Social status modulates the behavioral and physiological consequences of a chemical pollutant in animal groups

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Abstract. The social environment (i.e., the suite of social interactions that occur among individuals that can result in variation in social ranks) is a commonly overlooked aspect of biology when scientists evaluate the effects of chemical contaminants. The social environment, however, represents the arena in which individual-level performance shapes group- or population-level outcomes and may therefore mediate many of the ultimate consequences of chemicals for wildlife. Here, we evaluated the role that the social environment plays in determining the consequences of pollutant exposure. We exposed groups of juvenile brown trout (*Salmo trutta*) to an emerging pharmaceutical pollutant that is commonly detected in freshwaters (the benzodiazepine, oxazepam) and allowed them to form dominance hierarchies. Exposure affected dominant and subordinate fish differently, causing fish to become less aggressive at high doses and subordinate fish to become more competitively successful at low doses. These perturbations had further consequences for growth, fin damage, and survival. Exposure also modulated physiological stress in the hierarchy, and social status itself affected how much oxazepam was absorbed in tissues, potentially creating a dynamic feedback loop that further influences the asymmetric effects of exposure on differing social statuses. Many effects followed a "U-shaped" dose-response curve, highlighting the importance of nonlinear, low-dose effects. Altogether, we show that social structure in animal groups can interact with and modulate the effects of an environmental contaminant. We underscore the need to account for an organism's natural ecological context, including their social environment, in future experiments and environmental risk assessments to predict the effects of chemical contaminants on wildlife.

Key words: behavior; cortisol; dominance; ecotoxicology; exposure; fish; oxazepam; pharmaceutical; Salmo trutta; social status; trout.

Introduction

Pharmaceutical pollution is a widely recognized environmental problem (Boxall et al. 2012, Bernhardt et al. 2017, OECD 2019). Pharmaceuticals are specifically designed to have therapeutic effects on humans at low, non-toxic doses, and many of their biological targets (e.g., receptors) are conserved across vertebrate taxa (Gunnarsson et al. 2008). Consequently, there is mounting concern that these compounds could have similar, but wholly unintended, effects in exposed wildlife (Boxall et al. 2012, Saaristo et al. 2018). Pharmaceuticals enter the environment, especially aquatic habitats, via several routes (e.g., domestic wastewater, manufacturing) and are now being measured in surface waters on a global scale (aus der Beek et al. 2016). A growing body of evidence is showing that compounds like antidepressants, anxiolytics, and synthetic hormones can have sublethal effects on the physiology and behavior of aquatic animals, altering traits that are crucial for their survival and reproduction (Söffker and Tyler 2012, Saaristo et al. 2018). For example, the antidepressant fluoxetine has been shown to delay or disrupt antipredator responses in multiple fish species (Weinberger and Klaper 2014, Pelli and Connaughton 2015, Martin et al. 2017). Much of our understanding of how pharmaceutical contaminants affect aquatic wildlife is largely limited to studies on

individual organisms, often housed and tested in isolation. Yet, animals display a range of social phenotypes, with inter- and intraspecific interactions contributing to fitness outcomes in the wild (e.g., via cooperation, competition, dominance). The social environment, which constitutes the pattern of complex and non-random interactions among individuals (Kurvers et al. 2014), should therefore be an important aspect of biology when scientists evaluate the effects of pharmaceutical pollutants on wildlife.

Social environments are important because many animals live in groups or societies that are organized into hierarchies (Ellis 1995). Dominance hierarchies are a consequence of dyadic, usually aggressive, interactions among group members that determine an individual's social status (i.e., being subordinate vs. dominant; Drews 1993). Social statuses are associated with all or a combination of morphological, physiological, or behavioral traits (Creel 2001, Sloman and Armstrong 2002). For instance, in African wild dogs (*Lycaon pictus*), dominant males are more aggressive, have higher testosterone and cortisol levels, and monopolize reproduction when compared to subordinates (Creel et al. 1997). Dominance hierarchies can strongly influence individual fitness outcomes because hierarchy status determines access to key resources (e.g., food, habitat, mates), leading to considerable variation in growth, predation risk, mating opportunities, and survival across the hierarchy (Fretwell 1972, Huntingford and Turner 1987, Ellis 1995).

Pharmaceutical pollutants like antidepressants or anxiolytics are likely to disrupt dominance hierarchies because they are specifically designed to modify social behavior. Such pharmaceuticals exert their therapeutic effects by agonizing (enhancing) or antagonizing (reducing or blocking) pre-existing neural substrates like receptors or transporters that modulate natural physiology and behavior. For example, the antidepressant fluoxetine acts by blocking the reuptake of the neurotransmitter serotonin into neurons by the serotonin transporter, thereby causing an increase in serotonin concentrations. Fish exposed individually to antidepressants (e.g., SSRIs) and anxiolytics (e.g., benzodiazepines) have been shown to exhibit a dampened physiological stress response, altered androgen profiles in situ, reduced aggression, and increased activity and boldness (Fernandes et al. 2011, Brodin et al. 2013, De Abreu et al. 2014, McCallum et al. 2017). These exposure-induced changes to individual physiology and behavior could have consequences in a hierarchy context because aggression and stress dynamics often underpin social status and dominance relationships (Creel 2001). Oxazepam is a benzodiazepine anxiolytic that is highly prescribed to treat anxiety, sleep disorders, and muscle spasms (Farach et al. 2012). Benzodiazepines like oxazepam have a sedative or stress-relieving effect by agonizing the $GABA_A$ receptor, increasing its affinity for the inhibitory neurotransmitter, GABA, that hyperpolarizes neurons (Korpi et al. 2002). Oxazepam is of environmental concern because it persists in sediments and has been measured in surface waters at concentrations up to 1.8 µg/L (Loos et al. 2013, Klaminder et al. 2015, Fick et al. 2017); moreover, it is just one of a family of benzodiazepine compounds with the same mechanism of action that are measured in the environment together (Fick et al. 2017). A concentration of ~2 µg/L has been shown to affect certain fish species; for example, activity and risk-taking behavior in both the Eurasian perch (*Perca fluviatilis*) and the common roach (*Rutilus rutilus*), and migratory behavior in Atlantic salmon (Brodin et al. 2013, Hellström et al. 2016, Brodin et al. 2017). While the effects of oxazepam (and many other pharmaceuticals) on individual fish have been described (e.g., Brodin et al. 2013, 2017, Vossen et al. 2020), rarely has social status or social context, like a dominance hierarchy, been considered when assessing consequences of pharmaceutical pollution for aquatic wildlife. Previous research on metals and synthetic estrogens has indicated that social status can interact with pollutant exposure to affect animals in unique ways that would not have been revealed without considering the natural social context (e.g., Sloman et al. 2007, Coe et al. 2008, Filby et al. 2012).

Here, we fill this gap by testing the hypothesis that exposure to the pharmaceutical pollutant oxazepam will modulate physiological stress, disrupt dominance hierarchies, and have negative consequences for behavior and growth in groups of wild fish. We focused on juvenile brown trout, a well-described model system for understanding the causes and physiological consequences of dominance relationships (reviewed in Sloman and Armstrong 2002, Gilmour et al. 2005). Juvenile brown trout are territorial and form dominance hierarchies in the laboratory and in natural streams to access food and optimal habitat resources. Hierarchies form most strongly among young juveniles when such resources are limited, and hierarchies will dissolve when fish migrate to still waters as adults (i.e., lake or sea; Jonsson and Jonnson 2011). Dominant individuals typically display increased aggression, larger body size, and reduced physiological stress when compared to subordinates, and consequently they have been shown to grow faster and have increased survival and fitness (Höjesjö et al. 2002, Jonsson et al. 2010). The hierarchies formed by brown trout are characteristic of many salmonid species, and are generally linear, where aggression is mostly directed downward in the hierarchy (Jonsson and Jonsson 2011). We exposed groups of four fish to a control dose, an environmentally relevant dose, or a higher, therapeutic dose of oxazepam and allowed them to form dominance hierarchies over five days. Specifically, we evaluated how exposure and social status affected how much oxazepam was absorbed in muscle tissue, plasma cortisol levels, competitive foraging, and pairwise aggressive interactions. We then quantified the consequences of exposure and social status by measuring change in body mass, fin damage, and survival. Because oxazepam is designed to reduce anxiety and stress in humans (Mihic and Harris

2014), we predicted that exposure would reduce plasma cortisol levels, reduce pairwise aggression, and promote equal food access among group members, translating to more equal mass gain, reduced fin damage, and increased survival. However, exposure may also affect subordinate and dominant individuals asymmetrically because each status has distinct physiological and behavioral profiles. We therefore explored the interaction between exposure and social status in our measured endpoints.

METHODS

Collection and housing

We collected 160 juvenile brown trout (*Salmo trutta*) from Norrfors, near Umeå, Sweden (mean ± SD: 15 ± 1 cm standard length; 45.6 ± 11.5 g mass; ~1–2yr old), a site that does not receive any known sources of pharmaceutical pollution (e.g., wastewater or livestock effluents). We transported fish to Umeå University and housed them in large 1,000-L, aerated, flow-through tanks in high densities such that dominance hierarchies did not form. We fed fish commercial pellets (INICIO 917, BioMar, Brande, Denmark) until satiation once per day, and fish were held for at least 7 d before exposures began to ensure recovery from transport and normal feeding. We maintained the fish on a 12 light:12 dark cycle. This research was approved under ethical permit Dnr. A-18-15 to T. Brodin from Jordbruksverket.

Exposure and dominance hierarchy trials

We exposed fish to one of three oxazepam treatments: a control, low, or high dose (see *Analytical chemistry* for measured concentrations). These treatments were selected to represent a pharmaceutically polluted habitat (e.g., near a wastewater outfall) and a higher, human-therapeutic dose. We dissolved oxazepam (Merck, CAS ID: 604-75-1) in ultrapure water prepared by a Milli-Q Gradient water system (Millipore, Billerica, Massachusetts, USA) to make concentrated stock solutions to dose exposure tanks. New stock solutions were prepared weekly.

Before exposures, we anesthetized the fish (MS222) and measured their standard length, mass (to the nearest 0.01 g), and made a unique fin clip on the dorsal fin to facilitate identification during behavioral trials. We then scored damage to the caudal fin (and again at the end of the experiment) to quantify the physical costs of aggression using a previously established scale (Hoyle et al. 2007). Each fish was then transferred to an aerated individual exposure tank (12 L, plastic, black, opaque) and allowed to recover for 24 h before starting exposures. Fish were first exposed to oxazepam individually for 5 d to ensure fish absorbed the pharmaceutical to steady-state equilibrium (previously shown to take ~5 d; Brodin et al. 2013). After 5 d, we transferred individuals in groups of four to larger, 120-L glass aquaria equipped with an air stone and a small motor to create a current (Appendix S1: Fig. S1) where they continued to be exposed to the same treatment of oxazepam for another 5 d (fish were therefore exposed for 10 d in total). All groups were size matched by standard length. Variation (standard deviation) in mean group mass and mean group standard length did not differ between the assigned oxazepam treatments (linear model$_{mass}$ $F_{2,37} = 1.30$, $P = 0.29$; linear model$_{SL}$ $F_{2,37} = 0.29$, $P = 0.75$). Moreover, size-matching was effective given that neither body mass nor standard length predicted whether a fish would be assigned as dominant or subordinate in their groups (binomial generalized linear mixed effects model: mass, [estimate ± SE] 0.03 ± 0.02, $Z = 1.80$, $P = 0.07$; standard length, 0.003 ± 0.14, $Z = 0.89$, $P = 0.37$). In both the individual exposure tanks and the group exposure tanks, we took water samples 1 h after adding the dose and again on day 5 and froze them at $-20°C$ for further analysis. We monitored temperature using thermologgers (range: 16–19°C; iButtons, Maxim Integrated Products, San Jose, CA, USA) and pH of the water used was ~8.0 (reported by the municipality, Vakin Umeå).

We video recorded (2.65 MP/1080p, Sony HDR-PJ50) group behaviors from the side of the tank once on the first day between 14:00 and 17:00 and then twice per day, between 8:00 and 11:00 and 14:00 and 17:00, respectively. We recorded nine behavioral trials in total (across 5 d) in each group. Recordings were taken behind an opaque barrier to limit experimenter influence. During each trial, we recorded video for 10 minutes in total, adding three or four food pellets halfway through (i.e., after 5 minutes). Adding food halfway through ensured that we would capture aggressive interactions among the individuals, regardless of whether aggression occurred primarily before or during food competition. We introduced food via a PVC tube connecting the top of the tank to the opaque blind concealing the experimenter. The fish were fed pellets until satiation after the afternoon trials.

Two hours after the final behavioral recording, we quickly netted all fish from the same tank and euthanized them with an overdose of MS222 (in <2 minutes). We sampled blood via caudal vein puncture using chilled, EDTA-coated needles and syringes, centrifuged the blood for 6 minutes (16,000 g, Eppendorf 5415C, Hamburg, Germany) and drew off the plasma supernatant. We removed the brain (for other analyses), and both plasma and brain samples were frozen and stored at $-80°C$. Fish were weighed, caudal fin damage was scored, and whole fish were frozen at $-20°C$.

Videos were later scored blind to fish ID and oxazepam treatment for all aggressive interactions and feeding behavior following an ethogram (Appendix S1: Table S1). We recorded the aggressor and receiver of each interaction, allowing us to tally the total number of aggressive acts performed and the total aggressive acts

received by each individual. Individual fish were assigned a social status as either dominant (one per group) or subordinate (three per group) based on their ranking for aggression given to others minus aggression received from others summed across all trials. We assigned social rank in a binary fashion, dominant vs. subordinate, as opposed to assigning linear ranks to all individuals in each group (as in Sloman et al. 2008). We did this because we deemed the variation among subordinates in our data set to be too narrow to reliably assign relative ranks to these individuals. Fish assigned the dominant status in this way also typically occupied the most valuable position in the water column, with the other fish being restricted to the tank periphery, reaffirming their social status ranking (Sloman and Armstrong 2002).

ELISA

Cortisol concentrations in plasma samples were analyzed with a commercial enzyme linked immunosorbent assay (ELISA) kit (product #402710; Neogen Corporation, Lexington, Massachusetts, USA). Following the manual's instructions, all samples were diluted $50\times$ using extraction buffer included in the ELISA kit, and plates were read on a microplate reader (Eon, BioTek, Winooski, VT, USA). Each sample was run in duplicate during a single assay with an average intra-assay coefficient of variation of 3.33%. The assay manufacturer states that this kit has cross-reactivity with Cortisone (15.7%), 11-Deoxycortisol (15%), and Corticosterone (4.81%). This kit has been used previously to quantify plasma cortisol in salmonids (e.g., pink salmon, *Oncorhynchus gorbuscha* [McConnachie et al. 2012]; rainbow trout, *Oncorhynchus mykiss* [Schjolden et al. 2009, Backström et al. 2011]) as well as in other fishes (e.g., *Neolamprologus pulcher* [Culbert et al. 2018, 2019]; *Perca fluviatilis* [Heynen et al. 2016]).

Analytical chemistry

The extraction of oxazepam from muscle tissue and preparation of water samples for chemical analysis followed protocols described previously (McCallum et al. 2019). Briefly, a sample of muscle tissue (0.1 ± 0.01 g) was taken from the dorsal flank of each fish in a 2 mL polypropylene (PP) tube. We added 50 ng of internal standard (Oxazepam-D5, CAS-ID 65854-78-6, Merck; 50 µL of 1 µg/mL in methanol), zirconium beads, and 1.5 mL of acetonitrile, and then homogenized the sample for 4 minutes at 42,000 oscillations per minute (Mini Beadbeater, Biospec, Bartlesville, Oklahoma, USA) and centrifuged at 17,500 g for 10 minutes (Beckman Coulter Microfuge 22R Centrifuge, Indianapolis, IN, USA). The eluent was removed and the process repeated a second time (but without adding new internal standard or beads). Eluents were combined, evaporated under an air stream to dryness, and reconstituted in 130 µL of

methanol. Final extracts were transferred to 2-mL glass autosampler vials equipped with 200-µL inserts and frozen at $-18°C$ (for a minimum of 24 h). Samples were centrifuged again directly before analysis to separate precipitated proteins from supernatant and analyzed using method of liquid chromatography tandem mass spectrometry (LC-MS/MS). Water samples were defrosted at room temperature, 3 mL of each sample was weighed, filtered through a 0.45-mm syringe filter (Filtropur S, Sarstedt, Nümbrecht, Germany) into the 10-mL autosampler glass vial, and 5 ng of internal standard was added. Water samples were analyzed using method based on an online solid phase extraction system coupled with liquid chromatography-tandem mass spectrometry (SPE LC-MS/MS). Further details of the instrumental analysis are presented in the appendix (Appendix S1: Section S1, Tables S2–S4). Isotope dilution was used to quantify oxazepam in water and in muscle tissue samples. No water oxazepam concentrations above the limit of quantification (1 ng/L) were detected in any control tank, and the average exposure concentrations for low and high treatment tanks were (mean $\pm$ SD): 0.7 ± 1.9 µg/L and 0.8 ± 0.7 µg/L (for individual and group exposure tanks, respectively) and 15.5 ± 1.9 µg/L and 18.5 ± 2.6 µg/L (for individual and group exposure tanks, respectively). Concentrations did not decrease over the exposure period (see Appendix S1: Table S5; Fig. S2).

Statistical analyses

All statistical analyses were conducted using R (version 3.6.1; R Core Team 2018). All analyses were conducted using the *glmmTMB* package (Brooks et al. 2017), and model assumptions were visually inspected using quantile-quantile and residuals-vs.-fitted plots (*DHARMa* package; Hartig 2019). We used linear mixed-effects models (LMM) to test for effects of oxazepam exposure and social status on plasma cortisol concentrations and oxazepam bioconcentration in muscle tissue. In these analyses, we ln-transformed both response variables to meet parametric assumptions, and control fish were not included in the analysis of oxazepam in muscle tissues because all tested control tissues were below the limit of quantification. We used negative binomial generalized linear mixed effects models (GLMM.nb) to test for effects of oxazepam exposure (control, low, or high), social status (dominant or subordinate), and trial number (numeric, 1–9) on individual aggression (total aggression given per trial) and foraging (number of food pellets consumed). We next evaluated how oxazepam exposure affected the aggression that was directed between individuals of different social statuses within each group (i.e., subordinate to dominant, dominant to subordinate, subordinate to subordinate). To do this, we summed the total number of aggressive acts of each dyad type (i.e., all subordinate to dominant aggression, all dominant to subordinate aggression, all

subordinate to subordinate aggression) within each trial per group, and analyzed these using the GLMM.nb models. We used LMMs to test for effects of oxazepam exposure and social status. We used a Bayesian binominal GLMM (blme package; Dorie 2016) to analyze the effect of exposure and social status on the proportion of fish surviving. For this analysis, a Bayesian approach was needed because of perfect separation in the response variable (Mansouria et al. 2018). In this model, fish survival was used as the response variable, while exposure, social status and the standard deviation in group body mass were included as fixed effects, and we included a weak prior based on a multivariate normal distribution. A random intercept of group ID was also included to account for covariation among individuals from the same group. In all models, we tested for interactions between social status and exposure (and also between exposure and trial number for behavioral measures), and removed these interactions to simplify the models when nonsignificant. We included a random intercept of tank ID and a random slope of trial ID where appropriate. Full model descriptions, syntax, and output are detailed in the supplementary materials (Appendix S1: Section S2).

RESULTS

Physiological effects of exposure depend on social status

Subordinate fish absorbed more oxazepam in their muscle than dominant fish (Fig. 1A; LMM, $N = 103$, estimate $\pm$ SE 0.34 ± 0.06, $Z = 5.42$, $P < 0.0001$), and this was not driven by their body mass (-0.0002 ± 0.004, $Z = -0.05$, $P = 0.96$). High-dose fish absorbed more oxazepam than low-dose fish (3.19 ± 0.063, $Z = 50.47$, $P < 0.0001$).

Fish exposed to the high dose of oxazepam tended to have lower basal cortisol concentrations than control fish (Fig. 1B; LMM, $N = 144$, estimate $\pm$ SE -0.84 ± 0.44, $Z = -1.90$, $P = 0.058$; control vs. low, -0.36 ± 0.44, $Z = -0.81$, $P = 0.42$; high vs. low, 0.48 ± 0.44, $Z = 1.10$, $P = 0.27$), and dominant fish, regardless of treatment, had lower basal cortisol than subordinate fish (0.78 ± 0.27, $Z = -2.89$, $P = 0.004$). Basal cortisol levels for dominant fish in the high, low, and control groups were 3.5 ± 9.5, 9.2 ± 23.6, and 7.6 ± 19.9 ng/mL, respectively. Basal cortisol levels for subordinate fish in the high, low, and control groups were 7.2 ± 22.8, 14.5 ± 39.1, and 53.3 ± 95.9 ng/mL, respectively.

Aggression and feeding are modulated by exposure and social status

Fish exposed to the high dose of oxazepam were less aggressive than fish exposed to the low dose of oxazepam, regardless of social status (Fig. 2A; GLMM.nb, $N = 158$, estimate $\pm$ SE, -0.41 ± 0.20, $Z = -1.99$, $P = 0.047$), while neither high-dose or low-dose fish differed from controls (control vs. high, -0.33 ± 0.21,

$Z = -1.56$, $P = 0.12$; control vs. low, 0.075 ± 0.20, $Z = 0.35$, $P = 0.72$). Dominant fish were more aggressive than subordinate fish (Fig. 2A; 3.00 ± 0.18, $Z = 17.07$, $P < 0.0001$), and aggression declined over time (-0.06 ± 0.03, $Z = -2.24$, $P = 0.025$).

The observation that aggression was dampened in the high-dose group appears to be in part driven by less aggression from subordinates toward dominants in the high-dose group when compared to the low-dose group (GLMM.nb, $N = 360$, estimate $\pm$ SE -1.53 ± 0.56, $Z = -2.73$, $P = 0.006$). However, subordinate-to-dominant aggression did not clearly differ between the low-dose group and the control group (control vs. low, 0.64 ± 0.55, $Z = 1.17$, $P = 0.24$) nor the high-dose group and the control group (control vs. high, -0.88 ± 0.58, $Z = -1.53$, $P = 0.13$). Subordinate-to-dominant aggression did not change significantly over time (-0.07 ± 0.09, $Z = -0.72$, $P = 0.47$). In contrast, both dominant-to-subordinate aggression and subordinate-to-subordinate aggression showed little change with treatment (all exposure contrasts $P > 0.1$, see Appendix S1: Tables S10, S11), but both dominant-to-subordinate aggression (-0.18 ± 0.06, $Z = -2.86$, $P = 0.004$) and subordinate-to-subordinate aggression (-0.17 ± 0.05, $Z = -3.30$, $P = 0.0009$) declined across time.

While dominants tended to eat more than subordinates, the difference in the amount of food eaten by dominants and subordinates was significantly smaller in the low-dose group than the high-dose group (Fig. 2B; GLMM.nb, $N = 158$, exposure $\times$ status interaction, estimate $\pm$ SE 1.59 ± 0.57, $Z = 2.78$, $P = 0.005$). That is, subordinates fed relatively more successfully in the low-dose group compared to the high-dose group. This difference was also detectable between the low-dose group and the control group, though it did not reach statistical significance (1.02 ± 0.58, $Z = 1.76$, $P = 0.07$), and there was no effect in the high-dose group compared to the control group (Fig. 2B; -0.57 ± 0.61, $Z = -0.93$, $P = 0.35$). Feeding success for subordinates and dominants did not change significantly over time (-0.002 ± 0.01, $Z = -0.11$, $P = 0.91$).

Physical consequences of exposure and social status

Dominant fish incurred more damage to their caudal fins relative to subordinate fish in the low-exposure group when compared to the control group (Fig. 3A; LMM, $N = 147$, exposure $\times$ status interaction, estimate $\pm$ SE -0.80 ± 0.24, $Z = -3.36$, $P = 0.0008$) and high exposure (-0.62 ± 0.24, $Z = -2.66$, $P = 0.0079$), while fin damage among subordinates and dominants did not differ between the control and high-exposure groups (-0.19 ± 0.24, $Z = -0.77$, $P = 0.44$).

Dominant fish lost more mass relative to subordinate fish in the low-exposure group when compared to the control exposure group (Fig. 3B; LMM, exposure $\times$ status interaction, $N = 147$, estimate $\pm$ SE, 1.82 ± 0.87, $Z = 2.09$, $P = 0.037$) and high-exposure group ($2.28 \pm$
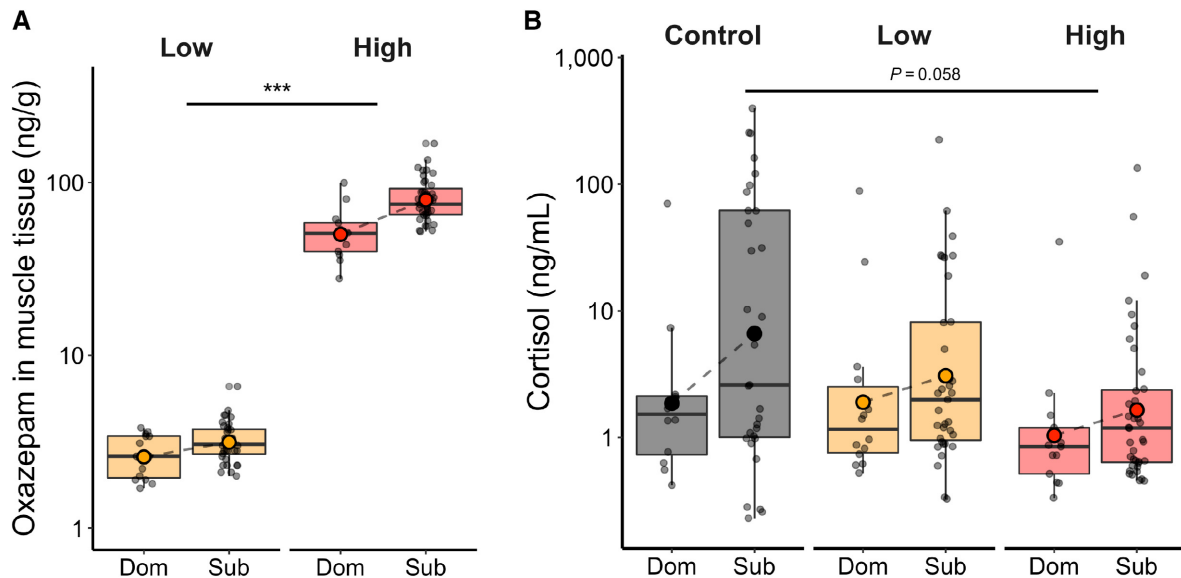
Fig. 1. (A) Concentrations of oxazepam in muscle tissue plotted by social status (dom, dominant; sub, subordinate) and grouped by treatment. The asterisks *** indicate that treatments are significantly different at $P \leq 0.001$. (B) Cortisol concentrations in plasma plotted by social status, grouped by treatment. All plots show raw data points, along with box plots (showing medians and the interquartile range) and means for each group (large colored circle), and do not visualize other covariates or random effects.

0.85, $Z = 2.69$, $P = 0.007$), while change in mass among subordinates and dominants did not differ between control and high exposure (-0.46 ± 0.88, $Z = -0.52$, $P = 0.60$).

Fish exposed to the low-dose tended to have higher mortality than fish exposed to the high dose, though this did not reach statistical significance (Fig. 3C; Bayesian binomial GLMM $N = 158$; high vs. low, estimate $\pm$ SE 2.10 ± 1.11, $Z = 1.90$, $P = 0.057$; control vs. high, 1.12 ± 1.74, $Z = 1.18$, $P = 0.24$; control vs. low, -2.42 ± 1.74, $Z = -1.39$, $P = 0.17$). Survival did not differ clearly between social statuses (1.94 ± 1.12, $Z = 1.73$, $P = 0.08$), although there was never mortality among the dominant fish.

DISCUSSION

The trout in our study, despite being size matched, quickly formed dominance hierarchies with dominant fish displaying the most aggression, monopolizing access to food resources, and having lower cortisol levels than subordinates, which are characteristic for this species (Sloman et al. 2000). Generally, the hierarchies also stabilized over time as aggression declined across trials. Exposure to oxazepam, however, disrupted the relationship between dominant and subordinate fish, particularly in the low-dose group. We predicted that exposure would lead to a reduction in stress and aggression for all group members in the hierarchy. Indeed, we found that fish were overall less aggressive in the high-dose group relative to the low-dose group, which could indicate an aggression-reducing effect of oxazepam when exposed in

high doses. However, our results regarding foraging, mass loss, and caudal fin damage showed that subordinates were also more competitively successful in the low-dose group relative to the high-dose group, which could indicate behavioral disinhibition of subordinates at low doses. Certain anxiolytics like oxazepam have been shown to disinhibit subordinate individuals' behavior towards dominants (reviewed in Miczek et al. 2003, Albrecht et al. 2014), however, the effects of benzodiazepines on aggression in fish have not been extensively studied (see Hellström et al. 2020 for a reproductive context). The idea that low-dosage exposures caused behavioral disinhibition of subordinates is further supported by our observation that subordinate fish in the low-dose group became comparatively more successful relative to the dominant individual at securing food in the competitive foraging task. Altogether, our study suggests that the disinhibitory effects of oxazepam can depend on an organism's social status.

Oxazepam exposure did not completely abolish the formation of dominance hierarchies as behaviorally dominant and subordinate fish were still identifiable, despite the aggressive and foraging behaviors of the group members being altered. However, the net benefit of becoming the dominant individual in the social hierarchy appears to shrink when exposed to oxazepam; dominant fish incurred more fin damage and lost more mass relative to subordinates in the low-dose group when compared to the other treatment groups. Survival also tended to be lower for subordinates in the low-dose group. These consequences are in line with the behavioral disinhibition of subordinate fish, leading
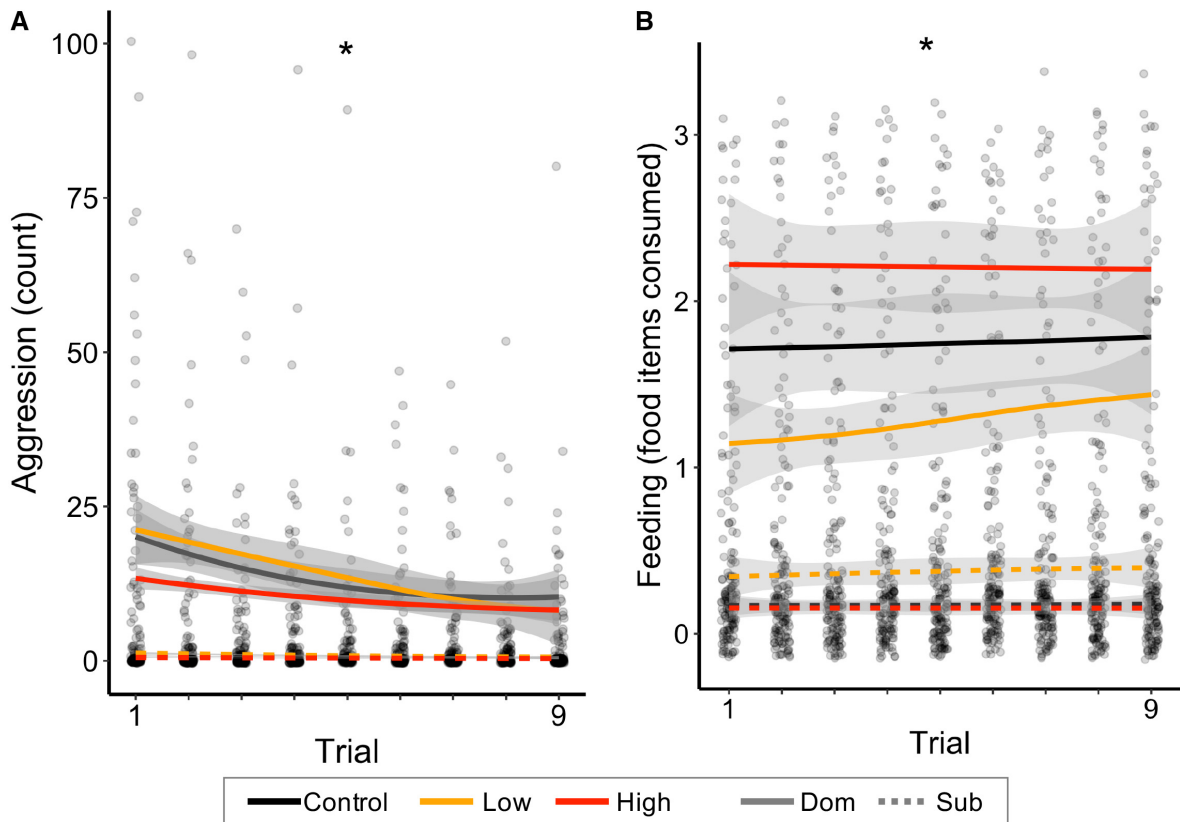
FIG. 2. (A) Aggression (count) plotted over trial, by treatment and social status. An asterisk indicates a significant effect of treatment ($P \leq 0.05$). (B) Feeding (count) plotted over trial, by treatment and social status. An asterisk indicates a significant effect of treatment by social status interaction ($P \leq 0.05$). All plots show predicted model values, with means and the 95% confidence intervals around the mean.

subordinates to incur less fin damage and to capture more food items (dominant fish also lost more mass). Thus, while social hierarchies did not collapse under exposure to oxazepam, our results suggest that the social relationships between dominants and subordinates became disrupted and the distinction between fish of both social statuses becomes less clear. While subordinates achieved greater benefits (though possibly at a greater risk), dominants often incurred more costs, potentially shrinking the cost–benefit asymmetries across social ranks.

Exposure to benzodiazepines like oxazepam could have fitness implications for these animals in the wild if exposure reduces the asymmetry between dominant and subordinate individuals. As brown trout mature, one potential consequence of exposure is a reduction in the variance exhibited across individuals in their competitive abilities, either in terms of their resource holding potential (known to be associated with body size and body condition; Hurd 2006) or in their adoption of alternative reproductive tactics (ARTs). In species expressing ARTs, like many salmonids, the determination of which tactic to adopt is often based on a conditional strategy whereby individuals employ one tactic or another,

dependent on their expression of traits relative to critical thresholds (i.e., threshold traits; see Roff 1996). Traits like body size, condition, and growth rate are prime candidates for driving ART decisions (Taborsky and Brockmann 2010), and these are also traits that could be affected by oxazepam exposure.

Many of the effects of oxazepam in our study were most apparent between the low- (measured ~0.8 µg/L) and high-dose (measured ~18 µg/L) groups and the control group was intermediate. Many anxiolytic pharmaceuticals exhibit a biphasic or inverted U-shaped behavioral response in mammalian study systems, where low doses cause disinhibition (i.e., individuals become more active or bold [less anxious] and more aggressive) and higher doses lead to sedation (Miczek et al. 2003, Calabrese 2008). Our results are consistent with such a pattern, and this type of response has been reported in prior studies with fish exposed to benzodiazepines (De Abreu et al. 2014, Lorenzi et al. 2016, Brodin et al. 2017). For instance, Lorenzi et al. 2016 found that fathead minnow (*Pimephales promelas*) reproduction was increased when fish were exposed to 0.1 µg/L of diazepam and not the higher doses. It is clear that plasma cortisol, one mechanism by which oxazepam may induce its
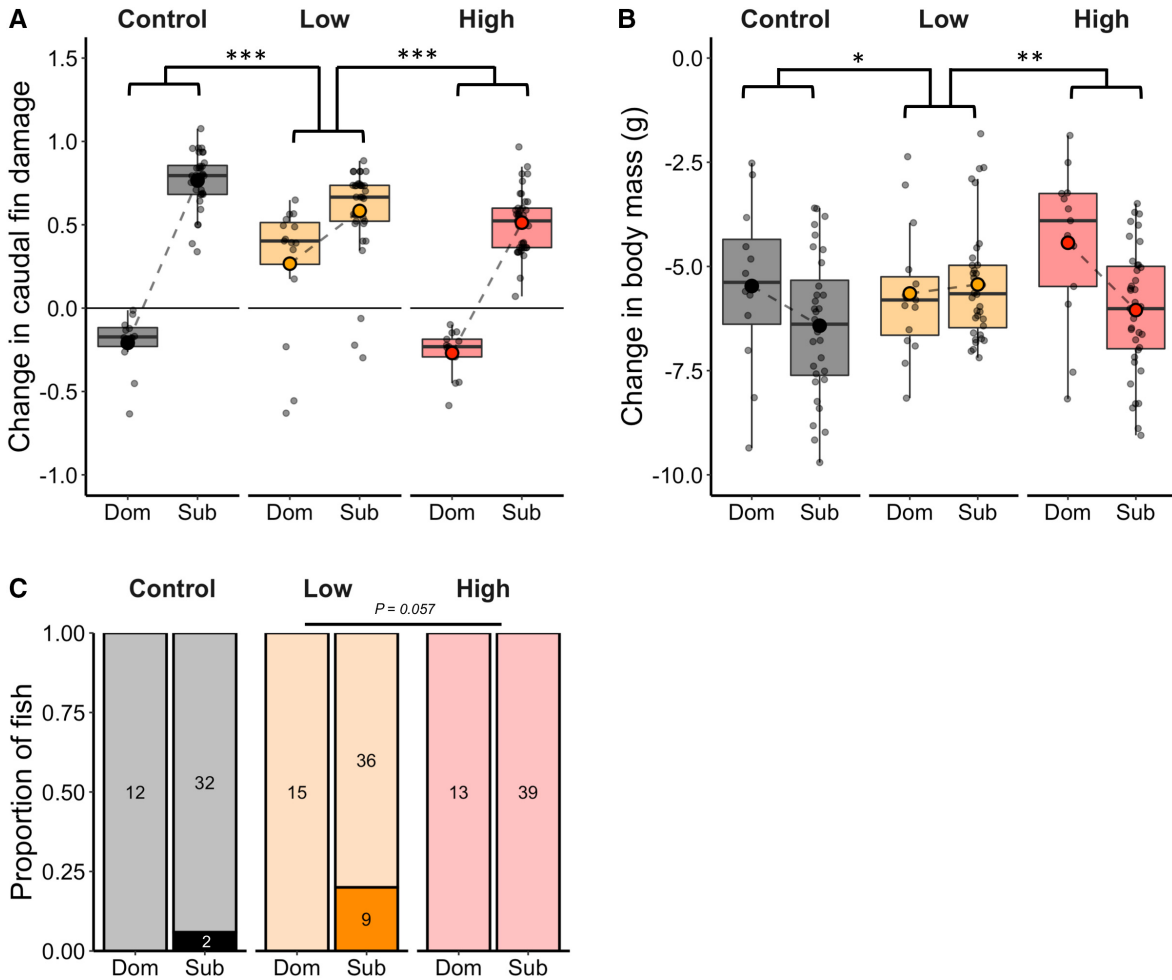
FIG. 3. (A) Change in caudal fin damage plotted by social status and facetted by treatment, showing social status by treatment interactions (a lower score means a more intact fin). (B) Change in body mass plotted by social status and facetted by treatment, showing social status by treatment interactions. Box plots show medians and the interquartile range, and means for each group (large colored circles). (C) Proportion of fish surviving plotted by social status and grouped by treatment. Saturated colors indicate fish that did not survive. Panels A and B show predicted model values.

effects. does not linearly drive the behavioral and morphological changes we observed in this study. Cortisol followed a more linear pattern and was the highest in control fish and lowest in high-dose fish. Benzodiazepines can have therapeutic effects through a number of mechanisms in addition to their inhibition of the hypothalamic-pituitary-interrenal (HPI) axis, including disinhibiting the dopaminergic system (Van Der Kooij et al. 2018). Future work with more concentrations and additional molecular approaches is needed to clarify the cause of the biphasic behavioral effects we found here.

This is the first study to report that social status itself can be associated with the uptake of a pharmaceutical pollutant in fish tissues: subordinate fish absorbed more oxazepam in their muscle tissue than dominant fish despite both experiencing the same ambient exposure conditions. These findings are in line with previous research showing that social status can modulate heavy metal uptake in rainbow trout (reviewed in Sloman 2007). There are several possible mechanisms underlying this effect. First, subordinate fish in all treatments had higher cortisol levels than dominant fish, which may cause subordinates to increase oxazepam uptake via higher ventilation and respiration. Brown trout subordinates in dominance dyads show increased standard metabolic rates (Sloman et al. 2000a, b, Culbert and Gilmour 2016), and higher standard metabolic rates have been associated with increased contaminant uptake in killifish (Blewett et al. 2013). Alternatively, it is possible that the fish that absorbed more oxazepam in the individual exposure phase of our experiment were predisposed to become subordinates in the group-exposure phase. This finding certainly requires further investigation to identify the causal mechanism driving our results. If social status can modulate the magnitude of an internal exposure (or vice versa), then this may allow feedback loops to form that could

exacerbate the behavioral and physiological effects of exposure; for example, if subordinates bioaccumulate more oxazepam in their tissues, then they may be more subject to behavioral perturbations than dominants, and these changes may in turn affect subordinate metabolic rates and pharmaceutical uptake.

In conclusion, we provide one of the first comprehensive examinations of how a pharmaceutical pollutant can disrupt the natural social structures of aquatic wildlife. We found that the behavioral and physiological consequences of being exposed to the benzodiazepine oxazepam differed by social status, with dominant fish incurring additional costs and subordinate fish accruing more benefits at low doses. We are also the first to show that the amount of pharmaceutical itself that is bioaccumulated in tissues varies with social status, and this might contribute to the asymmetrical behavioral and physiological effects that we observed between dominants and subordinates. A natural next step for this work will to be disentangle the cause–effect relationship between social status and oxazepam absorption. It will also be fruitful to test if the present findings are similar to those measured in a more ecologically realistic environment such as experimental mesocosms or ponds, because brown trout dominance hierarchies measured in the laboratory can be more aggressive than those measured in the wild (Sloman and Armstrong 2002). It is also important to study this over longer timescales to better clarify the effects of habituation. Altogether, our findings add to a newly growing interest in increasing the ecological and evolutionary relevance of environmental chemical regulation (Martin et al. 2019, Straub et al. 2020), and they underscore the importance of considering an organism's natural social environment when evaluating the effects of chemical pollutants on wildlife.

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Supporting Information

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The code and datasets supporting this article are uploaded as part of the supplementary files (Data S1).