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Dear Femy M.,

Further to the email I sent you on 31/05/19, I wanted to check if you would still like to continue with your submission. I would appreciate if you could confirm receipt of this email, and respond to our outstanding comments.

I'm afraid the data files included with your submission must be uploaded to a structured genetic repository, such as the [Sequence Read Archive](#). As such, your article does not adhere to F1000Research's [FAIR and open data policy](#) and we cannot continue with your submission. Please see our [data guidelines](#) for more information.

In order to continue with your submission, we ask that you please deposit the underlying data to the [Sequence Read Archive](#) and add a [comprehensive data availability statement](#) at the end of the manuscript.

Please see our [data guidelines](#) and [FAIR and open data guide](#) for further guidance. Please don't hesitate to get in touch should you have any questions.

If there's anything we can help you with regarding your submission, please do let me know. I look forward to hearing from you within the next few days.

The Editorial Team, F1000Research

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Femy Sahami <femysahami@ung.ac.id>
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Min, 9 Jun 2019 21:33 ☆ ↶ ⋮

Dear Jonathan
(Editorial Team of F1000Research)

I'll improve it as soon as possible according to your comments.
Thank you very much for your quick response.

Best regards,
Femy M. Sahami





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Jum, 5 Jul 2019 22:54 ☆ ↶ ⋮

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Dear Femy,

I am writing to ask how you're proceeding with addressing the below comments? Please could you let us know if you are encountering any difficulties that we can assist you with.

Best wishes,
Jonathan

Jonathan Threlfall
Senior Assistant Editor

F1000Research
<https://f1000research.com>

Email: jonathan.threlfall@f1000.com
F1000 Research House, 34-42 Cleveland St London W1T 4LB UK

Femy Sahami <femysahami@ung.ac.id>
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23 Jul 2019 08:53 ☆ ↶ ⋮

Dear Jonathan
(Editorial Team of F1000Research)

We have just completed the submission of sequence data into NCBI Directory; <https://www.ncbi.nlm.nih.gov/nucleotide/>
with these accession number:

BankIt2234047 N1_LEATO_1	Accession number MN065178
BankIt2234572 N2_LEATO_1	Accession number MN069305
BankIt2234576 N3_LEATO_1	Accession number MN069306
BankIt2234578 N4_LEATO_1	Accession number MN069307
BankIt2234582 N5_LEATO_1	Accession number MN069308

Therefore, please continue the submission process.

Best regards,
Femy M. Sahami



Manuscript 19501 conditionally accepted for publication

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24 Jul 2019 22:54 ☆ ↶ ⋮

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Dear Femy M.

What species structuring the fish fish assemblages at macrotidal estuary, in Gorontalo Bay, Indonesia?

Sahami PM, Kapal RC, Oti AH and Prastika SB

Thank you for your submission to F1000Research. We have noted a few issues with your manuscript (below) - once these are addressed we will be pleased to accept your article for publication.

Abstract: Please include 1-2 sentences describing the conclusions of your study in the abstract.

Methods: Please include more detail in the methods. In particular, please describe the splitting of fish into different groups and what these groups represented, what was sequenced and all post-sequencing processing methods (including all software with version numbers).

Tables/Figures: Please note that tables cannot contain images. The content of all tables/figures must be described in the text - they cannot stand alone.

Grant information: Please state who funded the work. If the work was not funded by any grants, please state: "The authors declare that no grants were involved in supporting this work".

Acknowledgements: Please confirm that you have notified the people you have acknowledged by name in the manuscript.

Reviewers: As you know, F1000Research operates an author-driven publication model. This means that you will be responsible for suggesting suitable reviewers, whom we invite on your behalf, giving you an opportunity to ensure that appropriate experts review your article. Our transparent peer review process means that the peer review reports, together with the reviewers' names, will be published alongside your article.

To avoid delay to the publication process, we need you to provide us with at least five potential reviewers who meet our reviewer criteria before we can publish your article - please be aware that it is likely we will need to request further reviewer suggestions after publication. Please go to your [Suggest Reviewers](#) page, where you will find a useful tool to help you find reviewers; use this page to track the progress of the peer review process for your article. You can access this page directly via the article's record under My Research >> Submissions. See also our [reviewer criteria](#) and [tips for finding reviewers](#).

Please remember that suggested reviewers should have appropriate level of experience and the right expertise to judge your article; they must be able to provide an unbiased report (e.g. they must not be recent collaborators or colleagues in your institution). All reviewer suggestions are checked by the editorial team and will be rejected if they do not meet our criteria.

Payment: As F1000Research is open access, we will require payment of the Article Processing Charge (APC) to be able to complete the processing of your submission. As your article is currently classified as a small article, the APC is \$150.00. Please provide us with the name, postal address, e-mail address and VAT number (if applicable) of the person or single organization taking responsibility for making this payment. Please also let us know which currency you prefer to pay in (USD, GBP or Euros). Our Accounts department will be in touch regarding payment.

We have also lightly copyedited your article - please [download the document](#) and check you are happy with the amendments and then address the queries detailed above using track changes in Word. Please return your revised manuscript to the e-mail address above. Please note that this is your final opportunity to make any changes to the content of your manuscript. Once the typeset PDF of your manuscript has been created, we will send you a final PDF proof for checking prior to publication.

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28 Agu 2019 23.11 ☆ ↶ ⋮

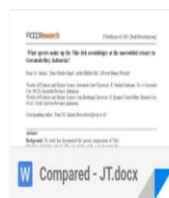
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Dear Femy,

Thank you for your message. There are still outstanding issues with your manuscript that need to be resolved before we can proceed.

My message of 13 August contained an updated version of the file, which I have attached here. Please respond to each query in this updated version of the manuscript.



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Sel, 27 Agu 2019 21.19 ☆

Dear Femy M., We have not received a response to our previous email so I wanted to check if you have received it. I would appreciate if you c...

Femy Sahami <femysahami@ung.ac.id>
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Kam, 29 Agu 2019 14.18 ☆ ↶ ⋮

Dear, Jonathan

I have attached the revised manuscript according to your comments. (find it in attachment).
Hopefully, it could meet your comments.

Best regards,
Femy M. Sahami



 **Femy Sahami** <femysahami@ung.ac.id>
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1 Sep 2019 21.47 ☆ ↶ ⋮

Dear, Jonathan

Thank you for your reply.
I have attached the revised manuscript according to your comments. (find it in attachment).
Hopefully, it could meet your comments.

Best regards,
Femy M. Sahami



Your article 19501 is now accepted 📄 Kotak Masuk x

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Sen, 2 Sep 2019 13.48 ☆ ↶ ⋮

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Dear Femy M.

What species make up the Nike fish assemblages at the macrotidal estuary in Gorontalo Bay, Indonesia?
Sahami FM, Kepel RC, Olii AH and Pratasik SB

We have now accepted your article for publication in F1000Research. It will be sent to the typesetters and a member of the Production team will send you a proof in due course.

In addition, one of our editorial team will be assisting you with the peer review process of your article, and will be your main contact once the article is published.

Best wishes,

Jonathan
The Editorial Team, F1000Research



Femy Sahami <femysahami@ung.ac.id>
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Sen, 2 Sep 2019 17.36 ☆ ↶ ⋮

Dear Jonathan,

Thank you for the good news. I will be here to wait for the next progress of our publication.

Best wishes,
Ferry M. Sahami



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

Femy M. Sahami^{1*}, Rene Charles Kepel², Abdul Hafidz Olii¹, Silvester Benny Pratasik²

¹Faculty of Fisheries and Marine Science, Gorontalo State University, Jl. Jendral Sudirman. No. 6, Gorontalo City, 96128, Gorontalo Province, Indonesia;

²Faculty 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*

Keywords: Nike-fish, gorontalo, melanophores pattern, genetic, morphology

Comment [JT1]: Please use this section to provide 1-2 concluding sentences for your abstract, per our article guidelines

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).

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 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.

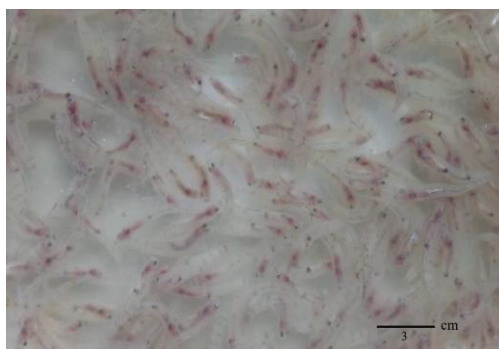


Figure 1. Nike fish assemblages.

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).

Comment [JT2]: Please provide references

Comment [JT3]: Please consider providing location using degree coordinates

Comment [JT4]: By whom? Please describe separation of the fish into different groups and how/why you assumed them to be different species

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.

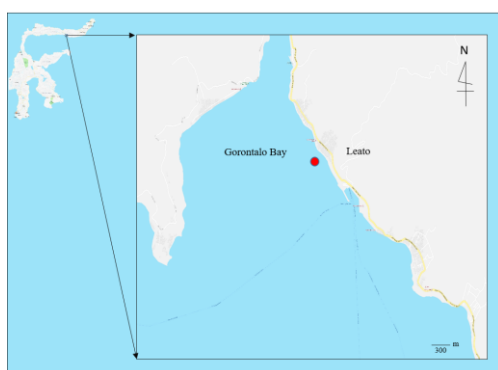


Figure 2. Study site. The red dot indicates the position of fishing ground where the samples were collected from fishermen.

Results

Melanophores pattern

Nike-fish schools consisted of various 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).

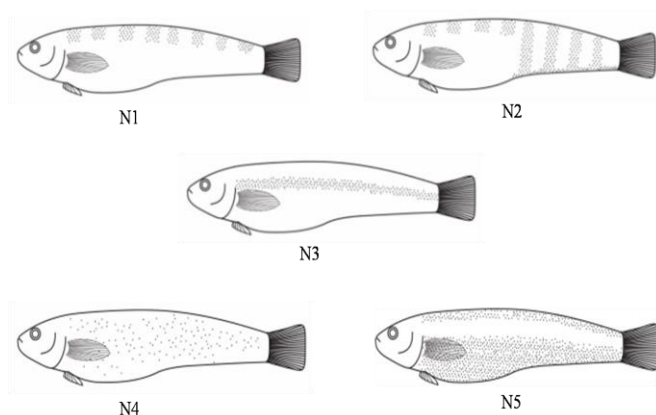


Figure 3. Nike-fish with different melanophores pattern.

Comment [JT5]: Please provide further information. Did you perform any further processing of the samples before sending them for sequencing?

What method was used to sequence them? What was sequenced? From your data, it seems that CO1 was sequenced.

What post-sequencing processing was performed? Please state all software used alongside version number.

How did you generate Figure 3?

Comment [JT6]: This section contains insufficient detail – you must at least state all of the species identified

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*.

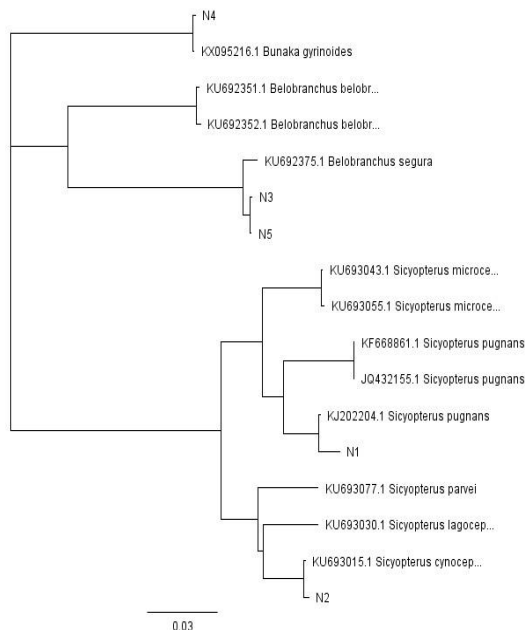


Figure 4. Phylogenetic tree of individuals with different melanophore patterns.

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 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 compos Nike-fish schooling. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.

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](#).

Grant information

The authors declare that no grants were involved in supporting this work

Comment [JT7]: Please see email

Acknowledgements

The authors would like to thank La Nane, Sitty Ainsyah Habibie, and Nuralim Pasingi for technical support during this research.

Comment [JT8]: Please see email

References

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Wolok, T., Fachrusyiah, Z. C., & Yantu, I. (2019). Technical And Economic Analysis Of Catching Equipment Totaluo In Nike Fishing (*Awaous Melanocephalus*) In Gorontalo City. *Jambura Science of Management*, 1(2), 65–71.

Yamasaki, N., Kondo, M., Maeda, K., & Tachihara, K. (2011). 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. *Cybiurn, International Journal of Ichthyology*, 35(4), 345-360.

Zakaria, Z. (2018). Analisis Morfometrik Schooling Ikan Nike di Perairan Laut Pesisir Kota Gorontalo. *Jambura Journal of Educational Chemistry*, 13(1), 77-80.

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

Femy M. Sahami^{1*}, Rene Charles Kepel², Abdul Hafidz Olii¹, Silvester Benny Pratasik²

¹Faculty of Fisheries and Marine Science, Gorontalo State University, Jl. Jendral Sudirman. No. 6, Gorontalo City, 96128, Gorontalo Province, Indonesia;

²Faculty 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.

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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 Gorontalo Bay. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.

Keywords: Nike-fish, gorontalo, melanophores pattern, genetic, morphology

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Introduction

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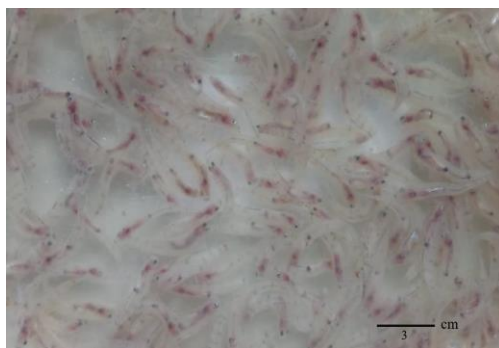


Figure 1. Nike fish assemblages.

Methods

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to the lab for measurement. Immediately after collection, samples were identified visually by their melanophore pattern display (Yamasaki et al., 2011).

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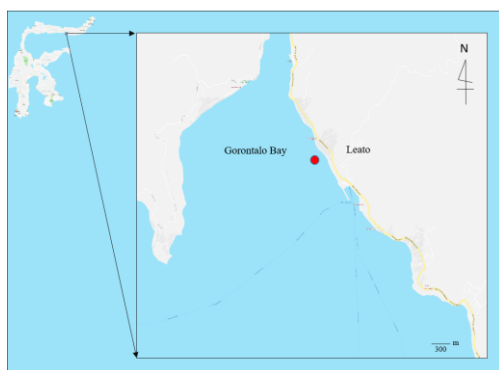


Figure 2. Study site. The red dot indicates the position of fishing ground where the samples were collected from fishermen.

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Comment [JT1]: By whom? Please describe separation of the fish into different groups and how/why you assumed them to be different species

Comment [JT2]: Please provide this information

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Comment [JT3]: Please state:
•Which gene was sequenced
•The software used to generate Figure 4

Comment [JT4]: Which alcohol – ethanol?

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Comment [JT6]: Please list all of the species identified in this section, and state which pattern they have

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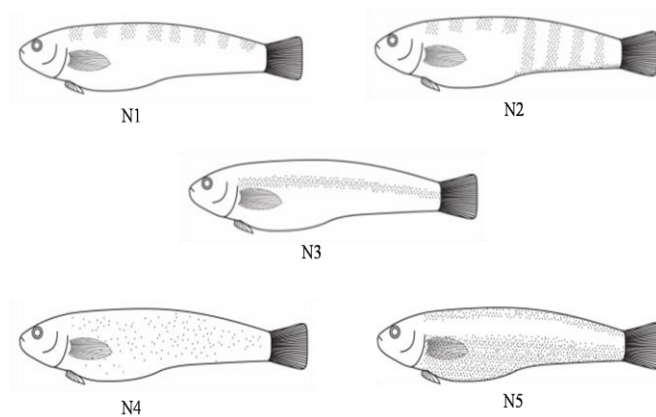


Figure 3. Nike fish with different melanophore patterns.

Genetic identification

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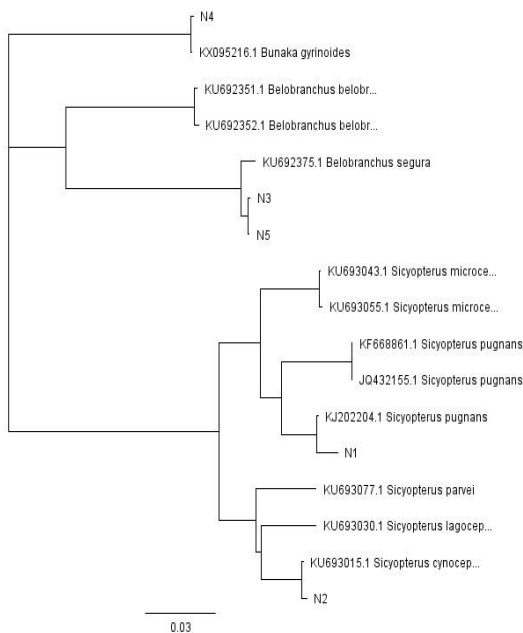


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Discussion

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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 Kepel², Abdul Hafidz Olii¹, Silvester Benny Pratasik²

¹Faculty of Fisheries and Marine Science, Gorontalo State University, Jl. Jendral Sudirman. No. 6, Gorontalo City, 96128, Gorontalo Province, Indonesia;

²Faculty 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 there are only four species that compose the Nike fish schooling in Gorontalo Bay. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.

Keywords: Nike-fish, gorontalo, melanophores pattern, genetic, morphology

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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 (Pasingi & 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.

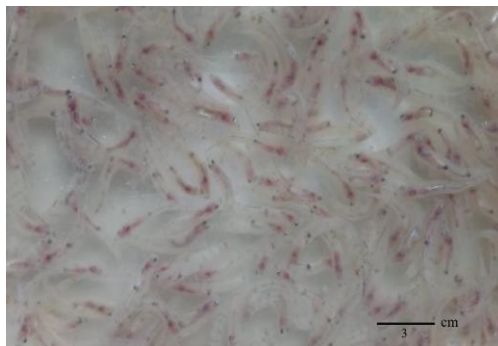


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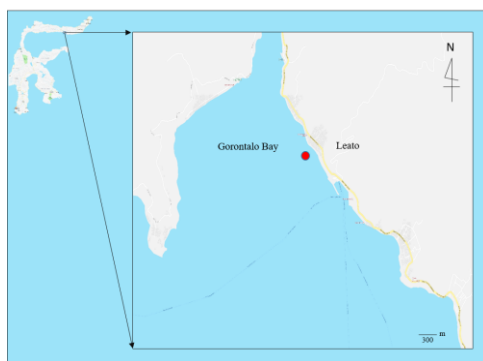


Figure 2. Study site. The red dot indicates the position of fishing ground where the samples were collected from fishermen.

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Five unspecified individuals of Nike-fish that have identified morphologically by melanophores differences showing in Figure 3. N1 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 the each group showing in figure 4.

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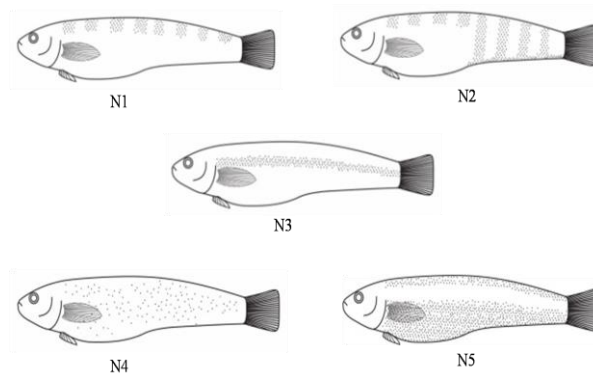


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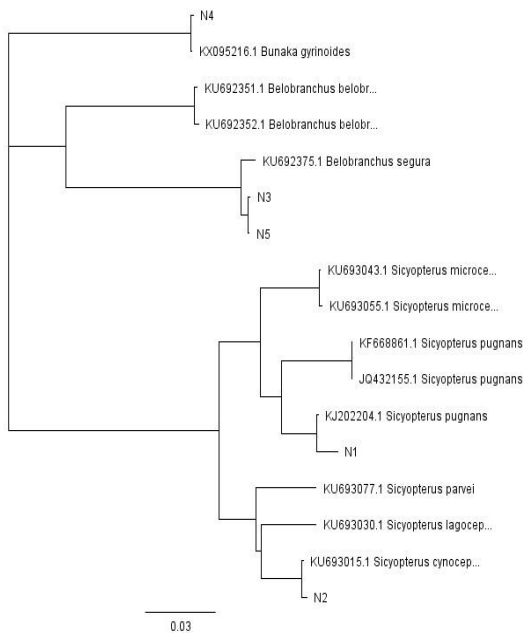


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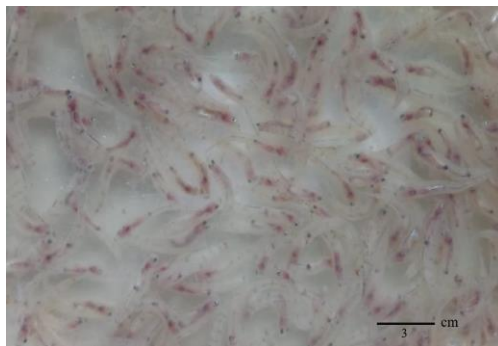


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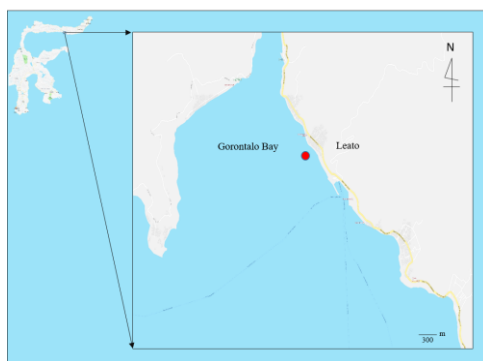


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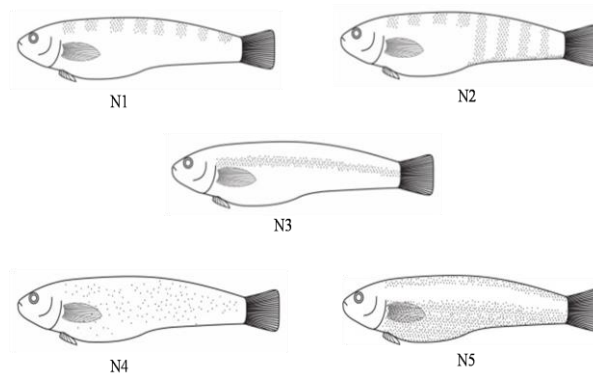


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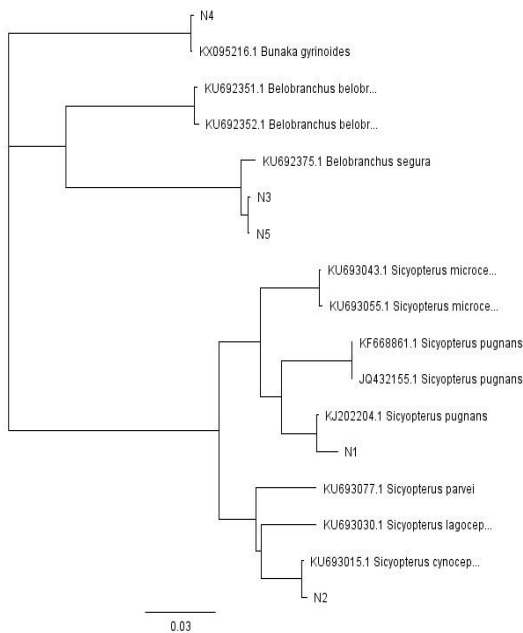


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Group N5, *Belobranchussegura* isolate N5_LEATO_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN069308](#).

Grant information

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