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Effects of waterborne nitrite on hematological parameters and stress indicators in olive flounders, *Paralichthys olivaceus*, raised in bio-floc and seawater

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HIGHLIGHTS

- Hematological paramaters of *P. olivaceus* were significantly changed by nitrite.
- Stress indicators of *P. olivaceus* raised in bio-floc and seawater were significantly increased by nitrite.
- Toxic effects on *P. olivaceus* exposed to nitrite were more prominent in the fish raised in seawater.

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ABSTRACT

Juvenile olive flounders, *Paralichthys olivaceus* (mean weight 2.69 ± 0.31 g), were raised in bio-floc and seawater for six months, these *P. olivaceus* (mean weight 280.1 ± 10.5 g, mean length 28.37 ± 2.3 cm) were exposed to different concentrations of waterborne nitrite (0, 25, 50, 100, and 200 mg NO_2^-/L) for 7 days. None of the *P. olivaceus* individuals exposed to bio-floc and seawater containing waterborne nitrite concentrations of 200 mg/L for 7 days survived. Hematological parameters (hemoglobin and hematocrit) were significantly reduced by nitrite exposure. Regarding plasma components, the concentrations of glucose, glutamic oxalate transaminase (GOT), and glutamic pyruvate transaminase (GPT) increased significantly in response to nitrite exposure, whereas cholesterol concentrations significantly decreased. Stress indicators, including concentrations of plasma glucose, cortisol, and liver and gill concentrations of heat shock protein 70 (HSP70) were significantly increased by nitrite exposure. The results of the study indicate that nitrite exposure affected the hematological parameters and stress indicators of *P. olivaceus* raised in bio-floc and seawater, and these changes were more prominent in the *P. olivaceus* raised in seawater than those raised in bio-floc.

1. Introduction

The Korean aquaculture industry has developed rapidly since the 1970s, but has recently experienced difficulties as aquaculture farms effluents exceed the absorptive capacity of the environments. This has resulted in decreased immunity and an increase in the frequency of disease emergence in aquaculture species (Bae et al., 2017). There is an increasing need for a sustainable, eco-friendly aquaculture system to address such environmental problems. Of the various aquaculture systems, bio-floc technology (BFT) is one of the most innovative technologies for minimizing environmental contamination, due to its no water exchange system (Najdegerami et al., 2016). In particular, the advantage of BFT is that it can be reused without discarding the water, thereby making it sustainable and avoiding contamination of the sea. Therefore, BFT can be a good alternative for sustainable aquaculture practices.

Nitrite, a natural substance of the nitrogen cycle in aquatic environments, generally occurs at low concentrations in marine environments. However, water contamination with industrial waste can increase marine nitrite concentrations through ammonia oxidation (Avilez et al., 2004). In addition, excessive use of high

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protein feed in high-density aquaculture environments produces unnaturally high nitrite and fecal concentrations, resulting in an imbalance of nitrification and denitrification, resulting in nitrite accumulation (Chen et al., 2011; Guo et al., 2016). The accumulation both of nitrite from unutilized feed and excrement in high-density farms results in high nitrite concentrations, which are toxic to fish (Wang et al., 2018).

Nitrite exposure affects fish physiology, and causes ion-regulatory disturbances. Nitrite exposure affects Cl^-/HCO_3^- exchange in fish due to its affinity for branchial Cl^- uptake, which substitutes NO_2^- uptake for Cl^- uptake (Jensen, 2003). In addition, nitrite exposure affects the K^+ balance of fish, through stimulating K^+ loss from skeletal muscle. The increase in extracellular K^+ induces cardiac failure and nervous function loss, thus affecting the heart's metabolism and function, and other excitable tissues (Knudsen and Jensen, 1997). In addition, high concentrations of nitrite are toxic to aquatic animals, due to their toxic effects on physiological processes (including stress induction, growth inhibition, and immunosuppression). Furthermore, high concentrations of nitrite result in higher concentrations of reactive oxygen species (ROS), which increase oxidative damage and result in functional impairment (Ciji et al., 2012; Lin et al., 2018).

Hematological parameters are considered reliable indicators of the physiology and health status of fish exposed to toxic substances (Kim and Kang, 2014, 2017a). The blood is a major target site of nitrite action in fish exposed to waterborne nitrite (Ciji et al., 2012). Nitrite enters the plasma through chloride cells in fish gills (Sun et al., 2012). Therefore, nitrite oxidizes hemoglobin in red blood cells to methemoglobin (metHb) by competing with chloride ions, resulting in heme ion oxidation, hypoxia, and hemolytic anemia (Silva et al., 2018). Hematocrit is a reliable indicator of nitrite toxicity because its decrease in fish exposed to nitrite generally occurs through blood cell lyses (Yildiz et al., 2006). Nitrite exposure induces nitrite accumulation in blood plasma, which affects the plasma components (Grosell and Jensen, 2000). Thus, hematological parameters and plasma components are critical factors in assessing toxic effects of nitrite exposure in fish.

Stress responses in fish are induced by alterations in the external environment or exposure to toxic substances, and stress indicators are used to detect these toxic effects (Kim et al., 2017a). High levels of nitrite exposure cause considerable stress in fish, and its toxicity manifests in physiological change, tissue damage, and cell injury (Das et al., 2004a). Of the various stress indicators, glucose is important because glucose concentrations are generally elevated by the increase in carbohydrate metabolism of fish exposed to toxic substances (Kim and Kang, 2015). The utilization of glucose and glycogen by aquatic animals is a mechanism for detoxification, and glucose concentrations generally increase under stressful conditions (Kim and Kang, 2017a). Plasma cortisol is a major steroid hormone in fish, and cortisol secretion is generally stimulated by various stressors, such as toxicity exposure and environmental change (Kim and Kang, 2016a). Heat shock proteins (HSPs) are stress proteins induced by various stressors, and serve as reliable stress indicators in fish exposed to nitrite (Deane and Woo, 2007). Many authors suggest that HSPs are closely related with a cyto-protective function and that their regulation in cells and tissues serve as a stress response (Basu et al., 2002; Deane et al., 2006).

Fish have been considered a reliable biomarker in assessing the effects of toxicity exposure in aquatic ecosystems (Kim and Kang, 2017b). *Paralichthys olivaceus* is a widely used aquaculture species with a yellowish-brown body, large mouth and sharp teeth. They usually live on sandy bottoms that do not exceed 200 m in depth. As juveniles, they eat small prawns and fish fry, and as adults, crustaceans, mollusks and small fishes. In Korea, they account for over 50% of fish production through aquaculture. However, since 2005, high mortalities (approximately 40%) caused by contamination in dense farms have become an obstacle to a sustainable *P. olivaceus* aquaculture industry. The development of *P. olivaceus* culture techniques using BFT may address the problems currently facing the industry. Nitrite is a critical source of toxicity in the aquaculture environment. Therefore, the purpose of this study was to evaluate nitrite toxicity criteria in *P. olivaceus* raised in bio-floc and seawater through analysis of hematological parameters and stress indicators, and to compare the nitrite tolerance between the two groups via LC_{50} values.

2. Materials and methods

2.1. Experimental fish and design

Juvenile *P. olivaceus* (mean weight 2.69 ± 0.31 g) were obtained from a local fish farm in Taean, Chungnam, Korea. Fish were cultivated for 6 months in the bio-floc and seawater (running water), respectively. After 6 months, 60 fish (2 groups (bio-floc and seawater) x 6 fish × 5 tanks, mean weight 280.1 ± 10.5 g, mean length 28.37 ± 2.3 cm) were selected to conduct the waterborne nitrite exposure experiment. Juvenile *P. olivaceus* were exposed to different waterborne nitrite (0, 25, 50, 100, and 200 mg NO_2^-/L) in 500 L circular tanks. Nitrite standard stock solution was prepared at a concentration of 20,000 mg NO_2^-/L using sodium nitrite ($NaNO_2$), and diluted in each tank to adjust the concentrations. The measured concentrations of nitrite in bio-floc and seawater are demonstrated in Table 1. The nitrite concentrations were determined using nitrite test assay kit (Merck & Co., Inc., NJ, USA). At the end of the experimental exposure (7 days), the blood and tissues were collected after sufficient anesthesia using MS-222 (Sigma Chemical, St. Louis, MO, USA). The plasma separated from the blood and tissues were stored at −80 °C until analysis.

2.2. Survival rate and lethal concentration 50% (LC_{50})

Dead individuals by nitrite exposure were daily observed, and the LC_{50} values after 7 days were determined using the probit test within the SPSS/PC + statistical package (SPSS Inc., Chicago, IL, USA).

2.3. Hematological parameters

Dead individuals by nitrite exposure were daily observed, and the LC_{50} values after 7 days were determined using the probit test within the SPSS/PC + statistical package (SPSS Inc., Chicago, IL, USA). Blood samples were collected within 35–40 s through the

Table 1
Analyzed waterborne nitrite concentration (NO_2^- mg/L) from each source.

Waterborne Nitrite concentration (NO_2^- mg/L)					
Waterborne Nitrite levels	0	25	50	100	200
Measured Bio-floc Nitrite levels	0.19	28.1	54.6	109.4	213.5
Measured Seawater Nitrite levels	0.24	29.4	53.1	107.8	210.6