Effects of antidepressant fluoxetine on personality in the freshwater isopod *Asellus aquaticus*

Shiori Kitano

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The widespread use of pharmaceuticals raises an anthropogenic issue in natural environment. Selective serotonin reuptake inhibitors (SSRI) used as antidepressants, pass through most wastewater treatment plants and enter natural waters. Their impact on personality of aquatic animals is poorly investigated, especially in invertebrates. In the current study, the impact of fluoxetine (an SSRI) on animal personality was investigated in the freshwater isopod *Asellus aquaticus*. To investigate responses, isopods were exposed for 1 day to fluoxetine of 10 ng L\(^{-1}\). Boldness, exploration, activity and escape behaviour (running and freezing) were tested on male and female isopods of two phenotypes of pigmentation (dark and light). The isopods showed consistency of behaviour responses in assays; one of the prerequisites for the existence of personality traits. Fluoxetine exposure reduced activity level, but had no effect on the other personality traits measured. This study thus provides some support for the idea that an environmentally relevant concentration of fluoxetine affects personality in *A. aquaticus*.
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1. Abstract

The widespread use of pharmaceutics raises an anthropogenic issue in natural environment. Selective serotonin reuptake inhibitors (SSRI) used as antidepressants, pass through most wastewater treatment plants and enter natural waters. Their impact on personality of aquatic animals is poorly investigated, especially in invertebrates. In the current study, the impact of fluoxetine (an SSRI) on animal personality was investigated in the freshwater isopod *Asellus aquaticus*. To investigate responses, isopods were exposed for 1 day to fluoxetine of 10 ng L$^{-1}$. Boldness, exploration, activity and escape behaviour (running and freezing) were tested on male and female isopods of two phenotypes of pigmentation (dark and light). The isopods showed consistency of behaviour responses in assays; one of the prerequisites for the existence of personality traits. Fluoxetine exposure reduced activity level, but had no effect on the other personality traits measured. This study thus provides some support for the idea that an environmentally relevant concentration of fluoxetine affects personality in *A. aquaticus*. 
2. Introduction

The comprehension of impacts of pharmaceutical substance leakage in the natural environment is a highly demanded subject in biology. Especially, there have been increasing concerns about the effects of pharmaceutical contamination in the aquatic environment (Bossus et al. 2014). In recent years, the consumption of antidepressants has increased dramatically, while most of current waste water treatment plants are technically inadequate to remove pharmaceutical compounds. Hence, a large proportion drains out in natural aquatic systems (Boxall et al. 2012; Swedish Environmental Protection Agency 2018). Traditionally, drugs used to be designed to treat diseases in humans and domestic animals, but not in aquatic animals. Therefore, the ecological and evolutionary consequences of anti-depressive substance leakage are unknown; even less is known about its impact on behaviour of both prey and predators (Brooks et al. 2005; Saaristo et al. 2017).

Among anti-depressive drugs, consumption of selective serotonin reuptake inhibitors (SSRI) multiplies worldwide (Bossus et al. 2014). Serotonin is a neuro-transmitter that is present in a wide range of taxa, from worm to mammal (Hay-Schmidt 2000), and a change of serotonin levels causes alteration in biological processes such as growth, metabolism, locomotion and reproduction, with consequences for individual fitness and population dynamics (Frazer & Hensler 1999; Bean et al. 2014). Also animal behaviour and animal personality may be influenced by anti-depressive drugs modifying serotonin levels (Bean et al. 2014). Among the behavioural studies, animal personality research has increased in the recent decades, since many scientists have observed individual behavioural variation in animals as in humans (MacKay & Haskell 2015). Dall et al. (2004) defined animal personality as behavioural characteristics that differ between individuals but are relatively consistent over time and across situations (Gosling 2001). Broadly, personality can be categorized into five traits with ecological validity; boldness, exploration, activity, sociability and aggressiveness (Réale et al. 2007). However, it is not simple to provide standardized measurements of personalities. Accordingly checking consistency of behaviour responses is fundamental. Furthermore, investigating linked personalities within population should be a subject of consideration (e.g. high boldness versus high degree of exploration), namely how behavioural traits are correlated within population, and it is often called behaviour syndrome among behavioural ecologists (Sih et al. 2004; Bell 2005; Réale et al. 2007). It is important to study the impact of antidepressant leakage in natural environments on animal personality because the alteration of animal personality and behavioural patterns can alter the ecological balance and put the whole ecosystem at risk (Saaristo et al. 2017).

Antidepressant fluoxetine is one of several SSRI drugs to treat mental illnesses such as depression in humans. Hence, several studies particularly focus on fluoxetine to look into impact of the antidepressants in the aquatic environments because of its widespread use and stability regarding bio- and photo-degradation (Brooks et al. 2003; Know 2006; Bossus et al. 2014; Saaristo et al. 2017). Impacts of fluoxetine on animal personality have been shown a
wide range of taxa. Especially in fishes, behavioural researches have shown; less boldness in males, weakened aggression, low activity and altered social behaviour (Henry & Black 2008; Winder et al. 2012; Barry 2013; Dziewczynski et al. 2016; Saaristo et al. 2017). A recent review of behaviour studies with focus on aquatic invertebrates concludes that there was a negative impact on productivity and activity of crustaceans. A conclusion was, however, that a large number of antidepressant effects on behaviour are still unknown in invertebrates (Fong & Ford 2014).

Macroinvertebrates play a vital role in freshwater ecosystems. The freshwater isopod *Asellus aquaticus* (*Isopoda, Crustacea*) has an important role in the nutrient cycling of aquatic ecosystems as decomposer and as benthic-pelagic food chain component. It is spread over Europe and is a common benthic organism which inhabits shallows as well as lakes, ponds, and caves (Johansson 2005). Being a very common species it has a quantitative importance for ecosystem process (Hargeby et al. 2004). It is also a very easy one to handle, and it appears in many studies (Wallace & Webster 1996; Hargeby et al. 2004; Harris et al. 2011; Augusiak & Van den Brink 2016; Ginneken et al. 2017; Plahua et al. 2017). Therefore, it is interesting to learn more about potential changes in personality of *Asellus* when exposed to SSRI.

The current study aims to research the effects of antidepressant fluoxetine on the personalities of wild caught *A. aquaticus*. I have investigated consistency of behavioural responses to test if isopods have personality. I carried out four behavioural assays to measure boldness, exploration, activity and additionally escape behaviour; as antipredator behaviour. An environmentally relevant concentration (10 ng L\(^{-1}\)) of fluoxetine was applied in this study. Today fluoxetine concentration in nature typically ranges between 0.15 and 32 ng L\(^{-1}\) (Bean et al. 2014; Dziewczynski 2016). As we learn from a previous study in crustacean (Fong & Ford 2014), the isopods in this study might be expected to be negatively affected regarding activity, while impact on the other traits may be difficult to predict due to the lack of analogous studies.
3. Materials and methods

3.1. Study organism and holding condition

Adult wild caught *Asellus aquaticus* used in the experiment were collected beneath snow covered ice on the 7th of March 2018 in Lake Tåkern (X: 6468580 Y: 1441910). The lake bottom consisted soft sediment and was covered by *Chara* sp. vegetation. A rapid local morphological differentiation of freshwater isopod populations has been observed in Lake Tåkern: reduced pigmentation on ancestral dark isopods lead to the appearance of lighter phenotype, as a result of switching habitat vegetation from original reeds to *Chara* stands in the past twenty years, i.e. local adaptive evolution (Hargeby et al. 2004; Johansson 2005). About 100 isopods were placed in five containers (10 cm × 25 cm × 45 cm: H × D × W). The containers were filled with filtrated lake water from Fröberget, Tinnerö nature reserve, Linköping, Sweden and kept in 5 °C and darkness for almost one month before the experiment started. Water was changed every week and oxygenated with an air pump. The isopods were fed decaying alder (*Alnus alutinosa*) and elm (*Ulmus glabra*) leaves which had been colonised by natural microbial film during four weeks before the experiment.

Five days prior to the experiments, containers with the isopods were moved to another cold room, where they were kept for three days (room temperature: 15 °C and water temperature: 13.4 °C, 12:12 h /light: dark). This procedure with the stepwise acclimatization to temperature and light conditions was used to reduce stress and heat shock caused by increased water temperature, which could affect behaviour of the animals (Lagerspetz 2003). On the fourth day isopods were chosen randomly in combination of sex (male and female) and two phenotypes of pigmentation (dark and light): dark male, light male, dark female and light female. Sexing was based on the length of legs and antenna, and the presence of eggs on the animals’ ventral side (Bertin & Cezilly 2003). Moreover, isopod pigmentation is a quantitative trait in Lake Tåkern (Johansson 2005), but in the current study the isopods were classified only as dark and light phenotypes. They were placed singly in white round plastic bins (ø 10 cm × 3.5 cm) with food and filled with Linköping tap-water instead of Lake Frökärret natural water. Tap-water was oxygenated and dechlorinated in the current room temperature and water temperature of 13.4 °C.

3.2. Experimental treatment

After the sorting, the isopods were moved to the experimental laboratory and kept there for 1 day to acclimatize to a new temperature; 12 hours in light (7:00 – 19:00) and 12 hours in dark, at a room temperature of 20 °C. Each isopod was exposed to 24 hours prior to the behavioural assays, to two treatments; (1) control, i.e. tap-water (2) fluoxetine (10 ng L⁻¹). Due to the lack of information on the fluoxetine concentration in Lake Tårkern itself, I referred to a study of nearby Lake Roxen in Sweden, where a sample of surface water in 2008 showed 20 ng L⁻¹ (Helmfrid et al. 2010). Each isopod was placed in a plastic bin filled with treatment solution.
without food. In the centre of the bin 3 small stones (\(\varnothing 5 - 10\) mm) were placed as a refuge to minimalize stress factors because the isopods need something to grip on to. Oxygenated and dechlorinated tap-water was used as control solution, and therefore fluoxetine solution was prepared by diluting fluoxetine stock solution (1 \(\mu\)g L\(^{-1}\)) with dechlorinated tap-water just before the experiment. The stock solution was prepared by diluting another solution (1 mg L\(^{-1}\)), which was obtained from PhD. Robin Abbey-Lee, Linköping University, Sweden. The solution (1 mg L\(^{-1}\)) was made in 10\(^{th}\) November 2017 and stored in darkness at 5 °C. This solution (1 mg L\(^{-1}\)) was prepared by dissolving crystal formed fluoxetine (F132- Fluoxetine hydrochloride solid; Sigma Aldrich) in buffer solution (Phosphate Buffer Solution pH 7.5; Sigma Aldrich). Fluoxetine solution has 277 days half-time when fluoxetine is dissolved in buffer solution of pH 7 (Kwon & Armbrust 2006). Therefore, there was not any risk of minimalized effect since experiment proceeded until 19\(^{th}\) March.

3.3. Behavioural assays

Behavioural assays were initiated after the 24 h exposure period. All assays were performed in sequence in a certain order: boldness, exploration, activity and escape behaviour in the morning (8:00-12:00) and the afternoon (13:00-17:00). The order of choosing an individual to experiment was done randomly. The individual that failed maximum time were given the maximum score. Behavioural assays were performed on a total of 111 freshwater isopods (A. aquaticus). The length of isopods was measured individually after all tests. To do this, a graph paper (1 mm × 1 mm) was placed under the dish (males: 12.38 ± 0.96 mm (mean ± SD) and female: 9.53 ± 0.99 mm). There was no difference in length between treatment groups for any of the sexes (Kruskall-Wallis test; males: \(H = 0.002, p = 0.966, n = 55\) and females: \(H = 0.051, p = 0.475, n = 56\)).

3.3.1. Boldness test

To investigate boldness, latency to leave a refuge was measured, which was recognized as the individual’s risk-taking behaviour (Réale et al. 2007; Harris et al. 2010; Tremmel & Müller 2012). A small beige stone was placed in the centre of a glass petri-dish (\(\varnothing 8\) cm; white background) that was filled up with homogeneity solution as in the exposure bin to the level of 15 mm. The stone was of the same size and texture as in the exposure bin and thus regarded as a familiar refuge. Before an isopod was introduced to the experimental area, an opaque tube (\(\varnothing 3\) cm, height 7 cm) was put over the stone, thus isolating the stone from the rest of arena. An individual was gently relocated from its exposure bin into the tube, which was carefully lifted when the isopod took refuge. The recording was started just as the isopod had taken refuge until the last limb of isopod let go of the refuge. The maximum observation time was limited to 7 minutes. After the test the stone was removed and the arena open for further behavioural assays.
3.3.2. Exploration test

After the boldness test, an exploration test was performed in the same petri dish in which the isopod left the refuge. An open field test was used to investigate the exploratory behaviour by recording the total time the isopod took to visit all zones in novel area within at maximum 10 minutes (Perals et al. 2017). To identify the zones, a white paper marked with 9 areas (one centre and eight equal-sized peripheral areas) was placed under the transparent petri-dish. A visit to new zone recognized when the whole body was entered into it. Each individual was left to rest in the same petri-dish for 5 min prior to the next test.

3.3.3. Activity test

The activity was investigated by counting the total number of times the isopod changed zone during 2 minutes. This measure was regarded as a similar test to, the total area covered, which is a measure commonly used to estimate an individual’s activity (Réale et al. 2007; Saaristo et al. 2017).

3.3.4. Escape behaviour test

A simulated predator attack was performed to investigate the escape behaviour (Eroukhmanoff & Svensson 2009; Harris et al. 2011). The individual was poked with a silicon tube (Ø 1 mm) with air for 3 seconds to simulate a predator attack. The total amount of time (i) the isopod continued running to escape from the simulated attack and (ii) how long it maintained a still position after the running event, were recorded (Max 5 min each).

3.4. Consistency of behavioural responses

To investigate temporal consistency of behavioural responses (boldness, exploration, activity and escape behaviour) within individual, all tests were repeated 3 days after the first trial on 28 isopods which represent both sexes and phenotypes. After the first trial, the isopods were placed in a tap-water filled plastic bin with feed to repose two days. The following 24 hours before the second trial isopods were removed to a new plastic bin to achieve the same condition as the first trial. The second trial was carried out only in control treated isopods because fluoxetine effect was unknown on responses.

3.5. Statistical analysis

All statistical analyses were performed in SPSS (version 24).

I. Consistency in behaviour

The consistency of behavioural responses within individuals and between individuals was tested using Spearman’s rank correlation, because the behavioural data was not observed for normality of distribution or homogeneity of variance.
II. Fluoxetine effect
General liner models (GLM) were used to analyse effects of fluoxetine on personality traits. The data from all behavioural tests were not normally distributed and therefore “the two step approach” was used, that way the non-normally distributed variables were transformed into normally distributed ones. The first step involves transforming the original variable toward statistical uniformity (fractional rank). To avoid errors during the second step, it is necessary to replace any resulting 1’s with .9999. The second step is to transform uniform probabilities to normal using the inverse normal distribution function (Templeton 2011). After the transformation homogeneity of variance was confirmed. Effects of fluoxetine, sex, phenotype as single factors and interactions of these factors were also checked. The length was excluded from the analyses because length was not correlated with any of the behavioural responses (Pearson’s rank test: boldness, exploration, activity, running, freezing; \( p = 0.06, 0.523, 0.072, 0.146, 0.388 \), respectively).

4. Results

4.1 Consistency in behaviour over time

Behavioural responses of isopods were consistent between day one and day four for all personality traits (Table 1). Especially boldness and activity showed strong consistency over time.

<table>
<thead>
<tr>
<th>Escape behaviour</th>
<th>Boldness</th>
<th>Exploration</th>
<th>Activity</th>
<th>Running</th>
<th>Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>( r_s )</td>
<td>0.709</td>
<td>0.693</td>
<td>0.822</td>
<td>0.469</td>
<td>0.381</td>
</tr>
<tr>
<td>( p )</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.050</td>
<td>0.012</td>
</tr>
</tbody>
</table>

Table 1. Consistency in \( A. aquaticus \) behaviour over time in the five behaviour assays, tested with Spearman rank correlation (\( n = 28 \), Running: \( n = 27 \)).
4.2. Behavioural responses

4.2.1 Boldness

Fluoxetine exposure had no impact on the duration of leaving a refuge (Table 3). There were sex differences in the total amount of time, specifically females took longer time to leave the refuge than males (Table 3 and Fig. 1).

Table 3. GLM results on factors and interactions affecting the total amount of time A. aquaticus acquired to leave a refuge, p < 0.05 is bolded (n =111).

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>1</td>
<td>1.28</td>
<td>0.261</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>12.36</td>
<td>0.001</td>
</tr>
<tr>
<td>Phenotype</td>
<td>1</td>
<td>2.33</td>
<td>0.130</td>
</tr>
<tr>
<td>Fluoxetine*Sex</td>
<td>1</td>
<td>0.02</td>
<td>0.899</td>
</tr>
<tr>
<td>Fluoxetine*Phenotype</td>
<td>1</td>
<td>2.85</td>
<td>0.094</td>
</tr>
<tr>
<td>Sex*Phenotype</td>
<td>1</td>
<td>0.55</td>
<td>0.814</td>
</tr>
<tr>
<td>Fluoxetine<em>Sex</em>Phenotype</td>
<td>1</td>
<td>0.79</td>
<td>0.377</td>
</tr>
</tbody>
</table>

Fig. 1. The total amount of time (s) A. aquaticus acquired to leave a refuge a) between males and females b) between treatment groups in terms of 4 types of isopods. M/D indicates dark male, M/L; light male, F/D; dark female and F/L; light female. Columns show mean ± SE a) Male: n= 55, Female: n= 56 b) M/D: n=14, M/L: n =13, F/D: n =14, F/L: n= 14.
4.2.2 Exploration

Fluoxetine exposure did not affect the duration that isopods acquired to visit all zones (Table 4). Furthermore, there were no sex, phenotype differences neither interactions within or between control and fluoxetine (Table 4 and Fig. 2).

**Table 4.** GLM results on factors and interactions affecting the total amount of time *A. aquaticus* acquired to visit all zones in novel area, p < 0.05 is bolded (n =111).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>1</td>
<td>0.67</td>
<td>0.415</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.15</td>
<td>0.702</td>
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<tr>
<td>Phenotype</td>
<td>1</td>
<td>0.49</td>
<td>0.487</td>
</tr>
<tr>
<td>Fluoxetine*Sex</td>
<td>1</td>
<td>0.01</td>
<td>0.934</td>
</tr>
<tr>
<td>Fluoxetine*Phenotype</td>
<td>1</td>
<td>2.00</td>
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</tr>
<tr>
<td>Sex*Phenotype</td>
<td>1</td>
<td>0.24</td>
<td>0.625</td>
</tr>
<tr>
<td>Fluoxetine<em>Sex</em>Phenotype</td>
<td>1</td>
<td>0.43</td>
<td>0.512</td>
</tr>
</tbody>
</table>

**Fig. 2.** The total amount of time (s) *A. aquaticus* acquired to visit all zones in the experimental area between treatment groups in terms of 4 types of isopods. M/D indicates dark male, M/L; light male, F/D; dark female and F/L; light female. Columns show mean ± SE. M/D: n = 14, M/L: n = 13, F/D: n = 14, F/L: n = 14.
4.2.3 Activity

Isopods exposed to fluoxetine changed zones fewer times than control treated individuals (Table 5). However, activity responses differed between dark and light isopods. Furthermore, there was an interaction between sex and phenotype which the result being that light males had higher scores than other males (Table 5 and Fig. 3).

**Table 5.** GLM results on factors and interactions affecting the total number *A. aquaticus* changed zones in family environment, p < 0.05 is bolded (n = 111).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoxetine</td>
<td>1</td>
<td>4.14</td>
<td><strong>0.044</strong></td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>2.96</td>
<td>0.088</td>
</tr>
<tr>
<td>Phenotype</td>
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<td>7.97</td>
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<td>Fluoxetine*Sex</td>
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<tr>
<td>Fluoxetine*Phenotype</td>
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<tr>
<td>Sex*Phenotype</td>
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<td>4.56</td>
<td><strong>0.035</strong></td>
</tr>
<tr>
<td>Fluoxetine<em>Sex</em>Phenotype</td>
<td>1</td>
<td>0.04</td>
<td>0.841</td>
</tr>
</tbody>
</table>
Fig. 3. The total number *A. aquaticus* changed zones a) between treatment groups b) sex and phenotype interaction c) between treatment groups in terms of 4 types of isopods. M/D indicates dark male, M/L; light male, F/D; dark female and F/L; light female. Columns show mean ± SE. a) Control: n = 56, Fluoxetine: n = 55 b) M/D: n = 28, M/L: n = 27, F/D: n = 28, F/L: n = 28 c) M/D: n = 14, M/L: n = 13, F/D: n = 14, F/L: n = 14.
4.2.4 Escape behaviours

I. Running
Fluoxetine exposure did not affect to running phase after simulated attack (Table 6). Furthermore, there were sex differences the total amount of time in running which the result being that females ran longer than males after the attack (Table 6 and Fig. 4).

Table 6. GLM results on factors and interactions affecting the total amount of time *A. aquaticus* ran after a simulated predator attack in family environment, , p < 0.05 is bolded (n = 111).

<table>
<thead>
<tr>
<th></th>
<th>df</th>
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<th>p</th>
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<tbody>
<tr>
<td>Fluoxetine</td>
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<tr>
<td>Sex</td>
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<td>Phenotype</td>
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<td>Fluoxetine*Sex</td>
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<td>Fluoxetine*Phenotype</td>
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<td>Sex*Phenotype</td>
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<td>0.18</td>
<td>0.675</td>
</tr>
<tr>
<td>Fluoxetine<em>Sex</em>Phenotype</td>
<td>1</td>
<td>0.01</td>
<td>0.921</td>
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</table>

Fig. 4. The total amount of time (s) *A. aquaticus* ran after a simulated predator attack a) between males and females b) between treatment groups in terms of 4 types of isopods. M/D indicates dark male, M/L; light male, F/D; dark female and F/L; light female. Columns show mean ± SE. a) Male: n = 55, Female: n = 56 b) M/D: n = 14, M/L: n = 13, F/D: n = 14, F/L: n = 14.
**II. Freezing**

Fluoxetine exposure had no effect on the duration of the isopods’ freezing behaviour after the running event. However, there was a tendency isopods exposed to fluoxetine spent less time freezing (Table 7). Dark isopods remained in a freeze longer than light individuals (Table 7 and Fig. 5).

**Table 7.** GLM results on factors and interactions affecting the total amount of time *A. aquaticus* froze after the running event, *p < 0.05* is bolded (*n* = 111).

<table>
<thead>
<tr>
<th>Factor</th>
<th>df</th>
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<th><em>p</em></th>
</tr>
</thead>
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</tr>
<tr>
<td>Fluoxetine<em>Sex</em>Phenotype</td>
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<td>1.56</td>
<td>0.215</td>
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</tbody>
</table>

**Fig. 5.** The total amount of time (s) *A. aquaticus* froze after the running event a) between dark and light isopods b) between treatment groups in terms of 4 types of isopods. M/D indicates dark male, M/L: light male, F/D: dark female and F/L: light female. Columns show mean ± SE.  a) Dark: *n* = 56, Light: *n* = 55  b) M/D: *n*= 14, M/L: *n* = 13, F/D: *n* = 14, F/L: *n* = 14.
4.3 Correlation between behavioural responses

Responses between individuals across the assays were related in control treatment (Table 8). Boldness was positively correlated with exploration and negatively correlated with activity, and therefore exploration was negatively associated with activity, activity was correlated negatively with freezing from escape behaviour under the predator risk. That is, individuals that left the refuge sooner were more explorers in novel environment and less active in a familiar environment, and vice versa. Slow explorer visited fewer zones. In addition, individuals that froze longer under predator risk also visited fewer zones.

Fluoxetine exposed individuals were similarly related across the assays as control group (Table 8). Boldness was positively related with exploration and negatively with activity. Exploration was negatively correlated with activity, i.e. such as control treated individuals.

Table 8. Spearman correlations between behavioural assays within each of the treatments (control: n = 56; fluoxetine: n = 55). Statistically significant correlation is bolded where p-values were < 0.05 after Bonferroni correction. Values for control treatment are above the diagonal, values for fluoxetine treatment are below the diagonal.

<table>
<thead>
<tr>
<th></th>
<th>Boldness</th>
<th>Exploration</th>
<th>Activity</th>
<th>Running</th>
<th>Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boldness</td>
<td></td>
<td><strong>0.49</strong></td>
<td><strong>-0.48</strong></td>
<td>0.09</td>
<td>0.25</td>
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<tr>
<td>Exploration</td>
<td><strong>0.40</strong></td>
<td></td>
<td><strong>-0.49</strong></td>
<td>0.26</td>
<td>0.28</td>
</tr>
<tr>
<td>Activity</td>
<td><strong>-0.43</strong></td>
<td><strong>-0.60</strong></td>
<td></td>
<td>-0.10</td>
<td><strong>-0.42</strong></td>
</tr>
<tr>
<td>Running</td>
<td>0.06</td>
<td>0.22</td>
<td>-0.31</td>
<td>-</td>
<td>0.07</td>
</tr>
<tr>
<td>Freezing</td>
<td>0.14</td>
<td>0.14</td>
<td><strong>-0.14</strong></td>
<td>-0.08</td>
<td>-</td>
</tr>
</tbody>
</table>
5. Discussion

The current study demonstrated weakly that short-term and environmental relevant concentration of fluoxetine affected activity traits in the freshwater isopods *A. aquaticus*. Fluoxetine treated individuals were less active than individuals in control solution. Furthermore, there was a tendency that fluoxetine exposed isopods spent shorter time in a freezing position. Besides, the total duration to leave the refuge differed between the sexes; males were faster to leave the refuge than females. There was an interaction between sex and phenotype in activity, light males changed zones more than the others. Regarding the escape behaviour, females remained a running longer than males after the attack. There were phenotype differences in freezing, dark isopods froze longer than light isopods.

In accordance to expectations based on a review of effects of SSRI on crustaceans (Fong & Ford 2014), fluoxetine exposure reduced activity. Altogether the fluoxetine effect was scarcely observed upon personality traits in this experiment. *A. aquaticus* is well introduced in ecological research because of its ecological significance for aquatic ecosystems as decomposer and as in benthic-pelagic food chain (Wallace & Webster 1996; Hargeby et al. 2004; Harris et al. 2011; Augusiak & Van den Brink 2016; Ginneken et al. 2017; Plahuta et al. 2017). Nevertheless, there are few behaviour focused researches about *A. aquaticus* (Eroukhmanoff & Svensson 2009; Harris et al. 2011). Besides, there are remarkably few investigations into antidepressant impacts on *A. aquaticus* (Plahuta et al. 2017; Ford & Fong 2014). Thus it is difficult to compare my results with other investigations on how fluoxetine may alter behavioural responses.

Ecotoxicological studies show that, the isopod is not sensitive toward several environmental pollutions such as acidification, insecticide and metal pollution (Augusiak & Van den Brink 2016; Ginneken et al. 2017; Plahuta et al. 2017). In other words, if the freshwater isopods have contamination tolerance it might be expected that they are not easily affected by pharmaceutical compounds in wastewater i.e. fluoxetine. There is also a discussion about which concentration of fluoxetine that should be used to estimate behavioural alteration. Regrettably, I did not find any research on freshwater isopod short term exposure to fluoxetine. However, some studies have observed the impacts of lower concentrations of fluoxetine on the behaviour of invertebrates. The freshwater amphipod *Gammarus pulex* (*Crustacea*) is phylogenetically close to the isopod, the research on *G. pulex* shows low activity and alteration of feeding behaviour at 100 ng L\(^{-1}\) in a 14 days exposure. Other studies on amphipod *Echinogammus marinus* display significant behaviour alteration in 10-100 ng L\(^{-1}\), whereas there was no impact on behaviour in higher concentration 1-10 µg L\(^{-1}\) (Guler & Ford 2010; Bossus et al. 2014; De Castro-Catalá et al. 2017). These instances from the amphipod studies could explain why the concentration of fluoxetine used in the current study had effects on activity traits in the freshwater isopod.
Except for the fluoxetine impacts, there were a few sex and phenotype differences in behavioural responses. Previous studies of escape behaviour show that dark isopods from reed habitat escaped faster but used a shorter period of time escaping from simulated predator attack than light isopods from *Chara* habitat (Eroukhmanoff & Svensson 2009; Harris *et al.* 2011). However, the results of escape behaviour in the current study do not match the pattern of the previous studies. There were no phenotype differences in the running phase, and dark isopods remained in a freeze longer than light individuals. Furthermore, Harris *et al.* (2011) also tested latency to emerge from the shelter; females took longer time before emerging from shelter than males but there were no differences between phenotypes from the two habitats. The result of Harris *et al.* 2011 corresponds with the one in this study, namely that there were sex differences in latency to leave the refuge. Nevertheless the refuge in this study was constructed differently from the shelter that Harris *et al.* (2011) used. It should also be considered that the isopods in this study were not caught in different habitats, but all came from the same place. Unfortunately there is a lack of studies about activity and exploration traits in the isopod. It has been difficult to compare if sex differences and phenotype differences act differently upon activity and exploration. However, previous studies do report differentiated escape behaviour responses between dark and light individuals caused by differentiated predator pressure between reed and *Chara* habitats (Eroukhmanoff & Svensson 2009; Harris *et al.* 2011). In other words, perhaps the dark and light isopods in this study should not be regarded as the equivalent of the phenotypes of the previous studies. One might also suspect that the background colour of the experimental area could have influenced the result of the current study, i.e. this perhaps caused the dark isopods to act in freezing behaviour as if more scared than the light isopods. Even if there are no available observations of colour specific habitat choices between different pigmentation of *A. aquaticus* (Johansson 2005), the light coloured background could in fact act differently upon the stress levels of the two phenotypes. The current experiment was performed in a white colour background, although the same as in the previous studies, still the results of phenotype differences in the current study do not match the pattern of the previous studies.

Regarding the personality traits in this study, consistency in behavioural responses were shown over time within individuals, that stands for existence of personality in the isopods. In the current study, decline in activity might cause less foraging dispersal and lead to a decline in reproductive success. (Dzieweczynski *et al.* 2016). An interesting piece of research on *C. elegans*, observed that the worm waste energy trying to reproduce instead of foraging in scanty nutritional environment, when the serotonin level in brain is increased deliberately. The original evolutionary benefit of the neurotransmitter serotonin has been suggested to be that the animal “knows” if it finds itself in a benign or non-benign environment. (Dempsey *et al.* 2005). On the basis of the study on *C. elegans*, fluoxetine could cause the isopods to become desensitised to its environment e.g. a risky situation will deceivingly seem less risky. Anyhow, lesser activity in females seems to be beneficial. Females with eggs should be especially careful since they have little to gain from activity if it is associated with increased predation risk or ending up in a worse habitat.
In the current study there were correlations between such behavioural responses as boldness, exploration and activity within isopods. The individuals in both treatments that left the refuge sooner were more explorers in novel environment and less active in familiar environment. However, these correlations might not yet be considered as behaviour syndrome; there is lack of variety of assays that vary in different situations and/or contexts (e.g. low versus high risk situation).

Accordingly, the biological phenomenon that is called animal personality is quite complicated. It is necessary to pay attention to the great variation among animal personality researches. Gosling (2001) alerts behavioural researchers who investigate personalities that the same trait might be measured by different methods. And vice versa, the same method might measure different traits (e.g. boldness can be measured under the risk situation in novel area or while feeding (Bell 2007)). It is also difficult to design standard methods because of differences between organisms (Réale et al. 2007). I suggest recording the full movements of the experimental objects, that way one might gain more insight in abundant and precise data on animal behaviour and personality as the movement is a manifestation of behavioural responses to environmental and physiological conditions (Gurarie et al. 2009).

As summarized in this thesis, observations of the effects of antidepressant fluoxetine on A. aquaticus are still vague and therefore its ecological consequences remain unclear. A wide range of fluoxetine concentrations is observed in natural waters and we are therefore in need of more tests with different concentrations. However, reducing fluoxetine substrate leakage in aquatic environment is the best thing to consider. I would like to expect that the individuality of animals will be made clearer in the future by emphasizing the evolutionary perspective further.

5.1 Social and ethical considerations

The amount of antidepressant used has been rapidly increasing in recent years. Medicines can come into the environment from variable sources of emissions; pharmaceutical production, clinical, chemical laboratories, hospitals and especially sewage from household. Thus, understanding effects of SSRI on animal behaviour is important for ecosystem function and service. Raising awareness in society about the disposal of medicines will be required. According to Swedish legislation (Jordbruksverkets författningssamling (2015:38), 2 cap 5§), there is no ethical requirement for the use of invertebrate studies. Nevertheless, behavioural trials can still stress the animals. Therefore an ethically correct treatment of them is important.
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6. References


