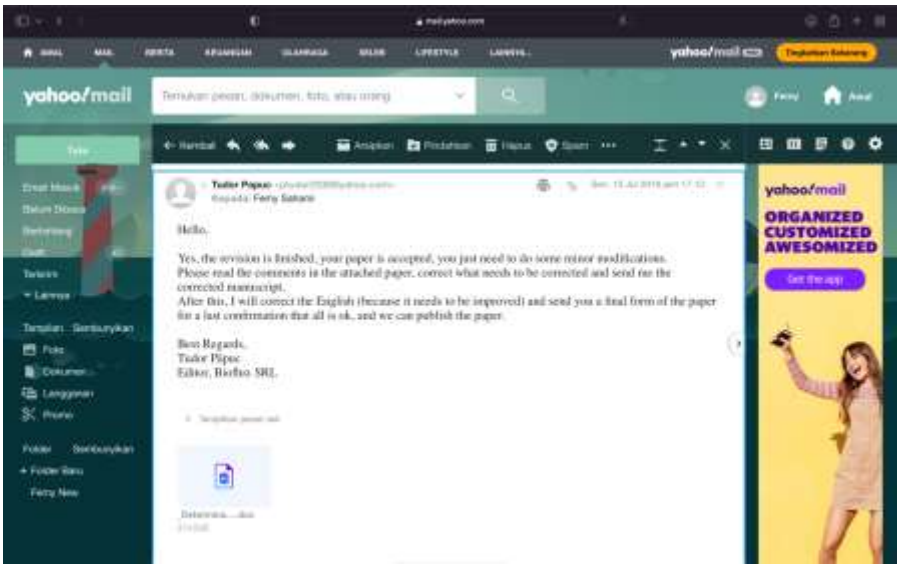

Korespondensi

Determination of Morphological Alteration Based on Molecular Analysis and Melanophore Pattern of Nike Fish from Sea to Estuary Area of Gorontalo Bay, Indonesia

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Abstract. Nike is a term for minuscule fish that frequently appear in Gorontalo waters. This type of fish belongs to the amphidromous goby group. This study aims to describe the morphological alterations of nike fish from the sea to the estuary area of Gorontalo waters based on molecular analysis and different of melanophore pattern. The small size of this fish (larval stage/post-larva) causes it be tough to analyze morphometric and meristic aspects. Therefore, the melanophore pattern approach and molecular analysis using the CO1 gene were used to identify these fish species. Sampling was carried out on 5 to 11 October 2018 in the Gorontalo Bay area to the mouth of Bone Bolango river when this type of fish appeared. Samples of nike fish were grouped based on differences in melanophore patterns approach in the body and we found there were five different groups and coded N1, N2, N3, N4, and N5. The molecular analysis using the CO1 gene were used to identify these fish species. Genetic investigation showed that from five groups of samples, there were found two groups of samples identified (N3 and N5) that had g different melanophore patterns were the same species, *Belobranchus segura*. Morphological changes have occurred due to an increase in the melanofor pattern of nike species when they migrate from the sea to the river mouth.
Key Words: melanophore, molecular, amphidromus gobie, nike fish

Introduction. Nike is the name of a small type of fish that is around 2-4 cm at length which appears in the waters in a large number carried out by Gorontalo people and its surroundings. These fish do not appear every day in the sea throughout the year but only once a month. Its emergence is unpredicted usually occurs at the end of each month in the calendar of the Hijri year or in the new moon time. The duration of the appearance of this fish at the location of its arrest is also not fixed, which is around three until seven days per the appearance period.

Scientific information about this nike is still not widely publicized. Scientific studies of this type of fish in Gorontalo waters are still rare. Thus far, information about the existence of these fish species has only been obtained from the community. Important aspects of the existence of these fish species can be a source of biodiversity whose existence needs to be preserved.

Nike in Gorontalo waters consists of two species, namely *Awaous melanocephalus* and *Eleotris frusca*. Among the schooling, *A. melanocephalus* is the main constituent species that is 99%, while *E. frusca* is only a supporter species. Furthermore, the inhabited area is from the coast to the upstream (Tantu 2001). *A. melanocephalus* is an amphidromous fish. When these adults spawn in freshwater, the eggs are placed on the substrate at the bottom of the water, after the eggs hatch the larvae drift into the sea, then after a while in the waters, the juvenile returns to the river where the parent originates (Yamasaki et al 2011). Fish from the gobii group in Hawaiian waters lived and developed in the waters of the sea, initially the fish hatched larvae in the river, and by river currents, the larvae were carried into the sea, living and developing into juveniles, then back to their habitat in freshwater (Maie et al 2009). The distribution of nike fish larvae that move from sea waters to river mouths is influenced by internal factors and external factors.

Gobiidae is one of the largest acanthomorph fish groups, which number approximately 1120 species from 30 genera that have been described and many more that have not been described (Thacker & Roje 2011). A large amount of gobi fish causes genetic similarity and can allow the occurrence of fertilization naturally. This fish in its life cycle passes through several environments that can allow morphological changes, but might be morphologically dissimilar but still the same species.

The high demand for these fish causes high fishing activity and exploitation. And the improper management will accelerate extinction as well. All waters cannot be managed directly (Sahami et al 2017). The response of organisms to the environment becomes significant information in an effort to manage waters. The differences in the topography of Nike fishing grounds can allow for differences in adaptation and ecology and behavior that may be expressed through changes in color and size. Due to their small size and often cryptic ecologies, the full extent of gobiid diversity often goes unnoticed (Thacker & Roje 2011). An effort to hypothesize relationships among the gobioid groups have been hampered by the prevalence of reductive evolution among goby species, such reduction can make identification of informative morphological characters particularly difficult (Thacker 2003).

Molecular and morphological studies can help determination of characteristics of nike fish in the Gorontalo Bay, making it easier for management. Genetic characters can provide fish genetic information and support morphological characters data (Purnama et al 2019). This study aims to determine morphological changes based on molecular analysis and melanophore patterns along the body of nike fish that migrate from the sea to estuaries in Gorontalo waters using molecular analysis.

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Material and Method

Sampling. The study started from October 2018 to January 2019. The sampling of these fish from fishmen was carried out randomly during the period of capture in Leato waters from 5 to 11 October 2018. A map of the sampling location is shown in Fig.1. Sampling was carried out at the Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Gorontalo State University by sorting and grouping it according to the appearance of the melanophore pattern on the fish's body. Samples of nike fish were grouped based on differences in melanophore patterns in the body and we found there were five different groups and coded N1, N2, N3, N4, and N5. From each group of fish, 5 individuals were taken and filled in the sample bottles which were preserved in 70% alcohol. Furthermore, samples were analyzed for genetic identification in the Papua State University genetic laboratory, Manokwari. The small size of this fish (larval stage/post-larva) causes it be tough to analyze morphometric and meristic aspects. Therefore, the melanophore pattern approach and molecular analysis using the CO1 gene were used to identify these fish species.

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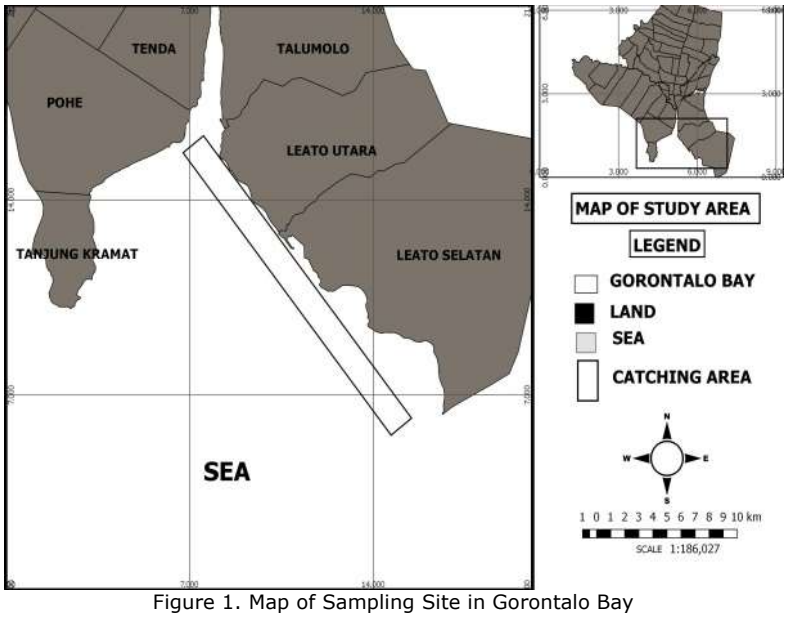

Figure 1. Map of Sampling Site in Gorontalo Bay

Molecular Analysis. DNA genome isolation of samples was conducted using an Isolation kit, which was produced by Geneaid (Genomic DNA Mini Kit (Tissue)). The isolation method carried out refers to the product standard protocol. The PCR process was carried out using the primary pair (Baldwin et al 2008), namely (Forward) BCL Fish: 5 '-TCAACYAATCAYAAAGATATYGGCAC-3' and (Reverse) Fish BCH: 5 '-ACTTCYGGGTGRCCRAARAATCA-3'. PCR products were then electrophoresed and photographed above UV Transilluminator (Pacific image, Electronic). The nucleotide sequencing cycle is a method for determining the sequence of nucleotides contained in DNA. The DNA samples that had been amplified and electrophoresed were subsequently sequenced. The sequencing process was carried out at the first Base Laboratory in Malaysia through PT Genetics Science Indonesia by sending samples consisting of 30 µl of PCR DNA products, 10 µl of forward primer and 10 µl reverse primer. Editing and proofreading sequences were performed using MEGA 6.0 software. The proofreading results from the forward and reverse sequences were combined into a sequence. Then the sequence results were analyzed to see samples that had genetic similarity. To find out the relationship level between samples,

further analysis was carried out using phylogenetic trees using Maximum Likelihood Method with 1000 bootstraps using MEGA 6.0 software.

Results and Discussion. The results indicate that nike fish schooling consists of 2 groups which morphologically are dissimilar and have different melanophore pattern yet genetically are the same species. The proofreading results from the forward and reserve sequences which were combined with the sequence of the two samples (N3 and N5) are presented in Table 1.

Table 1
Proofreading results of forward and reserve sequence of samples N3 and N5

Sample code	Proof reading results
N3	CCTTTATCTTGTCTTCGGTGCCTGAGCCGGGATAGTGGGCACCGCTTTAAGC CTACTTATCCGCGCTGAACTAAGTCAACCTGGCGCACTCCTAGGAGATGACC AAATCTATAATGTTATCGTTACCGCCCACGCGTTCGTAATAATTTTCTTTATAG TAATACCAATTATGATTGGCGGATTTGGTAACTGACTAATCCCCTTAATGATT GGCGCCCCAGACATGGCCTTCCCACGAATAAACAACATAAGTTTCTGACTTC TCCCGCCATCTTTCCTCCTCCTTTTAGCATCCTCTGGAGTAGAAGCAGGGGC CGGAACAGGGTGAACCGTCTACCCGCCCCTAGCGGGCAACCTCGCCCACGC AGGCGCCTCTGTGGACCTAACAATCTTTTCACTACACCTAGCAGGGGTGTCC TCAATTCTTGGAGCAATTAATTTCATTACCACAATTATTAACATAAAACCTCCG GCAATTTCCCAATACCAAACGCCCTTGTTCGTCTGAGCCGTTCTAATTACAGC CGTCTTATTACTATTATCCCTTCCCGTACTTGCTGCTGGCATCACAATGCTACT TACAGATCGAAATTTAAATACGACGTTCTTTGACCCGGCCGGGGGTGGGGAC CCAATCTTATACCAACACCTTTTC
N5	CCTTTATCTTGTCTTCGGTGCCTGAGCCGGGATAGTGGGCACAGCTTTAAGC CTACTTATCCGCGCTGAACTAAGTCAACCTGGCGCACTCCTAGGAGATGACC AAATCTATAATGTTATCGTTACCGCCCACGCGTTCGTAATAATTTTCTTTATAG TAATACCAATTATGATTGGCGGATTTGGTAACTGACTAATCCCCTTAATGATT GGCGCCCCAGACATGGCCTTCCCACGAATAAACAACATAAGTTTCTGACTTC TCCCGCCATCTTTCCTCCTCCTTTTAGCATCCTCTGGAGTAGAAGCAGGGGC CGGAACAGGGTGAACCGTCTACCCGCCCCTAGCGGGCAACCTCGCCCACGC AGGCGCCTCTGTGGACCTAACAATCTTTTCACTACACCTAGCAGGGGTGTCC TCAATTCTTGGAGCAATTAATTTCATTACCACAATTATTAACATAAAACCTCCG GCAATTTCCCAATACCAAACGCCCTTGTTCGTCTGAGCCGTTCTAATTACAGC CGTCTTATTACTATTATCCCTTCCCGTACTTGCTGCTGGCATCACAATGCTACT TACAGATCGAAATTTAAATACGACGTTCTTTGACCCGGCCGGGGGTGGGGAC CCAATCTTATACCAACACCTTTTC

The sequences produced are then compared with sequences contained in bank gene deposits (NCBI nucleotide databases). The results are presented in Table 2.

Table 2. Comparison sequences of sample and NCBI nucleotide database

No	Species	Gen	Accession Number	Max Score	Query cover	Identity
	Sequence N3:					
1	*Belobranchus segura*	COI	KU692375.1	1166	99%	99%
2	*B. segura*	COI	KU692374.1	1166	99%	99%
3	*B. segura*	COI	KU692367.1	1166	99%	99%
4	*B. segura*	COI	KU692362.1	1166	99%	99%
5	*B. segura*	COI	KU692372.1	1160	99%	99%
	Sequence N5:					

Commented [u6]: since the identification of B. segura as a member of a schooling nike is the first to be carried out in the world, there should be more emphasize on the finding.

1	*Belobranchus segura*	COI	KU692375.1	1171	99%	99%
2	*B. segura*	COI	KU692374.1	1171	99%	99%
3	*B. segura*	COI	KU692367.1	1171	99%	99%
4	*B. segura*	COI	KU692362.1	1171	99%	99%
5	*B. segura*	COI	KU692372.1	1166	99%	99%

Table 2 shows that the two samples based on genetic testing with mitochondrial CO1 are the same species as *Belobranchus segura*. Further analysis was carried out with the phylogenetic tree to show the kinship relationships between samples (N3 and N5) and several species available in the NCBI database as presented in Fig.2.

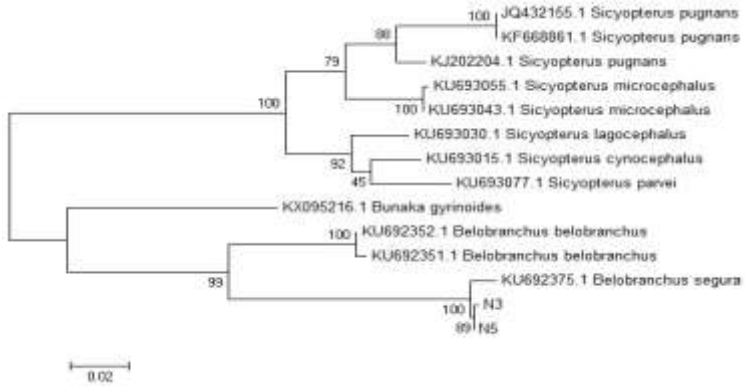

Figure 2. Phylogenetic trees of N3 and N5 samples were compared with several species in the NCBI database.

The genetic analysis illustrates that the two samples are similar species although they had different morphology particularly the melanophore patterns. This modification might be an adaptation of species when migrating from sea water to fresh water which is part of its life development. When entering a river mouth, post-larva undergoes changes in morphology, physiology, and behavior (Keith et al 2008). The difference in melanophore patterns from samples N3 and N5 is schematically presented in Figure 3.

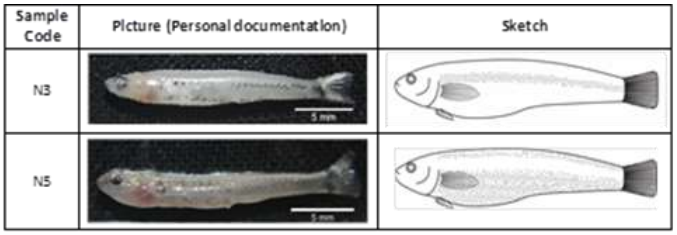

Fig.3. Schematic morphological alterations in the samples N3 and N5 based on the shape of the melanophore pattern

In addition, geographically, N3 and N5 are found in different locations. N3 (N 00°30,122' E 123°03,895'26.2) was found in the sea area, while N5 (N 00°30,305' E123° 03,739'26.2) was found at the mouth of the river (Fig. 4). Sample N5 is N3 that has developed and has undergone alterations in the melanophore structure. This can be possible because the N3 sample was taken from the catch on 8 October 2018, while the N5 sample was found in the sample conducted on 11 October 2018. This supports Valade et al (2009) statement that there has been a change in the appearance of chromatophore in the body *Sicyopterus langocephalus* larvae, which starts from the head area then spreads throughout the body along with the larvae age. The results of this study illustrate that there has been a variation in the pattern of melanophore from the

species of nike *Belobranchus segura* when migrating from sea water to the mouth of the river. When still in the sea the melanophore arrangement is still minor, but when it will enter the river mouth the melanophore arrangement has spread along its body.

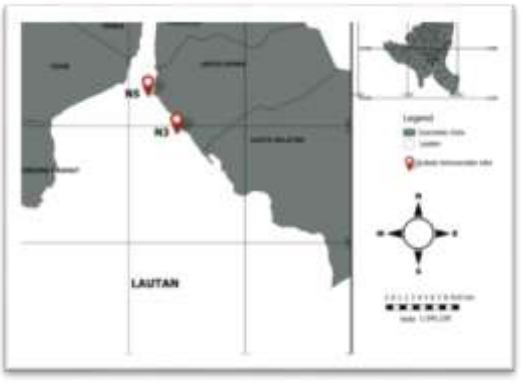

Figure 4. Area sampling of N3 and N5

Hawaiian gobioid fish are amphidromous and have one phase of life in the sea (Hobson & Smith 2007) They are in the pelagic phase of the sea for several months before juveniles in the river (Teichert et al 2016). Nike fishing in the Gorontalo Bay from the first, second and third days has a tendency to shift closer to the river mouth which indicates the migration of nike from sea water to fresh water (Olii et al 2017, Pasisingi & Abdullah 2018).

Changes in color morphology or melanophore patterns of nike species *Belobranchus segura* from the sea with melanophore content which becomes slightly more when approaching the estuary is a form of adaptation in order to enter a new aquatic environment or is part of the stages of its development. Distribution of species along the river is determined by postlarva color aggregation (Nishimoto & Fitzsimons 1986)

The occurrence of migratory behavior has a genetic basis in freshwater fish, although it is clear from various studies that genetic signals for migratory behavior may be strongly influenced by environmental and developmental factors (Lucas & Baras 2001). Although the lives of amphidromous fish are strongly related to the conditions of the oceans and rivers, they vary in ecology and behavior and the causative factors that drive juveniles to move upstream into the adult habitat are not fully understood. This migration is related to their phase of development (Fitzsimon & McRae 2007). Post-larvae return to rivers where they are recruited and grown to reproductive stages (Ellien et al 2016).

The distribution of nike fish tends to approach the estuary since its appearance time until it disappears (Olii et al 2017). Based on the results of this study it can be concluded that in fact, nike fish do not disappear from the waters as what nike fishing community consider. There had been a morphological alteration in color due to an increase in the number of melanophores.

Conclusions. In conclusion, samples N3 and N5 that have different melanophore patterns are genetically the same species, *Belobrancianus segura*. The morphological changes of nike fish of this species are indicated by differences in melanophore patterns, that was an increase in the number and spread of melanophore on the surface of the body when this species has entered the river mouth.

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Please use the full name of the journal. (ex: not J of Morpho, but Journal of Morphology)

At the end of the manuscript, there should be a text box with the full name of the authors and the affiliations. Please check again the model paper (available on our site, in the left menu), copy the text box from the model paper, paste it at the end and change the names and affiliations, exactly like it is there (in that order). The rest of the text will be changed, but the names and affiliations need to be completed by you.

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