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No evidence of increased growth or mortality in fish exposed to oxazepam in semi-natural ecosystems

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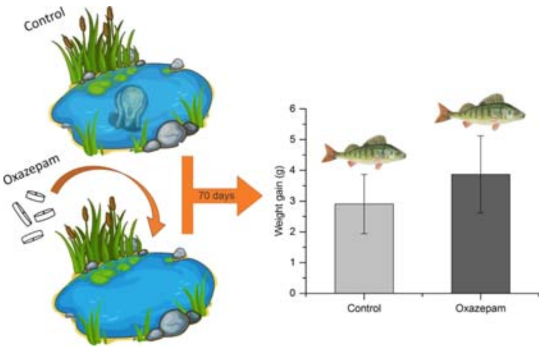
HIGHLIGHTS

- Laboratory results show that oxazepam can alter ecologically important behaviors of fish.
- We studied oxazepam effects on perch growth and survival in a semi-natural ecosystem.
- Mean values for growth of exposed fish was higher than the control but not significant.
- There was no significant difference between exposed and control fish in mortality.
- An additional lab study showed a reduced prey capture efficiency in the exposed predator.

GRAPHICAL ABSTRACT



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ABSTRACT

An increasing number of short-term laboratory studies on fish reports behavioral effects from exposure to aquatic contaminants or raised carbon dioxide levels affecting the $GABA_A$ receptor. However, how such GABAergic behavioral modifications (GBMs) impact populations in more complex natural systems is not known. In this study, we induced GBMs in European perch (*Perca fluviatilis*) via exposure to a GABA agonist (oxazepam) and followed the effects on growth and survival over one summer (70 days) in replicated pond ecosystems. We hypothesized that anticipated GBMs, expressed as anti-anxiety like behaviors (higher activity and boldness levels), that increase feeding rates in laboratory assays, would; i) increase growth and ii) increase mortality from predation. To test our hypotheses, 480 PIT tagged perch of known individual weights, and 12 predators (northern pike, *Esox lucius*) were evenly distributed in 12 ponds; six control (no oxazepam) and six spiked ($15.5 \pm 4\ \mu g\ l^{-1}$ oxazepam [mean ± 1 S.E.]) ponds. Contrary to our hypotheses, even though perch grew on average 16% more when exposed to oxazepam, we found no significant difference between exposed and control fish in growth (exposed: 3.9 ± 1.2 g, control: 2.9 ± 1 g [mean ± 1 S.E.], respectively) or mortality (exposed: 26.5 ± 1.8 individuals $pond^{-1}$, control: 24.5 ± 2.6 individuals $pond^{-1}$, respectively). In addition, we show that reduced prey capture efficiency in exposed pike may explain the lack of significant differences in predation. Hence, our results suggest that GBMs, which in laboratory studies impact fish behavior, and subsequently also feeding rates, do not seem to generate strong effects on growth and predation-risk in more complex and resource limited natural environments.

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1. Introduction

Behavioral studies are becoming an increasingly important tool for assessing non-lethal effects of pollutants on aquatic organisms (Weis et al., 2001; Hellou, 2011; Melvin and Wilson, 2013; Arnold et al., 2014; Brodin et al., 2014; Hellstrom et al., 2016; Pyle and Ford, 2017). The rationale behind this is that behavioral tests have been reported to be 10–100 times more sensitive than tests using lethal endpoints (Gerhardt, 2007), and in general also more sensitive than tests assessing effects on reproduction or development (Melvin and Wilson, 2013). Over the past two decades, studies simulating aquatic pollution and environmental change have used behavioral tests, originally advanced from research focusing on animal personality, for ecological predictions (Reale et al., 2007). Many tests have been focusing on behaviors, such as activity and boldness, that have ecological and evolutionary consequences (Huntingford, 1976; Wilson et al., 1993; Wilson, 1994). A variety of environmentally disrupting compounds, such as pharmaceuticals (Brodin et al., 2013; Dzieweczynski et al., 2016; Giacomini et al., 2016; Hedgespeth et al., 2016), raised carbon dioxide (CO_2) concentrations (Jutfelt et al., 2013; Jutfelt and Hedgarde, 2013; Munday et al., 2013; Hamilton et al., 2014), mercury (Pereira et al., 2016), and organic pollutants (Vignet et al., 2015) have been shown to modify activity or boldness in several fish species. Increased boldness and activity caused by a group of psychoactive contaminants (benzodiazepines), as well as similar effects resulting from increased CO_2 concentrations in waters, most likely have a comparable mechanistic explanation: a lower action potential of the $GABA_A$-receptor (Braestrup and Squires, 1978; Nilsson et al., 2012). Benzodiazepines (e.g. oxazepam and diazepam) are GABA (the main inhibitory neurotransmitter in the brain) agonists known to alter anti-anxiety-like behaviors (such as activity and boldness/exploration) in fish (Gebauer et al., 2011; Brandao et al., 2013; Brodin et al., 2013; Giacomini et al., 2016), via a chain of events as the drugs target the $GABA_A$ receptor and depolarize the neuron leading to a calming effect as it reduces the communication between the neurons (Argyropoulos and Nutt, 1999). When marine fish are exposed to high CO_2 levels an ion-regulation in blood and tissues, to avoid acidosis, it affects the transmembrane gradients in some neurons, leading to an accumulation of HCO_3^- and a compensatory reduction in Cl^- (Nilsson et al., 2012). Nilsson et al. (2012) argue that these regulatory changes alter the behavior of fish because the changes in HCO_3^- and Cl^- levels result in $GABA_A$-receptors becoming depolarized and excitatory.

The ecological consequences of increased boldness and activity derived from GABAergic behavioral modifications (GBMs), as observed in laboratory assays, remain debated. Increased boldness and activity levels, as a consequence of GBMs, are associated with higher risk taking that can result in higher mortality (Lima and Dill, 1990; Werner and Anholt, 1993; Anholt and Werner, 1998; Brodin and Johansson, 2004), which in turn may result in decreasing or even collapsing populations (Munday et al., 2010). Yet, improved fitness can also be expected from GBMs as increased activity and boldness allow for higher foraging rates and, as a consequence, increased growth rates and survival under low predation pressure (Werner and Anholt, 1993; Biro et al., 2004, 2006; Klaminder et al., 2014). Moreover, increased mortality among individuals experiencing a GBM may reduce intraspecific competition and stimulate growth among surviving fish through a mechanism referred to as thinning (Brodin and Johansson, 2002). Effects from pharmaceuticals are also most likely species-specific (Brodin et al., 2014) suggesting that in food webs, the effects may be asymmetric if some species are affected and others are less- or unaffected, making it hard to draw conclusions about implications for food web structures and ecosystem functions from single-species studies.

Difficulties in extrapolating results on behavioral endpoints, measured in artificial settings, to natural environments was recognized long ago (Sprague, 1971), and the use of behavioral tests for detecting effects of contaminants has recently been questioned, due to lack of standardization and support from repeated studies so far (Harris and Sumpter, 2015). However, results from three recent fields studies indicate that GBMs are indeed important in complex aquatic ecosystems, by altering home-range size and habitat choice (Klaminder et al., 2016), migration rates (Hellstrom et al., 2016) and diurnal rhythm activity patterns (Hasler et al., 2016) in fish exposed to contaminants. All studies were, however, relatively short term (<12 days) and provided only information about fish movement and positions, hence data on direct fitness correlates, such as growth rates are still lacking. Furthermore, predators were not exposed to contaminants in these studies, as they would be in contaminated environments, meaning that the results must be interpreted with caution. To our knowledge there are thus so far, no long-term studies that have looked at the ecological consequences of GBMs in natural ecosystems where both the prey and predator were exposed to contaminants with the potential to alter behaviors. Therefore, such studies should be highly prioritized.

In this study, we assess if GBMs, in replicated aquatic ecosystems containing two fish species; the prey fish European perch (*Perca fluviatilis*) and the predator northern pike (*Esox lucius*), affect growth rate and overall survival of perch over a 70-day period. The GBMs were induced by additions of an anxiolytic pharmaceutical (oxazepam, a $GABA_A$-receptor agonist; the most prescribed benzodiazepine in Sweden and a metabolite of diazepam (e.g. valium), which is another commonly prescribed benzodiazepine. Given that exposure to oxazepam in laboratory environment has been shown to generate anti-anxiety like behaviors and increased foraging rates in fish (Brodin et al., 2013; Klaminder et al., 2014; Brodin et al., 2017), and subsequently potentially increased predation risks, we hypothesized that the GBMs should result in; i) a higher growth rate and ii) increased predation leading to a lower population-size in the exposed ponds. In addition to the field experiment, we present findings from a laboratory assay on how GBMs affect northern pike's ability to catch prey, to support our interpretations of results and conclusions.

2. Materials and methods

2.1. Pond experiment

2.1.1. Study site

The study was conducted in a semi-natural pond system in Röbäck (63°48.572′N; 20°14.584′E), Umeå, Sweden. The system consists of two large ponds (36 × 10 m) that are divided into eight smaller compartments (4.5 × 10 m, maximum depth: ~90 cm), henceforth referred to as ponds. The two outermost small ponds in each large pond were excluded from the study, resulting in a total of 12 smaller ponds for the experiment. Each small pond has a slight inflow from the Umeå municipality groundwater source (pH: 8.05 ± 0.09, P: 3.8 ± 1.3 $\mu g\ l^{-1}$, N: 69 ± 4.6 $\mu g\ l^{-1}$, and DOC: 1.06 ± 0.03 mg/l^{-1}, (average ± SE), for more water chemistry details see Hedström et al., 2016). Dominating aquatic vegetation is quillworts (*Isoetes*), broad-leaved pondweed (*Potamogeton natans*), and duckweed (*Lemna minor*). Prior to the experiment, all ponds were prepared manually using a rake to remove much of the existing vegetation to generate a homogenous amount of aquatic vegetation between the ponds. The benthic fauna has previously been described and consist mainly of Ephemeroptera, Planorbidae, and Chironomidae (Byström and Andersson, 2005; Lagesson et al., 2016).

2.1.2. PIT tagging fish

Juvenile perch (*Perca fluviatilis*) were caught in Rovågern (63° 44.007′N; 20° 32.063′E), close to Umeå city (63° 49.783′N; 20° 16.017′ E), Sweden, during the last week of May 2015 using a beach seine net. The fish were transported to Umeå University in oxygenated containers within 2 h of capture. The perch were then kept in an oxygenated holding tank (approximately 85 × 150 × 150 cm) with ~13 °C aged tap water and a constant drip of tap water flow through. During the following acclimatization period, fish were fed with thawed red chironomidae