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Behavioral response of juvenile rainbow trout exposed to an herbicide mixture

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ABSTRACT

Fish are capable of sensing water-borne chemicals at sub-lethal concentrations. Inadequate behavioral responses to physiological and environmental stimuli owing to adverse effects of aquatic toxicants can have serious implications for survival.

In this study we exposed juvenile rainbow trout (*Oncorhynchus mykiss*) during 5 days to a low-concentration mixture of three co-occurring herbicides: atrazine, linuron and metolachlor, at maximum concentrations of 4.5, 4.9 and 13.4 μg L^{-1}, respectively. Our hypothesis was that fish behavior – swimming activity and interactions between individuals – would be modified due to exposure to the mixture. We studied these behaviors by observing fish twice-daily throughout the exposure period at 30-s intervals for 5 min, registering the vertical distribution of fish in the water column and the number of agoniztic acts between all individuals.

Fish exposed to the mixture of herbicides were hypoactive and spent more time in the lower parts of the aquaria in comparison to non-exposed controls, reflecting inhibited swimming activity. Average swimming height of exposed fish decreased significantly with the number of agoniztic acts, whilst in control groups there was no significant relationship between the two behaviors. Overall, behavior of fish exposed for a short time to the herbicide mixture was altered in comparison to control-fish behavior. The behavioral endpoints chosen here were easily observed, simple to quantify, and of ecological relevance.

1. Introduction

A mixed-crop farmland will typically be treated with a broad range of substances with different modes of action. Thus, adjacent water bodies can carry a mixture of chemicals that are applied aiming at specific organisms but may present risks to non-target species. Herbicides, designed to inhibit germination, development and persistence of weeds, may indirectly affect aquatic fauna through disturbance of communities at the lower end of the trophic-chain, such as phytoplankton and macrophytes (Belden et al., 2007; Daam et al., 2009). However, more information is needed on the direct effect of herbicides on invertebrates, fish, and other aquatic vertebrates, and given that they are commonly applied simultaneously, studying the effects of mixtures is more environmentally relevant.

Behavior is the result of the interactions of an organism with its external environment, integrating physiological, biochemical and metabolic processes with the environmental factors that stimulate behavioral responses (Grue et al., 2002). Inadequate behavioral responses to physiological and environmental stimuli owing to adverse effects of aquatic toxicants can have serious implications for survival (Weber and Spieler, 1994): if an organism is not minimally in tune with the surrounding physical or biological conditions, basic necessities may become jeopardized. Although behavioral responses are not as contaminant-specific as other biomarkers of lower complexity (Peakall, 1994), their attractiveness relates to a higher sensitivity regarding dosage and response time, and therefore a potential as early-warning signals of effect (Hellou, 2011). Indeed, fish are capable of detecting, and sometimes responding by avoiding, water-borne chemicals at sub-lethal concentrations (Folmar, 1976; Scott and Sloman, 2004).

Fish are convenient models for behavioral ecotoxicology studies as many of their behaviors that are easily observed and

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quantified under controlled conditions are per se more ecologically relevant than lower level biomarkers (Scott and Sloman, 2004). For example, social interactions such as schooling, courtship, and dominance hierarchies are directly linked to fitness. Disruption of those behaviors may jeopardize the chances of succeeding in fundamental processes that are crucial to individual, and eventually population, continuity (Grue et al., 2002).

Dominance hierarchies are a key factor in ensuring enough resources (food and shelter) for individual fish optimal growth, and are established via intraspecific competition (Chapman, 1966). Alterations in agoniztic acts due to the presence of toxicants may lead to either failure to maintain a territory or metabolic fatigue (Triebskorn et al., 1997). The action of toxic substances can also directly interfere with feeding and predator recognition-and-escape behaviors through physiological interaction with sensory organs/cells (Saglio and Trijasse, 1998; Tierney et al., 2007b). Fish swimming activity might also be altered in the presence of contaminants (Little et al., 1990; Zhou and Weis, 1999; Steinberg et al., 1995).

The three herbicides studied here-atrazine, linuron and metolachlor-are representatives of three chemical groups and two modes of action. Due to their application to the same crops (e.g., corn, sorghum, soybeans), they have been reported to co-occur in environmental water samples collected from different watersheds (Gilliom, 2007; Faggiano et al., 2010).

Atrazine is part of the s-triazine chemical group and inhibits photosynthesis by blockage of electron transport in the photosystem II (van Rensen, 1989). It is included in the EU priority substance list due to its high mobility and persistence in the environment (Directive 2008/105/EC of the European Commission, 2008) and has been banned from use on most crop types in the EU since 2004 (Directive 2004/248/EC of the European Commission, 2004). The Canadian Water Quality Guideline (WQG) for the protection of freshwater life against atrazine has set a maximum allowed limit of 1.8 μg L^{-1} (CCME, 1999). A lowest observed effect concentration of $\leq$5 μg L^{-1} has been reported for swimming behavior of zebrafish (Steinberg et al., 1995). Saglio and Trijasse (1998) reported increased surfacing activity, decreased grouping behavior, and decreased sheltering in response to an alarm signal in goldfish exposed to 5 μg L^{-1} for 24 h. A 30-min exposure to 1 μg L^{-1} atrazine eliminated preference behavior for a natural odorant in rainbow trout, and 10 μg L^{-1} atrazine significantly reduced L-histidine-evoked olfactory sensory responses (Tierney et al., 2007a). Atrazine at 1 μg L^{-1} significantly reduced male Atlantic salmon olfactory response to a female pheromone (Moore and Waring, 1998; Moore and Lower, 2001).

Linuron is a phenylurea that acts upon photosynthesis in a similar way as atrazine (van Rensen, 1989). The Canadian WQG for the protection of freshwater life against linuron has set the maximum allowed limit to 7 μg L^{-1} (Caux et al., 1998). Linuron is suspected to alter olfactory-mediated behaviors in fish (Tierney et al., 2007b). The structurally similar herbicide diuron was reported to alter olfactory-based behaviors in goldfish, such as the decrease of grouping behavior in the presence of an alarm signal, after a 24-h exposure to 5 μg L^{-1} (Saglio and Trijasse, 1998). Tierney et al. (2007b) detected reduction of L-serine-evoked olfactory sensory responses in rainbow trout exposed to linuron at 10 μg L^{-1} for 15 min, suggesting that linuron has the potential to disturb predator avoidance and food location in salmonid fish.

Metolachlor is a chloroacetanilide that promotes the inhibition of cell division in seedling shoots and roots (Takacs et al., 2002). The Canadian Council of Ministers of the Environment has established the WQG limit at 7.8 μg L^{-1} for the protection of aquatic life (CCME, 1999). This herbicide has been reported to affect the perception of chemical stimuli by the crayfish *Orconectes rusticus*, leading to inappropriate decisions regarding detection of food and response to an alarm signal (Wolf and Moore, 2002), as well as interfere with the ability of crayfish to respond to social signals involved in agoniztic behaviors when exposed to 80 μg L^{-1} for 96 h (Cook and Moore, 2008).

In the present study we investigated the effect of a mixture of three herbicides on the behavior of juvenile trout (*Oncorhynchus mykiss*). Occupation of the water column, number of movements, and number of agoniztic acts, observed at regular intervals throughout the experiment, were compared between exposed and control organisms.

2. Material and methods

2.1. Herbicide mixture selection

Three herbicides, each belonging to 3 different chemical groups (*s*-triazines, acetanilides and phenylureas) were selected to be assessed in the mixture toxicity tests. The selection was based on the pesticide concentrations measured by the AEAG throughout the Adour-Garonne river basin during the year 2007 (140 sampling sites × 5 or 6 samplings per site). Hierarchical clustering analysis was performed in R using complete linkage and Euclidean distances between the maximum concentrations of contaminants that were detected in more than 5% of the samples. The contaminants were thus grouped according to their co-occurrence and concentration, i.e. the mixture occurs in the natural environment with all 3 herbicides co-occurring in 22% of sampling sites. The selected herbicides were atrazine, linuron and metolachlor.

2.2. Herbicide concentrations

Test concentrations of each compound were established according to the highest concentration found in the year 2007 (*C*max) in the Adour-Garonne river basin, multiplied by 50 for atrazine and linuron and by 5 for metolachlor. The difference in the multiplication factor aimed to avoid metolachlor (*C*max=9 μg L^{-1}) dominating the mixture – Metolachlor contributed to 70% of the total contamination of 2009-spring flood water in a medium-sized river of the Adour-Garonne river basin (Polard et al., 2011) –, and thus possibly determining fish response regardless of the presence of atrazine (*C*max=0.2 μg L^{-1}) and linuron (*C*max=0.3 μg L^{-1}). A more representative mixture of year-round pesticide concentrations would be one in which metolachlor was not dominating, but to nevertheless maintain the weight of metolachlor contamination in the environment, increasing the concentrations of the other two pesticides was chosen over decreasing that of metolachlor. The final nominal concentrations of each chemical in the mixture was thus 10, 15 and 45 μg L^{-1} for atrazine, linuron and metolachlor, respectively. Although the tested concentrations do not copy the *C*max found in the environment, they closely reflect levels that have been studied previously and that induced sub-lethal effects in fish.

2.3. Exposure setup

A flow-through system (Fig. 1) was constructed in a closed room with air at a constant temperature of 16 °C and light:dark regime of 16:8 h. Four control (only water) and four treated 40.4 L (28 × 38 × 38 cm^3) glass aquariums received a continuous flow of filtered (25 μm, DomSource-COMAP, Lyon, France), aerated and refrigerated tap water. A multichannel peristaltic pump (Watson-Marlow 205U, La Queue Lez Yvelines, France), equipped with silicone tubes (63 μm internal diameter) delivered the mixture of herbicides to each mixing vessel at the desired rate. Water inflow, regulated by flow meters (Cole Parmer, Chicago, USA), was