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Abstract

Fluoxetine is a serotonin re-uptake inhibitor, generally used as an antidepressant. It is suspected to provoke substantial effects in the aquatic environment. This study reports the effects of fluoxetine on the life cycle of four invertebrate species, *Daphnia magna*, *Hyalella azteca* and the snail *Potamopyrgus antipodarum* exposed to fluoxetine spiked-water and the midge *Chironomus riparius* exposed to fluoxetine-spiked sediments. For *D. magna*, a multi-generational study was performed with exposition of newborns from exposed organisms. Effects of fluoxetine could be found at low measured concentrations (around 10 µg L^{-1}), especially for parthenogenetic reproduction of *D. magna* and *P. antipodarum*. For daphnids, newborns length was impacted by fluoxetine and the second generation of exposed individuals showed much more pronounced effects than the first one, with a NOEC of 8.9 µg L^{-1}. For *P. antipodarum*, significant decrease of reproduction was found for concentrations around 10 µg L^{-1}. In contrast, we found no effect on the reproduction of *H. azteca* but a significant effect on growth, which resulted in a NOEC of 33 µg L^{-1}, expressed in nominal concentration. No effect on *C. riparius* could be found for measured concentrations up to 59.5 mg kg^{-1}. General mechanistic energy-based models showed poor relevance for data analysis, which suggests that fluoxetine targets specific mechanisms of reproduction.

Keywords : invertebrates, fluoxetine, pharmaceuticals, sublethal effects, multi-generational test, mechanistic models.

1. Introduction

For the past few years, there has been a growing concern about ecotoxicological risk of pharmaceuticals. Indeed, human medicines have been detected in many countries in sewage treatment plant effluents, surface waters, seawaters, groundwater and some drinking waters (Fent *et al.*, 2006). As pharmaceuticals are present at relatively low levels in the environment, risk for acute toxic effects is unlikely, but chronic environmental toxic effects cannot be excluded (Carlsson *et al.*, 2006). However, little is known about the chronic effects of these substances. Moreover, environmental risk assessment based on acute data is inappropriate (Ferrari *et al.*, 2004). For instance, carbamazepine and propanolol would be inaccurately identified as having negligible risks in France and Germany. There is a real lack of long term effects studies, in particular chronic data on the entire life cycle and investigation of multigenerational effects (Fent *et al.*, 2006).

Fluoxetine is a serotonin re-uptake inhibitor. It is apparently the most acute toxic human pharmaceuticals reported so far (Fent *et al.*, 2006), which makes necessary more studies about risks of low levels of exposure. In terms of environmental concentrations, Kolpin *et al.* (2002) estimated at 0.012 µg L^{-1} the median concentration of fluoxetine in U.S. streams. In terms of chronic effects, the recently published data on the effects of fluoxetine to invertebrates provide contradictory information. Flaherty and Dodson (2005) found an enhancement of reproduction for *Daphnia magna* exposed to a concentration of 36 µg L^{-1}. In contrast, Brooks *et al.* (2003) found a reproduction decrease for *Ceriodaphnia dubia* with a NOEC of 56 µg L^{-1} and a LOEC of 112 µg L^{-1}. Henry *et al.* (2004) also found reproduction decrease for the same species with a NOEC of 89 µg L^{-1} and a LOEC of 447 µg L^{-1}. Brooks *et al.* (2003) derived growth LOECs for *Chironomus tentans* and *Hyalella azteca* of respectively 1.3 and 5.6 mg kg^{-1} sediment, but Nentwig (2007) did not find any significant effect on

Chironomus riparius for concentrations up to 5.86 mg kg^{-1} sediment. This author obtained an extremely low LOEC of 0.47 µg L^{-1} for the reproduction of the snail *Potamopyrgus antipodarum*, comparable to concentrations measured in surface water.

Toxic effects of fluoxetine on invertebrates are consequently still worth being studied. We selected four invertebrates, with the following criteria: first, we tested species for which at least one test result is available in the literature to allow comparisons between our data and data from other studies ; second, as past studies indicated that the sublethal effects of fluoxetine are likely to be on reproduction, we tried to have different reproduction strategies (sexual reproduction and parthenogenesis). We consequently used a large test battery encompassing several phylogenetic groups. The species selected were then *Chironomus riparius* for sediment borne exposure, the crustacean *Daphnia magna* and *Hyalella azteca*, and the mollusc gastropod *Potamopyrgus antipodarum* concerning water borne exposure. This selection for water borne exposure is relevant to cover a large range of invertebrate sensitivity for organic compounds, as presented by Wogram and Liess (2001). Indeed, these authors showed that, relative to organic compounds, amphipoda are significantly more sensitive than daphnids and that gastropoda are significantly less sensitive than daphnids. We plan here to investigate effects on all the components of the life cycle of our species. In particular, effects on adults survival and reproduction as well as effects on juveniles survival and growth have been assessed. Moreover, for one of the species (*Daphnia magna*), fitness of the newborns produced during exposure and exposed themselves at the same concentration as their mother was assessed, to account for subtle effects on reproduction (like malformation) which would be undetectable with results expressed only in terms of numbers of newborns. Finally, the growth and reproduction data were analyzed using energy-based models, DEBtox (Kooijman and Bedaux, 1996). These models permit to estimate parameters not dependent on time, which is valuable to assess effects for long term exposure, and they allow insides

relative to the physiological mode of action of the compound (Kooijman and Bedaux, 1996; Péry *et al.*, 2003).

2. Materials and Methods

2.1. Chemical substance

Fluoxetine-HCL was used for all ecotoxicity tests (CAS 59333-67-4). It was purchased from Interchim (Montluçon, France ; product number 09674, batch number RD0001).

2.2. Chironomus riparius *tests*

Larvae were exposed to fluoxetine-spiked sediment. Sediment has been taken from Port-Galland, a tributary of the river Ain (France). This sediment has been monitored for years by Cemagref. Chemicals concentration are at low level and it has never been toxic to sediment organisms. Sediments were 2 mm sieved and homogenised before use. Organic carbon content is 3%, organic nitrogen content is 0.35%.Water was constituted of ¼ water from a spring, situated under our laboratory, added to demineralised water, resulting in pH of 7.8 and conductivity of 450 $\mu S\ cm^{-1}$. Sediments were spiked with fluoxetine 10 days before starting the tests. Fluoxetine (Interchim, Montluçon, France) was introduced in 0.5 L water at the concentration corresponding to the chosen nominal exposure concentrations and mixed with 1.5 kg wet sediment. Tested exposure concentrations were 1.2, 3.7, 11, 33, 100 and 1000 $mg\ kg^{-1}$ d.w., the latest being the concentration for which a compound is considered as safe if no significant effect occur at this exposure concentration (OECD, 2004). Sediments were transferred in beakers three days before starting tests. There were ten beakers per concentration. We put 0.1 L sediment and 0.4 L water in these beakers. The beakers were set in a water bath at 21°C with a 16:8 h light:dark photoperiod. Conductivity, temperature, pH,

amount of dissolved oxygen were measured daily. We used an aeration system (air introduced through a Pasteur pipette in each beaker) to maintain oxygen level.

The experiment was initiated with two-day-old larvae (end of first instar) from our laboratory culture. We put ten larvae per beaker. Length at the beginning of the test, measured on 20 organisms, was 1.7 +/- 0.1 mm. Organisms were fed daily with 0.6 mg per larva Tetramin® fish food.

At day 7, survivor and growth were monitored by sampling five beakers per concentration. Individuals were counted, killed using formaldehyde, then length was measured using a binocular microscope. Emergence was monitored for the five remaining beakers per concentration which had been covered to prevent organisms from escaping. The females were then put into 1 L mating chambers, with 0.1 L water, with males from laboratory culture in a ratio of three males per female as described by Péry *et al.* (2002). After mating and oviposition, each egg mass was removed and put into a 5 mL tube with 2 mL H_2SO_4, 2N overnight and the number of eggs was counted.

2.3. Hyalella azteca *tests*

Amphipods were exposed to fluoxetine-spiked water. Experimental protocol was the same as in Péry *et al.* (2005) : amphipods were exposed in beakers and an artificial nylon-shelter was introduced at the bottom of each beaker. The beakers were set in a water bath at 21 °C with a 16:8 h light:dark photoperiod. Test water in the beakers was continuously renewed (four renewals a day): for each concentration, there was a continuous pumping of clean water (same as for *C. riparius*) and stock solution at a speed calculated to obtained the required exposure concentration and then mixed in a bottle. Stock solution was protected from light and renewed every three days. The nominal exposure concentrations were 3.7, 11, 33 and 100 µg L^{-1}. Specific conductivity, temperature, pH, dissolved oxygen were measured

daily. Organisms were fed daily with 0.16 mg Tetramin® per individual. Two experiments were conducted. In the first one, young organisms (between 7 and 9 days old, mean length 1.69 +/- 0.17 mm) selected in our laboratory culture were exposed (ten per beaker) and length was measured every 7 days to assess effects on growth. Length measurements were performed on the dorsal side from the base of the first antenna to the end of the next to last segment, using an image analysis method (Sigma Scan Pro 5.0, SPSS Inc., Chicago). In the second one, we exposed five precopula (one male and one female, resulting in ten organisms per beaker) taken from our laboratory culture per beaker during 28 days, and we monitored reproduction every week. For each experiment, there was four replicates per concentration.

2.4. Daphnia magna *tests*

Organisms were exposed individually in 100 mL-bottles which contained 80 mL of solution. There were ten replicates per concentration. Tested fluoxetine concentrations were 0, 3, 10, 30, 100 and 300 µg L^{-1}. Test duration was 21 d, temperature was maintained at 20 °C by putting the bottles in temperature-controlled chambers, water (M4 medium, as recommended by ISO 10706) was renewed every day. Daphnids length was measured at days 7, 14 and 21 using image analysis and reproduction (number of newborns) was monitored every day. Length of the newborns was measured for the third brood. Food was algae *Pseudokirchneriella subcapitata* from our laboratory culture. Each organism received 10^7 algal cells per day the two first days, 2. 10^7 algal cells per day the three following days, 3. 10^7 algal cells per day the two following days and 4.10^7 algal cells per day until the end of the test. These feeding conditions are *ad libitum* conditions, as it has been chosen by previous tests (unpublished results).

To assess effects on two generations, an experiment in the same conditions was performed with newborns from the fifth brood. This experiment with the newborns started

exactly the day when the experiment with their mother ended. There was not enough surviving newborns to start this new test for nominal concentration 300 µg L^{-1}.

2.5. Potamopyrgus antipodarum *tests*

Snails from the species *Potamopyrgus antipodarum* came from our laboratory culture. The test beakers were filled with 0.5 L fluoxetine-spiked water (the same as for amphipods), three days before the beginning of the tests. The beakers were set in a water bath at 21 °C with a 16:8 h light:dark photoperiod. The exposure system was the same as for *H. azteca*. The nominal exposure concentrations were 3.7, 11, 33 and 100 µg L^{-1}. Specific conductivity, temperature, pH, dissolved oxygen were measured daily. Organisms were fed with 0.6 mg Tetramin® fish food (Tetrawerke, Melle, Germany) per individual per day. We performed two experiments, one to assess effects on growth, the other one to assess effects on reproduction. Growth was monitored every week through shell length measurements using a binocular. At the beginning of the growth test, each beaker contained ten organisms, which had been selected in the culture according to their length (0.48 +/- 0.026 mm). Reproduction was monitored once a week, by counting and removing all newborns using a binocular. The test was initiated with individual adult length superior to 4 mm at the beginning of the test, and also ten individuals per replicate. For each experiment, there were three replicates per concentration. The experiments lasted six weeks.

2.6. Analytical Procedures

Spiked water was sampled for all exposure concentrations in the *D. magna* and *P. antipodarum* tests at day 10 for daphnids and day 42 for snails. We could not have chemical measurements for amphipod tests. To get enough volume to perform chemical measurements,

waters for all replicates of a given concentration were pooled. Spiked sediments were sampled for all concentrations at the end of the toxicity test.

Water samples (10-250 mL), adjusted to pH 3 with sulphuric acid, were spiked with the surrogate standard fluoxetine-d5 (Isotec, Miamisburg, USA). Samples were enriched at a flow rate of 10-20 mL min^{-1} (ca. 200 mbar) with OASIS HLB SPE cartridges (200 mg, 30 µm, Waters, Milfort, USA) and the SPE material was dried for 1 h under a nitrogen stream. Fluoxetine was eluted using 4 x 2 mL of methanol/acetic acid (98/2, v/v). After blowing down to 100 µL the samples extracts, they were reconstituted to 1 mL of the LC eluent A (see below).

Sediment samples (1 g) were spiked with the surrogate standard fluoxetine-d5 and extracted by pressurized liquid extraction (PLE) with MeOH/water/acetic acid (49:49:2) at 100 bar and 120 ºC during two static cycles of five min. Afterwards, the extract was made up to 50 mL and one aliquot of 0.1-1 mL diluted in 500 mL of groundwater. SPE clean-up was carried out with OASIS HLB cartridges eluted with MeOH-MTBE (95:5).

The sample extracts were measured by LC tandem MS (Agilent 1100 with degasser, quaternary pump and autosampler, Agilent Technologies, Waldbronn, Germany/API 4000 with ESI ionization, Applied Biosystems, Foster City, CA, USA) operating in the positive ion mode using multiple reaction monitoring (MRM). Chromatographic separation took place at room temperature by means of a Synergi Polar RP 80A column (150 x 3 mm, 4µm) (Phenomenex®, Aschaffenburg, Germany). A mixture of 20 mM ammonia solution (pH 5.7 adjusted with acetic acid): acetonitrile (98:2) (A) and a mixture of A:acetonitrile (2:3) (B) were used as mobile phases. Two MRM transitions were monitored for each substance for identification and quantification of the analytes (fluoxetine: 310/44 and 310/148 amu; fluoxetine-d5: 315/44 and 315/143 amu).

205 Calibration curves showed a good correlation in the range 5-2000 ng.mL^{-1}. Limits of
206 quantification for fluoxetine in sediment and water samples were 10 ng.g^{-1}and 5 ng.L^{-1},
207 respectively.
208
209 *2.7. Statistical analysis*
210 To analyse the data, we used standard methods (ANOVA, Dunnett-t tests) but also
211 DEBtox models (See a complete description in Kooijman and Bedaux, 1996 and in the OECD
212 guideline about statistics in ecotoxicology (OECD, 2006)). These models are based on the
213 DEB theory (Kooijman, 2000), which describes growth and reproduction as a function of
214 bioenergetics parameters like for instance costs of maintenance or food assimilation rate.
215 Effects on growth and reproduction are described as the consequences of effects on one of
216 these bioenergetics parameters. These effects are proportional to the difference between
217 accumulated compound concentration and a threshold concentration, called the NEC (No
218 Effect Concentration). The estimate of this threshold concentration, obtained through
219 maximum likelihood methods, does not depend on the duration of the test.
220
221 **3. Results**
222
223 *3.1.* Chironomus riparius *tests*
224 Temperature was constant (21+/-1 °C) so as pH (7.9 +/-0.3). Conductivity was 490 +/-
225 35 µS cm^{-1}, and the percentage of dissolved oxygen was always above 90 %. Growth (length
226 of 12.1 mm at 7 days) and survival (72%) in the control were enough to validate the test.
227 Chemical measurement showed a recovery of 63 +/- 4% for fluoxetine spiked on the
228 sediments. Traces of fluoxetine near detection limit (0.1 mg kg^{-1}) were found in the control.

There was no significant effect on *Chironomus riparius* growth, emergence and reproduction for concentrations up to 59.5 mg kg^{-1} (ANOVA, $p>0.05$). Final length for all these concentrations were between 11.9 and 12.1 mm and total number of eggs per female were between 426 and 456. For measured concentration 666 mg kg^{-1}, there was no emergence, survival at day 7 was low (34%) and growth at day 7 was very significantly reduced ($p<0.01$, Dunnet-t test), by 31%.

3.2. Hyalella azteca *tests*

Temperature was constant (20.9+/-0.4 °C) so as pH (7.55 +/-0.2). Conductivity was 391 +/- 17 µS cm^{-1}, and the percentage of dissolved oxygen was always above 90%.

No adult died for any of the concentrations during the test. There was no significant effect of fluoxetine on reproduction (ANOVA, $p>0.5$), with mean number of newborns per female from 12.8 to 15.9. For the young organisms, more than 87.5% amphipods survived in all the concentrations. Effects on growth were significant for nominal concentration 100 µg L^{-1} at days 14, 21 and 28 ($p<0.01$, Dunnet-t test), as presented by Figure 1. This resulted in a LOEC of 100 µg L^{-1} and a NOEC of 33 µg L^{-1}. We used DEBtox models, with the three possible physiological modes of action for growth (effects on food assimilation, on growth energy costs or on maintenance energetic costs), and with a Von Bertalanffy growth rate of 0.08 d^{-1} (parameter required by the software, estimated with a least square method using control data). Growth was very low the first week, so we used DEBtox only from day 7 to 28, but taking into account that compound accumulation has started from the very first day. The best fit was obtained for the mode of action "increase of energetic costs for growth", the two other modes of action leading to estimations significantly different from the data at day 28 obtained for nominal concentration 100 µg L^{-1}. To propose a rough explanation for that, we should point that, in the DEBtox context, "increase of energetic costs for growth" is

characterized by effects on growth rate but no effect on ultimate length. By looking at Figure 1, it seems that all growth curves tend to reach the same ultimate length. The NEC estimated by DEBtox was 19 µg L^{-1}, but the software was unable to provide a confidence interval, which means that all numbers between 0 and infinity were in this confidence interval.

3.3. Daphnia magna *tests*

Temperature was constant (19.9 +/- 0.34 °C) so as pH (7.9 +/- 0.27) and conductivity (642 +/- 30 µs cm^{-1}). Chemical measurements showed a recovery of fluoxetine in the exposure system from 80 to 102%.

In the first test, a significant effect on growth was found at day 7 for concentrations 102 and 241 µg L^{-1}. At days 14 and 21, this effect was only significant for exposure concentration 241 µg L^{-1}. Moreover, there was 40% mortality for this concentration at day 21 and a significant decrease of reproduction by 32%. No effect on reproduction was found for the other concentrations. The measurements of the newborns length for the third brood of the first test showed significant effects of fluoxetine for exposure concentrations 31, 102 and 241 µg L^{-1} (Figure 2). This parameter is the most sensitive to fluoxetine, resulting in a NOEC of 8.9 µg L^{-1} and a LOEC of 31 µg L^{-1}. Concerning the second test, effects were much more pronounced than for the first one, but with the same LOEC and NOEC. 70 % of the newborns were found dead at 21d for exposure concentration 102 µg L^{-1}. Moreover, reproduction was significantly reduced for exposure concentration 31 µg L^{-1} (by 18%) and length was significantly lower than the control for exposure concentrations 31 and 102 µg L^{-1}.

3.4. Potamopyrgus antipodarum *tests*

Temperature was constant (20.8+/-0.4 °C) so as pH (7.6 +/-0.3). Conductivity was 400 +/- 24 µS cm^{-1}, and the percentage of dissolved oxygen was always above 90%. Chemical

measurements showed a bad recovery of fluoxetine in the exposure system (from 27 to 69%), with measured exposure concentrations : 1, 4.2, 13 and 69 µg L^{-1}.

There was no significant effect of fluoxetine on growth for all weekly measurements (ANOVA, $p>0.5$). As for reproduction, we observed a significant decrease at 69 µg L^{-1} (Figure 3) but no significant effect at lower concentrations (Dunnett-t tests, $p<0.05$), resulting in a NOEC of 13 µg L^{-1} and a LOEC of 69 µg L^{-1}. We used DEBtox models to analyse data on reproduction. We selected the physiological mode of action « increase of the energetic costs of reproduction ». Indeed, the selection of effects on reproduction due to effects on growth would have no sense here, because we exposed adults. We obtained a NEC of 5 µg L^{-1} with 95% confidence interval 4.3-10.4 µg L^{-1}. All values in this confidence interval are lower than the estimated NOEC.

4. Discussion

The chemical measures for the tests with daphnids and chironomids showed a correct spiking with fluoxetine. In contrast, the chemical measurements for the tests snails showed an irregular, sometimes low efficiency of water spiking (especially for low concentrations), despite a continuous renewal of the solution. Recently Kwon and Armbrust (2006) have demonstrated that fluoxetine is hydrolitycally and photolytically stable in aqueous solutions including natural waters. Fluoxetine may thus not be degradated in our system. Our hypothesis is thus that fluoxetine, which is likely to sorb very quickly on the sediments, could sorb very quickly on the fish food provided to the snails and the amphipods in our test or to the plastic tubes of the renewing system. As the exposure system was the same for *H. azteca* as for *P. antipodarum*, and as the tests for the snails were performed immediately after the tests with amphipods, we could consider that the actual concentrations that have been

304 measured for snails exposure to fluoxetine are also valid for amphipods exposure to
305 fluoxetine. The NOEC of 33 µg L^{-1} expressed in nominal concentration for growth of
306 amphipods would be a NOEC of 13 µg L^{-1} expressed in measured concentration.

307 The classification of sensitivity for our species was quite different from our expectations based
308 on the works of Wogram and Liess (2001) on effects on organic compounds on invertebrates,
309 which classified amphipods, daphnids and snails in order of decreasing sensitivity, which
310 confirms the necessity to treat pharmaceuticals specifically among all organic compounds. In our
311 tests, the most sensitive species is *P. antipodarum*, with a NEC of 5 µg L^{-1} relative to
312 reproduction. Moreover, the effects of fluoxetine were on reproduction for daphnids and snails
313 (juveniles fitness and total amount of newborns respectively), whereas they were on growth for
314 *H. azteca*. Our choice of tested species was consequently relevant to capture a large range of
315 different types of responses.

316 For daphnids, the highest effects were found on the development of the embryos, with smaller
317 newborns resulting in significant effects on their future reproduction. Exposure to fluoxetine
318 has thus consequences on the fitness of the newborns, which energy-based models like
319 DEBtox are not able to account for. Indeed, they assume that the total amount of energy
320 invested per newborn is not concentration dependent, so that newborns should have the same
321 length and ability to resist to toxic exposure. This suggests a direct action of fluoxetine on the
322 development of newborns, which may not be the consequence of energy depletion in the adult
323 female. Consequently, no result from modeling was presented for daphnids toxicity tests, for
324 our models cannot account for fluoxetine mode of action. For *H. azteca*, the use of DEBtox
325 models was also irrelevant. DEBtox was unable to provide a confidence interval, which
326 means that all numbers between 0 and infinity were in this confidence interval. This suggest
327 that energy-based models like DEBtox are unable to account accurately for the observed

effects, and that, probably, the main target of fluoxetine in *H. azteca* is not the dynamics of energy.

We can compare our results with other studies from the literature. Fluoxetine appeared to have different effects on growth, fecundity and reproduction depending on species. The freshwater snail *P antipodarum* has shown to be the most sensitive invertebrate species for reproduction as a NOEC of 3.2 µg L^{-1} (56 days) has been reported by Nentwig (2007). This is very coherent with the NEC of 5 µg L^{-1} we found in this project. For *H. azteca*, fluoxetine treatments inhibited growth with a NOEC of 33 µg L^{-1} expressed in nominal concentration. Brooks *et al.* (2003) also showed an inhibition of growth due to fluoxetine exposure. We have contradictory results compared to the data from Flaherty and Dodson (2005) who found an enhancement of reproduction for *Daphnia magna* exposed to a concentration of 36 µg L^{-1} fluoxetine. In contrast, our results are coherent with Brooks *et al.* (2003) who found a reproduction decrease for *Ceriodaphnia dubia* with a NOEC of 56 µg L^{-1} and a LOEC of 112 µg L^{-1}. Henry *et al.* (2004) also found reproduction decrease for the same species with a NOEC of 89 µg L^{-1} and a LOEC of 447 µg L^{-1}. Nentwig (2007) found a LOEC of 1.12 mg kg^{-1} (measured value) when studied fluoxetine effects on *C. riparius* emergence which was associated to a significant increase of number of eggs per clutch. However, he observed no effect on growth for concentrations up to 5.86 mg kg^{-1} and he recommends confirming the potential reduced emergence and increased clutch size observed after fluoxetine exposure. In our study, we observed no effect for concentrations below 59 mg kg^{-1}. Fluoxetine is consequently very unlikely to have effects in the field on *Chironomus riparius*.

To conclude, data sets on acute and chronic toxicity of the selected case study pharmaceuticals have been derived in our study. Fluoxetine seems to interact with growth and reproduction processes in invertebrates. Depending on the tested species, effects of fluoxetine can be found at low exposure concentrations, around 10 µg L^{-1}. The fact that the second

generation of daphnids was more sensitive than the first one highlights the need for investigation of the effects of pharmaceuticals on at least two generations of invertebrates. Energy-based models were developed and used to describe effects on growth and reproduction, but were not relevant to estimates threshold effect for fluoxetine.

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Figure 1. Length of the young *H. azteca* as a function of time and nominal concentration (control : black diamonds, 1.2 µg L^{-1}: black squares, 3.7 µg L^{-1} : black triangles, 11 µg L^{-1} : white squares, 33 µg L^{-1} : white triangles, 100 µg L^{-1} : white diamonds). Asterisk accounts for significant difference with the control ($p<0.05$, Dunnet-t test).

Figure 2. Length of *Daphnia* newborns from the third brood as a function of fluoxetine nominal concentration. Asterisk indicates significant difference from the control ($p<0.05$, Dunnett-t test).

Figure 3. Number of newborns per *P. antipodarum* adult (mean value and standard deviation) as a function of fluoxetine nominal concentration. Asterisk indicates significant difference from the control ($p<0.05$, Dunnett-t test).

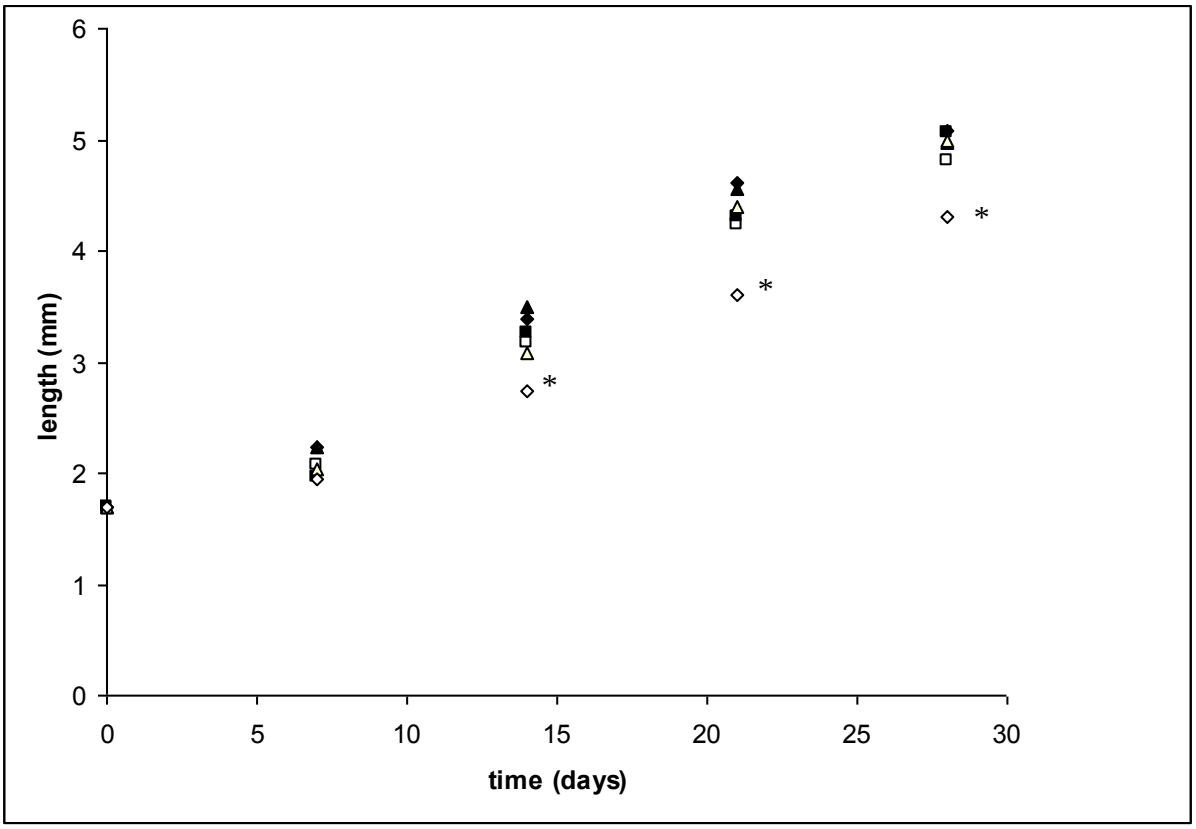

Figure 1.

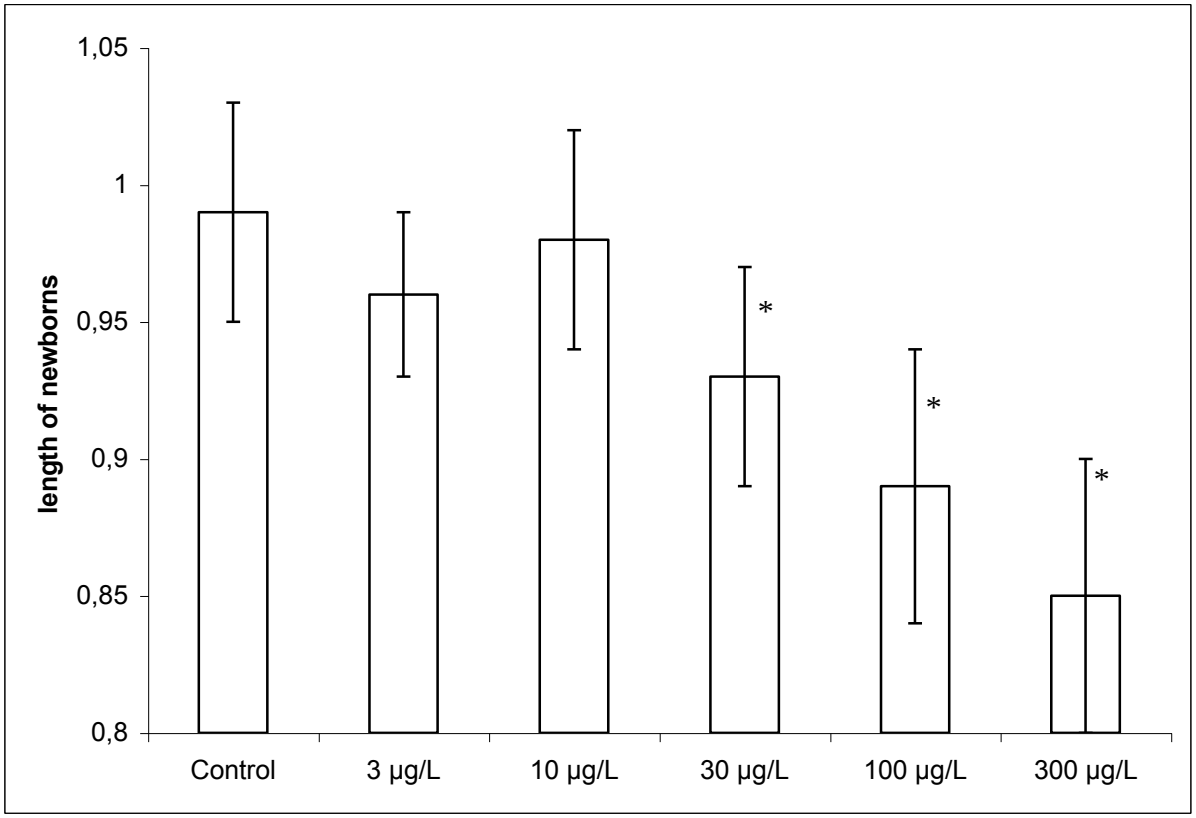
1,05
1
0,95
0,9
0,85
0,8
length of newborns
*
*
*
Control
3 µg/L
10 µg/L
30 µg/L
100 µg/L
300 µg/L

Figure 2.

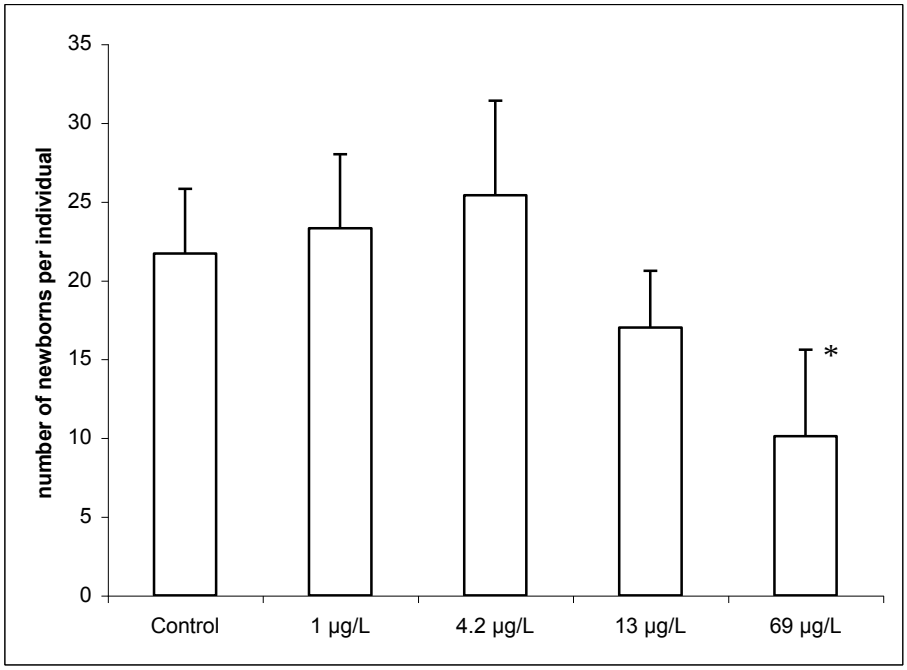
number of newborns per individual
35
30
25
20
15
10
5
0
*
Control
1 µg/L
4.2 µg/L
13 µg/L
69 µg/L

Figure 3.