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Environmental levels of atrazine and its degradation products impair survival skills and growth of red drum larvae

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Abstract

Red drum larvae (*Sciaenops ocellatus*) were exposed to environmentally realistic and sublethal levels of the herbicide atrazine (2-chloro-4-ethylamin-6-isopropylamino-*S*-triazine) to evaluate its effects on ecologically critical traits: growth, behavior, survival potential, and resting respiration rate. Settlement size larvae (7 mm total length) were given an acute exposure of atrazine at 0, 40, and 80 $\mu g\,l^{-1}$ for 4 days. Tests of 96 h survival confirmed that these naturally occurring concentrations were sublethal for red drum larvae. Growth, routine swimming, antipredator responses to artificial and actual predators, and resting respiration rate were monitored 1 and 3 days after onset of exposure. Atrazine exposure significantly reduced growth rate. Atrazine-exposed larvae also exhibited significantly higher routine swimming speeds, swam in more convoluted paths, and were hyperactive. Responses to artificial and actual predators were not affected by atrazine exposure nor were resting respiration rates. The higher rate of travel (86% higher in atrazine-treated larvae) resulted in higher predicted encounter rates with prey (up to 71%) and slow moving predators (up to 63%). However, hyperactivity and faster active swimming speeds of exposed larvae indicated that naturally occurring sublethal levels of atrazine will result in an elevated rate of energy utilization (doubling the total metabolic rate), which is likely to increase the risk of death by starvation. Moreover, atrazine effects on growth will prolong the larval period, which could reduce the juvenile population by as much as 24%. We conclude that environmentally realistic levels of atrazine induce behavioral and physiological effects on fish larvae that would compromise their survival expectations.

Keywords: Atrazine; Fish larvae; *Sciaenops ocellatus*; Behavior; Respirometry; Metabolism; Endocrine disrupting chemical

1. Introduction

Atrazine (2-chloro-4-(ethylamino)-6-(isopropylamino)-*S*-triazine) is a widely used herbicide in the United States. It was first registered in 1958 and the U.S. Environmental Protection Agency has estimated that between 34 and 36 million kilograms of atrazine were used in 2001 (Kiely et al., 2004). Due to its high use and its relatively high mobility in soils, atrazine is frequently detected in surface and ground waters. Atrazine levels in runoff can reach very high levels in the first rain events after application (Southwick et

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al., 2003). Thurman et al. (1992) showed that atrazine levels in storm runoff could reach levels of 40 $\mu g\,l^{-1}$. Reported levels in South Texas coastal waters have reached 65 $\mu g\,l^{-1}$ (Pennington et al., 2001).

Commercially and ecologically important fish species such as red drum (*Sciaenops ocellatus*) can be affected by contaminated runoffs entering estuaries. Red drum spawn in coastal areas and they reach the estuaries still in the larval stage. Contaminants in the environment can impair growth and development of larvae and may ultimately lead to mortality (e.g., Weis and Weis, 1976, 1995; Faulk et al., 1997; Zhou and Weis, 1998; Beg et al., 2001; McCarthy et al., 2003). Although atrazine was developed to inhibit photosynthesis in plants, it has multiple effects on animals. For example, atrazine is a classified endocrine disrupting chemical that affects steroidogenesis in alligators and frogs (Crain et al., 1997; Hayes et al., 2002; Goulet and Hontela, 2003) and olfactory-mediated endocrine function in salmon parr (Moore and Lower, 2001). Atrazine exposure has been shown to produce altered social and antipredator behavior in goldfish (*Carassius auratus*) (Saglio and Trijasse, 1998). This study assesses the effects of environmentally realistic levels of atrazine on red drum larvae at the size they enter contaminated nursery areas by evaluating ecologically important behaviors, growth, and the energetic cost of exposure.

2. Materials and methods

2.1. Experimental animals

Red drum eggs were obtained from three sources: University of Texas Fisheries and Mariculture Laboratory (Port Aransas, TX), Texas Parks and Wildlife Department CCA/CPL Marine Development Center (Corpus Christi, TX), and Perry R. Bass Marine Fisheries Research Station (Palacios, TX). Broodstocks were induced to spawn by manipulating ambient temperature and photoperiod (Arnold, 1988). Eggs were collected within 12 h of spawning and hatched in conical tanks in 20 l of sea water. From day 5 after hatching, the volume was gradually increased to 100 l over approximately a 5 d. Larvae were reared with flow-through circulation until experimentation. Temperature and salinity in the rearing tanks were maintained at about 27 °C and 27 PSU. Larvae were fed 10 rotifers (*Brachionus plicatilis*) $ml^{-1}\,d^{-1}$ from day 1 until about 10 d posthatching when their diet was gradually shifted to *Artemia* nauplii over a 3 d period. At the time of the chemical exposure fish were completely weaned from rotifers and onto nauplii. *Artemia* were enriched overnight with Algamac 2000 (Aquafauna Bio-Marine Inc., CA) and added to rearing tanks in the morning so that they reached a concentration of 5 nauplii ml^{-1}. Fish were fed at approximately 08:30 h daily and allowed to feed for about 1 h before moving them to the experimental chambers.

Since the highest levels of atrazine in surface waters are found in estuarine areas, exposures were conducted on larvae at the size of settlement to estuarine seagrass beds, about 7–8 mm total length (TL) (Rooker and Holt, 1997; Herzka et al., 2002). Red drum larvae reached settlement size within 15–20 days under rearing conditions.

2.2. Exposures

Atrazine (with a guaranteed purity of 98%) was purchased from Chem Service Inc. (West Chester, PA). Atrazine (approximately 24 mg) was dissolved in 3 ml of acetone and added with gentle stirring to the trial tanks to the desired concentration. An equal amount of acetone was also added to the control group.

Survival experiments were performed to evaluate whether environmentally realistic doses were within the sublethal range for settlement size red drum. Groups of 50 settlement size larvae were transferred to 1.5-l exposure watch bowls (20 cm diameter) in a temperature-controlled room. Salinity and temperature were maintained at 27 °C and 27 PSU. Atrazine dissolved in acetone or acetone alone was added to the watch bowls 24 h after transfer. Doses tested were 0, 40, 80, and 500 μg atrazine l^{-1}. Fish were fed a ration of 5 nauplii $ml^{-1}\,d^{-1}$. Survival was recorded 96 h after the herbicide was added to the water. Six replicates for 40 and 500 $\mu g\,l^{-1}$ exposures, and 12 for control and 80 $\mu g\,l^{-1}$ exposures were performed with larvae from five different spawns. The proportions of surviving larvae from the atrazine-exposed groups were compared to the survival rate of control groups.

Settlement-size red drum larvae (7–8 mm TL) were transferred to six 50-l fiberglass tanks on experimental day −1 at a density of 10 larvae l^{-1} and allowed