Impact of the antidepressant citalopram on the behaviour of two different life stages of brown trout

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Background. Over the last two decades, there has been a constant increase in prescription rates of antidepressants. In parallel, neuroactive pharmaceuticals are making their way into aquatic environments at increasing concentrations. Among the antidepressants detected in the environment citalopram, a selective serotonin reuptake inhibitor, is one of the most commonly found. Given citalopram is specifically designed to alter mood and behaviour in humans, there is growing concern it can adversely affect the behaviour on non-target wildlife

Methods. In our study, brown trout were exposed to citalopram (nominal concentrations: 1, 10, 100, 1000 µg/L) in two different life stages. Larvae were exposed at 7 and 11 °C from the eyed ova stage until 8 weeks post yolk sac consumption, and juvenile brown trout were exposed for 4 weeks at 7°C. At both stages we measured mortality, weight, length, tissue citalopram concentration, behaviour during exposure and behaviour in a stressfull environment. For brown trout larvae additionally hatching rate and heart rate, and for juvenile brown trout the tissue cortisol concentration were assessed.

Results. During the exposure, both larvae and juvenile fish exposed to the highest test concentration of citalopram (1 mg/L) had higher swimming activity and spent longer in the upper part of the aquaria compared to control fish, which is an indicator for decreased anxiety. Most probably due to the higher swimming activity during the exposure, the juveniles and larvae exposed to 1 mg/L citalopram showed decreased weight and length. Additionally, in a stressful artificial swimming measurement device, brown trout larvae displayed the anxiolytic effect of the antidepressant by reduced swimming activity during this stress situation, already at concentrations of 100 µg/L citalopram. Chemical analysis of the tissue revealed rising citalopram tissue concentrations with rising exposure concentrations. Tissue concentrations were 10 times higher in juvenile fish compared to brown trout larvae. Fish plasma concentrations were calculated, which exceeded human therapeutic levels for the highest exposure concentration, matching the behavioural results. Developmental parameters like hatching rate and heart rate, as well as mortality and tissue cortisol content were unaffected by the antidepressant. Overall, we could trace the pharmacological mode of action of the antidepressant citalopram in the non-target organism brown trout in two different life stages.

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Abstract

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Introduction

Pharmaceuticals like psychotropic drugs are widely distributed in the environment and can be found in most human-influenced surface waters and within most trophic levels, from algae to fish (Alvarez-Munoz et al. 2015; aus der Beek et al. 2016). During the last decades, psychotropic drugs, especially antidepressants, increasingly detected in rivers and surface waters (Acuna et al. 2015; Grabicova et al. 2017; Hughes et al. 2013; Schultz et al. 2010) due to the increasing numbers of diagnosed mental disorders (Destatis 2017) and the consequent rising numbers of antidepressant prescriptions (Schwabe & Paffrath 2016). Within the group of antidepressant drugs, the selective serotonin reuptake inhibitors (SSRIs) make up the largest part of the prescription rates (Schwabe & Paffrath 2016). Their mode of action is based on their binding to the serotonin transporter (SERT) in the presynaptic membrane, thereby inhibiting the reuptake of serotonin into the presynaptic neuron causing increased serotonin levels in the synaptic cleft (Hyttel 1994). These 5-HT reuptake transporters are highly conserved in the animal kingdom and can be found in all phyla (Gunnarsson et al. 2008; Verbruggen et al. 2018). One of the most important antidepressants of the SSRI class is citalopram, which is the most prescribed

67   antidepressant in Germany, with 306.8 million defined daily doses (DDD) in 2015 (Schwabe &
68   Paffrath 2016). Assuming a DDD of 20 mg per patient, this leads to a minimum consumption of
69   6.1 t/year in Germany alone.

70   Citalopram has been found in U.S. surface waters in concentrations ranging from 4 ng/L to 219
71   ng/L (Schultz et al. 2010). In different effluents of wastewater treatment plants, citalopram was
72   found in concentrations range from 44 ng/L to 431 ng/L (Himmelsbach et al. 2006; Nodler et al.
73   2010; Silva et al. 2014). Furthermore, surface water citalopram was detected at concentrations of
74   up to 76 µg/L downstream of a wastewater treatment plant in India, close to Hyderabad and up to
75   840 µg/L in the effluent of a drug manufacturer (Fick et al. 2009; Larsson et al. 2007). In
76   contrast environmental concentrations of other antidepressants like fluoxetine are lower within a
77   range of 1 to 43 ng/L (Acuna et al. 2015; Paiga et al. 2016; Schultz et al. 2010). With regard to
78   the critical effect concentrations (CEC) published by Fick et al. (2010), it can be seen that
79   citalopram has a very low CEC of 141 ng/L compared to other antidepressants like fluoxetine
80   (CEC: 489 ng/L) or venlafaxine (CEC: 6112 ng/L). Despite the fact that citalopram is one of the
81   most commonly prescribed antidepressants and is frequently detected in surface waters, most
82   studies on the effects of SSRIs in fish were performed with other pharmaceuticals like fluoxetine
83   (Airhart et al. 2007; Foran et al. 2004; Henry & Black 2008; Martin et al. 2017); as a result, there
84   is a deficit in knowledge concerning the effects of citalopram in fish. At present, the impact of
85   citalopram on fish have been mixed: Kellner et al. (2016) showed an increase in the swimming
86   activity in three-spined sticklebacks and a longer time spent in the upper aquaria, caused by
87   exposure to 1.5 µg/L citalopram for 21 days. In addition, anxiolytic effects like reduced freezing
88   behaviour in the novel tank diving test and increased curiosity in the novel object test were
89   observed after exposure to 15 and 1,5 µg/L of citalopram respectively for 21 days. Whereas,
90   Kellner et al. (2017) exposed three-spined sticklebacks in a developmental stage for 30 days to
91   1,5 µg/L citalopram with a subsequent 120 days recovery phase and showed a reduced
92   swimming activity in the novel tank diving test as well as increased aggressive behaviour.
93   Though, neither freezing behaviour and latency or number of transitions spent in the upper half
94   were influenced. But, Olsen et al. (2014) could show anxiolytic effects in the novel tank diving
95   test, like reduced freezing behaviour and faster and longer time spent in the upper aquaria in
96   Endler's guppies exposed to 2,3 and 15 µg/L of citalopram for 21 days. With regard to feeding
97   behaviour, Kellner et al. (2015) showed that three-spined sticklebacks had reduced food intake
98   when exposed to 0.15 µg/L citalopram for 21 days. But in contrast, Kellner et al. (2017)
99   observed increased food intake in sticklebacks exposed to 1.5 µg/L citalopram for 30 days during
100 developmental stages, and a subsequent 120 days recovery phase. Keysomi et al. (2013) also
101 proved a decrease in plasma cortisol level in rainbow trout (*Oncorhynchus mykiss*) exposed to 5
102 µg/L citalopram for 10 days. Due to these diverse outcomes, it is important to assess different
103 endpoints in different life stages to detect the diverse effects that citalopram can have on aquatic
104 organisms. In our case, two life stages of the native fish species brown trout (*Salmo trutta* f.
105 *fario*) were chosen because of the species´ sensitivity and high ecological relevance in central
106 Europe (Klemetsen et al. 2003). To assess the effects of citalopram on developmental parameters
107 like mortality, time to hatch and heart rate, we exposed brown trout eggs to citalopram at 7 and
108 11°C for 5 months until 8 weeks post yolk consumption. Also, juvenile brown trout were
109 exposed to citalopram for 4 weeks at 7 °C, and the apical endpoints mortality, weight and length

110   were evaluated. The cortisol content of the juvenile fish was assessed. Cortisol is a
111   glucocorticoid hormone which has various effects in fish like the regulation of hydro-mineral
112   balance and energy metabolism (Wendelaar Bonga 1997), and it can be used as an indicator of
113   stress. In addition, in both experiments behavioural parameters were recorded during the
114   exposure period. The preference of the fish to stay in the upper aquaria part in the exposure tanks
115   was assessed and swimming behaviour in a stressful environment was recorded using an
116   artificial swimming measurement device. The aim of this study was to evaluate effects of
117   citalopram on developmental and behavioural endpoints. Furthermore, the study aimed at
118   showing whether these effects occur under chronic exposure at environmentally relevant
119   concentrations.

Material and methods

Fish
122   Brown trout (*Salmo trutta* f. *fario*) eggs and juveniles were purchased from a trout farm in
123   Southern Germany (Forellenzucht Lohmühle, Alpirsbach-Ehlenbogen, Germany). This
124   commercial fish breeder is listed as category I (disease-free) according to the EC Council
125   Directive (2006). The eggs were obtained in the eyed ova stage in December 2016 and directly
126   transferred into the experiment. The juveniles were acclimatised to laboratory conditions for two
127   weeks prior to exposure in a 200 L tank (filtered tap water, aerated) and exposed afterwards in
128   August 2017. Fish were kept under a 10:14 light:dark regime and fed daily with commercial
129   trout feed (8 mm, Inico Plus, Biomar, Brande, Denmark). All animals were approved by the
130   animal welfare committee of the Regional Council of Tübingen, Germany (ZO 2/16).

Test Substance
132   Citalopram hydrobromide ($C_{20}H_{21}FN_2O \cdot HBr$, CAS: 59729-32-7) was purchased from Sigma
133   Aldrich (Steinheim, Germany). It was dissolved in distilled water to obtain stock solutions of 100
134   mg/L and 1 mg/L citalopram. The citalopram concentrations refer to citalopram free base
135   ($C_{20}H_{21}FN_2O$). To achieve the respective nominal concentrations, test solutions were prepared
136   with appropriate volumes of the equivalent stock solutions and aerated, filtered tap water (iron
137   filter, active charcoal filter, particle filter).

Experiment with brown trout larvae
139   Brown trout eggs in the eyed ova stage (37 days post fertilisation (dpf)) were exposed in a semi
140   static three block setup to 0, 1, 10, 100, 1000 µg/L citalopram at both 7°C and 11°C, in order to
141   reveal influences of temperature on the effects investigated. Additionally, we had one tank in
142   each of the three replicate blocks with 100 µg/L citalopram but without fish, which served as a
143   control for ingestion, photolytic and microbial degradation of the chemical. Aquaria containing
144   10 L test solution and 30 fish each were set up in triplicate in a randomised order. Twice a week,
145   50% of the test solution were replaced with freshly prepared test solution. A 10:14 light:dark
146   cycle was set and the tanks were covered with black foil to protect them from direct light. Fish
147   were fed daily (0.5 mm, 0.8 mm, Inico Plus, Biomar, Brande, Denmark) from the day the yolk-
148   sac was consumed (for 7°C: 52 d post hatch; for 11°C: 35 d post hatch) with a defined amount
149   of food (3% body weight) adjusted to the developmental state of the fish. Exposure ended 8
150   weeks (total exposure time 7°C: 135 d; 11°C: 107 d) after yolk-sac consumption. During the

151 exposure, time to hatch and mortality were recorded daily. At 7 days post-hatch, the heart rate of
152 5 individuals of each control and the highest concentration tank was measured and, whenever a
153 difference was revealed, the other treatments were also assessed. Two weeks before sampling,
154 pictures of the photographable tanks were taken on a daily basis to assess the number of fish in
155 the upper and lower aquaria part during exposure. One week before the last sampling, the
156 swimming behaviour was recorded using an artificial swimming measurement device (ASMD).
157 Here, 5 fish from each tank were transferred into small glass aquaria and swimming behaviour
158 was recorded for 18 minutes. Terminally, fish were sampled 8 weeks after yolk sac consumption.
159 When sampling took place, the fish were anaesthetised by an overdose of the fish anaesthetic
160 MS222 (tricaine mesylate, 1 g/L, buffered with NaHCO$_3$) followed by a cervical spine cut. After
161 individual determination of the weight and the total length fish were dissected, and tailfins were
162 frozen in liquid nitrogen and stored at -80°C for further analysis of citalopram tissue content.
163 Water conditions (temperature, conductivity, pH, oxygen content) were tested at the beginning,
164 twice during the experiment and at the end.

165 **Experiment with juvenile brown trout**
166 Juvenile brown trout (8 months post hatch) were exposed at 7°C in a semi-static three block
167 setup to 0, 1, 10, 100, 1000 µg/L citalopram for 28 days. The treatments were setup in triplicate
168 in a randomised order in aquaria containing 15 L of the test solution and 10 fish each. Twice a
169 week, 50% of the test solution were renewed. The test was conducted under a 10:14 light:dark
170 regime at 7°C, and the tanks were covered with black foil to protect them from direct light. Fish
171 were fed daily with a defined amount (3% body weight) of commercial trout feed (0.8 mm, Inico
172 Plus, Biomar, Brande, Denmark). Mortality was recorded daily. From two weeks before the
173 sampling took place until the end of the experiment, daily pictures of the photographable tanks
174 were taken to assess the number of fish in the upper and lower aquaria portion during the
175 exposure. In addition, three fish from each tank were used for swimming behaviour
176 measurements in the artificial swimming measurement device (ASMD) explained further below
177 and sampled afterwards. At the end of the experiment, 7 of the 10 fish per tank were
178 anaesthetised and killed by an overdose of the fish anaesthetic, MS222 (Tricaine mesylate, 1 g/L,
179 buffered with NaHCO$_3$) followed by a cervical spine cut. Prior to dissection, the weight and total
180 length of fish were determined. The dorsal part and tailfin of the fish were frozen in liquid
181 nitrogen and stored at -80°C for further analysis of cortisol and citalopram tissue content. Water
182 conditions were tested at the beginning and end of the experiment, and water samples for
183 chemical analyses were taken right before the start of the experiment, after 2 weeks and at the
184 end of the experiment.

185 **Chemical analyses**
186 Water samples were taken at the beginning, in the course and at the end of the experiments.
187 Sampling during the exposure period took place regularly before and after water exchange.
188 Water samples from triplicate aquaria were pooled and stored at -20°C until further processing.
189 For tissue analysis, at the end of the experiments, tailfin samples of the fish were taken to
190 determine the citalopram concentration in the muscle.

Water analysis

The real water concentrations were determined using LC-MS with a 1290 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany) and a triple quadrupole mass spectrometer (6490 iFunnel Triple Quadrupole LC/MS, Agilent Technologies, Santa Clara, CA, USA) in ESI (+) mode. An Agilent Poroshell-120-EC-C18 column (2.1 × 100 mm; 2.7 µm particle size) was used at a flow rate of 0.4 mL/min for separation, and column temperature was maintained at 40°C. Eluent A and B were water (+0.1% formic acid) and acetonitrile (+0.1% formic acid), respectively. Gradient elution was used: 0–1 min 5% B, linear increase to 100% B within 7 min, hold for 7 min at 100% B. After switching back to the starting conditions, a reconditioning time of 3 min was employed. Samples were kept in the autosampler at 10°C. The injection volume was 1 or 10 µl (dilution factor 0–100). The limit of detection of citalopram (mass transition m/z 325 → 109) for undiluted samples was 10 ng/L (10 µL injection volume). Further details on the operating parameters of the triple quadrupole are provided in the supplement.

Tissue analysis

The citalopram concentrations in the tissues of brown trout larvae and juveniles were determined by liquid chromatography–mass spectrometry (LC–MS). For sample extraction, a miniaturised and optimised QuEChERS procedure was applied. Fish samples (tailfin samples containing mainly muscle tissue) originating from all exposure concentrations were analysed. For each exposure group, tissue samples of 10 individuals per treatment were pooled. Frozen fish samples (−20°C) were first homogenised by grinding using a mortar and pestle under liquid nitrogen. Aliquots of the homogenised samples were transferred to an Eppendorf tube, and 0.25 mL acetonitrile and 0.75 mL water were added. For extraction, samples were shaken with a vortex device for 30 sec., after which 30 mg sodium chloride and 120 mg anhydrous magnesium sulfate were added; and the sample was immediately shaken for 30 sec. After centrifugation for 15 min at 13 000 rpm, 0.1 mL of the acetonitrile phase were evaporated to dryness under a gentle stream of nitrogen and the concentrated residue was resolved in 0.3 mL methanol. The extracts were diluted to reach concentrations compatible with the calibration range established for citalopram, and filtered for LC-MS analysis. Matrix matched calibration was performed between 1 and 20 µg/L. The limit of detection was 0.06 ng/g. Further details can be found in the supplementary material.

All analyses were performed using a 1260 Infinity LC system coupled to a 6550 iFunnel QTOF mass spectrometer (Agilent Technologies, Waldbronn, Germany and Santa Clara, CA, USA) with an electrospray ionisation source (ESI). Aliquots of 10 µL sample were injected onto a Zorbax Eclipse Plus C18 column (2.1 x 150 mm; 3.5 µm particle size, narrow bore, Agilent Technologies, Waldbronn, Germany) at a column temperature of 40°C. A gradient elution at a flow rate of 0.3 mL/min using water and methanol, containing 0.1% formic acid, was used. Details on the LC-MS method are given in the supplementary material.

Swimming behaviour in exposure aquaria

For quantification of the swimming behaviour during the exposure, photos of tanks were taken and the number of fish in the upper and lower aquaria part was counted. This was not possible for all aquaria due to their position in the climate chamber. Nevertheless, the selection of

232 photographable tanks was representative for the entire number of aquaria. Pictures were taken
233 with a Panasonic DMC-TZ56 camera 5 minutes after the black foil cover was removed; a white
234 sheet of paper was placed in the back of the aquarium to provide a bright background for better
235 contrast. For experiments with brown trout larvae, 3 pictures were taken per day of each
236 photographable tank at an interval of 5 minutes, from one-week prior to the experiment until
237 sampling (Apr 03 – Apr 13, 2017). In the juvenile brown trout experiment, 3 pictures were taken
238 every day of each photographable tank at an interval of 5 minutes, from two weeks before
239 sampling until sampling (Aug 21 – Sep 03, 2017). The pictures were analysed manually and the
240 number of fish located in the lower and upper half of the aquaria was recorded. Data for all
241 pictures taken from one tank on the same day were averaged.

242 **Artificial swimming measurement device (ASMD)**
243 The recording of the brown trout larvae took place one week before the second sampling of the
244 fish. Small aquaria (17*17*8.5 cm) were filled with 500 ml of the respective test solution at an
245 appropriate temperature, and five brown trout larvae were placed in there. The testing of juvenile
246 fish was scheduled after the sampling, where the swimming behaviour of the three leftover
247 juvenile brown trout from each tank was recorded. One litre of the respective test solution was
248 added to the small aquaria, before the three juvenile fish were transferred to them and recorded.
249 Each of the four small aquaria was equipped with a camera (Basler acA 1300-60 gm, 1.3 MP
250 resolution, Basler AG, Ahrensburg, Germany, lens: 4.5-12.5 mm; 1:1.2; IR 1/2") placed 32 cm
251 above the water surface. The setup was arranged on a table in the climate chamber and enclosed
252 by white polystyrene plates on each side and on top. Inside the enclosure, 4 lamps (2700 K, 1521
253 lm each) were placed, one in each corner facing the top polystyrene plate to obtain indirect
254 illumination. The bright illumination, lack of aeration of the ASMD-aquaria and the transfer
255 process of the fish led to stressful conditions for them. Locomotion was recorded for 20 minutes,
256 but the first 2 minutes were ignored to account for acclimatisation. During the remaining 18 min,
257 video sequences were taken and each of the four aquaria was analysed individually. Fish were
258 centre-point tracked individually, and the total distance moved plus the average velocity were
259 logged with the EthoVision 12 XT (Noldus Information Technology bv, Wageningen,
260 Netherlands). A manual correction of some of the tracked data was essential due to difficulties in
261 automatic tracking.

262 **Cortisol content**
263 Cortisol content was determined in juvenile brown trout exposed under stressful conditions in the
264 ASMD as well as in fish not exposed to such stress. The cortisol content was measured with the
265 commercially available Fish cortisol ELISA Kit by Cusabio Technology LCC (Houston, Texas,
266 USA). The dorsal parts (muscle and kidney tissue) of juvenile brown trout were manually
267 homogenised in 1xPBS buffer (tissue/buffer ratio 1:11 w/v) with a pestle. After 2 freeze-thaw
268 cycles at -20°C and room temperature, the samples were centrifuged (5000 x g, 5 min, 4°C) and
269 the supernatant stored at -20°C until analysis. Before pipetting the assay, the supernatant was
270 diluted with sample buffer provided in the Kit (supernatant/buffer ratio 1:10 v/v). The assay was
271 conducted in a pre-coated 96 well plate provided by the manufacturer. Each well contained 50
272 µL antibody and either 50 µL of standard or 50 µL of sample, before being incubated for 40 min
273 at 37°C. After 3 washing cycles with washing buffer, 100 µL of HRP-conjugate was added and

274   incubated for 30 min at 37°C. Following 5 washing cycles with washing buffer, 90 µL of TMB
275   (3,3',5,5'-tetramethylbenzidine) substrate were added and incubated for 20 minutes at 37°C.
276   Then, 50 µL of stop solution were added to each well and the plate was measured
277   photometrically at 450 nm and for wavelength correction at 570 nm. Concentrations were
278   calculated with blanked and wavelength corrected data to a four parameter logistic standard
279   curve fit. Concentrations of cortisol are expressed in ng/mL (see Table 1).

280   **Statistical analysis**
281   Statistical analyses were performed with SAS JMP 14 and R 3.5.0 (packages: lme4). Mortality
282   and time to hatch were analysed by nested Cox proportional hazards model, using replicate
283   aquaria as a nested factor. Length, weight and Data of the ASMD were analysed by a nested
284   ANOVA, using replicate aquaria as nested factor, and a post hoc Dunnett's test. If necessary,
285   data were transformed to achieve normal distribution and homogeneity of variance. If no normal
286   distribution could be achieved, data were evaluated with a nonparametric Kruskal-Wallis test
287   post-hoc Steel method with control. The difference in cortisol content was analysed with a
288   Linear Mixed Model with replicate as random factor and subsequently post-hoc Dunnett's test.
289   Data for swimming behaviour during the exposure were evaluated with a Generalized Linear
290   Mixed Model (binomial distribution, aquarium identity as random factor) and supsequently post-
291   hoc Dunnett's Test. The α-level was set to 0.05. Comparison of the results for different climate
292   chambers was only descriptive to prevent the problem of pseudo-replication due to missing
293   climate chamber replicates. Statistical details are given in the supplementary material.

294   **Criteria for reporting and evaluating ecotoxicity data (CRED)**
295   Criteria for reporting and evaluating ecotoxicity data (CRED) are given in the supplementary
296   material (Moermond et al. 2016). CRED is important to improve the reproducibility, relevance
297   and transparency of aquatic ecotoxic research between the different institutions (Moermond et al.
298   2016).

299   # Results
300   **Water conditions**
301   Temperature, conductivity, pH and oxygen content were measured at the beginning and end of
302   both experiments. In the brown trout larvae experiment, water quality parameters were assessed
303   at 2 additional time points (18.01.2017, 06.03.2017). All water quality parameters were in an
304   acceptable range (brown trout larvae: mean temperature 7°C: 7.1 ± 0.32°C; 11°C: 10.47 ±
305   0,24°C; mean conductivity 7°C: 472.6 ± 9.9 µS/cm; 11°C: 478.3 ± 7.2 µS/cm; mean pH 7°C:
306   8.08 ± 0.41; 11°C: 7.96 ± 0.46; mean oxygen content 7°C: 10.77 ± 0.3 mg/L; 11°C: 9.94 ± 0.5
307   mg/L; juvenile brown trout: mean temperature: 7.15 ± 0.41°C; mean conductivity: 493.7 ± 17.5
308   µS/cm; mean pH: 8.09 ± 0.01; mean oxygen content: 11.22 ± 0.1 mg/L). Further details are
309   given in the supplementary materials.

310   **Chemical analyses**
311   Regarding water analysis, citalopram could not be detected in any of the control samples. In
312   most of the treatments, aqueous citalopram concentrations measured were lower than nominal
313   concentrations, except for the treatments with the highest concentrations in the brown trout
314   larvae experiment and the exposure at 1 µg/L of the juvenile brown trout experiment. The

315   recovery rate was about 80%. The citalopram concentrations in the controls for photolytic and
316   microbial degradation were slightly higher (79.84 ± 2.50 µg/L) than in the 100 µg/L exposure
317   tank (70.50 ± 11.11 µg/L). Overall, the measured citalopram concentrations in water samples
318   were in good accordance with the nominal concentrations (Table 1). Further details on water
319   concentrations are given in the supplementary materials.

320   Regarding biota analysis, citalopram determined in tissue samples was in the µg/g range.
321   Citalopram could not be detected in the muscle tissue of brown trout in any of the control
322   samples. Tissue concentrations of citalopram were shown to correlate with water concentrations
323   with the highest values in fish exposed to 1000 µg/L citalopram. Tissue concentrations of brown
324   trout larvae exposed at 7°C were higher than those of brown trout larvae exposed at 11°C.
325   Juvenile brown trout accumulated at least 20 times more citalopram in muscle tissue than brown
326   trout larvae.

Experiment with brown trout larvae

328   Table 1

329   The mortality of brown trout larvae was not affected by citalopram (11°C Cox Regression: df=4,
330    $\chi 2$=4.2743, p=0.3701; 7°C Cox Regression: df=4, $\chi 2$=6.9203, p=0.1402). However, the mortality
331   of larvae exposed at 11°C was higher in all treatments, including the controls, from day 51 to day
332   86 of exposure (11°C mean mortality 29.11%). During this exposure time, fish were 43 d – 71 d
333   post hatch which is the critical phase of complete yolk sac consumption and first food intake.
334   The mortality of fish exposed to 7°C ranged from 0 – 10% (7°C mean mortality 4.69%). Also,
335   time to hatch did not differ between treatments (11°C: Cox Regression: df=4, $\chi 2$=0, p=1; 7°C:
336   Cox Regression: df=4, $\chi 2$=2.42E-0.9, p=1). The fish exposed at 11°C hatched approximately 10
337   days earlier than fish exposed at 7°C. The heart rate of brown trout larvae was not affected when
338   exposed to 1000 µg/L citalopram at 11°C (nested ANOVA: df=1, F=0.3968, p=0.5347) and 7°C
339   (nested ANOVA: df=4, F=2.6161, p=0.0447; post-hoc Dunnett's test no difference to control).
340   The heart rate of the fish exposed at 11°C was about 20 beats per minute higher on the average
341   than in fish exposed at 7°C. The weight and length of fish exposed to 1000 µg/L citalopram was
342   significantly lower compared to the control at both temperatures (Table 1) (11°C weight: nested
343   ANOVA: df=4, F=12.8137, p<0.0001; post-hoc Dunnett's test [0 µg/L|1000 µg/L] p<0.0001;
344   11°C length: nested ANOVA: df=4, F=13.1786, p<0.0001; post-hoc Dunnett's test
345   [0 µg/L|1000 µg/L] p<0.0001; 7°C weight: nested ANOVA: df=4, F=9.7415, p<0.0001; post-hoc
346   Dunnett's test [0 µg/L|1000 µg/L] p<0.0001; 7°C length: nested ANOVA: df=4, F=22.0216,
347   p<0.0001; post-hoc Dunnett's test [0 µg/L|1000 µg/L] p<0.0001).

348   Swimming behaviour during exposure
349   We could show that citalopram had an effect on the stay of the fish in the upper half of the
350   aquaria at both temperatures. Significantly more fish exposed to 1000 µg/L citalopram stayed
351   close to the water surface compared to the control fish at both temperatures. Fish exposed to 1
352   and 10 µg/L citalopram concentrations at 7°C stayed slightly more often in the upper half of the
353   aquaria than control fish (Table 1) (11°C: Generalized Linear Mixed Model, df=4, F= 12.4141,
354   post-hoc Dunnett's [0 µg/L|1000 µg/L] p<0.0001; 7°C: Generalized Linear Mixed Model, df=4,

355   F= 106.9664, post-hoc Dunnett's [0 µg/L|1µg/L] p=0,0265, [0 µg/L|10 µg/L] p=0.0165, [0
356   µg/L|1000 µg/L] p<0.001).

357   ASMD
358   Figure 1

359   Overall, fish exposed at 11°C swam further and faster in the ASMD than fish exposed at 7°C.
360   Furthermore, the citalopram treatments also revealed an effect on the total distance moved and
361   the mean velocity during the recordings in the ASMD. Fish exposed to 1000 µg/L citalopram at
362   11°C swam significantly less and slower than control fish (distance moved: nested ANOVA:
363   df=4, F=4.7551, p=0.0021; post-hoc Dunnett's test [0 µg/L|1000 µg/L] p=0.0008; velocity:
364   nested ANOVA: df=4, F=4.7552, p=0.0021; post-hoc Dunnett's test [0 µg/L|1000 µg/L]
365   p=0.0009). Also, fish exposed at 7°C showed significantly less total distance moved and had a
366   lower mean velocity when exposed to 100 µg/L or 1000 µg/L citalopram, compared to control
367   fish (Table 1, Figure 1, A, D) (distance moved: nested ANOVA: df=4, F=7.8214, p<0.0001;
368   post-hoc Dunnett's test [0 µg/L|100 µg/L] p=0.0212 [0 µg/L|1000 µg/L] p<0.0001; velocity:
369   nested ANOVA: df=4, F=7.8214, p<0.0001; post-hoc Dunnett's test [0 µg/L|100 µg/L] p=0.0212
370   [0 µg/L|1000 µg/L] p<0.0001). Furthermore, it can be seen that control brown trout larvae have
371   an increased time of no movement at the beginning of the experiment, which decreases overtime.
372   Contrary mean velocity at the beginning of the experiment is lower and increases with time and
373   has its peak at 12 minutes. In contrast brown trout larvae exposed to the highest treatment have a
374   constant lower mean velocity over time and a constant longer time of no movement over
375   recordingtime (Figure 1, B-C, E-F).

376   **Experiment with juvenile brown trout**
377   Table 2

378   No mortality occurred during the experiment. Weight and length were significantly lower in fish
379   exposed to 1000 µg/L citalopram compared to control fish (weight: nested ANOVA: df=4,
380   F=3.2964, p=0.0013; post-hoc Dunnett's test [0 µg/L|1000 µg/L] p=0.0234; length: nested
381   ANOVA: df=4, F=4.6661, p=0.0015; post-hoc Dunnett's test [0 µg/L|1000 µg/L] p=0.0193).

382   Swimming during exposure
383   There was a strong effect of the highest citalopram concentration on the swimming behaviour of
384   fish: About 25% of the 1000 µg/L citalopram-treated fish stayed in the upper half of the aquaria,
385   in contrast to the control and other treatments, where no fish stayed in the upper aquaria part
386   (Generalized linear mixed model, df=4, F= 7.3259, post-hoc Dunnett's [0 µg/L|1000 µg/L]
387   p=0,00107) (Table 2).

388   ASMD
389   In the artificial swimming measurement device, neither the total distance moved nor the mean
390   velocity of the exposed fish differed significantly to the control (Table 2, Figure 1, G)(distance
391   moved: nested ANOVA: df=4. F=1.0846. p=0.3818; velocity: nested ANOVA: df=4, F=1.0846,
392   p=0.3818). Mean total distance moved and averaged mean velocity of the exposed fish were
393   about 70% of the fish from the control; however, this was not significant. The behaviour pattern

394 over time does not differ strongly between control and treated fish. At the beginning juvenile
395 brown trout have a reduces mean velocity, which increases over time. Opposed to this time of no
396 movement is decreasing over time (Figure 1, H-I).

397 Cortisol
398 Figure 2

399 Tissue cortisol concentrations did not differ between exposed and control fish. The citalopram
400 exposed fish did not show significant differences between the treatments (Table 2, Figure 2)
401 (Linear Mixed Model: df=4,68.638, F=3.7625, p=0.007959, post-hoc Dunnett's Test revealed no
402 difference between control and treatments). However, there was a significant increase in tissue
403 cortisol content in fish tested in the ASMD (mean cortisol content: 26.66 ± 18.57) compared to
404 fish not tested in the ASMD (mean cortisol content: 15.22 ± 10.16) (Linear Mixed Model:
405 df=1,95.378, F=16.7132, p<0.0001) (Figure 2).

Discussion

407 This study shows that citalopram affects the swimming behaviour and growth of brown trout in
408 different life stages. Effect concentrations were close to citalopram concentrations measured in
409 wastewater effluents (Fick et al. 2009; Larsson et al. 2007; Nodler et al. 2010; Vasskog et al.
410 2006).

Bioconcentration

412 It has been shown that citalopram can accumulate in the liver, kidney and brain of fish
413 (Grabicova et al. 2017; Grabicova et al. 2014). In the present study, we analysed tail fin tissue
414 samples (muscle) since all other organs were used for biomarker analyses. The obtained data
415 made evident that even early life stages of brown trout but, more intensely, juveniles accumulate
416 citalopram in their muscle tissue. In both life stages, citalopram tissue concentrations rose
417 slightly with increasing exposure concentrations of 1 to 100 µg/L (Table 1 and Table 2). When
418 comparing the aqueous with the tissue concentration there is a linear relationship between
419 exposure concentrations and internal concentration (Supplemental material, Figure 1).
420 Citalopram concentrations in fish exposed at 7°C (135 days) were about 3 times higher than
421 those in fish exposed at 11°C (107 days), possibly due to the longer exposure time of about 4
422 weeks. The muscle tissue concentration of juvenile brown trout was about 10 times higher than
423 the muscle tissue concentration of brown trout larvae possibly based on a more intense
424 citalopram uptake due to the ongoing development of the gastro-intestinal system and/or gills of
425 larvae. Sackerman et al. (2010) showed a bioconcentration of 115 ± 37 ng/g citalopram in the
426 brain and 193 ± 33 ng/g in the muscle tissue of zebrafish exposed to 24.3 µg/L for only 3
427 minutes. Brown trout exposed to an effluent-dominated stream (citalopram water concentrations:
428 4.5-58 ng/L) in the Czech Republic showed citalopram concentrations up to 31 ± 11 ng/g in liver
429 and 164 ± 19 ng/g in kidney tissue; however, no citalopram could be detected in brain and
430 muscle tissue (Grabicova et al. 2017). Likewise, rainbow trout exposed to the effluent of a
431 Swedish wastewater treatment plant (citalopram water concentration: 260±60 ng/L) had most
432 citalopram in the liver and brain, with concentrations of 12 ± 5 ng/g and 2.2 ± 1.3 ng/g,
433 respectively. In contrast, no citalopram was detected in plasma and muscle tissue (Grabicova et
434 al. 2014). The reason for finding citalopram being accumulated in muscle tissue in our study

435 might either be due to the fact that the exposure time was longer and the test concentrations were
436 higher than those used in other studies (Grabicova et al. 2017; Grabicova et al. 2014; Sackerman
437 et al. 2010) or result from a diverging accumulation pattern in brown trout compared to other fish
438 species (Du et al. 2016).

Mortality

440 Mortality of larvae and juvenile fish was not influenced by citalopram exposure. Although the
441 overall mortality increased to 29% in brown trout larvae exposed at 11°C. This higher mortality
442 relates to the fact that brown trout larvae exposed to 11°C had a higher metabolism and that the
443 beginning of exogenous feeding in salmonids is associated with higher mortality risks
444 (Klemetsen et al. 2003). Also in a study by Kellner et al. (2016), in which sticklebacks have been
445 exposed for 21 days to 1.5 and 15 µg/L citalopram, no differences in mortality were found.
446 Likewise, time to hatch was not affected by citalopram. This is in accordance with other studies
447 that did not reveal differences in hatching success and time for zebrafish or Japanese medaka
448 exposed to the SSRI fluoxetine (Foran et al. 2004; Wu et al. 2017). With regard to the
449 developmental parameter heart rate of brown trout larvae, no effect was seen in fish exposed to
450 either temperature and any citalopram concentration corroborating finding for other SSRIs by
451 Airhart et al. (2007), who exposed zebrafish larvae to 1,39 mg/L of the SSRI fluoxetine.

Fish growth

453 In our experiment, we visually observed more food leftovers in the exposure tanks with 1000
454 µg/L citalopram, which allowed us to conclude that there was a lower food intake in fish exposed
455 to 1000 µg/L citalopram; however, a quantification of this effect was not possible. Known side
456 effects of citalopram are anorexia and weight loss in humans (Information of HEXAL (2012))
457 and published data on fish revealed a decreased food intake in sticklebacks exposed to 0.15 µg/L
458 citalopram (Kellner et al. 2015). Reduced food intake in fish was also shown for two other
459 antidepressants, sertraline and fluoxetine, in European perch and goldfish (Hedgespeth et al.
460 2014; Mennigen et al. 2010). In contrast three-spined stickleblacks exposed for 30 days to 1,5
461 µg/L citalopram with a subsequent 185 days recovery phase showed increased attacks on a
462 chironomid larvae (Kellner et al. 2017). Mechanistically, the increased swimming activity and
463 the conceivably decreased food intake of the fish make the observed decrease in weight and
464 length of the fish exposed to 1000 µg/L citalopram is reasonable to result from exposure to
465 citalopram, especially as exposed fish were in a period of intense growth. Decreased weight has
466 also been shown in goldfish and decreased length in zebrafish exposed to 54 and 10 µg/L of the
467 SSRI fluoxetine, respectively (Mennigen et al. 2010; Wu et al. 2017).

Behaviour during exposure

469 Control brown trout preferably stayed at the bottom of the tank when kept in the exposure tanks
470 (Table 1 and 2). Fish exposed to 1000 µg/L citalopram showed an increased preference for the
471 upper half of the aquaria independent of their life stage. In general, the test design used in the
472 present study for this parameter implies parts of the novel tank diving test, the scototaxis test and
473 shoaling. Stewart et al. (2012) described the novel tank diving test for the measurement of
474 anxiety, where single zebrafish are placed in tanks. Time spent in the upper aquaria portion is
475 recorded along with other parameters like the number of transitions into the upper aquaria
476 portion or number of freezing bouts. When the fish in a novel tank stay in the lower aquaria part

477 (geotaxis) it is a sign for anxiety behaviour and in contrast the transition into the upper aquaria
478 part is a sign of boldness and therefore anti-anxiety behaviour (Stewart et al. 2012). Maximino et
479 al. (2010) described the scototaxis test, where fish are transposed into a novel tank and can freely
480 swim into the dark or bright zone. Preferring the dark part of the aquaria and avoiding the
481 brighter side is a clear sign of anxiety (Maximino et al. 2010). Furthermore, Stewart et al. (2012)
482 described fish with lower anxiety to have a greater tendency to break away from the shoal in
483 zebrafish. It is obvious that in our case swimming behaviour of the shoal of brown trout was
484 assessed and not individual swimming behaviour. Therefore, the stressor not being in a shoal,
485 like in the novel tank and scototaxis test is not present in our case. Additionally, brown trout
486 were not transposed into novel tanks, but the tanks were covered the most of the exposure time
487 with black foil, only when the photos were taken, the foil was removed, which lead to a higher
488 illumination from the top. Overall an increased stay in the upper aquaria part is a preference for
489 the brighter illuminated water column and water surface in contrast to the darker aquaria bottom.
490 Additionally, these fish recede from the shoal at the bottom of the tank. In our experiments, up to
491 80% brown trout larvae and 25% of juveniles exposed to 1000 µg/L citalopram stayed in the
492 upper aquaria section compared to the control animal. This can be explained by decreased
493 anxiety and an altered swimming behaviour characterised by a higher activity of the exposed fish
494 (Maximino et al. 2010; Stewart et al. 2012). The stronger effect of citalopram on the vertical
495 distribution of the brown trout larvae can be explained by a 5 times longer exposure time
496 compared to the juvenile individuals. Also, different sensitivities of the life stages can come into
497 play, and has already be shown for diclofenac (Schwarz et al. 2017). The significant difference in
498 quantity spent in the upper aquaria part of the brown trout larvae exposed to 1 µg/L citalopram at
499 7°C could also be due to the fact that only one of the three replicate aquaria was photographed
500 and therefore a single individual has a higher impact on the relative number spent in the upper
501 aquaria part. For this reason and the inherent variation in this setup for behaviour measurement
502 the biological relevance of the slight effect in the 1 and 10 µg/L treatment has to be confirmed
503 with a bigger sample size. An anxiolytic effect of citalopram in the novel tank test was also
504 shown for other fish species like Endler's guppies, three-spined sticklebacks and zebrafish, even
505 at decidedly lower concentrations of citalopram down to 1.5 µg/L, which all spent more time in
506 the upper aquaria part during the novel tank test (Kellner et al. 2016; Olsen et al. 2014;
507 Sackerman et al. 2010). Furthermore, Kellner et al. (2017) discovered increased transitions to the
508 bright side in sticklebacks exposed to 1,5 µg/L citalopram for 30 days with subsequent 120 days
509 recovery phase. But latency to first transition and time spent in the brighter zone was not
510 influenced. Also sticklebacks exposed did not show any difference to control fish in the novel
511 tank test, except of decreased acitivty (Kellner et al. 2017). Kellner et al. (2016) also observed
512 increased swimming activity in fish exposed to 1.5 µg/L citalopram. This effect has not only
513 been shown in response to citalopram but has also been found for other antidepressants like
514 fluoxetine or amitriptyline, which seem to reduce anxiety and increase the stay of the fish in the
515 upper part of the aquarium (Demin et al. 2017; Henry & Black 2008; Meshalkina et al. 2018)..

516 **Behaviour in a stressful environment and cortisol measurements**
517 In contrast to the increased swimming activity under minor stress conditions, brown trout larvae
518 exposed to citalopram showed a decreased swimming activity in the stressful artificial swimming
519 measurement device (ASMD). This effect can be due to the anxiolytic and soothing effect of the

520 antidepressant. Based on the measurements of the tissue cortisol level, it is evident that the
521 ASMD creates a rather stressful environment for the fish (Figure 2) (Wendelaar Bonga 1997).
522 The transfer of fish into an ASMD leads to a stress reaction in larvae. This stress reaction is
523 characterised by freezing behaviour at the beginning followed up by increased velocity and
524 escape behaviour (Figure 1). Having a look on the time dependant velocity and time of no
525 movement, it is clear that the control larvae are swimming faster and have reduced time of no
526 movements over recording. In contrast larvae exposed to 1 mg/L citalopram do not show this
527 pattern, but have a constant low mean velocity and longer time of no movement over the total
528 recording time. A similar pattern can be seen in fish exposed to 100 µg/L citalopram but not as
529 distinct as in fish exposed to 1 mg/L. Since citalopram is an anxiolytic drug and reduces anxiety,
530 fish are reasonably soothed when exposed to 100 µg/L or 1000 µg/L citalopram as they swam
531 slower than control fish over the total recording time. Therefore, the total distance they moved
532 and their mean swimming velocity were lower than that of the controls. Additionally, the time
533 dependant behaviour pattern is not given in fish exposed to 1 mg/L citalopram. It is also possible
534 the reduced mean velocity and increased time of no movement is due to a sedative effect of the
535 antidepressant. Furthermore, it cannot be excluded, that the increased time of no movement of
536 fish exposed to 1 mg/L citalopram represents freezing and therefore anxiogenic behaviour.
537 Nevertheless, with regard to the results of the behaviour during the exposure, it is more likely
538 that the effect is due to a reduction of anxiety in brown trout larvae which resulted in calmed
539 behaviour. This reduction in activity due to citalopram in the ASMD could not be seen in
540 juvenile fish. Neither total distance moved nor the pattern over time differed between the
541 treatment and the control animals. Nevertheless, the total distance moved and the mean velocity
542 of swimming in all citalopram-treated juveniles was 70% lower than in controls, but these
543 differences were not significant. One explanation for this difference between juvenile fish and
544 larvae can be the shorter exposure time of juvenile fish (larvae were exposed about 5 times
545 longer than juveniles). Another factor contributing to the observed difference between the two
546 life stages could also be a divergent sensitivity to the antidepressant and was already shown for
547 diclofenac by Schwarz et al. (2017). It has been reported previously that antidepressants reduce
548 swimming activity. Three-spined sticklebacks exposed to 1,5 µg/L citalopram for 30 days at a
549 developmental stage with subsequent 160 days recovery phase showed a decrease in swimming
550 activity (Kellner et al. 2017). Zebrafish embryos exposed to either 1 mg/L venlafaxine or 3 mg/L
551 sertraline showed reduced swimming behaviour in the zebrabox (Sehonova et al. 2019). With
552 regard to the sedative effect of antidepressants, for example, western mosquitofish exposed to 0.5
553 µg/L fluoxetine over 91 days showed increased lethargy similar to fish exposed to 53 µg/L for 7
554 days (Henry & Black 2008). Furthermore, guppies exposed to 16 ng/L fluoxetine for 28 days
555 showed increased freezing time and time spent under cover (Saaristo et al. 2017). Male bluehead
556 wrasse injected intraperitoneal with 6 µg/g bw fluoxetine showed decreased activity levels
557 (Perreault et al. 2003). But also meagre exposed to 20 µg/L venlafaxine showed reduced
558 swimming activity (Maulvault et al. 2018). But it could also be shown that antidepressants
559 reduces anxiety in exposed fish: Painter (2009) showed a decreased escape behaviour in fathead
560 minnow larvae exposed to 250 ng/L of the SSRI fluoxetine, resulting in reduced swimming
561 velocity. Likewise, fathead minnow larvae exposed to the serotonin and noradrenalin reuptake

562   inhibitor (SNRI) venlafaxine revealed reduced anxiety, indicated by a reduced escape response
563   (Painter 2009).

564   **Calculated plasma concentrations**
565   Therapeutic human plasma concentrations in patients treated with doses of 20-60 mg citalopram
566   per day are 117 ± 95 µg/L (Le Bloc'h et al. 2003). In contrast, Schreiber et al. (2011) reported a
567   maximum blood plasma concentration of only 21.1 µg/L in patients after drug administration
568   with a maximum daily dose of 60 mg. Considering measured human therapeutic plasma
569   concentrations of citalopram, calculated plasma concentrations in fish exposed to 1, 10, 100 and
570   1000 µg/L at pH 8 with the fish plasma model (Supplement, Table 8) (Fu et al. 2009; Huggett et
571   al. 2003; Schreiber et al. 2011) revealed that the calculated concentrations in fish exposed to 100
572   µg/L citalopram or higher exceeded the human therapeutic plasma concentrations according to
573   Le Bloc'h et al. (2003). When referred to the human plasma concentrations in the study
574   conducted by Schreiber et al. (2011), when even exposed to 10 µg/L fish plasma concentrations
575   would exceed the concentrations in human plasma. However, Holmberg et al. (2011) showed
576   that 2 out of 5 rainbow trout exposed to 10 µg/L citalopram revealed a plasma citalopram
577   concentration of 0.044 µg/L and 0.08 µg/L after exposure for only 24 h. The lack of behavioural
578   effects in the study of Holmberg et al. (2011) and also our results obtained for the lower
579   treatments 1 µg/L and 10 µg/L citalopram, suggests that citalopram plasma concentrations in fish
580   below human therapeutic plasma concentrations do not to affect the fish. Though, our results on
581   behaviour and growth of brown trout provide evidence that citalopram plasma concentrations in
582   fish higher than human therapeutic plasma concentrations can have severe impact on brown trout
583   in different life stages.

584   # Conclusion
585   Our results clearly show that citalopram affects brown trout according to its mode of action
586   known for humans. Under stressful conditions, fish showed reduced swimming behaviour when
587   exposed to at least 100 µg/L citalopram. Furthermore, in the exposure tanks, an increased
588   swimming activity during exposure was observed for fish exposed to 1 mg/L citalopram, which
589   can be linked to the anti-depressant effect of the drug. The behavioural changes were stronger in
590   early life stages, which cannot exclusively be associated with the longer exposure time, but also
591   different sensitivities can play a role. In addition, side-effects of the antidepressant known from
592   human applications could be detected, like reduced weight and length, in both juvenile brown
593   trout and brown trout larvae exposed to 1000 µg/L citalopram. Our results confirm similar
594   findings for citalopram exposure to those reported for other aquatic species. To conclude,
595   citalopram, as a widely distributed drug, severely alters the behaviour and growth of brown trout
596   in different life stages, at concentrations higher than current environmentally relevant levels. And
597   the 10 times stronger accumulation of citalopram in juveniles makes evident that an increase in
598   surface water concentration of citalopram could have severe impact on specific life stages of
599   fish. Nevertheless, considering safety factors up to 10^3 that have to be included in environmental
600   risk assessment and also additive effects of pharmaceuticals affecting similar pathways,
601   citalopram is far from being an environmentally safe pharmaceutical and has to be considered
602   carefully with respect to risk for the aquatic environment.

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780

Results for brown trout larvae exposed to citalopram.

Data are shown as arithmetical means ± standard deviation. Asterisks represent significant differences to the respective control (*p<0.05; **p<0.01; ***p<0.001). Abbreviations: n.a.=not assessed; dpf=day post-fertilisation; LoD=limit of detection.

1 *Table 1:*
2 **Results for brown trout larvae exposed to citalopram**.
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Temperature	11°C					7°C				
Treatment (µg/L)	0	1	10	100	1000	0	1	10	100	1000
Mortality (%)	26.67 ± 2.72	32.22 ± 1.57	28.89 ± 8.75	35.56 ± 4.16	22.22 ± 3.14	2.22 ± 3.14	4.44 ± 4.16	3.37 ± 2.72	6.74 ± 2.72	6.67 ± 2.72
Weight (g)	0.449 ± 0.139	0.484 ± 0.169	0.447 ± 0.140	0.487 ± 0.143	**0.306 ± 0.146 *****	0.327 ± 0.089	0.413 ± 0.113	0.335 ± 0.101	0.337 ± 0.091	**0.247 ± 0.066 *****
Length (cm)	3.76 ± 0.37	3.83 ± 0.42	3.73 ± 0.39	3.74 ± 0.40	**3.24 ± 0.43 *****	3.33 ± 0.27	3.38 ± 0.32	3.30 ± 0.32	3.25 ± 0.28	**2.91 ± 0.23 *****
Heart rate (bpm)	76 ± 3.74	n.a.	n.a.	n.a.	75 ± 4.52	49.93 ± 1.5	51.8 ± 3.19	49.2 ± 3.25	50 ± 3.03	49 ± 2.37
Time to hatch (dpf)	49.69 ± 0.93	49.64 ± 1.11	49.74 ± 1.02	49.22 ± 0.96	49.14 ± 0.84	59.38 ± 1.27	58.68 ± 1.65	59.07 ± 1.31	58.60 ± 1.31	59.38 ± 1.17
Aquaria photographed	2	n.a.	2	2	2	2	1	2	1	2
Sojourn in upper aquaria half (%)	7.29 ± 11.11	n.a.	5.71 ± 10.36	10.68 ± 13.24	**66.52 ± 11.28 *****	0.14 ± 0.68	**2.3 ± 3.73 ***	**2.65 ± 3.22 ***	1.30 ± 2.47	**79.36 ± 7.19 *****
ASMD: total distance moved (cm)	2700 ± 1405	1987 ± 755	2501 ± 815	2252 ± 1353	**1133 ± 1015 *****	2473 ± 1016	1591 ± 1045	1792 ± 1320	**1361 ± 1033 ***	**568 ± 697 *****
ASMD: mean velocity (cm/s)	2.50 ± 1.30	1.84 ± 0.70	2.32 ± 0.76	2.085 ± 1.253	**1.15 ± 0.94 *****	2.29 ± 0.94	1.47 ± 0.97	1.66 ± 1.22	**1.26 ± 0.96 ***	**0.53 ± 0.65 *****
Aqueous citalopram concentration (µg/L)	<LoD	0.97 ± 0.20	8.30 ± 1.17	65.74 ± 5.77	973.98 ± 180.64	<LoD	0.83 ± 0.27	8.74 ± 0.48	70.50 ± 11.11	1017.97 ± 125.84
Tissue citalopram concentration (µg/g) (wet weight)	<LoD	0.07 ± 0.014	0.69 ± 0.1	1.57 ± 0.451	55.87 ± 12.972	<LoD	0.2 ± 0.042	0.97 ± 0.235	5.63 ± 2.0	142.15 ± 44.961

Results for juvenile brown trout exposed to citalopram. <!--[if !supportLineBreakNewLine]--> <!--[endif]-->

Data are shown as arithmetical mean ± standard deviation. Asterisks indicate significant differences to the respective controls (*p<0.05; **p<0.01; ***p<0.001). LoD=limit of detection.

1 *Table 1*
2 *Results for juvenile brown trout exposed to citalopram.*
3 *Data are shown as arithmetical mean ± standard deviation. Asterisks indicate significant differences to the respective controls*
4 *(*p<0.05; **p<0.01; ***p<0.001). LoD=limit of detection*

Treatment (µg/L)	0	1	10	100	1000
Mortality (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Weight (g)	2.75 ± 0.84	2.85 ± 0.85	2.86 ± 1.16	2.74 ± 0.92	**2.17 ± 0.53 ***
Length (cm)	6.41 ± 0.65	6.59 ± 0.61	6.46 ± 0.71	6.50 ± 0.75	**5.93 ± 0.49 ***
Aquaria photographed	2	2	1	2	2
Sojourn in upper aquaria half (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0	**25.42 ± 19.11 ** **
Total distance moved (cm)	1371 ± 1057	847 ± 785	878 ± 1004	939 ± 571	980 ± 473
Mean velocity (cm/s)	1.27 ± 0.98	0.78 ± 0.73	0.81 ± 0.93	0.87 ± 0.53	0.91 ± 0.44
Cortisol content in fish extract (ng/mL)	19.06 ± 14.80	15.51 ± 7.48	12.50 ± 9.58	20.76 ± 15.14	23.66 ± 17.81
Aqueous citalopram concentration (µg/L)	<LoD	1.41 ± 0.22	9.20 ± 0.59	81.51 ± 2.39	864.93 ± 51.54
Tissue citalopram concentration (µg/g) (wet weight)	<LoD	8.2 ± 4.37	38.3 ± 30.71	340.63 ± 124.74	2966.83 ± 1556.77

Figure 1

Results for brown trout in the ASMD.

Results of total distance moved are shown as boxplot (A, D, G), Results of mean velocity (B, E, H) and time of no movement (C, F, I) are shown as bar plot, with depicted mean values over time. A-C: Results for brown trout larvae exposed at 7 °C, D-F: Results for brown trout larvae exposed at 11°C, G-H: Results for juvenile brown trout.
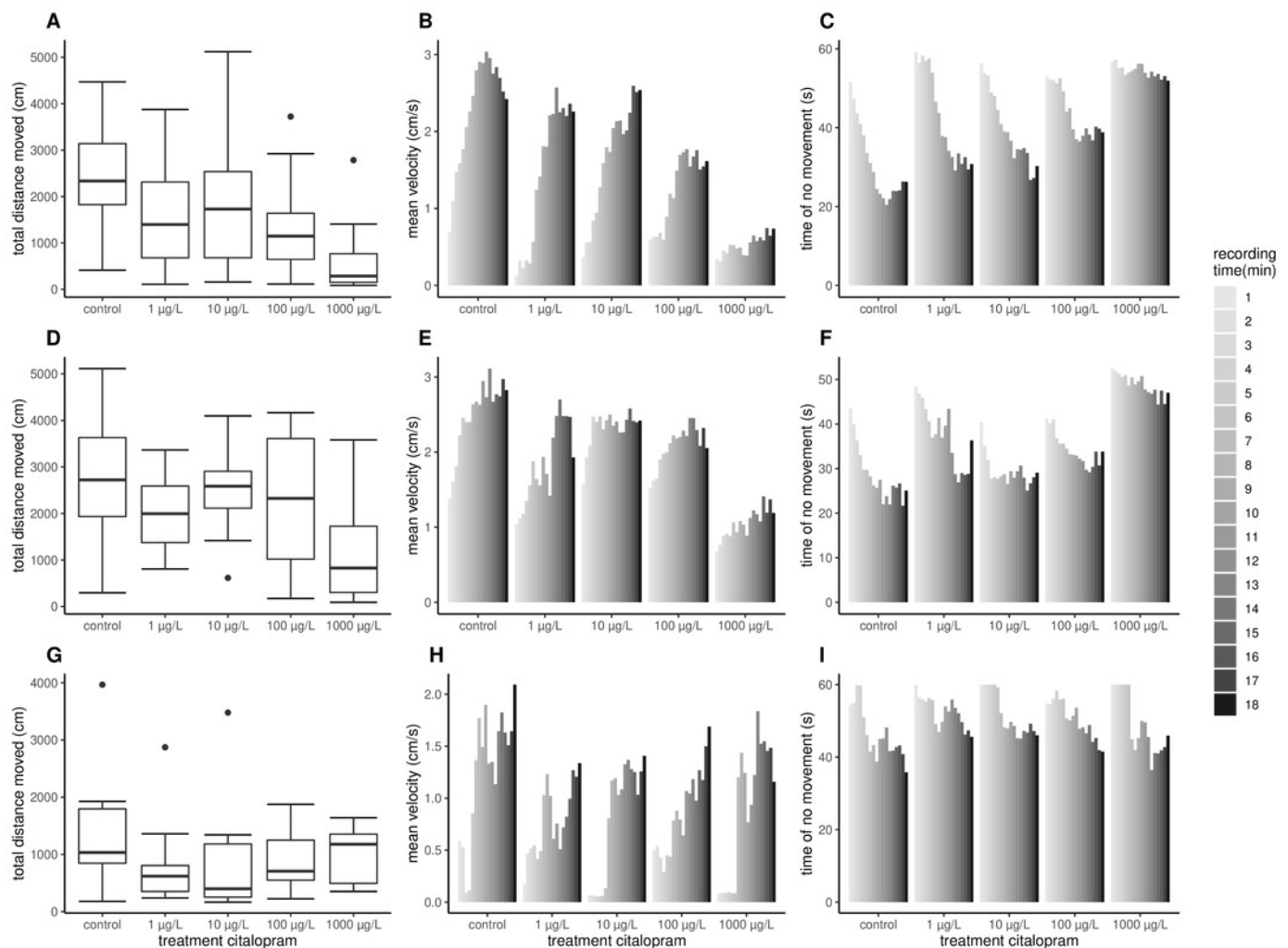
Figure 2

Cortisol content of juvenile brown trout.

Cortisol content of the fish assessed in the ASMD compared to whose swimming behaviour was not assessed in the ASMD. Results are shown as boxplots.