



First report on the seasonal and spatial variation of the species composition of amphidromous goby fry schools in Tondano River and Poigar River estuaries, North Sulawesi, Indonesia

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Abstract. There has been no published report on the seasonal and spatial variation of the species composition of goby fry schools in Tondano River and Poigar River estuaries, North Sulawesi, Indonesia. The aim of this study is to document the species composition of goby fry schools in Tondano River and Poigar River estuaries, North Sulawesi, Indonesia, at different times of emergence of the schools. Goby fry samples were collected in January 2021 from the Poigar River estuary, while from Tondano River estuary the goby fries were sampled in January and February 2021. Collected samples were sorted and grouped according to the melanophore patterns. Five individuals from each group were selected for further molecular genetic analysis. Among all goby fry in collected samples, three melanophore patterns were identified, which were then encoded M1, M2 and M3. The phylogenetic molecular analysis procedures proved that sample M1 is *Awaous grammepomus*, M2 is *Sicyopterus pugnans* and M3 is *Sicyopterus lagocephalus*. The goby fry schools in Poigar River estuary composed of two species, i.e. *Awaous grammepomus* (35% of the total number) and *Sicyopterus pugnans* (65% of the total number); while those in Tondano River estuary, at the first sampling from January 2021, composed merely of one species, *Sicyopterus lagocephalus* (100%) and at the second sampling, February 2021, composed of three species, i.e. *Awaous grammepomus* (7.5% of the total number), *Sicyopterus pugnans* (40% of the total number) and *Sicyopterus lagocephalus* (52.5% of the total number). This study shows that the species composition of the goby fry schools varies by either season or location in the two studied estuaries of Poigar and Tondano rivers, in North Sulawesi, Indonesia.

Key Words: Gobiidae, melanophore, migration, molecular phylogenetics.

Introduction. Amphidromy is a distinct form and the most common type of diadromous migration of aquatic organisms (McDowell 2007; Milton 2009; Egan et al 2019). Noticeable characters of amphidromous fish are the following: mature fish spawn in fresh water, and the eggs or newly hatched larvae drift downstream to the sea where they go through a planktonic life, growing for a few months, and then, as postlarvae or juveniles they return to freshwater to grow to adult phase and reproduce (McDowall 1997; Bell 1999; Keith et al 2003; Valade et al 2009; Augspurger et al 2016).

Most of the goby species belonging to the Gobiidae family are amphidromous (Keith 2003; McDowell 2007; Milton 2009; Augspurger et al 2016). Ruber and Agoretta (2011) stated that gobies are small fishes that display a wide variety of morphology, behavior, and ecology. They live in freshwater, brackish water and seawater (Milton 2009; Ruber & Agoretta 2011; Ramadhian et al 2016). Most of them occupy shallow waters around mangrove ecosystems and estuarine muddy habitats (Yokoo et al 2009; Redjeki 2013; Latifa et al 2015). Gobies are widely distributed in tropical and sub-tropical islands of the Indo-Pacific region and make a key component of the freshwater

ichthyofauna on many tropical islands (Keith et al 2003; Milton 2009; Watanabe et al 2011; Teichert et al 2013).

Several studies showed that, the juveniles of amphidromous gobies migrate back from the sea to the river on a monthly regular basis. These large schools of goby fries are identified while they are entering the river mouth through the estuarine waters (Bell 1999; Valade et al 2009; Vedra & Ocampo 2014; Ollie et al 2017; Pasingi & Abdulah 2018; Nurjirana et al 2019; Sahami et al 2019a,b; Sahami et al 2020; Pasingi et al 2020). In many countries, these schoolings have been regularly fished, making the goby-fry fishery to grow consistently in many parts of the Indo-Pacific region (Bell 1999; Valade et al 2009; Vedra & Ocampo 2014; Asis et al 2013; Sahami et al 2020; Pangemanan et al 2020).

As a tropical country positioned in the centre of the Indo-Pacific region, Indonesia has numerous species of Gobiidae. With high economic value, "nike" (local name in North Sulawesi) or "penja" (local name in West Sulawesi), are schools of fish composed of many species of goby fish (Sahami et al 2019a,b; Sahami et al 2020; Pangemanan et al 2020; Pasingi et al 2020; Nurjirana et al 2019). Fishermen catch the "nike" or "penja" fish when they are in post-larva or juvenile stage (Sahami et al 2019a,b; Sahami et al 2020; Pangemanan et al 2020; Pasingi et al 2020; Nurjirana et al 2019). As in other parts of the Indo-Pacific region, goby fries in Indonesia have high economic value as well, due to the large market needs. Consequently, the exploitation level is consistently increasing, which may put these fish species in danger of extinction (Bell 1999; Keith 2003). If the schools of post-larva and juvenile fish are uncontrollably caught at every season of their appearance, eventually the existence of these fish species will be threatened, as hardly any individuals have a chance to grow up to reproduce (Keith 2003; Teichert et al 2013).

Conservation and management of these amphidromous species is urgently required (Keith 2003; Valade et al 2009; Vedra & Ocampo 2014; Asis et al 2013; Egan et al 2019) in all parts of the Indo-Pacific region where the goby fry fishery is active, including North Sulawesi. Information concerning the species composition of the schooling fries is necessary for basic data in conservation plan (Asis et al 2013). Several studies revealed that the schools appearing in different seasons and different estuaries may be represented by dissimilar species (Bell 1999; Ollie et al 2017; Ollie et al 2019; Sahami et al 2019; Nurjirana et al 2019; Pasingi et al 2020; Pangemanan et al 2020). The only information on the biodiversity of the goby fry schools from a North Sulawesi estuary was reported by Pangemanan et al (2020) and shows that the schools in Poigar River estuary consisted of 5 species of Gobiidae, namely *Sicyopterus cynocephalus*, *Sicyopterus lagocephalus*, *Sicyopterus pugnans*, *Stiphodon semoni* and *Awaous ocellaris*. There was no information regarding this issue from other estuaries and in different seasons of appearance. Therefore, the species composition of goby fry schools in the estuaries of Poigar River and Tondano River from North Sulawesi, Indonesia, is investigated in this study. Identification is based on the melanophore pattern examination and molecular analysis.

Material and Method

Sample collection. The two sampling points for collecting the goby fry is shown in Figure 1. Data from the Poigar River estuary (1°00'40"N - 124°17'51"E) was compared with that from Tondano River estuary (1°29'58"N - 124°50'25"E). In terms of different seasons comparison, data collected days prior to new moon lunar phase in January 2021 from Poigar River estuary, coded "Pg", was compared with the data from Pangemanan et al (2020). In Tondano River estuary, samples were collected at two different seasons of the fry school appearance, first days prior to new moon lunar phase in January and

secondly days prior to new moon lunar phase in February 2021, coded 'Tn-1' and 'Tn-2' respectively. A triangle scoop net measuring around 1,2 m on each side was used in sampling of the goby fry from the shallow waters adjacent to the banks of the river mouth. 100 grams of collected samples were kept in plastic containers with cold preservation. Forty individuals were then randomly picked from each sample, sorted, and grouped by the melanophore pattern. Five individuals from each group were selected and preserved in 70% alcohol for further molecular genetic analysis.

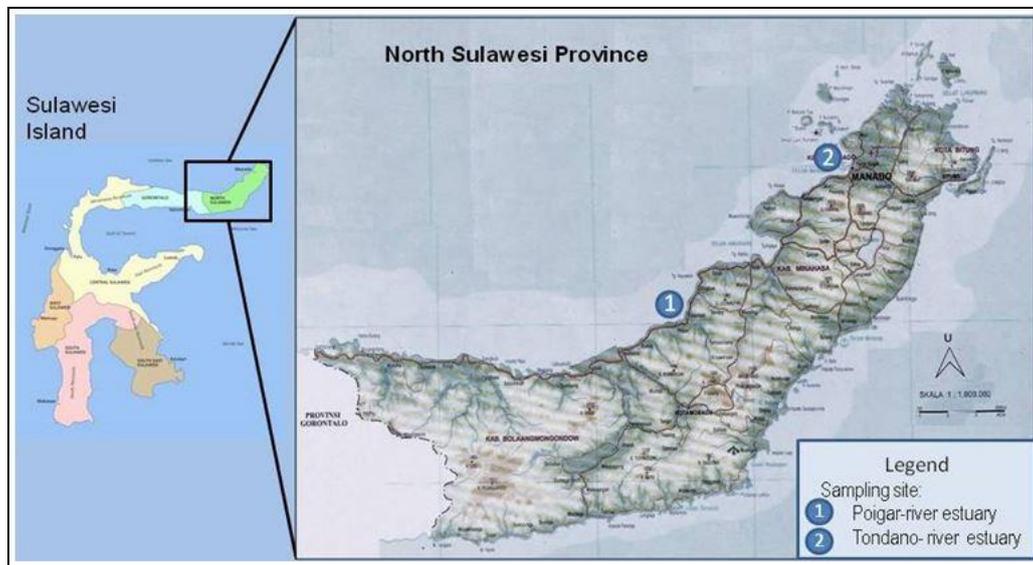


Figure 1. Sampling sites in Poigar river estuary (1) and Tondano river estuary (2) (figure generated using Adobe Photoshop; map from www.alnturambi.wordpress.com).

Molecular analysis. The molecular analysis was conducted in the Laboratory of Bionesia (Indonesian Biodiversity), Denpasar, Bali, Indonesia. The tissue of the fish sample was prepared and extracted to isolate DNA using the Qiagen Blood & Tissue Kit protocol. DNA multiplication at the cytochrome oxidase I (COI) locus used the BIONESIA PCR protocol with F1/R1 primers. Data was analyzed by means of phylogenetic tree analysis and then compared with the database from GenBank (KU692311.1, KJ202204.1, MK496948.1) (NCBI 2021). Phylogenetic analysis was performed using the neighbor-joining (NJ) method with 1000 times bootstrap replication. The value of the genetic distance was analyzed using the p-distance.

Results and Discussion. Among all fish in the three bunches of collected samples (coded Pg, Tn-1 and Tn-2), three melanophore patterns were identified which were then encoded M1, M2 and M3, as shown in Table 1. The proportion of fish with each melanophore pattern was also presented.

The results of the nucleotide sequencing cycle procedure are presented in Table 2, which details the sequence of nucleotides contained in DNA of three different samples. The average length of the three tissue samples nucleotides was ± 670 bp. Table 3 shows the results of the procedure for matching the nucleotide sequence contained in DNA of three different samples with the data available in GenBank (KU692311.1, KJ202204.1, MK496948.1) (NCBI 2021) by the means of BLAST (Basic Local Alignment Search Tool).

Figure 2 shows the result of the phylogenetic tree analysis, where the resulted DNA data from the three samples was compared with the database from GenBank (KU692311.1, KJ202204.1, MK496948.1) (NCBI 2021). The two phylogenetic molecular analysis procedures proved that the BIOSUB112.001 (M1) sample is *Awaous*

grammepomus, sample BIOSUB112.002 (M2) is *Sicyopterus pugnans* and sample BIOSUB112.003 (M3) is *Sicyopterus lagocephalus*.

Table1

Three melanophore patterns identified from the three bunches of collected samples (coded Pg, Tn-1 and Tn-2) and the fish proportion of each melanophore group from the total number of samples

Melanophore code	The morphology of the three goby fries with clearly different melanophore pattern	Proportion of each melanophore at each sampling location		
		Poigar estuary (Pg)	Tondano estuary 1 st sampling (Tn-1)	Tondano estuary 2 nd sampling (Tn-2)
M1 (<i>Awaous grammepomus</i>)		35%	0	7.5%
M2 (<i>Sicyopterus pugnans</i>)		65%	0	40%
M3 (<i>Sicyopterus lagocephalus</i>)		0	100%	52.5%

It can be inferred from the data in Table 1 and Table 2, the goby fry schools in Poigar River estuary are composed of two species, i.e., *Awaous grammepomus* (35% of total number) and *Sicyopterus pugnans* (65% of total number); while those in Tondano River estuary at the first sampling represent only one species, *Sicyopterus lagocephalus* (100%) and at the second sampling three species are identified, i.e. *Awaous grammepomus* (7.5% of total number), *Sicyopterus pugnans* (40% of total number) and *Sicyopterus lagocephalus* (52.5% of total number). According to Milton (2009), these three species are part of the approximately 55 species of Gobiidae inhabiting the Indo-Pacific to the eastern Atlantic, whereas Indonesian waters is in the tropical central area of the Indo-Pacific region.

The data of the species composition of the goby fry schools appearing in different seasons at the estuary of Tondano River, Manado, North Sulawesi, Indonesia, are the first ever recorded. This study shows that, the species composition varies by either season or location of the appearance of the schools. There were two species, *Awaous grammepomus* and *Sicyopterus pugnans*, that comprised the schools appearing in January 2021, at Poigar river estuary, in this study, which was quite different from the finding of Pangemanan et al (2020) that identifies five species, i.e., *Sicyopterus cynocephalus*, *Sicyopterus lagocephalus*, *Sicyopterus pugnans*, *Stiphodon semoni* and *Awaous ocellaris* in the schools at the similar location in May 2020. The common species from these two different studies is *Sicyopterus pugnans*. The species composition of the school at the Tondano River estuary emerging in January 2021, which comprised of only one single species, *Sicyopterus lagocephalus*, was different from that of February 2021 consisting of three species, i.e., *Awaous grammepomus*, *Sicyopterus pugnans* and *Sicyopterus lagocephalus*. By location of appearance, goby fry schools sampled in Tondano River estuary comprise of three species, *Awaous grammepomus*, *Sicyopterus*

pugnans and *Sicyopterus lagocephalus*, while those in Poigar River estuary only compose of two species without *Sicyopterus lagocephalus*.

Table 2

The sequence of nucleotides contained in DNA of three different samples

<i>Sample code</i>	<i>Sequence result</i>
BIOSUB112001_M1	CTATACCTGGTATTTGGTGCTTGAGCTGGAATAGTAGGCGCAGCTCTTAGC CTTCTAATCCGAGCTGAACCTAGCCAACCTGGGGCTCTTTAGGAGACGAC CAAATCTATAATGTCATTGTAACAGCACATGCATTTGTAATAATTTTCTTTAT AGTAATACCAATTATGATTGGTGGCTTTGGGAACTGACTAATCCCCCTAATG ATTGGTGGCCCTGACATGGCCTTCCCTCTAATGAATAACATGAGCTTTTGAC TTCTCCCTCCTTCATTCTGCTTCTCCTATCATCATCAGGGGTTGAAGCTGG AGCAGGGACTGGTTGAACTGTCTACCCCCACTAGCAGGGAACCTTGCCCA TGCTGGAGCTTCTGTAGACCTAACAATTTTCTCACTTCACCTGGCAGGTGTC TCATCAATTTTAGGTGCAATTAACCTCATTACAACCATCCTAAATATGAAACC CCCTGCAATTTACAATACCAACACCCCTGTTGTTGAGCCGTTCTAATC ACAGCAGTCTACTACTTCTTTCCCTACCAGTCCTTGCTGCCGGCATTACAA TGCTACTAACAGACCGCAACCTGAACACGACCTTCTTTGACCCGTCGGGTG GAGGAGACCCAATCCTTTACCAACACTTGTCTGATTCTTTGGCCACC
BIOSUB112002_M2	CTATACCTGTTTTCGGTGCCTGAGCAGGAATAGTAGGGACTGCCCTCAGC CTACTCATCCGAGCTGAATTAAGTCAACCTGGAGCTCTTCTAGGGGACGAC CAAATTTACAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTTCTTTAT AGTAATACCAATCATGATTGGAGGCTTTGGGAACTGACTTATCCCCTAATG ATCGGTGCCCTGATATGGCCTTTCCTCGAATAAATAACATAAGCTTTTGAC TTCTCCCCCTTCATTCTTCTCCTCCTAGCATCTTCCGGTGTGAAGCAGG GGCCGGAACCTGGCTGAACAGTATATCCTCCTCTGGCAGGAAACCTTGACA TGCAGGAGCTTCTGTTGACTTAACTATTTTCTCCCTCCACCTGGCAGGTATT TCATCAATTTCTGGGGCAATTAATTTTATTACAACCATCCTAAACATGAAACC CCCTGCAATTTACAATATCAAACACCTCTATTTGTGTGAGCTGTTCTTATTA CAGCAGTCTCCTACTTCTCTCCCTCCCTGTCCTTGACAGCTGGCATTACAAT GCTACTAACAGACCGAAACCTTAACACAACCTTCTTTGACCCATCAGGAGG AGGTGACCCAATTCTCTACCAACATCTATTCTGATTCTTTGGCCACC
BIOSUB112003_M3	CTGTACCTGTTTTCGGTGCCTGAGCAGGAATGGTAGGAACTGCCCTAAGC CTACTTATCCGAGCTGAATTAAGTCAACCTGGGGCTCTTCTAGGAGACGAC CAAATTTACAATGTAATTGTTACTGCACATGCCTTTGTAATAATTTTCTTTAT AGTAATACCAATCATGATTGGAGGCTTTGGGAACTGACTCATCCCCCTAATG ATTGGTGGCCCAGATATAGCCTTCCCCGAATGAACAACATAAGCTTTTGAC TCCTCCCCCTTCATTCTTCTCCTCCTAGCATCTTCAGGTGTTGAGGCAGG AGCTGGGACTGGCTGAACAGTCTACCCCCCTTAGCAGGAAACCTTGCCCA TGCAGGGGCTTCTGTTGATTTAACTATTTTCTCCCTTACCTAGCAGGTATT TCATCAATTTCTGGTGCAATTAATTTTATCACAACCTATTCTAAATATGAAACC TCCTGCAATTTACAATATCAGACACCCCTTTTCGTCTGAGCTGTCCTTATC ACAGCAGTCTACTACTTCTTTCCCTCCCAGTTCTTGACAGCAGGCATTACAA TGCTACTGACAGACCGAAACCTCAACACAACCTTCTTTGACCCGTCAGGGG GAGGTGACCCAATTCTCTACCAACATCTATTCTGATTCTTTGGCCACC

The findings of this study are in accordance with the reports on the goby fry schools composition at Gorontalo waters and other regions of Sulawesi Island, which is also seasonally and spatially varied. In Gorontalo waters, Olii et al (2017) identified only *Awaous melanocephalus* comprising the school, but soon after that, Olii et al (2019) added up *Sicyopterus longifilis* as part of the school. Sahami et al (2019a) reported that, *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka*

gyrinoidea were present in the schools, however in the next finding Sahami et al (2020) identified a significantly different composition of the school consisting of five new species, *Sicyopterus parvei*, *Sicyopterus longifilis*, *Sicyopterus lagocephalus*, *Stiphodon semoni* and *Belobranchus belobranchus*, without *Sicyopterus pugnans*, *Belobranchus segura*, and *Bunaka gyrinoidea* which were found in the school before at similar location. The common species in each school was *Sicyopterus pugnans*. The latest data on the goby fry school in Gorontalo waters was reported by Pasingi et al (2020) indicating *Awaous ocellaris* and *Belobranchus belobranchus* comprised the school. In Leppangan River, West Sulawesi, Nurjirana et al (2019) found nine species composing the goby fry schools, i.e., *Sicyopterus lagocephalus*, *Sicyopterus longifilis*, *Stiphodon semoni*, *Stiphodon atropurpureus*, *Sicyopus zosterophorum*, *Smilosicyopus leprurus*, *Schismatogobius* sp., *Eleotris fusca* and *Eleotris* sp.

Table 3

The identified species based on the comparison procedure for the nucleotide sequence results with the GenBank (KU692311.1, KJ202204.1, MK496948.1) (NCBI 2021) data using BLAST

No	Code sample	Species	Accession no. GenBank	Query cover	Identity index (%)
1	BIOSUB112.001 (M1)	<i>Awaous grammepomus</i>	KU692311.1	97	98.31
2	BIOSUB112.002 (M2)	<i>Sicyopterus pugnans</i>	KJ202204.1	100	99.70
3	BIOSUB112.003 (M3)	<i>Sicyopterus lagocephalus</i>	MK496948.1	100	99.85

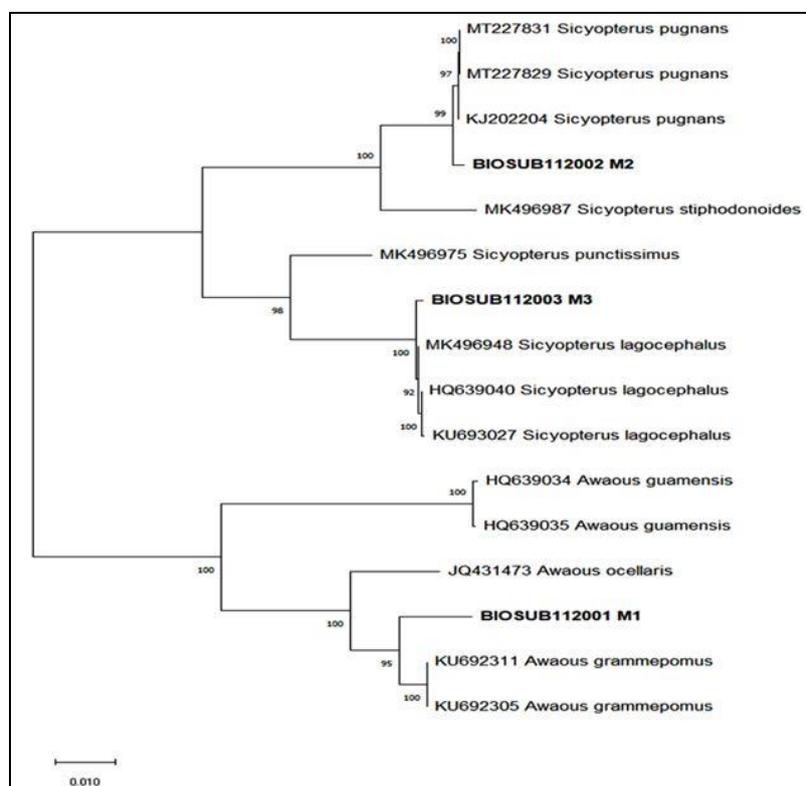


Figure 2. Phylogenetic tree of the three identified species compared with several relative species in the GenBank (KU692311.1, KJ202204.1, MK496948.1) (NCBI 2021) database.

In his review, Keith (2003) indicated that, natural events such as, rainfall, drought, floods, and typhoons, can have a profound effect on the reproduction, spawning and the dispersal of larvae, which means these physiological aspects are generally synchronized with the environmental character. Undoubtedly, temperature is the main factor regulating most of the character of the environment. This hypothesis is most likely to be the reason that the species composition of goby fry schools varies by either season or location of the appearance of the schools. Furthermore, numerous studies show that, the reproductive behavior was suspected as the determinant factor affecting the seasonal and spatial existence of goby post-larvae and juvenile occurrences. The reproduction behavior of goby fish varies according to the tropic-level of the region. In the northern sub-tropical Indo-Pacific, Yamasaki et al (2011) reported that, the active reproduction time of the gobies predominantly started right before summer and ended up in autumn. Spawning seasons of *Sicyopterus japonicus* and *Awaous melanocephalus* in Japan were May to August and June to November, respectively, while *Stenogobius* sp. can spawn throughout the year except from January to April, when the temperature was very low. On the other hand, in the Southern sub-tropical Indo-Pacific region where the summer occurs around December–February, Teichert et al (2013) reported that reproduction period of *Sicyopterus lagocephalus* mainly started in October–November when the first signs of ovarian growth appeared. The spawning season started in December, when the first spent females were identified, and the intensive reproductive period is February to May. This spawning season was not significantly different with that of in the tropical Indo-Pacific region, where the spawning of gobies was observed at the onset of the rainy season, from October to November (Vedra & Ocampo 2014). This was agreed by Milton (2009) stating that many studies have shown that the wet season is the primary growth and reproductive season for fishes in tropical areas.

Most of the published reports on the goby fry fishery and the biology of the fry schools in Sulawesi Island waters are based on the studies of the estuaries of Gorontalo waters. The study sites were in the vicinity of the Leato estuary, which is located on the southern coast of Gorontalo province, right to the west of the North Sulawesi province in the Minahasa Peninsula. Interestingly, no reports based on the study at the estuaries of Gorontalo on the northern coastline of Minahasa Peninsula. On the contrary, this study found that the goby fry schools appear seasonally at the two studied estuaries of the North Sulawesi waters in the northern part of Minahasa Peninsula. At other estuaries along the northern coastline of North Sulawesi, based on the local community's information, were used to be the host of goby fry schools. Furthermore, no information is available, even from local communities, regarding of the goby fry appearance at estuaries of North Sulawesi waters on the southern coastline of Minahasa Peninsula nowadays. This issue might be the object for future studies regarding migration distance and reproduction behavior of the gobies in Gorontalo and North Sulawesi waters.

The predominant species found in this study is *Sicyopterus lagocephalus* (100% and 55% portion in Tn-1 and Tn-2 samples, respectively). This finding agreed with the report of Teichert et al (2013) stating that in La Réunion Island (southwest Indian Ocean), *Sicyopterus lagocephalus* also dominates the fish assemblages and represents a major economic activity for local fishermen. In their review on *Sicyopterus lagocephalus*, Teichert et al (2013) stated that, this species is the most widely distributed gobiid, living from the Western Indian Ocean to the Eastern Pacific, inhabiting the short and oligotrophic coastal streams of continents and islands. This statement was corroborated by Keith (2003), Watanabe et al (2011), Vedra and Ocampo (2014) reporting the vast existence of *Sicyopterus lagocephalus* in all the Indo-Pacific region, either in the northern sub-tropical or southern sub-tropical, and in tropical Indo-Pacific. This species was also reported in the schools in all other studied areas of Sulawesi waters, i.e., North Sulawesi,

Gorontalo, Central Sulawesi, and West Sulawesi (Nurjirana et al 2019; Sahami et al 2020; Pangemanan et al 2020; Gani et al 2020).

The picture of the goby fry sample in Table 1 shows that, the size of *Sicyopterus lagocephalus* is much larger than that of the two other species. These three samples closely represent the size of all samples collected. The large body size *Sicyopterus lagocephalus* is most likely due to the extended oceanic larval period duration of this species. When the larvae of *Sicyopterus lagocephalus* migrate from the river to the sea, they spend much longer time in the ocean than other amphidromous goby species do. Watanabe et al (2011) stated that *Sicyopterus japonicus* and *Sicyopterus lagocephalus* have the longest oceanic larval duration, ranging 131-266 days in various tropical and subtropical regions, compared to any other species of the Sicydiinae. This extended sea-life time give them the chance to grow larger, and when they migrate back to the river, their body size is significantly larger than other goby species.

Interestingly, our study shows that, *Awaous grammepomus* is included in the schools appearing in both Poigar and Tondano estuaries. This is the first report on the existence of this species in the waters of either North Sulawesi or neighboring Gorontalo. The numerous studies on the species composition of goby fry school carried out in Gorontalo and West Sulawesi waters found various goby species, but not *Awaous grammepomus*. Likewise, there has been no record of this species constituting the goby schools in other Indonesian waters, except the adult specimen found in the river of Luwuk Banggai (Gani et al 2020). Rajan and Sreeraj (2013) reported that, *Awaous grammepomus* is native to Andaman waters, India, and is rarely found recently. They stated further that this species distributes widely from India, Andaman Islands to the Philippines. While Jahan et al (2017) working on molecular analysis of *Awaous* spp., reported that rivers in Bangladesh are hosting *Awaous grammepomus*. The existence of this species in Indonesian waters needs further investigation.

Conclusion. This study shows that the species composition of the goby fry schools seasonally and spatially varied at the 2 studied estuaries, of Poigar and Tondano rivers, in North Sulawesi, Indonesia. Two species, *Awaous grammepomus* and *Sicyopterus pugnans*, comprised the schools appearing in January 2021 at Poigar River estuary, which was quite different from the finding of Pangemanan et al (2020) identifying five species, *Sicyopterus cynocephalus*, *Sicyopterus lagocephalus*, *Sicyopterus pugnans*, *Stiphodon semoni* and *Awaous ocellaris* in the schools at the similar location in May 2020. The species composition of the schools at the Tondano River estuary emerging in January 2021, which is of only a single species, *Sicyopterus lagocephalus*, is different from that in February 2021 composing of three species, *Awaous grammepomus*, *Sicyopterus pugnans* and *Sicyopterus lagocephalus*. By location of appearance, goby fry schools sampled in Tondano River estuary comprise of three species, *Awaous grammepomus*, *Sicyopterus pugnans* and *Sicyopterus lagocephalus*, while that in Poigar River only compose of two species, without *Sicyopterus lagocephalus*.

Conflict of Interest. The authors declare no conflict of interest.

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