Science of the Total Environment 648 (2019) 1257–1262

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

High-speed imaging reveals how antihistamine exposure affects escape behaviours in aquatic insect prey

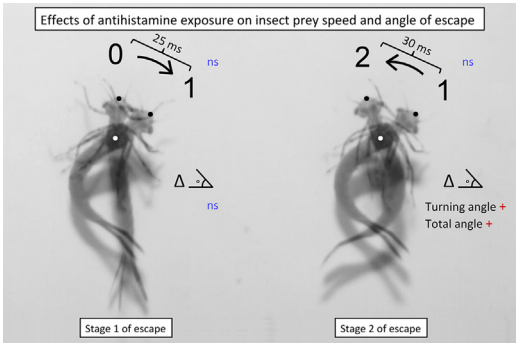
Micael Jonsson [a,*], Magnus Andersson [b], Jerker Fick [c], Tomas Brodin [a,d], Jonatan Klaminder [a], Susanna Piovano [e]

[a] Department of Ecology and Environmental Science, Umeå University, SE 90187 Umeå, Sweden
[b] Department of Physics, Umeå University, SE 90187 Umeå, Sweden
[c] Department of Chemistry, Umeå University, SE 90187 Umeå, Sweden
[d] Department of Wildlife, Fish, and Environmental Studies, SLU, Umeå, Sweden
[e] School of Marine Studies, The University of the South Pacific, Laucala Bay Road, Suva, Fiji

HIGHLIGHTS

- High-speed imaging was successful at capturing fast escape responses.
- Antihistamine exposure altered prey escape responses.
- The changed behaviour indicates a reduced ability to evade predator attacks.
- High-speed imaging should be used in environmental risk assessment.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 21 June 2018
Received in revised form 16 August 2018
Accepted 17 August 2018
Available online 18 August 2018

Editor: Daniel Wunderlin

Keywords:
Damselfly larvae
Diphenhydramine
Escape response
Pharmaceutical pollution

ABSTRACT

Aquatic systems receive a wide range of pharmaceuticals that may have adverse impacts on aquatic wildlife. Among these pharmaceuticals, antihistamines are commonly found, and these substances have the potential to influence the physiology of aquatic invertebrates. Previous studies have focused on how antihistamines may affect behaviours of aquatic invertebrates, but these studies probably do not capture the full consequences of antihistamine exposure, as traditional recording techniques do not capture important animal movements occurring at the scale of milliseconds, such as prey escape responses. In this study, we investigated if antihistamine exposure can impact escape responses in aquatic insect, by exposing damselfly (*Coenagrion hastulatum*) larvae to two environmentally relevant concentrations (0.1 and 1 μg L^{-1}) of diphenhydramine. Importantly, we used a high-speed imaging approach that with high-time resolution captures details of escape responses and, thus, potential impacts of diphenhydramine on these behaviours. Our results show overall weak effects of antihistamine exposure on the escape behaviours of damselfly larvae. However, at stage 2 of the C-escape response, we found a significant increase in turning angle, which corresponds to a reduced swimming velocity, indicating a reduced success at evading a predator attack. Thus, we show that low concentrations of an antihistamine may affect behaviours strongly related to fitness of aquatic insect prey – effects that would have been overlooked using traditional recording techniques. Hence, to understand the full consequences of pharmaceutical contamination on aquatic wildlife, high-speed imaging should be incorporated into future environmental risk assessments.

* Corresponding author.
E-mail address: micael.jonsson@umu.se (M. Jonsson).

https://doi.org/10.1016/j.scitotenv.2018.08.226
0048-9697/

1. Introduction

Freshwater systems receive a wide range of pharmaceutical substances, primarily via wastewater effluent, with potential adverse impacts on aquatic wildlife (Boxall et al., 2012; Rosi-Marshall and Royer, 2012; Brodin et al., 2014). The nature of these potential impacts is still poorly understood, but there are studies that have found changed animal behaviours following exposure to pharmaceuticals, such as benzodiazepines (Brodin et al., 2013; Klaminder et al., 2016), selective serotonin reuptake inhibitors (Valenti Jr. et al., 2012), and antihistamines (Jonsson et al., 2014). Such changes, albeit initially non-lethal, can over the longer term reduce individual fitness and thus survival, with potential consequences for populations, communities, and ecosystem functioning in freshwaters (Kidd et al., 2007; Rosi-Marshall and Royer, 2012; Brodin et al., 2014).

It is well known that freshwater insects can be sensitive to changes in environmental conditions, and insect abundance and community composition are therefore often used to determine effects of a changed freshwater environment (Rosenberg and Resh, 1993). Studies thus far have focused on effects of changed pH, eutrophication, and metal pollution, whereas much less is known when it comes to effects of pharmaceutical contamination (Rosi-Marshall and Royer, 2012). Moreover, focus has been on lethal effects, whereas pharmaceuticals in freshwaters are found mostly at concentrations (i.e. ng to μg L^{-1}) well below those found to be lethal in laboratory assays (Kolpin et al., 2002). There are, however, studies showing that environmentally relevant concentrations of pharmaceuticals can modify behaviours in freshwater animals without having direct lethal effects (Brodin et al., 2014). Therefore, there is a need to further develop standardized methods that efficiently detect behavioural changes following exposure to non-lethal concentrations of chemical substances, such as pharmaceuticals.

Among the wide range of pharmaceutical compounds found in surface waters, there are several types of antihistamines that are found at low concentrations, 1–20 ng L^{-1}, but concentrations up to μg L^{-1} have also been detected (Stackelberg et al., 2007; Kosonen and Kronberg, 2009; Gros et al., 2012; López-Serna et al., 2013; Kristofco and Brooks, 2017). As insects use histamines for neurotransmission (Hashemzadeh-Gargari and Freschi, 1992; Rosi-Marshall and Royer, 2012), exposure to antihistamines, even at low concentrations, has the potential to cause changes in physiology and behaviours of freshwater insects. This may be especially true, as bioconcentration of antihistamines in freshwater invertebrates (including insects) have been found to be very high, both in laboratory (Jonsson et al., 2014; Jonsson et al., 2015) and field (Lagesson et al., 2016) studies. Accordingly, one study found that predator-escape behaviours in damselfly larvae were altered following exposure to the antihistamines hydroxyzine or fexofenadine, but whether effects were detected or not depended on the specific behaviour measured and differed between the studied antihistamines (Jonsson et al., 2014).

Thus far, studies have mainly used rather long and imprecise, albeit standardized, methods (i.e. personal observations or analyses of movements recorded with an ordinary camera) to quantify behavioural change following exposure to pharmaceuticals. Hence, effects on rapid (i.e. at the scale of milliseconds) animal movements – that still are crucial for determining escape success – may therefore have been overlooked. Hence, image analysis using a high-speed camera to record escape behaviours may be required (e.g. Janssens and Stoks, 2012; Janssens et al., 2014), but, to our knowledge, this has thus far never been done to assess effects of pharmaceuticals on aquatic insect behaviours. In this study, we therefore wanted to use a high-speed imaging approach to quantify potential behavioural changes in response to pharmaceutical exposure. We did this by exposing larvae of the damselfly *Coenagrion hastulatum* to two different, environmentally relevant concentrations of the antihistamine diphenhydramine (DPH), while keeping one control group unexposed, and measured escape response using high-speed imaging. Damselfly larvae show a rapid predator escape response (Brackenbury, 2003), making them relevant test objects for high-speed imaging experiments. Further, the larvae of damselflies are common in lake and pond ecosystems globally (Kalkman et al., 2008), are easy to hold in laboratory conditions, and therefore a suitable organism for behavioural and ecotoxicological studies.

2. Material and methods

2.1. Experimental setup

For this study, we collected 200 similar-sized damselfly (*C. hastulatum*) larvae with a sweep net in lake Nydalasjön, that is marginally affected by anthropogenic contaminants, in Umeå, northern Sweden, in August 2013, three days prior to the beginning of the experiment. Groups of 50 damselfly larvae were kept in containers filled with 5.0 L of aged, non-chlorinated tap water in a climate-controlled room, at 20 ± 0.5 °C and 15:9 light:dark regime, to mimic outdoor conditions during this time of the year. The larvae were fed ad libitum twice a day with zooplankton (*Daphnia* sp. and *Artemia salina*) cultivated at Umeå University.

To ensure steady state of internal concentrations, exposure to DPH lasted for seven days (Heynen et al., 2016), during which each damselfly was housed individually in a single plastic container of the size 11 × 11 × 6 cm (height, width, depth) containing 0.1 L of aged tap water. All the other conditions were kept as during acclimation. DPH was added to 20 randomly chosen containers (replicates) to a concentration of 0.1 μg L^{-1} (low treatment), and to 20 randomly chosen containers to a concentration of 1.0 μg L^{-1} (high treatment), i.e. concentrations that were predicted to result in an internal concentration corresponding to 0.25 and 2 times the human therapeutic concentration, respectively, while 20 containers were kept clean from DPH (control treatment). Treated and untreated water was sampled 30 min after DPH addition and at the end of the 7-day exposure. The chosen concentrations were lower than the 2.2 μg L^{-1} of the antihistamine fexofenadine that previously was found to affect behaviours in damselfly larvae (Jonsson et al., 2014), but similar to, and lower then, the predicted no-effect concentration of DPH for the aquatic invertebrate *Daphnia magna* (i.e. 0.8 μg L^{-1}; Berninger et al., 2011), and lower than the highest DPH concentrations detected in freshwater surface waters (i.e. 1.4 μg L^{-1}; Kristofco and Brooks, 2017).

To investigate whether escape response of damselfly larvae changes following DPH exposure, we recorded the behaviour of 60 larvae (20 from each treatment) before and immediately after exposure. To be able to measure potential behavioural changes at the scale of milliseconds, we used a high-speed camera (Mikrotron MotionBLITZ EoSens Cube7), equipped with a 50 mm Nikon AF Nikkor lens that was mounted on a tripod to allow for top-view imaging of the arenas. We acquired images of 1696 × 1710 pixel resolution at a sampling rate of 500 Hz using the software Mikrotron MotionBLITZDirector2 v. 1.4.3. The pixel-to-mm conversion factor was determined by imaging a ruler in the field-of-view and measuring the number of pixels per mm using the software ImageJ. The pixel-to-mm conversion factor was 0.05747 mm.

To measure the escape response, each damselfly larva was carefully placed in the middle of the longest side of a grey plastic arena with a size of 40.0 × 60.0 × 13.5 cm (height, width, depth), filled with 3.0 L of aged tap water. The damselfly larva was left in the arena for 15 s, after which it was touched at the tip of its lamellae (tactile stimulation), with the point of a 15 cm long stick, which elicits escape response in damselfly larvae (Brackenbury, 2002). After these response experiments, damselflies were individually weighed with a scale (DeltaRange Mettler PE 360, precision 0.001 g) and then frozen for later DPH concentration analysis.

2.2. Determination of DPH

To assess the bioconcentration of DPH, tissue concentrations (full body) were analyzed after 7 days of exposure (n = 20, for each treatment). Samples were dried; surrogate standards were added (50 ng of D6 amitriptyline); then extracted sequentially with 1.5 mL acetonitrile twice. Samples were homogenized for 4 min at 42,000 oscillations per minute, using a Mini Beadbeater (Biospec. Bartlesville, USA) with zirconium beads and then centrifuged at 14,000 rpm for 10 min. Both supernatants were combined, evaporated to 20 μL and reconstituted in 100 mL methanol. Absolute recovery was 92% (n = 6). Bioconcentration factors (BCFs) were estimated by dividing individual full body concentrations with measured water concentration in the corresponding individual aquarium.

Analytical methods to measure the pharmaceuticals in water samples have been published previously (Lindberg et al., 2014). In short, the samples were determined by chemical analysis using a triple stage quadrupole MS/MS TSQ Quantum Ultra EMR (Thermo Fisher Scientific, San Jose, CA, USA) coupled with an Accela and a Surveyor LC pump (Thermo Fisher Scientific, San Jose, CA, USA), and a PAL HTC autosampler (CTC Analytics AG, Zwingen, Switzerland) were used as analytical system. For a detailed description of the analysis and pretreatment see Lindberg et al. (2014). Biota samples were pre-treated as described previously (Brodin et al., 2013; Lagesson et al., 2016). In short, full-body analysis of pooled samples was used and biota samples were homogenized and extracted sequentially with acetonitrile. Five-point standard curves were used for quantification and linearity was 0.999.

2.3. Escape response

Damselfly larvae typically show two types of escape responses (Brackenbury, 2003): a rapid flex response comparable to the C-escape of fish, and a twist one, which involves pitch and roll over planes. Based on kinematics, C-escape response can be divided into two stages (Domenici and Blake, 1991; Lefrançois and Domenici, 2006): stage 1 (s1), which involves a strong, unilateral, muscular contraction bending the body into a C-shape, defined as the first detectable movement of the head, and stage 2 (s2), in which a change in the turning direction of the anterior body midline is observed and the tail bends in the opposite direction to that in stage 1 (Fig. 1). In this study, we investigated behavioural (responsiveness and directionality), kinematics (turning angles, total escape angle, and angular velocity), and distance-related components (escape duration and total distance) of the C-escape response in damselfly larvae.

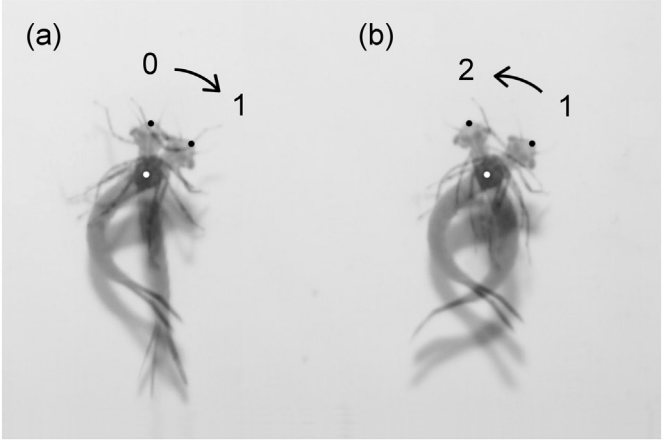


Fig. 1. Method for determining escape-response stage 1 (a) and stage 2 (b) turning angles by overlapping frames extracted from video recording at time 0 (0), time 1 (1), and time 2 (2).

Responsiveness was categorized as escape response, or lack of escape response, to the stimulus. Directionality was categorized according to if the first movement of the head in s1 was oriented away from or towards the stimulus (Domenici and Blake, 1993). For turning angles (Domenici and Blake, 1993), s1 turning angle (s1a) was determined by measuring the angle between the straight lines passing from the centre of the mesonotum to the tip of the head at rest (frame 0) and at the end of s1 (Fig. 1a), and s2 turning angle (s2a) was determined by measuring the angle between the end of s1 and the end of s2 (Fig. 1b). Total escape angle was identified as the angle between the body axis at rest (frame 0) and body orientation at the end of s2. Larvae body positions at rest, s1, and s2 were extracted with ImageJ (Rasband, 2016), overlapped and aligned at the centre of the mesonotum with GIMP 2.8 (www.gimp.org), and then the angles were measured with ImageJ. Angular velocity was calculated for s1 and s2. Escape-response duration was calculated separately for s1 and s2 and as a total, which was the sum of the distances travelled during s1 and s2.

2.4. Statistical analyses

Upon performing mixed design analysis of variance (ANOVA) with compound symmetry correlation structure, no significant difference was found among treatments (all $p > 0.4$), nor for the interaction between treatment and time (all $p > 0.1$). Hence, we split the analysis in two separate parts of interest. The first analysis focused on individuals after exposure and aimed at comparing the treatments (low, high, control). For this purpose, linear statistics (Kruskal-Wallis test) and, for angles, circular statistics (Fisher's Nonparametric Test for a common median direction; Fisher, 1995) were used (Domenici et al., 2011). The second analysis focused on time (after vs. before) and aimed at comparing the escape components of the individuals within each separate treatment. For this purpose, linear statistics (Wilcoxon Signed-Rank test) and, for angles, circular statistics (Moore's test for a common distribution for paired samples) were used. Analyses were performed in R version 3.3.1 (R Core Team, 2017), with packages “exactRankTests” (Hothorn and Hornik, 2017) and “circular” (Agostinelli and Lund, 2017), and new functions from Pewsey et al. (2013).

3. Results

3.1. Diphenhydramine concentrations

Concentrations of DPH in water varied substantially among replicates, but were still higher in the high than in the low treatment, and absent in the controls (Table 1). Over the 7-day period, mean DPH concentrations dropped by 22.8% and 21.0% in the low and high treatment, respectively. The damselfly larvae exhibited a rather low level of bioconcentration factors that did not differ between the low and high treatment (Table 1).

3.2. Behavioural analyses

Survival during exposure and during the behavioural assays was 100% in all treatments. Before the exposure, all individuals reacted to

Table 1
Mean concentrations (±1 SE; n = 20) of diphenhydramine (DPH) in water and damselfly tissue, in low (0.1 μg L^{-1}) and high (1.0 μg L^{-1}) treatment, and control (no DPH added), and bioconcentration factor (BCF) in damselfly tissue. LOQ = limit of quantification; for water 1.0 ng L^{-1} and for tissue 0.1 ng g^{-1}.

	Treatment		
Measured variables	Low	High	Control
DPH in water, day 1 (μg L^{-1})	0.13 ± 0.59	0.83 ± 0.44	<LOQ
DPH in water, day 7 (μg L^{-1})	0.10 ± 0.23	0.65 ± 0.20	<LOQ
DPH in damselfly tissue (μg kg^{-1})	1.5 ± 0.7	8.0 ± 5.0	<LOQ
BCF of DPH in damselfly tissue	15.2 ± 6.8	13.3 ± 8.3	

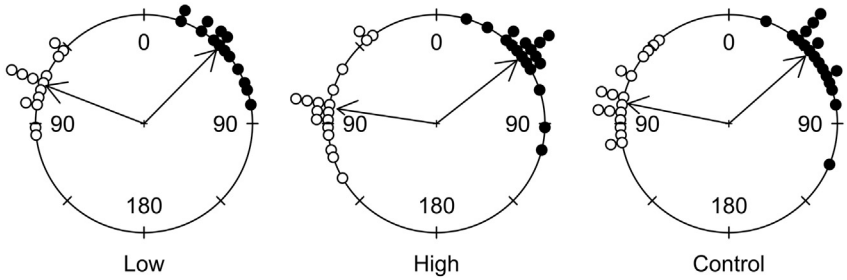


Fig. 2. Frequency of turning angles at stage 1 (black colour) and stage 2 (white colour) of escape response recorded on each treatment after exposure. Points are grouped in 90 bins. Arrows represent the sample median direction (from 0° to 180°) and resultant length.

the tactile stimulus with an escape response. All but two individuals oriented the head away from the stimulus, and C-escape was observed in 87% of the escape responses. After the exposure, all but four individuals (1 in the low treatment, 2 in the high treatment, and 1 in control) reacted to the stimulus with an escape response. Among the ones that showed responsiveness, all but one individual in the low treatment oriented the head away from the stimulus. C-escape was observed in 91% of the escape responses.

3.3. Effects among treatments after exposure

Following exposure to DPH, turning angle at s2 was significantly different among treatments ($p = 0.009$), with a greater turning angle in the high- than in the low-concentration and control treatment (Fig. 2), while no significant difference among treatments was found for turning angle at s1 ($p = 0.273$) or in the total escape angle ($p = 0.121$; data not shown). With respect to angular velocity (Fig. 3a), duration (Fig. 3b), and total distance travelled (Fig. 4), no statistical differences were found among the three treatments after exposure (all $p > 0.15$).

3.4. Within-treatment effects (after vs. before)

In the low-concentration treatment, we found no statistical difference before and after exposure, with respect to turning angles, total escape angle, angular velocity, duration, or total distance travelled (all $p > 0.16$). However, in the high-concentration treatment, we found significant increases after exposure in the turning angle of the second stage (from a median of 64.7° to a median of 83.3°; $p = 0.019$) and in the total escape angle (from a median of 117.4° to a median of 139.4°; $p = 0.035$). However, there were no differences in the first stage turning angle, angular velocity, duration, or total distance travelled (all $p > 0.10$). In the control treatment, there were significant increases in the first stage duration (from a mean of 22 milliseconds to a mean of 26 milliseconds; $p = 0.024$) and in the total distance travelled (from a mean of 6.3 mm to a mean of 8.7 mm; $p < 0.001$), but not in turning angles, total escape angle, angular velocity, or in second stage and total durations (all $p > 0.14$, except for total duration, which was marginally significant at $p = 0.078$).

4. Discussion

Diphenhydramine (DPH) exposure showed overall weak effects on damselfly escape behaviour, which could be a consequence of rather low level of bioconcentration observed in this study. Antihistamines tend to differ in their propensity to bioconcentrate in aquatic invertebrates (Lagesson et al., 2016), with DPH showing much lower BCF than, for example, hydroxyzine, which also is the antihistamine previously found to affect the behaviour in damselfly larvae (Jonsson et al., 2014). There was also high degree of variation in antihistamine

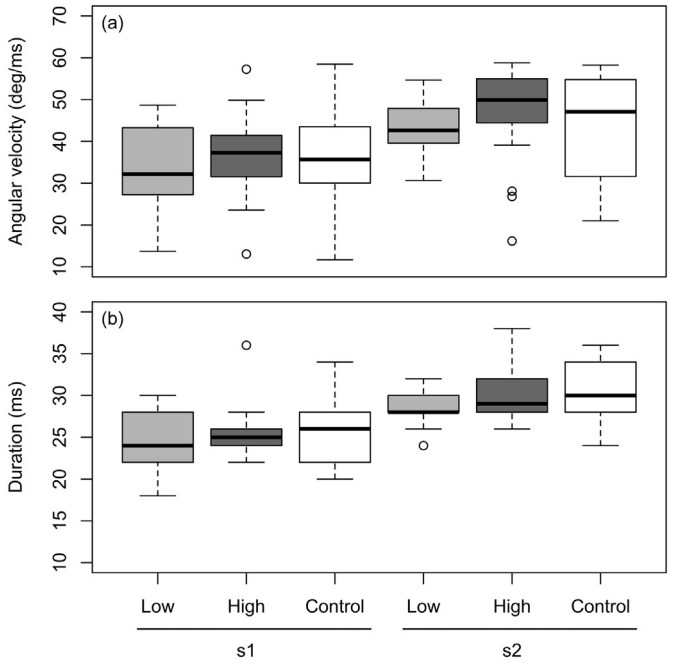


Fig. 3. Angular velocity (a) and escape-response duration (b) recorded at stage 1 and stage 2 of escape response recorded on each treatment after exposure. The thick black line shows the median and the box is defined by the upper and lower quartile (i.e. 25% of the data are higher and lower, respectively). The box whiskers represent the greatest and least values, excluding outliers, which are denoted by open circles.

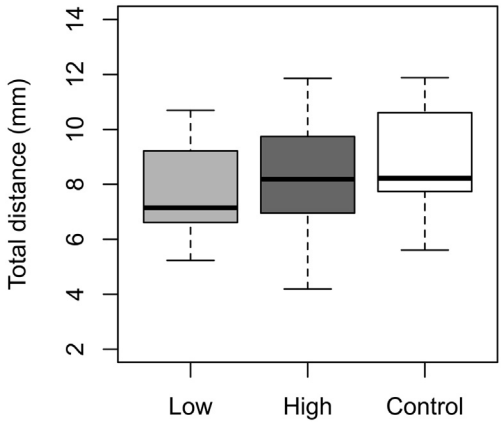


Fig. 4. Total escape-response distance recorded for each treatment after exposure. The thick black line shows the median and the box is defined by the upper and lower quartile (i.e. 25% of the data are higher and lower, respectively). The box whiskers represent the greatest and least values, excluding outliers, which are denoted by open circles.

concentrations in both water and damselfly larvae, which has been seen in previous studies (Jonsson et al., 2014, 2015), suggesting that uptake and/or depuration rates can vary extensively among insect individuals. However, this variation was not due to the different DPH concentration in the two treatments, which showed very similar BCFs. Nevertheless, the observed decrease in antihistamine concentration over the course of the experiment likely reduced the impact of DPH on damselfly behaviour. In the laboratory, antihistamines show low persistence unless added continuously (Jonsson et al., 2015), whereas they can show pseudo persistence in the field due to continuous input via treated wastewater (Daughton, 2003). Hence, compared to field conditions, our study likely underestimates potential effects of DPH on insect prey escape behaviours.

Despite rather weak effects of DPH exposure on escape behaviour, the significantly higher turning angle at stage 2 after, compared to before, exposure in the high-exposure treatment, and compared to the low and control treatments after exposure (along with higher total escape angle), indicate that DPH can influence the way in which an aquatic prey react to a predatory attack. It is important to note that these effects were found without any lethal effects of exposure, which underscores the importance of investigating non-lethal (i.e. behavioural) effects of pharmaceuticals on aquatic wildlife (Brodin et al., 2014). Moreover, these effects would not have been detected in observational studies using traditional approaches including naked-eye observations and ordinary camera equipment tracking behaviour over seconds to minutes. Our finding of subtle behavioural changes highlights the need to develop standardized methods using a high-speed imaging approach to better understand the full consequences of pharmaceutical effects on organisms in natural environments.

In a C-type escape response, the bend at stage 2 is considered to be the propulsive stroke, whereas the bend at stage 1 prepares the animal for escape (Weihs, 1973). Whether or not changes in turning angle at these stages influence success of predatory escape is still debated, but studies have found that it may affect swimming performance and thus, most likely, survival probability of escaping individuals (Walker et al., 2005; Hitchcock et al., 2015). More specifically, an increased turning angle at stage 2 has been shown to be associated with lower escape velocities in fish, while turning angle at stage 1 is more constrained (Hitchcock et al., 2015) and thus less likely to be influence by external factors, such as changed environmental conditions or exposure to contaminants. Therefore, the higher turning and total escape angles we found in the high-exposure treatment, compared to in the low and control treatment, and following exposure in the high-exposure treatment, respectively, suggest that the ability to evade an attacking predator would have been lowered following exposure to DPH. The ability of a prey to evade predator attacks has obvious consequences for prey fitness (Dill, 1987; Ludwig and Rowe, 1990), and as aquatic insects are important, and ubiquitous, components of freshwater food webs (Merritt et al., 1984; Covich et al., 1999), such pharmaceutically driven alterations of behaviours can potentially have ecosystem-scale consequences.

By using high-speed imaging, we were able to detect changes in damselfly behaviours following exposure to an antihistamine that is found at low concentrations in natural systems. As these changes would have gone unnoticed, using personal observations or ordinary camera equipment, our results highlight the advantages of considering high-speed imaging as a tool for environmental risk assessment of pharmaceutical impacts. In this study, we used environmentally relevant concentrations of one antihistamine, but wastewater effluent brings several types of antihistamines that each could have additional effects, along with several other types of pharmaceuticals (Vasquez et al., 2014), in a continuous manner, resulting in pseudo persistence of otherwise rapidly degrading compounds (Daughton, 2003). As such, our results are probably underestimations of effects in contaminated natural systems, although net effects of pharmaceutical cocktails on aquatic wildlife in systems receiving wastewater effluent are immensely difficult to understand and thus predict.

Acknowledgements

The Kempe Foundations provided funds that financed the high-speed camera equipment, and financial support to S.P. was provided by C.M. Lerici Foundation.

References

Agostinelli, C., Lund, U., 2017. R package "circular": Circular statistics. https://cran.r-project.org/web/packages/circular/circular.pdf.

Berninger, J.P., Du, B., Connors, K.A., Eytcheson, S.A., Kolkmeier, M.A., Prosser, K.N., Valenti Jr., T.W., Chambliss, C.K., Brooks, B.W., 2011. Effects of the antihistamine diphenhydramine on selected aquatic organisms. Environ. Toxicol. Chem. 30, 2065–2072. https://doi.org/10.1002/etc.590.

Boxall, A., Rudd, M.A., Brooks, B.W., Caldwell, D.J., Choi, K., Hickmann, S., Innes, E., Ostapyk, K., Staveley, J.P., Verslycke, T., Ankley, G.T., Beazley, K.F., Belanger, S.E., Berninger, J.P., Carriquiriborde, P., Coors, A., DeLeo, P.C., Dyer, S.D., Ericson, J.F., Gagné, F., Giesy, J.P., Gouin, T., Hallstrom, L., Karlsson, M.V., Larsson, D.G.J., Lazorchak, J.M., Mastrocco, F., McLaughlin, A., McMaster, M.E., Meyerhoff, R.D., Moore, R., Parrott, J.L., Snape, J.R., Murray-Smith, R., Servos, M.R., Sibley, P.K., Straub, J.O., Szabo, N.D., Topp, E., Tetreault, G.T., Trudeau, V.L., Van Der Kraak, G., 2012. Pharmaceuticals and personal care products in the environment: what are the big questions? Environ. Health Perspect. 120, 1221–1229. https://doi.org/10.1289/ehp.1104477.

Brackenbury, J., 2002. Kinematics and hydrodynamics of an invertebrate undulatory swimmer: the damsel-fly larva. J. Exp. Biol. 205, 627–639. http://jeb.biologists.org/content/205/5/627.

Brackenbury, J., 2003. Escape manoeuvres in damsel-fly larvae: kinematics and dynamics. J. Exp. Biol. 206, 389–397. http://jeb.biologists.org/content/206/2/389.

Brodin, T., Fick, J., Jonsson, M., Klaminder, J., 2013. Dilute concentrations of a psychiatric drug alter behavior of fish from natural populations. Science 339, 814–815. https://doi.org/10.1126/science.1226850.

Brodin, T., Piovano, S., Fick, J., Klaminder, J., Heynen, M., Jonsson, M., 2014. Ecological effects of pharmaceuticals in aquatic systems – impacts through behavioural alterations. Philos. Trans. R. Soc. B 369, 20130580. https://doi.org/10.1098/rstb.2013.0580.

Covich, A.P., Palmer, M.A., Crowl, T.A., 1999. The role of benthic invertebrate species in freshwater ecosystems. Bioscience 49, 119–127. https://doi.org/10.2307/1313537.

Daughton, C.G., 2003. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. II. Drug disposal, waste reduction, and future directions. Environ. Health Perspect. 111, 775–785. https://doi.org/10.1289/ehp.5948.

Dill, L.M., 1987. Animal decision making and its ecological consequences: the future of aquatic ecology and behaviour. Can. J. Zool. 65, 803–811. https://doi.org/10.1139/z87-128.

Domenici, P., Blake, R.W., 1991. The kinematics and performance of the escape response in the angelfish (*Pterophyllum eimekei*). J. Exp. Biol. 156, 187–205. http://jeb.biologists.org/content/156/1/187.

Domenici, P., Blake, R.W., 1993. Escape trajectories in angelfish (*Pterophyllum eimekei*). J. Exp. Biol. 177, 253–272. http://jeb.biologists.org/content/177/1/253.

Domenici, P., Blagburn, J.M., Bacon, J.P., 2011. Animal escapology II: escape trajectory case studies. J. Exp. Biol. 214, 2474–2494. https://doi.org/10.1242/jeb.053801.

Fisher, N.I., 1995. Statistical Analysis of Circular Data. University Press, Cambridge.

Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analyses of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 204–221. https://doi.org/10.1016/j.chroma.2012.05.084.

Hashemzadeh-Gargari, H., Freschi, J.E., 1992. Histamine activates chloride conductance in motor neurons of the lobster cardiac ganglion. J. Neurophysiol. 68, 9–15. https://doi.org/10.1152/jn.1992.68.1.9.

Heynen, M., Fick, J., Jonsson, M., Klaminder, J., Brodin, T., 2016. Effects of bioconcentration and trophic transfer on realized exposure to oxazepam in 2 predators, the dragonfly larvae (*Aeshna grandis*) and the Eurasian perch (*Perca fluviatilis*). Environ. Toxicol. Chem. 35, 930–937. https://doi.org/10.1002/etc.3368.

Hitchcock, A.C., Chen, T., Connolly, E., Darakanada, K., Jeong, J., Quist, A., Robbins, A., Ellerby, D.J., 2015. Trade-offs between performance and variability in the escape responses of bluegill sunfish (*Lepomis macrochirus*). Biol. Open 4, 743–751. https://doi.org/10.1242/bio.201511577.

Hothorn, T., Hornik, K., 2017. exactRankTests: exact distributions for rank and permutation tests. https://cran.r-project.org/web/packages/exactRankTests/exactRankTests.pdf.

Janssens, L., Stoks, R., 2012. How does a pesticide pulse increase vulnerability to predation? Combined effects on behavioral antipredator traits and escape swimming. Aquat. Toxicol. 110–111, 91–98. https://doi.org/10.1016/j.aquatox.2011.12.019.

Janssens, L., Van, K.D., Debecker, S., Bervoets, L., Stoks, R., 2014. Local adaptation and the potential effects of a contaminant on predator avoidance and antipredator responses under global warming: a space-for-time substitution approach. Evol. Appl. 7, 421–430. https://doi.org/10.1111/eva.12141.

Jonsson, M., Fick, J., Klaminder, J., Brodin, T., 2014. Antihistamines and aquatic insects: bioconcentration and impacts on behavior in damselfly larvae (Zygoptera). Sci. Total Environ. 472, 108–111. https://doi.org/10.1016/j.scitotenv.2013.10.104.

Jonsson, M., Ershammar, E., Fick, J., Brodin, T., Klaminder, J., 2015. Effects of an antihistamine on carbon and nutrient recycling in streams. Sci. Total Environ. 538, 240–245. https://doi.org/10.1016/j.scitotenv.2015.08.061.

Kalkman, V.J., Clausnitzer, V., Dijkstra, K.-D.B., Orr, A.G., Paulson, D.R., van Tol, J., 2008. Global diversity of dragonflies (Odonata) in freshwater. Hydrobiologia 595, 351–363. https://doi.org/10.1007/s10750-007-9029-x.

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick, R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. U. S. A. 104, 8897–8901. https://doi.org/10.1073/pnas.0609568104.

Klaminder, J., Hellström, G., Fahlman, J., Jonsson, M., Fick, J., Lagesson, A., Bergman, E., Brodin, T., 2016. Drug-induced behavioral changes: using laboratory observations to predict field observations. Front. Environ. Sci. 4, 81. https://doi.org/10.3389/fenvs.2016.00081.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999–2000: a national reconnaissance. Environ. Sci. Technol. 36, 1202–1211. https://doi.org/10.1021/es011055j.

Kosonen, J., Kronberg, L., 2009. The occurrence of antihistamines in sewage waters and in recipient rivers. Environ. Sci. Pollut. Res. 16, 555–564. https://doi.org/10.1007/s11356-009-0144-2.

Kristofco, L.A., Brooks, B.W., 2017. Global scanning of antihistamines in the environment: analyses of occurrence and hazards in aquatic systems. Sci. Total Environ. 592, 477–487. https://doi.org/10.1016/j.scitotenv.2017.03.120.

Lagesson, A., Fahlman, J., Brodin, T., Fick, J., Jonsson, M., Byström, P., Klaminder, J., 2016. Bioaccumulation of five pharmaceuticals at multiple trophic levels in an aquatic food web – insights from a field experiment. Sci. Total Environ. 568, 208–215. https://doi.org/10.1016/j.scitotenv.2016.05.206.

Lefrançois, C., Domenici, P., 2006. Locomotor kinematics and behaviour in the escape response of European sea bass, *Dicentrarchus labrax* L., exposed to hypoxia. Mar. Biol. 149, 969–977. https://doi.org/10.1007/s00227-006-0261-0.

Lindberg, R.H., Östman, M., Olofsson, U., Grabic, R., Fick, J., 2014. Occurrence and behaviour of 105 active pharmaceutical ingredients in sewage waters of a municipal sewer collection system. Water Res. 58, 221–229. https://doi.org/10.1016/j.watres.2014.03.076.

López-Serna, R., Jurado, A., Vázquez-Suñé, E., Carrera, J., Petrović, M., Barceló, D., 2013. Occurrence of 95 pharmaceuticals and transformation products in urban groundwaters underlying the metropolis of Barcelona, Spain. Environ. Pollut. 174, 305–315. https://doi.org/10.1016/j.envpol.2012.11.022.

Ludwig, D., Rowe, L., 1990. Life history strategies for energy gain and predator avoidance under time constraints. Am. Nat. 113, 686–707.

Merritt, R.W., Cummins, K.W., Burton, T.M., 1984. The role of aquatic insect in the processing and cycling of nutrients. In: Resh, V.H., Rosenberg, D.N. (Eds.), The Ecology of Aquatic Insects. Praeger Scientific, New York, pp. 134–163.

Pewsey, A., Neuhäuser, M., Ruxton, G.D., 2013. Circular Statistics in R. Oxford University Press, Oxford; New York.

R Core Team, 2017. R: a language and environment for statistical computing (Vienna, Austria: R Foundation for Statistical Computing). https://www.r-project.org.

Rasband, W.S., 2016. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA https://imagej.nih.gov/ij/.

Rosenberg, D.M., Resh, V.H., 1993. Introduction to freshwater biomonitoring and benthic macroinvertebrates. In: Rosenberg, D.M., Resh, V.H. (Eds.), Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman and Hall, New York, pp. 1–9.

Rosi-Marshall, E.J., Royer, T.V., 2012. Pharmaceutical compounds and ecosystem function: an emerging research challenge for aquatic ecologists. Ecosystems 15, 867–880. https://doi.org/10.1007/s10021-012-9553-z.

Stackelberg, P.E., Gibs, J., Furlong, E.T., Meyer, M.T., Zaugg, S.D., Lippincott, R.L., 2007. Efficiency of conventional drinking-water-treatment processes in removal of pharmaceuticals and other organic compounds. Sci. Total Environ. 377, 255–272. https://doi.org/10.1016/j.scitotenv.2007.01.095.

Valenti Jr., T.W., Gould, G.G., Berninger, J.P., Connors, K.A., Keele, N.B., Prosser, K.N., Brooks, B.W., 2012. Human therapeutic plasma levels of the selective serotonin reuptake inhibitor (SSRI) sertraline decrease serotonin reuptake transporter binding and shelter-seeking behaviour in adult male fathead minnows. Environ. Sci. Technol. 46, 2427–2435. https://doi.org/10.1021/es204164b.

Vasquez, M.I., Lambrianides, A., Schneider, M., Kümmerer, K., Fatta-Kassinos, D., 2014. Environmental side effects of pharmaceutical cocktails: what we know and what we should know. J. Hazard. Mater. 279, 169–189. https://doi.org/10.1016/j.jhazmat.2014.06.069.

Walker, J.A., Ghalambor, C.K., Griset, O.L., McKenney, D., Reznick, D.N., 2005. Do faster starts increase the probability of evading predators? Funct. Ecol. 19, 808–815. https://doi.org/10.1111/j.1365-2435.2005.01033.x.

Weihs, D., 1973. The mechanisms of rapid starting of slender fish. Biorheology 10, 343–350.