What species make up the Nike fish assemblages at the macrotidal estuary in Gorontalo Bay, Indonesia? [version 1; peer review: 2 approved with reservations]

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Abstract
Background: No study has documented the species composition of Nike fish (fam: Gobiidae) schools. The aim of this study is to document the species composition of the Nike-fish schooling.

Methods: All samples were collected randomly from fisher’s catch during the fishing season on 5th–11th October 2018 at macrotidal area in Leato. Then, all specimens were identified morphologically by melanophore pattern differences. Subsequently, all identified-samples by melanophores pattern differences were sent to the genetic laboratory for identification.

Results: The morphological results show there are five individuals with a different melanophores pattern. On the contrary, the genetic results only show four species from those five individuals. They are Sicyopterus pugnans, S. cynocephalus, Belobranchus segura, and Bunaka gyrinoides.

Conclusions: Our findings show that there are only four species that compose the Nike fish schooling in Gorontao Bay. They are Sicyopterus pugnans, Sicyopterus cynocephalus, Belobranchus segura, and Bunaka gyrinoides.

Keywords
Nike-fish, Gorontalo, melanophores pattern, genetic, morphology
Introduction

Estuaries are a crucial habitat for biota and small fish, in particular juveniles of commercially relevant species. They are considered as the most productive and dynamic ecosystem in the world (Cantera & Blanco, 2001; Lahjie et al., 2019; McHugh, 1967; Sreekanth et al., 2017). They also perform the most crucial role in the population dynamic for a lot of invertebrate and fish species. These ecosystems also significantly contribute to provide some ecological services such as nursery ground, feeding ground and breeding habitats for both freshwater and marine species (Beck et al., 2001; McLusky & Elliott, 2004; Sun et al., 2019). The most well-known species that occupy the seas and estuary area in Gorontalo Bay is Nike fish.

Nike (pronounced nee-K) is a local name for transparent juvenile of unknown fish. These fish are approximately 2–4 cm in length; they appear seasonally and fished at estuary waters around the Gorontalo Bay. These juvenile fish has been fished and marketed traditionally for a long time. They are preferable for consumption by the local people than other fisheries products. As a consequence, fishing activity has increased over time to supply local demand for Nike (Wolok et al., 2019).

However, the impact of fishing activities is unknown. A recent paper concerning Nike only reports the seasonal appearance during the fishing season (Pasisingi & Abdullah, 2018), total length and morphometric measurements (Zakaria, 2018), nutrition content (Liputo et al., 2013), and mercury contamination of these fish (Salam et al., 2016). To our knowledge, no studies have documented the species diversity that composed the schooling of Nike. Although, Yamasaki et al. (2011) have reported that species in juvenile form can be determined by its melanophores pattern and genetic determination.

The objective of the present study is to address this lack of knowledge by identifying the fish species that composed a Nike fish schooling. This information is very urgent and required for fisheries management. Therefore, we aimed to identify the species that composed the schooling of Nike fish in Gorontalo Bay by melanophores pattern and genetic identification.

Methods

This study was conducted in October 2018 at Leato (0°30’0.58”N, 123°3’55.42”E), Gorontalo Bay, Indonesia (Figure 2). Approximately 100 g of the Nike-Fish Assemblages (Figure 1) were collected randomly from the fishermen’s catch at fishing grounds.
during the catch-season (on October 5th–11th). All samples were transported using a cool-box to the lab for measurement. Immediately after collection, all samples were identified visually according to Yamasaki et al. (2011), and the specimens with different melanophore patterns were separated according to their melanophore display. We assumed that those separated individuals were different on species.

Then, we selected one individual from each group and labeled these as N1, N2, N3, N4, N5, for genetic identification. Images of the selected samples were captured using Canon EOS 100d with 58 mm pro Digital Wide Converter 0.45X Lens and subsequently converted to black and white using CorelDraw Graphic Suite 2019.

After selection, all of the individuals with different melanophores were preserved with ethanol 70% in a separate bottle and sent to the Genetics Laboratory at Manokwari for genetic identification by Sanger sequencing. The DNA cytochrome oxidase subunit I (CO1) of the sample was isolated with a Geneaid™ DNA Isolation Kit. Editing, and proofreading of sequences, and construction of the phylogenetic tree was generated with MEGA 5.0 software.

Results
Five unspecified individuals of Nike-fish were identified morphologically by melanophore differences, as shown in Figure 3. N1 was revealed as Sicyopterus pugnans; N2 as Sicyopterus cynocephalus; N3 and N5 as Belobranchus segura; and N4 as Bunaka gyrinoides. The specimens with melanophores differences of each group is shown in Figure 4.

Melanophores pattern
Nike-fish schools consist of various species with the same body-shape, but different melanophore displays. Moreover, from 100 g (~145 individuals) of the total specimens that we identified, only five individuals with different melanophore patterns were identified (Figure 3).

Genetic identification
Figure 3 shows the genetic identification among the individuals (species). The outcomes of genetic identification for N3 and N5 shows that both samples are the same species: Belobranchus segura.

Discussion
Although the melanophore patterns in N3 and N5 are different, their genetics are identical, meaning they are the same species (Belobranchus segura). This dissimilarity might be affected by the changes of melanophore during the development of the larvae. Valade et al. (2009) report that such melanophores change on Sicyopterus langocephalus during the larvae stage. These changes could represent a problem for morphological identification. We can not count the species by morphological differences. Therefore, for the next examination we strongly recommended determining the species composition of the Nike fish schools by genetic rather than morphological identification because for that reason.

Conclusion
Our findings show that there are four species that compose Nike fish schooling. They are Sicyopterus pugnans, Sicyopterus cynocephalus, Belobranchus segura, and Bunaka gyrinoides.
Figure 4. Phylogenetic tree of individuals with different melanophore patterns.

Data availability
Underlying data
Group N1, *Sicyopterus pugnans* isolate N1_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number MN065178.

Group N2, *Sicyopterus cynocephalus* isolate N2_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number MN069305.

Group N3, *Belobranchus segura* isolate N3_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number MN069306.

Group N4, *Bunaka gyrinoides* isolate N4_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number MN069307.

Group N5, *Belobranchus segura* isolate N5_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number MN069308.

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References
Beck MW, Heck KL, Able KW, et al.: A better understanding of the habitats that serve as nurseries for marine species and the factors that create site-specific variability in nursery quality will improve conservation and management of these areas. *BioScience.* 2001; 51: 633–641.

Cantera JR, Blanco JF: The estuary ecosystem of Buenaventura bay, Colombia. In *Coastal marine ecosystems of Latin America.* Springer, Berlin, Heidelberg, 2001; 265–268.

Lahjie AM, Nouval B, Lahjie AA, et al.: Economic valuation from direct use of mangrove forest restoration in Balikpapan Bay, East Kalimantan, Indonesia [version 2; peer review: 2 approved]. *F1000Res.* 2019; 8: 9.

Liputo SA, Berhimpon S, Fatimah F: Analisa Nilai Gizi Serta Komponen Asam
Amino dan Asam Lemak dari Nugget ikan Nike (Awaous melanocephalus) Dengan Penambahan Tempe. CHEMISTRY PROGRESS. 2013; 6(1).

Reference Source
McHugh JL: Estuarine nekton. In Lauff GH, (Ed.), Estuaries, American Association for the Advancement of Science Special Publication, Washington, DC, 1967; 83, 581–620.

Reference Source
McLusky DS, Elliott M: The Estuarine Ecosystem. Ecology, Threats and Management. Third ed. Oxford University Press, 2004; 214.

Publisher Full Text
Pasisingi N, Abdullah S: Pola kemunculan ikan Nike (Gobiidae) di Perairan Teluk Gorontalo, Indonesia. DEPIK Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan. 2018; 7(2): 111–118.

Reference Source
Salam A, Sahami FM, Panigoro C: Nike (Awaous melanocephalus) Fishery and Mercury Contamination in the Estuary of BoneBolango River. Omnia-Akuatika. 2016; 12(2).

Publisher Full Text
Sreekanth GB, Lekshmi NM, Singh NP: Temporal patterns in fish community structure: environmental perturbations from a well-mixed tropical estuary. Proc Natl Acad Sci India Sect B Biol Sci. 2017; 87(1): 135–145.

Publisher Full Text
Sun Z, Sokolova E, Brittain JE, et al: Impact of environmental factors on aquatic biodiversity in roadside stormwater ponds. Sci Rep. 2019; 9(1): 5994.

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Valade P, Lord C, Grondin H, et al: Early life history and description of larval stages of an amphidromous goby, Sicyopterus japonicus (Gobioidei: Sicydinae). Cybium. 2009; 33(4): 309–319.

Reference Source
Wolok T, Fachruisyah ZC, Yantu I: Technical And Economic Analysis Of Catching Equipment Totaluo In Nike Fishing (Awaous Melanocephalus) in Gorontalo City. Jambura Science of Management. 2019; 1(2): 65–71.

Reference Source
Yamasaki N, Kondo M, Maeda K, et al: Reproductive biology of three amphidromous gobies, Sicyopterus japonicus, Awaous melanocephalus, and Stenogobius sp., on Okinawa Island/Biologie de la reproduction de trois gobies amphidromes de l’île d’Okinawa: Sicyopterus japonicus, Awaous melanocephalus et Stenogobius sp. Cybium, International Journal of Ichthyology. 2011; 35(4): 345–360.

Reference Source
Zakaria Z: Analisis Morfometrik Schooling ikan Nike di Perairan Laut Pesisir Kota Gorontalo. Jambura Journal of Educational Chemistry. 2018; 13(1): 77–80.

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Reviewer Report 23 October 2019

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If the nike-fish material was composed of *Sicyopterus*, *Bunaka*, and *Belobranchus* species, the larvae must represent different shapes, for example, standard length, head length, preanal length, length of caudal peduncle, fin-ray counts (especially second dorsal, anal and pectoral fins), and fin shapes (especially pelvic fin). Although arrangements of the pigments scattering upon the body surface were shown in the Figure 3, melanophores along the dorsal and ventral midlines are more useful for identification. Morphological identification of these taxa are not difficult at least to the genus level. Please observe the morphologies of the specimens carefully before the molecular identification.

**Introduction**

Second paragraph:

- The first sentence should be “Nike (pronounced nee-k) is a local name for transparent postflexion larvae of fish, but it has not been identified to the species as well as the genus or family level.”

- If you or the local people actually used to know what it is, for example, they are young gobies, please write it.

- Does the “length” mean standard length or total length? Please specify, they are significantly different.

Third paragraph:

- Yamasaki et al. (2011) provided key morphological characters (not only the melanophore patterns) to identify species of the newly hatched larvae (not for postflexion larvae and juveniles) of goby. They did not use genetic characters for the larval identification.

**Methods**
First paragraph:
- How did you identify the samples visually according to Yamasaki et al. (2011)? They described the morphologies of newly hatched larvae, not the postflexion larvae and juveniles. See the comment above.
- Was the collection site the sea, not the estuary? According to the Figure 2, it is marine environment, 150-200 m off from the coast.

Second paragraph:
- Because the images have not been used in the manuscript, you don't need to write the second sentence.

Results

First sentence:
- Please replace “five unspecified individuals” with “five unspecified types”.
- *Sicyopterus pugnans* is a species in Polynesia. So probably it is a misidentification. Please remind that the information in the database is not always correct. Indeed the *Sicyopterus pugnans* in the figure 4 is divided into two clades. If they are different species, at least one of them is not the *S. pugnans*. Please consider the meaning of the results before trusting the information of the database blindly. Please suggest the possibility of misidentification in the Discussion.

Melanophore patterns:
- As I wrote above, if the nike material was composed of *Sicyopterus*, *Bunaka*, and *Belobranchus* species, the larvae can be identified at least at the genus level by their morphologies. Please observe the specimens carefully before saying “same body shape”.

Discussion
- I don't agree with the last two sentences.

Figure 1
- Please write status of the larvae. Are they living, on ice, or fixed in 70% ethanol?
- The scale bar must be an error. The larvae are too big, if the bar indicates 3 cm. Please confirm.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly
If applicable, is the statistical analysis and its interpretation appropriate?
Not applicable

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** larval biology of goby

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 17 October 2019

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**Dini Wahyu Kartika Sari**
Department of Fisheries, Faculty of Agriculture, Gadjah Mada University, Yogyakarta, Indonesia

**Methods:**
1. Do not show clearly how many samples of the Nike either for morphological analysis or molecular analysis.

2. No information about the size of the Nike.

3. What is the mean of "The DNA cytochrome oxidase subunit I (CO1) of the sample was isolated with a Genaeid DNA isolation kit"? It should be genomic DNA.

4. No primer information used in this study.

5. No information about the PCR mix and the PCR condition.

6. No information about how the authors got the sequence result? Sequencing done by who?

**Is the work clearly and accurately presented and does it cite the current literature?**
Partly
Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
Partly

If applicable, is the statistical analysis and its interpretation appropriate?
Partly

Are all the source data underlying the results available to ensure full reproducibility?
Partly

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Fish Genetics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Comments on this article

Reader Comment 06 Jun 2020
Rahmi Ami, Reader, Gorontalo, Indonesia

This journal is very helpful. I can improve my knowledge about nike fish. thank you!

Competing Interests: No competing interests were disclosed.

Reader Comment 06 Jun 2020
jonathan prasetya, Reader, Gorontalo, Indonesia

Thank you for posting this journal, this journal is gonna help many people

Competing Interests: No competing interests were disclosed.
Reader Comment 06 Jun 2020
Nabila N, Reader, Gorontalo, Indonesia
this journal has increased my knowledge, thank you!

*Competing Interests:* No competing interests were disclosed.

Reader Comment 06 Jun 2020
pita zara, Reader, Gorontalo, Indonesia
Thank you so much. This journal is very interesting and also very helpful to me.

*Competing Interests:* No competing interests were disclosed.

Reader Comment 06 May 2020
tesya Tesa, Reader, Indonesia
selama ini pemahaman masyarakat ikan nike hanya merupakan ikan satu jenis saja. Dan jurnal ini sangat membantu masyarakat untuk bisa tahu lebih dalam tentang ikan nike

*Competing Interests:* No competing interests were disclosed.

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