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SHORT COMMUNICATION

Remarkably high ingestion ratio of acidic food in juvenile marble goby, *Oxyeleotris marmorata*

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Abstract Behavioral test was conducted to determine the level of food pH which was preferable by the juvenile marble goby, *Oxyeleotris marmorata* (50 individuals or replicates; total length 6.6–7.0 cm) using agar gel pellets. Eight pH levels of agar gel pellets were prepared (pH 2.4, 3.0, 3.2, 3.4, 4.1, 4.9, 5.1, and the pure agar gel pellet without pH modification—pH 5.9). The ingestion ratio for each pH treatment of agar gel pellet was calculated, and the binary data (ingested or rejected) was analyzed using binomial test. Negative relationship was found between the ingestion ratio and pH level of the agar gel pellets. The highest ingestion ratio was found in the agar gel pellet with pH 2.4 (ingestion ratio, 94%), following by pH 3 (85%), pH 3.2 (3% agar gel powder; 65%), pH 3.2 (2% agar gel powder; 58%), pH 3.4 (24%), pH 4.1 (6%), pH 4.9 (6%), pH 5.1 (6%), and pH 5.9 (2%). The ingestion ratio of pH 2.4 agar gel pellet was significantly higher ($P < 0.05$) than that in the other treatments except that of pH 3.0. These results confirmed the taste preference of *O. marmorata* for acidic foods, and the most preferred was the one with pH 2.4–3.0.

Keywords Taste preference · Feeding · Dietary pH · Behavior · Weaning · *Oxyeleotris marmoratus*

Abbreviations

FT pellet	For-training pellet
PAG pellet	Pure agar gel pellet
TS pellet	Test substance pellet
HCl	Hydrochloric acid
NaCl	Sodium chloride
CaCl	Calcium chloride
CI	Confidence interval

Introduction

Marble goby, *Oxyeleotris marmorata* is a freshwater fish species with high commercial value, especially in Southeast Asia (Asia-Pacific Fishery Commission 2014). It is also a popular candidate for aquaculture in many Southeast Asian countries including Malaysia, Thailand, and Vietnam (Cheah et al. 1994; Lin and

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Kaewpaitoon 2000; Luong et al. 2005). However, it was reported that this fish generally rejects formulated feeds (Rojtinnakorn et al. 2012) and this results in its poor growth and survival. In addition to that, information on the nutritional requirement of *O. marmorata* is still limited up-to-date (Cheah et al. 1994; Lin and Kaewpaitoon 2000; Yong et al. 2015), and there is still no compounded feed commercially available for this fish. For these reasons, there is a need to find way to improve the acceptability of formulated feeds to *O. marmorata*. In the previous studies, amino acids mixture and nucleotides were reported as the suitable feeding stimulant through behavioural assays (Lim et al. 2015, 2016, 2017) and dietary supplementation of these taste substances can be practiced to improve the weaning of *O. marmorata* (Lai et al. in press). However, amino acid mixture and nucleotides are expensive and supplementation of these taste substances in the practical diet for *O. marmorata* may not be cost-effective.

According to Kasumyan and Døving (2003), fish has sensation to sour taste and its preference for food acidity is species-specific. This fact provides us the rationale to determine the possibility of improving the acceptability of formulated feeds to *O. marmorata* by manipulating the feed's pH to the fish preferable level, which is also a simple and cheaper way (by means of adding in acid) than the dietary supplementation of feeding stimulant mentioned earlier. However, information on the taste preference of *O. marmorata* for feeds with different pH levels and the level of pH in the feed most preferred by *O. marmorata* is unknown. Therefore, the present study was conducted to determine the pH level of food which is preferred by the *O. marmorata* juveniles.

Methods

Preparation of agar gel pellets

In this study, behavioral test was conducted and agar gel pellet was used as the food medium in the test. The types and composition of agar gel pellet prepared for the test are shown in Table 1. Two types of agar gel pellets were prepared: (1) the For-Training (FT) pellet that contained the extract of the commercial pellet, size 3.1 mm (Otohime, EP3 type, Nisshin Co., Tokyo, Japan), and (2) the Test Substance (TS) pellets which contained distilled water and different amounts of diluted or non-diluted hydrochloric acid—HCl (37%, 16 M, MERCK, NJ, USA). The pure agar gel (PAG) pellet that contained only distilled water (without HCl) was used as the negative control in the present study. In general, all agar gel pellets were prepared by dissolving

Table 1 Types of agar gel pellet prepared for the behavioral test in the present study with its ingredients and compositions

	Distilled water	Otohime extract	Agar gel powder[a]	Red food dye[b]	HCl[c] (molarity)
Treatments					
For-training (FT) pellet—pH 5.6	N/A	50 ml	1.0 g	0.05 g	N/A
Test-substance (TS) pellets					
(1) Pure agar gel (PAG) pellet—pH 5.9	50 ml	N/A	1.0 g	0.05 g	N/A
(2) 2% agar powder—pH 5.1	50 ml	N/A	1.0 g	0.05 g	10 µl (0.1 M)
(3) 2% agar powder—pH 4.9	50 ml	N/A	1.0 g	0.05 g	10 µl (1 M)
(4) 2% agar powder—pH 4.1	50 ml	N/A	1.0 g	0.05 g	10 µl (3 M)
(5) 2% agar powder—pH 3.4	50 ml	N/A	1.0 g	0.05 g	10 µl (16 M)
(6) 2% agar powder—pH 3.2	50 ml	N/A	1.0 g	0.05 g	18 µl (12 M)
(7) 3% agar powder—pH 3.2	50 ml	N/A	1.5 g	0.05 g	15 µl (12 M)
(8) 3% agar powder—pH 3.0	50 ml	N/A	1.5 g	0.05 g	34 µl (12 M)
(9) 3% agar powder—pH 2.4	50 ml	N/A	1.5 g	0.05 g	100 µl (12 M)

[a]Mermaid, Bangkok, Thailand

[b]Ponceau 4R, Mibo, Sabah, Malaysia

[c]Hydrochloric acid (37%, 16 M, MERCK, NJ, USA)

the agar gel powder (Mermaid, Bangkok, Thailand) (2% of the water volume) and red food dye (Ponceau 4R, Mibo, Sabah, Malaysia) (0.1%) into 50 ml distilled water. The mixture was heated on a hot plate until it boiled. Subsequently, the mixture was poured into a glass petri dish for cooling and hardening. The hardened mixture were then cut into uniform pellet size (approximately 2 mm × 2 mm × 2 mm) and stored in a refrigerator (4 °C) until further use (Lim et al. 2015, 2016, 2017).

For preparing the FT pellet, the distilled water was replaced by the commercial pellet (Otohime EP3) extract. The extract was obtained by soaking 30 g of the pellet (Otohime EP3) in 100 ml distilled water for approximately 15 min with occasional hand-stirring. When the water color turned dark brown, the homogenate was then filtered through a 60 µm mesh net to obtain the aqueous extract. The extract was prepared only prior to the preparation of the FT pellet to maintain its freshness.

To prepare the TS pellets with different pH levels, various amounts of HCl which were preliminary determined (see Table 1) were added into the mixture (distilled water + agar gel powder + red food dye) after the mixture was boiled. In total, eight types of TS pellet with pH levels of 2.4, 3.0, 3.2, 3.4, 4.1, 4.9, 5.1, and 5.9 (the PAG) were prepared. The pH levels of the TS pellets were determined following the method by Sathe (1999). In brief, a few pieces of agar gel pellet from each treatment were dropped in 100 ml distilled water, crushed with spatula, and the mixture was stirred frequently for about 3 min then the measurement was done using a pH meter (Oakton EcoTestr® pH 2, Eutech Instruments, Singapore). As the TS pellets with the extremely low pH levels (pH 2.4 and 3.0) could not harden naturally like the others, 3% of agar gel powder (instead of 2%) was used to prepare these TS pellets. To confirm that such act will not cause any bias to the results, the TS pellet with pH 3.2 was prepared with 2 and 3% agar gel powder and fed to the fish for comparison.

Experimental fish

Fifty Otohime pellet-trained *O. marmorata* juveniles (total length = 6.0–7.7 cm) was obtained and transferred from the fish hatchery to the wet laboratory of Borneo Marine Research Institute (BMRI), Universiti Malaysia Sabah (UMS). Each individual of fish was stocked in 7-L acrylic-made aquaria supplied with 4-L water and aeration, and this was done in replicate. Before the behavioral test started, all the fish were conditioned to accept agar gel pellet using the FT pellet. The fish conditioning protocol was the same as described by Lim et al. (2016, 2017). Each individual of fish was conditioned once daily at 04:00 p.m. with three pieces of FT pellet. If the fish ingested the FT pellets, they were rewarded with the commercial pellet (Otohime EP3) at the end of the daily conditioning session. The fish were starved on that day if none of the FT pellet given were ingested, and the conditioning continued on the next day. The fish were considered well-conditioned and ready for the behavioral test when it accepted all three FT pellets continuously for 3 days. In the present study, all fish were successfully conditioned to accept the FT pellets within 16 days and no mortality occurred. All experimental fish were cared and handled following the Researcher's Guideline on Code of Practice for the Care and Use of Animals for Scientific Purposes, implemented by the Universiti Malaysia Sabah (UMS).

Behavioural assays

The procedures of the behavioral test used in the present study were adopted from Lim et al. (2016, 2017). The test was started at 04:00 p.m. daily. Each fish was first fed with a FT pellet to stimulate its desire to feed, followed by a TS pellet, and then another FT pellet. The final piece of FT pellet was served to remind the fish that agar gel pellet was palatable, in case the TS pellet given was a deterrent to them. During the test, feeding responses (ingestion or rejection of the TS pellet given) were examined through naked eye and manually recorded. Each treatment of TS pellet was given only once to each fish, and only one treatment of TS pellet was tested daily. At the end of the daily test, each fish was fed with three commercial pellets (Otohime EP3) as the reward to compensate its nutritional requirement. Feces and the uneaten feed in all aquariums were syphoned out and about 20% of water was exchanged on the next day morning. The cleaning and water exchanging work was done daily at least 4 h before the behavioral test started to prevent the fish from stress. The behavioral test was conducted under indoor condition. During the test, the water temperature was at about 28 °C. The water pH before and after the test was at about 8.0–8.1.

Data collection and analysis

The total number of the ingested TS pellet from each pH level treatment was counted. Then, the ingestion ratio [total number of the ingested pellet/(total number of the given pellet, which was 50) × 100%] was calculated and expressed as the representative data. As binary data (ingested or rejected) was collected in the present study, the one-tailed binomial test was used to determine the significance of difference among the treatments. The 95% confidence interval (CI) of the total ingestion of each pH level of TS pellet was calculated and the ranges in the values were compared to each other. Significant difference ($P < 0.05$) was assumed if the range of the CI values between two treatments was not overlapped and vice versa (Sauro and Lewis 2012).

Results and discussion

In the present study, the ingestion ratio of TS pellets by the *O. marmorata* juveniles was clearly affected by the TS pellet's pH level. The ingestion ratio increased when the pH level decreased, suggesting a negative relationship between the ingestion ratio and the pH level of TS pellet. Figure 1 shows the ingestion ratio of the TS pellets by the juvenile *O. marmorata*. The highest ingestion ratio was found in the TS pellet with pH 2.4 (ingestion ratio 94%), following by pH 3 (85%), pH 3.2 (3% agar gel powder; 65%), pH 3.2 (2% agar gel powder; 58%), pH 3.4 (24%), pH 4.1 (6%), pH 4.9 (6%), pH 5.1 (6%), and pH 5.9 (2%). These results evidenced that the *O. marmorata* indeed has taste preference for food pH and they preferred the acidic food. In addition, no significant difference ($P > 0.05$) was observed between the ingestion ratio of TS pellets at pH 3.2 with 2 and 3% of agar gel powder. This result confirmed that the high ingestion ratio of the TS pellets with pH 2.4 and 3.0 was not due to the pellet's texture. In fact, many fish species were also reported to prefer the acidic food, including *Tilapia zilli* (Adams et al. 1988), European grayling *Thymallus thymallus* (Kasumyan 1997), tench *Tinca tinca* (Kasumyan and Prokopova 2001) and most of the salmonids and poecilids (Kasumyan and Døving 2003). On the other hand, acidified foods have also been reported to evoke negative response in many fish species including the puffer *Fugu pardalis* (Hidaka et al. 1978), acipenserids and many cyprinids (see Kasumyan and Døving 2003). Erteken and Nezaki (2002) also reported that the favorable food pH for the Black Sea turbot *Psetta maxima* was in the range of 7.2–7.4. Therefore, results of the present study were in agreement with the findings of Kasumyan and Døving (2003) that fish can sense sour taste and their taste preference for pH of the food is species-specific.

According to Kasumyan and Døving (2003), the classical taste substances that contained Cl^- ion such as sodium chloride (NaCl) and calcium chloride (CaCl) could act as the feeding stimulant for some fish species. In the present study, HCl was used to adjust the pH level of the TS pellets. When the amount of HCl added to

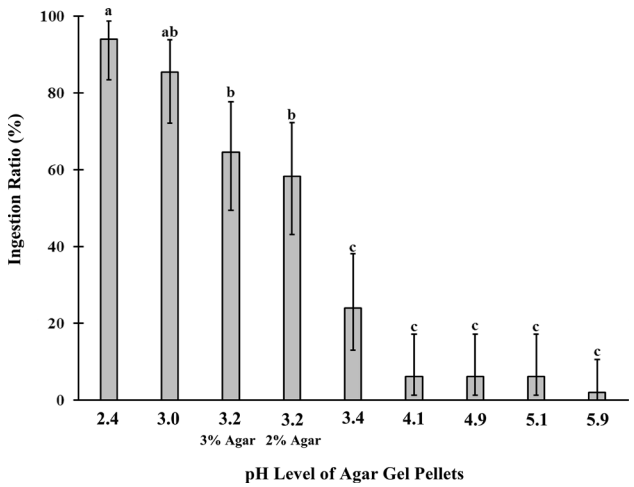

Fig. 1 Ingestion ratio of the agar gel pellets with different pH levels by the *O. marmorata* juveniles. Vertical bars show the 95% confidence interval of the data

the TS pellets increased (pH level decreased), the amount of Cl^- ion was also increased. Fish intraoral taste system can be sensitive to the Cl^- ion as examined electro-physiologically (Konishi and Niwa 1964). Therefore, the high ingestion ratio of the highly acidic TS pellets in the present study also could be contributed by the Cl^- ion. However, such hypothesis could not have the vigor of the experimental trials because NaCl and CaCl were also reported as the taste-indifferent substance for many fish species (Kasumyan and Døving 2003), including *O. marmorata* (Lim et al. 2017).

Conclusions

In conclusion, the preference of *O. marmorata* juveniles for acidic food was confirmed, and the food's pH preferred by the fish was in the range of 2.4–3.0. Evaluation of the weaning performance of *O. marmorata* juveniles with the HCl-acidified feed is recommended in a future study.

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Author contributions C-FT provided ideas to refine the experimental methods, conducted the experiments, collected and analyzed the data, and wrote the manuscript. L-SL contributed the original idea of this study, designed the experimental methods, supervised the experimental data collection and analysis, and revised the manuscript. GK involved in designing the statistical analysis and proof-read the manuscript.

Compliance with ethical standards

Conflict of interest No competing interests.

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