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Toxic responses of swimming crab (*Portunus trituberculatus*) larvae exposed to environmentally realistic concentrations of oxytetracycline

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HIGHLIGHTS

- We investigated the effects of OTC on *Portunus trituberculatus* larvae.
- Exposure to OTC suppressed the antioxidant system of *P. trituberculatus* larvae.
- OTC affected genes and enzymes related to detoxification.
- Exposure to OTC induced biomolecule damage in *P. trituberculatus* larvae.

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ABSTRACT

Oxytetracycline (OTC) is the most commonly used antibiotics for bacterial treatment in crustacean farming in China, and because of their intensive use, the potential harmful effects on aquatic organisms are of great concern. The aim of this study was to investigate the effects of oxytetracycline (OTC) on the antioxidant system, detoxification progress, and biomolecule damage in *Portunus trituberculatus* larvae. In this study, larvae that belonged to four zoeal stages were exposed to four different concentrations of OTC (0, 0.3, 3, and 30 μg/L) for 3 days. The results showed that the exposure to OTC significantly suppressed the antioxidant system of, especially, zoea I (Z1) and zoea II (Z2) larvae. OTC inhibited the transcriptional expression of phase I (*CYP2* and *CYP3*) and phase II detoxification genes (*GST*) in a dose-dependent manner and altered the expressions of their corresponding enzymes, namely, aminopyrine *N*-demethylase, erythromycin *N*-demethylase, and glutathione *S*-transferase. Moreover, 0.3 μg/L OTC activated the transcription of ATP-binding cassette (ABC) transporter subfamily B (ABCB) and subfamily G (ABCG) in the Z1 and Z2 larvae, while 3 and 30 μg/L OTC suppressed all of them. Additionally, malondialdehyde content exhibited a dose- and zoea-effect relationship to some extent, but no significant differences were observed in the *F* values of the Z3 and Z4 larvae, except for the 30 μg/L OTC treatment. Thus, the Z3 and Z4 larvae were less sensitive to OTC exposure than the Z1 and Z2 larvae.

1. Introduction

Recently, diseases caused by *Vibrio* spp. have become a major issue, resulting in economic losses for crab culture (Wang et al., 2006); therefore, solutions for the survival of crabs with pathogen infections are necessary. Oxytetracycline (OTC) is a tetracycline antibiotic with broad-spectrum activity; it is the most commonly used antibiotic in aquaculture (Boleas et al., 2005; Ferreira et al., 2007). OTC inhibits the association of aminoacyl-tRNA and bacterial ribosomes, thus preventing protein synthesis (Reemtsma and Jekel, 2006).

In China, OTC has been used extensively as a chemotherapeutic agent to treat crustaceans. However, excessive use of OTC has several adverse effects on both crustaceans and the environment (Gharred et al., 2016). Therefore, appropriate use of OTC is strongly recommended. In 2016, the US Food and Drug Administration and

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the US Environmental Protection Agency approved five antibiotics, including OTC, for the treatment of crustaceans.

Portunus trituberculatus is found along the coast of China, Japan, Korea, and other Southeast Asian countries, and it is a common edible marine crab species in China (Hao et al., 2015). The larval development of *P. trituberculatus* experienced two stages: Zoea stage and megalopa stage. Under suitable conditions, it takes 10–12 days for the Zoea metamorphosis to megalopa after four time molting. After 5–6 days, megalopa metamorphosis to the juvenile crab stage I (Fig. 1) (Sun and Yan, 1984). Although the toxic effects of OTC on aquatic organisms have been studied in detail (Botelho et al., 2015), there is limited information on the ecological toxicity and exact mechanism underlying the toxicity of OTC to crustaceans. Therefore, it is necessary to evaluate the risks of residual OTC of this species.

Drug-metabolizing enzymes (DMEs) have a major role in the metabolism, elimination, and/or detoxification of xenobiotics in organisms (Ku et al., 2014). To minimize the potentially adverse effects of xenobiotics, most tissues and organs have diverse and abundant DMEs, for example, phase I and II detoxification enzymes and phase III transporters, present either at the basal uninduced level and/or inducible at elevated levels after exposure to xenobiotics. Cytochromes P450 (CYP) belong to a superfamily of heme-containing monooxygenases, and they aid in phase I biotransformation. CYPs are frequently used as biomarkers in environmental toxicological studies because of their sensitivity and inducibility under exposure. The CYP2 and CYP3 subfamilies have important roles in xenobiotic metabolism in crustaceans. Our laboratory constructed the *P. trituberculatus* transcriptome (Accession Number: SRR1168416); *P. trituberculatus* appears to have orthologs of several key enzymes involved in phase 0 and III detoxification. This provides strong evidence for vertebrate-type enzymatic detoxification progress in *P. trituberculatus.*

OTC has been shown to induce DNA damage of rainbow trout (*Oncorhynchus mykiss*) (exposure dose, 0.5 mg/L) (Rodrigues et al., 2016) and increase the malondialdehyde (MDA) level in *Oncorhynchus mykiss* (fish fed with 2% of the fish body weight per day) (Yonar, 2012). The above mentioned studies focused on acute effects. However, because of dilution, antibiotics are found at μg/L and ng/L levels in aquatic environments; therefore, the observed effects are chronic. Thus, environmental concentrations should be used for toxicological evaluations, to ensure representative findings. To our knowledge, no studies on the responses of *P. trituberculatus* to environmental OTC concentrations have been conducted.

The aim of this study was to determine the potential effects of OTC at environmentally relevant concentrations on *P. trituberculatus* at a whole-organism level at zoea I to zoea IV stages and evaluate the responses of the antioxidant system and detoxification enzymes and genes involved in the phase I (*CYP2* and *CYP3*), phase II (*GST*), phase 0 *(ABCB*), and phase III (*ABCG*) detoxification process. The levels of biomolecule damage parameters (DNA damage and MDA content) were also measured. Our findings will provide a potentially practical approach to health management and the use of biomarkers for checking the health of crab larvae.

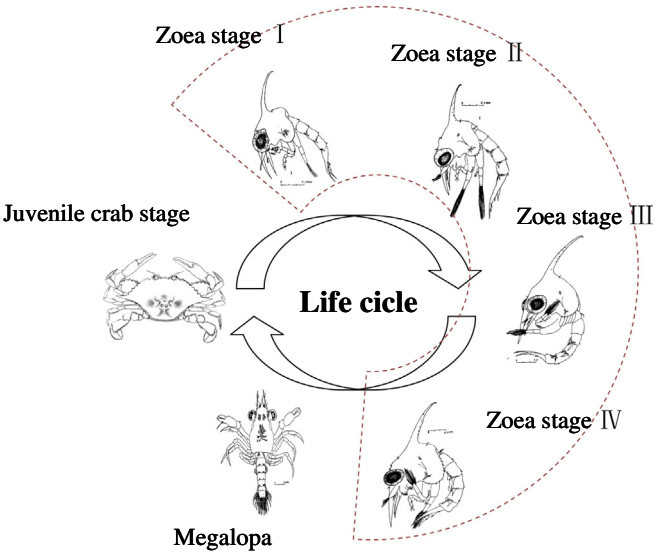

Fig. 1. Developmental stages of *Portunus trituberculatus*. Zoea stage I (0–4 days), zoea stage II (4–8 days), zoea stage III (8–12 days), zoea stage IV (12–16 days), and megalopa stage (16–20 days).

2. Materials and methods

2.1. Chemicals

OTC hydrochloride (95% purity) was purchased from Sigma Aldrich (Saint Louis, MO, USA).

2.2. Larval culture

The present experiment was conducted at the hatchery of Changyi Haifeng Aquiculture Ltd., Weifang, Shangdong Province, China. *P. trituberculatus* stocks were obtained from Laizhou Bay (37°17′7″N, 119°35′10″E), Shandong Province, China. Parent spawning and larval rearing were conducted according to Wu et al. (2010). The larvae from four zoea developmental stages (Fig. 1) were collected: zoea stage I (Z1), 1 day post-hatching (dph); zoea stage II (Z2), 5 dph; zoea stage III (Z3), 9 dph; and zoea stage IV (Z4), 14 dph. The larvae were observed under a microscope to ensure developmental synchrony, and only more than 90% larvae at the same developmental stage were randomly used for the OTC treatment.

2.3. Exposure system

OTC is a less toxic substance, and LC_{50} of the larvae at the four zoea developmental stages at 48 h was higher than 100 mg/L (Unpublished). Therefore, no acute toxicity test was performed before this study. On the basis of the preliminary experiment (Chen et al., 2015; Zou et al., 2011), the larvae from each developmental stage were exposed to four OTC concentrations (0, 0.3, 3, and 30 μg/L), and three replicates were used for each treatment at a density of 0.1 individual (ind.)/mL. The larvae were cultured in 3.0 × 3.0 × 1.5-m cement pools with aerated sand-filtered seawater (32‰ salinity) at 22 ± 1 °C and 12 light:12 dark cycle. The larvae were daily fed rotifers (L type, *Brachionus plicatilis*; density, 20 ind./mL). Sodium phosphate (Na_2HPO4:NaH_2PO4 = 19:1) was added to the immersion bath to maintain the pH at ~7.5. During the OTC exposure period, 50% of the test solution was renewed once every 24 h.

2.4. Sample collection

Forty-eight samples from each stage (4 developmental stages × 4 treatments × 3 replicates) were collected after 72 h of OTC exposure. For each replicate, the samples were ground in liquid nitrogen immediately, and the powder was divided into several centrifuge tubes for different parameter assays. For enzyme and biomolecule damage analyses, the samples were stored at −80 °C. For gene transcription analysis, the samples were placed in 1.5-mL RNA-clean plastic centrifuge tubes filled with RNAiso Plus