# Dilute Concentrations of a Psychiatric Drug Alter Behavior of Fish from Natural Populations

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Environmental pollution by pharmaceuticals is increasingly recognized as a major threat to aquatic ecosystems worldwide. A variety of pharmaceuticals enter waterways by way of treated wastewater effluents and remain biochemically active in aquatic systems. Several ecotoxicological studies have been done, but generally, little is known about the ecological effects of pharmaceuticals. Here we show that a benzodiazepine anxiolytic drug (oxazepam) alters behavior and feeding rate of wild European perch (*Perca fluviatilis*) at concentrations encountered in effluent-influenced surface waters. Individuals exposed to water with dilute drug concentrations (1.8 micrograms liter$^{-1}$) exhibited increased activity, reduced sociality, and higher feeding rate. As such, our results show that anxiolytic drugs in surface waters alter animal behaviors that are known to have ecological and evolutionary consequences.

Among pharmaceuticals, anxiolytics (pharmaceuticals used to treat anxiety) are a frequently prescribed class of psychotherapeutic drugs of which benzodiazepines are the most commonly used globally (*1*). Benzodiazepines persist in wastewater effluent and can therefore be found at concentrations ranging from 0.01 to several μg liter$^{-1}$ in treated effluent (*1*–*4*). Further, several benzodiazepines are also quite resistant to photodegradation (*5*), which enables them to persist in aquatic environments, and have been found at concentrations ranging from 0.001 to 0.4 μg liter$^{-1}$ in rivers and streams (*2*, *3*). Because benzodiazepines are designed to alter behavior by binding to γ-aminobutyric acid (GABA) receptors, which are found in a wide range of animal species, it is possible that organisms in aquatic environments receiving treated wastewater effluent are experiencing behavioral modifications (*6*). Behavior is a crucial determinant for important fitness correlates, such as growth, reproduction, and survival (*7*, *8*). Hence, pharmaceuticals such as benzodiazepines, which are designed to alter behavior, could have evolutionary and ecologically important effects through modifications of fish behavior that, over time, influence aquatic community compositions and, consequently, the functioning of aquatic systems. It is therefore surprising that ecotoxicological research thus far has not assessed how psychotherapeutic drugs frequently found in aquatic ecosystems may affect key behaviors of aquatic organisms.

In a screening of Swedish surface waters, we found concentrations of a common benzodiazepine, oxazepam, of 0.73 μg liter$^{-1}$ in treated wastewater effluent and 0.58 μg liter$^{-1}$ in a mid-sized stream (River Fyris) receiving input of treated wastewater (Table 1). These concentrations are comparable to those of benzodiazepines reported in other European and American waters (*1*–*4*). The concentration of oxazepam in muscle tissue of European perch (*Perca fluviatilis*) from River Fyris was more than six times that in the water, indicating bioaccumulation of this drug in the fish (Table 1). To assess how the presence and subsequent uptake of dissolved oxazepam may affect fish behavior, we exposed naturally spawned juvenile perch to water with two different concentrations of oxazepam: a low, environmentally relevant concentration of 1.8 μg liter$^{-1}$ and a high concentration of 910 μg liter$^{-1}$. After 7 days of exposure, fish treated with low concentrations had accumulated oxazepam in their muscle tissue at concentrations overlapping with those found in fish from River Fyris (Table 1), indicating that the treatment with low concentrations represents an environmentally relevant oxazepam contamination level. To investigate if oxazepam alters fish behavior, we quantified the behavioral traits boldness, activity, and sociality (*9*) of perch individuals before and after they were exposed to either of the two chosen concentrations. These behavioral traits, sometimes referred to as personality traits (*10*), are known for being both ecologically and evolutionarily important and are used to predict how individuals respond to changed environmental conditions (*11*–*14*). The studied behavioral traits of untreated and treated fish were quantitatively measured with standardized protocols including video surveillance and subsequent image analysis (*9*). Activity was defined as number of swimming bouts (>2.5 cm) during the observation period (600 s). Boldness was calculated as the inverse of an individual's latency to enter a novel area during the observation period (900 s). Sociality was measured as an individual's spatial use, during 600 s, in relation to a group of conspecifics.

We found strong effects of oxazepam on fish behavior (Fig. 1, A to C). Individuals that were exposed to low concentrations became more active ($F_{1,46}$ = 4.2, $P$ = 0.047) and less social ($F_{1,46}$ = 14.4, $P$ = 0.0001) than fish that were not exposed, whereas boldness was largely unaffected. The reliability of the observed effects at the low concentration was strengthened by similar effects in the high-concentration treatment where all studied behavioral traits showed significant changes; exposed fish became more active ($F_{1,46}$ = 21.8, $P$ = 0.0001), bolder ($F_{1,46}$ = 29.9, $P$ = 0.0005), and more asocial ($F_{1,46}$ = 17.6, $P$ = 0.0001) compared to unexposed individuals.

To assess more direct ecological effects of oxazepam exposure, we measured individual feeding rate, a fundamental fitness correlate (*15*–*19*). This was done by recording the time it took for each individual to initiate feeding on, and deplete, a resource consisting of 20 zooplankton (*Daphnia pulex*), both before and after pharmaceutical exposure (*9*). There was no significant difference in feeding rate between perch allocated to the three different treatments (control, low, and high) before exposure. However, the drug-induced change in feeding rate of fish

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**Table 1.** Measured concentrations and relative standard deviations (RSD) of oxazepam in water and muscle-tissue samples and corresponding estimated bioaccumulation factors (BAF).

| Sample | Water | | Fish muscle tissue | | | BAF* |
|---|---|---|---|---|---|---|
| | μg liter$^{-1}$ | RSD (%) | μg kg$^{-1}$ | RSD (%) | Range (μg kg$^{-1}$) | |
| River Fyris | 0.58† | | 3.6‡ | 121 | 0.39–13§ | 6.2 |
| High (1000 μg liter$^{-1}$) | 910‖ | 11 | 4900¶ | 33 | 1500–8500# | 5.3 |
| Low (1 μg liter$^{-1}$) | 1.8‖ | 46 | 18¶ | 39 | 6.6–36# | 9.7 |
| Control (0 μg liter$^{-1}$) | >LOQ** | | >LOQ†† | | | |

*Estimated bioaccumulation factors based on average values in water and fish muscle tissue from field and experimental measurements. †Single grab sample. ‡Average value, $n$ = 10. §Range from minimum to maximum in μg liter$^{-1}$, $n$ = 10. ‖Average value, $n$ = 49. ¶Average value, $n$ = 25. #Range from minimum to maximum in μg liter$^{-1}$, $n$ = 25. **Below limit of quantification (LOQ), $n$ = 49. ††Below limit of quantification, $n$ = 25. For more details, see supplementary materials.

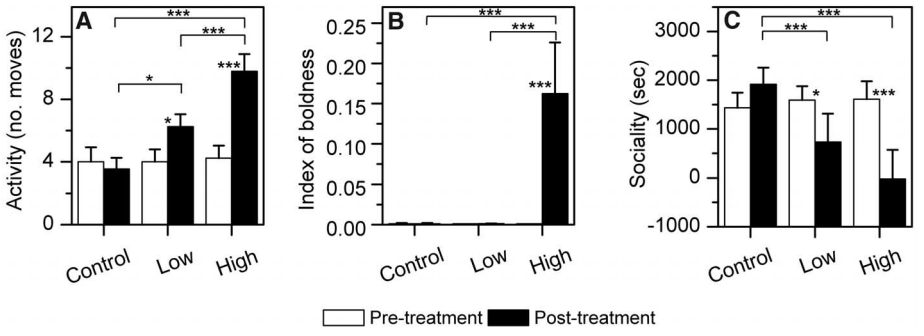


**Fig. 1.** Fish behavioral response to two concentrations (low: 1.8 μg liter$^{-1}$; high: 910 μg liter$^{-1}$) of dissolved oxazepam compared to control treatment (0 μg liter$^{-1}$). (**A**) Activity, measured as number of swimming bouts (>2.5 cm) during 10 min. (**B**) Boldness, measured as the inverse of latency to enter a novel area during the total trial time (900 s). (**C**) Sociality, measured as the cumulative time (in seconds) spent close to a group of conspecifics. Error bars represent ±1 SE ($n$ = 25 in all treatments); statistically significant differences between the pre- and posttreatments are indicated (*$P$ < 0.05 or ***$P$ < 0.001).

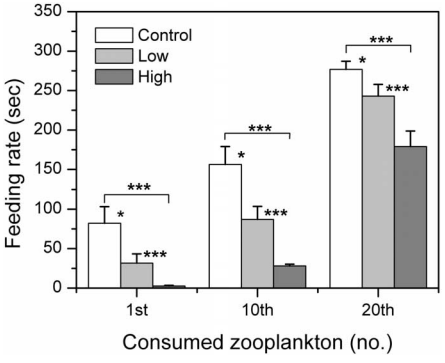


**Fig. 2.** Feeding rate of perch after oxazepam treatments. Feeding rate is expressed as the latency to capture the first zooplankton, the 10th zooplankton, and the 20th zooplankton. Error bars represent ±1 SE ($n$ = 25 in all treatments); statistically significant differences between the control and treatments are indicated (*$P$ < 0.05 or ***$P$ < 0.001).

was pronounced (Fig. 2). Individuals exposed to the high concentration started feeding earlier and depleted the food resource faster than those exposed to the low concentration ($F_{1,48}$ = 46.3, $P$ = 0.0001 and $F_{1,48}$ = 25.0, $P$ = 0.0001, respectively) and the unexposed individuals ($F_{1,48}$ = 79.6, $P$ = 0.0001 and $F_{1,48}$ = 30.1, $P$ = 0.0001, respectively). This enhanced feeding rate fits well with the observed drug-induced increases in boldness and activity (*7*, *20*). Moreover, also fish exposed to the low concentration initiated feeding earlier ($F_{1,49}$ = 5.9, $P$ = 0.019) and depleted the food resource faster ($F_{1,49}$ = 6.0, $P$ = 0.018) than unexposed fish. That is, fish exposed to oxazepam concentration similar to that found in River Fyris showed altered foraging behavior that resulted in an accelerated depletion of the food resource. Correlations between the different behavioral traits and between the behavioral traits and feeding rate (table S1) suggest that oxazepam exposure induced bolder behavior that, in turn, increased feeding rate. Before exposure, none of the behavioral traits were significantly correlated with each other or with feeding rate, suggesting that oxazepam affects not only individual traits but also how the traits are correlated—relationships referred to as behavioral syndromes (*12*). Considering that the concentrations of oxazepam in muscle tissue of perch exposed to the low concentration are similar to those in wild-caught fish (Table 1), it is likely that behavior and feeding rates are modified also in wild fish exposed to dilute concentrations of anxiolytic drugs.

Changes in fish feeding rate may, over time, have ecosystem-level consequences, as fish are known to influence the structure of aquatic communities (*21*). Given that we found pharmaceutical effects on ecologically important behavioral traits, at environmentally relevant concentrations in water and muscle tissue, ecosystem-level consequences in natural systems seem likely. For example, increased feeding rate, as we observed, may result in top-down effects on primary production (algae) via suppression of primary consumers (zooplankton), especially because some organisms (e.g., zooplankton and algae) lack GABA receptors—a prerequisite for the pharmaceutical effects of benzodiazepines (*6*). However, increased activity and boldness, and reduced sociality (lower prevalence of shoaling), may also increase predation risk (*22*, *23*), making the net outcome of these pharmaceutical effects difficult to predict. Regardless of this uncertainty, it seems likely that fish fitness and food-web structures are altered in oxazepam-contaminated waters.

That environmentally relevant concentrations of a single benzodiazepine affect fish behavior and feeding rate is alarming, considering the cocktail of different pharmaceutical products that are found in waters worldwide (*1*, *4*, *24*). It should also be emphasized that there are several benzodiazepines, and direct additive effects from these compounds on behavior traits cannot be excluded. Further, increasing concentrations of pharmaceutical residues in aquatic systems can be expected, as pharmaceutical use is projected to increase as they become more available for the growing global population (*25*). Our results highlight ecologically important, previously underappreciated effects of psychotherapeutic drugs that enter aquatic ecosystems, and call for new test protocols to examine the full environmental impact of pharmaceutical residues.

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## Supplementary Materials

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Materials and Methods
Table S1
Additional Data
Reference (*26*)

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Editor's Summary

**Unintended Recipients of Antidepressants**

Pharmaceuticals are used to treat a wide variety of ailments and conditions in humans. However, many animal species share physiologies, receptors, and pathways that may be acted upon by pharmaceutical compounds. Increasingly, pharmaceuticals are being found in natural aquatic systems. Such pharmaceutical pollution can cause mortality and alter development and reproduction of aquatic animals. **Brodin *et al.*** (p. 814) report that excreted drugs may also have far more subtle, yet eventually significant, impacts in natural systems. Benzodiazepines, which reduce anxiety in humans, alter social and foraging behavior in fish. European perch exposed to oxazepam were bolder, more active, less social and fed more rapidly.