University of South Bohemia

Faculty of Science

The impact of antidepressant (sertraline) on *Daphnia magna* life-history traits under different food levels

Bachelor thesis

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České Budějovice 2017

Šefferová M. 2017: The impact of antidepressant (sertraline) on *Daphnia magna* life-history traits under different food levels. Bc. Thesis, in English – 32 p., Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

Annotation

The chronic toxicity of one of the most prescribed antidepressants, sertraline, on life-history traits of *Daphnia magna* was evaluated under high and low food level. Reproduction, body length and survival of the animals were examined. The results indicate that sertraline can impact reproduction and body length of *Daphnia magna*; however, only at the concentrations exceeding current concentrations of sertraline reached in the environment.

Affirmation

Acknowledgements

I would like to thank the whole Department of Ecosystem Biology for giving me the opportunity to work there on my bachelor thesis. Special thanks go to Mgr. Jana Zemanová, who guided me through the whole work and was always ready to help me with any problem. I am also thankful to RNDr. Michal Šorf, Ph.D. for his professional advice. Further thanks go to doc. Ing. MgA. David Boukal, Ph.D. for allowing me to work in his laboratory; to Mgr. Kateřina Zadinová for her technical support; to prof. RNDr. Jaroslav Vrba, CSc. for his insightful notes about my English and presentation of the thesis; and to everyone in the lab who have helped me or who contributed to the friendly atmosphere.

Content

1 Introduction

In the modern world, pharmacologically active substances are widely used to treat or prevent diseases (Castiglioni, 2005). Through human excretion and incomplete elimination in waste water treatment plants, the parent substances themselves and their metabolites are excreted into the environment (Castiglioni, 2005; Buchberger, 2007; Ge and Lee, 2013). Studies from around the world have examined waste and surface water and have reported the occurrence of both pharmaceuticals and illicit drugs (Halling-Sørensen et al., 1998; Fedorova, 2014).

The presence of drugs in aquatic ecosystems might have effects on non-target aquatic organisms. The pharmaceuticals can possibly change animal behavior, reproduction, and survival (Fedorova, 2014). Consequently, they can affect not only the individual, but also ecology and evolution of the whole system. One of the studied drugs are antidepressants – drugs, which are targeted to influence nervous system in humans. Their consumption grows rapidly, which also increases their concentrations in surface waters (e.g. Vasskog et al., 2006; Metcalfe et al., 2010). One of the most prescribed antidepressants worldwide is sertraline; however, its impact on aquatic organisms is not yet fully known (Cheng et al., 2012; Shoja et al., 2016).

Key organisms in freshwater ecosystems are *Daphnia* species. Not only do they influence the whole ecosystems due to high filtering abilities, which increase water transparency, but they are also reasonably sensitive to chemicals in the environment. With short generation time and relatively easy cultivation requirements in laboratory conditions, they are suitable model organism for wide variety of studies (Seda and Petrusek, 2011).

Despite growing interest in assessing the ecotoxicology of antidepressants, only a few studies combining sertraline and *Daphnia* species have been published (Henry et al., 2004; Christensen et al., 2007; Minagh et al., 2009; Lamichhane et al., 2014; Minguez et al., 2015). Furthermore, usually only one stress factor – the chemical – is used in these studies neglecting the possibility of other stress factors coming into play. Additionally, the studies of changes in reproduction, growth and other life-history traits induced by sertraline exposure do not present consistent outcomes. The fecundity is either increased or decreased; the body length is either suppressed or not affected at all. Due to this fact, this topic offers wide range of experiments and their combinations to be investigated.

2 Aims

- To verify the impact of sertraline on *Daphnia magna* life-history traits and compare our results with very scarce published data.
- To study the combined effect of food and sertraline concentrations on *Daphnia magna*.

3 Literature review

3.1 Pharmaceuticals in the environment

In our modern society, the use of chemicals for personal care and for treating and preventing diseases is on the rise. The effects of these chemicals on human health are usually well-known, but the human body is not the only site where pharmaceuticals appear. The occurrence of chemicals has been proven in the environment repeatedly, however, the fate and the effects on ecosystems are not yet fully investigated.

Research about pharmaceuticals and personal care products present in the environment has been rapidly increasing since the late 1990s (Boxall et al., 2012; Beek et al., 2016). The research focus is to determine origin, quality, and quantity of the substances in the environment, e.g. surface waters or soil. Furthermore, the studies focus on the impact of compounds, their combinations, and metabolites on various organisms (Kümmerer, 2009).

Pharmaceuticals enter the environment by direct introduction (e.g. flushing unused or outdated drugs in the toilet) or via feces and urine (Götz and Kiel, 2007; Kümmerer, 2009). Pharmaceuticals used by humans are usually released into a sewage system, pass the waste water treatment plants (WWTPs) and subsequently enter the water environment. However, drugs used in veterinary medicine, for instance on livestock and fish farms, are mostly excreted directly on the ground or into surface waters and do not pass through the WWTPs (Rivera-Utrilla et al., 2013). Unfortunately, even if the chemicals pass the WWTPs, they are not removed completely (Ternes, 1998; Ternes et al., 1999; Heberer, 2002). Conventional treatment plants are usually based on the use of microorganisms, which are not able to destroy complex organic compounds. Therefore, the percentage of removed chemical compounds can be even lower than 10% (Jones et al., 2005; Rivera-Utrilla et al., 2013).

Consequently, various pharmaceuticals are often found in $\mu g\ l^{-1}$ or $ng\ l^{-1}$ range in the surface waters from around the world (Halling-Sørensen et al., 1998; Kümmerer, 2009; Irvine et al., 2011). With increasing production of drugs, many countries in Europe routinely monitor their waste and surface waters and influents and effluents of WWTPs (Castiglioni et al., 2005; Buchberger, 2007; Kasprzyk-Hordern et al., 2008; Berset et al., 2010; Gros et al., 2010; Fedorova et al., 2014; Senta et al., 2013 etc.). Drugs, which are most often found in effluents of WWTPs, are antibiotics, antacids, steroids, antidepressants, analgesics, anti-inflammatories, antipyretics, beta-blockers, lipid-lowering drugs, tranquilizers, and stimulants (Rivera-Utrilla et al., 2013). However, not only the drugs themselves are introduced to the water system, but also other molecules resulting from these compounds take place in the environment. Metabolites, chemicals created from a parent compound within a human, animal or plant body, can also influence aquatic life. The same is valid for transformation products, compounds created after the excretion by transformation processes outside the human or animal body (Kümmerer, 2009). With addition of these products resulting from modifications of the parent substances, the number of chemicals to be investigated becomes enormous.

Obviously, because of high diversity of pharmaceuticals and their metabolites and transformation products, the impact of all these chemicals on the environment has not yet been assessed. However, a few interesting interactions were already found. For instance, estrogens present in water can alter fecundity in female fish, reduce testicular development in male fish and induce synthesizing vitellogenin in male fish (Sumpter, 1995; Jobling et al., 2003). Diclofenac used for treating livestock is even responsible for devastation of Gyps vulture species in India and Africa (Naidoo et al., 2009). Studies about the ecotoxicology of antidepressants are also increasing; however, the effects on the environment are not yet known.

3.2 Antidepressants and sertraline

Antidepressants are a group of drugs used to treat major depressive disorders, obsessive-compulsive disorder, panic disorder, social phobia, post-traumatic stress disorder, premenstrual dysphoric disorder, and generalized anxiety disorder (Sanz et al., 2005). Due to their wide field of use, the consumption of antidepressants has increased multiple times over the last decades, as illustrated by Figure 1 based on the OECD Health Data database (OECD, 2016).

As stated by Ge and Lee (2013), antidepressants can be divided into three categories according to their mode of action: monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), and selective serotonin re-uptake inhibitors (SSRIs). Sertraline, along with other substances such as citalopram, escitalopram, fluoxetine, fluvoxamine, or paroxetine, belongs to the class SSRIs. Low levels of serotonin, a neurotransmitter in human and animal body, are associated with mood swings and depression. In the central nervous system, SSRIs limit the reabsorption of serotonin into the presynaptic cell and therefore increase its level in the synaptic cleft, where it is available to bind to the postsynaptic receptors (Asberg and Mårtensson, 1993).

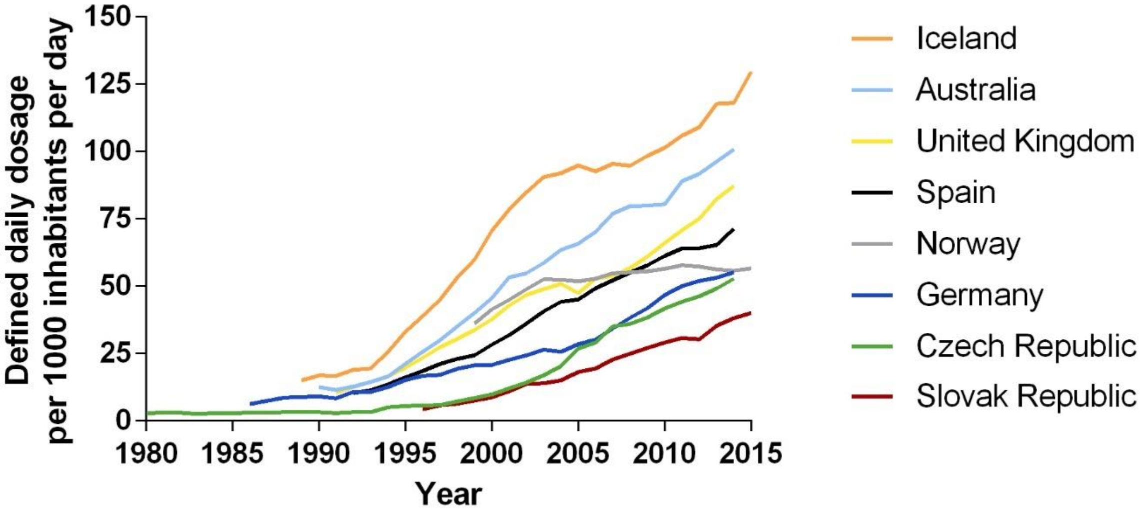

Figure 1: The development of antidepressant consumption in selected countries during the past 34 years.

Sertraline (IUPAC name (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydro-1-naphthalenamine; structure in Figure 2) is used to treat clinical depression, obsessive-compulsive, panic and posttraumatic stress disorders (Nouws et al., 2005). Sertraline hydrochloride is the active form, which is most often sold under the name Zoloft® with daily dose ranging from 50 to 200 mg of the active compound (Bosch, 2008). Due to minimal side-effects, sertraline is one of the most prescribed antidepressants worldwide (Cheng et al., 2012; Shoja et al., 2016). For instance, it has been the most prescribed antidepressant in the Czech Republic at least from 2011 to 2016 based on State Institute for Drug Control (SUKL, online). However, the amount of excreted unmodified compound from human body is only 1-2% (Christensen et al., 2007).

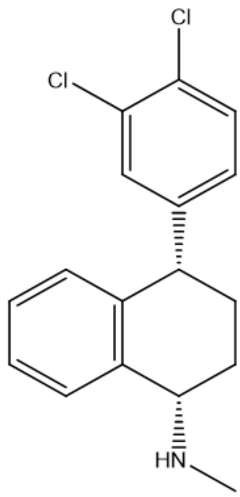

Figure 2: Structure of sertraline

Concentration of sertraline in surface waters in Europe and America range between 1 to 20 ng l^{-1} (Lamas et al., 2004; Himmelsbach et al., 2006; Vasskog et al., 2006; Metcalfe et al., 2010) with the highest concentrations around 50 ng l^{-1} found occasionally e.g. in Minnesota, USA by Schultz and Furlong (2008), or in Slovenia by Klancar et al. (2016). Furthermore, Wiegel et al. (2004) detected sertraline in concentration reaching 100 ng l^{-1} in a water effluent from psychiatric hospital in Norway. The predicted concentrations in exposed regions, such as untreated wastewater originating from a hospital, could even be as high as 17.1 μg l^{-1}

(Styrishave et al., 2011). WWTPs might, however, remove relatively high amount of sertraline; the level ranges between 40 and 80% in various studies (Styrishave et al., 2011; Golovko et al., 2014; Klancar et al., 2016).

In the Czech Republic, sertraline content was analyzed in samples from rivers from all over the country. The chemical was found on most of the analyzed sites in concentrations between 2 to 5 ng l^{-1} (Fedorova et al., 2014; Grabicova et al., 2014, 2015). Golovko et al. (2014) analyzed the amounts of sertraline in the WWTP in České Budějovice (Czech Republic) and found concentrations ranging from 7 to 27 ng l^{-1} in the influent and concentrations between 3 to 6 ng l^{-1} in the effluent of this WWTP. The concentrations varied depending on the time of year, with increasing consumption during winter as more people suffer from depression. Nevertheless, no research was done in exposed regions, such as hospital wastewater from psychiatric hospital, in the Czech Republic.

Increased concentrations of sertraline were also found in tissue of aquatic organisms (*Erpobdella*, *Hydropsyche*, *Oncorhynchus*; Grabicova et al., 2014, 2015). Based on the results, sertraline can be assigned as bioaccumulative pharmaceutical, as suggested also in Daughton and Brooks (2011). Furthermore, sertraline can enter animal body via ingestion due to accumulation in algae that serve as food for various organisms (Vannini et al., 2011; Minguez et al., 2015). Additionally, sertraline has relatively high sorption to sediment, where it remains stable (Kwon and Armbrust, 2008). In case that it is not incorporated in either tissue or sediment, the degradation to transformation products starts rather quickly. Based on the laboratory experiments, sertraline degradation starts immediately after its addition into water; for instance, only 80% of the nominal concentration remains in the solution (Minguez et al., 2015).

3.3 *Daphnia* spp.

Daphnia species (Arthropoda, Crustacea, Cladocera), are relatively large planktonic crustaceans, populating wide range of habitats, from temporary pools to large permanent lakes (Seda and Petrusek, 2011). Their high ability to filter and reduce the biomass of phytoplankton and therefore increase transparency of water makes them so called keystone species – species which have the prominent effect on their environment (Kalff, 2002; Sommer et al., 2012).

Daphnids are reasonable model organism for wide variety of scientific studies from aquatic ecology to biomedicine experiments due to their relatively easy cultivation and handling in laboratory conditions, sensitivity to chemicals in their environment, and short generation time (Seda and Petrusek, 2011). Both US National Institutes of Health and

OECD Guidelines for the Testing of Chemicals use *Daphnia magna* as model organism (NIH, 2004; OECD, online).

The reproduction of daphnids is parthenogenic most of the time during the season. Females lay many diploid eggs into the brood pouch and produce only females, which carry the same genetic information as their mother. Gametogenic reproduction, which occurs under unfavorable conditions, forms dormant eggs. These eggs, covered by resistant cases (known as ephippia), settle to the sediments to experience diapause, the latent period of life. However, the parthenogenetic reproduction can be almost indefinitely long under laboratory conditions (Kalff, 2002; Smirnov, 2014).

Daphnids are adaptable organisms, surviving fluctuation of temperature, presence of predators, drought, food starvation etc. by changing their behavior or appearance. For instance, under low food level, females produce fewer and smaller sized eggs and the body length of the animals decreases (Ebert and Yampolsky, 1992). Furthermore, the time to the first brood of daphnids increases in low food level, however, their first reproduction begins at smaller body length (Giebelhausen and Lampert, 2001). Interestingly, the life-span of the animals increases under poor feeding conditions (Ebert, 2005). Their physiological functions can be changed as well. For instance, the respiration rate and therefore oxygen consumption drops under low food levels (Schmoker and Hernández-León, 2003). The food deprivation also results in lower body size, which then influences the respiration and filtering rate (McMahon and Riegler, 1965; Burns, 1969; Heisey and Porter, 1977).

3.4 Influence of antidepressants on *Daphnia* spp.

Sertraline is the most toxic substance among SSRIs according to the acute toxicity tests conducted on *Daphnia magna* and *Ceriodaphnia dubia* (Henry et al., 2004; Christensen et al., 2007). EC_{50}, the concentration estimated to immobilise 50% of *Daphnia magna* animals within 48-h exposure period of juvenile daphnids, has been assessed to be close to 1 mg l^{-1} for sertraline in multiple publications (Christensen et al., 2007; Minagh et al., 2009; Minguez et al., 2014).

To our knowledge, only few studies about chronic effects of sertraline on cladocerans were published (Table 1). Unfortunately, the published data are not balanced, making it difficult to summarize the impact of sertraline on daphnid life-history traits. Sertraline can probably cause decrease in fecundity, body length of females and juveniles and prolongation of time to the first brood and interclutch period. According to Campos et al. (2013), SSRIs in general affect serotonin metabolism, neuronal developmental processes, and carbohydrate and lipid metabolism in daphnids. However, the exact mode of action of sertraline in crustaceans is not yet understood.

Table 1: The summary of published studies about impact of sertraline on crustacean life-history traits. Directions of arrows show predominant trend of examined factors, with the lowest effect concentrations in the brackets (mg l^{-1}).

Species	**Fecundity**	**Body length of female**	**Body length of juveniles**	**Time to the first brood**	**Interclutch period**	**Literature**
Ceriodaphnia dubia	↓ (0.045)	-	-	Not affected	-	Henry et al. (2004)
Daphnia magna	↓ (0.1)	-	-	Prolonged (0.1)	-	Minagh et al. (2009)
Ceriodaphnia dubia	↓ (0.053)	↓ (0.053)	↓ (0.053)	-	Prolonged (0.053)	Lamichhane et al. (2014)
Daphnia magna	↑ (0.03)	Not affected	-	-	-	Minguez et al. (2015)

3.5 Combination of stress factors

The concentration of certain chemical is not the only relevant disturbance in nature. Number of chemicals can influence the organism simultaneously, sometimes having antagonistic, additive, or synergistic effects. In addition, natural stress factors, such as food shortage, presence of predators, oxygen deficiency, or changes in temperature can alter toxicity of the given chemical. For instance, daphnids migrate downward in the mornings to avoid fish predators and upwards in the evenings to feed on algae in lakes. Therefore, they experience stress from food and oxygen shortage during the day, and can become more sensitive to chemicals than expected from toxicity tests carried out in laboratory (Forbes and Calow, 1999; Hanazato 2001).

Food levels can undeniably influence the toxicity of a certain chemical. Antunes et al. (2003) and Pavlaki et al. (2014) showed different responses of *Daphnia magna* to chemical lindane, insecticide imidacloprid and nickel under different food levels. According to Campos et al. (2011), daphnids exposed to another SSRI (fluoxetine) produce more offspring, but only at low food concentrations. The impact of sertraline concentrations combined with other stress factors (specifically food shortage) on *Daphnia magna* life-history traits has not yet been published.

4 Hypothesis

- Sertraline reduces the number of offspring per female.
- Sertraline reduces body length of females and juveniles.
- Time to the 1st brood and average interclutch period are prolonged under exposure to sertraline.
- Daphnids exposed to the low food level are more sensitive to sertraline.

5 Methods

5.1 Experimental set-up

Parent animals from long-term laboratory culture of *Daphnia magna* were acclimated for the test conditions from birth. For the experiments, juveniles aged less than 24 hours were randomly selected from this single-clone stock culture of parent animals. They were at least third brood progeny of these daphnids.

Sertraline hydrochloride (CAS No. 79559-97-0) was purchased from SIGMA-ALDRICH at analytical grade (purity ≥ 98%). Because of relatively high degradation and bioaccumulation in algae, sertraline stock solution was prepared freshly by dissolving approximately 1 mg of sertraline in dechlorinated aged tap water at each media change. The volume of the dechlorinated water for the stock solution was calculated after weighting solid sertraline to achieve concentration of 40 mg l^{-1}. No carrier solvent was used. Working solutions of sertraline were made by dilution of the stock solution with dechlorinated aged tap water and algae.

Algae *Desmodesmus subspicatus* (CCALA 668, Brinkmann 1953/SAG 86.81) cultured in a continuous chemostat system with Z medium (Staub, 1961) under 16:8h light-dark photoperiod was used as source of food for daphnids. Fresh algae were taken from continuous algal culture every day during the experiment.

Experiments were conducted in air-conditioned room at 18°C ± 1°C with natural photoperiod of 10:14h light-dark. Beakers (100 ml) were filled with 50 ml of the working solutions with 50 ml tilt pipette dispenser. Each vessel contained one animal in both preliminary and reproduction test. The beakers were covered with clear, non-colored acrylic glass during the experiment.

5.2 Preliminary test

A preliminary test for acute immobilisation was inspired by OECD methodology (OECD, 2004). The test was carried out to verify the sensitivity of our daphnids to different sertraline concentrations. The tested parameter was immobilization of daphnids after 24 h and 48 h. Animals were considered immobilised when they were not able to swim within 15 seconds after gentle agitation of the test vessel.

Daphnids were exposed to five nominal concentrations of sertraline: 0 μg l^{-1} (control), 0.3 μg l^{-1}, 30 μg l^{-1}, 600 μg l^{-1}, and 1200 μg l^{-1}. Apart from the sertraline concentration, daphnids were exposed to two levels of algal concentration by adding the calculated amount of concentrated algae directly to the test vessels: 0.3 mg C l^{-1} (low food level) and 3 mg C l^{-1} (high food level). Five replicates in each combination of studied factors were used, resulting in 50 test animals used in total. The test lasted 48 h.

5.3 Reproduction test

A *Daphnia magna* reproduction test was carried out based on modified OECD methodology (OECD, 2012). The observed parameters were: cumulative number of juveniles after 21 days per female; number of juveniles in each brood per female; body length of each test animal after 21 days; body length of juveniles aged less than 24 hours from last joint brood of the low and high food level (3rd brood); average interclutch period; time to the 1st brood; immobilisation/death; and any abnormal behavior.

Daphnids were exposed to three nominal concentrations of sertraline: 0 µg l^{-1} (control), 0.3 µg l^{-1}, and 30 µg l^{-1}. Apart from sertraline concentration, the animals were exposed to two levels of algal concentration: 0.3 mg C l^{-1} (low food level) and 3 mg C l^{-1} (high food level) per animal per day. Fifteen replicates in each combination of studied factors were used, resulting in 90 test animals used in total.

Media were changed every 48 hours. The test animals were carefully transferred to the new media using Pasteur pipettes. In case of occurrence of juveniles, the rest of the old media was transferred on a shallow plastic plate and the juveniles were counted, transferred to 1.5 ml Eppendorf flask and preserved in 4% formaldehyde solution. On days with no media change, animals were fed to achieve 0.3 mg C l^{-1} and 3 mg C l^{-1} for the low and high food concentration, respectively, by pipetting calculated amount of fresh concentrated algae directly to the wall of the beakers.

Positions of the treatments were regularly changed to avoid possible effect of light differences. Starting from day 5, solid cetyl alcohol was added on the surface of new media in each beaker to prevent animals from being trapped to the water surface by lowering the surface tension. Temperature was measured in separate vessel filled with dechlorinated tap water using temperature data logger during the whole experiment. Dissolved oxygen (DO) concentration and pH were measured once during the experiment with WTW 340i (with DO electrode CellOx 325 and pH-electrode SenTix 41, respectively) in fresh media and in treated media (i.e. solutions after 48-h exposure of test animal and its juveniles), in two vessels for each combination of food and sertraline parameters. The duration of the experiment was 21 days. At the end of the experiment, tested animals were preserved in 4% formaldehyde solution.

The body length of the test animals was measured using stereo microscope Olympus SZ51. Juveniles from the 3rd broods younger than 24 hours were measured using microscope Olympus CX 41.

Factorial ANOVA was used for statistical analysis of the data, testing the influence of food level and sertraline concentrations on daphnid life-history traits. Analysis were computed using statistical software STATISTICA 13 (Dell Inc., 2016). Graphs were designed using GraphPad Prism 6.

6 Results

6.1 Preliminary test

The immobilization test followed validity criteria of Guideline 202 (OECD, 2004). Even none of test animals in control (0 µg l^{-1} of sertraline in both low and high food level) was immobilized during the experiment. The test proved that concentrations above 1 mg l^{-1} are toxic for *Daphnia magna* in the 48-h acute toxicity test, and showed even higher, 100% immobilisation under sufficient feeding conditions. The concentrations of 0.3 µg l^{-1} and 30 µg l^{-1} did not cause any immobilisation in any combination of tested parameters, which approved these concentrations to be suitable for our reproduction test. Interestingly, the immobilisation of animals in the low food level in the two highest of tested concentrations (600 µg l^{-1} and 1200 µg l^{-1}) was lower than immobilisation in the high food level (Table 2). No statistics was applied to the test.

Table 2: The immobilisation of test animals after 24 and 48 hours of exposure. The results are presented in % of immobilised animals per each combination of studied factors after the given time period.

Sertraline (µg l^{-1})	**Low food**		**High food**	
	24 hours (%)	**48 hours (%)**	**24 hours (%)**	**48 hours (%)**
0	0	0	0	0
0.3	0	0	0	0
30	0	0	0	0
600	0	20	80	80
1200	40	40	40	100

6.2 Reproduction test

Average dissolved oxygen concentration and average pH in the fresh media was 7.98 ± 0.11 mg l^{-1} and 7.98 ± 0.07, respectively (Table 3). In the media after 48 hours of animal exposure, the average amount of dissolved oxygen was 7.88 ± 0.27 mg l^{-1} and pH 7.84 ± 0.07 (Table 3).

In the fresh media, the pH was significantly lower ($F_{2,6} = 7$; $p = 0.028$) in media containing sertraline compared to the control. Neither sertraline concentrations nor food level influenced the amount of dissolved oxygen in the fresh media.

Treated media showed lower amount of dissolved oxygen ($F_{1,12} = 45.2$; $p = 0.007$) and lower pH ($F_{1,12} = 53$; $p < 0.001$) compared to the fresh media. An increased amount of dissolved oxygen ($F_{1,6} = 45.2$; $p < 0.001$) and decreased pH ($F_{1,6} = 6$; $p = 0.045$) were recorded in the treated media with the high food level in comparison with the low food level. The amount of dissolved oxygen gradually increased with sertraline concentrations, but only in the high food level ($F_{2,6} = 30$; $p < 0.001$). The value of pH decreased with increasing sertraline concentration in both food levels ($F_{2,6} = 6$; $p = 0.036$). The combined impact of sertraline, food, and fresh/treated media on the amount of dissolved oxygen was also recorded ($F_{2,12} = 5{,}8$; $p = 0.017$).

Table 3: Mean values of dissolved oxygen (mg l^{-1}) and pH measured in two replicates for each combination of tested parameters (food level, sertraline concentration, fresh vs. treated media).

	Sertraline (µg l^{-1})	**Fresh media**		**Treated media**	
		O_2 (mg l^{-1})	**pH**	**O_2 (mg l^{-1})**	**pH**
Low food	0	8.15	7.99	7.8	7.925
	0.3	7.89	7.95	7.7	7.87
	30	8	7.965	7.7	7.83
High food	0	7.9	8.1	7.65	7.885
	0.3	7.95	7.9	8.07	7.5
	30	8	7.96	8.35	7.8

The reproduction test itself followed the validity criteria defined by the OECD Guideline 211: the mortality of animals from each combination of studied factors did not exceed 20% at the end of the test; the mean number of apparently live offspring produced per surviving parent animal was ≥ 60 in controls in the high food level at the end of the test. As expected, the mean number of apparently live offspring produced per female was < 60 in the low food level in controls. Because of very low mobility of the youngest juveniles, distinguishing between live and dead juveniles was uncertain. For this reason, the total number of juveniles was used for following analysis of observed parameters.

Generally, daphnid females produce fewer juveniles under low food levels. Specifically, cumulative number of juveniles per female and numbers of juveniles from the 1^{st}, 2^{nd}, and 3^{rd} brood per female in the low food level were lower than numbers of juveniles per female in the high food level (Figure 3, Table 4). The decrease of mean cumulative number of offspring per female was 87% in the low food level compared to the high food level. No impact of sertraline concentrations and their interactions with food was recorded (Figure 3). Fourth brood was not reached by females in the low food level, however, number of juveniles in the 4^{th} brood progeny in the high food level was significantly decreased by sertraline concentrations. The decrease was observed for 30 µg l^{-1} sertraline where the females produced 24% less neonates than the females in the control (Figure 3, Table 4).

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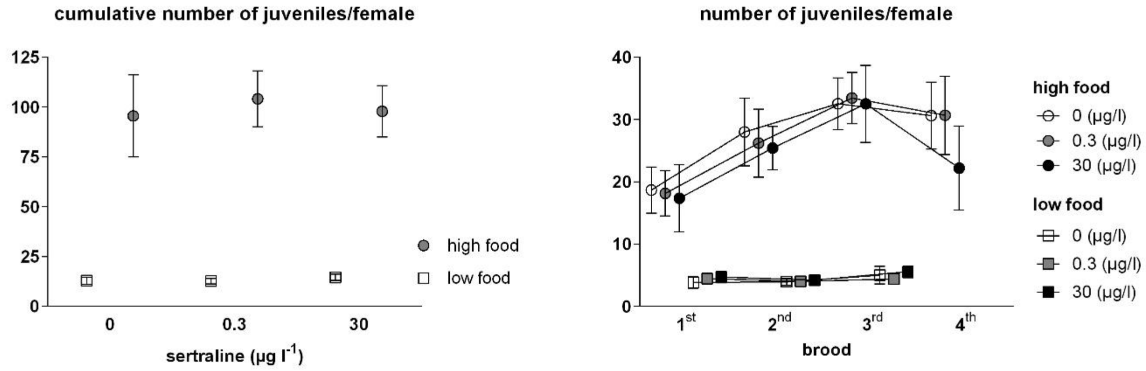

Figure 3: Cumulative number of juveniles per female (left) and number of juveniles per female per brood (right) in the three sertraline levels (0, 0.3, and 30 µg l^{-1}), expressed in mean ± SD. Low and high food levels are denoted with □ and ○, respectively, with white, grey and black color representing 0, 0.3 and 30 µg l^{-1} sertraline.

Females exposed to the low food level showed lower body length at the close of the experiment. The decrease of mean body length was 29% compared to the high food level. Furthermore, sertraline exposure also reduced the body length of the test animals (Figure 4, Table 4). The most pronounced change in the body length was recorded in the high food level for sertraline concentration 30 µg l^{-1} with reduction by 4% compared to the control in the high food level. The average length of juveniles from the 3rd brood was significantly affected by neither food level nor sertraline concentration (Figure 4).

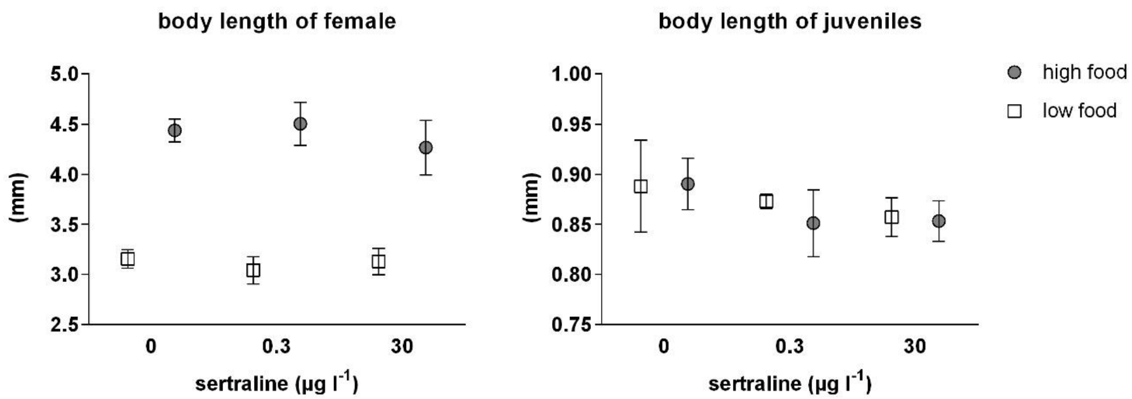

Figure 4: Body length of females after 21 days of the experiment (left) and body length of juveniles from the 3rd brood progeny aged less than 24 hours (right) in the three sertraline levels (0, 0.3, and 30 µg l^{-1}), expressed in mean (in mm) ± SD. Low and high food levels are denoted with □ and ○, respectively.

Likewise, days to the 1st brood and average interclutch period were significantly affected by the low food level, which prolonged the times by 1.8 days (15%) and 0.5 days (12%), respectively (Figure 5, Table 4). No impact of sertraline and its interaction with food was recorded. The values of observed parameters are summarized in Appendix (Table 5).

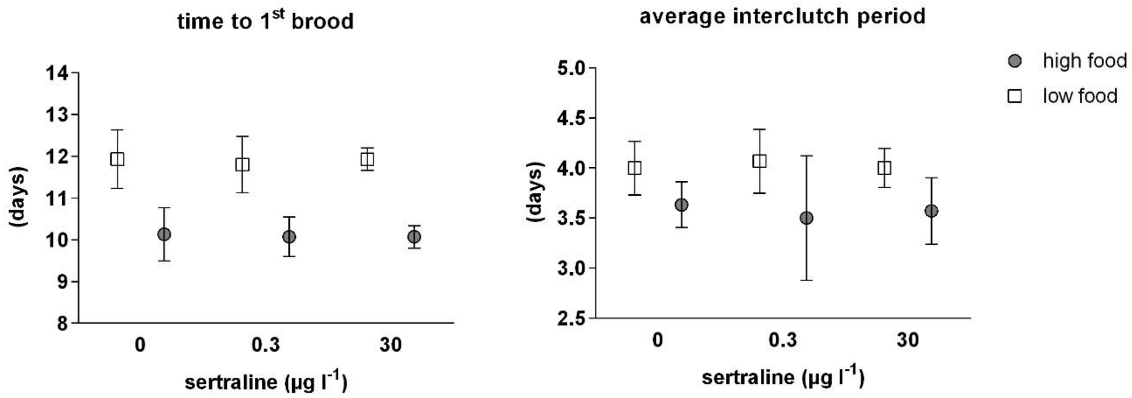

Figure 5: Average time to the 1st brood (left) and average interclutch period (right) in the three sertraline levels (0, 0.3, and 30 µg l^{-1}), expressed in mean number of days ± SD. Low and high food levels are denoted with □ and ○, respectively.

Table 4: Significant results of factorial ANOVA (df; F; p) examining the impact of food level and sertraline concentration on cumulative number of juveniles per female, number of juveniles in the 1st brood, number of juveniles in the 2nd brood, number of juveniles in the 3rd brood, number of juveniles in the 4th brood, time to the 1st brood, average interclutch period, and body length of females.

Observed parameter	**Significant effect of**	**df**	**F**	**p**
Cumulative number of juveniles per female	food	1	3611	< 0.001
Number of juveniles in the 1st brood	food	1	538	< 0.001
Number of juveniles in the 2nd brood	food	1	2287	< 0.001
Number of juveniles in the 3rd brood	food	1	1634	< 0.001
Number of juveniles in the 4th brood (only high food level)	sertraline	2	7.1	0.003
Time to the 1st brood	food	1	232	< 0.001
Interclutch period	food	1	75.8	< 0.001
Body length of females	food	1	1170	< 0.001
	sertraline	2	4.2	0.019
	food*sertraline	2	4.2	0.018

7 Discussion

To our knowledge, this is the first study evaluating the effects of sertraline on life-history traits of *Daphnia magna* in combination with food level. It showed that sertraline might not only influence daphnid reproduction, but also its body length under high food level. Furthermore, it seems that food level can play an important role in influencing daphnids by sertraline.

An interesting finding was also done during the evaluation of the preliminary, acute toxicity test. According to the published literature, the concentration of 1 mg l^{-1} of sertraline was considered to correspond to the EC_{50} value in 48-h acute toxicity tests (Christensen et al., 2007; Minagh et al., 2009; Minguez et al., 2014). Indeed, our results confirmed that the concentration of 1.2 mg l^{-1} of sertraline in 48-h exposure of the *Daphnia magna* animals was toxic for the daphnids. However, a complete (100%) immobilisation was observed at the high food level. This difference can probably be explained by modifications of the methodology, usage of other *Daphnia* clone, and the source of food.

On the other hand, the immobilisation of females in the low food level was lower compared to the high food level, only 40% of the daphnids in 1.2 mg l^{-1} of sertraline. Bioaccumulative properties of sertraline in general are well documented; it easily accumulates also in algae which serves as food for the daphnids (Daughton and Brooks, 2011; Vannini et al., 2011; Grabicova et al., 2014, 2015). The chemical could more likely remain stable when incorporated to the algae (Kwon and Armbrust, 2008). At higher food levels, the filtering rate of the daphnids increases (McMahon and Riegler, 1965), which means that more food is introduced to the animal body carrying the incorporated sertraline. It suggests that not only sertraline present in water can directly influence the animals, but also indirect introduction of the chemical via ingestion can play major role in their survival. More importantly, the introduction via ingestion can even outweigh the importance of sertraline present in water. That means that in certain conditions, daphnids could be more vulnerable at high food concentrations, which is in contrast with the expected effect (Hanazato, 2001).

We confirmed the main expectancy of our acute toxicity test that lower concentrations of sertraline, 0.3 µg l^{-1} and 30 µg l^{-1}, were not toxic for our daphnids and were suitable for the reproduction test. Therefore, no subsequent tests were conducted to confirm other theories.

In the chronic reproduction test, quality of the environment had to be evaluated first. It was found that the differences in amount of dissolved oxygen and pH were significantly influenced by sertraline concentration and food level. These changes were observed only in very small ranges, which should probably not influence the life-history traits (Smirnov, 2014).

Moreover, the values within both fresh and treated media followed recommendations in Guideline 211 (OECD, 2012). Nevertheless, the causes for these differences need to be explained.

Treated media in general displayed lower values of dissolved oxygen (7.88 ± 0.28 mg l^{-1}) compared to the fresh media (7.98 ± 0.09 mg l^{-1}) due to presence of the animals which reduce the amount by respiration. Simultaneously, higher amount of dissolved oxygen (8.02 ± 0.35 mg l^{-1}) was observed in the treated media with the high food level compared to the treated media with the low food level (7.73 ± 0.06 mg l^{-1}). This was not a surprising outcome as algae photosynthesize and therefore produce oxygen. The amount of dissolved oxygen gradually increased with sertraline concentrations, but only in the high food level. This may be linked to lower respiration rates of smaller-bodied females, which were present in this set of factors (Heisey and Porter, 1997). Dissolved oxygen was not significantly influenced in any of the fresh media by either sertraline concentrations or food level. This could be explained by short or none exposure of synthetizing algae and test animal, respectively.

A significant influence of food level and sertraline on pH was observed in both fresh and treated media. The value of pH is influenced not only by photosynthesis of the algae and sertraline concentration, but also by the presence of the living animals and transformation products of sertraline, especially in the treated media. Due to the complexity of the system, it is difficult to determine the exact contributors to the pH change. Nevertheless, these changes were in very small ranges which should not significantly influence the studied life-history traits of daphnids.

Effects of decreased food level alone on the *Daphnia* spp. were already well documented. Our experiment was consistent with published literature. Females produced fewer eggs and their body length decreased under the low food level (Ebert and Yampolsky, 1992). Furthermore, time to the 1st brood and average interclutch period were prolonged in poorly fed daphnids (Giebelhausen and Lampert, 2001).

Studies investigating the effects of sertraline on reproduction of test organisms, like *Daphnia magna* or *Ceriodaphnia dubia*, showed mostly reduction in the mean cumulative number of offspring (Henry et al., 2004; Minagh et al., 2009; Lamichhane et al., 2014). In our study, the decreased number of offspring occurred only in the 4th brood of the tested animals, and this 4th brood was only reached in the high food level due to limited duration of the experiment. This suggests that under longer duration of the test, the overall offspring production might be reduced at concentrations of 30 μg l^{-1} even in our conditions. This slight difference from the mentioned studies is not surprising, since all these experiments differ in many variables, including the test organism (i.e. different *Daphnia* clone or species), food level and source (with possible different levels of accumulation of sertraline in different algae), frequency of media change, photoperiod, temperature, the supplier of sertraline, or the chemical preparation (e.g. use or not of carrier solvent). Furthermore, it is important to note that number of offspring is a function of body length (Hanazato, 2001). Decrease in number of juveniles might be therefore linked to the decrease of body length. The sertraline concentration decreased body length of the tested animals in agreement with Lamichhane et al. (2014). The effect was observed especially at 30 $\mu g\ l^{-1}$ of sertraline at the high food level, which could support the theory of importance of the exposure via ingestion.

In the low food level, the number of offspring was not significantly affected by sertraline concentration. This could be explained by the following reasons: (i) the effect occurs at later broods (only three broods were reached in the low food level during the 21d test period); (ii) the sertraline exposure via environment is not as prominent as via ingestion; or (iii) there is no influence of sertraline. Further experiments are necessary to answer this question with e.g. longer duration of the experiment.

Apart from the reasons described above, there is one other important information to be noted. Sertraline is known to decompose to transition products rather quickly. No analysis of transition products was made in our study; however, the media were changed relatively frequently (48 h) to prevent high occurrence of transition products and to primarily expose daphnids to the parent compound. Even these frequent media changes might not prevent any sertraline from degradation. This raises further questions: Could transition products of sertraline have an effect as well? Could this effect be higher than the one of the parent compound? Unfortunately, most of the transition products of sertraline are not being analysed in the nature, and the data about their toxicity are not very well known either.

Finally, the influence of sertraline of daphnids was recorded, but only at concentrations significantly higher compared to current concentrations found in the nature. The environmental concentrations are perhaps not sufficient to cause significant effects on reproduction, body length or survival of the animals, but they may affect physiological processes that were not assessed in this experiment. Unfortunately, there is a lack of publications dealing with the exact mode of action of sertraline in the *Daphnia* spp. body, making it difficult to observe any effects on other physiological processes.

8 Conclusion

- Daphnids exposed to the low food levels were less sensitive to sertraline. This was in contrast with the expected outcome.
- Sertraline reduced the number of offspring per female, but only in the 4^{th} clutch of the high food level. The decrease was observed for 30 µg l^{-1} sertraline where the females produced 24% less neonates than the females in the control.
- Sertraline did reduce the body length of females, which was observed mainly in the high food level.
- Fewer juveniles were produced under the low food level, which is in accordance to literature. The decrease in mean cumulative number of offspring per female was 87% compared to the high food level.
- Daphnids reached smaller body length under the low food level, which is in accordance to literature. The decrease of mean body length was 29% compared to the high food level.
- Time to the 1^{st} brood and average interclutch period were prolonged by 1.8 days (15%) and 0.5 days (12%), respectively, under the low food level, which is in accordance with literature.
- However, the influence of sertraline on *Daphnia magna* life-history traits is still not very clear as even the published sertraline effects are partly contradictory and ambiguous.

9 Literature

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10 Appendix

10.1 Materials and methods – list of glassware

- Erlenmeyer flask, 25 ml, for sertraline stock solution
- Volumetric flask, 1000 ml, 3 pcs, for working solutions (media preparation)
- Bottle-top tilt pipette dispenser, 50 ml, with Erlenmeyer flask, 500 ml; 2 pcs, one for control media, one for sertraline media
- Beakers, 100 ml, 4 sets of 45 beakers; 2 sets for living animals, 2 sets for preparing fresh media
- Pasteur pipettes, plastic, multiple pieces, for transferring animals
- Pasteur pipettes, glass, multiple pieces, for transferring animals
- Plates of acrylic glass, 6 pcs, for covering beakers with test animals
- Others: beakers, Erlenmeyer flasks, plastic trays, 5 l plastic containers for dechlorinated tap water, …
- Note: Each piece of glassware was washed with dechlorinated tap water right after use. Glassware used for control was never contaminated with sertraline solution.

10.2 List of figures

10.3 List of tables

10.4 Table of results

Table 1: Summary of results of observed parameters. Cumulative number of juveniles, number of juveniles in each brood, time to the 1st brood, average interclutch period, body length of female, body length of juveniles from 3rd brood, all expressed in mean ± SD.

	High food			Low food		
Sertraline ($\mu g\ l^{-1}$)	**0**	**0.3**	**30**	**0**	**0.3**	**30**
Cumulative number of juveniles	96 ± 21	104 ± 17	98 ± 13	13 ± 2	13 ± 1	15 ± 2
Number of juveniles in the 1st brood	19 ± 4	18 ± 4	17 ± 5	4 ± 1	4 ±1	5 ± 1
Number of juveniles in the 2nd brood	28 ± 5	26 ± 5	25 ± 4	4 ± 1	4 ± 1	4 ± 1
Number of juveniles in the 3rd brood	33 ± 4	33 ± 4	33 ± 6	5 ± 1	4 ± 1	6 ± 1
Number of juveniles in the 4th brood	31 ± 5	31 ± 6	22 ± 7	-	-	-
Time to the 1st brood (days)	10.1 ± 0.6	10.1 ± 0.5	10.1 ± 0.3	11.9 ± 0.7	11.8 ± 0.7	11.9 ± 0.3
Interclutch period (days)	3.6 ±0.2	3.4 ± 0.2	3.6 ± 0.3	4.0 ± 0.3	4.1 ± 0.3	4.0 ± 0.2
Body length of female (mm)	4.44 ± 0.11	4.50 ± 0.21	4.27 ± 0.27	3.15 ± 0.09	3.04 ± 0.14	3.13 ± 0.13
Body length of juveniles in the 3rd brood (mm)	0.89 ± 0.03	0.85 ± 0.03	0.85 ± 0.02	0.89 ± 0.05	0.87 ± 0.01	0.86 ± 0.02