

Diversity of species in making up Nike fish schools and a new record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

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Abstract. Sahami FM, Habibie SA. 2021. Diversity of species in making up Nike fish schools and a new record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia. *Biodiversitas* 22: 5459-5467. Several estuaries in the waters of Tomini Bay are reported as the primary location for Nike fishing in Gorontalo; one of which is Paguyaman Bay. However, scientific information on Nike fish in the Tomini Bay waters is currently limited in Gorontalo Bay. The present study aims to determine the diversity of species in making up the Nike fish schools in Paguyaman Bay and show the types of constituent species whose distribution in Gorontalo waters has never been reported. A total of 1773 samples of Nike fish were collected from the sea to the estuary of Paguyaman Bay in one period of their emergence on April 8-10, 2021. The species were grouped based on the melanophore pattern's similarity and then analyzed morphometrically. The molecular identification of COI mitochondrial DNA was performed on species with different morphological appearances from those found in Gorontalo Bay. The Discriminant Function Analysis (DFA) indicated that the main distinguishing character of morphometrics is body depth. The morphological results suggested that the Nike fish schools in Paguyaman Bay consisted of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The first finding of *E. melanosoma* as a species making up the Nike fish schools in Paguyaman Bay was a new variant of the distribution of this species in Gorontalo waters and confirmed using morphology and molecular analysis. Further, based on the species composition, Nike fish schools in the waters of Paguyaman Bay show a typical species dominance trend during the recruitment process returning to freshwater, i.e., *S. longifilis* (52%) on the first day, *Belobranchus segura* (63.27%) on the second day, and *Stiphodon semoni* (83.43%) on the third day.

Keywords: *Belobranchus*, Eleotridae, Gobiidae, Sicydiinae, *Sicyopterus*, *Stiphodon*

INTRODUCTION

Tomini Bay is Indonesia's largest bay intersecting related with 14 districts in three provinces of Sulawesi, namely North Sulawesi, Central Sulawesi, and Gorontalo. Nike (local name, pronounced *nee-K*) are among the essential fishery commodities in Tomini Gorontalo Bay communities. They consist of amphidromous goby schools in the pelagic to juvenile larval stages in the recruitment process from marine waters to freshwater, and their emergence is seasonal. Their hatched embryos are carried by the river flow to the sea and develop as pelagic larvae before being recruited, returning to rivers and growing as adults and spawn (Thuesen et al. 2011; Yamasaki et al. 2011; Taillebois et al. 2012; Iida et al. 2017; Mennesson et al. 2019; Mennesson and Keith 2020; Sahami and Habibie 2020). The diversity of species making up Nike fish schools (post-larva goby) in Gorontalo waters is one of the essential studies that should be conducted continuously, considering the high economic value potential as a food commodity in Gorontalo with an R/C ratio of 2.68 (economically feasible) (Wolok et al. 2019). As of today, approximately 1120 species from 30 genera in the goby group are described. The goby group plays a vital role in ichthyofauna diversity with a wide area distribution among many species. These fish groups are dominantly found in the eastern part of Indonesia, with high species diversity

(Tweedley et al. 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

Geographically, there are several rivers in Gorontalo which empty into Tomini Bay. Some of these river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). Numerous studies have reported Nike fish in Gorontalo Bay (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020a; Sahami et al. 2020). Meanwhile, scientific data on Nike fisheries in the Paguyaman Bay estuary are not widely observed. Paguyaman Bay is part of the Tomini Bay areas located on the border of Girisa Village, Paguyaman Sub-district, Boalemo District with Bilato Village, Boliyohuto Sub-district, Gorontalo District. The bay is the estuary of the Paguyaman River, whose watershed flows across three districts, i.e., Gorontalo, Boalemo, and Pohuwato. The presence of the Paguyaman watershed as the second-largest watershed in Gorontalo and directly intersecting with three administrative districts draws the interest to observe the Paguyaman Bay estuary.

The demographic information, such as life history at early stages, recruitment, migration patterns, and other biological characteristics, is crucial for managing fish populations (Habibie et al. 2015). Information about the identification and composition of species is also essential in the management process. Hence, the management can be

carried out effectively. As currently reported, the types of species making up the Nike fish schools in the waters of Gorontalo Bay consist of nine species i.e., *Sicyopterus pugnans*, *S. longifilis*, *S. lagocephalus*, *S. cynocephalus*, *S. parvei*, *Bunaka gyrinoides*, *Belobranchus segura*, *B. belobranchus*, and *Stiphodon semoni* (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Although the goby is widely distributed in tropical Indo-Pacific waters (Keith et al. 2015; Lord et al. 2019), species diversity in each water location needs to be examined further. Therefore, the present study aims to find out the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species whose distribution in the waters of Gorontalo Bay has never been reported. The present work is also expected to provide significant scientific information for Nike fish resource management in Paguyaman Bay and to support and optimize Nike fisheries in the Gorontalo waters.

MATERIALS AND METHODS

Sampling

The samples of Nike fish were collected in Paguyaman Bay, Gorontalo, Indonesia (00°31'1.10"N, 122°39'0.73"E) (Figure 1) for three days in one period of emergence on April 8-10, 2021. This period was the only emergence period of Nike fish in Paguyaman Bay from January to May 2021. A total of 150 grams of Nike fish samples were taken from the fishermen. Nike fishing in Paguyaman Bay typically used Dudayahu and Totaluo fishing gear with excellent mesh designed for operation in shallow water

(Salam et al. 2016). Nike's schooling migration (Sahami et al. 2020) made it easy for fishers to catch this fish specifically. The catching process was carried out in the early morning. The samples were then brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University, for analysis. After that, the samples were grouped, identified, referred to Sahami et al. (2019b) and Sahami et al. (2020) methods, photographed for morphometric analysis, and calculated the number per group. Fish samples with different melanophore patterns from Nike fish that have been identified in the Gorontalo Bay (Sahami et al. 2019b; Sahami et al. 2020) were referred to as a new variant. Identifying a new variant was carried out genetically by placing five individuals into a sample bottle that already contained a 95% ethanol solution.

Morphometric characters

Ten morphometric characters of Nike fish referring to Sahami et al. (2020) (Figure 2, Table 1) were measured using the imageJ application with an accuracy of 0.001 cm.

Each measured morphometric character datum was then standardized following the allometric formula according to Elliott et al. (1995) as follows:

$$M_{adj} = M (L_s/L_0)^b$$

M_{adj} is the standardized morphometric data, M is the measured morphometric data, L_0 is the total length of fish, L_s is the average total length, parameter b is the slope of log-linear curve M to $\log L_0$ of all data.

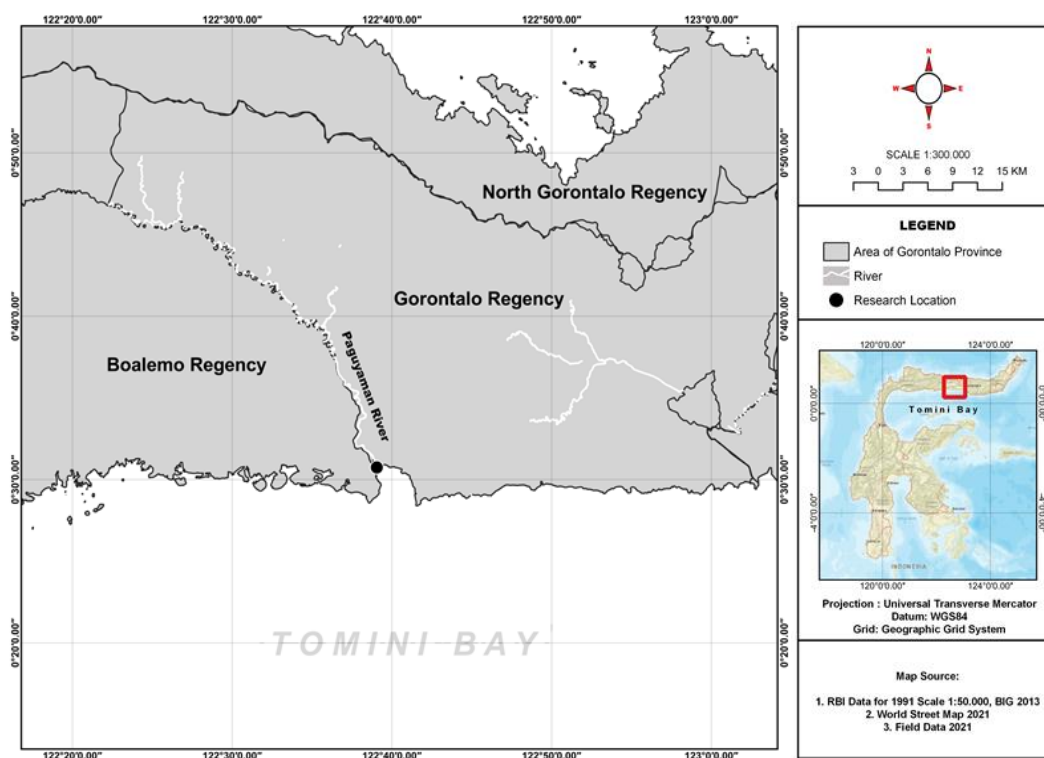


Figure 1. Research site map in Paguyaman Bay, Gorontalo, Indonesia

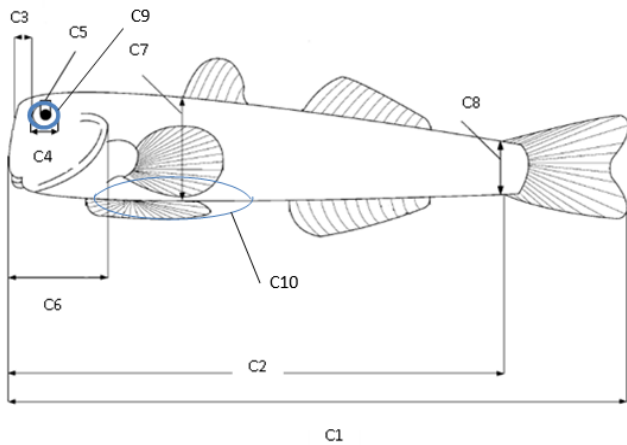


Figure 2. Morphometric characters of Nike fish (Sahami et al. 2020)

Table 1. Morphometric characters of Nike fish (Sahami et al. 2020)

Code	Morphometric characters
C1	Total length (TL)
C2	Standard length (SL)
C3	Preorbital length (PL)
C4	Eye diameter (ED)
C5	Eye lens diameter (EL)
C6	Head length (HL)
C7	Body depth (BD)
C8	Peduncle depth (PD)
C9	Eye area (EA)
C10	Yolk sac area (YS)

DNA extraction, PCR amplification, and sequencing

The target gene for molecular analysis was the mitochondrial Deoxyribonucleic acid (DNA) Cytochrome Oxidase Subunit 1 (COI) gene. COI gene is the best resolution of the intraspecific level than other core genes (Bellagamba et al. 2015; Hubert et al. 2015; Rodrigues et al. 2017; Bingpeng et al. 2018; Roesma et al. 2018, Yulianto et al. 2020). In addition, mitochondrial COI genes are also widely and reliably utilized to identify species in the goby group (Jeon et al. 2012; Viswambharan et al. 2013; Laskar et al. 2016; Lejeune et al. 2016; Wang et al. 2017; Linh et al. 2018; Olli et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020b; Roesma et al. 2020; Sahami et al. 2020). Following the protocol kit, 20 grams of fish meat tissue of a new variant individual was isolated using Qiagen Tissue and blood extraction kits for genetic analysis. The mitochondrial DNA COI gene was further amplified using the primer pair FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Samples were amplified at pre-denaturation temperature of 94°C for five minutes, denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 7 minutes. This Polymerase Chain Reaction (PCR) process lasted for 38 cycles.

Data analysis

The DNA samples were amplified and electrophoresed, and then the gene was sequenced using the Dideoxy Sanger Termination Method. Contig was done for the nucleotide sequences. After that, the results were matched with the data available in the GenBank database (www.ncbi.nlm.nih.gov) through the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was arranged by aligning the DNA sequences of identified samples with some DNA of gobies (accession number KF489573, KU232392, KU692483, KU692484, and KU692490) and the species of Nike fish schools in Gorontalo Bay (accession number MN069306, MN069307, MN069308, MT706639, MT706640, MT706641, MT706720, MT706721, MT706722, MT706723, MT706724, MT706725, MT706726, and MT706791) available in the GenBank database using the Maximum Likelihood 1,000 bootstrap method on MEGAX software (Kumar et al. 2018). The results of genetic identification were further confirmed morphologically, referring to Maeda and Tachihara (2005). Furthermore, the Discriminant Function Analysis (DFA) (Landau and Everit 2004) was performed to determine the main distinctive characters among species using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

New variant of species that makes up the Nike fish schools

The emergence of Nike fish in Paguyaman Bay does not have a regular pattern as in Gorontalo Bay, in which the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared in April with a 3-day emergence period (April 8-10, 2021) since observed from January and no longer appeared in May. Based on the NCBI database referring to the mitochondrial COI gene sequencing, a new melanophore pattern species was identified as *Eleotris melanosoma* (Figure 3). The nucleotide sequence of *E. melanosoma* was listed in the NCBI database with accession number MZ401475.

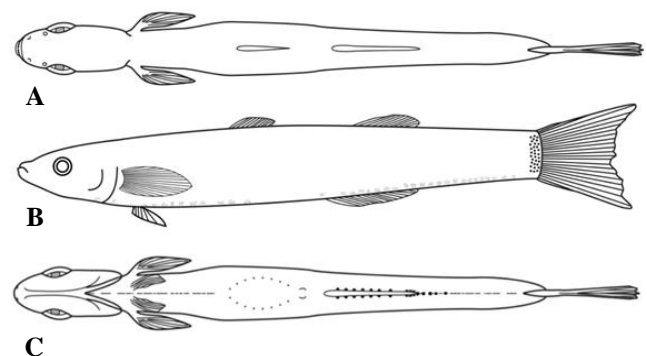


Figure 3. Pelagic larvae of *Eleotris melanosoma* in Paguyaman Bay, Gorontalo, Indonesia. A. Dorsal view; B. Lateral view; C. Ventral view

The caught species of *E. melanosoma* have a standard length of 19.05-20.04 mm with an average standard length of 19.55 mm. These species have a transparent body, an elongated and compressed body shape, and no scales. In addition, the body fins are not yet fully developed, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore spots distributed from head to body, and few melanophore spots accumulated at the base of the caudal fin. These morphological features correspond to the morphology of the pelagic larvae of *E. melanosoma* as reported by Maeda and Tachihara (2005).

The results of the alignment of the DNA sequences of *E. melanosoma* with the DNA sequences of the species that make up the Nike fish school in Gorontalo Bay and several *Eleotris* species available in the NCBI Genbank are displayed through a phylogenetic tree (Figure 4). The analysis results show that *E. melanosoma* is closely related to *Eleotris fusca* and is in the same monophyletic clade as *Belobranchus segura*, *B. belobranchus*, and *Bunaka gyrinoides*. The similarity of the clades in these five species is because they are members of the *Eleotridae* family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species *Stiphodon semoni*, *Sicyopterus longifilis*, *S. lagocephalus*, *S. parvei*, and *S. cynocephalus*. The second monophyletic clade also groups the species in

the genus *Sicyopterus* in the same sub-clade and separated from the genus *Stiphodon*.

Composition of species

A total of 1773 specimens were observed as constituents of Nike fish schools in Paguyaman Bay for three days of emergence in April 2021. Two families, i.e., Gobiidae and Eleotridae, were recorded to make up Nike fish schools. The Gobiidae family consisted of four species, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, and *Stiphodon semoni*. Meanwhile, the Eleotridae family consisted of three species, namely *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. Among seven species, three species in family Gobiidae, i.e., *S. longifilis*, *S. parvei*, and *Stiphodon semoni* were observed as species that made up Nike fish schools during their emergence. The species was consistently observed in considerable numbers (*S. longifilis*), whereas *B. segura* was observed only on the second day. On the third day of emergence, *B. belobranchus* and *E. melanosoma* were observed. *S. semoni* is the most abundant species observed (34.07%), meanwhile *S. parvei* and *B. segura* are at least 0.003%. Based on the species composition, species dominance was different each day. On the first, second, and third day, the school of Nike fish was dominated by *S. longifilis*, *Belobranchus segura*, and *Stiphodon semoni* with a composition value of (52%), 63.27%, and 83.43%, respectively (Figure 5).

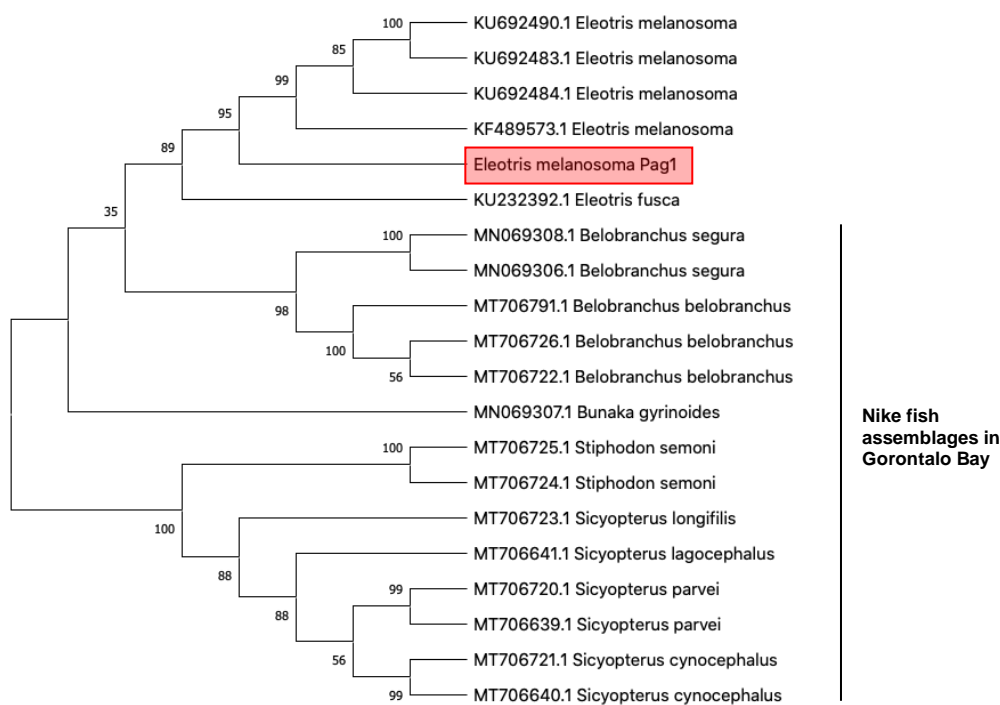


Figure 4. Phylogenetic tree of *Eleotris melanosoma* with several species of gobies and composers of Nike fish in Gorontalo Bay waters, Indonesia available in the NCBI database

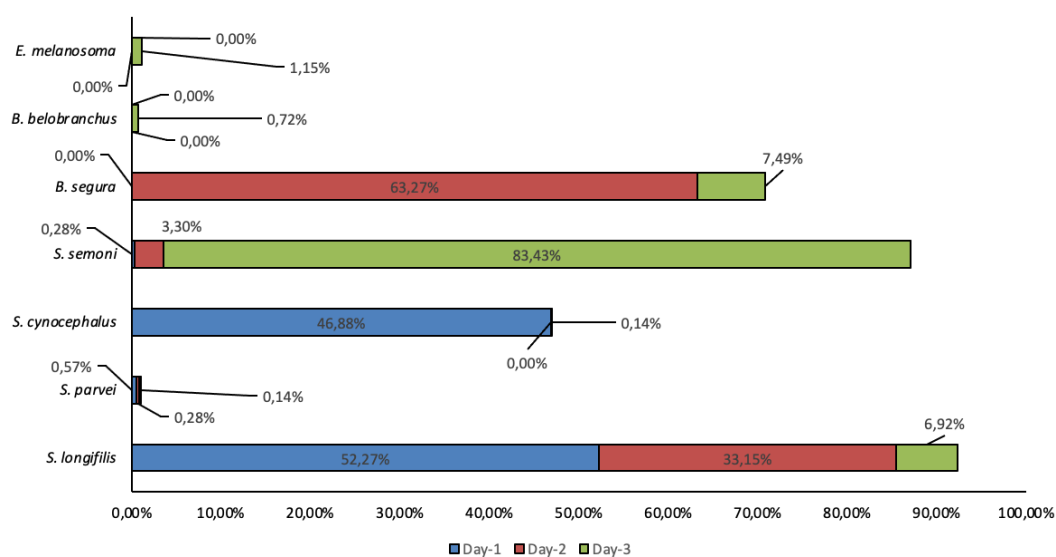


Figure 5. Nike fish schools composition in Paguyaman Bay waters, Gorontalo, Indonesia

Size distribution and morphometric characters

The total length of the collected Nike fish ranged from 1.523 to 3.572 cm (Table 2). The *Stiphodon semoni* (Gobiidae) and *S. parvei* (Gobiidae) species have the smallest and biggest size. The species with the widest size range is *S. cynocephalus* (2.422-3.572 cm), and the species with the smallest size range is *B. belobranchus* (2.290-2.403 cm).

The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary morphometric characters data standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Moreover, the analysis of discriminant function is presented in Figure 6. Two discriminant functions are able to explain 67.3% and 24.5% of the total variance of morphometric characters. Based on this work, the C7 character (body depth) is the main distinctive character of the Nike population in Paguyaman Bay. The tendency of Nike fish formation in Paguyaman Bay is clearly shown by a group of centroid in Figure 6. The species of *Sicyopterus longifilis*, *S. cynocephalus*, *S. parvei*, and *S. semoni* tend to overlap and adjacent since

four species are members of the Gobiidae family. Furthermore, *Belobranchus segura* and *Belobranchus belobranchus* are the most related spots because both species are from the same genus of *Belobranchus*. The *Eleotris melanosoma*, although was distributed in separate areas, is close to *B. belobranchus* because both species are members of the Eleotridae family. In general, species identification through canonical discriminant diagrams (Figure 6) is in line with the phylogenetic tree shown in Figure 4.

Table 2. Size range of each species making up Nike fish schools in Paguyaman Bay waters, Gorontalo, Indonesia

Species	Mean size (cm)	Size range (cm)
<i>Sicyopterus longifilis</i>	2.666	2.262-3.044
<i>Sicyopterus parvei</i>	3.036	2.831-3.403
<i>Sicyopterus cynocephalus</i>	2.775	2.422-3.572
<i>Stiphodon semoni</i>	2.137	1.523-2.586
<i>Belobranchus segura</i>	2.311	2.005-2.638
<i>Belobranchus belobranchus</i>	2.347	2.290-2.403
<i>Eleotris melanosoma</i>	2.377	2.304-2.448

Table 3. Morphometric character data of Nike fish schools in Paguyaman Bay waters, Gorontalo, Indonesia

Species	Unit of characters (Mean (cm)±SD)								
	SL	PL	ED	EL	HL	BD	PD	EA	YS
<i>S. longifilis</i>	2.054±0.05	0.111±0.02	0.136±0.02	0.060±0.01	0.518±0.04	0.381±0.03	0.208±0.02	0.013±0.00	0.026±0.00
<i>S. parvei</i>	2.086±0.03	0.128±0.03	0.133±0.02	0.062±0.01	0.535±0.03	0.376±0.01	0.205±0.01	0.012±0.00	0.031±0.01
<i>S. cynocephalus</i>	2.043±0.05	0.114±0.02	0.133±0.02	0.057±0.01	0.521±0.05	0.375±0.03	0.203±0.02	0.012±0.00	0.029±0.01
<i>S. semoni</i>	2.062±0.05	0.104±0.02	0.129±0.02	0.056±0.01	0.489±0.04	0.343±0.04	0.188±0.03	0.012±0.00	0.020±0.01
<i>B. segura</i>	2.094±0.04	0.126±0.02	0.129±0.02	0.061±0.01	0.556±0.05	0.392±0.03	0.221±0.02	0.012±0.00	0.035±0.01
<i>B. belobranchus</i>	2.051±0.05	0.140±0.03	0.128±0.02	0.067±0.01	0.579±0.03	0.325±0.01	0.213±0.01	0.015±0.00	0.021±0.00
<i>E. melanosoma</i>	2.033±0.02	0.123±0.01	0.129±0.00	0.058±0.01	0.535±0.04	0.280±0.02	0.208±0.01	0.012±0.00	0.013±0.00

Notes: SL: Standard Length; PL: Preorbital Length; ED: Eye Diameter; EL: Eye Lens diameter; HL: Head Length; BD: Body Depth; PD: Peduncle Depth; EA: Eye Area; YS: Yolk Sac area

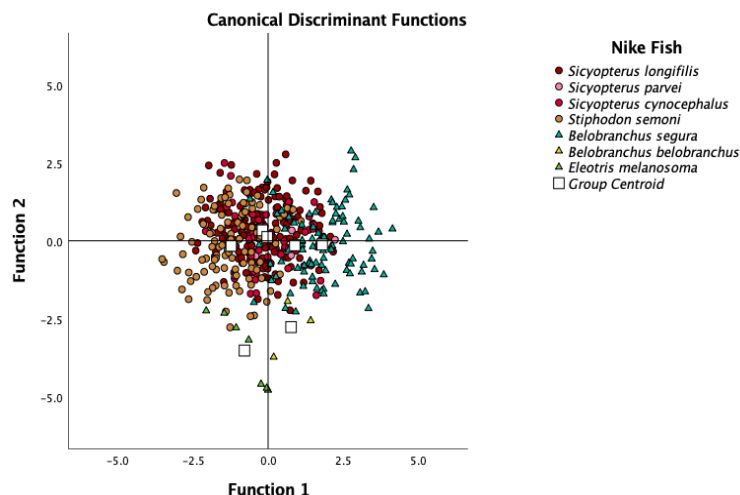


Figure 6. Diagram of the canonical discriminant function of Nike fish in Paguyaman Bay waters, Gorontalo, Indonesia

Discussion

The species that make up the Nike fish schools in Paguyaman Bay consist of seven species, fewer than the nine species reported in Gorontalo Bay (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies are *S. pugnans* and *Bunaka gyrinoides* are not observed as the constituents of Nike fish schools in Paguyaman Bay. However, the present study reports a new record of the distribution of *E. melanosoma*. Identifying *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay based on molecular and morphological analysis is a new record of the distribution of this species in Gorontalo waters. A total of eight species of *E. melanosoma* were caught as the constituents of the Nike fish schools in Paguyaman Bay when they began to approach the river estuary on the third day of their emergence.

The *E. melanosoma* species is one of the three main species of *Eleotris* reported to be distributed in the Indo-Pacific waters. Two of the other species are *E. fusca* and *E. acanthopoma* (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). The other *Eleotris* species can continue to be recorded when more studies focus on this topic in more locations in Indo-Pacific waters. As reported by Maeda and Tachihara (2005), the pelagic and newly settled larvae of *E. fusca* show a larger body size than *E. melanosoma* and *E. acanthopoma*. *Eleotris* pelagic larvae migrate to river areas by utilizing the rising tides at night. *E. melanosoma* and *E. acanthopoma* settle at the upper end of the tidal affected area, meanwhile *E. fusca* migrates to freshwater upstream against river water flow currents.

In terms of size, the species of *E. melanosoma* caught in Paguyaman Bay (average standard length 19.55 mm) should in the newly settled larval stage based on research Maeda and Tachihara (2005) in the Teima River estuary area, Okinawa Island, with increasingly numerous visible body pigmentation and completely pigmented caudal fin with no distal border. However, the morphology of *E. melanosoma* species in the site area still characterizes the

morphology of the pelagic larval stage, with the most conspicuous feature and no melanophore spots on its sides. Such a difference in feature is due to the difference in the aquatic habitat area (Habibie et al. 2018). Besides, the tropical area's stable temperature throughout the year has no significant effect on the fish growth rate (Habibie et al. 2015). The pelagic to juvenile larval stages of *E. melanosoma* species were also recorded in the estuary area of the Teima River, Okinawa Island (Maeda and Tachihara 2005) and Sri Lankan waters estuary (Batuwita et al. 2017). Meanwhile, the adult species were found in the fresh waters of Japan (Maeda et al. 2011); rivers on the Buton and Kabaena Islands (Tweedley et al. 2013); Opak River, Yogyakarta (Djumanto et al. 2013); freshwater of Sri Lanka (Batuwita et al. 2017); freshwater of West Sulawesi (Nurjirana et al. 2020); and Sanenrejo and Wonoasri River Resorts (Subchan et al. 2020).

Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidae) are the most common families discovered in estuaries and freshwater of the Indo-Pacific area. The Eleotrid species mostly inhabits the lower and middle parts of freshwater flows characterized by waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). *Eleotris* pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). On the other hand, the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, discrete fin ventral, has no lateral line on the sides of the body, and inconspicuous body color, mostly light brown, dark brown, or olive with some dazzled metallic (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species of the Gobiidae family are active swimmers. They have pelvic fins that are fused and form a sucking disc to attach themselves to the substrate while actively swimming against currents towards the upstream (Keith 2003; Taillebois et al. 2014). The genus *Sicyopterus* uses its

mouth as a secondary sucking disc that allows this genus to access areas upstream of waterfalls quickly (Keith 2003).

The Nike fish both in Paguyaman Bay and Gorontalo Bay is an amphidromous species. As an amphidromous species, the adults hatch in rivers, then larvae flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs in rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Iida et al. 2017; Mennesson et al. 2019; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively follow the river water currents after hatching in the afternoon and reach the estuary in the middle of the night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so that the larvae are carried downstream with lots of yolks, and the risk of starvation is minimized (McDowall 2009). One of the strategies employed is spawning in river areas near the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

The emergence period of Nike fish schools in the bay and moving into the estuary is pre-colonization phase. In this phase, gobies' post-larvae will swarm and prefer to swim near the shoreline because the water flowing is slower and can even be reversed by tides or waves (Keith et al. 2008). Making schools is a gobies strategy to avoid predators besides finding food (Keith 2003). The species' diversity and sizes making up the fish schools are strongly influenced by the characteristics of the species, season, and hatching time. According to Taillebois et al. (2012), the duration of the pelagic larvae is not the only factor determining species distribution; It is most likely interactions between larval behavior, environment, currents, and substrate preferences. Apart from these factors mentioned previously, the larger fish larvae possibly result from earlier hatching compared to smaller fish larvae (Mennesson et al. 2015). In addition, the rainy season will support the newly hatched larvae drifting into the sea faster (Keith 2003).

According to Landau and Everit (2004), the discriminant analysis determines the main distinctive character among populations. The present study confirmed that body depth is the main distinguishing characteristic among Nike fish populations in Paguyaman Bay. It is contrary to the main distinctive character of Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The difference in the main distinguishing characters between the two locations since the gobies species are able to develop various morphological specificities as adaptations strategy for their environment (Gani et al. 2019; Roesma et al. 2020). Thus, the different backgrounds are able to affect the morphological characters of species in their environment. Populations in different environments are likely to have different population structures. Differences in population structure will influence population size, which can be observed through differences in morphometric and meristic characters (Aisyah and Syarif 2018).

Based on their species composition, the Nike fish schools in Paguyaman Bay waters indicate a trend of typical species dominance every day during the recruitment

back to freshwater. On the first day of their emergence in Paguyaman Bay, the Nike fish schools were composed of species in the Gobiidae family and dominated by *S. longifilis* (52%). Furthermore, the species were dominated by *Belobranchus segura* (63.27%) on the second day and *Stiphodon semoni* (83.43%) on the third day when the fish schools started to enter the river estuary. This research is the first finding related to the pattern of Nike fish emergence and the composition of its constituent species in Paguyaman Bay. The emergence period of Nike fish in this location, which is not a regular pattern such as in Gorontalo Bay, limits the research sample. Therefore, species composition variation in the emergence period requires further studies. In general, the gobies adapt mostly to aquatic habitats, although with various abundance degrees (Gani et al. 2019).

In conclusion, the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. Species with the new melanophore pattern is *E. melanosoma*, confirmed through morphological and molecular analysis. A new variant of *E. melanosoma* species as the first species of the genus *Eleotris* composes the Nike fish schools in Paguyaman Bay and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of the Paguyaman River assumed to be the habitat of adult Nike fish should be performed to ensure the sustainability of Nike fish resources in the future. Although the adult species of Nike fish is an unvalued economic commodity, unfortunately the post larva stage when recruiting back into the river is often targeted by fishermen for consumption (Ellien et al. 2016; Roesma et al. 2020), including in Gorontalo waters. Identification of these species must be carried out continuously to prevent the loss of these fish species while catching along with commodity fishing. This research indicates many types of species constituents in Gorontalo waters may be collected when an extensive study is conducted later in the future, specifically in river estuarine locations that are not explored.

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