F1000Research

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What species make up the Nike fish assemblages at the macrotidal estuary in Gorontalo Bay, Indonesia?

Femy M. Sahami[1*], Rene Charles Kepel[2], Abdul Hafidz Olii[1], Silvester Benny Pratasik[2]

[1]Faculty of Fisheries and Marine Science, Gorontalo State University, Jl. Jendral Sudirman. No. 6, Gorontalo City, 96128, Gorontalo Province, Indonesia;
[2]Faculty of Fisheries and Marine Science, Sam Ratulangi University, Jl. Kampus Unsrat Bahu, Manado City, 95115, North Sulawesi Province, Indonesia.

Corresponding author: Femy M. Sahami (*femysahami@ung.ac.id*)

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 only four species that compos Nike fish schooling in Gorontao Bay. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*

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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 et al., 2001; Lahjie et al., 2019; McHugh, 1967; Sreekanth et al., 2017). They also perform the most important 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., 200; McLusky and 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).

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However, the impact of fishing activities is unknown. A recent paper concerning Nike only reports the seasonal appearance during the fishing season (Pasisingi and 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 determi\
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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.

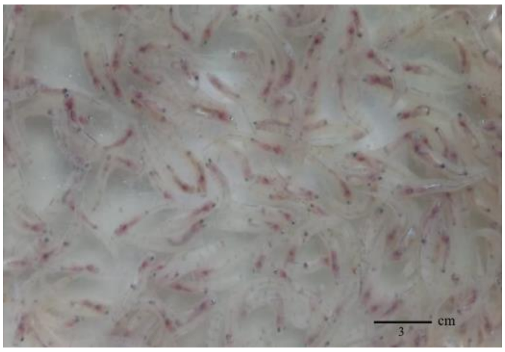

Figure 1. Nike fish assemblages.

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 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, samples were identified visually by melanophores pattern display (Yamasaki et al., 2011).

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Then, each selected individuals with different melanophores were separated from the samples and subsequently labeled as N1, N2, N3, N4, N5, and so on. All of the selected samples were pictured using Canon EOS 100d with 58 mm pro Digital Wide Converter 0.45X Lens and subsequently converted to black-and-white image using software CorelDraw Graphic Suite 2019.

After selection, all of the individual with different melanophores were preserved with alcohol 70% in a separate bottle and send to the Genetic Laboratory at Manokwari for genetic identification. The DNA of the sample was isolated with a Geneaid™ DNA Isolation Kit. PCR operation was performed with primary pairs, namely BCL Fish according to Baldwin et al.,2008. Moreover, the sequencing method was conducted with the protocol of Sanger dideoxy chain-termination (Sanger et al., 1977). Furthermore, editing and proofreading sequences were performed using MEGA 6.0 software.

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Figure 2. Study site. The red dot indicates the position of fishing ground where the samples were collected from fishermen.

Results

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Melanophores pattern

Nike-fish schools consisted of vaarious species with the same body-shape, but different on melanophore display. Moreover, from 100 g (~145 individuals) of the total specimens that have identified, we only found five individuals with a different melanophores pattern (Figure 3).

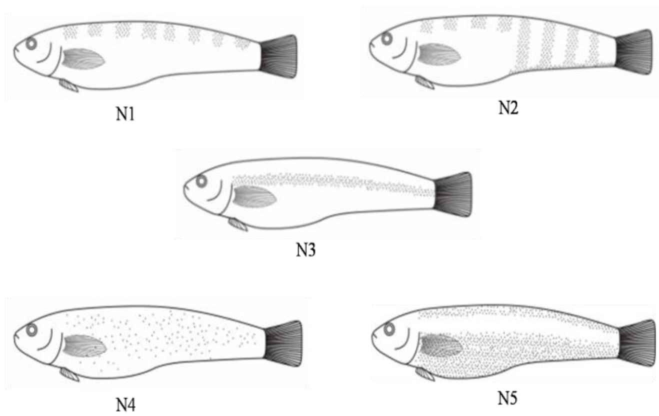

Figure 3. Nike-fish with different melanophores pattern.

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*.

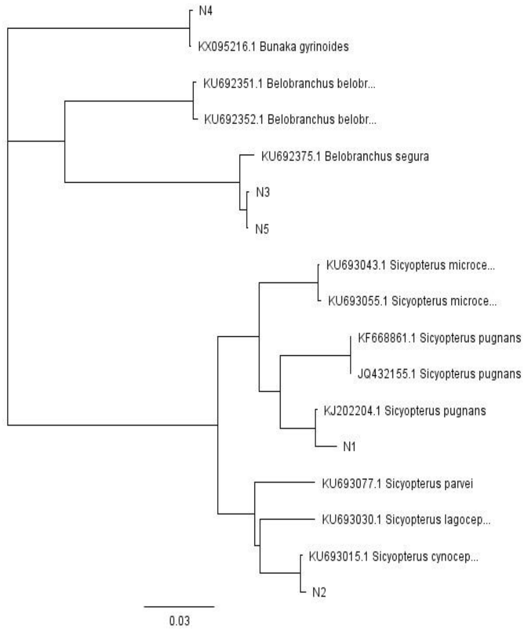

Figure 4. Phylogenetic tree of individuals with different melanophore patterns.

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 chang on *Sicyopterus langocephalus* during the larvae stage. These changes could represent a problem for morpholigical 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 compos Nike-fish schooling. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.

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References

Baldwin CC, Mounts JH, Smith DG, and Weigt LA, 2008. Genetic identification and color descriptions of early life-history stages of Belizean Phaeoptyx and Astrapogon (Teleostei: Apogonidae) with comments on identification of adult Phaeoptyx. Zootaxa. 1–22.

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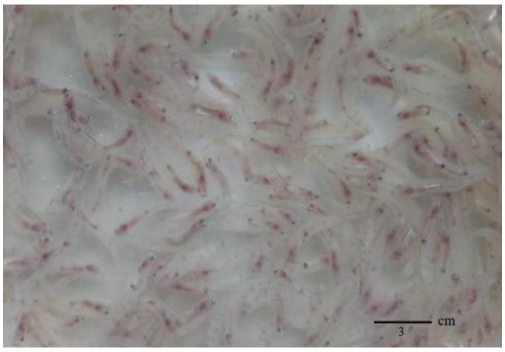

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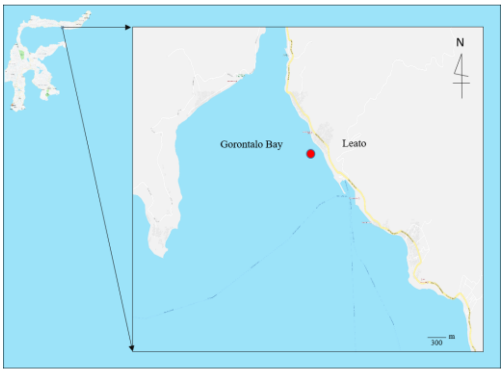

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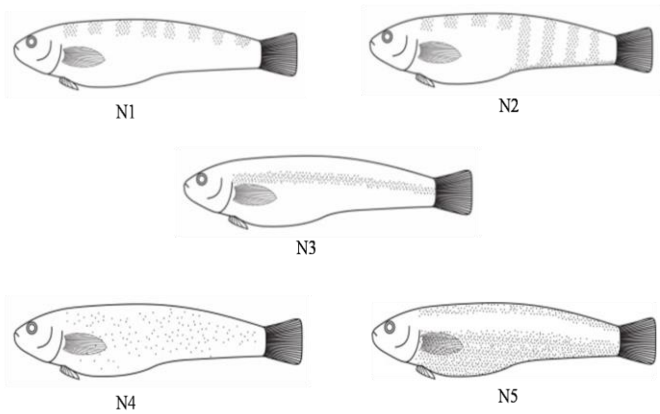

Figure 3. **Nike fish with different melanophore patterns.**

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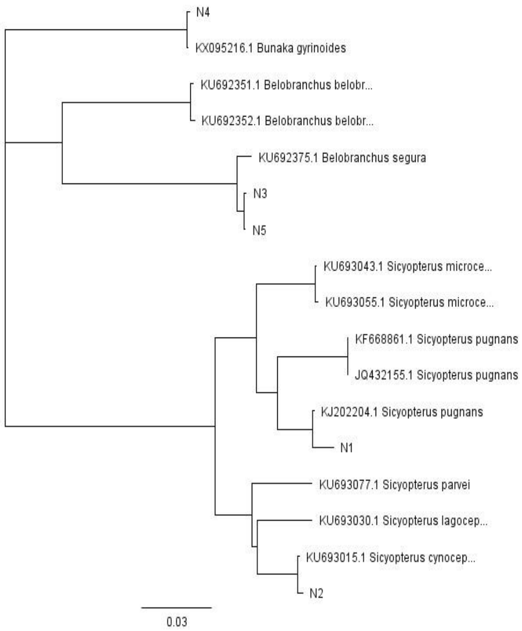

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