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Short communication

Effect of tributyltin on reproduction in Japanese whiting, *Sillago japonica*

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Abstract

We examined the effect of tributyltin (TBT) on reproduction in the Japanese whiting, *Sillago japonica*. Mature fish were placed in indoor 500-L polyethylene tanks (five males and three females per tank) with a flow-through system and received dietary exposure to tributyltin oxide at concentrations of 2, 20, or 200 μg/g for 30 days during the active spawning period. Eggs spawned from the fish were collected daily, and the floating egg rate, larval deformity, hatchability, and viable hatch were monitored. TBT concentration in eggs of 200-μg/g group ranged from 85.0 to 159.6 ng/g in the evaluation period (days 5–30). In this period, the floating egg rate (83.2%), viable hatchability (82.2%), and total number of viable larvae (422,000 larvae per 100 g of female) were all significantly decreased in the 200-μg/g group compared with the control group (93.0%, 91.9%, and 709,000 larvae, respectively). The rate of deformity (2.6%) in the 200-μg/g group was about three times that in the control group (0.8%), although this difference was not significant. From these results, the lowest observed effect concentration of TBT in eggs on reproduction in Japanese whiting was estimated to be less than 159.6 ng/g-eggs.

Keywords: Tributyltin; Marine fish; Japanese whiting; Reproduction

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Tributyltin (TBT) has been widely used as an antifouling agent in aquatic environments since the early 1960s (Clark et al., 1988). After regulation or restriction of its use from 1982 (Alzieu, 1998), TBT is still detected in marine environments (Arambarri et al., 2003; Ramaswamy et al., 2004). Previously, Suzuki et al. (1992) detected high concentrations of TBT (~431 ng/g) in eggs from hairtail, and Takahashi et al. (1997) detected 91 ng/g of TBT in eggs of cornet fish. Because hatchability and swim-up in Japanese medaka are adversely affected by TBT at a concentration of 123 ng/g in eggs (Nakayama et al., 2005), TBT pollution is suspected of having adverse effects on marine fish. However, there is no information on the reproductive effects of TBT in marine fish, especially important fish in fishery resources. We investigated the reproductive effects of TBT in Japanese whiting, *Sillago japonica*, and elucidated the relationship between TBT concentration in the eggs and reproductive measures.

Adult Japanese whiting were collected by fishing off the coast of Tsuyazaki, in Fukuoka Prefecture, Japan, in May 2000, at the beginning of the reproductive season. The fish were acclimatized in 12 indoor 500-L polyethylene tanks [five males (mean body weight, 38.3 g) and three females (mean body weight, 53.1 g) in each tank] for 3 weeks with a commercial diet (Himezakura, Higashimaru, Kagoshima, Japan) under natural photoperiod and water temperature (22.7–28.7 °C) in flowing (500 L/h) and aerated seawater. Phenotypic sex was determined by observation of release of sperm after applying abdominal pressure. After acclimatization, the fish received dietary exposure to tributyltin oxide (TBTO, >95%, Tokyo Kasei Kogyo, Japan) at concentrations of 0, 2, 20, or 200 μg/g for 30 days (three tanks per treatment group) from 26 July to 24 August under the same conditions as those in the acclimatization period, at a water temperature of 25.6–28.3 °C and salinity of 31–34 p.s.u. Each test diet was fed to each group at 5% of body weight. During the exposure period, 2 h after spawning, eggs were gathered in a collecting net fixed under the outlet pipe outside each test tank. The fish spawned almost daily between about 7 p.m. and 12 midnight. TBT concentrations in eggs were determined by a method described previously (Inoue et al., 2004) using gas chromatograph equipped with a mass spectrometer. Floating and sunken eggs were volumetrically estimated as 3400 eggs/mL in a measuring cylinder (normal eggs float, whereas poor quality, abnormal, or unfertilized eggs sink). About 100 floating eggs were transferred to a 1-L beaker filled with seawater and incubated at the same water temperature as in the main test tanks, to monitor hatched, deformed, and viable hatched larvae. Under the experimental conditions described above, fertilized eggs hatched about 20 h after fertilization. The numbers of hatched and deformed larvae were counted 24 h after hatching. Deformed larvae were defined as those in which the notochord or caudal portion was curved, crooked, or atrophied, and viable hatched larvae as those that were alive 24 h after hatching and were not deformed. Each endpoint was calculated by one of the following formulae:

$$\text{Floating egg rate } (\%) = 100 \times (\text{no. of floating eggs})/(\text{total no. of eggs spawned})$$

$$\text{Hatchability } (\%) = 100 \times (\text{no. of hatched larvae})/(\text{no. of eggs in test beaker})$$

$$\text{Deformity } (\%) = 100 \times (\text{no. of deformed larvae})/(\text{no. of hatched larvae})$$

$$\text{Viable hatchability } (\%) = 100 \times (\text{no. of viable hatched larvae})/(\text{no. of eggs in test beaker})$$