadweep. The results of the presently reported study are also the first estimates for *Hemiramphus archipelagicus* from the region. Similarly, the LWR estimates of bait fishes are being reported after two decades. The majority of the fishes studied are pelagic open ocean species with either limited geographic distribution (bait fishes and needlefishes) or are migratory (tunas) contributing substantially to the mainstay of commercial fisheries in the region. The latter is more affected by the changing oceanographic and climatic scenarios resulting in annual fluctuation of their stock. Results emerging from the presently reported study form the baseline information on the status of both straddling and migratory fish stocks of the Lakshadweep archipelago that in the future could be used as a yardstick to assess fishery stocks and also to develop sustainable fisheries management policies for the region.

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Length–weight relations of 14 fish species (Actinopterygii) from the Chalakudy River, Western Ghats, India

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Abstract

Length–weight relations of 14 fish species caught by small-scale fishery from the Chalakudy River of the Western Ghats biodiversity hotspot, India were analyzed from April 2018 to March 2019. The following species were studied: *Amblypharyngodon microlepis* (Bleeker, 1853); *Dawkinsia filamentosa* (Valenciennes, 1844); *Puntius mahecola* (Valenciennes, 1844); *Osteobrama bakeri* (Day, 1873); *Labeo dussumieri* (Valenciennes, 1842); *Channa marulius* (Hamilton, 1822); *Channa striata* (Bloch, 1793); *Horabagrus brachysoma* (Günther, 1864); *Mystus armatus* (Day, 1865); *Pangasianodon hypophthalmus* (Sauvage, 1878); *Heteropneustes fossilis* (Bloch, 1794); *Etroplus suratensis* (Bloch, 1790); *Megalops cyprinoides* (Broussonet, 1782); *Parambassis thomassi* (Day, 1870). The main fishing gear that was used in the data collection were gill nets, seine nets, and cast nets. The *b* values in the LWRs ranged from 2.649 (*L. dussumieri*) to 3.023 (*P. hypophthalmus*). This study reports the first LWR reference for five species and new maximum total lengths for five species. The results provide baseline information for the sustainable management and conservation of the studied species.

Keywords

LWRs, regression coefficients, maximum length, conservation

Introduction

Information on length–weight relations (**LWRs**) of fishes in a given geographical region is helpful in fisheries management and monitoring of environmental programs (Froese 2006; Hossain et al. 2019). LWRs plays a significant role in fishery research having a wide range of uses in fishery biology, ecology, fish stock and population assessments (Le Cren 1951; Froese 2006). It provides information on growth, life history, survival, as well as the overall condition of the fish (Le Cren 1951; Christensen and Walters 2004). It is widely used as a tool to calculate the biomass from the length data, converting individual lengths or mean lengths of a group of fishes to weights (Froese 2006; Baitha et al. 2018). Length–weight relations are also used to compare the well-being of individuals within the specific stocks or separate stocks of the same species (King 2007). The LWRs differ among fish species depending upon body shape and biological factors such as maturity and spawning (Schneider et al. 2000). The information regarding the LWR of fishes from the Western Ghats biodiversity hotspot of India is scarce. Therefore, the presently reported study aimed to provide the LWRs of 14 species representing 8 families, collected from small-scale fishery in the Chalakudy River flowing through the Western Ghats biodiversity hotspot in India. To our best knowledge, no LWRs are reported in FishBase (Froese and Pauly 2020) for five of the selected species.

Materials and methods

The Chalakudy River (10°10′–10°33′N, 76°17′–77°4′E) is the fifth-longest among the 44 perennial rivers of state of Kerala, India that originates from the Anamalai Hills of the Western Ghats biodiversity hotspot and empties in the Arabian Sea (Myers et al. 2000). The river harbors a rich and diverse fish fauna of 98 species, and many of them are endemic (36%) and threatened (33%) (Biju et al. 2000; Raghavan et al. 2008). Fish specimens were caught by gill nets (mesh, 1.5–12.0 cm), seine nets (mesh, 1.5–3.0 cm), and cast nets (mesh, 1.0–1.5 cm) operated in the river during the period of April 2018–March 2019. All specimens were identified following Talwar and Jhingran (1991) and Jayaram (2009) and scientific names were confirmed with FishBase (Froese and Pauly 2020). The total length (TL) was measured to the nearest 0.1 cm using a vernier caliper, and body weight (BW) was taken in the field to an accuracy of 0.01 g using an electronic balance. LWR was determined using the equation:

$$W = aL^b$$

and logarithmically transformed in to

$$\log W = \log a + b \log L$$

where W is the whole body weight [g], L is the total length [cm], and parameters a and b are the regression parameters (Le Cren 1951; Froese 2006). The 95% of confidence limits for the parameters of a and b and co-efficient of determination (r^2) were estimated. Extreme outliers were removed from the regression analysis. (Froese 2006).

Results and discussion

In total, 927 specimens from 14 fish species representing 13 genera and eight families were examined (Table 1). The following species were studied: *Amblypharyngodon microlepis* (Bleeker, 1853); *Dawkinsia filamentosa* (Valenciennes, 1844); *Puntius mahecola* (Valenciennes, 1844); *Osteobrama bakeri* (Day, 1873); *Labeo dussumieri* (Valenciennes, 1842); *Channa marulius* (Hamilton, 1822); *Channa striata* (Bloch, 1793); *Horabagrus brachysoma* (Günther, 1864); *Mystus armatus* (Day, 1865); *Pangasianodon hypophthalmus* (Sauvage, 1878); *Heteropneustes fossilis* (Bloch, 1794); *Etroplus suratensis* (Bloch, 1790); *Megalops cyprinoides* (Broussonet, 1782); *Parambassis thomassi* (Day, 1870). The sample size ranged from 25 (*M. cyprinoides*) to 134 (*P. mahecola*) individuals. Estimated parameters of length–weight relation including sample sizes (N), regression parameters a and b and their 95% confidence limits (CL), and the coefficient of determination (r^2) are given in Table 1. For all analyzed species LWRs were highly significant ($P < 0.001$), while r^2 values ranged from 0.950 (*P. mahecola* and *O. bakeri*) to 0.994 (*M. cyprinoides*) therefore suggesting a strong relation between total length and body weight. The values of coefficient a ranged from 0.006 (*O. bakeri*, *P. hypophthalmus*, and *M. cyprinoides*) to 0.018 (*E. suratensis*) and the values of exponent b ranged from 2.649 (*L. dussumieri*) to 3.023 (*P. hypophthalmus*). After comparing the results against the Bayesian approach, a parameters were within the probable range for all the species except *C. striata* ($a = 0.014$) possibly due to narrow length ranges (Froese and Pauly 2020). For all species, the estimated b values were within the normal range of 2.5 to 3.5, as suggested by Froese (2006). Variations in the b values are usually attributed to several factors such as sample size, habitat, season, sex, diet, and gonadal maturity (Bagenal and Tesch 1978; Froese 2006; Hanif et al. 2020). The size range covered for some species was narrow. Hence, the LWR for these species should be considered preliminary and need to be verified in future studies using larger sample sizes. Considering the somatic type of growth, two species showed isometric growth ($b = 3$) and twelve species exhibited negative allometric growth ($b < 3$). During the presently reported study LWR estimates of five species, *A. microlepis*,

Table 1. Descriptive statistics and estimated length–weight relation parameters of 14 fish species from the Chalakudy River, India.

Family	Species	N	TL range [cm]	TW range [g]	a	95% Cl of a	b	95% Cl of b	r²
Cyprinidae	*Amblypharyngodon microlepis**	65	8.6–**12.6**	6.70–20.70	0.007	0.006–0.009	2.960	2.848–3.071	0.977
	Dawkinsia filamentosa	108	8.5–14.5	8.40–35.30	0.008	0.006–0.010	2.944	2.838–3.050	0.966
	*Puntius mahecola**	134	7.8–**12.6**	6.00–24.00	0.008	0.006–0.010	2.952	2.846–3.058	0.958
	*Osteobrama bakeri**	61	10.3–**15.3**	9.50–27.30	0.006	0.004–0.008	2.989	2.825–3.153	0.958
	*Labeo dussumieri**	78	12.8–35.0	22.00–422.00	0.017	0.014–0.020	2.649	2.579–2.719	0.986
Channidae	*Channa marulius*	88	23.9–61.2	90.00–1800.00	0.014	0.012–0.018	2.658	2.577–2.739	0.980
	Channa striata	46	16.0–42.1	35.00–520.00	0.014	0.011–0.017	2.684	2.593–2.774	0.988
Bagridae	*Horabagrus brachysoma*	56	18.1–32.5	65.00–345.00	0.008	0.006–0.010	2.962	2.863–3.061	0.985
	*Mystus armatus**	26	10.1–12.6	8.90–17.00	0.012	0.007–0.019	2.697	2.469–2.925	0.963
Pangasiidae	*Pangasianodon hypophthalmus*	28	20.7–68.0	70.00–2300.00	0.006	0.004–0.008	3.023	2.898–3.148	0.990
Heteropneustidae	*Heteropneustes fossilis*	54	16.4–**41.2**	28.00–400.00	0.007	0.005–0.010	2.946	2.792–3.101	0.965
Cichlidae	*Etroplus suratensis*	83	10.4–31.6	18.00–41.40	0.018	0.014–0.022	2.701	2.603–2.800	0.973
Megalopidae	*Megalops cyprinoides*	25	19.4–37.0	60.00–394.00	0.006	0.005–0.008	2.978	2.884–3.072	0.994
Ambassidae	*Parambassis thomassi*	75	8.0–**13.6**	5.50–25.00	0.011	0.009–0.013	2.761	2.680–2.843	0.984

N, number of individuals; TL, total length, TW, total weight; a, intercept; b, slope; CL, confidence limits; r^2, coefficient of determination; first LWR report in FishBase (*); TL in bold, new maximum length observed in the FishBase.

P. mahecola, *O. bakeri*, *L. dussumieri*, and *M. armatus* are described the first time (Froese and Pauly 2020). Further, it has been found that *A. microlepis* (12.6 cm), *P. mahecola* (12.6 cm), *O. bakeri* (15.3 cm) *P. thomassi* (13.6 cm) and *H. fossilis* (41.2 cm) have a highest total length not previously reported in FishBase (Froese and Pauly 2020). To conclude, these results provide useful information for the conservation and sustainable management of these fishes.

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PENSOFT.

ACTA ICHTHYOLOGICA ET PISCATORIA

Length–weight relations for 14 fish species (Actinoptergii) from the coastal waters off Gwangyang Bay, South Korea

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Abstract

Length–weight relations were estimated for 14 fish species sampled from the coastal waters off the Gwangyang Bay in South Korea. The following species were studied: *Okamejei kenojei* (Müller et Henle, 1841); *Muraenesox cinereus* (Forsskål, 1775); *Thryssa adelae* (Rutter, 1897); *Thryssa kammalensis* (Bleeker, 1849); *Tribolodon hakonensis* (Günther, 1877); *Inimicus japonicus* (Cuvier, 1829); *Chelidonichthys spinosus* (McClelland, 1844); *Jaydia lineata* (Temminck et Schlegel, 1842); *Sillago japonica* Temminck et Schlegel, 1843; *Pholis nebulosa* (Temminck et Schlegel, 1845); *Favonigobius gymnauchen* (Bleeker, 1860); *Pampus echinogaster* (Basilewsky, 1855); *Cynoglossus joyneri* Günther, 1878; *Takifugu niphobles* (Jordan et Snyder, 1901).The length–weight relation of *Thryssa adelae* (Rutter, 1897), (Engraulidae) has not been previously reported. The new maximum total length of *Thryssa kammalensis* (18.0 cm) is now provided. The values of coefficient *a* ranged from 0.0007 to 0.0218, and the values of exponent *b* ranged from 2.82 to 3.52.

Keywords

Introduction

Length–weight relations (**LWRs**) are generally used to estimate the weight of an organism for a corresponding length, and this can then be applied to determine fish stocks and population assessments such as those involving information regarding the fish growth type and the determination of fish growth conditions (LeCren 1951; Ricker 1968). LWRs data are used for estimating the weight corresponding to a given length and can be affected by many factors including season, habitat area, gonad maturity, sex, diet, stomach fullness, and health (Bagenal and Tesch 1968). Thus, this contribution provides information concerning LWRs of 14 common fish species in the coastal waters of Gwangyang Bay. LWRs data involving *Favonigobius gymnauchen* (Bleeker, 1860); *Pampus echinogaster* (Basilewsky, 1855); and *Thryssa adelae* (Rutter, 1897) have not been registered in FishBase (Froese and Pauly 2020).

Material and methods

The fishes were collected at four sites from the coastal waters of Gwangyang Bay in South Korea (34°52′07″N, 127°47′50″E; 34°50′34″N, 127°46′56″E; 34°45′43″N, 127°49′31″E; 34°43′7″N, 127°47′41″E) between July 2018 and June 2019 (July, September in 2018; March,

Table 1. Descriptive statistics and estimated parameters of length–weight relations for 16 fish species in the coastal waters off the Gwangyang Bay, South Korea.

Family	Species	FB	n	TL [cm]	W [g]	a	95% CL of a	b	95% CL of b	r²
Rajidae	*Okamejei kenojei*	1	34	20.3–48.6	48.6–815.7	0.0036	0.0026–0.0048	3.18	3.09–3.27	0.994
Muraenesocidae	*Muraenesox cinereus*	2	32	35.5–61.7	50.1–276.9	0.0007	0.0003–0.0015	3.14	2.93–3.35	0.972
Engraulidae	*Thryssa adelae*	—	17	8.0–13.6	2.0–11.4	0.0030	0.0014–0.0063	3.11	2.80–3.42	0.975
	Thryssa kammalensis	3	141	8.0–**18.0**	3.1–36.5	0.0068	0.0051–0.0092	3.04	2.91–3.16	0.955
Cyprinidae	*Tribolodon hakonensis*	2	10	13.0–38.4	16.5–478.0	0.0074	0.0043–0.0128	3.04	2.87–3.20	0.994
Scorpaenidae	*Inimicus japonicus*	2	16	4.0–27.5	0.8–420.5	0.0041	0.0022–0.0076	3.52	3.31–3.73	0.989
Triglidae	*Chelidonichthys spinosus*	2	37	11.3–29.1	11.7–204.5	0.0053	0.0040–0.0071	3.17	3.08–3.26	0.993
Apogonidae	*Jaydia lineata*	1	86	4.0–14.8	0.8–40.0	0.0218	0.0181–0.0261	2.82	2.73–2.91	0.985
Sillaginidae	*Sillago japonica*	1	38	10.0–18.3	7.5–49.3	0.0085	0.0062–0.0116	2.95	2.84–3.06	0.990
Pholidae	*Pholis nebulosa*	2	13	4.0–27.5	0.1–106.8	0.0013	0.0010–0.0015	3.42	3.31–3.53	0.998
Gobiidae	*Favonigobius gymnauchen*	1	26	3.5–7.4	0.4–3.5	0.0061	0.0037–0.0100	3.11	2.81–3.40	0.955
Stromateidae	*Pampus echinogaster*	—*	43	8.9–21.5	12.9–140.2	0.0175	0.0103–0.0299	3.12	2.91–3.38	0.951
Cynoglossidae	*Cynoglossus joyneri*	6	67	10.3–28.9	12.5–99.6	0.0053	0.0034–0.0084	2.98	2.82–3.13	0.964
Tetraodontidae	*Takifugu niphobles*	2	13	8.0–15.9	9.1–83.3	0.0131	0.0087–0.0200	3.19	3.02–3.36	0.992

n = number of individuals, TL = total length, W = weight, a = intercept, 95% CI of a = 95% confidence intervals of a, 95% CI of b = 95% confidence intervals of b; r^2 = correlation coefficient; FB = the number of LWR data in FishBase (Froese and Pauly 2020); Bold prints denotes the newly recorded maximum total length (In reference to FishBase accessed on 26 June 2020); *published (Wu et al. 2018) but not yet registered in FishBase.

June in 2019; 4 times in total). The specimens were caught with small-bottom trawl nets (length 8 m, width 8 m, mesh wing and body 3 cm, mesh liner 1 cm) at the four study sites. The fishes were identified at the species level based on Kim et al. (2005), classified based on Nelson (2006), and validated according to FishBase (Froese and Pauly 2020). Then, they were anesthetized using 0.1 g L⁻¹ ethyl 3-aminobenzoate methanesulfonate salt (Sigma-Aldrich, Munich, Germany) before weight determination. Total length and body weight values were measured on site to the nearest 0.1 cm and 0.1 g, respectively. After measurement, fishes were kept in a recovery tank (100 × 100 × 80 cm) for recovery, and subsequently released. LWRs for each species were estimated using the equation:

$$\log W = \log a + b \log L$$

where a is the regression intercept and b is the regression slope for the species (Ricker 1973; Anderson and Gutreuter 1983; Jobling 2002). Prior to regression, obvious outliers were removed by the linear regression analysis of the log-transformed data (Froese 2006; Froese et al. 2011). Scientific names and family assignments were assigned based on FishBase designations. All analyses were performed using Sigma Plot 10.0 software (Systat Software, Inc., San Jose, U.S.A.).

Results

LWRs for a total of 573 individuals representing 14 fish species were calculated (Table 1). The following species were studied: *Okamejei kenojei* (Müller et Henle, 1841); *Muraenesox cinereus* (Forsskål, 1775); *Thryssa adelae*; *Thryssa kammalensis* (Bleeker, 1849); *Tribolodon hakonensis* (Günther, 1877); *Inimicus japonicus* (Cuvier, 1829); *Chelidonichthys spinosus* (McClelland, 1844); *Jaydia lineata* (Temminck et Schlegel, 1842); *Sillago japonica* Temminck et Schlegel, 1843; *Pholis nebulosa* (Temminck et Schlegel, 1845); *Favonigobius gymnauchen*;

Pampus echinogaster; *Cynoglossus joyneri* Günther, 1878; *Takifugu niphobles* (Jordan et Snyder, 1901). The number of individuals sampled for a given species ranged from 10 in cases of *Tribolodon hakonensis* to 141 in the case of *Thryssa kammalensis*. All species exhibited high correlation coefficients for LWRs, with r^2 values > 0.950. The values for parameter b ranged from 2.82 to 3.52, and a ranged from 0.0007 to 0.0218. A new maximum total length value of 18.0 cm for *Thryssa kammalensis* is provided herewith as a result of the presently reported study.

Discussion

Our data provided LWR parameters for 14 fish species from the coastal waters of Gwangyang Bay in South Korea. The b values ranged from 2.82 for *Jaydia lineata* to 3.52 for *Inimicus japonicus*. Our study provided the first LWR records for *Thryssa adelae*. The values identified for the LWR parameters may be explained by other factors, including gender, gonadal maturity, abiotic variables (e.g., temperature, hydrodynamics), or food availability (Araújo and Vicentini 2001; Froese 2006). The value of parameter a is indicative of the body shape of fish (Froese 2006); in this study, the fish exhibited a mixture of two morphological forms: *Okamejei kenojei* was eel-like and elongated; *Inimicus japonicus* was elongated and fusiform; and *Takifugu niphobles* was short and deep. Values of parameter a for *Pampus echinogaster* ranged from 0.0103 to 0.0299, which is a newly reported finding in this study. It is anticipated that our LWR results will contribute to fisheries management through future research.

Acknowledgments

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PENSOFT.

Length–weight relations of 11 goby species (Actinopterygii: Gobiiformes) from mangroves along the Ba Lat estuary of the Red River, Vietnam

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Abstract

Presently reported study examined the length–weight relations for 11 goby species collected from a mangrove estuary of the Red River, Vietnam. A total of 1097 individuals of the following species, representing three goby families, were analyzed: *Butis butis* (Hamilton, 1822); *Butis koilomatodon* (Bleeker, 1849); *Acentrogobius moloanus* (Herre, 1927); *Acentrogobius viridipunctatus* (Valenciennes, 1837); *Apocryptodon madurensis* (Bleeker, 1849); *Aulopareia unicolor* (Valenciennes, 1837); *Glossogobius giuris* (Hamilton, 1822); *Gobiopsis macrostoma* Steindachner, 1861; *Mugilogobius abei* (Jordan et Snyder, 1901); *Tridentiger barbatus* (Günther, 1861); and *Tridentiger trigonocephalus* (Gill, 1859). The regression slope values (*b*) ranged from 2.909 to 3.621. The majority of species had positive allometric or isometric growth pattern with $b \geq 3$, except for only one species (*G. giuris*) which had a negative allometric growth with $b = 2.909$. This study provided the first LWR information of four gobies that have not been reported in FishBase yet. Besides, the reference for LWRs of other gobies at an ecologically important area like Ba Lat Estuary is also provided.

Keywords

gobies, length–weight relations, LWRs, Xuan Thuy National Park

Introduction

The length–weight relation (**LWR**) of fishes is a crucial tool for fishery management, used to quantify the biomass while assessing the population dynamics and inferring the impact of the environment on fish (Tesch 1971; Froese 2006). The growth patterns of fishes can be estimated and compared between populations or species using the slope values of LWR regressions (Anderson and Gutreu-

ter 1983). Moreover, the body condition that reflects the relative wellness of fish populations in a given environment can also be estimated from the LWR (Froese 2006).

The Ba Lat Estuary, composed of well-developed mangrove forests of Xuan Thuy National Park and Tien Hai Wetland Nature Reserve, is an estuary with diverse biotopes. It is the habitat for many wild species, including some rare migration birds and many fishes (Hoang et al. 2013). Despite this importance, there is a lack of informa-
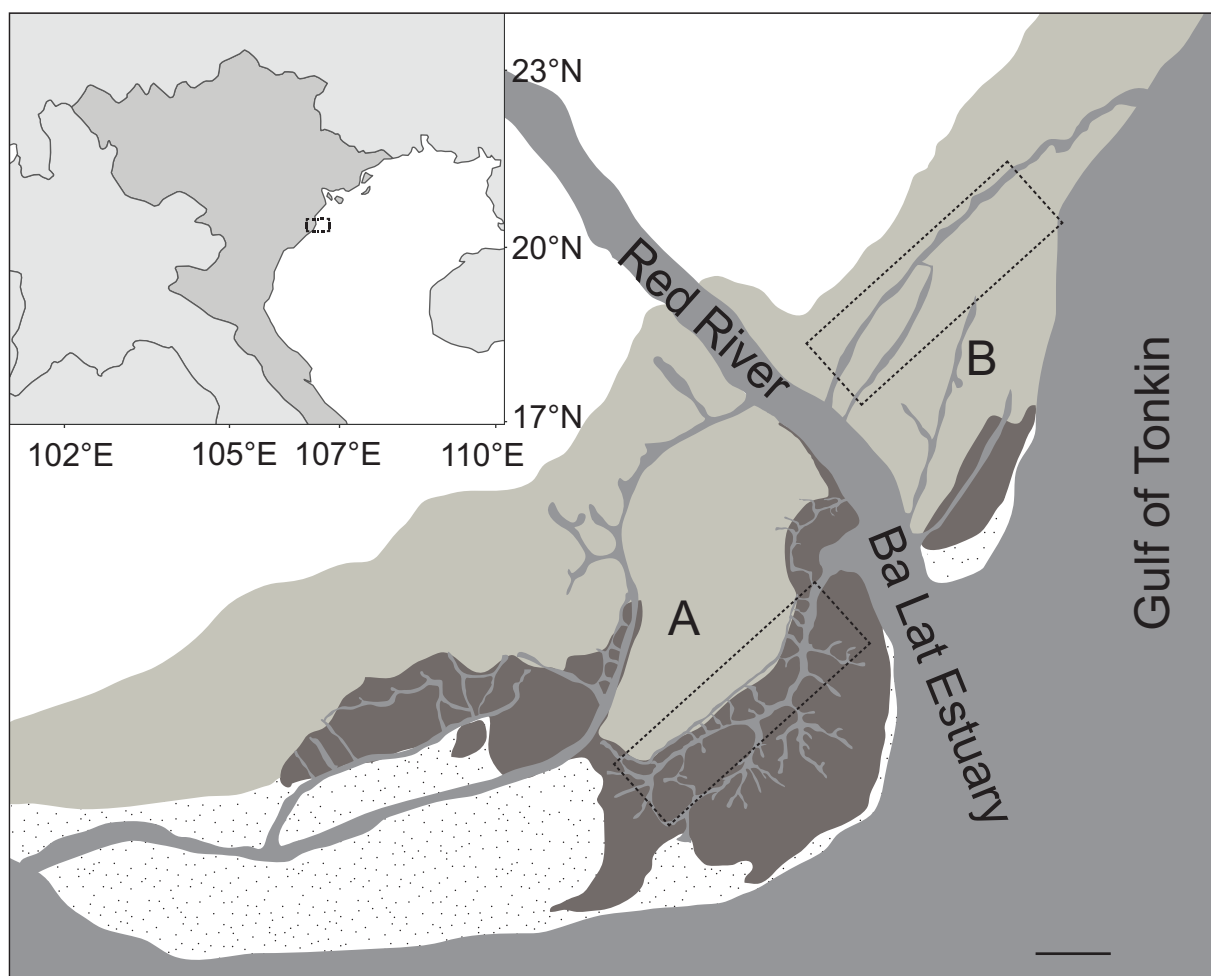
Figure 1. Map of the study area along Ba Lat Estuary, Vietnam. (**A**) Xuan Thuy National Park; (**B**) Tien Hai Wetland Nature Reserve; dashed rectangles indicate sampling sites. Scale bar: 2 km.

tion about LWRs of fishes in this area that may be useful for fisheries management.

This study intended to provide necessary information on LWRs for a better management of fishery resources in the area of Ba Lat Estuary.

Materials and methods

Fishes were collected monthly using hand nets and eight-hole fishing nets at Xuan Thuy National Park from March 2018 to February 2019 and Tien Hai Wetland Nature Reserve from March 2019 to February 2020 (Fig. 1). Total length (TL) and body weight (*W*) of fresh anesthetized fish samples were measured using a digital caliper and a digital balance to the nearest 0.01 cm and 0.1 g (except for *Mugilogobius abei* was measured to nearest 0.01 g), respectively.

The LWRs were estimated using the following formula:

$$W = a\text{TL}^b$$

where *W* is total body weight [g], TL the total length [cm], *a* is the regression intercept, and *b* is the slope. The

values of *a* and *b* were obtained from linear regression analysis using the natural logarithm of the variables, i.e., $\ln W = \ln a + b \times \ln \text{TL}$ (Ricker 1973). Student's *t*-tests were performed to examine the growth type of fishes by comparing *b* with 3 (isometric growth if *b* = 3, negative allometric if *b* < 3 or positive allometric if *b* > 3). Basic functions in R software version 4.0.2 were used to perform all statistical analysis (R Core Team 2020).

Results

A total of 1097 specimens belonging to 3 families (Butidae, Gobiidae, Oxudercidae), and representing 11 species were examined: *Butis butis* (Hamilton, 1822); *Butis koilomatodon* (Bleeker, 1849); *Acentrogobius moloanus* (Herre, 1927); *Acentrogobius viridipunctatus* (Valenciennes, 1837); *Apocryptodon madurensis* (Bleeker, 1849); *Aulopareia unicolor* (Valenciennes, 1837); *Glossogobius giuris* (Hamilton, 1822); *Gobiopsis macrostoma* Steindachner, 1861; *Mugilogobius abei* (Jordan et Snyder, 1901); *Tridentiger barbatus* (Günther, 1861); and *Tridentiger trigonocephalus* (Gill, 1859). Table 1 shows the estimated LWRs and related information.

Table 1. LWR parameters for 11 goby species collected from mangroves along Ba Lat Estuary, Vietnam.

Family/Species	n	Total length [cm]	Weight [g]	Regression parameters							b (in FishBase)	Length [cm] (on FishBase)
				a	95% CI of a	b	95% CI of b	b compare to 3 (P, t-test)	r²			
Butidae												
Butis butis	18	6.78–12.20	2.7–22.6	0.003	0.001–0.007	3.621	3.180–4.061	0.008	0.950	3.000[1]	8.6 TL[1]
Butis koilomatodon	65	3.96–9.24	0.8–12.8	0.010	0.006–0.016	3.208	2.936–3.480	0.132	0.898	3.260[2]	1.4–5.1 SL[2]
Gobiidae												
Acentrogobius moloanus	36	5.77–8.17	1.2–5.9	0.003	0.001–0.010	3.408	2.816–3.999	0.170	0.801	—	—
Acentrogobius viridipunctatus	109	2.34–13.75	1.8–29.3	0.008	0.006–0.010	3.164	3.067–3.262	0.001	0.975	3.230[1]	4.3–12.3 TL[1]
Apocryptodon madurensis	188	4.58–8.94	0.7–7.0	0.005	0.004–0.007	3.266	3.098–3.433	0.002	0.888	—	—
Aulopareia unicolor	196	4.43–10.74	0.8–15.8	0.004	0.003–0.006	3.451	3.298–3.605	< 0.001	0.910	—	—
Glossogobius giuris	270	5.81–27.00	1.4–162.0	0.009	0.008–0.011	2.909	2.843–2.974	< 0.001	0.966	2.682–3.298[3]	2.3–23.6 TL[3]
Gobiopsis macrostoma	56	5.35–9.90	1.7–12.0	0.005	0.003–0.009	3.391	3.144–3.637	0.002	0.934	—	—
Oxudercidae												
Mugilogobius abei	43	1.54–3.20	0.04–0.33	0.009	0.008–0.010	3.240	3.096–3.383	0.002	0.981	2.876[1]	1.2–5.0 TL[1]
Tridentiger barbatus	53	3.56–9.16	0.5–10.1	0.006	0.003–0.010	3.416	3.137–3.695	< 0.001	0.922	3.237[4]	3.5–10.4 SL[4]
Tridentiger trigonocephalus	63	5.21–8.88	1.4–10.3	0.008	0.004–0.015	3.291	2.949–3.633	0.094	0.859	3.000[1]	2.3 TL[1]

n = sample size, a = intercept, b = slope, CI = confidence interval, r^2 = coefficient of determination, TL = total length, SL = standard length; [1]Froese and Pauly (2020), [2]Lobato et al. (2018), [3]Hossain et al. (2009), [4]Xu et al. (2016).

The coefficients of determination r^2 ranged from 0.801 (for *A. moloanus*) to 0.981 (for *M. abei*). All 11 examined species had r^2 larger than 0.800 and were highly significant ($P < 0.001$ for all cases). The estimated values of intercepts (a value) ranged from 0.003 (*B. butis* and *A. moloanus*) to 0.010 (*B. koilomatodon*). The values of parameter b ranged from 2.909 for *G. giuris* to 3.621 for *B. butis* (Table 1), all remained within the expected 2–4 range (Tesch 1971), and mostly within the range 2.5–3.5 as suggested by Carlander (1969) or the range of 2.7–3.4 reported for 90% species examined by Froese (2006). The b values for the majority of the species were significantly higher than or at least equal to three, except for only *G. giuris* (Table 1).

Discussion

The LWRs of *A. moloanus*, *A. madurensis*, *A. unicolor*, and *G. macrostoma* were hitherto not available in FishBase (Froese and Pauly 2020). Hence, present study represents the first reference on LWRs for these four species. Besides these first records, we provided more robust data of LWRs for *B. butis*, and *T. trigonocephalus* than those were estimated with single data points on FishBase (Froese and Pauly 2020). For *B. butis*, we recommend another robust LWR data estimated by using 141 individuals at the size of approximately 4.5–15.5 cm TL from the Mekong Delta, southern Vietnam that has been reported with $b = 2.74$ lower than in the present study (Dinh 2017).

Additionally, the maximum lengths of *A. moloanus* (8.17 cm) and *A. unicolor* (10.74 cm) at present were longer than those reported on FishBase as 8.0 cm and 8.8 cm, respectively (Froese and Pauly 2020), constitute new records of these species' maximum length. Although the sample sizes were relatively small for the above-mentioned species, the LWRs from this study can be used confidently since the specimens were collected monthly covering a complete year and a wide size range of each species.

This study also reported supplementary information on LWRs of the remaining species, in which the b values

of *B. koilomatodon* (3.208), *A. viridipunctatus* (3.165), *G. giuris* (2.909), and *T. barbatus* (3.416) were quite similar to the b value listed on FishBase presented in Table 1 (Hossain et al. 2009; Xu et al. 2016; Lobato et al. 2018; Froese and Pauly 2020). Remarkably, the estimated b value for *M. abei* (3.240) was higher than the value reported as 2.876 by Li et al. (2013). From the Mekong Delta area, the proximal area of our study site, the LWR of *G. giuris* was reported with a higher b value than in the present study with $b = 3.407$ (Dinh 2014). Given that, many factors can affect the parameters of LWR of fish, such as season, gonad maturity, sex, preservation methods, and differences in environmental conditions (Froese 2006). The dissimilarities found between this study and the others may be due to the differences in habitat conditions.

Concerning the growth type, our result showed that only one species had negative allometric growth ($b < 3$, $P < 0.01$), three species had isometric growth ($b = 3$, $P > 0.05$ for all cases), seven species had positive allometric growth ($b > 3$, $P < 0.01$ for all cases). The positive allometric growth indicates that fish grow in body weight faster than growth in body length, and fish will possess a plump shape as they grow. At this same study area, we also observed the positive allometric growth for another goby namely *Periophthamus modestus* with general $b = 3.094$ (Tran et al. 2021).This suggests that the estuary composed of mangroves in northern Vietnam is suitable for these fishes as providing sufficient food for them to grow and gain robust body shapes.

Acknowledgment

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PENSOFT.

ACTA
ICHTHYOLOGICA
ET PISCATORIA

Non-invasive ploidy determination in live fish by measuring erythrocyte size in capillaries

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Abstract

Information about ploidy is important in both commercial and conservation aquaculture and fish research. Unfortunately, methods for its determination, such as karyology, determination of the amount of DNA in a cell using microdensitometry or flow cytometry and/or measuring erythrocytes in a blood smear can be stressful or even destructive. Some of these methods are also limited by the relatively large minimum size of the individual being measured. The aim of this study was to test a new low-stress method of determining ploidy by measuring the size of erythrocytes in the capillaries of a fish, including small individuals. First, we examined diploid and triploid loach (*Cobitis* sp.) and gibel carp, *Carassius gibelio* (Bloch, 1782), using flow cytometry and blood smears, with these results being used as a control. Subsequently, we measured the size of erythrocytes in the caudal fin capillaries of anesthetized fishes of known ploidy under a light microscope. For both the loaches and gibel carp, direct observation of the mean erythrocyte size in epithelial fin capillaries provided a consistent and reliable determination of ploidy when compared with the controls based on flow cytometry and blood smears. This new method allows for rapid determination of ploidy in living small fish, where collection of tissue using other methods may cause excessive stress or damage. The method outlined here simply requires the measurement of erythrocytes directly in the bloodstream of a live fish, thereby making it possible to determine ploidy without the need for blood sampling. The method described is sufficiently efficient, less demanding on equipment than many other procedures, can be used by relatively inexperienced personnel and has benefits as regards animal welfare, which is especially important for fish production facilities or when dealing with rare or endangered species.

Keywords

Introduction

Polyploidy, the multiplication of whole sets of chromosomes beyond the normal set of two, occurs independently in many groups of fish, from sharks to the higher teleosts. While there are several ways that a polyploid fish can develop, environmental change and hybrid stabilization may play a large role in the initiation of a new polyploid species. Polyploid fish could gain an advantage over diploid fish through increased heterozygosity, the divergence of duplicate genes, and/or increased expression of key physiological proteins (Leggatt and Iwama 2003). Hybridization and polyploidization thus became increasingly appreciated as important evolutionary mechanisms that even had a profound impact on mankind, such as increased crop yields, quality or pathogen-resistance (Mason and Batley 2015).

At the phenotypic level, the effects of polyploidization are often mild and idiosyncratic (Otto 2007). Cell volume generally rises with increasing genome size

(Cavalier-Smith 1978; Gregory 2001), although the exact relation between ploidy and cell volume varies among environments and taxa.

Further, although cell size typically is larger in polyploids, adult size may or may not be altered; as a rough generalization, polyploidization is more likely to increase adult body size in plants and invertebrates than in vertebrates (Otto and Whitton 2000; Gregory and Mable 2005).

Triploidy may be accompanied by morpho-anatomical changes to the organs. Changes may occur not only in proportion but also as anomalies or deformations that have clearly negative impacts on the individual. For example, in fish, negative changes may include gill defects such as missing gill filaments, leading to a reduction in gill surface area, as recorded in triploid *Salmo salar* by Sadler et al. (2001).

In addition to possible changes in organ structure, polyploid individuals may also show differences in physiology. Previous studies have tended to focus on differences in metabolism rates between diploid and triploid fish or the ability to survive in oxygen-poor environments. The results of these studies have tended to be ambiguous, however, showing variability within both species and developmental stages, depending on test conditions (e.g., Benfey and Sutterlin 1984; Lilyestrom et al. 1999; Ellis et al. 2013).

Polyploidy is especially common in loach (*Cobitis* sp.) (Papoušek et al. 2008), gibel carp, *Carassius gibelio* (see Vetešník et al. 2006) complexes and in artificial fish farming conditions (Piferrer et al. 2009; Preston et al. 2013; Fiske et al. 2019). These hybrid diploid polyploid complexes and polyploidization are currently intensively used in a whole range of studies, from evolutionary issues to fish production (Kotusz et al. 2014; Pakosta et al. 2018; Bartoš et al. 2019).

To accurately identify individual biotypes, it is necessary to gradually combine several diagnostic approaches: sequencing of mitochondrial and nuclear markers, allozyme analysis, and cytogenetic tools (e.g., karyotyping and C-banding), including the determination of degrees of ploidy (Bohlen and Ráb 2001).

Three basic methods were used in the presently reported study to detect polyploidy: i) karyology (e.g., Blaxhall 1975), the only method able to determine the exact number of chromosomes; ii) determination of the amount of DNA in a cell using microdensitometry (e.g., Gervai et al. 1980; Hardie et al. 2002) or flow cytometry (e.g., Thorgaard et al. 1982); or iii) comparison of whole erythrocyte or nucleus size between ploidies, using, for example, a microscope or an automated particle size analyzer (e.g., Thorgaard and Gall 1979; Benfey et al. 1984; Flajšhans 1997; Fiske et al. 2019). These methods usually have a number of limitations, including the necessity for specialized equipment, a need to kill (especially in karyology or flow cytometry of muscle tissue), or otherwise damage the fish (e.g., through fin clipping or removal of blood for flow cytometry or blood smears) and/or financial and time constraints.

In this paper, we present a new method for determining ploidy based on the measurement of erythrocyte size in caudal fin capillaries. The method is non-invasive, suitable for small fish that should not be killed, affordable, and does not require specialized equipment.

Methods

For this study, we examined 20 loaches (10× diploid *Cobitis elongatoides*, 10× triploid *C. elongatoides* × *C. tanaitica*; standard length [SL] 6.0–8.5 cm and 20 gibel carp (10× diploid, 10× triploid; SL 1.5–2.5 cm). Ploidy in these individuals was initially determined by flow cytometry (as DNA content using a Partec CCA flow cytometer; dyed with DAPI-CyStain DNA 1-step solution) on a blood sample (loach 2n = 103.6% (96.0–112.0); 3n = 153.8% (142.0–164.4); gibel carp 2n = 97.8% (94.4–104.4); 3n = (154.0% (146.6–162.8)) (Flajšhans et al. 2005), using the gold fish (*Carassius auratus*) as a standard (=100%), and a separate blood smear (Boroń 1994) for measurement of length erythrocyte (without staining). These data were used as a control for comparison with the new method.

The fish used for measurement of erythrocyte size in caudal fin capillaries were immobilized on the mechanical stage of an Olympus BX50 light microscope using a 36 × 125 mm 'pad' with two overlapping tiles glued to the underlying glass (Fig. 1). This allowed for the placement of individuals exceeding the length of the classical glass (76 mm) as well as allowing the pad to be fastened using stage clips and moved with the stage controls.

The individual being examined was first anesthetized with clove oil (0.05 mL in 1 L of water; Svoboda and Kolářová 1999) and then placed on its side on the underlying glass. The caudal fin was then stretched, allowing blood in the epithelial capillaries to be observed using a 40× lens. At the same time, an image was transferred to the computer screen using an Olympus DP70 camera. In vivo measurements (major axes) were obtained for 20 cells using Photoshop software. As the high velocity of the blood cells usually did not allow for high quality photographic capture, images were obtained from localities where blood flow was reduced to a minimum.

Results

For both the loaches and gibel carp, direct observation of mean erythrocyte size in epithelial fin capillaries provided a consistent and reliable determination of ploidy (Table 1; Figs 2–4), using flow cytometry and blood smears as controls.

The ratio of the mean fin capillary erythrocyte length was similar to that for blood smears, the lower absolute values observed using blood smears most likely being the result of cell shrinkage after drying on the surface of the glass or that larger values obtained using this new method

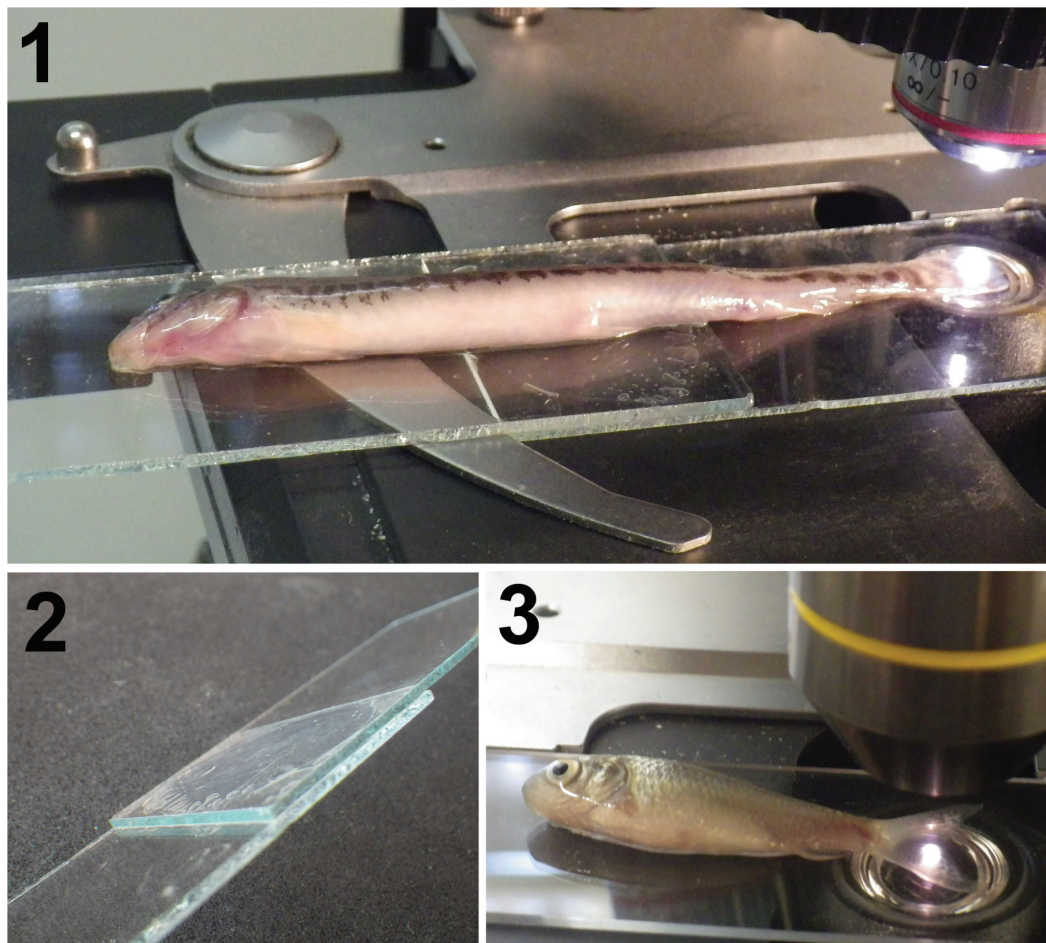**Figure 1.** Loach (**1**) and gibel carp (**3**) mounted on the light microscope using the pad made from two overlapping underlying glass (**2**).
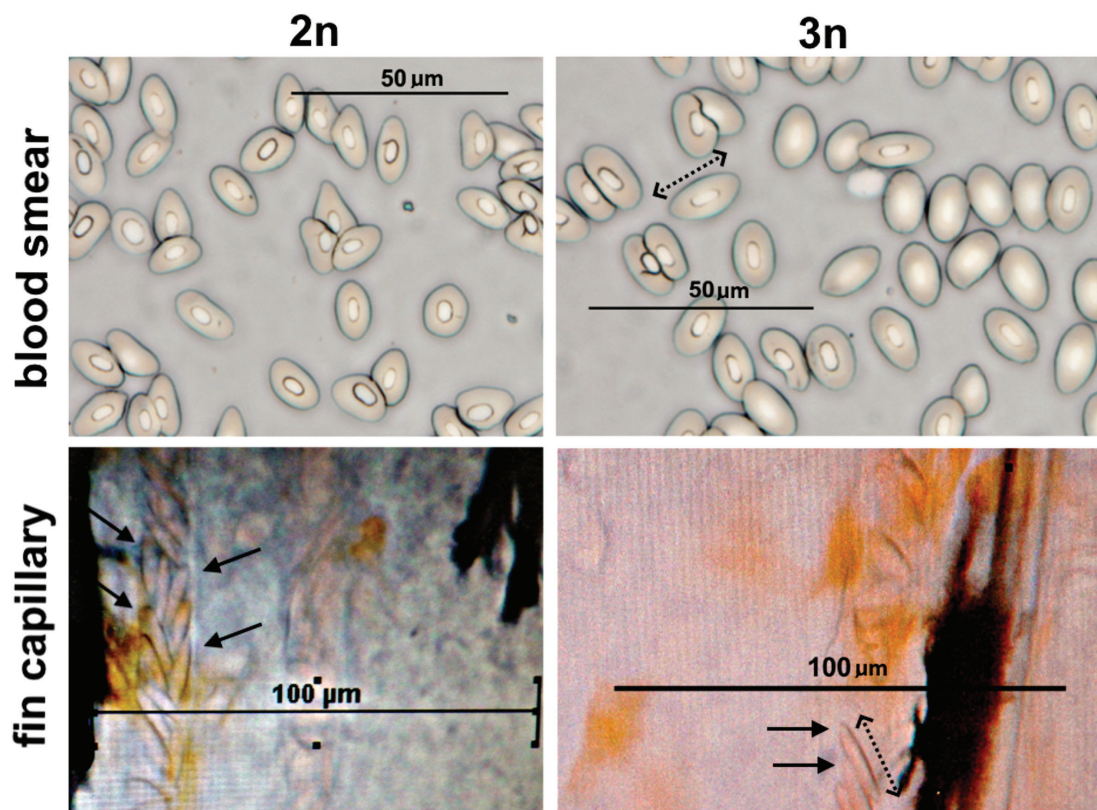

Figure 2. Erythrocytes of gibel carp; arrows = erythrocytes in capillaries, dotted line = erythrocyte length.
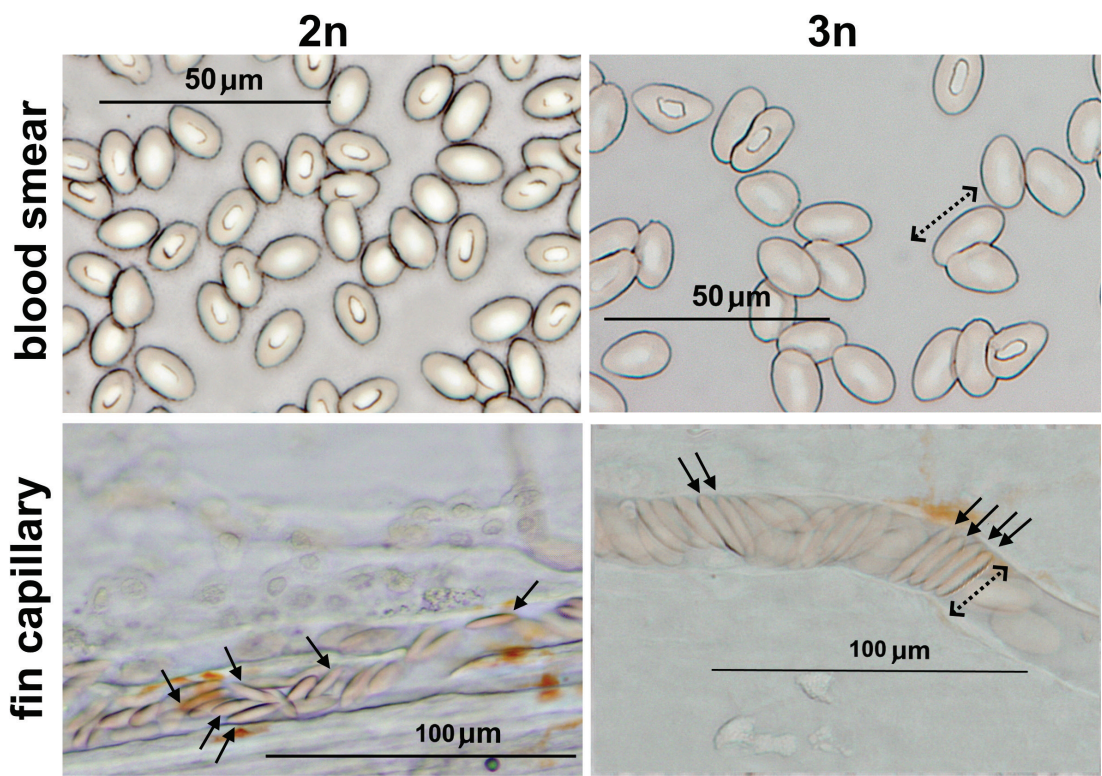
2n   3n

Figure 3. Erythrocytes of cobitids; arrows = erythrocytes in capillaries, dotted line = erythrocyte length.
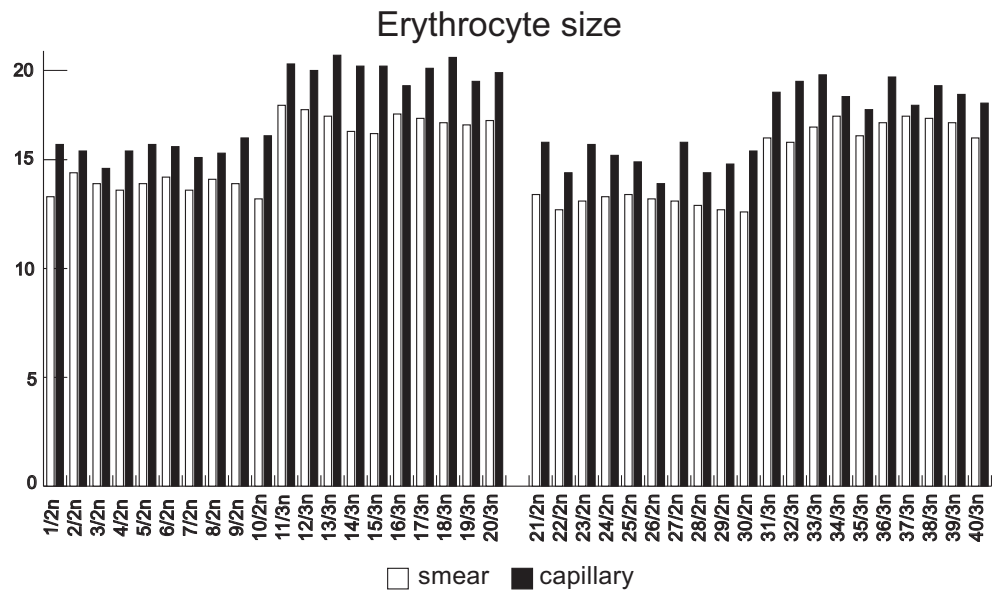

Figure 4. Erythrocyte length (mean in μm) for loach (1–10 = diploid/2n; 11–20 = triploid/3n) and gibel carp (21–30 = diploid; 31–40 = triploid), measured from blood smears and directly from fin capillaries.

Table 1. Erythrocyte length [μm] for loach (*Cobitis* sp.) and gibel carp (*Carassius gibelio*) measured from blood smears and direct from fin capillaries.

Species	Source	2n			3n			2n:3n ratio
		Mean	Range	SD	Mean	Range	SD	
Loach	Smear	13.8	13.1–14.4	0.35	16.9	16.2–17.5	0.42	1:1.22
	Capillary	15.5	14.6–16.1	0.39	19.3	18.4–19.8	0.43	1:1.25
Gibel carp	Smear	13.2	12.7–13.4	0.24	16.3	15.5–17.0	0.43	1:1.25
	Capillary	15.0	13.9–15.8	0.68	17.9	16.4–18.9	0.54	1:1.19

SD = standard deviation.

were due to cell deformation (stretching) of the cells by passage through a capillary.

Discussion

A range of methods have been used to identify polyploid fish; however, each has specific limitations. While chromosome preparation and counting are now considered inexpensive and require little specialized

equipment, it is not always easy to perform or successful. Further, while there are exceptions (see Kalous et al. 2010), the method requires that the fish be killed and the results take time, often up to 24 h (e.g., Kligermann and Bloom 1977; Felip et al. 2009). A second method frequently utilized is the measurement of DNA content in individual blood cells (Wolters et al. 1982; Kotusz 2008), muscle samples, or from a fin clip using either microfluorimetry, microdensitometry (Gervai et al. 1980), or flow cytometry (Thorgaard et al. 1982). In the case of blood samples and fin clips, while there is no need to kill the fish and the results are obtained relatively quickly (minutes to tens of minutes), the method is expensive and requires specialized equipment. Finally, ploidy has also been determined by measuring the length and width of whole red blood cells or (more often) their nuclei. By using a suitable nuclear staining technique (e.g., Giemsa, hematoxylin), the nucleus "area" can also be determined through image analysis (Cherfas 1966; Benfey et al. 1984; Felip et al. 2009). Though the results obtained from nucleus measurements tend to be statistically more significant, differences in the maximum whole-cell size tend to be sufficient to distinguish diploid and triploid individuals. While this method is relatively quick and inexpensive and does not require specialized equipment (aside from image analysis software) or chemicals, it does require a blood sample. Even in larger individuals (up to ca. 10 cm), removal of a blood sample can result in injury or even the death of the fish; and in small individuals (up to 1–2 cm), removal of a suitable blood sample may prove difficult.

In comparison, the method outlined here simply requires direct measurement of erythrocytes in the bloodstream of a live fish, thereby making it possible to determine ploidy without the need for a blood sample of any kind. Our results indicate that the difference in erythrocyte size between diploid and triploid individuals is perfectly sufficient to reliably determine ploidy. Equipment requirements are limited to a standard optical microscope with a 40× zoom lens and a camera/video attachment allowing an image of the blood cells to be captured and measured. The fish can then be returned to the water after recovering from the anesthetic. Further, the level of stress is relatively low, especially compared to some of the "invasive" methods mentioned above.

Conclusions

The method described is sufficiently efficient, less demanding on equipment than many other procedures (e.g., flow cytometry, microdensitometry), for especially small fish, can be used by relatively inexperienced personnel and has benefits as regards animal welfare, which is especially important for fish production facilities or when dealing with rare or endangered species.

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Genetic diversity of the yellowfin seabream, *Acanthopagrus latus* (Actinopterygii: Perciformes: Sparidae)—An enhancement species in Dongshan Bay

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Abstract

Dongshan Bay is located on the west side of the Taiwan Strait, which had abundant fishery resources in the past. With the increase in fishing pressure, resources have declined. To restore the fishery resources in Dongshan Bay and to increase fishing yield, much enhancement and release work has been carried out in Dongshan Bay. The yellowfin seabream, *Acanthopagrus latus* (Houttuyn, 1782), is an important enhancement species in Dongshan Bay that is also frequently captured. Due to yearly progress in enhancement and release, it is necessary to study the current status of the genetic diversity of yellowfin seabream in Dongshan Bay. The results show that all yellowfin seabream populations have high genetic diversity, which is mainly related to its breeding habits and growth rate, and this ensures a large recruitment stock in the natural seas. The current population has differentiated from the historical population due to a change in genetic structure, and many historical haplotypes have been lost. The results of this study provide a reference for fishery management departments to formulate management measures and conservation policies specifically for yellowfin seabream. In particular, yellowfin seabream is a hermaphroditic and protandrous species. Targeting an older age group as the main fishing subject is not conducive to its breeding protection and resource growth, and therefore, fishing of an older age group should be restricted in fishery production.

Keywords

Dongshan Bay, genetic diversity, resource decline, stock enhancement, yellowfin seabream

Introduction

Located on the west side of the Taiwan Strait, Dongshan Bay is a typical subtropical estuary semi-closed bay. The bay is affected by the offshore water of the South China Sea, by the Taiwan warm current in the summer, and by the Fujian–Zhejiang coastal current as well as the Taiwan Strait inversion thermocline in the fall. It originally had rich fishery resources and was a good place for various economic species to inhabit, reproduce, and grow. With the rapid development of industry and agriculture since the reform and opening up, the development and

utilization of sea areas and the continuously growing aquaculture industry have aggravated the ecological environment of the sea area in the bay, leading to a significant decline in fishery resources (Lin et al. 2013).

To restore the fishery resources in Dongshan Bay, the government of Dongshan County launched a program for enhancement and release some economically important shellfish and finfish species, *Penaeus japonicus*, *Penaeus penicillatus*, *Pagrus major* (Temminck et Schlegel, 1843); *Acanthopagrus schlegelii* (Bleeker, 1854); and *Acanthopagrus latus* (Houttuyn, 1782). By releasing a large number of artificially bred seedlings into natural seas to restore decreased populations and increase fishing yields, enhancement and release are of great significance for replenishing and restoring the population structure of biological resources, improving the ecological environment of waters, replenishing fishery resources, and increasing fishermen's income. However, the genetic impact of released species on wild resources has also received increasing attention (Helfman 2008). In general, although cultured populations may not be able to adapt well to the changing external environment, they may outperform wild populations in intraspecies competition in natural seas, resulting in a decline in the number of wild populations and a corresponding reduction in their genetic diversity (Tringali and Leber 1999). Moreover, the fishing yield will increase after enhancement and release, which will cause greater fishing pressure on the original wild populations, bringing about a further decline in the number of wild populations and, ultimately, a reduction in the genetic diversity of the species (Helfman 2008).

The yellowfin seabream, *Acanthopagrus latus*, an important economic fishery species in Dongshan Bay, is an important species for enhancement and release in this sea area. It is a warm water shallow coastal fish species that can adapt to rapid changes in salinity and generally does not migrate long distances. It is widely distributed in the East China Sea and South China Sea (Jiang et al. 2012; Iwatsuki 2013). With enhancement and release in Dongshan Bay in the past 10 years, the current status of the genetic diversity of the yellowfin seabream population in the bay is unclear. Has gene flow occurred between the introduced released population and wild populations, thereby changing the composition of the original populations and causing the original stock to become a mixed population consisting of released individuals, wild individuals, and hybrid offspring of the two stocks? Have the genetic diversity and genetic structure of the wild population also changed? It is necessary to evaluate the current status of the genetic diversity of the yellowfin seabream in Dongshan Bay to propose targeted fishery management measures.

Mitochondrial DNA (mtDNA), as an important genetic information library, has the advantages of maternal inheritance, a fast evolution rate, high copy numbers, and easy amplification. Therefore, it is often employed in research on species diversity and phylogeny with wide applications (Simon et al. 2006; Li et al. 2018, 2019). In this study, the first hypervariable region of the control region (CR) of mtDNA was used to study the genetic diversity and genetic structure of the yellowfin seabream in three populations collected from Dongshan Bay and in one population from Xiamen to evaluate the current status of its genetic resources. This study will provide a reference for the formulation of management measures and conservation policies for yellowfin seabream and the development of enhancement and release work.

Materials and methods

Sample collection

A total of 75 individuals of four yellowfin seabream populations, Dongshan I (DSI; wild individuals, 2009.11), Dongshan II (DSII; wild individuals, 2019.10), Dongshan III (DSIII; cultured individuals, 2019.11), and Xiamen (XM; wild individuals, 2019.10), were collected from November 2009 to November 2019 (Fig. 1, Table 1). Wild individuals were captured in the open waters of Dongshan Bay and Xiamen, and cultured individuals were captured in the farming waters of Dongshan Bay. Yellowfin seabream can be easily identified based on their yellow or pale-yellow ventral fin, anal fin, and lower lobe of the caudal fin. The main morphological identification was based on Nakabo (2013). All samples were accurately identified by morphology, and the dorsal muscles were stored in 95% alcohol for subsequent experiments, or fresh dorsal muscles were collected to directly extract genomic DNA.

Genomic DNA extraction, PCR amplification, and sequencing

A Qiagen DNeasy kit was used to extract the genomic DNA of yellowfin seabream. DNA with a quantified concentration was amplified by PCR. The amplification primers were DL-S (5′-CCCACCACTAACTCCCAAAGC-3′) and DL-R (5′-TTAACTTATGCAAGCGTCGAT-3′) (Gao

Table 1. Sampling sites, date, number, number of haplotype and genetic diversity indices for each population of *Acanthopagrus latus*.

Sampling site	ID	Collection date	Sample size	h	π	k	No. of haplotype
Dongshan I	DSI	Nov 2009	20	0.9895 ± 0.0193	0.0163 ± 0.0088	8.9632 ± 4.3104	18
Dongshan II	DSII	Oct 2019	22	0.9957 ± 0.0153	0.0178 ± 0.0095	9.7922 ± 4.6616	21
Dongshan III	DSIII	Nov 2019	14	0.9670 ± 0.0366	0.0129 ± 0.0072	7.0879 ± 3.5401	11
Xiamen	XM	Oct 2019	19	1.0000 ± 0.0171	0.0161 ± 0.0087	8.8246 ± 4.2585	19
Total			75	0.9960 ± 0.0030	0.0196 ± 0.0100	10.8090 ± 4.9731	65

h = haplotype diversity, π = nucleotide diversity, k = mean number of pair-wise differences; DSI = Dongshan I population (wild individuals, 2009.11), DSII = Dongshan II population (wild individuals, 2019.10), DSIII = Dongshan III population (cultured individuals, 2019.11), XM = Xiamen (wild individuals, 2019.10).
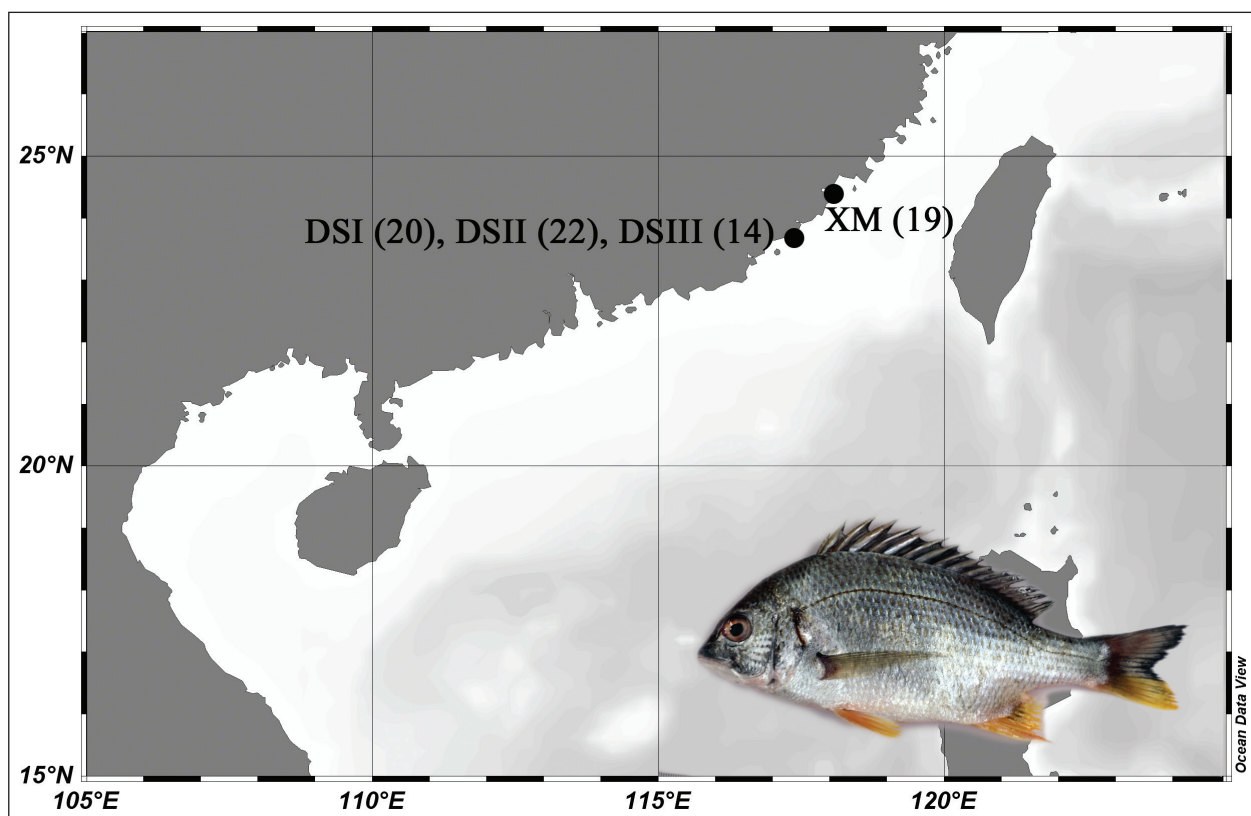
Figure 1. Sampling locations for *Acanthopagrus latus*.

et al. 2019). The PCR system required a total volume of 25 μL, including 17.25 μL of deionized water, 2.5 μL of 10× PCR buffer, 2 μL of dNTPs, 1 μL of each of the forward and reverse primers, 0.25 μL of Taq enzyme, and 1 μL of the DNA template. The PCR conditions were as follows: denaturation at 95°C for 5 min; 30 cycles of denaturation at 95°C for 45 s, annealing at 50°C for 45 s, and extension at 72°C for 45 s; and final extension at 72°C for 10 min. 3 μL of PCR product was used for 1.5% agarose gel electrophoresis. During PCR electrophoresis on agarose gel, different DNA ladder can be distinguished, so the agarose gel electrophoresis was often used to detect the length of PCR products. The qualified products were purified and bidirectional sequenced at the Personal Biotech Co., Ltd. The newly obtained CR haplotype sequences of yellowfin seabream have been submitted to the GenBank database, and the accession numbers are MT312258–MT312282, MT312289, MT312291, MT312298, MT312301, MT312308, MT312310, MT312313, MT312315–MT312321, MT312333–MT312341, MT312343–MT312351, MT312353–MT312358.

Data analysis

The same primers employed for PCR amplification, were used to manually edit and correct the raw sequences for the CR of the yellowfin seabream with SeqMan in the DNASTAR software package. The genetic diversity indexes, such as the mutation sites, haplotype numbers, and

population genetic diversity parameters, were calculated using ARLEQUIN 3.5 (Excoffier et al. 2005). *Acanthopagrus schlegelii* was included as an outgroup. Based on the yellowfin seabream haplotypes and Kimura's two-parameter (K2P) model, MEGA 5.0 software was employed to construct a neighbor-joining tree (NJ). The 1000 nonparametric self-expanding analysis was adopted for repetitive tests and calculation of the confidence of each branch of the phylogenetic tree (Tamura et al. 2011). The F_{ST} values between pairwise groups were calculated with ARLEQUIN 3.0 software (Excoffier et al. 2005), and a significance test was performed using 10 000 replacement tests. Analysis of molecular variation (AMOVA) of the parameters was calculated with ARLEQUIN software to determine the genetic structure of different yellowfin seabream populations, and the significance of covariance at different levels of genetic structure was tested using 5000 replacement tests.

Results

Genetic diversity

A total of 75 sequences were obtained from all populations. After manual alignment, the lengths of the obtained target fragments were 548–550 bp, of which only one sequence was 548 bp in length, 549-bp sequences were dominant (71), and three sequences were 550 bp long. There were 87 mutation sites in all sequences, 54 parsimony informative sites, 33 singleton variable sites, and

three insertions/deletions. The contents of each base were as follows: A, 34.67%; T, 32.04%; G, 13.79%; and C, 19.49%. The A + T content (66.71%) was higher than the G + C content, demonstrating a certain AT preference.

The 75 sequences defined 65 CR haplotypes. The number of haplotypes in each population ranged from 11 to 21. Four (6.15%) haplotypes were shared by two or more populations. There were 61 (93.85%) unique haplotypes. DSIII had a shared haplotype (Hap_11, Hap_17, and Hap_47, respectively) with each of the other three populations, DSI had a shared haplotype (Hap_11 and Hap_17, respectively) with DSIII and XM, and DSII had a shared haplotype (Hap_20) with DSIII; there was no shared haplotype between DSI and DSII (Table 2).

Table 2. Distribution of haplotypes in *Acanthopagrus latus* populations.

	DSI	DSII	DSIII	XM	Total		DSI	DSII	DSIII	XM	Total
Hap_1	1				1	Hap_34	1				1
Hap_2	1				1	Hap_35	1				1
Hap_3	1				1	Hap_36	1				1
Hap_4	1				1	Hap_37	1				1
Hap_5	2				2	Hap_38	1				1
Hap_6	1				1	Hap_39			1		1
Hap_7	1				1	Hap_40			2		2
Hap_8	1				1	Hap_41			1		1
Hap_9	1				1	Hap_42			1		1
Hap_10	2				2	Hap_43			1		1
Hap_11	1			1	2	Hap_44			1		1
Hap_12	1				1	Hap_45			2		2
Hap_13	1				1	Hap_46			1		1
Hap_14	1				1	Hap_47		1	1		2
Hap_15	1				1	Hap_48			1		1
Hap_16	1				1	Hap_49			1		1
Hap_17	1		1		2	Hap_50			1		1
Hap_18		1			1	Hap_51			1		1
Hap_19		1			1	Hap_52			1		1
Hap_20		1	2		3	Hap_53			1		1
Hap_21		1			1	Hap_54			1		1
Hap_22		1			1	Hap_55			1		1
Hap_23		1			1	Hap_56			1		1
Hap_24		1			1	Hap_57			1		1
Hap_25		1			1	Hap_58			1		1
Hap_26		1			1	Hap_59			1		1
Hap_27		2			2	Hap_60			1		1
Hap_28		1			1	Hap_61			1		1
Hap_29		1			1	Hap_62			1		1
Hap_30		1			1	Hap_63			1		1
Hap_31		1			1	Hap_64			1		1
Hap_32		1			1	Hap_65	1				1
Hap_33		1			1						

DSI = Dongshan I population (wild individuals, 2009.11), DSII = Dongshan II population (wild individuals, 2019.10), DSIII = Dongshan III (cultured individuals, 2019.11), XM = Xiamen (wild individuals, 2019.10).

The entire yellowfin seabream population demonstrated high haplotype diversity (0.9960 ± 0.0030) and low nucleotide diversity (0.0196 ± 0.0100). Among them, the wild XM population showed the highest diversity (1.000 ± 0.0171), followed by the current wild Dongshan population (0.9957 ± 0.0153), and the historical wild Dongshan population (0.9895 ± 0.0193); the current cultured Dongshan population showed the lowest diversity (0.9670 ± 0.0366).

Population genetic structure and differentiation

The NJ tree was constructed based on 65 mitochondrial CR haplotypes of yellowfin seabream, showing that two large haplotype lineages existed in the four yellowfin seabream populations, with low confidence. No pedigree structure corresponding to geographic locations was detected (Fig. 2). Lineage 1 consisted of 50 haplotypes (58 individuals), and lineage 2 consisted of 15 haplotypes (17 individuals) (Fig. 2). The haplotype composition of the two lineages was quite different. Thirteen of the 15 haplotypes of lineage
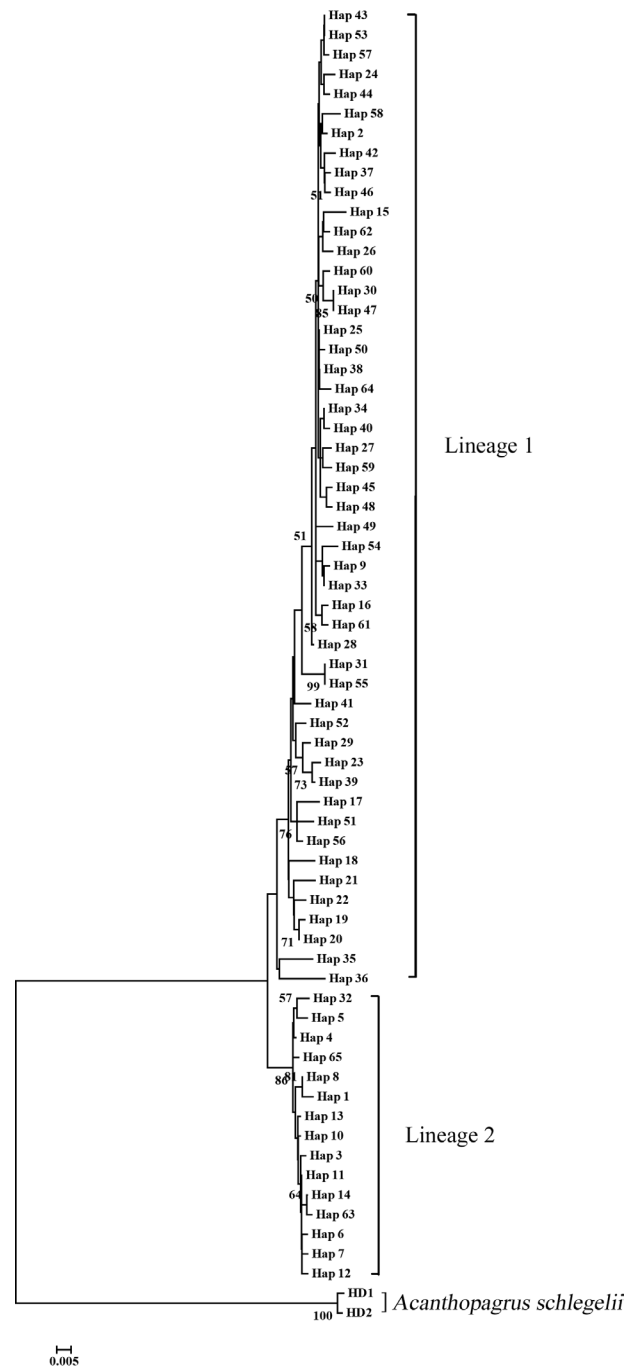

Figure 2. NJ tree of CR haplotypes in *Acanthopagrus latus*. Bootstrap supports > 50 in 1000 replicates are shown.

2 belonged to the DSI population, with one each belonging to the DSII and XM population. Lineage 1 was constituted by the remaining haplotypes of the populations, and there was no specific internal topological structure.

The pairwise F_{ST} values were estimated based on the mtDNA CR sequences, ranging from 0.0005 to 0.4288 (Fig. 3). The F_{ST} values between DSI and the other three populations were large, and statistical tests showed extremely significant differences, indicating significant genetic differentiation between DSI and the other three populations. However, the differences in F_{ST} values among DSII, DSIII, and XM were small, and the statistical tests were not significant, indicating less genetic differentiation among these populations.
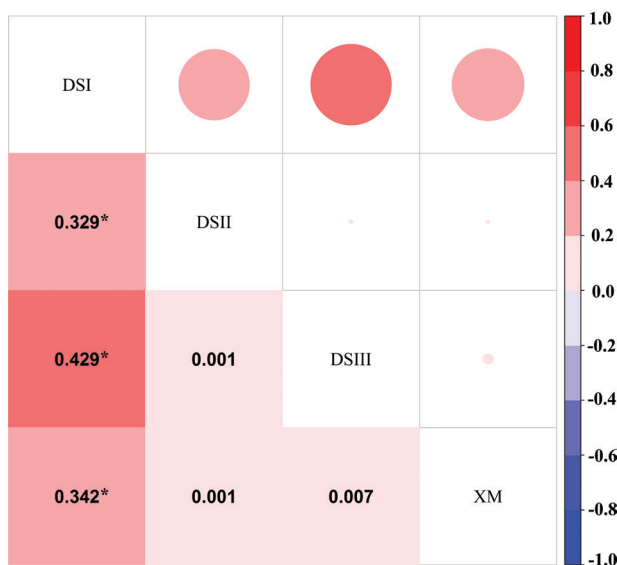

Figure 3. Matrix of pairwise F_{ST} values among four *Acanthopagrus latus* populations. *extremely significant at $P < 0.01$ by the permutation test.

AMOVA was performed to examine the population genetic structure of yellowfin seabream (Table 3). First, all yellowfin seabream populations were analyzed as a single gene pool. The results showed that the genetic differences in the yellowfin seabream populations mainly existed

Table 3. AMOVA of *Acanthopagrus latus* populations based on mtDNA CR sequences.

Source of variation	Percentage variation	F-Statistics	P
One gene pool (DSI, DSII, DSIII, XM)			
Among populations	22.92	0.2292	0.000 ± 0.000
Within populations	77.08		
Two gene pool (DSI, DSII, DSIII), (XM)			
Among groups	–19.17	–0.1917	1.000 ± 0.000
Among populations within groups	34.83	0.2923	0.000 ± 0.000
Within populations	84.34	0.1566	0.000 ± 0.000
Two gene pool (DSI), (DSII, DSIII, XM)			
Among groups	36.27	0.3627	0.253 ± 0.011
Among populations within groups	–0.09	–0.0015	0.420 ± 0.014
Within populations	63.83	0.3617	0.000 ± 0.000

DSI = Dongshan I population (wild individuals, 2009.11), DSII = Dongshan II population (wild individuals, 2019.10), DSIII = Dongshan III (cultured individuals, 2019.11), XM = Xiamen (wild individuals, 2019.10).

inside the population, accounting for 77.08% of all variation, while genetic differences among populations only accounted for 22.92%. To further confirm the population genetic structure of yellowfin seabream, (1) the four populations were grouped into two gene pools according to their geographical distribution, and the results showed that the genetic differences between groups were small with nonsignificant statistical test results ($P > 0.05$), and the genetic differences mainly existed within populations (84.34%) with very significant genetic differentiation ($P < 0.01$); however, the genetic differentiation among populations within groups was small while very significant ($P < 0.01$). (2) According to the F_{ST} values, the four yellowfin seabream populations were divided into two gene pools. The results showed that the genetic differences between groups were small and nonsignificant ($P > 0.05$); genetic differences mainly existed within populations (63.83%), with very significant genetic differentiation ($P < 0.01$), while the genetic differentiation among populations within groups was small and nonsignificant ($P > 0.05$).

Discussion

Due to the impact of ocean currents and climate and its unique geographical location, Dongshan Bay had relatively high biodiversity and rich fishery resources. However, in recent years, with the continuous growth of the human population, the development of surrounding areas and coastal industries, and the increasing development and utilization of sea areas, the impact on the ecology of this sea area has increased, and the habitats and spawning grounds of many economic species have been damaged. Moreover, the fishing pressure of this sea area is overloaded, resulting in a decline in fishery resources (Lin et al. 2013; Ye et al. 2018). To restore the fishery resources in Dongshan Bay, the government of Dongshan County organized the enhancement and release of a variety of economic species, yellowfin seabream is an important economic fish species. After nearly ten years of enhancement and release, to find the changes in the yellowfin seabream of Dongshan Bay, we selected yellowfin seabream samples in 2009 and 2019 for comparative analysis of genetic diversity. Additionally, in order to better reflect the changes in germplasm resources of yellowfin seabream in the past ten years, we also selected yellowfin seabream samples in an adjacent sea area, Xiamen, for reference comparison.

Under normal circumstances, when the number of artificially bred seedlings released into the natural seas is greater than the carrying capacity, an intraspecies competitive relation is formed within the released population, within the wild population, and between the released population and the wild population in which individuals compete for food and living space (Cooney and Brodeur 1998). Additionally, according to the previous study, a higher total allowable catch due to the expansion of coastal development in Dongshan Bay, especially the enhancement and release places more fishing pressure on the wild populations (Ye

et al. 2018). This pressure, to certain extent, further aggravates overfishing and may reduce the size of the wild populations, eventually leading to the loss of genetic and allelic diversity (Helfman 2008), such as *P. major* in the Gulf of Kagoshima, Japan (Hamasaki et al. 2010), and *A. schlegelii* in the Pearl River Estuary, China (Yang and Sun 2019).

However, the results of this study showed that the genetic diversity of yellowfin seabream was high in both wild and cultured populations. The genetic diversity of wild yellowfin seabream in Dongshan Bay in 2009 was largely the same as that in 2019, while that of the cultured population in 2019 was slightly lower (Table 1). The genetic diversity of the wild population in Xiamen in 2019 was basically the same as that in 2008 and slightly higher than that in 2002 (Xia et al. 2008). Such a high level of genetic diversity may be related to the biological reproduction habits of yellowfin seabream, which produce spawn in batches. The individual absolute fecundity of yellowfin seabream reaches as high as 300 000 to 2.38 million, with an average of 1.36 million, and the relative fecundity ranges from 740 to 5,757 spawn · g⁻¹, with an average of 2511 spawn · g⁻¹. Furthermore, its growth rate in natural waters is not lower than that of other fishes (Shi et al. 2012). These favorable characteristics ensure a large recruitment stock of yellowfin seabream in natural seas. Although artificial breeding of yellowfin seabream was successful in 1981, the current breeding seedlings of yellowfin seabream are mainly obtained by sea catch, and the proportion of artificial seedlings is very small (Jiang et al. 2012). Sea-caught seedlings are sold on the market every year from the end of November to December. The continuous seedling supply will ensure that the released population of yellowfin seabream has sufficient parents, avoiding a decline in genetic diversity caused by fewer parents and more offspring, which maintains a high genetic diversity of yellowfin seabream under the current situation of declining fishery resources.

Although the genetic diversity of yellowfin seabream in Dongshan Bay is high, the current fishery resources in Dongshan Bay have been critically overfished (Ye et al. 2018), and the situation is not optimistic. Yellowfin seabream is highly adaptable and can grow in both sea water and fresh water. However, it generally does not migrate long distances, and it is a hermaphroditic male precocious species that can maintain a certain proportion of male and female attributes in its life cycle, which may also be one of the reasons that this species can maintain high genetic diversity under the current situation of declining resources.

To date, many scholars have shown that breeding and release will induce certain genetic impacts on populations in natural seas and have discussed the advantages and dis-

advantages of the breeding and release of fishery resources from the perspective of protecting genetic diversity (Araki and Schmid 2010; Hamasaki et al. 2010). From the genetic structure of yellowfin seabream in this study, significant genetic differentiation can be seen between the 2009 population and the current population, and many historical haplotypes have been lost. Although the sampling strategy and the number of samples may have some impact on the results of this study, these results have revealed certain germplasm resource issues. The breeding and release work of Dongshan Bay placed a large number of seedlings in the natural seas, which restored the fishery resources and at the same time increased the fishing yield, resulting in greater fishing pressure on the yellowfin seabream and further damaged resources, forming a feedback loop. Although this fishing is not the 'sweeping' type, exogenous genetic influences, such as genetic changes caused by the lack of selection pressure and genetic drift, have led to the loss of many haplotypes and genetic and allelic diversity (Helfman 2008).

Campton (1995) proposed that if fisheries were not properly managed, the existing overfishing and continuous breeding and release would change the original population structure and eventually lead to irreversible genetic effects. The results of this study show that the genetic structure of yellowfin seabream in Dongshan Bay has changed. Although it still has high genetic diversity, fishery management of yellowfin seabream resources should attract the attention of management departments to develop specific measures and conservation policies for this species. In particular, yellowfin seabream is a hermaphroditic male precocious species. Targeting older fish as the main fishing subject is not conducive to its breeding protection and resource growth, and therefore, catching older fish should be restricted in fisheries.

Acknowledgment

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Effect of dietary lipid levels on growth, body composition, and enzyme activities of larvae of butter catfish, *Ompok bimaculatus* (Actinopterygii: Siluriformes: Siluridae)

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Abstract

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is a high-value catfish that has gained immense consumer preference in South-East Asia. However, information on the nutritional requirements of this species is scanty. Hence, an experiment was conducted to evaluate the effects of varying dietary lipid levels on growth, body composition, and activities of digestive and metabolic enzymes in larvae. Three isonitrogenous (40% crude protein) diets were formulated by supplementing fish and vegetable oil (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels (containing crude lipid 5.7%, 8.0%, and 10.45%, respectively in diets D1–D3) to a fish meal- and oilcake-based formulated diet. Experimental diets were fed to butter catfish larvae (0.15 ± 0.01 g) in triplicate groups for a period of 42 days. Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures (AOAC 2005). Digestive and metabolic enzyme activities were analyzed at the completion of the experiment by standard methodology. Butter catfish larvae fed the diet D2 (8% crude lipid) resulted in the best performance in terms of weight gain (final weight 1.40 ± 0.07 g), net weight gain (1.31 ± 0.06 g), specific growth rate ($5.50 \pm 0.05\% \cdot day^{-1}$), and protein efficiency ratio (2.39 ± 0.17). The highest lipid deposition ($2.90 \pm 0.12\%$) in the carcass was also recorded in fish reared on diet D2. The final weight, net weight gain, protein efficiency ratio, and specific growth rate were significantly ($P < 0.05$) higher in D2 having 8% lipid. Moisture and lipid contents of the whole body were significantly ($P < 0.05$) higher in larvae fed diet D2. Amylase activity in fish significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The maximum alkaline protease, pepsin, and lipase activities were noticed in the larvae fed diet D2. Progressive decrease in liver glucose-6-phosphate dehydrogenase activities and significant increase ($P < 0.05$) in the activities of neoglucogenic enzymes (glucose-6-phosphatase and fructose-1,6-bis phosphatase) were noticed with an increase in dietary lipid levels. Significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in the group fed diet D2. Results of the study indicated that 8% crude lipid in the diet could assure optimum growth and survival of butter catfish larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider application of formulated diets for the larviculture of *Ompok bimaculatus*.

Keywords

Introduction

The Indian butter catfish, *Ompok bimaculatus* (Bloch, 1794), is indigenous to the South East Asian countries (Giri et al. 2019) and has recently gained immense importance because of its good taste, high lipoprotein, low fat, soft bony structure, and competitive prices (Rawat et al. 2018). It is an excellent source of ω-3 and ω-6 fatty acids, vitamins, minerals, protein, and fat (Paul et al. 2018). The wild population of *O. bimaculatus* has sharply declined due to ecological changes and indiscriminate fishing. Thus, the species has been categorized under the "near threatened" category by the IUCN Red List and faces a risk of extinction in nature (Lakra et al. 2010; IUCN 2014). Considering high demand, price, and IUCN status, the species has been prioritized for diversification of aquaculture as well as for conservation and restocking programs (Debnath et al. 2016). Although its aquaculture potential has been realized, the species has not yet received adequate attention due to insufficient information on larval rearing and culture technology.

The successful culture of any fish species largely depends on the accessibility of nutritionally balanced practical diets. Although species of the genus *Ompok* have been generally recognized as carnivorous to omnivorous, reports on nutritional requirements of the species are scanty (Chakrabarti et al. 2012). Therefore, no commercially formulated diet has yet been available for this species. Since captive breeding of *O. bimaculatus* has been established (Raizada et al. 2013), it is necessary to develop larval diets to ensure growth and survivability of the species during the stages of early development, which is essential for reliable and regular supply of the fish for widespread commercial production. A previous study conducted on *O. bimaculatus* larvae determined a required level of 40% crude protein in the diets for this species (Paul et al. 2020).

However, dietary protein requirements are known to be affected by the amount of non-protein energy sources in the diet (NRC 2011). When non-protein energy is insufficient, a part of dietary protein may be catabolized to supply energy affecting the growth of the organism. Therefore, supplementation of energy-yielding nutrients, mainly lipid has been suggested as a strategy to improve protein utilization in fish (Sankian et al. 2017). Supplementation of lipid rather than carbohydrate as a source of non-protein energy is generally more effective for enhancing dietary energy level as lipid is an energy-dense nutrient that is readily metabolized by fish, particularly the carnivorous one (NRC 1993). Further, all-round development and well being of fish are known to be greatly influenced by dietary lipids that are not only important as an energy source but also for the supply of essential fatty acids as well as carrier of fat-soluble vitamins (Glencross 2009). Moreover, the incorporation of a proper amount of lipid seems to be important as lipid level determines the palatability of the diet (Boonyaratpalin 1991). Therefore, the presently reported study was conducted to determine the effects of dietary lipid levels on the growth, survivability, body composition, and activities of digestive as well as metabolic enzymes in butter catfish larvae.

Vegetable and fish oils are rich in different fatty acids, which were recognized as effective for diverse freshwater fish species (Paul et al. 2011). Hence, in the presently reported study, a practical diet was fortified with a combination of vegetable and fish oils (1:1) to have the desired lipid levels in the diets. The nutrient utilization and digestive physiology in fish are indicated by the activity of digestive and metabolic enzymes that ultimately affect the growth and development of fish (Chen and Zhang 2004; Wei et al. 2010). Therefore, the presently reported study considered an appraisal of digestive enzymes and some key metabolic enzymes to evaluate the effects of formulated diets with varying lipid levels. The results of the study could be helpful to provide some important information for feed formulation of *O. bimaculatus* larvae.

Materials and methods

Experimental diets

Three experimental diets were formulated by incorporating equal proportions of fish oil (cod liver oil) and vegetable oil (sunflower oil) (1:1) at 4.5% (D1), 7% (D2), and 9.5% (D3) levels to a basal mixture of fish meal (**FM**), soybean meal (**SBM**), and groundnut oil cake (**GNOC**). After analysis of lipid content of the feed, it was noticed to contain 5.7%, 8.0%, and 10.5% crude lipids, respectively. The amount of lipid sources used was adjusted at the expense of wheat flour. A vitamin–mineral premix was added to the diets as per Paul et al. (1997). Dietary ingredients were finely powdered, sieved to obtain uniform particle size (<400 μm in diameter), mixed thoroughly, and fortified with a calculated amount of vitamin-mineral premix and oil sources. The prepared powered feeds were stored in a freezer at –20°C until use.

Experimental fish and feeding trial

The experiment was conducted at the Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Rahara, Kolkata. Farm-raised larvae of the butter catfish were collected from ICAR-Central Institute of Freshwater Aquaculture, Kalyani Field Station, and acclimatized to the laboratory condition for one week in fiber-reinforced plastic (**FRP**) tanks with the provision of continuous aeration. During this period the larvae were fed a basal formulated diet and natural food (mixed zooplankton and chopped tubifex). After acclimatization, the larvae (mean weight 0.15 ± 0.02 g; length 22.65 ± 1.70 mm; 14 days old) were randomly distributed in 9 FRP tanks at a stocking density of 50 fish per tank. Thus, there were three replicates for each dietary group. The experiment was conducted in 150 L FRP tanks, each

containing 50 L of water, with continuous aeration and water exchange at every 5 days interval. The powdered feed mixtures were made to soft dough with distilled water and the fish were fed ad libitum to apparent satiation twice daily, at 10.00 and 16.00 h, for 42 days. Feed consumption and mortality in each tank were recorded separately, and the survival rate was calculated. During the experimental period, water quality parameters were monitored on weekly basis following the standard methods of the American Public Health Association (APHA 2005) and noticed to vary within the acceptable range (temperature 28–30°C; pH 6.8–7.7, dissolved oxygen 6.8–7.4 mg · L^{-1}, total alkalinity 230–240 mg · L^{-1}, ammonia 0.26–0.64 mg · L^{-1}, nitrite 0.001–0.003 mg · L^{-1}, nitrate 0.002–0.074 mg · L^{-1}).

Proximate composition of experimental diets and fish carcass

Proximate compositions of the experimental diets, as well as fish carcass, were analyzed using standard procedures portrayed by the Association of Official Analytical Chemists (AOAC 2005). Moisture content was determined by oven drying (initially at 100 ± 5°C for 30 min, thereafter at 60°C); crude protein (Nitrogen × 6.25), by a semi-automatic digestion system together with micro Kjeldahl distillation Unit (KelPlus-Elite Ex, Pelican Equipments, Chennai, India); crude lipid (ether extract; petroleum ether, 60–80°C), by a Soxhlet apparatus (Socsplus, Pelican Equipments, Chennai, India); and ash, by combustion at 550°C in a muffle furnace. Nitrogen-free extract (NFE) was calculated by subtracting the sum of values for crude protein, crude lipid, ash, crude fiber, and moisture from 100 (Maynard et al. 1979). The gross energy of the diets was measured with a bomb calorimeter (Lab-X, Kolkata, India). Proximate analyses of the fish carcass (whole body) were done on wet weight basis.

Growth parameters

At the end of the feeding trial fish were collected from each tank, weighed, and analyzed for calculating the growth parameters. Net weight gain [%], specific growth rate (SGR [%·day^{-1}]), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), and survivability [%] were calculated following standard methods described by Castell and Tiews (1980). The daily growth coefficient (DGC) was calculated as per Cowey (1992).

Estimation of digestive enzymes

Digestive enzymes (amylase, alkaline protease, pepsin, and lipase) of fish from each experimental set were estimated at the termination of the experiment. For each replicate, digestive tracts of 20 experimental fish from each tank were dissected out, washed thoroughly with chilled distilled water, taken on an ice-cooled Petri plate, and weighed. A 10% homogenate with chilled 0.1 (M) phosphate buffer (pH 7) was prepared and centrifuged at 10 000 rpm (10 min, 4°C). The ensuing supernatant was used as the enzyme extract to appraise the activities of the digestive enzymes. The protein content of the extract was estimated after Lowry et al. (1951) using bovine serum albumin (BSA) as standard. Amylase (α-amylase) activity was determined using dinitro salicylic acid (DNSA) reagent following Bernfeld (1955). Amylase activity (unit) was expressed as mg maltose liberated mg^{-1} protein h^{-1}. Alkaline protease activity was estimated using Hammerstein casein substrate according to Walter (1984). One unit of enzyme activity was defined as μg of tyrosine liberated mg^{-1} protein h^{-1}. Pepsin activity was resolved after Anson (1938) with minor modifications, using 2% hemoglobin as a substrate. The specific activity was expressed as μg of tyrosine liberated mg^{-1} protein min^{-1}. Lipase activity was determined with the olive oil substrate following Bier (1955). Lipase activity was expressed as μ mole of fatty acid liberated mg^{-1} protein h^{-1}.

Estimation of metabolic enzymes

Following the collection of the digestive tracts, hepatic tissues were removed, collected separately and a 10% homogenate was made in sucrose solution (0.25 M, pH 7.4). Remains of the cell along with nuclei were removed by centrifugation (1000 g, 30 min, 4°C), and the supernatants were further centrifuged (10 000 g, 15 min, 4°C) to get the mitochondrial pellets (Biswas et al. 2006). The resultant supernatant was again centrifuged (12 500 g, 1 h, 4°C) and the cytosolic fraction thus obtained was used as the crude enzyme extract for other metabolic enzyme assays. The mitochondrial pellet was treated with triton X-100 (0.1%), washed with PBS (0.1 M, pH 7.4) and the supernatant was used as crude extracts for mitochondrial metabolic enzyme assays. The tissue fractions were kept at –20°C until use. The soluble protein content of the crude enzyme extracts was determined following Lowry et al. (1951).

Hexokinase (HK) activity was measured by the reduction of NADP to produce NADPH according to Tranulis et al. (1996). Enzyme activity was expressed as μM of NADPH formed mg^{-1} protein h^{-1}. Pyruvate kinase (PK) activity was assayed after Driedzic and Almeida-Val (1996) with minor modification. Enzyme activity was presented as μmole of pyruvate converted to NADH mg^{-1} protein min^{-1}. Glucose-6-phosphatase (G6P) and fructose-1, 6-bis phosphatase (FBP) activities were measured by estimating the amount of phosphorus released from the substrates, glucose-6-phosphate (Marjorie 1964) and fructose-di-phosphate (Freeland and Harper 1959), respectively. Release of phosphorus by both the enzymes was estimated after Fiske and Subbarow (1925), and activities were expressed as μg of phosphorus released mg^{-1} protein min^{-1}.

Glucose-6-phosphate dehydrogenase (G6PD) activity was analyzed using glucose-6-phosphate (substrate) and NADP following Kornberg and Horecker (1955). Enzyme activity was expressed as µM of NADPH formed mg^{-1} protein h^{-1}. NADP-malic enzyme (NADP-ME) activity was determined using L-malic acid as substrate (Hsu and Lardy 1967, modified by Murphy and Walker 1974). Enzyme activity was presented as µM of NADPH formed mg^{-1} protein h^{-1}. Lipid peroxidation (LPO) activity was measured according to Okhawa et al. (1979). Enzyme activity was expressed as thiobarbituric reactive substance (TBARS) formed mg^{-1} protein min^{-1}.

Alanine transaminase (ALT) activity was determined using α-ketoglutarate and DL-Alanine as substrates (Reitman and Frankel 1957). ALT activity was expressed as µM of pyruvate formed mg^{-1} protein min^{-1}. Likewise, Aspartate transaminase (AST) activity was measured with the substrate solution containing α-ketoglutarate and DL-aspartic acid (Reitman and Frankel 1957). AST activity was expressed as µM of oxaloacetate formed mg^{-1} protein min^{-1}. Glutamate dehydrogenase (GDH) activity of the crude mitochondrial enzyme extract was measured using sodium glutamate and tetrazolium salt (Lee and Lardy 1965). Enzyme activity was expressed as µM of formazan formed mg^{-1} protein h^{-1}.

Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) as per Snedecor and Cochran (1994) to calculate the effect of dietary lipid level on growth performance and activities of the digestive as well as metabolic enzyme of fish and the least significance (LSD) was used for comparison of the mean values. Data are presented as treatment mean ± standard error of the mean (SE).

Results

The ingredients and proximate composition of the experimental diets are presented in Table 1. Experimental diets were isoproteinous (crude protein ≈ 40%). However, supplementation of fish oil and vegetable oil has led to varying crude lipid levels in the diets (D1–D3) as 5.7%, 8.0%, and 10.45%, respectively. All experimental diets were readily accepted by the *O. bimaculatus* larvae.

The growth performance of *O. bimaculatus* larvae fed varying levels of dietary lipid for 42 days is depicted in Table 2. The growth of the larvae was significantly ($P < 0.05$) affected by the dietary crude lipid levels. The net weight gain (%) of the larvae showed an increasing trend with increasing levels of the dietary lipid up to 8% and thereafter decreased. Butter catfish larvae fed diet D2 containing 8% crude lipid had the highest weight gain, which was significantly different ($P < 0.05$) from other dietary lipid levels. The highest values of PER and ANPU were recorded in fish fed diet D2. The value of FCR was

the lowest for fish fed diet D2, however, didn't differ significantly between the diets D1 and D3. Butter catfish larvae in all treatment groups survived well (more than 80%) during the experimental period, and there were no significant differences among the groups.

Proximate carcass compositions of the butter catfish larvae fed experimental diets are presented in Table 3.

Table 1. Feed formulation and proximate composition (% DM Basis) of the experimental diets.

Parameter	Experimental diet		
	D1	D2	D3
Fish meal	53.00	53.0	53.00
Groundnut oil cake	15.00	15.0	15.00
Soybean meal	10.00	10.0	10.00
Wheat flour	10.50	8.0	5.50
Carboxy methyl cellulose	2.00	2.0	2.00
Fish:Veg. oil (1:1)	4.50	7.0	9.50
Vitamin-mineral mix*	5.00	5.0	5.00
Proximate composition [% DM basis]			
Dry matter	92.85 ± 0.06	92.37 ± 0.23	92.06 ± 0.05
Crude protein	40.46 ± 0.06	40.18 ± 0.49	40.61 ± 0.83
Crude lipid	5.70 ± 0.20	8.00 ± 0.25	10.45 ± 0.45
Total Ash	14.40 ± 0.30	15.40 ± 0.20	16.50 ± 0.30
Nitrogen free extracts	29.50 ± 0.37	27.61 ± 2.05	21.80 ± 0.28
Crude protein:crude fat	7:1	5:1	4:1
Energy [kJ g^{-1}]	13.85 ± 0.02	14.09 ± 0.08	14.39 ± 0.08

*Vitamin-mineral premix contains: Vitamin A (as acetate) 5000 I.U., cholecalciferol 1000 I.U., thiamine monocitrate 10 mg, riboflavin 10 mg, pyridoxine hydrochloride 5 mg, cyanocobalamine 15 µg, nicotinamide 75 mg, calcium pantothenate 10 mg, ascorbic acid 150 mg, α-tocopheryl acetate 25 mg, biotin 5 mg, folic acid 5 mg, menadione 100 mg, choline chloride 50 mg, PABA 5 mg, myoinositol 10 mg, calcium lactate 0.125, magnesium oxide 60 mg, dried ferrous sulphate 30 mg, manganese sulphate 2 mg, copper sulphate 2 mg, zinc sulphate 2 mg, sodium molybdate 0.25 mg, sodium bortate 0.80 mg, potassium iodate 20 mg, bicalcium phosphate 0.10 g, cobalt chloride 20 mg (Paul et al. 1997).

Table 2. Growth performance in *Ompok bimaculatus* larvae fed with graded levels of lipid.

Parameter	Experimental diet		
	D1	D2	D3
Initial weight [g]	0.15 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
Final weight [g]	1.10 ± 0.12[a]	1.40 ± 0.07[b]	1.06 ± 0.03[a]
Net weight gain	0.95 ± 0.12[a]	1.31 ± 0.06[b]	0.91 ± 0.03[a]
Specific growth rate [%]	4.73 ± 0.35[a]	5.50 ± 0.05[b]	4.66 ± 0.22[a]
Daily growth coefficient	0.73 ± 0.02[a]	1.003 ± 0.05[b]	0.76 ± 0.09[a]
Survivability	83.85 ± 6.15	83.85 ± 6.15	79.55 ± 5.46
Number of dead fish	25	25	31
Feed conversion ratio	1.86 ± 0.10[b]	1.39 ± 0.05[a]	1.74 ± 0.07[b]
Protein efficiency ratio	1.31 ± 0.09[a]	2.39 ± 0.17[b]	1.30 ± 0.08[a]
Apparent net protein utilization	16.09 ± 0.92[a]	23.19 ± 1.10[b]	17.18 ± 0.82[a]

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Table 3. Carcass composition [g · 100 g^{-1}] of *O. bimaculatus* larvae fed different levels of lipid.

Constituent [g·100 g^{-1}]	Experimental diet		
	D1	D2	D3
Moisture	79.37 ± 0.09[a]	80.93 ± 0.22[b]	79.80 ± 0.17[a]
Crude protein	13.93 ± 0.09	14.40 ± 0.21	14.03 ± 0.08
Crude lipid	2.50 ± 0.06[a]	2.90 ± 0.12[b]	2.77 ± 0.07[b]
Ash	1.70 ± 0.06	1.97 ± 0.09	1.80 ± 0.06

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).
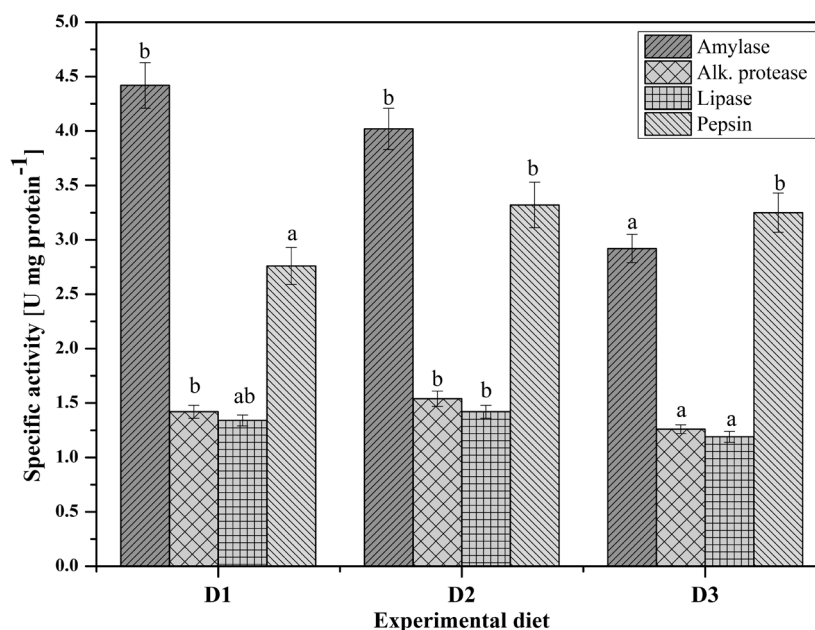
Figure 1. Specific activity of digestive enzymes of *Ompok bimaculatus* larvae fed varying levels of crude lipid. Mean values (± SE) with no common superscript letters are significantly different ($P < 0.05$).

Moisture and lipid contents of the whole body were significantly ($P < 0.05$) affected by the dietary lipid levels, being the highest in larvae fed diet D2 containing 8% crude lipid. However, varying dietary lipid had no significant effect on crude protein and ash contents of *O. bimaculatus* larvae at the tested lipid levels.

Digestive enzymes i.e., amylase, alkaline protease, lipase, and pepsin of the butter catfish larvae fed diets with varied lipid levels are given in Fig. 1. Overall, digestive enzymes were significantly ($P < 0.05$) affected by the dietary lipid levels. Amylase activity in *O. bimaculatus* larvae significantly ($P < 0.05$) decreased with increasing dietary lipid levels. The highest alkaline protease and lipase activities were noticed in the butter catfish larvae fed diet D2 consisting of 8% crude lipid, while it was not significantly ($P < 0.05$) different from the group fed diet D1. The highest pepsin activity was also documented in the fish fed diet D2, although it did not differ significantly from the larvae that received diet D3 with 10.45% dietary lipid.

Activities of the hepatic enzymes involved in the intermediary metabolism of carbohydrate, protein, and lipid are depicted in Table 4. Varying dietary lipid levels led to significant differences ($P < 0.05$) in the activities of PK, G6P, FBP, G6PD, LDH, ALT, and AST in *O. bimaculatus* larvae. While no significant differences were detected in the activities of HK, NADP-ME, GDH, and LPO, the activities of PK and two major neoglucogenic enzymes, G6P and FBP, significantly increased ($P < 0.05$) with the increase in the dietary lipid levels. The activity of G6PD, a key enzyme of lipogenesis, revealed a significant decrease ($P < 0.05$) with an increase in the dietary lipid level from 5.7% (D1) to 8% (D2). Further, significantly lower ($P < 0.05$) activities of LDH, ALT, and AST were recorded in *O. bimaculatus* larvae fed diet D2 with 8% dietary lipid.

Table 4. Specific activity [U mg protein^{-1}] of metabolic enzymes of *Ompok bimaculatus* larvae fed varying levels of lipid.

Enzyme	Experimental diet		
	D1	D2	D3
Hexokinase	9.82 ± 0.35	10.27 ± 0.47	10.54 ± 0.51
Pyruvate kinase	5.6 ± 0.24[a]	6.2 ± 0.27[b]	6.4 ± 0.29[b]
Lactate dehydrogenase	0.845 ± 0.03[c]	0.507 ± 0.02[a]	0.690 ± 0.03[b]
Malate dehydrogenase	2.35 ± 0.11	2.24 ± 0.09	2.20 ± 0.11
Glucose 6 phosphatase	4.05 ± 0.13[a]	4.38 ± 0.17[ab]	4.62 ± 0.22[b]
Fructose 1,6 bis phosphatase	3.10 ± 0.11[a]	3.42 ± 0.14[ab]	3.72 ± 0.15[b]
Alanine aminotransferase	3.88 ± 0.16[b]	3.55 ± 0.09[a]	3.78 ± 0.12[b]
Aspartate aminotransferase	6.55 ± 0.17[b]	6.15 ± 0.12[a]	6.45 ± 0.14[b]
Glutamate dehydrogenase	5.12 ± 0.20	5.20 ± 0.23	5.28 ± 0.27
Glucose-6-phosphate dehydrogenase	32.6 ± 0.81[b]	27.5 ± 0.76[a]	26.4 ± 0.72[a]
Lipid peroxidation	0.92 ± 0.06	0.96 ± 0.004	1.02 ± 0.006

Data are presented as mean ± standard error of the mean; mean values with different superscripts in a row differ significantly ($P < 0.05$).

Discussion

During the experimental rearing of the *O. bimaculatus* larvae, water temperature varied within a narrow range (28–30°C) that was considered suitable since a temperature of around 30°C was suggested as optimum for the growth of catfish (Paul and Giri 2016). Other water quality parameters were also within the acceptable range as recommended elsewhere (Paul et al. 2000; Debnath et al. 2016). Apart from environmental factors, rearing of early larval stages under captive condition depends mostly on the availability of suitable diets that are readily acceptable and consists of nutrients at the required level to support growth and well being of the fish. Different larval stages of fish may have specific nutritional requirements (Malla and Banik 2015). Digestive systems of fish larvae are immature and therefore they depend on live food organisms to a great extent for the supply of exogenous

enzymes. Generally, fish larvae do not prefer artificial diets, even if larviculture with formulated diets is essential for large-scale production of any species. The limited success of the dry formulated diets in larval rearing might be attributed to insufficient feed intake, imbalanced protein (non-protein energy sources), impaired digestive, as well as metabolic functions (Lee et al. 2002).

In the presently reported study, formulated diets were readily accepted by the 14 day old *O. bimaculatus* larvae. The study suggests that 8% lipid in a diet with 40% crude protein might support the growth and survivability of the butter catfish larvae during early development. The required lipid level detected in the presently reported study was close to the suggested lipid levels documented for other catfishes. For example, 6.5% and 7% optimum dietary lipid requirements were reported for *Ompok pabda* (Hamilton, 1822) fry (Paul et al. 2011) and *Mystus montanus* (Jerdon, 1849) (see Raj et al. 2007), respectively. Among carps, 6.5% lipid in the diets of *Ctenopharyngodon idella* (Valenciennes, 1844) (see Jin et al. 2013) and 7% lipid for the juveniles of common carp, *Cyprinus carpio* Linnaeus, 1758 (see Choi et al. 2015) supported maximum growth. On the contrary, elevated lipid requirements have also been suggested. For example, lipid levels of 10% for larvae of magur, *Clarias batrachus* (Linnaeus, 1758) (see BIS 2014b) and 17% for far eastern catfish, *Silurus asotus* Linnaeus, 1758 (see Kim et al. 2012) were reported. Therefore, the majority of the preceding studies suggested varying lipid requirement levels between 6% and 10% in diverse fish species, with few exceptions. Hence, the presently reported study considered this narrow level of variation for evaluation of the lipid levels. The ability of the fish to use lipid as a source of energy was noticed to vary among diverse fish species (Jauncey (1982)). Thus, different fish species at different life stages might require different dietary lipid levels and it needs to be evaluated separately for individual fish species. Our results were in agreement with some of the previous reports depicting 8% lipid requirement as optimal for a minor carp, *Barbonymus gonionotus* (Bleeker, 1849) (see Paul et al. 2010) and fingerlings of rohu, *Labeo rohita* (see Mishra and Samantaray 2004). BIS (2014a) also suggested an 8% crude lipid requirement for carp spawn and fry.

The presently reported study revealed that an increase in the dietary lipid level from 5.7% to 8% was associated with maximum growth and increased SGR [% · day−1] of the butter catfish larvae. Similarly, the lowest FCR and the maximum PER and ANPU values were recorded in the larvae fed diets with 8% crude lipid (D2). Our result was in compliance with the preceding reports indicating that increase in the dietary lipid up to a certain level might aid in efficient protein utilization that results in improved growth of the fish (Jauncey 1982; Kim et al. 2012). Similar results were recorded for the stinging catfish, *Heteropneustes fossilis* (Bloch, 1794) (see Akand et al. 1991) and rohu, *L. rohita* (see Mishra and Samantaray 2004). In contrast to these observations, high dietary lipid might cause to reduce fish growth, as documented for gibel carp,

Carassius gibelio (Bloch, 1782); and Chinese long snout catfish, *Leiocassis longirostris* Günther, 1864 (see Pei et al. 2004). In the presently reported study, the group of larvae fed diet D3 with 10.45% crude lipid was associated with poor growth, which was in agreement with Pei et al (2004). When the non-protein energy source in the diet becomes insufficient or inaccessible, the protein is used as a source of energy instead of growth (Mohanta et al. 2009). In the presently reported study, the groups reared with diets D1 (5.7% lipid) and D3 (10.45% lipid) portrayed relatively poor growth that might be indicative of poor utilization of the non-protein energy source (Wang et al. 2018). Further, in the presently reported study, around 80% survivability of the butter catfish larvae was achieved with the formulated diets during the feeding trial. Previously, 52.18% and 45.82% survivability of the *O. bimaculatus* larvae with egg custards and compound feed was documented by Malla and Banik (2015), which was relatively lower than the presently reported findings. Improved survivability accomplished in the presently reported study could be due to improved feed utilization by the larvae.

An increase in dietary lipid levels seems to be an important consideration for the food fishes as it might have a significant effect on the carcass quality (Cowey 1993). There might be a positive correlation between lipid levels in the diets and carcass lipid deposition (Cowey 1993), which was in harmony with the presently reported study. Similar observations have been recorded in several species, e.g., rockfish, *Sebastes schlegelii* Hilgendorf, 1880 (see Lee et al. 2002); Eurasian perch; *Perca fluviatilis* Linnaeus, 1758 (see Mathis et al. 2003); cobia, *Rachycentron canadum* (Linnaeus, 1766) (see Craig et al. 2006); and grouper, *Epinephelus malabaricus* (Bloch et Schneider, 1801) (see Williams 2007). On the contrary, Paul et al. (2011) could not find any difference in carcass lipid in another species of butter catfish, *O. pabda* by feeding different levels of lipid. In the presently reported study, carcass protein content was not significantly affected by the dietary lipid levels, which was consistent with previous reports on the juveniles of pike perch, *Sander lucioperca* (Linnaeus, 1758) (see Schulz et al. 2008) and cobia (Webb et al. 2010). Overall, the carcass composition of the *O. bimaculatus* larvae detected in the presently reported study was similar to the previous report by Debnath and Sahoo (2013).

Although the ontogeny of the digestive enzymes during the early development of the *O. bimaculatus* has been documented by some authors (Pradhan et al. 2013; Chowdhury et al. 2019), to the authors' knowledge, there is no information on the diet-related changes in the digestive enzymes in the butter catfish. Adaptations of the digestive system in different species exhibit close association with their diet (Fernandez et al. 2001). Thus, changes in digestive enzyme activity could be correlated with the biochemical composition of food and feeding behavior of fish (Kuzmina 1996). In the presently reported study, amylase activity in *O. bimaculatus* larvae was noticed to be significantly decreased with elevated dietary

lipid levels. Previously, amylase activity in gilthead sea bream, *Sparus aurata* Linnaeus, 1758, was noticed to be influenced by dietary lipid levels (Fountoulaki et al. 2005). While, maximum activities of the alkaline protease, pepsin, and lipase were recorded with the group that was fed 8% lipid (D2) and achieved the highest growth. Digestive enzymes might contribute towards efficient digestion of the dietary components, which could be reflected through the growth of the fishes. Thus, increased growth in fish (fed 8% dietary lipid) associated with enhanced digestive enzyme activities might be indicative of better nutrient utilization in fish as stated elsewhere (Mandal and Ghosh 2018).

The presently reported study appraised activities of some major metabolic enzymes to evaluate the effects of the varying dietary lipid levels. Activities of the amino acid catabolizing enzymes were influenced by the dietary lipid levels. The fish liver is the hotspot for transdeamination with ALT and AST as the major enzymes (Enes et al. 2006; Kumar et al. 2008). A decrease in the activities of ALT, AST, and LDH might suggest reduced protein catabolism in fish fed diet D2 with 8% lipid. LDH is the enzyme of the glycolytic pathway that mediates the bidirectional conversion of pyruvate to lactate. A hike in LDH activity could be noticed under stress (Chatterjee et al. 2006). Thus, reduced LDH activity in the fish reared on D2 might indicate no or negligible stress on the experimental fish. In the presently reported study, increased activities of the gluconeogenic enzymes (G6P and FBP) coincided with an increase in the dietary lipid levels. Gluconeogenesis is a major pathway for glucose homeostasis, where glucose is produced from non-carbohydrate precursors (e.g., amino acid, lactate, glycerols). Increased activity of the neoglucogenic enzymes associated with decreased activity of digestive amylase might be indicative of the production of glucose by gluconeogenesis to meet the energy demand in this carnivorous species. No significant variation was noticed in the activity of the major glycolytic enzyme, HK. G6PD is the key enzyme catalyzing the first step of the HMP-shunt (pentose phosphate pathway) that generates NADPH for lipogenesis and stress management (Pandolfi et al. 1995). In the presently reported study, the activity of the lipogenic enzyme (G6PD) was inhibited by an increase in the dietary lipid, which was similar to the observations recorded in juveniles of Senegalese sole, *Solea senegalensis* Kaup, 1858 (see Dias et al. 2004; Guerreiro et al. 2012). Further, NADP-ME, GDH, and LPO activities were more or less unaffected by the dietary lipid levels. NADP-ME is responsible for NADP-dependent oxidative decarboxylation (malate to pyruvate and carbon dioxide) with the generation of NADPH that may be utilized for lipid biosynthesis, while GDH had been considered as a sensitive indicator of stress (Susan et al. 2010). Therefore, the results of the presently reported study might suggest that increased dietary lipid levels are neither required to augment lipid biosynthesis by the fish nor to induced stress on the experimental fish.

Conclusion

Results of the presently reported study indicated that 8% crude lipid in the diet with 40% crude protein might assure optimum growth and survival of *Ompok bimaculatus* larvae during early development. An appraisal on growth, body composition, and digestive as well as metabolic function in the butter catfish larvae recorded in the study might provide some important information to consider the application of formulated diets for the larviculture of *Ompok bimaculatus*.

Acknowledgments

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PENSOFT.

ACTA ICHTHYOLOGICA ET PISCATORIA

Grunts (Actinopterygii: Perciformes: Haemulidae) of Bangladesh with two new distributional records from the northern Bay of Bengal assessed by morphometric characters and DNA barcoding

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Abstract

Grunts (family Haemulidae) are among the most commercially important fish in Bangladesh. This paper provides brief diagnostic characters of five previously reported grunt species: *Plectorhinchus pictus* (Thunberg, 1792); *Pomadasys andamanensis* McKay et Satapoomin, 1994; *Pomadasys argenteus* (Forsskål, 1775); *Pomadasys argyreus* (Valenciennes, 1833); *Pomadasys maculatus* (Bloch, 1793) and two new distributional records: *Pomadasys guoraca* (Cuvier, 1829) and *Plectorhinchus macrospilus* Satapoomin et Randall, 2000. The fishes were collected from the Saint Martin's Island coral reef-like ecosystem and the adjacent sea of the Sundarbans mangrove forest of Bangladesh. The examined specimens were identified and diagnosed based on their morphometric characters and DNA barcoding COI gene. The new records of *Pomadasys guoraca* and *Plectorhinchus macrospilus* from Bangladesh greatly extend their distributional range in the Bay of Bengal. An updated checklist of grunts of Bangladesh is provided.

Keywords

Saint Martin's Island, Sundarbans, Bangladesh, morphology, COI barcoding gene

Introduction

The family Haemulidae Gill, 1885 (commonly known as grunts), comprises 134 valid species representing 19 genera, distributed worldwide (Randall 1995; Nelson et al. 2016; Froese and Pauly 2020; Fricke et al. 2021a). They are known as grunts for producing sounds by rubbing their pharyngeal teeth together (Tavera et al. 2012). Grunts inhabit both hard and soft bottoms of nearshore tropical, subtropical, brackish, and warm temperate waters (McKay 1984; Randall 1995; Froese and Pauly 2020). Generally, they tend to gather during the day and forage at night (Tavera et al. 2012; Froese and Pauly 2020).

The Haemulidae comprises two subfamilies: Haemulinae Gill, 1885 which includes 92 valid species and the Plectorhinchinae Jordan et Thompson, 1912 which
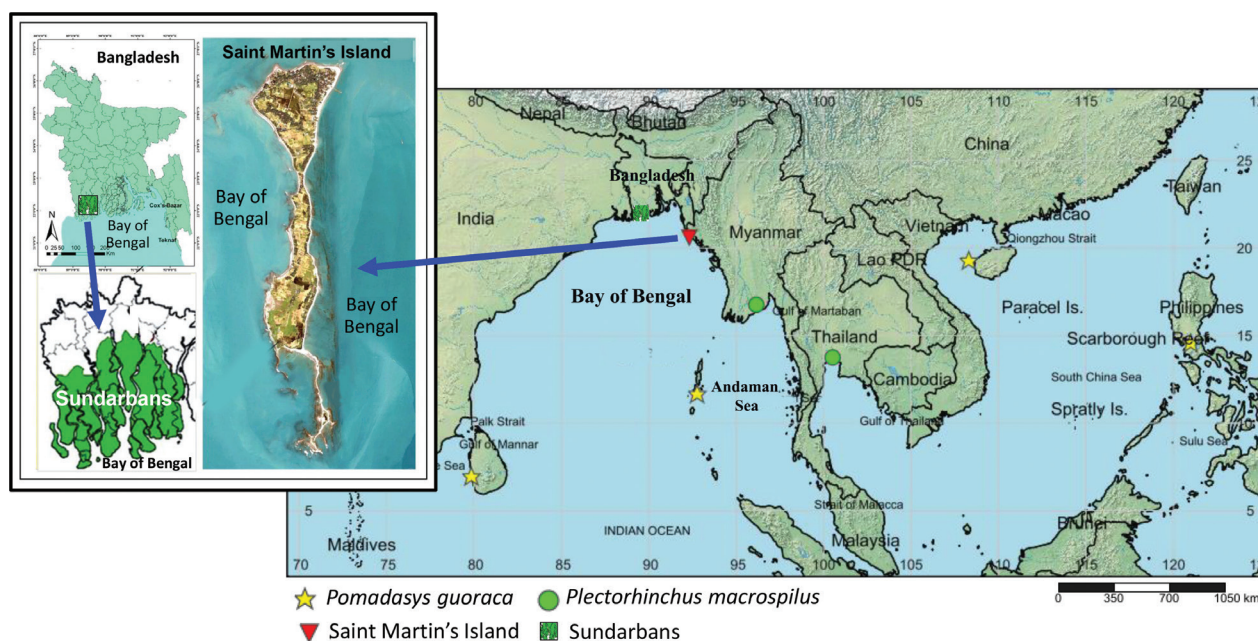
Figure 1. Map showing previous distribution of newly recorded fish species *Pomadasys guoraca* (★) and *Plectorhinchus macrospilus* (●) in the presently reported study, and the location of the Saint Martin's Island (▼) and Sundarbans (▧) of Bangladesh in the northern Bay of Bengal from where the species were collected.

has 42 species (Fricke et al. 2021a). These two groups differ greatly in diversity and distribution. Haemulines are identified by having a short dorsal fin with 12–16 soft rays in the majority of the genera; possess two chin pores and a median chin groove; generally, inhabit tropical and temperate reefs on sandy and muddy bottoms and are distributed in the New World (Johnson 1980; Smith and Heemstra 2012; McKay 2001). On the other hand, the Plectorhinchinae, generally known as sweetlips, are characterized by having a long dorsal fin with 15–26 soft rays, 4–6 chin pores without median pit; and are primarily found around coral and rocky reefs in the tropical Indo-Pacific, western Atlantic and Mediterranean (Talwar and Kacker 1984; McKay 2001; Smith and Heemstra 2012).

Ten valid species of grunts have been reported previously from the marine waters of Bangladesh (Hussain 1970; Rahman et al. 2009; Thompson and Islam 2010; Baki et al. 2017; Fanning et al. 2019; Froese and Pauly 2020; Naznin et al. 2020). Based on collected specimens, we herein provide brief diagnoses of seven of these grunts, including two new records for Bangladesh. Additionally, we provide a brief diagnosis of six of the seven collected species based on mitochondrial cytochrome c oxidase subunit I (COI) DNA barcode gene sequences.

Materials and methods

Sample collection

The presently reported survey was carried out from July 2016 through September 2018. The grunt specimens were collected from Saint Martin's (St. Martin's) Island and the

adjacent seaward side of the Sundarbans mangrove forest of Bangladesh (Fig. 1). All of the specimens were photographed and tagged on site. The collected specimens were then transferred and preserved at the Aquatic Bioresource Research Laboratory (ABR Lab), Sher-e-Bangla Agricultural University (SAU) in Dhaka, Bangladesh.

Morphological study and species deposition

The morphological study was undertaken using reference works of Talwar and Kacker (1984), McKay and Satapoomin (1994), Satapoomin and Randall (2000), and McKay (2001). All measurements were taken with Vernier calipers to the nearest 0.1 mm. After completing the morphological study, a piece of fresh muscle tissue was collected from each individual and preserved in 98% ethanol for molecular analysis. All of the examined specimens are deposited in the ABR Lab of SAU.

Genetic analysis

Genomic DNA was extracted from the collected muscle tissue using a TIANamp Marine Animals DNA Kit (TIANGEN) following the manufacturer's protocol. The partial fragment of the mitochondrial (mtDNA) cytochrome c oxidase subunit I (COI) gene was amplified with the primers FishF2 (5′-TCGACTAATCATA-AAGATATCGGCA-3′), and FishR2 (5′- ACTTCAGG-GTGACCGAAGAATCAGAA-3′) (Ward et al. 2005). Polymerase chain reaction (PCR) was performed in a 50 μL reaction mixture in 0.2 mL small reaction tubes in a

thermal cycler (2720 Thermal Cycler, Applied Biosystems). The PCR condition profile consisted of a preheating at 95°C for 2 min followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 54°C for 40 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR samples with a single and clear visible band were purified with the PCR Purification Kit (TIANGEN-Universal DNA Purification Kit). Sequencing was conducted with the same PCR primers by the Sanger standard method with automated sequencing (ABI 3730x1 DNA analyzer) at Macrogen Inc. (Korea). Nucleotide sequences were edited and aligned using the bioinformatics software MEGA-7 (Kumar et al. 2016). The obtained COI Sequences were checked using BLAST search engine provided by National Center for Biotechnology Information (NCBI) and Bold database. Finally, the consensus sequences obtained from collected specimens through DNA sequencing were submitted to GenBank.

Phylogenetic analysis was performed using Maximum likelihood (ML) methods through IQ Tree (Nguyen et al. 2015; Trifinopoulos et al. 2016) with bootstrap analysis of 10 000 replications. ML tree was visualized using Figtree v1.4.3 and edited by Adobe Illustrator. We used the evolutionary model TPM2u+F+G4 in the phylogenetic analysis obtained as the best-fit model using the program Modelfinder (Kalyaanamoorthy et al. 2017). This model was selected by applying the Bayesian information criterion. Two sequences of *Lethrinus nebulosus* (Forsskål, 1775) and *Gymnocranius griseus* (Temminck et Schlegel, 1843) retrieved from GenBank were used as outgroups in the phylogeny. Kimura-2 parameter (K2P) distance model (Kimura 1980) was used for calculating the genetic distance among the sequences using MEGA-7. Nucleotide and haplotype diversity and polymorphic sites were analyzed by DNASP (Librado and Rozas 2009).

Conservation status

The conservation status of many species of grunts has been published in the IUCN Red List of threatened species (https://www.iucnredlist.org/species/123439745/123494892) and are included here. The remaining assessments of species, listed here as 'not yet assessed', are scheduled for publication later in 2021.

Results

In the material collected within the presently reported study, we identified five previously recorded grunt species: *Plectorhinchus pictus* (Thunberg, 1792); *Pomadasys andamanensis* McKay et Satapoomin, 1994; *Pomadasys argenteus* (Forsskål, 1775); *Pomadasys argyreus* (Valenciennes, 1833); *Pomadasys maculatus* (Bloch, 1793) and previously unrecorded species, *Pomadasys guoraca* (Cuvier, 1829) and *Plectorhinchus macrospilus* Satapoomin et Randall, 2000. The former has not been recorded from the adjacent seaward coast of the Sundarbans mangrove forest of Bangladesh, while the latter is new to Saint Martin's Island. Diagnostic characters of all seven species collected by us are given below, and barcodes for six of the species from Bangladesh are provided for the first time.

Taxonomy

Plectorhinchinae Jordan et Thompson, 1912
Plectorhinchus Lacepède, 1801

Plectorhinchus pictus (Thunberg, 1792)

English common name: fork-striped slatey
Local common name: futki datina (Bangla)

Fig. 2a

Material examined. Bangladesh • 2 specimens; F1804SM-21 (110 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 April 2018, Amit Kumer Neogi, GenBank: MK340608; F1804SM-22(105 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 April 2018, Amit Kumer Neogi, GenBank: MK340609.

Diagnostic characters. Meristics: D-X, 23; P_1-17; P_2-I, 5; A-III, 7; C-18

Body deep and compressed. Profile of snout steep; lips fleshy; chin with six pores, but no median pit; lower jaw without longitudinal groove at midline. Caudal peduncle slender and long; scales small, ctenoid. Color varying greatly with size. Juveniles dark brown to black above, 4 broad longitudinal black bands on the body, silvery yellow below; dorsal and caudal fins yellow with black patches and broken stripes (Fig. 2a).

Remarks. This species has previously been included by the majority of authors in the genus *Diagramma* Oken, 1817, but recent phylogenetic analyses (Sanciangco et al. 2011; Tavera et al. 2012; Tavera et al. 2018) have shown that *Diagramma* (including type species *D. pictus*) is deeply nested within *Plectorhinchus* (and sister to its type species *Plectorhinchus chaetodonoides* Lacepède, 1801), and Tavera et al. (2018) have proposed that *Diagramma* should be considered a junior synonym of *Plectorhinchus* Lacepède, 1801. As pointed out by Parenti (2019), however, accepting *Plectorhinchus* as the senior synonym of *Diagramma* creates several nomenclatural problems: *Plectorhinchus pictus* (Tortonese, 1936) becomes a secondary homonym of *P. pictus* (Thunberg, 1792), and the next available name for *P. pictus* (Tortonese) is *Plectorhinchus cinctus punctatatus* Fang, 1942 (see Fricke et al. 2021b) which is secondarily preoccupied in *Plectorhinchus* by *Diagramma puncatatum* Cuvier, 1830 (see Fricke et al. 2021b). This problem was long ago recognized by Whitley (1951), who anticipated *Diagramma* as a synonym of *Plectorhinchus* and accordingly proposed the name *Plectorhinchus fangi* Whitley, 1951, as a replacement for *P. cinctus punctatus* Fang (a species not yet recorded from the Bay of Bengal). Thus, we
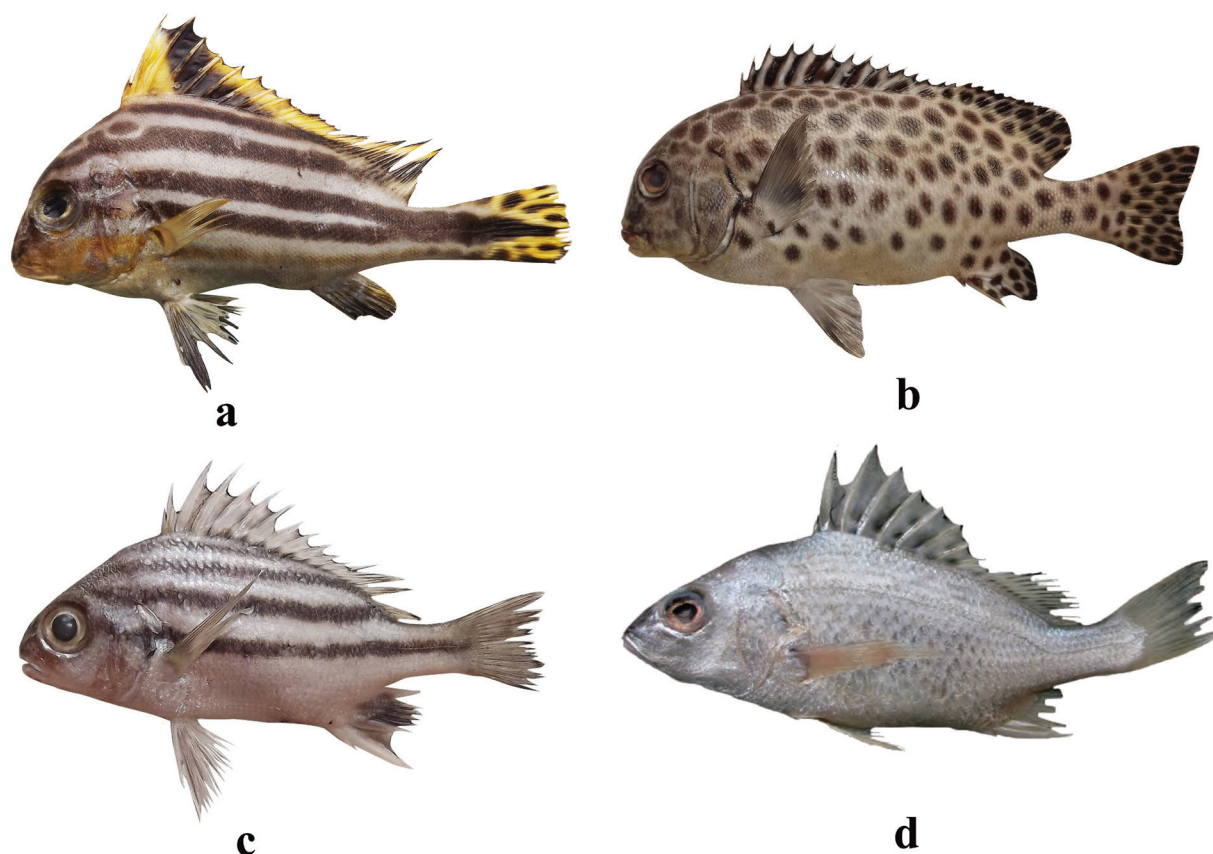
Figure 2. Lateral view of collected specimen, **a.** *Plectorhinchus pictus*, subspecies *cinerascens* (F1804SM-22; 105 SL mm), striped juvenile phase; **b.** *Plectorhinchus macrospilus* (F1803SM-67; 335 mm SL); **c.** *Plectorhinchus andamanensis*, (F1803SM-19; 137 mm SL); **d.** *Pomadasys argenteus* (F1602Sb-01; 132 mm SL).

regard *Plectorhinchus pictus* (Thunberg, 1792) as the correct name for the species described here from Bangladesh.

Specimens of *P. pictus* from the Bay of Bengal are part of a distinct subspecies, *Plectorhinchus pictus cinerascens* Cuvier, 1830—one of five geographically separate subspecies recognized by Johnson et al. (2001, as *Diagramma*): *P. pictus pictus* (Thunberg), *P. pictus labiosum* Macleay, *P. pictus punctatum* Cuvier, and *P. pictus centurio* Cuvier). *Plectorhinchus pictus*, subspecies *cinerascens* of Johnson et al. (2001) differs from all others in the configuration of the body stripes of juveniles, size of spots, and nature of the lines on the cheeks and operculum of large juveniles to small adults. Juveniles of about 150 mm TL often have broad, clearly defined body stripes, not yet beginning to break into broken lines or numerous spots; and progress from a striped to a fully spotted phase at 180–240 mm TL (Johnson et al. 2001: fig. 5). A more detailed genetic study is necessary to determine whether this geographically distinct color variety should be afforded separate species recognition (Johnson et al. 2001).

Distribution. *Plectorhinchus pictus cinerascens* , occurs from the Bay of Bengal to the Arabian/Persian Gulf (Johnson et al. 2001).

Conservation status. Not yet assessed, not listed in the IUCN Red List of Threatened Species (https://www.iucnredlist.org/species/123439745/123494892).

Plectorhinchus macrospilus Satapoomin et Randall, 2000

English common name: largespot sweetlip

Local common name: dagi datina (Bangla)

Fig. 2b

Material examined. Bangladesh • 1 specimen; F1803SM-67 (335 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 27 March 2018, Md. Jayedul Islam and Kazi Ahsan Habib, GenBank: MK340677.

Diagnostic characters. Meristics: D-XII, 21; P_1-17; P_2-I, 5; A-III, 8; C-18; LL-59; GR- 5 + 15.

Body compressed; dorsal profile of head strongly convex. Small mouth with fleshy lips, moderately thick; chin with 6 pores and no median pit. Dorsal fin slightly notched. Caudal fin truncate. Scales ctenoid; absent in front of snout, lips, and chin. Color of body whitish to grayish ground color on most parts of body; contrasting with many large, irregularly rounded black spots on body, nape, and soft portions of median fins, and smaller black spots on head. Posterior edge of opercle slightly serrate, margin of subopercle and interopercle smooth (Fig. 2b). Lateral line continuous. Meristic measurements are given in Table 1 and Table 2.

Remarks. *Plectorhinchus macrospilus* is one of six species of its genus that have numerous dark spots in adults;

Table 1. Meristic counts of the two new records of *Plectorhinchus macrospilus* and *Pomadasys guoraca* collected in the presently reported study, compared with reference data.

Character	*Plectorhinchus macrospilus*		*Pomadasys guoraca*	
	This study $n = 1$	Satapoomin and Randall 2000	This study $n = 3$	Talwar and Kacker 1984
Dorsal-fin spines	XII	XII	XII	XII–XIII
Dorsal-fin soft rays	21	21	14	14
Pectoral-fin soft rays	17	17	17	—
Pelvic-fin spines	I	I	I	—
Pelvic-fin soft rays	5	5	5	—
Anal-fin spines	III	III	III	III
Anal-fin soft rays	8	8	7	7–9
Caudal-fin rays	18	17	17–20	—
Gill rakers	5 + 15	—	—	—

Table 2. Morphometric measurements of two new records of *Plectorhinchus macrospilus* and *Pomadasys guoraca* collected in the presently reported study.

Measurements	*Plectorhinchus macrospilus* $n = 1$	*Pomadasys guoraca* $n = 3$
Total length [mm]	335	148–197
Standard length [mm]	300	116–159
Percentage of standard length		
Body depth	40.0	42.70–49.10
Head length	26.6	32.70–36.80
Inter orbital wide	9.0	7.70–8.20
Pre orbital length	8.3	9.40–10.60
Post orbital length	12.6	13.80–14.70
Eye diameter	7.3	10.06–11.48
Snout length	9.3	44.03–60.34
Caudal peduncle length	14.0	12.30–12.93
Dorsal-fin base length	60.6	55.35–61.48
largest 5th dorsal-fin length	11.0	16.98–18.03
Pectoral-fin base length	7.0	—
Pectoral-fin length	20.6	29.56–33.61
Pelvic-fin base length	19.6	5.03–5.74
Pelvic-fin length	20.3	22.64–27.05
Anal-fin base length	11.3	15.09–14.75
Anal-fin length	14.3	22.41–22.13
Caudal-fin base length	13.0	11.95–13.11
Caudal-fin length	20.3	30.17–30.30
Pre dorsal length	35.0	—
Pre pectoral length	27.6	—
Pre pelvic length	32.3	—
Pre anal length	60.6	—

others are *Plectorhinchus chaetodonoides*; *Plectorhinchus gaterinus* (Forsskål, 1775); *Plectorhinchus picus* (Cuvier, 1828); *Plectorhinchus pictus* (Thunberg, 1792); and *Plectorhinchus cinctus* (Temminck et Schlegel, 1843). The dark spots of *P. macrospilus* are generally larger than those of the other five species, and *P. macrospilus* also has greater number of dorsal soft rays (21 vs. 15–20 for other species). Subadult *P. chaetodonoides* are similar in coloration to *P. macrospilus* but are easily distinguished by gill raker count (9–12 + 28–33 versus 5 + 15 for *P. macrospilus*).

Distribution. *Plectorhinchus macrospilus* is known to occur from Thailand (Satapoomin and Randall 2000) and Myanmar (Yangoon and Myiek Archipelago) (Allen and Erdmann 2012; Russell 2016; Psomadakis et al. 2019). This study confirms its occurrence also in the northern Bay of Bengal.

Conservation status. Not yet assessed, not listed in the IUCN Red List of Threatened Species (https://www.iucnredlist.org/species/123439745/123494892).

Haemulinae Gill, 1885
Pomadasys Lacepède, 1802

Pomadasys andamanensis McKay et Satapoomin, 1994

English common name: andaman grunt

Local common name: dagi datina (Bangla)

Fig. 2c

Material examined. Bangladesh • 1 specimen; F1803SM-19 (137 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 April 2018, Amit Kumer Neogi, GenBank: MK340687.

Diagnostic characters. Meristics: D-XII, 13-14; P1-18; P2-I, 5; A-III, 8; C-18; LL-50

Body deep, compressed, depth 2.7 in standard length. Snout rounded, scales extending to nostrils; dorsal mouth small, terminal, without fleshy lips; 2 pores and a median pit on the chin. Lateral line single and complete; total gill rakers on first arch 17. Silvery white with 4 horizontal dark brown stripes on the dorsal half of body; anal fin with a dark brown streak covering anterior two-thirds of the soft-rayed portion (Fig. 2c).

Remarks. *Pomadasys andamanensis* is sometimes confused with *P. furcatus*, but can be distinguished by having four undivided dark brown longitudinal bands versus six to seven longitudinal brown bands in *P. furcatus* that frequently bifurcate anteriorly and after division longitudinally may number 10 thinner bands (Psomadakis et al. 2019).

Distribution. *Pomadasys andamanensis* is known to occur from Phuket Island, Andaman Sea, Thailand (McKay and Satapoomin 1994) and Andaman Sea off Myanmar (Psomadakis et al. 2019). Recently recorded from Saint Martin's Island, Bangladesh (Naznin et al. 2020).

Conservation status. Not yet assessed, not listed in the IUCN Red List of Threatened Species. (https://www.iucnredlist.org/species/123439745/123494892)

Pomadasys argenteus (Forsskål, 1775)

English common name: silver grunt

Local common name: rupali datina (Bangla)

Fig. 2d

Material examined. Bangladesh • 1 specimen; F1602Sb-01 (132 mm SL), Alorkol, Sundarbans, Bagerhat, 21°42.35′N, 89°35.24′E, 12 February 2016, Amit Kumer Neogi.

Diagnostic characters. Meristics: D-XII, 14; P_1-I, 16; P_2-I, 5; A-III, 7

Body ovate, compressed, depth 2.7 in standard length. Dorsal profile of head steep, mouth small, maxilla reaching to eye; lips not thickened. Chin with two pores and a median pit. Lateral line with 47 scales; 5 scales between lateral line and dorsal-fin origin. Body color silver-mauve above and white below; scattered charcoal scale spots on back and upper sides; spots only on body, absent on head and snout; snout dark brown (Fig. 2d).
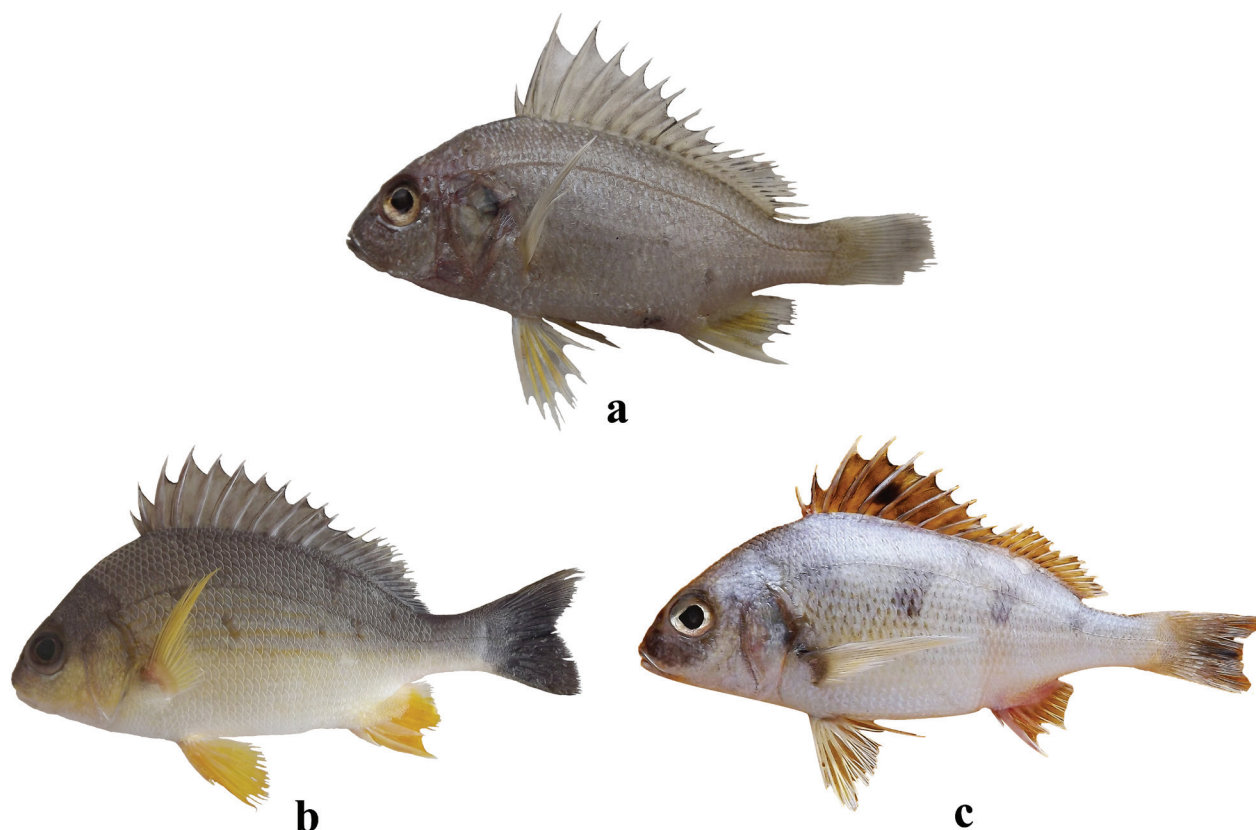
Figure 3. Lateral view of a collected specimen, **a.** *Pomadasys argyreus* (F1712SM-14.; 129 mm SL); **b.** *Pomadasys guoraca* (F1709SM-09; 197 mm SL); **c.** *Pomadasys maculatus* (F1708SM-10; 96 mm SL).

Distribution. *Pomadasys argenteus* is known to occur in Bangladesh (Hussain 1970; Rahman et al. 2009), Myanmar (Hla 1987), and the Andaman and Nicobar Islands (Rajan et al. 2011). Elsewhere in the Indian Ocean from the Red Sea and Persian Gulf (Wright 1988; Randall 1995; Froese and Pauly 2020) and India (Talwar and Jhingran 1991; Bijukumar and Sushama 2000). In the western Pacific from southern Japan (Masuda et al. 1984) and the Philippines to northern Australia (Johnson 2010), and New Caledonia (Thollot 1996).

Conservation status. Listed as 'Least Concern' in the IUCN Red List of Threatened Species (Dahanukar et al. 2012).

Pomadasys argyreus (Valenciennes, 1833)

English common name: bluecheek silver grunt
Local common name: rupali datina (Bangla)

Fig. 3a

Material examined. Bangladesh • 1 specimen; F1712SM-14 (129 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 12 December 2017, Amit Kumer Neogi, GenBank: MK340688.
Diagnostic characters. Fin Formula: D-XII, 13; P_1-I, 14; P_2-I, 5; A-III, 7
Body ovate, laterally compressed, depth 2.4 in standard length; head blunt, upper profile convex; mouth

small; maxilla reaching to eye, lips not thickened; chin with 2 pores and a median pit. Scale on lateral line 47, 5 scale rows between lateral line and dorsal fin origin. Color of body silvery, darker above; fins yellowish-brown; a large blue-black blotch on the operculum (Fig. 3a).

Distribution. *Pomadasys argyreus* is known to occur in Bangladesh (Hussain 1970), elsewhere in Indo-West Pacific: Pakistan, India, Sri Lanka eastward to Southeast Asia and south to Papua New Guinea (McKay 2001; Psomadakis et al. 2015; Froese and Pauly 2020).

Conservation status. Listed as 'Least Concern' in the IUCN Red List of Threatened Species (Al Abdali et al. 2019).

Pomadasys guoraca (Cuvier, 1829)

English common name: silver-grunt
Local common name: rupali datina (Bangla)

Fig. 3b

Material examined. Bangladesh • 3 specimens; F1709SM-08 (156 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 29 September 2017, Amit Kumer Neogi, GenBank: MK340689; F1709SM-09 (148 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 29 September 2017, Amit Kumer Neogi, GenBank: MK340690; F1710SM-03 (197 mm SL),

Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 October 2017, Kazi Ahsan Habib, GenBank: MK340691.

Diagnostic characters. Meristics: D-XII, 14; P_1-17; P_2-I, 5; A-III, 7; C-18-20; LL-52-53

Body elongate and compressed, rounded; eye diameter 3.3 in head length; snout 0.7 in head length. Mouth small, lips slightly thick. Maxilla extending below front edge of eye. Villiform teeth. Scales ctenoid. Body silvery, slightly darker on back. Yellow stripes present below lateral line. Dorsal fin silvery; pectoral, pelvic and anal fin yellowish; caudal fin black with white edge (Fig. 3b). Meristic measurements are given in Table 1 and Table 2.

Remarks. *Pomadasys guoraca* is distinguished from the related species, *Pomadasys aheneus* McKay et Randall, 1995, by having yellow stripes below lateral line (vs. no stripes); yellow anal and paired fins (vs. dusky anal and paired fins); caudal fin dusky with narrow white margin (vs. caudal fin dusky without white margin).

Distribution. *Pomadasys guoraca* is known to occur on the eastern coast of Africa, Oman, Thailand, Philippines, Madagascar (Roux 1986; Stiassny and Raminosoa 1994; GBIF 2020; Froese and Pauly 2020; Orrell 2020), Sri Lanka (Orrell 2020), Andaman and Nicobar Islands (Rajan et al. 2011), India (Talwar and Kacker 1984). This study confirms the occurrence of this species in the northern Bay of Bengal, Bangladesh for the first time.

Conservation status. Listed as 'Least Concern' in the IUCN Red List of Threatened Species (Borsa et al. 2019).

Pomadasys maculatus (Bloch, 1793)

English common name: saddle grunt
Local common name: guti datina (Bangla)

Fig. 3c

Material examined. Bangladesh • 4 specimens; F1602sb-38-2 (91 mm SL), Alorkol, Sundarbans, Bagerhat, 21°42.35′N, 89°35.24′E, 10 February 2016, Amit Kumer Neogi, GenBank: MF588665; F1708SM-10 (96 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 29 August 2017, Amit Kumer Neogi, GenBank: MK340692; F1708SM-11 (98 mm SL), Cox's Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 August 2017, Kazi Ahsan Habib, GenBank: MK340693; FCC1901SB-14 (101 mm SL), Cox`s Bazar, Bay of Bengal, Saint Martin's Island, 20°36′39.6″N, 92°19′37.2″E, 20 January 2019, Md. Jayedul Islam, GenBank: MN458364.

Diagnostic characters. Meristics: D-XII, 14; P_1-17; P_2-I, 5; A-III, 7; C-18

Body compressed; head blunt and dorsal profile convex; mouth small and slightly oblique; maxilla reaching to eye; narrow bands of small pointed teeth in the jaws. Scales ctenoid, moderate; present on head excluding snout. Chin with two pores and a median pit. Lateral line

slightly arched. Body color silvery white, nape and back with a series of incomplete variable cross bars on the upper half of the body; spinous dorsal fin large with black blotch; dorsal and caudal fins edged with black, other fins yellowish (Fig. 3c).

Distribution. *Pomadasys maculatus* is reported in Bangladesh; elsewhere from east coast of Africa, Madagascar, Red Sea, Gulf of Aden, Persian Gulf, Pakistan, India, Sri Lanka to northern half of Australia from Shark Bay to Moreton Bay, New Guinea, Philippines to southern Japan (McKay 2001; Habib et al. 2020).

Conservation status. Listed as 'Least Concern' in the IUCN Red List of Threatened Species (Collen et al. 2010).

Genetic description. We successfully barcoded six of the seven collected grunt species viz. *Plectorhinchus macrospilus*, *Plectorhinchus pictus*, *Pomadasys andamanensis*, *Pomadasys argyreus*, *Pomadasys guoraca*, and *Pomadasys maculatus*, and submitted to GenBank (NCBI) and BOLD system. The COI sequences of *Plectorhinchus macrospilus*, *Pomadasys andamanensis*, and *Pomadasys guoraca* were submitted for the first time to GenBank as reference DNA barcode sequence. We identified 11 COI barcode sequences of 6 species. For *Pomadasys argenteus*, we were unable to obtain a clear sequence. Sequence alignment of COI gene yielded about 602 nucleotide base pairs after removing the ambiguous sequences near primer ends. The COI sequences of 11 individuals of 6 species comprised 11 haplotypes with 174 polymorphic sites. The estimated mean ratio of transition and transversion was 2.88. The sequence analysis revealed that the mean nucleotide compositions in 11 COI sequences of 6 species were A = 22.5% ± 0.52%, T = 28.11% ± 0.59%, C = 30.45% ± 0.63%, G = 18.95% ± 0.71%. The overall GC content was 49.39%. The nucleotide diversity was calculated as 0.134 and the haplotype diversity was 1.0 for the sequences. The mean interspecific distance was 23.4% among the six species studied. The overall genetic distance among the sequences of COI gene was 16.3%. Among the six grunt species of the presently reported study, the highest pairwise genetic distance was found as 23.36% between *Plectorhinchus pictus* and *Pomadasys argyreus*, and the lowest distance (9.9%) was found between *Plectorhinchus macrospilus* and *Plectorhinchus pictus*.

In the phylogeny, we used 11 COI sequences of six species obtained in the presently reported study and three other sequences of *Pomadasys maculatus*, *Pomadasys argyreus*, and *Plectorhinchus pictus* retrieved from GenBank. The phylogenetic tree showed six clades, each belonging to the separate species (Fig. 4). No valid conspecific sequence of *Plectorhinchus macrospilus*, *Pomadasys andamanensis*, and *Pomadasys guoraca* was found in GenBank for comparison. However, the COI sequence of these three species clearly formed three separate clades from other species of grunt in the constructed ML tree with over 90% bootstrap value.
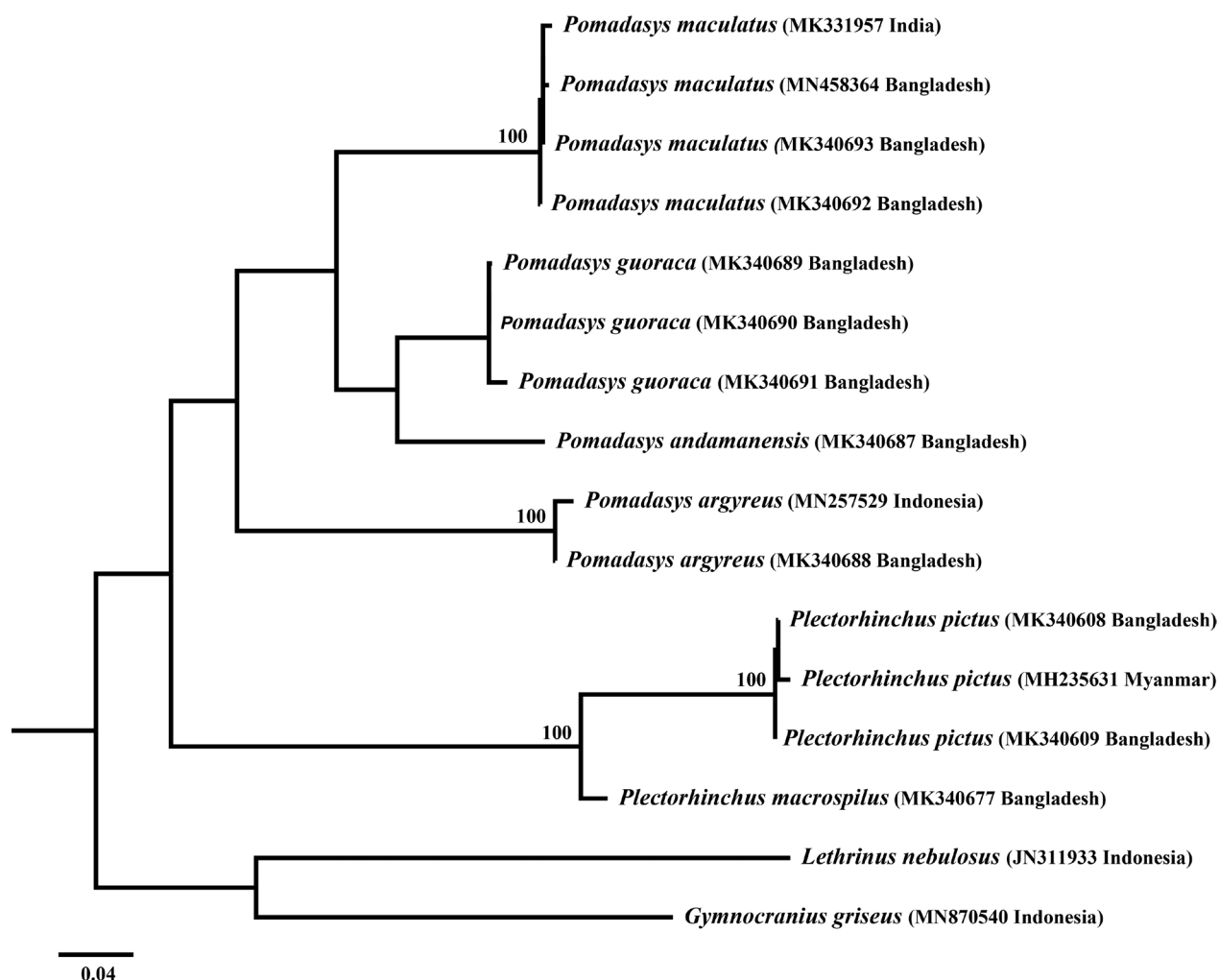
Figure 4. Maximum-likelihood tree constructed for COI barcode sequences of grunt species obtained in the presently reported study (Bangladesh) and for conspecifics reported in GenBank. The GenBank accession numbers and country of origin are given within parenthesis beside species name. Numbers on nodes represent support values for Maximum-Likelihood (bootstrap). Bootstrap support of >70% are shown above branches. Scale bar indicates number of nucleotide substitutions per site. Sequences of *Lethrinus nebulosus* and *Gymnocranius griseus* were used as outgroups.

Discussion

Grunts are one of the most commercially important fish groups in Bangladesh. Ten species of haemulids were previously recorded from Bangladeshi marine waters (Hussain 1970; Rahman et al. 2009; Thompson and Islam 2010; Baki et al. 2017; Fanning et al. 2019; Habib and Islam 2020; Naznin et al. 2020). Recent studies on the identification of haemulid species in Bangladesh (Baki et al. 2017; Fanning et al. 2021) were conducted mainly by morphological analysis, and except for the work of Naznin et al. (2020), genetic analysis using DNA barcoding (Floyd 2002; Tautz 2003; Ward 2005) was not applied. Our study is the first attempt to identify the grunts (Haemulidae) of Bangladesh based on both morphology and DNA Barcoding.

In the presently reported study, we identified seven species of grunts, confirmed by morphology and DNA barcoding. Among these, two species (viz. *Pomadasys guoraca* and *Plectorhinchus macrospilus*) were not previously recorded from Bangladesh.

Pomadasys guoraca is widely distributed in the Indo-West Pacific from the east coast of Africa to the Philippines (Roux 1986). Recently, this species was reported from Andaman and Nicobar Islands, the eastern boundary of the Bay of Bengal (Rajan et al. 2011). Previously, the species was not reported in the marine waters of Bangladesh: our record of *P. guoraca* from Saint Martin's Island of Bangladesh has confirmed its distribution in the northern Bay of Bengal.

Plectorhinchus macrospilus was previously known only from the Andaman Sea, off south-western Thailand (Satapoomin and Randall 2000). More recent studies also recorded it from Yangoon and the Myiek Archipelago of the Andaman Sea (Allen and Erdmann 2012; Russell 2016). Our study extends the range of *P. macrospilus* from the Andaman Sea into the northern Bay of Bengal.

Table 3. Grunt species (Actinopterygii: Perciformes: Haemulidae) recorded from Bangladesh.

English common name	Scientific name	References	IUCN Red List status
Painted sweetlips	*Plectorhinchus pictus* (Thunberg, 1792)	Hussain 1970 Rahman et al. 2009 This study	Not yet assessed
Crescent sweetlips	*Plectorhinchus cinctus* (Temminck et Schlegel, 1843)	Hussain 1970 Rahman et al. 2009	Not yet assessed
Largespot sweetlip	*Plectorhinchus macrospilus* Satapoomin et Randall, 2000	This study	Not yet assessed
Indian Ocean oriental sweetlips	*Plectorhinchus vittatus* (Linnaeus, 1758)	Hussain 1970 Rahman et al. 2009	Least Concern
Andaman grunt	*Pomadasys andamanensis* McKay et Satapoomin, 1994	Naznin et al. 2020 This study	Not yet assessed
Silver grunt	*Pomadasys argenteus* (Forsskål, 1775)	Hussain 1970 Rahman et al. 2009 This study	Least Concern
Bluecheek silver grunt	*Pomadasys argyreus* (Valenciennes, 1833)	Hussain 1970 This study	Least Concern
Banded grunter	*Pomadasys furcatus* (Bloch et Schneider, 1801)	Baki et al. 2017	Least Concern
Silver-grunt	*Pomadasys guoraca* (Cuvier, 1829)	This study	Least Concern
Javelin grunter	*Pomadasys kaakan* (Cuvier, 1830)	Baki et al. 2017	Not yet assessed
Saddle grunt	*Pomadasys maculatus* (Bloch, 1793)	Hussain 1970 Tomascik 1997 This study	Least Concern
Cock grunter	*Pomadasys multimaculatus* (Playfair, 1867)	Fanning et al. 2019	Least Concern

Two previously recorded species, *Plectorhinchus cinctus*, reported by Hussain (1970) and *Plectorhinchus vittatus* (Linnaeus, 1758), reported by Rahman et al. (2009), were not found in the presently reported survey. Based on other valid reports, we have compiled an updated list of grunts found in Bangladesh (Table 3) which brings the total known species to 12, including the two species newly recorded here. The conservation status of Bangladesh species of grunts also is included in Table 3. Of those species included in the IUCN Red List of threatened species, all those assessed are listed as 'Least Concern', but assessments of five species from Bangladesh remain to be published. Further research may reveal additional species of haemulids in Bangladeshi marine waters.

Acknowledgments

Conflict of interest

The authors declare that they have no conflict of interest.

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Dietary live yeast (*Debaryomyces hansenii*) provides no advantages in tropical gar, *Atractosteus tropicus* (Actinopterygii: Lepisosteiformes: Lepisosteidae), juvenile aquaculture

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Abstract

Tropical gar, *Atractosteus tropicus* Gill, 1863, is an ancient freshwater fish that is commercially cultivated in southern Mexico. Currently, there is a specific diet for its culture; however, the addition of probiotics has not been investigated. The objective of this study was to evaluate the supplementation of live yeast *Debaryomyces hansenii* for *A. tropicus* juveniles on growth, productive parameters, survival, somatic index, digestive enzyme activity, and immune system gene expressions (interleukin 10, *il-10*, Transforming growth factor β1, *tgf-β1*, and β2 microglobulin, *b2m*). Three experimental diets increased the dose of live yeast (0.5, 1.0, and 1.5%; 10^{14}, 10^{15}, and 10^{16} CFU g diet^{-1}, respectively) and a control diet (CD; without yeast) were designed. Daily weight gain and specific growth rate were higher in fish fed with CD and 0.5% *D. hansenii*. High activities of trypsin, chymotrypsin LAP, and α-amylase, as well as overexpression of *il-10* in the spleen, were detected in fish feed 0.5% *D. hansenii*. The inclusion of *D. hansenii* had no positive effect on aquaculture for *A. tropicus*, lower doses should be tested to optimize the diet.

Keywords

digestive physiology, enzymes, gar, immune system, nutrition, probiotics

* Both authors contributed equally.

Introduction

Fish production worldwide is facing challenges related to disease control and nutrition improvement through food optimization, where probiotics show beneficial effects for the host, showing several advantages in the aquaculture production (Akhter et al. 2015), including the immune system, maturation of the digestive system, and host metabolism (Hai 2015; Angulo et al. 2017) and antagonistic capacity to possible pathogenic organisms (Navarrete and Tovar-Ramírez 2014). Yeast *Debaryomyces hansenii* is a halotolerant, non-pathogenic ubiquitous yeast capable of growing and proliferating in a variety of environments, including marine fish (Raggi et al. 2014) and freshwater fish gut (Andlid et al. 1995) and several studies demonstrate positive effects of *D. hansenii* on digestive maturation, increase in survival, the activity of pancreatic and luminal digestive enzymes, increase in resistance to infection by the improvement of immunity and resistance as well to generate a positive effect on the antioxidative status in several fish species fed yeast such as European sea bass, *Dicentrarchus labrax* (Linnaeus, 1758); gilthead seabream, *Sparus aurata* Linnaeus, 1758; leopard grouper, *Mycteroperca rosacea* (Streets, 1877), to name just a few (Tovar-Ramírez et al. 2004, 2010; Reyes-Becerril et al. 2008; Reyes-Becerril et al. 2011). Moreover, the inclusion of probiotics has been evaluated through the immune system gene expression such as interleukins, where Il-10 stands out, which is an immunosuppressive cytokine that prevents immune-mediated damage to the host by dampening inflammatory responses of the immune response (Howes et al. 2014), the β2 microglobulin (*b2m*) is classified as part of the set of the immunoglobulin superfamily (IgSF) and play important roles in the adaptive immune system (Chen et al. 2010). The immunological function of *b2m* is to assist classical major histocompatibility complex (MHC) class I (i.e., MHC-I) molecules in assembling endogenous antigen peptides, forming a trimolecular complex (i.e., pMHC-I) and then presenting them to the surface of antigen-presenting cells (APCs); through interaction with T cell receptors (TCRs), specific cytotoxic T lymphocyte (CTL) immunity is thus induced (Flajnik and Kasahara 2001; Li et al. 2020), and the transforming growth factor β1 (*tgf-β1*), which is an important multifunctional cytokine involved in the regulation of cell proliferation, differentiation, survival, migration, and apoptosis under physiological and pathological conditions (Qi et al. 2016). For example, *tgf-β1* acts as an immunosuppressive mediator and inhibits nitric oxide production in TNF-γ-activated macrophages in fish (Wang et al. 2017). For instance, the positive effects and benefits of *D. hansenii* are well known; however, beneficial effects depend on the supplemented concentration (Hai 2015), where overdosing or underdosing results in lower efficacy and unnecessary costs, with low efficiency of the probiotic (Sharifuzzaman and Austin 2017). Therefore,

the evaluation of the possible impact of probiotic inclusion through the understanding of physiological and metabolic changes is part of a compressive understanding of the new emerging aquaculture species (Angulo et al. 2020).

Tropical gar, *Atractosteus tropicus* Gill, 1863, is an ancestral, carnivorous, freshwater fish species native to the south-eastern Mexico and Central America that possess ecological, biological, and economic importance (Márquez-Couturier and Vázquez-Navarrete 2015). Recently, this species has been used as a nutritional fish model, with several nutritional studies focused on the characterization of digestive enzymes during larval and juvenile stages (Guerrero- Zárate et al. 2014; Frías Quintana et al. 2015), development of microparticulate and microencapsulated diets for larvae (Saenz de Rodrigáñez et al. 2018), the inclusion of starch sources (Frías-Quintana et al. 2016, 2017), lipogenic metabolism characterization (Jiménez-Martínez et al. 2019), among others. Recently, Nieves-Rodríguez et al. (2018) and Nájera-Arzola et al. (2018) evaluated β-glucans and mannan oligosaccharides during the juvenile stage in *A. tropicus*, finding positive effects on growth performance, digestive enzymes activity, and immune system. The objective of this study was to evaluate the supplementation of live yeast *D. hansenii* for *A. tropicus* juveniles on growth, productive parameters, survival, somatic index, digestive enzyme activity, and gene expressions of *il-10*, *tgf-β1*, and *b2m*.

Material and methods

Juveniles rearing

Tropical gar juveniles were obtained from the División Académica de Ciencias Biológicas (DACBiol) from Universidad Juárez Autónoma de Tabasco (UJAT). Spawning was induced using a female and three tropical gar males. The larval development was carried out according to previously described methodologies by Saenz-Rodrigáñez et al. (2018). At the required size (0.18 ± 0.01 g), the organisms were used for the experimental.

Probiotic yeast and cell viability

Yeast *D. hansenii* strain CBS 8339 was provided by CIB-NOR, S.C. This strain was isolated from the trout intestine (Andlid et al. 1995) and was produced according to the protocol of Tovar et al. (2002). The strain was cultured in yeast peptone dextrose (YPD, Sigma-Aldrich) at 25°C with constant aeration until the early stationary phase (24 h). The cell suspension was centrifuged (1000 *g* for 5 min at 4°C) and the recovered pellet was immediately incorporated into the diet ingredients in different concentrations as shown in Table 1. The viability of the yeast biomass was determined as colony forming units (CFU)

Table 1. Ingredient content, proximate analysis, and gross energy content of the experimental diets supplemented with *Debaryomyces hansenii*.

Ingredient [%]	Treatment (Diet)			
	CD	D1	D2	D3
Fish meal[a]	40.7	40.7	40.7	40.7
Renderer meal[a]	30.0	29.5	29.0	28.5
Corn starch[b]	15.4	15.4	15.4	15.4
Fish oil[a]	6.9	6.9	6.9	6.9
Soybean lecithin[c]	4.0	4.0	4.0	4.0
Grenetin[f]	2.0	2.0	2.0	2.0
Vitamin c[d]	0.5	0.5	0.5	0.5
Vitamin and mineral premix[e]	0.5	0.5	0.5	0.5
D. hansenii concentration % and	0.0	0.5	1.0	1.5
CFU g of diet[-1]	0.0	6.3×10^{14}	1.2×10^{15}	1.9×10^{16}
Proximate composition [%]				
Energy [kj g[-1]]	17.7	17.7	17.7	17.7
Protein	43.6	44.2	43.3	43.0
Ether extract	15.0	14.8	14.9	15.1
Ash	15.0	14.9	14.6	15.2
NFE[1]	26.4	26.1	27.1	26.7

CD = control diet, D1, D2, and D3 are experimental diets featuring increasing content of *D. hansenii*; Renderer meal is a poultry by-product; [a]Marine and agricultural proteins S.A. de C.V., Guadalajara, Jalisco; [b]IMSA Corn Industrializer S.A de C.V. Guadalajara, Jalisco, México; [c]Pronat Ultra, Mérida, Yucatán, México; [d]ROVIMIX® STAY-C® 35 – Vitamins – Products – DSM México, Guadalajara, Jalisco, México and [e]Trouw Nutrition México S.A. de C.V. (by courtesy). [f]D'gari Productos alimenticios y dietéticos Relámpago, Tlalpan, Edomex, Mexico. NFE[1] = Nitrogen-free extract: 100 – (%protein – % ether extract – % ash); CFU = colony forming units.

by plating on YPD plates supplemented with antibiotics (chloramphenicol, 1 mg L^{-1}; polymyxin sulfate B, 1.6 mg L^{-1}; amoxicillin, 2.5 mg L^{-1}, Sigma-Aldrich). The plates were incubated for 48 h at 30°C before colony counting.

After 35 days of culture, yeast cell counts were taken using the trypan blue dye exclusion test to also measure the cell viability, and percent survival was calculated using the total viable yeast cell. A 1:10 dilution (w/v) was performed and mix 500 µL of 0.4% (w/v) trypan blue (Sigma, Aldrich) and 500 µL of dilution. Allow mixture to incubate for 3 min at room temperature. 10 µL of the dilution was taken to load the Neubauer chamber, and the microscope was observed in 400× magnification, where live cells (unstained) and dead cells (stained blue) were counted and the viable cell [%] was determined by mL with the following equation:

$$\text{Viable cells [\%]} = \frac{\text{Total number of viable cells per aliquot [mL]}}{\text{Total number of cells per aliquot [mL]}} \times 100$$

Experimental design and diet manufacture

The experiment designed consisted of four formulated diets, using diet reported by Frías-Quintana et al. (2016) a control diet (CD, without *D. hansenii*) and three diets supplemented with high increasing levels of yeast *D. hansenii* (D1 with 0.5%; D2 with 1.0 and D3 with 1.5% of *D. hansenii*) as shown in Table 1, being isocaloric and isolipidic. The experimental diets were manufactured following the protocol proposed by Alvarez-González et al. (2001). The diets obtained were dried at 40°C for 12 h. The experiment was performed in 70 L plastic tanks, connected to a recirculation system with a settler and biofilter, randomizing 20 juveniles per tank. All treatments were performed in triplicate. Water quality was monitored daily (mean ± standard deviation, SD), with mean values of temperature (26.9 ± 0.4°C) and dissolved oxygen (5.1 ± 0.4 mg L^{-1}) determined by an oximeter (YSI 85; OH), and pH (7.2 ± 0.1) with a potentiometer (HANNA HI991001, Romania).

Growth, somatic index, and sampling

Biometrics was performed every 15 days during 45 days of experimentation, recording wet weights and total length. At the end of the experiment, three juveniles per tank (nine per treatment) were euthanized with an overdose of clove oil dissolved in ethyl alcohol in 1:1 ratio and then dissected to record individual organ weight (stomach, intestine, and liver); additionally, mesenteric fat for each fish was removed and intestine length (from the pylorus until the anus) was measured. For digestive enzyme activity analysis (from the same fishes), the stomach and intestine were removed, rinsed with distilled water, and frozen at –80°C until the enzymatic process. For gene expression analysis, two fish per tank (six per treatment) were sacrificed. The liver, intestine, and spleen samples were fixed in RNA Later® (Thermo Fisher Scientific, Waltham, MA, USA) and frozen at –80°C for future treatment.

Based on the data obtained from feed consumption, growth, weight, and survival the following parameters and somatic indexes were calculated:

$$\text{Daily weight gain} \left(\text{DWG [g Day}^{-1}]\right) = \frac{\text{Final biomass [g]} - \text{Initial biomass [g]}}{\text{Number of days}}$$

$$\text{Specific growth rate} \left(\text{SGR [\%Day}^{-1}]\right) = \frac{\text{Ln final mean weight} - \text{Ln initial mean weight}}{\text{Number of days}} \times 100$$

$$\text{Feed conversion rate (FCR)} = \frac{\text{Total food consumed [g]}}{\text{Final biomass [g]} - \text{Initial biomass [g]}}$$

$$\text{Protein conversion rate (PER)} = \frac{\text{Final biomass [g]} - \text{initial biomass [g]}}{\text{Protein ingested [g]}}$$

$$\text{Survival (S [\%]} = \frac{\text{Final number of organisms}}{\text{Initial number of organisms}} \times 100$$

$$\text{Hepatosomatic index (HSI)} = \frac{\text{Liver weight [g]} \times 100}{\text{Body weight [g]}}$$

$$\text{Viscerosomatic index (VSI)} = \frac{\text{Viscera weight [g]} \times 100}{\text{Body weight [g]}}$$

$$\text{Mesenteric fat index (MSI)} = \frac{\text{Mesenteric fat weight [g]} \times 100}{\text{Body weight [g]}}$$

$$\text{Condition factor (CF)} = \frac{\text{Body weight [g]}}{\text{Body length [cm]}^3} \times 100$$

$$\text{Relative intestine size (RIL)} = \frac{\text{Intestine length [cm]} \times 100}{\text{Body length [cm]}}$$

Digestive enzymes techniques

The stomach and intestine were homogenized separately in distilled water in 1:5 ratio (w:v) with an Ultra Turrax (IKA T18 basic, Wilmington, USA), under cold conditions (4°C), then centrifuged at 16 000 *g* by 15 min at 4°C and the supernatant was recovered to be stored at –80°C until the analyzes were performed. Soluble protein concentration in the stomach and intestine multienzymatic extracts were determined with Bradford (1976) technique using bovine serum albumin as the standard protein.

Acid protease activity (stomach homogenate) was determined according to Anson (1938) using hemoglobin (1%) as a substrate in 100 mmol L^{-1} glycine-HCl buffer at pH 2, where the absorbance of the reaction was measured at 280 nm. Alkaline protease activity (intestine homogenate) was determined according to Walter (1984) using casein (1%) as substrate in buffer Tris-HCl 100 mmol L^{-1}, 10 mmol L^{-1} $CaCl_2$ at pH 9, where the absorbance of the reaction was measured at 280 nm. Trypsin activity was determined according to Erlanger et al. (1961) using 1 mmol L^{-1} BAPNA (Nα-Benzoyl-DL-arginine-p-nitroanilide) as substrate, dissolved in 50 mmol L^{-1} Tris-HCl buffer, 10 mmol L^{-1} $CaCl_2$ at pH 8.2, and the absorbance of the reaction was measured at 410 nm. The chymotrypsin activity was determined according to Hummel (1959) using N-benzoyl-L-tyrosine ethyl ester (BTEE) 5 mmol L^{-1} as the substrate dissolved in Dimethyl sulfoxide (DMSO) in 100 mmol L^{-1} Tris buffer, 100 mmol L^{-1} $CaCl_2$ at pH 7.8, and the absorbance was determined at 256 nm. The leucine aminopeptidase (LAP) activity was determined according to Maroux et al. (1973) using leucine p-nitroanilide (1.2 mmol L^{-1}) as substrate in 50 mmol L^{-1} sodium phosphate buffer at pH 7.2, and the absorbance was measured at 405 nm. The α-amylase activity was determined according to Robyt and Whelan (1968), using potato starch (2%) as substrate, dissolved in 100 mmol L^{-1} citrate-phosphate buffer and 50 mmol L^{-1} sodium chloride at pH 7.5, and reducing sugars were measured at 600 nm. The lipase activity was determined according to Versaw et

al. (1989), using β-naphthyl caprylate (200 mmol L^{-1}) as a substrate dissolved in buffer 50 mmol L^{-1} Tris-HCl and 100 mmol L^{-1} sodium cholate at pH 7.2, and the absorbance was measured at 540 nm. The alkaline phosphatase activity was determined according to Bergmeyer (1974), 4-nitrophenyl phosphate (2.4%) as substrate, dissolved in buffer 100 mmol L^{-1} glycine-NaOH at pH 10.1, and the absorbance was measured at 405 nm. All the techniques were performed at 37°C.

One unit (U) of enzymatic activity was defined as the amount of enzyme that produced 1 µmol of product released per minute. Total activity was calculated applying equation

$$\text{Total activity [Units mL}^{-1}] = \frac{\Delta abs \times \text{ reaction final volume [mL]}}{\text{MEC} \times \text{ time [min]} \times \text{extract volume [mL]}}$$

where Δabs represent the increase in absorbance, and MEC represents the molar extinction coefficient.

Specific digestive enzyme activity was calculated using equation

$$\text{U mg protein}^{-1} = \frac{\text{Units mL}^{-1}}{\text{mg protein mL}^{-1}}$$

where mg protein^{-1} is determined by Bradford method (1976).

RNA isolation and Quantitative Reverse Transcription PCR (RT-qPCR)

Total RNA from each tissue (liver, intestine, and spleen) was extracted individually using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The concentration and purity of the RNA samples were assessed by the ratio of the absorbance at 260/280 nm using a spectrophotometer (NanoDrop 2000). The RNA integrity of the samples was verified by visualization of 28S and 18S rRNAs after 1% agarose gel electrophoresis. The cDNA synthesis was performed using the Improm II Reverse Transcription System (Promega, Madison, WI, USA) following the manufacturer´s recommendations. On the ice, 0.5 µg of experimental RNA was combined with 1 µL of Oligo dT in nuclease-free water for a final volume of 5 µL. The primer/template mix was thermally denatured at 70°C for 5 min and chilled on ice. Subsequently, 15 µL of the reverse transcription reaction mix (5 × reaction buffer, 2 mM $MgCl_2$, 0.5 mM dNTPs, 1 µL reverse transcriptase, and 20 u ribonuclease inhibitor) was added in a final volume of 20 µL. The reaction mix was incubated at 25°C for 5 minutes following at 42°C for 60 min. The synthesized cDNA was diluted 1:3 (v/v) and stored at –80°C until later use.

The RT-qPCR was performed in a CFX96 Real-Time System (BioRad, Hercules, CA, USA) using 10 µL of IQ™ SYBR Green Supermix (BioRad), 1 µL primers mix, and 9 µL of diluted cDNA for a final volume of 20 µL. The cycles in the RT-qPCR program used was the following: 50°C for 2 min, 95°C 10 s, followed by 40

cycles at 95°C 15 s, and 62°C 1 min. As a reference, the gene the elongation factor (*ef1*) was used. Relative gene expression was calculated as fold-change compared with control and calculated by means of the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen 2001). Design of specific oligonucleotides for Interleukin 10 (*il-10*), β2 microglobulin (*b2m*), Transforming growth factor β1 (*tgf-β1*) were obtained from the *A. tropicus* transcriptome (Martínez-Burguete et al. 2021, Accession: PRJNA395289), shown in Table 2, and Elongation factor (*ef1*) was obtained as previously reported (Jiménez-Martinez et al. 2019).

Table 2. Primers designed for Interleukin 10, β2 microglobulin, Transforming growth factor β1 and Elongation factor genes for qPCR of *Atractosteus tropicus*.

Gene name	Symbols	Oligo	Primers sequence (5′–3′)	Temp. [°C]
Interleukin 10	*il-10*	–F	GCTGCCGAAGGTACTTCTCTT	60.03
		–R	GTCTGATAATGGGGAAATCCTG	59.67
β2 microglobulin	*b2m*	–F	AAGAACAAGCAGCAGATGGAG	59.63
		–R	TTTACATGTCAGGTTCCCAGGT	60.64
Transforming growth factor β1	*tgf-β1*	–F	TTCGATAAGACCAGAGGGGATA	59.92
		–R	CACACAGCAGTTTTCCATCTTC	59.78
Elongation factor	*ef1*	–F	CCTGCAGGACGTCTACAAGATCG	62.86
		–R	GACCTCAGTGGTCACGTTGGA	61.97

Statistical analysis

Data of growth, productive performance, enzymatic activities, and gene expression were analyzed for postulates of normality (KS) and homoscedasticity (Levene). One way (ANOVA) was performed and a posteriori Tukey test, if required. All tests were carried out using a level of significance of 95% in the Sigma Plot program (analytical software, AZ, USA).

Results

Cell viability in all experimental diets was $95.24 \pm 8.90\%$ at the end of the experiment without differences between treatments ($P > 0.05$). Fish fed with the CD and D1 obtained higher DWG and SGR compared to those fed D2 and D3 ($P < 0.05$). Feeding intake (FI) and survival did not show significant differences between treatments ($P > 0.05$); additionally, FCR showed a higher value for fish fed D3, while PER showed a lower value for fish fed D3 ($P < 0.05$) (Table 3). The HSI, VSI, and CF did not show significant differences between treatments ($P > 0.05$), while MSI and RIL showed lower value for fish fed D3 (Table 3).

The digestive enzymatic activity showed that acid proteases, total alkaline proteases, and lipase did not present significant differences between treatments ($P > 0.05$), while trypsin and chymotrypsin activities showed the lowest values for fish fed D3. LAP showed lower activity in fish fed D2 and D3 compared with fish fed CD and

Table 3. Productive values, survival and somatic indexes of *Atractosteus tropicus* juveniles fed with experimental diets supplemented with *Debaryomyces hansenii*.

Parameter	Treatment (Diet)			
	CD	D1	D2	D3
DWG	0.070 ± 0.002ᵃ	0.053 ± 0.003ᵃᵇ	0.047 ± 0.003ᵇ	0.037 ± 0.001ᶜ
SGR	5.19 ± 0.14ᵃ	4.82 ± 0.14ᵃᵇ	4.85 ± 0.08ᵇ	4.25 ± 0.28ᵇ
FCR	1.83 ± 0.13ᵃ	1.85 ± 0.16ᵃ	1.98 ± 0.14ᵃ	2.58 ± 0.26ᵇ
PER	1.72 ± 0.18ᵃ	1.25 ± 0.14ᵃ	1.10 ± 0.07ᵃᵇ	0.96 ± 0.11ᵇ
S	90.5 ± 4.5	81.6 ± 5.8	86.7 ± 7.6	76.7 ± 5.8
FI	4.28 ± 0.47	4.57 ± 0.32	4.59 ± 0.36	5.29 ± 0.97
HSI	3.36 ± 0.52	3.75 ± 0.47	3.51 ± 0.52	3.11 ± 0.45
VSI	7.49 ± 2.24	7.83 ± 0.89	7.46 ± 0.42	7.17 ± 1.75
MSI	2.17 ± 0.34ᵃ	1.52 ± .0.38ᵃᵇ	1.85 ± 0.49ᵃᵇ	1.34 ± 0.65ᵇ
CF	0.33 ± 0.06	0.29 ± 0.02	0.29 ± 0.01	0.30 ± 0.05
RIL	33.58 ± 5.99ᵃ	29.91 ± 4.64ᵃᵇ	28.89 ± 2.32ᵃᵇ	25.56 ± 5.55ᵇ

All weights expressed in grams [g], all lengths in centimeters [cm]; DWG = daily weight gain; SGR = specific growth rate; FCR = feed conversion rate; PER = protein conversion rate; S = survival; HSI = hepatosomatic index; VSI = viscerosomatic index; MSI = mesenteric fat index; CF = condition factor; RIL = relative intestine size. Values are mean ± standard deviation ($n = 9$). Different superscript letters within rows indicate significant ($P < 0.05$).

D1 treatments, α-amylase showed the lowest activity in fish feed D2, while alkaline phosphatases showed higher activity in fish fed CD ($P < 0.05$) (Table 4).

Table 4. Digestive enzyme activities of *Atractosteus tropicus* juveniles fed with experimental diets supplemented with *Debaryomyces hansenii*.

Total activity [U mg protein⁻¹]	Treatment (Diet)			
	CD	D1	D2	D3
Acid proteases	299.46 ± 31.10	403.59 ± 57.79	305.71 ± 38.40	305.71 ± 38.40
Alkaline proteases	38.80 ± 2.66	44.69 ± 3.28	30.80 ± 2.52	29.10 ± 3.39
Trypsin	32.40 ± 1.76a	38.24 ± 0.51a	28.76 ± 0.41a	21.42 ± 0.30b
Chymotrypsin	111.3 ± 19.8a	158.4 ± 13.0a	86.1 ± 14.8ab	62.6 ± 23.2b
LAP*	68.43 ± 4.08a	75.79 ± 4.57a	46.27 ± 0.82b	44.84 ± 1.22b
Lipase	31.69 ± 2.11	47.66 ± 8.17	29.59 ± 3.05	34.92 ± 2.92
α-amylase	27.69 ± 4.71a	30.72 ± 5.11a	11.76 ± 4.20b	20.21 ± 4.07ab
Alkaline phosphatases	2335.9 ± 98.0a	2118.1 ± 213.0b	1124.2 ± 34.1.0c	1495.0 ± 110.70b

*Leucine aminopeptidase. Values are mean ± standard deviation ($n = 6$). Different superscript letters within rows indicate significant ($P < 0.05$) differences.

On the other hand, gene expression of *il-10* showed significant differences between all treatments ($P < 0.05$), where all yeast supplemented treatments showed down-regulation in the liver (Fig. 1A) and intestine (Fig. 1B) with increasing yeast level dose; however, spleen showed up-regulation only in fish fed D1 (Fig. 1C). The *b2m* gene expression showed significant differences between all treatments ($P < 0.05$), where fish fed yeast supplemented diets treatments had down-regulation in the liver (Fig. 2A) and intestine (Fig. 2B), but in the spleen, the increment of yeast dose showed up-regulation in fish fed D3 (Fig. 2C). Finally, gene expression of *tgf-β1* showed that fish fed yeast supplemented treatments showed down-regulation in the liver (Fig. 3A) and intestine (Fig. 3B) ($P < 0.05$); nevertheless, when increasing yeast dose, the spleen (Fig. 3C) did not show significant differences between treatments ($P > 0.05$).
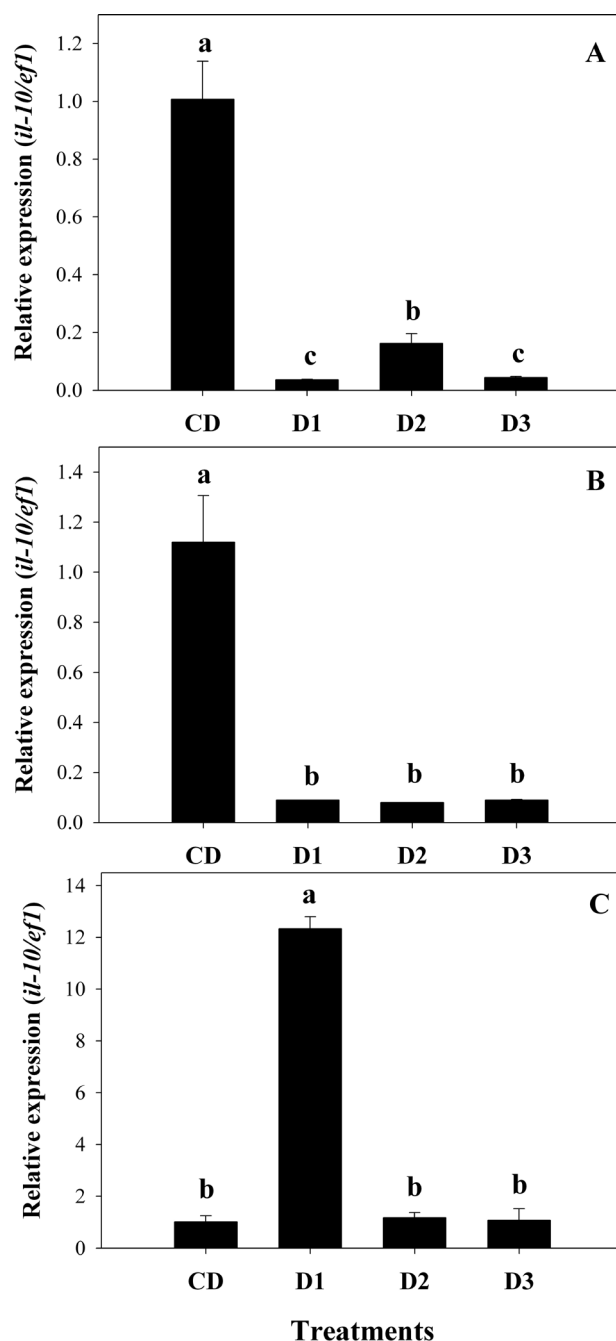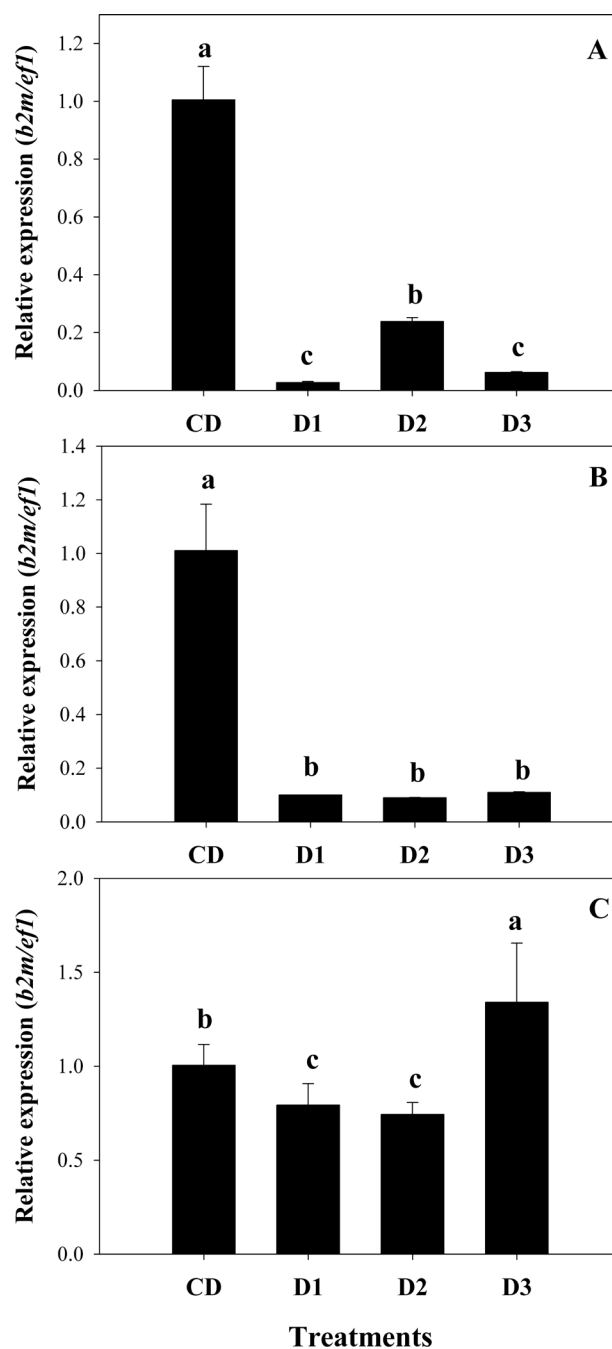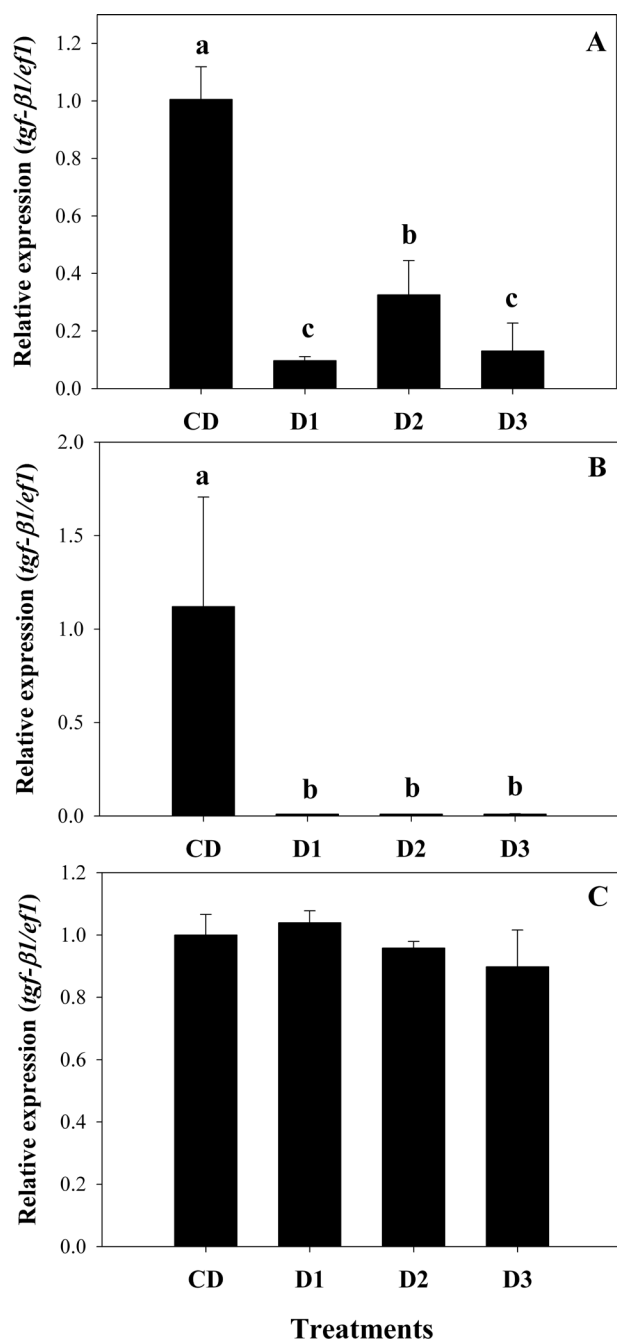
Figure 1. Relative expression of *il-10* in liver (1A) intestine (1B) and spleen (1C) of *Atractosteus tropicus* juveniles fed with experimental diets supplemented with *Debaryomyces hansenii*. Values are mean ± standard deviation ($n = 4$). Different superscript letters indicate significant ($P < 0.05$).

Figure 2. Relative expression of *b2m* in liver (2A) intestine (2B) and spleen (2C) of *Atractosteus tropicus* juveniles fed with experimental diets supplemented with *Debaryomyces hansenii*. Values are mean ± standard deviation ($n = 4$). Different superscript letters indicate significant ($P < 0.05$).

Discussion

During the feeding trial, *A. tropicus* juveniles fed CD, and D1 showed the same DWG and SGR. In this regard, the incorporation of yeast *D. hansenii* had no positive effect on growth, including the dose of 0.5% (10^{14} CFU g diet^{-1}). Meanwhile, higher doses of *D. hansenii* (1.0 and 1.5%, 10^{15} and 10^{16} CFU g diet^{-1}) could be highly excessive than 0.5%, which was reflected in some parameters such as

productive values (FCR and PER), somatic indexes (HSI, VSI, CF, and MSI) and digestive enzymes (acid and alkaline proteases, trypsin and chymotrypsin). It is well known that probiotics' positive effect depends on the concentration (Tovar-Ramírez et al. 2004). However, the appropriate concentration of probiotic levels depends on the probiotic type, fish species, physiological status, rearing conditions, and the specific goal of the applications, where 10^5 CFU mL^{-1} is commonly accepted probiotic density (Hai 2015). Nevertheless, reports in rainbow trout, *Oncorhynchus mykiss*

Figure 3. Relative expression of *tgf-β1* in liver (3A) intestine (3B) and spleen (3C) of *Atractosteus tropicus* juveniles fed with experimental diets supplemented with *Debaryomyces hansenii*. Values are mean ± standard deviation (*n* = 4). Different superscript letters indicate significant (*P* < 0.05).

(Walbaum, 1792), fed a probiotic (*Lactobacillus rhamnosus*) diet in a range of 10^9 or 10^{11} CFU g^{-1}, that can be considered as high doses, showed benefits on head kidney leukocyte phagocytic activity and improved serum lysozyme and alternative complement activity in a group that received the probiotic at 10^{11} CFU g diet^{-1}, as well to (Panigrahi et al. 2004). Nevertheless, to our knowledge, doses of live yeast as a probiotic over 10^{14} CFU g diet^{-1} are not still reported.

As characteristics, *D. hansenii* shows high adherence to fish gut mucosa (Tovar et al. 2002), being part of the

two main microbes of the microbiome core of wild and reared carnivore marine fishes, regardless of host species, domestication status, geographic location, and water salinity (Raggi et al. 2014). Our study showed that trypsin, chymotrypsin, LAP, and α-amylase had high activities in *A. tropicus* feed control diet and 0.5% *D. hansenii*, compared with higher inclusion (1.0 and 1.5%). Although it should be noted that the inclusion of 0.5% yeast was not statistically different from fish fed the control diet, it is observed that the digestive enzyme activities in fish supplemented with 0.5% *D. hansenii* are slightly higher than the control treatment. Furthermore, *D. hansenii* is associated with high production of polyamines (spermine, spermidine, and putrescine), considered as natural growth factors (Bardócz et al. 1993). Even polyamines play a role in promoting intestinal maturation and increasing the ability of enterocytes to absorb nutrients, in high concentrations decrease the absorption of nutrients (Sousadias and Smith 1995; Tovar et al. 2002, Tovar-Ramírez et al. 2004), where polyamines enter to enterocytes and induce a hormonal cascade that affects pancreas and liver (Peulen et al. 2000). As previously reported in *D. labrax* larvae, a low performance by feed high dose inclusion of *D. hansenii* is related to the high release of polyamines in the intestinal lumen (Tovar-Ramírez et al. 2004).

The beneficial effects of probiotics are consequences of several microbe properties, associated with the immune stimulation by providing molecules such as β-glucans, chitins, mannans, polyamines, among others (Akhter et al. 2015; Angulo et al. 2020), the production of inhibitory compounds that compete against pathogens bacteria for nutrients and adhesion sites and improving the microbial balance (Reyes-Becerril et al. 2017). These properties have been demonstrated in fish aquaculture, for example, cytokines as protein mediators contribute to cell growth, differentiation, and defense mechanisms of the host, where probiotics can modulate pro-inflammatory cytokines such as IL-10 and TGF-β1 in many species (Munir et al. 2016; Román et al. 2013). Accordingly, the liver and intestine showed a down-regulated effect of *il-10* and *tgf-β1* with yeast's inclusion; however, *il-10* expression was up-regulated in the spleen for *A. tropicus* feed 0.5% live yeast. Therefore, down-regulation of *il-10* and *tgf-β1* for fish feed 1.0 and 1.5% of live yeast corresponds to a negative effect to protect cells from an eventual inflammatory response (Kokou et al. 2015). On the other hand, *b2m* is involved in antigen presentation for the production of antibodies and has been reported to be regulated by diet composition (Murray et al. 2010). In our experiment, this gene was down-regulated in the liver and intestine and up-regulated in the spleen in *A. tropicus* feed 0.5%. Therefore, down-regulation could indicate the immune system's low ability to act against antigens (Kokou et al. 2015); however, this aspect should be verified by pathogen challenge in *A. tropicus*.

We hypothesize that incorporation of high dietary doses of *D. hansenii* (10^{14}, 10^{15}, and 10^{16} UFC g diet^{-1}) in diets for *A. tropicus* juveniles promote hyper colonization in the digestive tract with the concomitant high production of polyamines and the adverse effects on

growth, pancreatic and intestinal enzyme activities, as well as an immune-suppression of the immune systems (Tovar-Ramírez et al. 2004; Hai 2015). In this sense, fish feed at 0.5% (10^{14} UFC g diet^{-1}) of *D. hansenii* had better performance than fish feed higher doses; however, this dose of yeast cannot be recommended for the culture of *A. tropicus* either. Other studies, such as adherence bioassays and histological analyses of target tissues, clarify possible organ alterations or damage, as well as consider more immune-related gene targets.

Conclusions

Our results provide new evidence that the high inclusion of yeast *D. hansenii* (strain CBS 8339) (10^{14}, 10^{15}, and 10^{16} CFU g diet^{-1}) is not suitable for *A. tropicus* juveniles diet. These yeast concentrations affect growth, digestive enzymatic activity, and gene expression. For this reason, it is necessary to explore lower doses to optimize the inclusion of this probiotic and improve the growth and survival of this species.

Acknowledgments

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First record of *Monotaxis heterodon* (Actinopterygii: Perciformes: Lethrinidae) from the lagoon waters of Mischief Reef, South China Sea

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Abstract

Monotaxis heterodon (Bleeker, 1854) is widespread in the Indo-Pacific Ocean, however, it was reported that *Monotaxis grandoculis* (Forsskål, 1775) was the single species of *Monotaxis* in this area. We collected four *M. heterodon* specimens from the lagoon waters of Mischief Reef in South China Sea. A morphometric study was taken to confirm the occurrence of *M. heterodon* in the seawaters of the South China Sea and thoroughly separate them from *M. grandoculis*. In addition, DNA barcoding was taken for the classification of specimens. The mean genetic distance within *M. heterodon* group was 0.24 percentage points, group mean distance between *M. heterodon* and *M. grandoculis* was 8.71 percentage points. The phylogenetic analysis confirmed the existence of *M. heterodon* in the lagoon waters of the South China Sea. This study will contribute to species identification within this genus distributed in the South China Sea.

Keywords

Introduction

The family Lethrinidae consists of 5 genera and 43 species, including 28 species of *Lethrinus*, 11 species of *Gymnocranius*, 2 species of *Monotaxis*, and one each of *Gnathodentex* and *Wattsia* (see Chen and Borsa 2020; Fricke et al. 2021). However, only 4 genera and 33 species have been recorded from China (Chen and Zhang 2015).

There are some controversies on the taxonomy of *Monotaxis heterodon* (Bleeker, 1854), some authorities (Carpenter and Allen 1989; Nakabo 2002) indicated that it was a junior synonym of *Monotaxis grandoculis* (Forsskål, 1775), while others confirmed that the two species can be completely distinguished (Randall 2005; Senou et al. 2007; Chen and Borsa 2020).

Our purpose here was to confirm the existence of *M. heterodon* in the seawaters of the South China Sea and separating them from *M. grandoculis*. In addition, a mitochondrial DNA barcoding approach was also employed in order to better solve the identification problems of *M. heterodon* at the genetic level. This study reports for the first time *M. heterodon* from the South China Sea.

Material and methods

A total of 4 individuals of *Monotaxis heterodon* were collected from the lagoon waters of Mischief Reef (09°54′29″N, 115°32′05″E). All collected specimens were identified based on morphological characteristics used by Randall (2005).

For the genetic study, a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol to carry out DNA extraction. The classical phenol-chloroform technique was used for DNA extraction. PCR was subsequently conducted. The primer sequences used for COI amplification were 5′-TCGACTAAT-CATAAAGATATCGGCAC-3′ and 5′-ACTTCAG-GGTGACCGAAGAATCAGAA-3′ (Ivanova et al. 2007). PCR was carried out in a 25 µL reaction mix containing DNA template (1 µL, 50 ng · uL⁻¹), forward primer (F, 1 µL, 10 uM · L⁻¹), reverse primer (R, 1 µL, 10 uM · L⁻¹), dNTPs (2 µL, 2.5 mM · L⁻¹ each), Easy-Taq DNA Polymerase (0.15 µL, 5 U · µL⁻¹) and 10 × PCR buffer (2.5 µL, 25 uM · L⁻¹). A Biometra thermal cycler (Göttingen, Germany) with the following given procedure: one initial denaturation (95°C, 5 min), thirty-five cycles consisting of denaturation (94°C, 35 s), annealing (54°C, 35 s), and extension (72°C, 35 s), and one final extension (72°C, 10 min), was employed to put PCR amplification into effect. PCR products were preserved at 4°C. After agarose gel electrophoresis, the PCR products were sequenced by a biotechnology company. All 4 individuals' original sequences were successfully obtained and revised by DNASTAR software (DNASTAR Inc., Madison, WI, USA). Six COI sequences of *M. grandoculis* and two COI sequences of *M. heterodon* were downloaded from NCBI for phylogenetic study (Table 1). *Lethrinus miniatus* (Forster, 1801) (see Muallil et al. 2020) (EU148534.1) was chosen as the out-group to root the tree. MEGA X (Kumar et al. 2018) was used to construct a neighbor-joining (NJ) tree under the Kimura 2-parameter (K2P) model.

Table 1. GenBank accession numbers of related COI sequences downloaded from NCBI for phylogenetic tree study.

Species	GenBank accession number	Reference
Monotaxis grandoculis	MN870095.1	Lakra 2011
	MN870602.1, MN870297.1	Limmon et al. 2020
	JQ431909.1, JQ431910.1	Hubert et al. 2012
	JF952794.1	Zhang et al. 2011
Monotaxis heterodon	MN870117.1, MN869984.1	Limmon et al. 2020
Lethrinus miniatus	EU148534.1	Muallil 2020

Results

The general morphological features of *Monotaxis heterodon* are shown in Fig. 1. Body oblong and compressed laterally; eye very large; The operculum, cheek, and inner surface of pectoral fin base covered with scales; 5 rows of scales between lateral line and base of middle dorsal spines; greenish-gray dorsally, shading to silvery white ventrally. All color descriptions are in accordance with Randall (2005).

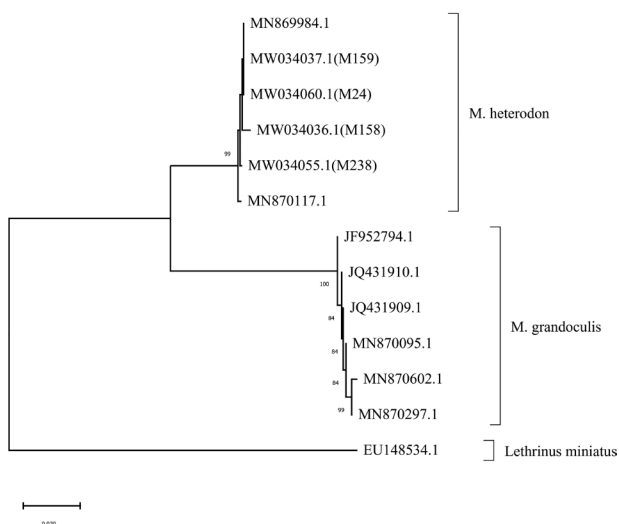**Figure 1.** Left lateral view of *Monotaxis heterodon* (No. M238; length 186 mm SL).

Figure 2. Phylogenetic tree based on neighbor-joining analysis of COI sequence. *Lethrinus miniatus* (EU148534.1) was chosen as the out-group to root the tree. Numbers above branches indicate neighbor-joining bootstrap percentages. Only Bootstrap values >50 percentage points are shown in the NJ tree.

All the sequences have been deposited in GenBank. The neighbor-joining phylogenetic tree is shown in Fig. 2. Specimens of *M. heterodon* in the presently reported study clustered in a group, and those of *M. grandoculis* and *Lethrinus miniatus* clustered in another two groups, respectively. The genetic distance of the COI sequence within *M. heterodon* group was only 0.24 percentage points, and the mean distance between *M. heterodon* and *M. grandoculis* was up to 8.71 percentage points (Table 2). Together, both the morphological and genetic analysis strongly supported the validity of *M. heterodon* as a new record in Mischief Reef.

Discussion

Located in the center of the Indo-western Pacific region, the South China Sea is the largest semi-closed sea in the western tropical Pacific Ocean with extremely rich biodiversity. With more than 1120 species of reef fish species, the South China Sea is one of the world's major centers of coral reef diversity (Zhang et al. 2021). Mischief Reef is oval and located in the eastern and central waters of the Nansha Islands with a large lagoon. Benefiting from the tropical monsoon climate and warm waters, Mischief Reef is well suited for marine fishing (Yu et al. 2006). In addition, Mischief Reef

area is a good spawning and nursery ground for many fish species like skipjack tuna, *Katsuwonus pelamis* (Linnaeus, 1758); yellowfin tuna, *Thunnus albacares* (Bonnaterre, 1788); blue round scad, *Decapterus maruadsi* (Temminck et Schlegel, 1843); and kawakawa, *Euthynnus affinis* (Cantor, 1849) (see Ma et al. 2016). Sun et al. (2019) found 37 species of fishes and 7 species of invertebrates collected with gill nets and trawls in Mischief Reef. Shan et al. (2021) identified 209 individuals using morphological and molecular techniques in Mischief Reef, including 101 species in 62 genera, 27 families, 8 orders, and 1 class. Biodiversity is based on species diversity and is closely related to ecosystems and human well being (Barman et al. 2018). The presently reported survey of Mischief Reef will hopefully contribute to a better understanding of this complex ecosystem.

Monotaxis heterodon is widespread in the Indo-Pacific Ocean, with records from the Seychelles, the Maldives, Sri Lanka, Indonesia, Malaysia, New Guinea, Great Barrier Reef, Marshall Islands, and New Caledonia (Randall 2005); the Tawi-Tawi Islands (Muallil et al. 2020), New Ireland (Fricke et al. 2019), the Andaman and Nicobar Islands (Devi et al. 2015), Madang (Fricke et al. 2014), and Japan (Senou et al. 2007; Akita et al. 2016). Previous studies on the fishes of the South China Sea have not reported *M. heterodon*, presumably because the controversies on the taxonomy of this species. *Monotaxis heterodon* and *M. grandoculis* are very similar, but morphologically, the two species can be distinguished by some characteristics. The first one is the number of transversal scale rows on the flank: the count of scales below the lateral line to origin of anal fin is 12.5 for *M. heterodon* while it is 13.5 for *M. grandoculis*. *Monotaxis heterodon* lacks the black spots at the base of the dorsal and anal fins. Furthermore, *M. heterodon* usually have brown or reddish lips but *M. grandoculis'* lips are often yellowish (Randall 2005; Chen and Borsa 2020). Although the lip color of our samples cannot be recognized, the count of scales below the lateral line to origin of anal fin is 12.5 and there are no spots at the base of the dorsal and anal fins of our samples.

Monotaxis heterodon was once thought to be a different color morph of *M. grandoculis* (see Bilecenoglu 2007) but Chen and Borsa (2020) recently confirmed the occurrence of the two species in the genus *Monotaxis*. Some different methods were adopted as the indicator of speciation. Such as the "2 percentage points rule" proposed by Ward et al. (2009), that is, individuals were much more likely to be congeneric than conspecific if that at a distance level of 2 percentage points or higher. The mean evolutionary distance within the species *M. heterodon* was 0.24 percent-

Table 2. Pairwise genetic distances (K2P) within (bold font) and between (normal font) species.

	MN870602.1	MN870297.1	JF952794.1	JQ431910.1	JQ431909.1	MN870095.1	MN870117.1	MN869984.1	M24	M158	M159	M238
MN870117.1	0.0980	0.0912	0.0853	0.0879	0.0879	0.0879						
MN869984.1	0.0941	0.0876	0.0849	0.0876	0.0876	0.0876	**0.0032**					
M24	0.0941	0.0876	0.0818	0.0844	0.0844	0.0844	**0.0031**	**0.0000**				
M158	0.0921	0.0857	0.0799	0.0826	0.0826	0.0826	**0.0062**	**0.0032**	**0.0031**			
M159	0.0941	0.0876	0.0818	0.0844	0.0844	0.0844	**0.0031**	**0.0000**	**0.0000**	**0.0031**		
M238	0.0961	0.0895	0.0836	0.0862	0.0862	0.0862	**0.0015**	**0.0016**	**0.0015**	**0.0046**	**0.0015**	

*Since the variation of COI sequence is very small, many sequences within species have no variation, so the genetic distance between *Monotaxis grandoculis* is omitted.

age points, the use of the 2 percentage points rule suggests that *M. heterodon* was here believed as a valid species.

The presently reported study constitutes the first record of *M. heterodon* and increases the number of *Monotaxis* species known to occur in the South China Sea.

Competing interests

The authors have declared that no competing interests exist.

Acknowledgments

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Population characteristics of the non-indigenous round goby, *Neogobius melanostomus* (Actinopterygii: Perciformes: Gobiidae), in the eastern Gulf of Finland

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Abstract

The round goby, *Neogobius melanostomus* (Pallas, 1814), is a fish of Ponto-Caspian origin that has been invading the Baltic Sea since the 1990s. Currently, it is abundant and commercially important in some areas of the sea. This species was first reported in the eastern Gulf of Finland (GoF) in 2012. Its occurrence increased thereafter, however it has remained largely unstudied in this region. The aim of this study was to investigate the population characteristics of the round goby in the eastern GoF to better understand its expansion trend and whether it will become abundant enough to be exploited by the local fishery. Fish were caught using multi-mesh gillnets (12–60 mm mesh) and a beach seine (0.5–10 mm mesh). Occurrence, density, catch per unit effort, biomass per unit effort, relative number and biomass in catches, as well as age, size and sex ratio were studied. The species regularly occurred in samplings along the southern coastline of the GoF, as well as some central areas and along the northern coast. Within 2012–2019, its occurrence in catches increased, with the highest frequency in 2015–2019 in shallow waters (<1.5 m) of Koporye Bay (70%) and in the deeper waters of Narva Bay (74%). Similarly, the highest density in the shallow waters was also observed in Koporye Bay (10.0 ind. · 100 m^{-2}), and offshore in Narva Bay. Relative abundance and biomass usually did not exceed 23%, although it reached 93% in Narva Bay. The oldest specimen was five years old. Young-of-the-year (YOY) juveniles predominated in the shallow waters (85%), while three-year-olds prevailed in deeper waters (75%). Among the fish older than two years, females were more predominant (mean ratio 3 to 1), and males were larger than females. Specimens in all life stages were found in the eastern GoF, and their abundance increased annually, suggesting that the round goby has successfully colonized this region of the Baltic Sea. However, compared to other areas of the Baltic Sea inhabited by longer-established populations, its population size is still relatively low.

Keywords

Introduction

The round goby, *Neogobius melanostomus* (Pallas, 1814), is considered to be one of the most invasive fish species in the Baltic Sea (Kornis et al. 2012). Expansion of this Ponto-Caspian fish began in the 1990s when it was transferred to the Baltic Sea via ballast waters (Sapota and Skóra 2005). The high adaptive ability and tolerance to different environmental conditions, along with its aggressive behavior and high reproductive potential, have resulted in the successful colonization of this species in new habitats (Charlebois et al. 1997). The first population of the

round goby in the Baltic Sea was established along the coastline of Poland, where the species became abundant in the early 1990s (Sapota 2004). The fish then expanded its distribution both eastward and westward, where it was later caught along the coastlines of Lithuania, Latvia, and Germany (Kotta et al. 2016). Thus far, the round goby has been established in all the sub-basins of the Baltic Sea, including coastal waters of Denmark, Estonia, Finland, Russia, and Sweden (Puntila et al. 2018).

In some newly invaded areas, the round goby became so abundant that management actions were applied for its commercial fishing (Ojaveer et al. 2015). These actions resulted in sharp increases in Latvian catches, from less than 1 ton in 2011 to over 500 tons in 2016; this species accounted for approximately one-third of all catches (Puntila et al. 2018). The round goby was first found along the Estonian coastline in 2002 (Ojaveer 2006). By 2016, its catches exceeded 100 tons (Järv et al. 2018).

The increase in non-indigenous round goby populations can lead to competition with the local fauna of the Baltic Sea. Round gobies mainly feed on bivalve mollusks, although other benthic organisms can also be included in their diet (Smirnov 1986). Therefore, the round goby can compete for food with native benthivorous fish species (Karlson et al. 2007; Rakauskas et al. 2013; Ustups et al. 2016). In addition, the round goby can directly influence native fish populations by feeding on their eggs and/or juveniles, including commercial species (Wiegleb et al. 2018). Native piscivorous fish, in turn, can limit the abundance of the round goby through top-down control (Rakauskas et al. 2013). Therefore, studies on round goby populations in newly invaded areas are very important for understanding the dynamics of fish populations, including commercially important species.

In the Russian territorial waters of the eastern Gulf of Finland (GoF), the round goby was first observed in 2012 (Uspenskiy and Naseka 2014). Its abundance has since increased, and the species has become an unintentional by-catch. During the late 20th–early 21st centuries, local fisheries were mainly focused on herring, *Clupea harengus* Linnaeus, 1758; smelt, *Osmerus eperlanus* (Linnaeus, 1758); and sprat, *Sprattus sprattus* (Linnaeus, 1758), (see Lajus et al. 2015). The appearance of the round goby in the eastern GoF can essentially change the target species for fisheries, as observed in other parts of the Baltic Sea.

The aim of this study was to investigate the population characteristics of the non-indigenous round goby in the eastern GoF in order to better understand its expansion trend and whether it will become abundant enough to be exploited by the local fishery.

Materials and methods

Sampling sites. The fish community in the eastern GoF has been monitored annually since 1998 by the State Research Institute on Lake and River Fisheries (Gos-NIORKh, Saint-Petersburg), using multi-mesh gillnets. In total, 1627 samplings were carried out, mainly in

Vyborg Bay and the adjacent waters (539 catches since 1998), Neva Bay (531 catches since 2002), and Luga Bay (245 catches since 2001) (Fig. 1). The Inner Estuary and Central Area were monitored less frequently (164 and 88 catches since 1998, respectively). In Koporye Bay, gillnet sampling was carried out only in 2014 and 2015. Gillnet sampling in Narva Bay began in 2016; to date, 57 catches have been conducted. An additional 179 beach seine samplings were organized between 2010 and 2019 at 48 coastal shallow sites of the eastern GoF (Fig. 1). All samplings were carried out during the ice-free season from April to November.

Fishing gears. Sampling gear included a hand-towed beach seine that reached a maximum depth of 1.2 m and gillnets that reached a sampling depth between 2.0 and 24.0 m. The length of the beach seine was 10 × 1.5 m; the mesh size was 10 mm in the wings and 0.5–4.0 mm in the cod end. On average, the mouth width of the beach seine while seining was 6.0 m. The trawling distance was 25–90 m according to the depth and bottom features of the sampling location. The demersal multi-mesh gillnet (48 × 1.8 m) included 8 monofilament net sections (6 m in length each) with mesh size 12, 15, 20, 25, 30, 35, 45, and 60 mm (Appelberg 2000). All fish specimens caught in the multi-mesh gillnet were combined into one sample.

Estimates of the abundance. Estimates of the abundance included: frequency of occurrence (V), density and biomass for beach seine catches, catch per unit effort (CPUE) and biomass per unit effort (BPUE) for the net catches, and relative abundance and biomass (RN and RB).

Frequency of occurrence (V) [%] in samples was estimated as:

$$V = 100 \cdot a \cdot A^{-1}$$

where, a represents the number of samplings where the species was caught, and A is the total number of the samplings. The species was classified as "accidental", "rare", "common", and "constant" for V values < 15%, 15%–40%, 40%–70%, and > 70% respectively (Žiliukas et al. 2012).

Density (D) [ind. · 100 m⁻²] and biomass (B) [g · 100 m⁻²] for the beach seine were estimated as number (N_i) [ind.] and wet weight (W_i) [g] of individuals per 100 m² of the sampled area (S) [m²] (Žiliukas et al. 2012):

$$D = 100 \cdot N_i \cdot S^{-1}$$

and

$$B = 100 \cdot W_i \cdot S^{-1}$$

where, S was calculated by multiplying the hauling distance and mouth width of a beach seine while seining; the distance was estimated by an optical laser distance meter (accuracy of 1 m).

CPUE [ind. · 12 h^{-1}] and BPUE [g · 12 h^{-1}] for the gill-nets were estimated as number (N_i) [ind.] and wet weight (W_i) [g] of individuals caught by one net for 12 hours of fishing (Appelberg 2000):

$$\text{CPUE} = N_i \cdot 720 \cdot t_f^{-1}$$

and

$$\text{BPUE} = W_i \cdot 720 \cdot t_f^{-1}$$

where, 720 represents the number of minutes in 12 hours and t_f is the actual duration of fishing (in minutes).

Relative abundance (RN) [%] and biomass (RB) [%] in a sample were estimated as:

$$\text{RN} = 100 \cdot N_i \cdot N_{total}^{-1}$$

and

$$\text{RB} = 100 \cdot W_i \cdot W_{total}^{-1}$$

where, N_i and W_i are the number [ind.] and wet weight [g] in a sample, respectively, and N_{total} and W_{total} are the total number [ind.] and wet weight [g] of all fish in a sample, respectively. A species was classified as "dominant", "abundant", "moderate in number", "few in number" and "scarce" for RN over 50%, 50%–10%, 10%–1%, 1%–0.1%, and less than 0.1%, respectively (Terešenko and Nadirov 1996).

Size, age, sex ratio. Fish which were examined to size, age and sex were collected at five sites in the shallow waters (<1.5 m depth) along the southern coastline ($n = 109$ ind.) and in two deeper (6–8 m depth) stations in Narva Bay ($n = 1093$ ind.) (Table 1).

Standard length (SL) and total (wet) weight (TW) were estimated with a ruler to the nearest mm and a lab-

Table 1. Number (N_i) of the round goby from the eastern Gulf of Finland analyzed in relation to length (L), mass (M), age (A), sex (S). Listed stations are depicted at Fig. 1.

Fishing gear	Depth [m]	Sampled stations	Date of catch	Studied population characteristics	N_i
Beach seine	0.0–1.2	1b, 2b, 3b, 4b, 5b	2012–2017	L, M, S, A	109
Gillnet	6.0–8.0	3n, 5n	06.2018	L, M	1093
		5n	06.2018	L, M, S, A	172

oratory scale (GP1200-G, Sartorius, Germany; accuracy of 0.01 g), respectively. TW of the goby specimens in the net samples was rounded to the nearest 0.1 due to the larger size. Age was estimated by examining otoliths cleared in glycerol (Kostûčenko 1961). Although sectioned and stained otoliths were recently recommended for age reading in the goby (Florin et al. 2018), earlier studies were mainly conducted using whole otoliths (Kostûčenko 1961; Sokołowska and Fey 2011; Azour et al. 2015; Bose et al. 2018). Hence, we also applied whole sagittal otoliths to obtain comparable data with

previous studies. The age of goby specimens with visible growth zones of the current season was marked with "+" (Kostûčenko 1961). Sex was assigned according to the shape of the urogenital papilla (Kornis et al. 2012). Sex and age were both studied using an MBS–10 (LZOS, Russia) stereomicroscope.

Statistical comparisons were conducted with the use of Statistica 12 and PAST Statistics software. The abundance of the round goby in different areas was compared using ANOVA. The abundance data were log-transformed to achieve normal distribution, which was checked with Shapiro–Wilk test. Males and females' length were compared using t-test after checking for normality with the use of chi-square test. Non-normally distributed length data was compared by means of non-parametric Mann–Whitney U Test. Spearman's rank correlation was used for the RN and RB relation analysis since normal distribution was not confirmed.

Results

Distribution. The round goby was first observed in the eastern GoF in 2012. Since then, its distribution area has increased (Table 2).

In shallow waters, gobies were caught at nine stations, i.e., 18.7% of all studied locations (stations 1b–9b, Fig. 1, Table 2). The first specimen was caught in Luga Bay (Fig. 1, station 1b). The easternmost finding was in 2015, collected by the St. Petersburg flood-prevention facility complex (SPb FPFC dam) (station 5b). In 2019, juveniles were first collected in the shallow waters along the northern coast (station 9b). No specimens were caught in the shallows of Vyborg and Neva Bay.

This species has occurred in gillnet catches since 2015, after its first finding in Luga Bay (stations 6n and 7n, Fig. 1, Table 2). In 2016, gobies were also caught in Narva Bay and close to Seskar Island (stations 2n and 12n). In 2017, it was first caught along the northern coast by cape Stirsudden (station 13n), and in Vyborg Bay in 2019 (station 15n). No specimens were caught by nets in Neva Bay.

Since 2012, the round goby has been regularly observed in catches along the southern coastline of the GoF, both in eastward and northward directions.

Frequency of occurrence (V) varied across the years, increasing from 2012 to 2019 (Table 2). The round goby was first caught in shallow waters with a beach seine in 2012, and three years later (2015) it was caught in deeper waters with gillnets. In beach seine catches between 2012 and 2019, V ranged from 2% to 50% (12% in total). Accordingly, the species was classified as "accidental" in 2012 and 2014, "rare" in 2015–2017, and "common" in 2019. In gillnets between 2012 and 2019, V ranged from 3% to 81% (26% in total) (Table 2). Accordingly, the species was "accidental" in 2015–2017, and "constant" in 2018–2019. This data includes the results for all sampled areas (including Neva Bay, where the species was not

Table 2. Number of beach seine and gillnet catches and frequency of the round goby occurrence in different parts of the eastern Gulf of Finland between 2012 and 2019 (see also Fig. 1).

Year	Luga Bay				Narva Bay				Koporye Bay				Inner Estuary*, Central and Island Area				Vyborg Bay, Berezovye Islands' Area				The entire eastern GoF		
	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i
Beach seine																							
2012	1	100	1	1b	2	0	0						10	0	0		1	0	0		17	6	1
2013	2	0	0		4	0	0						12	0	0		5	0	0		35	0	0
2014	2	50	2	1b	2	0	0						29	0	0		3	0	0		52	2	2
2015	1	0	0						2	50	1	2b	5	20	3	5b					11	18	4
2016	1	100	1	1b					1	100	40	2b	11	18	31	4;5b	2	0	0		19	21	74
2017									5	60	16	2;3b	5	40	12	4;5b					13	38	28
2018																					0	0	0
2019	2	50	4	7b	1	100	8	6b	2	100	8	2;8b	4	50	3	5;9b	1	0	0		12	50	23
Total	9	44	8		9	11	8		10	70	65		76	9	49		12	0	0		159	12	132
Multi-mesh gillnets																							
	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i	Stns.	A	V	N_i
2012	34	0	0										9	0	0		3	0	0		82	0	0
2013	8	0	0										23	0	0		7	0	0		100	0	0
2014	8	0	0						1	0	0		22	0	0		2	0	0		76	0	0
2015	20	15	4	6;7n					2	0	0		24	0	0		6	0	0		87	3	4
2016					14	14	11	2n					20	15	10	12n					48	8	21
2017	3	67	3	7n	3	33	1	2n					24	4	1	13n	23	0	0		37	11	5
2018	21	76	35	6–10n	40	98	1395	1;3–5n					9	44	7	11n	5	0	0		75	81	1437
2019					101	74	714	1;3–5n					18	78	51	12–14n	6	17	1	15n	122	74	766
Total	94	22	42		158	74	2121		3	0	0		149	15	59		52	2	1		627	26	2233

Data is presented only for the areas where the species occurred in catches ever. Abbreviations: A – total number of catches; V – frequency of the round goby occurrence; N_i – number of the specimens caught; Stns. – stations where the species was caught (see also Fig. 1).

* For sampling with the beach seine only data for the Inner Estuary is presented.

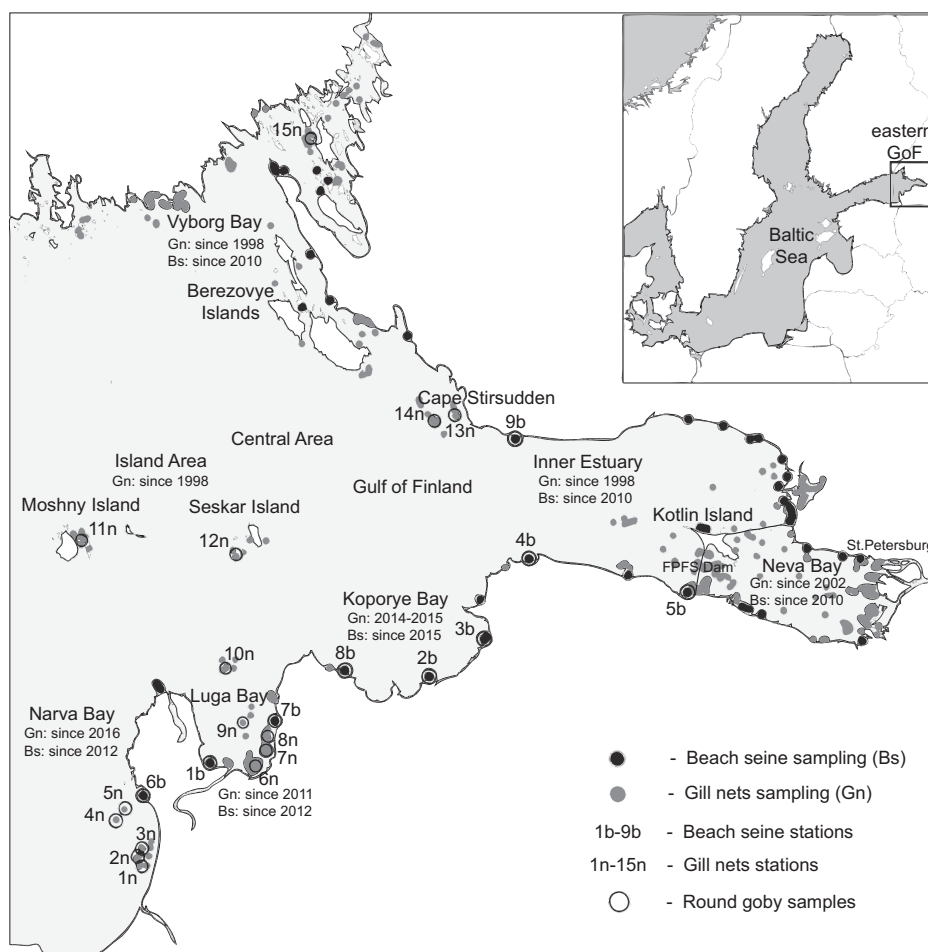** Mean V are estimated for each location from pool of all samplings.

Figure 1. Stations in the eastern Gulf of Finland where round goby, *Neogobius melanostomus*, was sampled within 1998–2019 using multi-mesh gillnets (Gn, grey dots) and a beach seine (Bs, black dots) between 2010–2019. Stations, where the round goby was caught, are numbered and marked with circles: 1n–15n are the gillnet stations, 1b–9b are the beach seine stations. Years indicates sampling periods.

caught) for the period since it was first observed there. Due to the differences in numbers of catches and localities between years, V values are presented separately by the sampling area in Table 2.

V was highest between 2012 and 2019 in the shallow waters of Koporye Bay (70%); a relatively high occurrence was also observed in Luga Bay (44%). In the deeper waters, V was highest in Narva Bay (74%) (Table 2). In these areas, the species was classified as "constant" (Narva Bay) and "common" (Koporye and Luga Bays).

Density, biomass, and catch per unit effort. The density (D) of the round goby ranged from 0.1 to 10.0 ind. $\cdot$ 100 m^{-2} (mean $\pm$ SE = 1.9 $\pm$ 0.68) between 2012 and 2019, and biomass ranged from 0.1 to 9.4 g $\cdot$ 100 m^{-2} (mean $\pm$ SE = 1.7 $\pm$ 0.52). For 58% of the samples, density did not exceed 1 ind. $\cdot$ 100 m^{-2} (Table 3). In the shallow waters, the highest catches per 100 m^2 was observed in the Inner Estuary (max 10.0 ind. $\cdot$ 100 m^{-2}, mean $\pm$ SE = 2.0 $\pm$ 1.35) in Koporye Bay (max 9.5 ind. $\cdot$ 100 m^{-2}, mean $\pm$ SE = 2.6 $\pm$ 1.29).

The round goby was not found in gillnets before 2015, and was rare until 2018; CPUE and BPUE ranged

from 0.3 to 4.8 ind. per 12 hours of fishing, and from 6.2 to 163.8 g per 12 hours of fishing, respectively (Table 4). It was first abundant in June 2018 in the catches of Narva Bay (means $\pm$ SE were 31.9 $\pm$ 5.71 for CPUE and 888.0$\pm$177.59 for BPUE, respectively). The highest catch throughout the monitoring period was 133.2 ind. $\cdot$ 12 h^{-1}, which was also in June 2018. CPUE was higher in Narva Bay (16.6 $\pm$ 2.58) (log-transformed data, ANOVA, $F = 45.9$, $P < 0.001$) than in other areas (1.9 $\pm$ 0.41), although the catches in Narva Bay varied between stations and seasons. In May–June 2018, catches per 12 h on stony bottom of the station 5n exceeded (log-transformed data, ANOVA, $F = 47.2$, $P < 0.001$) that at sandy bottom stations (87.6 $\pm$ 11.03 vs. 14.8 $\pm$ 1.91 ind. $\cdot$ 12 h^{-1}, respectively). In April–June 2019, the mean CPUE for Narva Bay was 18.2 $\pm$ 5.40 ind. $\cdot$ 12 h^{-1}, although station 5n was not sampled. Hence, mean CPUE was similar in spring and early summer in both 2018 and 2019 at the sandy bottom stations. Later in 2019, this value did not exceed 8.9 $\pm$ 1.42 ind. $\cdot$ 12 h^{-1}, although different grounds were observed.

Relative abundance (RN) and biomass (RB) in catches. The round goby was found to occur with 23 and 25 other fish species in the beach seine and gillnet catches, respectively. The most common species (arranged in ascending order of occurrence) were: gudgeon, *Gobio gobio* (Linnaeus, 1758); roach, *Rutilus rutilus* (Linnaeus, 1758); bleak, *Alburnus alburnus* (Linnaeus, 1758); common goby, *Pomatoschistus microps* (Krøyer, 1838); perch, *Perca fluviatilis* (Linnaeus, 1758); tubenose goby, *Proterorhius marmoratus* (Pallas, 1814); in the beach seine catches. In the gillnet catches, the most common species were perch, sprat, ruffe, *Gymnocephalus cernua* (Linnaeus, 1758), herring, and smelt.

In the beach seine catches within 2012–2019, RN and RB ranged from less than 0.1% to 11.1% (mean $\pm$ SE = 3.2 $\pm$ 0.70) and from 0.1% to 23.0% (mean $\pm$ SE = 5.1 $\pm$ 1.35), respectively (Fig. 2A). According to the mean RN

Table 3. Density (D) [ind. $\cdot$ 100 m^{-2}] and biomass (B) [g $\cdot$ 100 m^{-2}] of the round goby in catches of beach seine in the eastern Gulf of Finland.

Date	Narva Bay		Luga Bay		Koporye Bay		Inner Estuary	
	D	B	D	B	D	B	D	B
Jul 2012			0.1	0.4				
Jun 2014			0.4	1.0				
Aug 2015					0.3	1.7		
Sep 2015							0.3	0.1
Aug 2016			0.2	0.7	9.5	2.8	10.0	2.3
Sep 2016							0.3	4.9
Jul 2017					1.1	3.2		
Aug 2017					2.6 $\pm$ 2.16	5.1 $\pm$ 4.22	1.1 $\pm$ 0.74	1.5 $\pm$ 0.86
Aug 2019	2.7	0.2	1.1	0.1	1.0 $\pm$ 0.64	0.6 $\pm$ 0.39	0.7	0.1
Sep 2019							0.2	0.1

Mean values $\pm$ SE are given when two or more stations were sampled within one area (Fig. 1, Table 2).

Table 4. Catch per unit effort (CPUE) [ind. $\cdot$ 12 h^{-1}] and biomass per unit effort (BPUE) [g $\cdot$ 12 h^{-1}] of the round goby in catches of multi-mesh gillnets in the eastern Gulf of Finland. Mean values $\pm$ SE are given when two or more stations were sampled within one area (Fig. 1, Table 2).

Date	Narva Bay		Luga Bay		Seskar Island and Moshny Island*		Cape Stirsudden		Vyborg Bay	
	CPUE	BPUE	CPUE	BPUE	CPUE	BPUE	CPUE	BPUE	CPUE	BPUE
May 2015			1.0	56.0						
Jun 2015			1.0	23.0						
Aug 2015			2.0	103.0						
Aug 2016	4.8	163.8								
Sep 2016					1.4 $\pm$ 0.28	31.4 $\pm$ 8.22				
Jun 2017			1.5 $\pm$ 0.48	38.7 $\pm$ 18.72						
Sep 2017	0.3	6.2					0.5	8.8		
Jun 2018	31.9 $\pm$ 5.71	888.0 $\pm$ 177.59			2.4*	32.0*				
Oct 2018			1.8 $\pm$ 0.50	94.8 $\pm$ 24.91	1.3 $\pm$ 0.31	53.2 $\pm$ 13.52				
Nov 2018			1.0 $\pm$ 0.23	40.4 $\pm$ 14.22						
Apr–Jun 2019[1]	18.2 $\pm$ 5.40	490.4 $\pm$ 160.54								
Jul 2019	8.9 $\pm$ 1.42	233.5 $\pm$ 41.43			7.2 $\pm$ 4.44	222.6 $\pm$ 107.6	0.8 $\pm$ 0.04	49.1 $\pm$ 8.57		
Sep 2019					4.9 $\pm$ 2.68	243.3 $\pm$ 158.8	1.0 $\pm$ 0.20	41.0 $\pm$ 13.91		
Oct–Nov 2019	4.8 $\pm$ 1.28	229.3 $\pm$ 67.20							1.0	48.0

[1] Data obtained using gill nets of different mesh size (14, 18, and 20 mm) than in other lines, where multi-mesh gill nets of mesh size 12, 15, 20, 25, 30, 35, 45, and 60 mm were used. An asterisk * indicates a catches on Moshny Island, data without asterisks in such columns refers to Seskar Island.
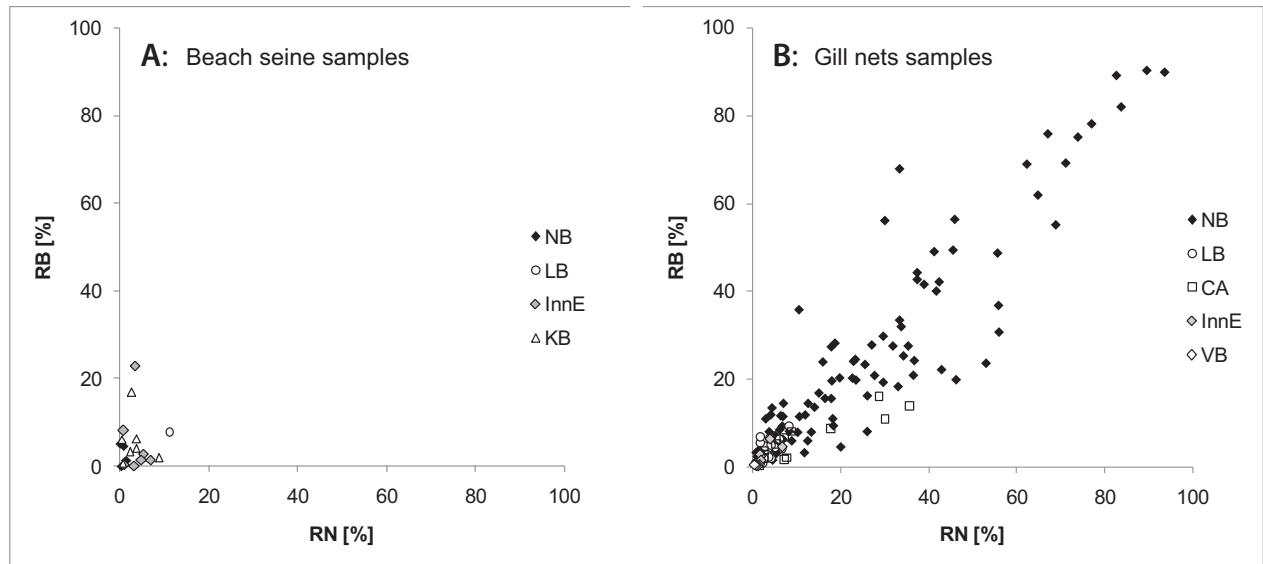
Figure 2. Relative abundance (RN) and relative biomass (RB) of the round goby, *Neogobius melanostomus*, in the eastern Gulf of Finland between 2012–2019. **A** Coastal beach seine catches. **B** Multi-mesh gillnet catches. Areas: LB = Luga Bay; NB = Narva Bay; KB = Koporye Bay; InnE = Inner Estuary; CA = Central and Island Area; VB = Vyborg Bay.

value, the species is considered as "moderate in number" in coastal stations.

Within 2015–2019, RN and RB in gillnet catches ranged from 0.3% to 93.4% (mean ± SE = 18.5 ± 1.76) and from 0.2% to 90.3% (mean ± SE = 17.5 ± 1.75), respectively (Fig. 2B). From 2015 to 2017 the RN and RB increased from 6.7 ± 1.81 and 3.5 ± 0.91 (mean ± SE), respectively. In 2018, these respective values increased to 23.4 ± 3.43 and 21.9 ± 3.31. In 2019, these values were 16.5 ± 2.02 and 16.2 ± 2.10, respectively. Accordingly, the species became "abundant" in gillnet catches in 2018.

The estimated RN was higher in Narva Bay (mean ± SE = 23.8 ± 2.25; log-transformed data, ANOVA, F = 16.1, $P < 0.001$) than in the other areas (Fig. 2B). In June of 2018 and 2019, the mean values (± standard error of the mean, SE) of RN in Narva Bay was 33.1 ± 4.33 and 11.0 ± 4.98, respectively, which is at least 3–4 times higher than in the later months.

In the gillnet catches, RN and RB were strongly correlated (Spearman's $R = 0.92$, $P < 0.001$), unlike the beach seine catches (Spearman's $R = 0.11$, $P = 0.66$). Therefore, in the shallow waters, round goby specimens never had high masses, even if they were numerous. However, it

was more predominant in the deeper waters, in terms of both mass and numbers. Such correlation is explained by the differences in round goby size composition between shallow waters and offshore biotopes.

Size, age, and sex ratio. Juveniles of the age 0+ (85.3%), 1+ (13.8%), and 2+ (0.9%) were caught in the shallow waters of the southern coast (Table 5). Sex identification was possible only for fish with SL > 18 mm. The sex ratio was equal in age classes 0+ and 1+, while older specimens were sporadic in the shallow waters.

In late summer, young-of-the-year (YOY) gobies reached 42 mm (mean ± SE = 22.3 ± 0.48) SL and 1.69 g (mean ± SE = 0.27 ± 0.02) TW. The following July, length of yearlings (1+) ranged from 37 to 60 mm SL, and reached 72 mm SL in late August (Table 5). Males and females were not different in SL at age of 0+ and 1+ ($t = 0.227$, df = 55, $P = 0.821$, and $U = 33.000$, $Z = -0.241$, $P = 0.810$, respectively).

In June 2018, round gobies caught by gillnets at depths of 6–8 m in Narva Bay ranged from 60 to 170 mm SL, and from 6.3 to 133.0 g TW ($N_i = 1093$, mean ± SE were 100.0 ± 0.11 and 30.4 ± 0.52, respectively). The age of the 172 specimens from this sample ranged from two to

Table 5. Age, length and mass of the round goby in the eastern Gulf of Finland.

Age*	Male				Female				Sex not determined		
	N_i	SL [mm]	TW [g]		N_i	SL [mm]	TW [g]		N_i	SL [mm]	TW [g]
Beach-seine survey, N_i = 109. June–August 2012–2017											
0+	28	18–31	24.7 ± 0.61	0.12–0.63	0.34 ± 0.02	29	18–42	25.0 ± 0.78	0.12–1.69 0.36 ± 0.05	34	12–22 18.1 ± 0.42 0.04–0.25 0.13 ± 0.01
1+	9	36–72	55.5 ± 3.87	1.00–9.80	4.5 ± 1.02	8	41–71	54.8 ± 3.00	1.39–8.24 3.9 ± 0.71		
2+	1		81		14.80						
Gillnet survey, N_i = 172. June 2018											
2	4	77–90	81.0 ± 3.02	6.9–14.0	9.7 ± 1.51	10	68–77	71.2 ± 1.02	5.6–8.1 6.7 ± 0.29		
3	29	85–145	120.5 ± 3.00	10.9–73.5	43.5 ± 3.18	94	73–114	88.9 ± 0.80	6.6–27.9 13.6 ± 0.36		
4	7	106–155	132.6 ± 5.99	22.5–99.2	56.3 ± 9.20	22	106–145	124.8 ± 2.17	24.9–70.1 44.5 ± 2.50		
5	1		141		65.0	5	122–145	136.4 ± 4.91	43.0–91.6 65.4 ± 9.14		

Abbreviations: N_i – number of individuals. SL – standard length. TW – total weight. * The specimens marked + were collected during the period of active growth, which had the clearly visible zone of the current vegetation season in the otoliths (usually from the second half of June) (Kostûčenko 1961).

five years, with the age ratio 8.1%, 71.5%, 16.9%, and 3.5% for two-, three-, four-, and five-year-olds, respectively (Table 5). In the otoliths of gobies caught by nets in June, the growth zone of the current season has not formed, and the age ring of the last year had not yet been discerned. Therefore, the age of these specimens was marked without "+" (Table 5).

The sex ratio was female-biased, with the mean value of 3 females to 1 male, and this ratio increased with age. For example, the sex ratio was 2.5, 3.2, 3.1, and 5.0 females to 1 male at the age of two-, three-, four- and five-years-old (Table 5). Males were larger than females at the age of two ($U = 0.500$, $Z = -2.687$, $P = 0.007$) and three ($U = 221.000$, $Z = -6.801$, $P = 0.000$) years (Fig. 3, Table 5). In older fish, the differences were not significant, mainly due to the small number of individuals ($U = 47.000$, $Z = -1.503$, $P = 0.133$ for 4-years-olds and $U = 0.000$, $Z = 0.000$, $P = 1.000$ for 5-years-olds).

52.3% of all males (in the sample where age was esti-
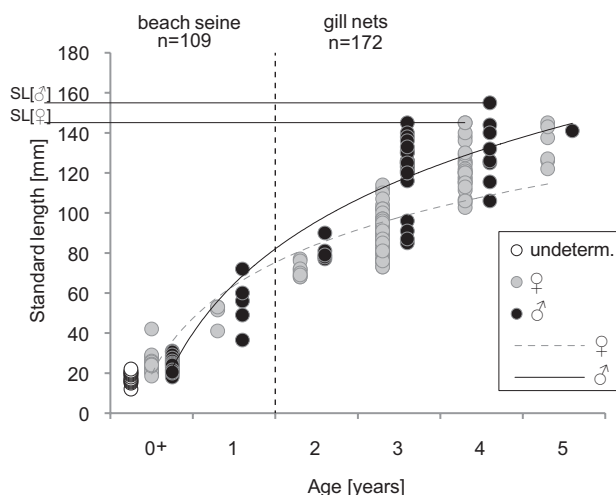
Figure 3. The size-at-age characteristics of round goby, *Neogobius melanostomus*. Fish caught by the beach seine and multimesh gillnets in the eastern Gulf of Finland between 2012 and 2018. Abbreviations: undeterm. = juveniles of undetermined sex; Sl [♂] = max SL of males; Sl [♀] = max SL of females. Curved lines are the log trends.

mated) had black spawning coloration.

Discussion

Since the first reported occurrence of the round goby in the eastern Gulf of Finland (GoF) in 2012 (Uspenskiy and Naseka 2014), this non-indigenous species has increased its distribution range, abundance, and frequency of occurrence in catches in this area. Currently, it is caught mainly along the southern coastline, and to a lesser extent in the Island area and along the northern coast, but not within Neva Bay. Young-of-the-year (YOY) juveniles, yearlings, and adults up to five years old—including spawners—were caught, suggesting that the species has successfully

colonized and is reproducing in these areas. In the following, we consider its population characteristics and the possible impacts on the ecosystem and fisheries.

Life span. The round goby is a fish with a short lifespan, with a predominance of younger age groups (Trifonov 1955). Although it can live up to 6 years, its average lifespan is 3–4 years (reviewed by Sokołowska and Fey 2011). This is consistent with the gobies sampled in the eastern GoF, where the maximum age was estimated to be 5 years old. YOY specimens were predominant in the shallow waters, while further from the coast in deeper habitats, 3-year-olds were the most abundantly sampled.

Sex ratio is almost equal in YOY specimens, but there was a female bias in fish older than two years. For gobies between the ages of 2 to 5 years old, the number of females increased from 2.5 to as many as 5 per male. A similar female-biased ratio was also observed in other areas and considered to be the result of males' mortality after the spawning season (Kostûčenko 1961; Charlebois et al. 1997). Nevertheless, male-biased populations have also been found (Corkum et al. 2004; Sokołowska and Fey 2011), which can be partly explained by fishing gear selectivity (Brandner et al. 2013b; Žák et al. 2018). In the presently reported study, both active and passive fishing gears were used, hence the female-biased ratio is more likely a feature of the studied population rather than the gear.

Growth. Male gobies were larger than females after the age of 2 years old, both in the eastern GoF and in other areas (MacInnis and Corkum 2000a; Kornis et al. 2012), including the Baltic Sea (Sokołowska and Fey 2011; Azour et al. 2015). This result is generally associated with the slower growth of females after maturation (Kostûčenko 1961).

In late summer, gobies of age 0+, 1+, and 2+ reached the standard length of 42, 72, and 81 mm, respectively. Overall, this corresponds well with the data of gobies in their native range. In the Sea of Azov, YOY also reach the length of 40–50 mm (SL), and rarely exceed 60 mm SL (Kostûčenko 1961). In the Bulgarian waters of the Black Sea, the mean length (SL) of the juveniles at this age were 65, 80, and 90 mm, respectively (Corkum et al. 2004). However, the round goby grows faster in other parts of the Baltic Sea. For example, in the waters of Sweden, gobies reached the mean total length of 100 and 130 mm at the ages of 1 and 2 years old, respectively (Florin et al. 2018). Along the Danish coastline, gobies of the age 0, 1, and 2 years reached 7, 15, and 19 cm (TL), respectively (Azour et al. 2015), i.e., these were apparently larger. In the presently reported study, the mean lengths (SL) of gobies at the age 2, 3, 4, and 5 years were 74, 96, 127, and 137 mm, respectively, which is lower than in other areas of the Baltic Sea and in the majority of the locations in their native range (see review Sokołowska and Fey 2011).

The higher growth rates have been previously attributed to newly established populations (Brandner et al. 2013a; Azour et al. 2015). Although the population in the eastern GoF can also be considered newly established, the growth rates reported here are apparently lower than

in other newly established populations. The low salinity in the eastern GoF (0‰–5‰) may be a factor contributing to the slower growth rates. Indeed, round gobies in fresh and brackish Bulgarian waters and in the Laurentian Great Lakes were smaller than those in the Black Sea (MacInnis and Corkum 2000b; Corkum et al. 2004). However, the round goby was larger in brackish waters of the Gulf of Gdańsk (Baltic Sea) compared to those in marine waters of the Ponto-Caspian area (Sokołowska and Fey 2011). Moreover, the gobies reached a higher size-at-age in the Kuibyshev reservoir (Shemonaev and Kirilenko 2009) than in the eastern GoF. Apparently, other environmental factors are more important to growth. It is important to note that the population studied here is the northernmost of all populations mentioned above.

Spatial distribution and possible ways of the invasion. It is commonly assumed that the Ponto-Caspian round goby was translocated to the Baltic Sea via ballast waters, where it then spread further into the basin and established local populations (Sapota and Skóra 2005). Indeed, larvae and early juveniles migrate to the pelagic zone during the night and hence can be transferred with ballast waters and/or water currents (Hayden and Miner 2009). The round goby was first caught in Luga Bay of the eastern GoF in 2012 (Uspenskiy and Naseka 2014). Early life stages of the species could have been translocated there with ballast waters released in the nearly situated port Ust'-Luga and/or with currents from western areas. The prevailing circulation pattern in the GoF, which is eastward along the southern coast and westward along the northern coast (Zimin et al. 2011; Raateoja and Setälä 2016), can facilitate the spread of larval stages from western populations along the southern coastline.

In Muuga Bay (Estonia), the species was first observed in 2002 and has become abundant (Järv et al. 2011; Puntila et al. 2018). Notably, Muuga Bay is 230–250 km away from Luga Bay (along the coastline). Thus, the species may have naturally spread there during this period, given that the invasion front advances about 30 km per year (Azour et al. 2015). Three years after the first reported incidence in the GoF (2015), juveniles were caught by SPb FPFC roughly 110 km east of Luga Bay. The species was not previously found to the east of the dam (SPb FPFC), which can limit its easterly spread to Neva Bay. Along the northern coast of the eastern GoF, the species was caught occasionally in 2017–2019. The closest sample to the west was near the Kotka area (Finland) in 2010–2013 (Puntila et al. 2018), roughly 240 km away. Currently, the round goby is rarely found along the northern coastline, likely due to westward currents and/or lack of favorable conditions there.

The species occurrence was the highest along the southern coast of the GoF, where it is classified as "common" or "constant". Indeed, the conditions may be more appropriate there, as the coastal and seafloor topography is less fragmented and patchier than along the northern coast (Kotilainen et al. 2016). The location of the Lenin-grad nuclear power plant (NPP) on the southern coast and the associated release of heated water increase the water temperature in the adjacent areas (Dvornikov et al. 2017), which can facilitate the survival and self-spreading of the goby. For example, sampling from the Leningrad NPP outflow channels in 2017 and 2019 shows that the round goby CPUE (not less than 48 ind. · 100 m^{-2}) greatly exceeded that in the areas beyond the warm water (Uspenskiy unpublished data).

Wave exposure is also an important factor affecting the round goby distribution in the Baltic, as it is more likely to occur in areas with low exposure (Kotta et al. 2016). Relatively low wave exposure sites, classified as "sheltered", are situated in Vyborg, Neva, and Luga Bays; in contrast, the northern coast of the Inner Estuary and Narva Bay are "moderately exposed" (Wijkmark and Isæus 2010). In the first two areas (Vyborg and Neva Bays), the species is rare or has not been caught. However, the goby is common in Luga Bay and abundant in deeper waters, but scarce in shallow waters of Narva Bay. Thus, the species distribution in the eastern GoF does not seem to be markedly influenced by wave exposure.

In the eastern GoF, sandy and muddy bottom habitats were more prevalent in offshore areas, while the bottom diversity is rather high in coastal areas. Round gobies were caught with beach seine and nets on all types of bottom substrates, such as sandy, stony, and mixed bottom. In the Gulf of Gdańsk, the round goby prefers artificial biotopes and stony substrates, while the adjacent sandy areas were colonized to a lesser degree (Sapota and Skóra 2005). However, in the Sea of Azov, the round goby avoids stony substrates and vegetated biotopes (Smirnov 1986). Therefore, bottom substrate preference seems to be highly variable in different regions of the round goby distribution. The current study did not have enough data to analyze substrate preferences in the eastern GoF, hence we reserve this for future research.

Vertical distribution. YOY and 1+ gobies prevailed at depths less than 1.5 m, while three-year-olds predominated in catches at depths from 6 to 8 m. Beach seine sampling is not size-selective for gobies (Jůza et al. 2018), i.e., if adults inhabited the shallow waters, they would have been caught. Although gillnets may underestimate the number of smaller gobies (Jůza et al. 2018), we used gillnets with a 12 mm mesh size, which is small enough to capture juveniles over 60 mm SL but too large to collect smaller individuals. Thus, our result is more likely to be a consequence of the age group distribution than of the fishing gear selectivity. The observed pattern implies the migration of older gobies from shallow plains into deeper waters.

Abundance in the eastern GoF. The density of the round goby in shallow waters was higher along the southern coast, especially in Koporye Bay and the Inner Estuary. However, it never exceeded 10 ind. · 100 m^{-2}, which is essentially lower than in some other areas of the Baltic Sea. In the Estonian waters, goby abundance ranges from 1 to 9 ind. · m^{-2}, but has been estimated to increase to 20

ind. · m^{-2} (Puntila et al. 2018). Comparable densities are observed in some Danish areas with 2 ind. · m^{-2} (Azour et al. 2015). Relatively low catches in the eastern GoF suggest that the species has not reached its peak abundance yet.

CPUE for the gillnets was also the highest along the southern coast, in Narva Bay since 2018. Catches increased in June, and were higher at the stony biotopes (e.g., station 5n in Fig. 1), which are assumed to be spawning grounds since the male gobies there had spawning coloration. In the Gulf of Gdańsk, the latest gonad stages were observed in April and July, when spawning intensifies (Tomczak and Sapota 2006).

Relative abundance (RN) of the round goby was higher in offshore stations, where it markedly increased during 2018–2019 and hence became "abundant" in catches. In the shallow waters, the species may be considered as "moderate in number".

Potential impacts on the ecosystem. The growing role of this new species in the food webs of the eastern GoF can be seen by its increasing abundance and incorporation in the diets of the great cormorant, *Phalacrocorax carbo* (Busun and Uspenskiy unpublished data) and the grass snake, *Natrix natrix* (Bogdanov unpublished data). Similarly, the round goby is part of the great cormorant and grey heron, *Ardea cinerea*, diets in Curonian Lagoon (Rakauskas et al. 2013). In the Gulf of Gdańsk, it was estimated to contribute to up to 60% of the great cormorant diet (Bzoma 1998). Thus, cormorants can provide some top-down control of the invasive goby population. In addition, perch and pike-perch, *Sander lucioperca* (Linnaeus, 1758), feed on round gobies, and may also reduce its numbers (Oesterwind et al. 2017). In the Baltic Sea, the round goby occasionally occurred in the diet of pike, *Esox lucius* Linnaeus, 1758; shorthorn sculpin, *Myoxocephalus scorpius* (Linnaeus, 1758); turbot, *Scophthalmus maximus* (Linnaeus, 1758); European eel, *Anguilla anguilla* (Linnaeus, 1758); burbot, *Lota lota* (Linnaeus, 1758); cod, *Gadus morhua* Linnaeus, 1758 (see Wallin 2019). The grey seal, *Halichoerus grypus*, and the harbour seal, *Phoca vitulina*, also prey on the round goby in the Baltic Sea (Scharff-Olsen et al. 2018; Keszka et al. 2020).

The relative abundance of the round goby increased annually, which can also increase competition with other fish for benthos. Round goby juveniles feed on different benthic organisms, such as crustaceans, polychaetes, and chironomids, while adults become primarily molluscivorous and feed on any bivalves abundant in the region (Smirnov 1986; Skóra and Rzeźnik 2001). In the Baltic Sea, the round goby was found to feed mainly on zebra mussels, *Dreissena polymorpha*, and bay mussels, *Mytilus trossulus*; isopods, *Idotea balthica*, and chironomids are also abundant in stomachs (Skóra and Rzeźnik 2001; Rakauskas et al. 2013). In the Baltic Sea, food competition with native ruffe, flounder, *Platichthys flesus* (Linnaeus, 1758), and turbot was considered to be significant (Karlson et al. 2007; Rakauskas et al. 2013;

Ustups et al. 2016), although the latter two are rare and of low commercial value in the eastern GoF. The ruffe is usually abundant in catches in coastal areas (Lajus et al. 2015), but has a low commercial value. Since the round goby diet may overlap with some demersal benthivorous fish species (Skóra and Rzeźnik 2001), diet competition can be expected with the roach, vimba bream, *Vimba vimba* (Linnaeus, 1758), and bream, *Abramis brama* (Linnaeus, 1758), which are important commercial species in the eastern GoF (Kuderskij 1999). The round goby is also known to feed on eggs of the herring and smelt (both are the main commercial species), potentially leading to a decrease in their populations (Wiegleb et al. 2018).

Conclusions

Since the first report of the round goby's occurrence in the eastern Gulf of Finland (GoF) in 2012, its abundance and distribution range have continued to increase. Currently, the species is common in some areas along the southern coastline. The finding of juveniles and pre-spawning adults suggests that this invasive species has successfully colonized this area, leading to our prediction of future expansion in this basin. However, its abundance in catches greatly fluctuates inter-annually, seasonally, and between the different areas of the GoF. Furthermore, the population size remains relatively low in most of the gulf area when compared to longer-established populations from other areas of the Baltic Sea. In the long term, the round goby can be considered a target species for commercial fisheries in the eastern GoF if the population size increases and the landings are profitable, as in the other Baltic regions. In the meantime, we recommend annual monitoring of the round goby population and its impact on the regional ecosystem and fisheries.

Acknowledgments

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