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

T. Brodin, J. Fick, M. Jonsson, J. Klaminder

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 micrograms 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 micrograms liter\(^{-1}\) in rivers and streams (2, 3). Because benzodiazepines are designed to alter behavior by binding to \(\gamma\)-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 micrograms liter\(^{-1}\) in treated wastewater effluent and 0.58 micrograms 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 micrograms liter\(^{-1}\) and a high concentration of 910 micrograms 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

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Water µg liter(^{-1})</th>
<th>RSD (%)</th>
<th>Fish muscle tissue µg kg(^{-1})</th>
<th>RSD (%)</th>
<th>Range (µg kg(^{-1}))</th>
<th>BAF*</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Fyris</td>
<td>0.58†</td>
<td></td>
<td>3.6‡</td>
<td>121</td>
<td>0.39–13§</td>
<td>6.2</td>
</tr>
<tr>
<td>High (1000 µg liter(^{-1}))</td>
<td>910</td>
<td></td>
<td></td>
<td>11</td>
<td>4900¶</td>
<td>33</td>
</tr>
<tr>
<td>Low (1 µg liter(^{-1}))</td>
<td>1.8‖</td>
<td>46</td>
<td>18‖</td>
<td>39</td>
<td>6.6–36#</td>
<td>9.7</td>
</tr>
<tr>
<td>Control (0 µg liter(^{-1}))</td>
<td>&gt;LOQ**</td>
<td></td>
<td>&gt;LOQ†</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*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, \(n = 49\). #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.
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.

References and Notes
9. Materials and methods are available as supplementary materials on Science Online.

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Supplementary Materials
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Table S1
Additional Data
Reference (26)
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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) Sociability, 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).
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.