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Fluctuating Asymmetry of Paired Fins of *Channa striata* Exposed to Different Cadmium Exposure

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Abstract

Fluctuating asymmetry (FA) is a useful ecological indicator of trace metal exposure in plants and animals. This study was conducted to observe the effects of different levels and duration of cadmium exposure on developmental instability of freshwater fish, *Channa striata,* indicated by assessing the FA of its pectoral and pelvic fins. In this study, four treatment groups of *C. striata* were exposed to different concentrations of cadmium at 0.000 mg/L (control), 0.005 mg/L, 0.010 mg/L and 0.015 mg/L, respectively for 16 weeks. The FA of paired fin lengths were measured after the first four weeks and subsequent biweekly measurement for 12 weeks. There was an increasing trend observed in average FA associated with the increased of cadmium level and duration of exposure in *C. striata*, although they were not statistically significant. While FA has been shown to be a useful tool to indicate developmental instability in fish, this study found that it is not a significantly effective model to be applied to *C. striata* in order to study cadmium pollution. The results suggested that higher level and duration of cadmium exposure may influence higher FA of paired fin lengths of *C. striata*.

Keywords: *Channa striata*; Fluctuating asymmetry; Paired fins, Cadmium

1. Introduction

Cadmium is a highly toxic trace metal and tends to bioaccumulate in living organisms which its concentration increases in higher trophic levels (Zhang *et al.,* 2016). Cadmium is water soluble which makes it readily absorbed by the fish from the water environment and through ingested food by eating other aquatic organisms (Govind and Madhuri, 2014). However, cadmium is poorly metabolized by organisms, where it can lead to oxidative stress, liver and kidney injuries, developmental and morphological instability in fish according to Perera *et al.* 2015 and eventually lower their survival and growth rate (Hazrat *et al.,* 2019).

The most common method to analyse the impact of developmental instability on a particular morphological feature is FA (Benitez *et al.,* 2018). Fluctuating asymmetry measures the small random deviations occurring between the right and left sides of bilaterally symmetrical traits of an organism (Omar *et al.,* 2015). Since the symmetrical traits in fish is more sensitive towards induced environmental stressor as compared to other animals, thus, fish serve as useful bio-indicator to detect negatively impact environmental quality of aquatic ecosystems (Lutterschmidt *et al.,* 2016). According to Trokovic *et al.* (2012), higher FA levels are expected in marine fish under environmental stresses when compared to control populations.

Since cadmium is not degradable in nature, it is possible for humans to be exposed to such toxic pollutant through food ingestion by eating aquatic organisms.

This can affect and endanger human health. Thus, the importance of this study is to protect human from health hazards caused by consuming foods containing cadmium (Renieri *et al.,* 2019). While numerous studies of FA in fishes as a bioindicator for various environmental stressors has been published there was no previous study has been conducted specifically measuring FA of *C. striata* upon exposure to cadmium contamination. Therefore, it is a novel finding. The snakehead fish (*C. striata*) is an economically important fish in Malaysia and Southeast Asia. According to Muntaziana *et al.* (2013), snakehead is considered as a valuable fish because of its good taste, nutrition and pharmaceutical values. The fish is also a carnivorous species (Talde *et al.,* 2004), hence making it a predatory species that is important in population control of prey species to maintain ecological balance in the freshwater aquatic ecosystem.

In this study, *C. striata* or Snakehead fish has been chosen as a sentinel species for cadmium contamination based on a few criteria which are 1) not threatened by extinction, 2) ubiquitous, 3) suitable size, 4) has a large distribution area and 5) has a stable population ((IUCN, 2019). The aims of this study are to measure and to compare the FA of pectoral and pelvic fin length of *C. striata* exposed to various level and duration of cadmium exposure. It should be noted that the LC_{50} of cadmium chloride for 96 h exposure to *C. striata* was found to be at 10 ppm (Radhakrishnan *et al.,* 2000).

2.Materials and Methods

2.1 Sample collection

The 500 Snakehead fish fingerlings with an average length of 6.5 ± 0.41 cm and average body weight of 2.21 ± 0.027 g were acclimatised to laboratory conditions for three days in dechlorinated tap water prior to any treatment.

2.2 Treatment groups

The fingerlings were then divided into four treatment groups of different cadmium concentration; 0 mg/L (control), 0.005 mg/L (low exposure), 0.010 mg/L (medium exposure) and 0.015 mg/L (high exposure) for 16 weeks. Fingerlings were stocked at 5 individuals/L (Kok, 1981) into 5 separate 5 L aquarium for each treatment group. The tops of the aquariums were closed with nets to prevent the fingerlings from moving outside of the experimental aquariums.

2.3 Rearing conditions

The fingerlings were fed chicken liver *ad libitum* twice a day with the total for each feed did not exceed the recommended feeding rate of 3% from the body weight (Yakupitiyage, 2013). Excess leftover feed was removed after feeding and water-change were carried out every week to ensure the water is suitable for fish to survive and grow without affecting the intended cadmium exposure conditions. Dechlorinated treated water was used for the purpose of this experiment in all treatment groups. The average laboratory ambient temperature recorded was 26.1 ± 0.3 °C and natural light was utilized during the entire experimental process conducted. The rearing process of fingerlings utilized natural ventilation without aeration installation. Only surviving fingerlings are sampled and the number of dead fingerlings before sampling were not taken into consideration

2.4 FA measurement

The lengths of the paired fins which are pectoral and pelvic fins were measured using digital vernier caliper in cm and their FA were calculated for the first four months and subsequent biweekly measurement for 12 weeks. All FA measurements were made exclusively by one person to avoid possible inter-observer variability (Kozlov *et al.,* 2017). FA is measured as the percentage of measurement deviation of a paired structure from the average values (Wilkins *et al.,* 1995). Data were analysed with SPSS version 25.0 program using two-way analysis of variance (ANOVA).

3. Results and Discussion

Figure 1 shows the comparison of FA for paired fin lengths of *C. striata* exposed to various levels of cadmium concentrations over 16 weeks period. The results show that the level of FA for paired fin lengths does not significantly ($p > 0.001$) increase with the increase in concentration and exposure duration to cadmium. However, there were a noticeable increasing trend of FA observed in both pectoral and pelvic fin lengths. Hence, this indicates that developmental instability could occur when the fish population is exposed to increased cadmium concentration, and also to increased exposure duration. It should be noted that the increase in FA in the exposure groups also did not differ significantly ($p > 0.001$) from the control group. This is also applicable when comparison among the three cadmium-exposed group (low, medium and high exposure) were made ($p > 0.001$). Snakehead fingerlings exposed to 0.015 mg/L Cd after 16 weeks showed the highest FA value while those in non-exposed group (control) after 4 weeks (shortest duration of exposure) showed the lowest FA value for both pectoral and pelvic fin lengths.

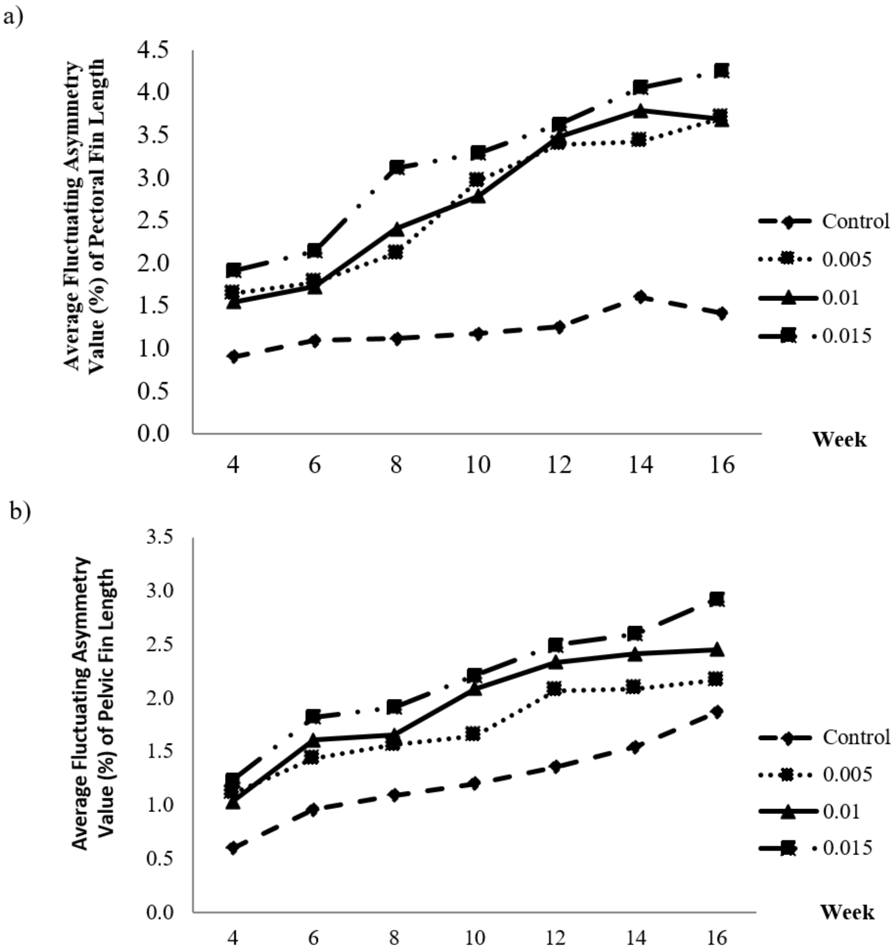

Figure 1. Fluctuating asymmetries of a) Pectoral fin length b) Pelvic fin length of *C. striata* exposed to various level and duration of cadmium exposure

The increased FA of the paired structures was not obvious when comparisons were made between the level and duration of cadmium exposure because *C. striata* has a slow growth rate. Based on several growth studies conducted, *C. striata* on average weighed only around 60 g by 12 weeks past the fingerling stage (Mehrajuddin *et al.,* 2011; Talpur *et al.,* 2014). According to Kok (1981), this species requires a long raising period of seven to ten months before harvesting depending on the optimum conditions achieved during the breeding season. Since this study was conducted only for 16 weeks, the manifestation of the effects of exposure to cadmium at different concentrations and time examined were subtle due to its slow growth rate. However, the increasing trend of fluctuating asymmetries of the paired structures observed as the levels and duration of exposure to surrounding ecological stress increases signify the animal to grow less symmetrically (De Coster *et al.,* 2013). Perhaps, a longer exposure duration may yield a different result and conclusions.

According to Authman *et al.* (2015), the growth rate of fishes is reduced as a result of exposure to cadmium. This is evident when studies conducted by Heydarnejad *et al.* (2013) and Zinia *et al.* (2018) reported significant decrease in growth rate of Tilapia fish (*Oreochromis niloticus*) which were exposed to cadmium as compared to the control group. As the growth rate of fish reduced or started to slow down, the animal's ability to cope with environmental stress increases over time (Michaelsen *et al.,* 2015). Thus, it explained why the FA of paired fin lengths observed in this study were not significant. In addition, the slow growth rate of Snakehead fish (Mehrajuddin *et al.,* 2011; Talpur *et al.,* 2014) further reduced the manifestation of the effects on developmental instability of *C. striata* upon environmental stressors including trace metal contamination (De Coster *et al.,* 2013). Therefore, this may suggest that exposure to cadmium may further reduce the growth rate of *C. striata* and thereby explain the subtle increment of FA value when comparison between the level and duration of cadmium exposure were made.

In contrast, some researchers suggested that developmental stability will serve to reduce FA in traits that are under strong stabilizing selection because of their functionality is highly significance to the organism (Graham *et al.,* 2010; Zakharov *et al.,* 2020). For instance, a study conducted by Michaelsen *et al.* (2015) recorded a significantly higher FA in eye diameter but not in length of paired fins of Menidia beryllina after an oil spill at Gulf of Mexico. It was suggested that asymmetries in the paired fins would have a greater effect on the overall organism performance than other paired organs such as the eyes. Since pectoral and pelvic fins play an important role for locomotion of *C. striata*, small asymmetries would have significance functional cost to the organism and thereby, the traits are highly stable towards environmental stressors such as cadmium contamination. While the increased FA value for paired fins length observed in this study was not statistically significant, the trend is still observable.

While numerous studies suggested that higher developmental instability may reflect the presence of ecological stress within their surrounding environment and thereby, mirror the level of stress to which they are imposed (Ducos and Tabugo, 2014). Willmore *et al.* (2007) reiterated that deviations from the regular conditions (genetic or environmental stress) encountered during the development would be buffered by mechanisms that ensure the developmental process of an organism will produce the predetermined phenotype under given genetic and environmental condition. In this case, if the developmental mechanism giving rise to a symmetric phenotype is disturbed (developmental instability), then it probably led to asymmetric morphological characters consisting asymmetric and irregular growth increments. Thus, animal with higher developmental instability tend to grow less symmetrically due to increasing exposure to surrounding ecological stress (De Coster *et al.,* 2013). This is consistent with our findings that noted an increasing trend of FA for paired fin lengths of *C. striata* across increasing level and duration of cadmium exposure.

The lowest FA has been detected in control population for both fins. The presence of these may be attributed to minor inconsistencies during development such as quality and quantity of food, temperatures variations, possible parasites infestation, diseases and behavioural stress imposed by interactions with conspecifics which can occur in normal developmental processes (Jawad *et al.,* 2020). Theoretically, stable development shall result in identical left and right sides (perfect symmetry) of paired traits in an organism in the absence of environmental stressor (Swaddle, 2003). However, organisms rarely develop with perfect symmetry as developmental disturbances caused by various random perturbations results in low levels of asymmetry (Hingabay *et al.,* 2016). Thus, low bilateral asymmetry value recorded for paired fin lengths of *C. striata* in the control group may have been resulted from such variations.

4. Conclusion

In summary, the present study found that FA is not a significantly effective tool to indicate the effect of cadmium on developmental instability of *C. striata* However, the trend of the results in this study may suggest that higher concentration and duration of cadmium exposure may induce higher FA for paired fin lengths of *C. striata*, hence affecting their developmental instabilities. If *C. striata* is to be used as a sentinel species for cadmium exposure in future studies, it is recommended that study duration be extended up to 12 - 24 months. In addition, instead of monthly measurements, FA should be measured bimonthly or quarterly. Longer duration would allow the fish grow bigger and show significant changes of the FA on the paired external structures of *C. striata*. It is also noted that FA could be an effective tool to indicate developmental instability in freshwater fish should these conditions are rectified.

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