Identifying Populations of the Marble Goby, *Oxyeleotris marmorata* (Bleeker, 1852) by Otolith Shape Analysis and Their Spawning Seasons in Likas Bay, Sabah, Malaysia

Gunzo Kawamura, Annita Seok Kian Yong, Difa Dhaniah Zharfan binti Engcong, Kai Ying Chieng, Audrey Daning Tuzan and Leong-Seng Lim *

Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

**Corresponding author: leongsen@ums.edu.my*

Received 27 May 2021 | Reviewed 6 January 2022 | Accepted 3 January 2024 | Published 31 December 2024

DOI: 10.51200/bjomsa.v8i.3094

Abstract

The marble goby, *Oxyeleotris marmorata* is native to fresh and brackish waters of Southeast Asia. The yield of wild *O. marmorata* has been decreased drastically and urgent conservation actions are required to prevent extinction. In the Likas Bay, *Oyeleotris marmorata* is caught throughout the year by local fishermen using various fishing gears. In fisheries management, population is a fundamental biological unit and population identification of a species is important. The population structure of this marine stock was determined by otolith shape analysis and their spawning season in the Likas Bay was determined by ovarian observations. A total of 147 specimens were procured between 2018 and 2019; size ranged from 443 g to 3,264 g BW. The curvature index of the otoliths was analyzed by Bhattacharya's method and found that the frequency distribution of curvature indices is a mixture of two normal components, indicating two different populations. The specimens with mature or spent ovaries were found in samples collected in January – February and September, showing two spawning seasons. The age of each specimen was estimated by counting the vertebral annuli. The estimated age ranged from 5 to 14 years. The minimum size and age of spawners was 338 mm TL, 499 g BW and 5 years old. The findings obtained in this study provide basic biological parameters of *O. marmorata* populations in Likas Bay.

Keywords: fish population, otolith shape analysis, Bhattacharya's method, spawning season

Introduction

The marble goby, *Oxyeleotris marmorata* (Bleeker, 1852) is a member of the family Eleotridae and native to fresh and brackish waters of Southeast Asia. *Oxyeleotris marmorata* is a highly sought-out food fish and has a large market demand domestically and internationally. The yield of wild *O. marmorata* has been decreased drastically due to habitat destruction and overfishing (Cheah et al., 1994). Tavarutmaneegul and Lin (1988) reported successful commercial production of *O. marmorata* of 147,300 juveniles in a year with high survival of 60 to 99% at Non-Sua Fisheries Station in Thailand. Nevertheless, the production of *O. marmorata* reduced drastically in Thailand during the 1990s, resulting mainly from outbreaks of disease (Jiwyam, 2008). The culture currently relies heavily on seed collection from the wild, subsequently, the supply of juveniles, traditionally recruited from natural waters, has become limited and imposes a severe deterrent to grow-out production (Teoh et al., 2019). This species is currently listed as a species of Least Concern on the IUCN Red List (Larson, 2019). However, in Indonesia, it is reported that the species has already been fished almost to the point of population collapse (Herawati et al., 2017).

Conservation actions for wild *O. marmorata* is an urgent requirement, however biological information required for monitoring wild *O. marmorata* populations is limited (Lestari et al., 2019). In fisheries management and assessment, population is the fundamental biological unit and population identification in the species is very important (Reiss et al., 2009). Only with understanding the geographical variation and reproductive biology, it is possible to effectively manage and subsequently conserve endemic fish populations (Al-Saleh et al., 2012). Otolith morphometrics have been shown to provide a practical basis for population identification and subsequent fisheries management (Keating et al., 2014, Libungan et al., 2015, Vasconcelos et al., 2018).

During fish market surveys in Kota Kinabalu, Sabah, Malaysia, *O. marmorata* was found often caught by small scale local fishermen using various fishing gears, mainly bottom gill nets, throughout the year in Likas Bay, Kota Kinabalu. Likas Bay is a marine bay with an average seawater salinity of 32.0 ± 2.1 ppt (Azad & Jinau, 2020). The objectives of this study are to identify the population structure of the marine *O. marmorata* and to determine their reproduction cycle. The findings obtained in this study provide basic biological parameters of *O. marmorata* populations in Likas Bay.

Materials and Methods

Study area and fish specimen collection

Likas Bay, faces the South China Sea (Figure 1), has shallow water and is partly fringed with mangrove forest and sandy beach. The shallow waters and man-made beaches of the Bay are popular among recreational visitors and serve as an important fishing ground for small-scale fishers. During the sampling period, we procured 147 specimens (male: female sex ratio was 73: 74) caught by the small-scale fishers. Systematic samplings were not conducted as the landings of *O. marmorata* in Likas Bay were irregular. As such, there were no specimens obtained between February and August 2018. Additionally, dead specimens of *O. marmorata* were procured from the Kota Kinabalu fish market from January 2018 to November 2019.

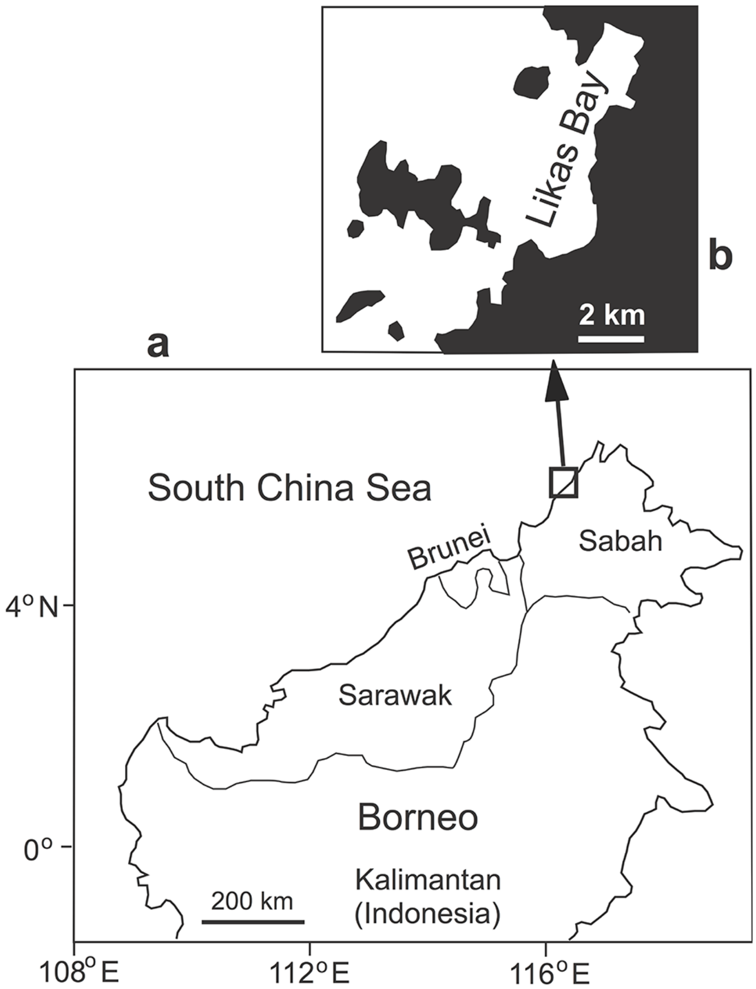

Figure 1. Map showing the location of Borneo (a) and Likas Bay (b), Sabah, Malaysia.

The body size of the fish specimens ranged from 328 mm in total length (TL) and 443 g in body weight (BW) to 594 mm TL and 3,264 g BW. After photographing the urogenital papilla and weighing the body weight to the nearest 1 g, the body cavity of each specimen was dissected, and the intact gonads were photographed. The gonads and alimentary canal contents were weighed to the nearest 0.1 g with a digital balance (TANIT, Tokyo, Japan). As there is no difference in gonad development between sites within the same gonad (Smith & Walker 2004), a piece of frontal part of gonads was sampled and preserved in 10% formalin for histological examination of the gonadal developmental stages.

Otolith collection and otolith shape analysis

The fish head was ventrally dissected and the sagittal otoliths of 147 specimens were removed. The sagittae were photographed with a smartphone camera (Huawei Nova 7i, Huawei, China) and the length and width of the right sagitta were measured (Figure 2) with a digital caliper (Shinwasokutei, Sanjo, Japan) to the nearest 0.01 mm. The measurement was done three times for one otolith and the median value was adopted. Then the ratio of width to length of the otolith was calculated. This ratio is the curvature index of the otolith; a bulky otolith has a large curvature index and vice versa.

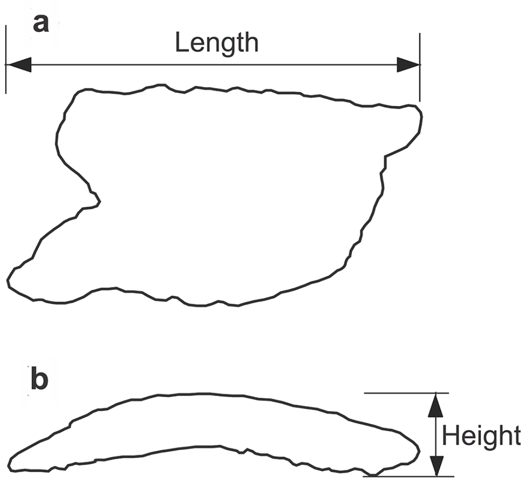

Figure 2. Illustration of dimensions of sagittal otolith of *Oxyeleotris marmorata* showing measurement of length and width.

The frequency distribution of a morphometric character in a biological population is usually skew and polymodal; in many cases, a mixture of normal components. Bhattacharya's method (Bhattacharya, 1967) is simple and useful for separating the normal components. Method, $y(x)$ denotes the observed frequency in the class with x as its mid-point and h denote the class interval. The log differences, $\Delta \log y = \log y(x + h) - \log y(x)$, are plotted against x on an ordinary graph paper, and regions where the graph looks like a straight line with negative slope was determined. The number of straight lines of such regions is the number of normal components (Bhattacharya, 1967).

Age estimation

Three vertebrae, from 3rd to 5th vertebrae of each specimen, were sampled and preserved in 10% formalin for the later counting of the vertebral rings. Clear rings on the cleaned wet vertebrae can be observed by naked eye without sectioning or staining (Figure 3). The rings on the vertebrae indicate the annuli, and age was estimated by counting the number of opaque rings observed on the inner surface of the cones in three vertebrae for each specimen. The counting was facilitated by placing a vertebra vertically on a slide glass and illuminating the vertebra upwards from the bottom with a light guide (LS-JHS. Sumita Optical Glass Inc., Saitama, Japan). The counting was done without the information on the fish size to avoid nuisance factors in the counting.

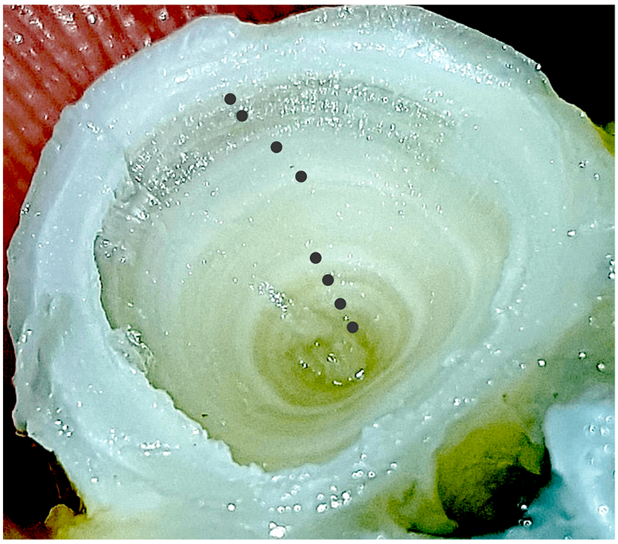

Figure 3. Vertebra from an interpreted 8 years old *Oxyeleotris marmorata.* Closed circles represent opaque rings on the inner surface of the cones.

Gonad development analysis

The ovary development stages were determined macroscopically and histologically. For the histological study, the gonad samples were dehydrated in ascending series of ethanol, embedded in paraffin, sectioned in 7 μm thick, and stained with haematoxylin and eosin. The gonadosomatic indices (GSI) was calculated as the percentage (%) ratio between gonad weight and body weight which did not include the weight of the alimentary canal contents and gonads.

Results and Discussion

Otolith shape analysis and population structure

The results of the otolith curvature analysis by the Bhattacharya's method is shown in Figure 4. The graph of logarithmic differences of the class-frequencies against the mid-point of the classes shows two straight lines with negative slope (Figure 4). This shows that the frequency distribution of the curvature index is a mixture of two normal components; indicating the presence of two different populations in Likas Bay.

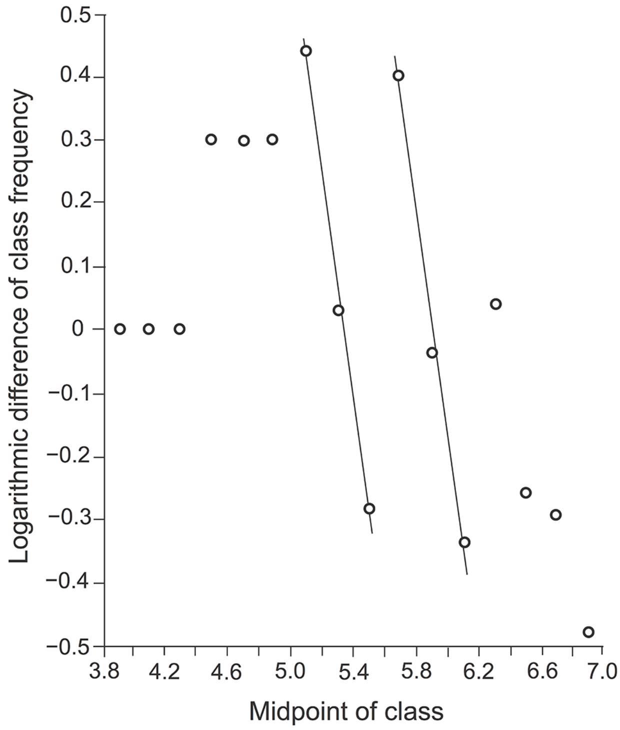

Figure 4. Graph of logarithmic differences of the class-frequencies against the mid-point of the classes. Two straight lines with negative slope indicate two normal components.

Ha et al. (2011) examined mitochondrial DNA of 85 *O. marmorata* specimens sampled from 14 locations in Southeast Asia and reported three genetically differentiated populations, i.e. Ayutthaya in Thailand, Dong Nai in Vietnam, and Sabah in East Malaysia. As geographically close samples were pooled and treated as a single population in their study, fine population structure was not detected.

Age structure of sample fish

There was no difference in the three counting of the vertebral rings in all specimens. The age of *O. marmorata* sampled ranged from 5 to 14 years. The age frequency distribution was skewed and polymodal with the first peak at 6 years and the second peak at 8 years (Figure 5). This age frequency distribution may not accurately represent the true population structure, as smaller specimens were likely under-sampled due to the size selectivity of the gill nets. Two specimens of 530 mm TL and 670 mm TL had a clear net-mark at the posterior part of the head, indicating a large mesh size of the gill net used.

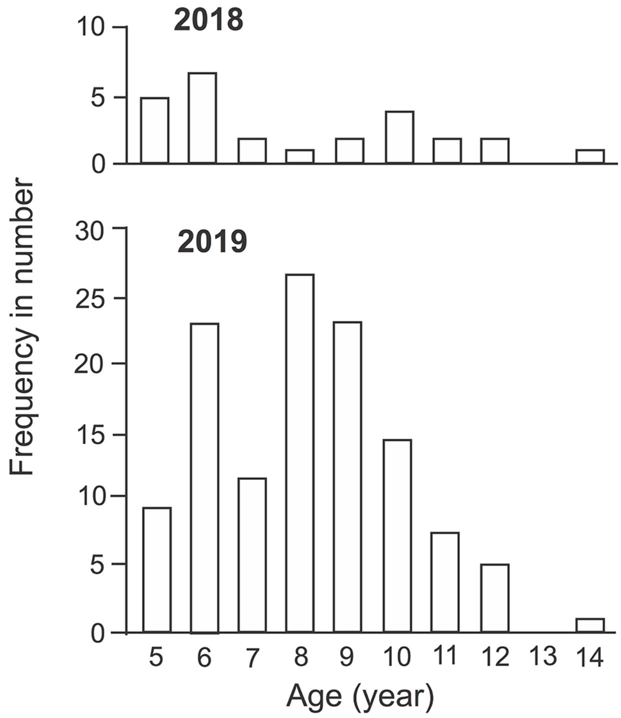

Figure 5. Frequency distribution of age of *Oxyeleotris marmorata* sampled in 2018 (*n* = 26) and 2019 (*n* = 121).

Ovary development and spawning season

The maturation stages were determined only for females since the testes were very small (GSI ranged from 0.03 to 1.76) and mature ones were not recognized macroscopically. Generally, there are three stages of the ovary development: maturing, mature and spent. Figure 6 shows photographs and microphotographs of histological sections of ovaries from two specimens sampled in January 2018. The mature ovary is yellowish in colour, distinctly bulging and large (Figure 6a) and filled with well-developed mature oocytes (Figure 6b) while oocytes did not run out when light pressure was applied. The spent ovary was slender, bloodshot and flaccid (Figure 6c). In the histological sections, spent ovaries contained scattered granular atretic oocytes and post ovulatory follicle (Figure 6d). Unspawned large ova and small ova were not visually recognized in all spent ovaries.

While the GSI is commonly used for staging gonadal development, these may be biased when samples of fish of different body weight are compared, as pointed out by West (1990). Indeed, GSI does not show the presence of spent ovaries. GSI of spent females did not give quantitative information on the spawning season. Therefore, the spawning season was estimated based on the presence of females with mature ovary or spent ovary. Figure 7 shows the distribution of GSI of female *O. marmorata* by sampling month. Specimens with mature ovaries were sampled only in September 2018, and in January and February 2019. Specimens with spent ovaries were sampled in January and September 2018, and in January, February and September 2019.

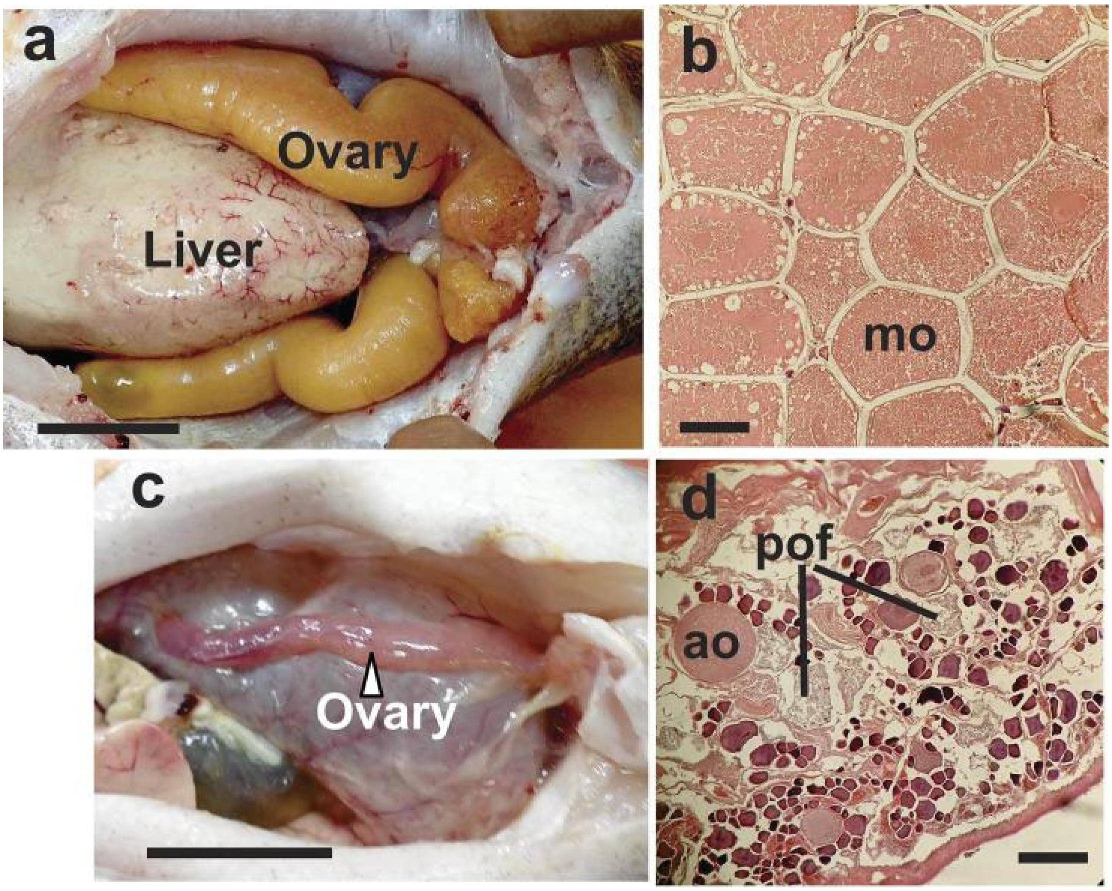

Figure 6. Photographs of ovaries (a & c: scale bar, 2 cm) and microphotographs (b & d: scale bar, 0.2 mm) of histological sections of ovaries of mature female [(a) and (b)], and spent female [(c) and (d)] of *Oxyeleotris marmorata*. (a) and (b), sampled on 26 September, 2018, BW 2,325 g, GW 52.4 g, GSI 2.254; (c) and (d), sampled on 11 January, 2018, BW 945 g, GW 2.6 g, GSI 0.275; mo, mature oocyte; ao, atretic oocyte; pof, post ovulatory follicle.

Based on the appearance of specimens with the mature or spent ovaries, it is concluded that the spawning of *O. marmorata* in Likas Bay took place twice, in January-February and September in 2018 and 2019. Tavarutmaneegul and Lin (1988) reported that each female spawned at least three times annually in the earthen ponds at Nong-Sua Fisheries Station in Thailand. In the present study, the spent ovaries of the samples did not contain unspawned large ova which indicates that *O. marmorata* are single spawners. Therefore, it is concluded that the spawners are from two different populations. Even though the two populations are sympatric in the small bay (insufficient spatial isolation), the different spawning seasons with a half-year time interval (sufficient temporal isolation) might be sufficient to prevent gene flow between the two populations and hence enable self-sustaining each other.

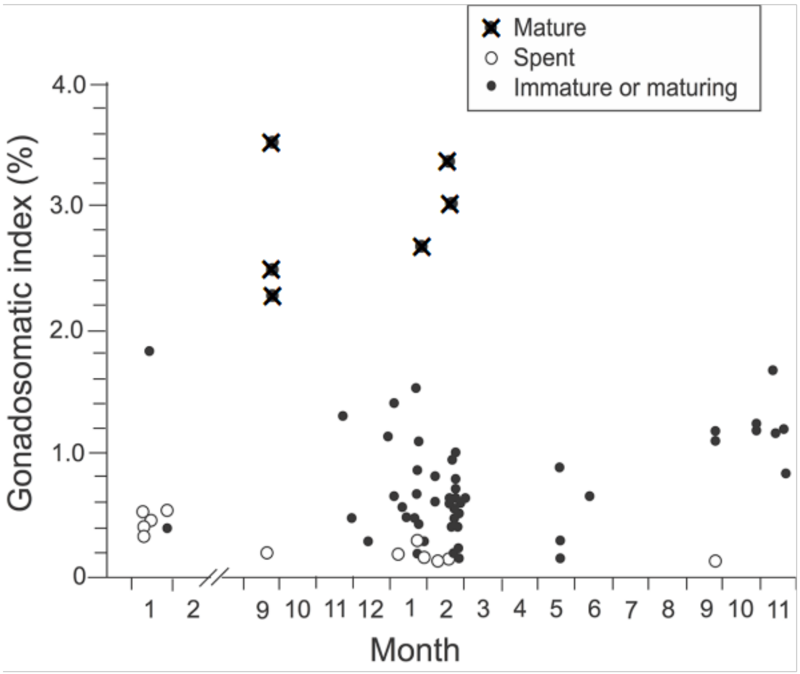

Figure 7. Distribution of gonadosomatic indices (GSI) of female *Oxyeleotris marmorata* by sampling month.

It is reported that the sexes of freshwater *O. marmorata* are distinguishable by the morphology of the urogenital papilla; the males have a small, flat triangular structures while the females have a large finger-shaped protrusion which become reddish especially at the tip during the spawning season (Tavarutmaneegul & Lin 1988; Cheah et al., 1994; Idris et al., 2012). However, this method of sex identification was not able to apply to the marine populations of *O. marmorata*. We found 20 out of 73 males with red urogenital papilla (Figure 8). Thus, the biological traits relevant to reproduction of marine populations of *O. marmorata* are different from those of freshwater populations.

The inhabitance of marine *O. marmorata* is not restricted to Likas Bay, but appears to be in the wider region in Borneo. Hatchery-produced *O. marmorata* juveniles tolerate a wide range of water salinity up to 30 ppt in the laboratory, although growth performance and survival are low compared to rearing in 0 and 10 ppt (Darwis et al., 2008). Recreational fishers occasionally catch *O. marmorata* in the coastal waters of Sarawak, Borneo (G. Kawamura, personal observation). Such capture information and population study are important for the better understanding of *O. marmorata* ecology.

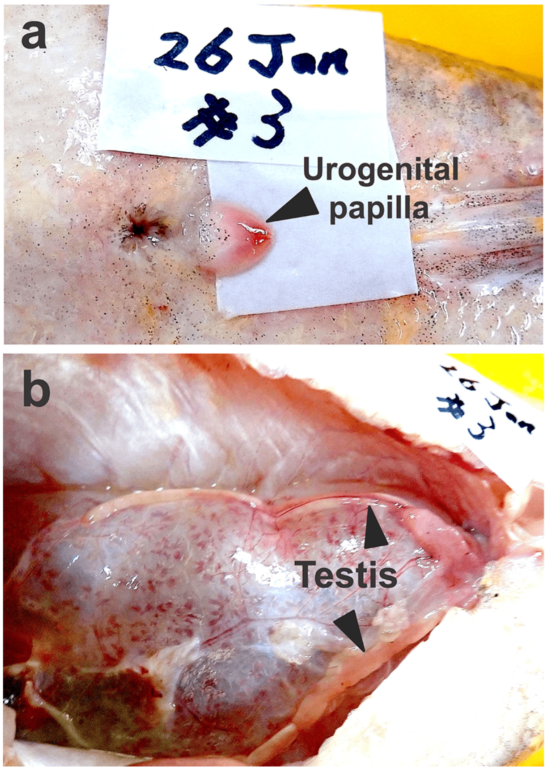

Figure 8. Photographs showing the urogenital papilla (a) and the testis (b) from the same male *Oxyeleotris marmorata.*

Conclusion

This study provided the first biological data on the *O. marmorata* collected from the marine ecosystem of Likas Bay, Sabah, Malaysia. The population structure of this marine stock determined by otolith shape analysis by the Bhattacharya's method indicated two different populations. The specimens with mature or spent ovaries were found in samples collected in January–February and September, showing two spawning seasons. The age of each specimen was estimated by counting the vertebral annuli. The minimum size and age of the spawner was 338 mm TL, 499 g BW and 5 years old. The findings obtained in this study provide basic biological parameters for the conservation of marine *O. marmorata* populations in Likas Bay.

Author Declaration on Animal Research Studies

The present study adhered to the Researcher Guideline outlined in the Code of Practice for the Collection, Care, and Use of Animals for Scientific Purposes established by Universiti Malaysia Sabah.

Acknowledgement

This study was supported by a Universiti Malaysia Sabah research grant (grant code: SBK0409-2018).

References

Al-Saleh, F., Hammoud, V., Hussein, A., & Alhazzaa, R. (2012). On the growth and reproductive biology of asp, *Aspius vorax*, population from the middle reaches of Euphrates River. *Turkish Journal of Fisheries and Aquatic Sciences, 12*, 149–156. https://doi.org/10.4194/1303-2712-v12_1_17

Azad, S. A., & Jinau, V. J. (2020). Spatial distribution of dissolved inorganic nutrients and phytoplankton around Kota Kinabalu Wetland, Sabah, Malaysia. *Advances in Biological Chemistry, 10*(2), 113–126. https://doi.org/10.4236/abc.2020.104009

Bhattacharya, C. G. (1967). A simple method of resolution of a distribution into Gaussian components. *Biometrics, 23*(1), 115–135. https://doi.org/10.2307/2528285

Cheah, S. H., Senoo, S., Lam, S. Y., & Ang, K. J. (1994). Aquaculture of a high-value freshwater fish in Malaysia: The marble or sand goby (*Oxyeleotris marmoratus*, Bleeker). NAGA, The *ICLARM Quarterly, 17*(3), 22–25.

Darwis, M., Shaleh, S. R. M., & Senoo, S. (2008). Effect of different salinity levels on growth and survival of marble goby, *Oxyeleotris marmoratus* juvenile. *Aquaculture Science, 56*(3), 433–439. https://doi.org/10.11233/aquaculturesci.56.433

Ha, H. C., Senoo, S., Tsunemoto, K., Nakagawa, Y., Miyashita, S., Murata, O., & Kato, K. (2011). Population structure of marble goby *Oxyeleotris marmorata* (Bleeker) in Southeast Asia inferred from mitochondrial DNA. *Aquaculture Science, 59*(3), 383–391.

Herawati, T., Putra, M. A., Rostini, I., Nurhayati, A., Yustiati, A., & Subhan, U. (2017). Marble goby (*Oxyeleotris marmorata* Bleeker, 1852) habitat mapping on Cirata Reservoir in West Java Province, Indonesia. *Proceedings of the Pakistan Academy of Sciences: B. Life and Environmental Sciences, 54*(4), 341–352.

Idris, H. B., Ambak, M. A., & Ikhwanuddin, M. (2012). Sex determination in *Oxyeleotris marmorata* (Bleeker, 1852) based on morphometric features. *Advances in Natural and Applied Sciences, 6*(6), 763–771.

Jiwyam, W. (2008). *Oxyeleotris marmorata*: Predator or by-product in integrated aquaculture ponds. *Pakistan Journal of Biological Sciences, 11*(4), 532–538. https://doi.org/10.3923/pjbs.2008.532.538

Keating, J. P., Brophy, D., Officer, R. A., & Mullins, E. (2014). Otolith shape analysis of blue whiting suggests a complex stock structure at their spawning grounds in the Northeast Atlantic. *Fisheries Research, 157*, 1–6. https://doi.org/10.1016/j.fishres.2014.03.009

Larson, H. (2019). *Oxyeleotris marmorata*. The IUCN Red List of Threatened Species 2019: e.T181009A1688022. http://dx.doi.org/10.2305/IUCN.UK.2019-2.RLTS.T181009A1688022.en

Lestari, W., Rukayah, S., & Jamilatun, T. (2019). Ikan introduksi *Oxyeleotris marmorata,* (Bleeker, 1852): populasi, kaju exploitasi dan pengendaliannya di Waduk Sempor. *Majalah Ilmiah Biologi Biosfera: A Scientific Journal, 36*(3), 132–138.

Libungan, L. A., Óskarsson, G. J., Slotte, A., Jacobsen, J. A., & Pálsson, S. (2015). Otolith shape: a population marker for Atlantic herring *Clupea harengus*. *Journal of Fish Biology, 86*(4), 1377–1395. https://doi.org/10.1111/jfb.12647

Reiss, H., Hoarau, G., Dickey-Collas, M., & Wolff, W. J. (2009). Genetic population structure of marine fish: mismatch between biological and fisheries management units. *Fish and Fisheries, 10*(4), 361–395. https://doi.org/10.1111/j.1467-2979.2008.00324.x

Smith, B. B., & Walker, K. F. (2004). Spawning dynamics of common carp in the River Murray, South Australia, shown by macroscopic and histological staging of gonads. *Journal of Fish Biology, 64*(2), 336–354. https://doi.org/10.1111/j.0022-1112.2004.00293.x

Tavarutmaneegul, P., & Lin, C. K. (1988). Breeding and rearing of sand goby (*Oxyeleotris marmorata*, Blk.) fry. *Aquaculture, 69*(3&4), 299–305. https://doi.org/10.1016/0044-8486(88)90337-7

Teoh, C-. F., Lim, L-. S., Kawamura, G., Yong, A. S-. K., & Shapawi, R. (2019). Growth performance and feed utilization of juvenile marble goby (*Oxyeleotris marmorata*) fed acidified diets. *Borneo Journal of Marine Science and Aquaculture, 3*(2), 41–47. https://doi.org/10.51200/bjomsa.v3i2.1703

Vasconcelos, J., Vieira, A. R., Sequeira, V., Gonzáles, J. A., Kaufmann, M., & Gordo, L. S. (2018). Identifying populations of the blue jack mackerel (*Trachurus picturatus*) in the Northeast Atlantic by using geometric morphometrics and otolith shape analysis. *Fishery Bulletin, 116*(1), 81–92. https://doi.org/10.7755/FB.116.1.9

West, G. (1990). Methods of assessing ovarian development in fishes: a review. *Australian Journal of Marine and Freshwater Research, 41*(2), 199–222. https://doi.org/10.1071/MF9900199