



SCIENTIFIC FINAL REPORT

Regnr Östersjöstiftelsen: 13/15

Project manager: Håkan Olsén, prof.

Project title: **Effects of SSRI exposures early in life on juvenile and adult behavior in three-spine stickleback (*Gasterosteus aculeatus*) and possible effects in the Baltic Sea**

1. Purpose of the project

Several studies have shown that pharmaceuticals of various kind are not completely degraded in sewage treatment plants. The physiological active compounds are released into the environment. Knowledge about their effects on fish and other animals are lacking. Compounds that are used in treatment of depression and psychological problems in humans can also have effects in the brain and the nerve system of fish. The serotonergic (serotonin) system is a conserved system important in fish and in all other vertebrates. It is important in regulation of hormones and various behaviors connected to reproduction and stress. Selective serotonin re-uptake inhibitors (SSRI) are a group of antidepressant pharmaceuticals that exert their effects by blocking the re-uptake of serotonin in the presynaptic cleft and by that elevated levels of serotonin in the synaptic cleft. There are different re-uptake inhibitors used to treat depression in humans. Fluoxetine was the first SSRI detected in wild fish ("Fish on Prozac"). Fluoxetine and setraline are two SSRI that have side effects that is not connected to serotonin. The third SSRI, citalopram, that we decided to use in our project has more specific effects on the serotonergic system. It is also the most commonly used SSRI in Sweden and it is found in sewage treatment effluents. As citalopram affect parts of the brain it is a risk that it also disturbs fish behavior. Our initial studies with adult three-spine stickleback (*Gasterosteus aculeatus*) demonstrated that behaviors connected to stress, feeding and aggression were affected after 21 days exposure to citalopram. **The next step was to study the effects of exposure to citalopram early in life and if the effects are permanent. Are there different effects when sticklebacks are exposed early or as adults?**

2. The three most important results of the project and what conclusions can be drawn from them

a. In the initial study the exposure of sticklebacks started two days after fertilization and continued for 30 days. After the exposure the young fish were placed in clean water. Behavior tests were run after 100 days in clean water. In contrast to adults exposed to citalopram the juveniles exposed were more aggressive and they attacked and ate the food (mosquito larvae) more frequently compare to unexposed individuals (c.f. Kellner et al. 2015. *Aquatic Toxicology* 158, 165-170; Kellner et al. 2016. *Aquatic Toxicology* 173, 19-28; exposed adult fish eat less, are less anxious and more active). Further, they were less active and avoided a white area in the test yard. One fish at the time had in this test the opportunity to choose between a white and black bottom area (scototaxis behavior).



Anxious fish prefer to stay over the black area. **The experiment shows that effects of citalopram on the brain can continue for long time when three-spine sticklebacks are exposed early in life.** Corresponding results have been shown with rodents exposed to SSRI early in life. The effects are different when exposed as adult or young individuals. The study is published (Kellner, M., Porseryd, T., Porsch-Hällström, I., Borg, B., Roufidou, C. & Olsén, K. H. 2018. Developmental exposure to the SSRI citalopram causes persistent behavioral effects in the three-spine stickleback (*Gasterosteus aculeatus*). *Ecotoxicology* 27, 12 - 22).

b. The next study started during the autumn 2018. The exposures and behavior tests were run during September-December 2018. We wanted to answer the question if exposure to citalopram can affect bold (proactive) and more “anxious” and stress sensitive individuals (reactive) differently. Proactive individuals are also more aggressive than those that are reactive. Does citalopram affect personality traits? We also wanted to answer the question if citalopram affects males and females differently. Adult individuals (not in spawning conditions) were individually exposed to environmental relevant citalopram concentrations for 21 days. Feeding and aggressive behavior were studied before and after the exposure. As the fish were kept separated it was possible to get individual data before and after exposure. Is there a change in behavior? Scototaxis tests were run only after the exposure. The fish were sacrificed and sexed after the scototaxis test. According to our hypothesis citalopram should make reactive individuals bolder and by that increase feeding and become more aggressive. **The results showed that citalopram had effects on feeding and aggressive behavior. Feeding was suppressed only in the males, but not in females. Both males that had high frequency of eating and those with low frequency decreased eating. The frequency of aggressive acts decreased after citalopram exposure in both males and females. High aggressive and low aggressive individuals had the same relationship after exposure, high and low, respectively, but at lower levels, that is all fish were less aggressive. This indicates that both proactive and reactive fish changed their behavior after exposure.** To get information about the effects of citalopram on growth the exposure should be for longer time. It should also be interesting to study the effects competition between exposed and unexposed fish. The study is published in *Archives of Environmental Contamination and Toxicology* (Kellner & Olsén 2020. Divergent response to the SSRI citalopram in male and female three-spine stickleback (*Gasterosteus aculeatus*). <https://doi.org/10.1007/s00244-020-00776-1>).

c. The following study was run at the Swedish University of Agricultural Sciences (SLU) in Uppsala. Extensive construction work on MD1, where the aquarium room is located, forced us to move aquaria and other equipment to SLU. The study started in January 2019 and continued for five months. We exposed sticklebacks to a mixture of citalopram and tramadol. Tramadol is synthetic opioid and is used to relief pain. It is also smuggled into



Sweden and used as a drug. Tramadol, as citalopram, is present in sewage treatment effluents. A combination of tramadol and citalopram can be deadly in people as the compounds interact and can give very high serotonin levels in the brain. Citalopram and tramadol are frequently present in the same water and there is a risk that they interact also in fish and tramadol can decrease the safe citalopram concentrations, i.e. lower concentrations affect behavior. Unfortunately, the experiment did not work properly. There were problems with fish and water quality. We were, though great efforts, not able to finish the study. We could not rely on the results.

d. Martin Kellner was invited to participate in another study with zebrafish embryos and larvae at SLU. Exposure to tramadol, citalopram and a mixture of both started directly after hatching and continued for 144 hours. **Behavior registrations revealed that both tramadol and citalopram decreased the behavior activity of larvae, but when the compounds were mixed together higher concentrations of both were needed to get the same effect. The results indicate that tramadol and citalopram have antagonistic effects.** The study was published (Bachour et al. 2020. Behavioral effects of citalopram, tramadol, and binary mixture in zebrafish (*Danio rerio*) larvae. *Chemosphere* 238. <https://doi.org/10.1016/j.chemosphere.2019.124587>.)

e. We have during the project done a study with Prof. Eric Baatrup and his group members at Aarhus University, Denmark. Males and females of zebrafish were exposed during three weeks to escitalopram that is the S-form of citalopram and is the most potent of the two stereo-isomers, S- and R-form. Fish was directly after exposure placed in a test aquarium and their behavior was automatically registered during 30 min. Weight and length were measured after the behavior tests. Females exposed to escitalopram had lower weight and were shorter than control females. Exposed males were shorter in length compare to control males. These results suggest that feeding was reduced in exposed fish. The exposure also decreased the stress response in females as they were longer time in an open space compare to controls. Both sexes had reduced swimming activity. **The results show that citalopram affect zebrafish in the same as three-spine stickleback. Their general behavior and especially feeding are changed.** (Nielsen et al. 2018. The psychoactive drug escitalopram affects swimming behaviour and increases boldness in zebrafish (*Danio rerio*). *Ecotoxicology* 27, 485 - 497).

3. The project's contribution to the international research frontline

All results stated above and published are new. Nobody as far as we know has done this before. The studies are important as they are cited in scientific publications. Not much is known about the effects of pharmaceuticals in fish and other aquatic organisms,



4. New research questions that the project has led to

Low concentrations of citalopram, at the same levels as found in sewage treatment effluents, have effects on three-spine stickleback behaviors that affect their ability to survive. It is especially problematic that citalopram affect feeding. This can result in lower growth and lower fitness or no reproduction at all. Our results with zebrafish show that the effects are not only in sticklebacks. The results from exposures of stickleback larvae show that the effects of citalopram are present long time after exposure. This can indicate permanent effects on the brain. As shown in rodents, the effects in young individuals are also different compare to adults. The effects on the brain is probably different during early development. It would be valuable to study the effects of citalopram for even longer time. For how long time will the effects on the brain and behavior continue? It should also be interesting to know the effects on growth and fitness when adults are exposed to citalopram and if exposed fish can compete with fish not exposed to citalopram.

5. The contribution of the research to the knowledge of the Baltic Sea Region and Eastern Europe

Pharmaceuticals are physiological active compounds and released into natural waters can give serious problems when affecting fish and other aquatic organisms. It is especially problematic when the compounds have effects on the brain and the rest of nervous system. Knowledge about their effects are lacking. The results from the present study show that the general behavior of three-spine stickleback and probably other Baltic Sea fish species can be affected. It is important that the methods to degrade pharmaceuticals and other compounds are improved in sewage treatment plants. The degradation is not effective enough.

6. Dissemination of the results of the project within and outside the research community

Original publications

Kellner, M., Porseryd, T., Porsch-Hällström, I., Borg, B., Roufidou, C. & **Olsén, K. H.** 2018. Developmental exposure to the SSRI citalopram causes persistent behavioural effects in the three-spine stickleback (*Gasterosteus aculeatus*). *Ecotoxicology* 27, 12 – 22. <https://doi.org/10.1007/s10646-017-1866-4> Open access

(*Ecotoxicology*, impact factor: 2.535)



Nielsen, S.V., **Kellner, M.**, Henriksen, P.G., **Olsén, H.**, Hansen, S.H. & Baatrup, E. 2018. The psychoactive drug escitalopram affects swimming behaviour and increases boldness in zebrafish (*Danio rerio*). *Ecotoxicology* 27, 485-497.

<https://www.researchgate.net/publication/323766663> The psychoactive drug Escitalopram affects swimming behaviour and increases boldness in zebrafish *Danio rerio* Open access.

Bachou, R.-L., Golovko, O., **Kellner, M.** & Pohl, J. 2020. Behavioral effects of citalopram, tramadol, and binary mixture in zebrafish (*Danio rerio*) larvae. *Chemosphere* 238. <https://doi.org/10.1016/j.chemosphere.2019.124587>. Open access.

(*Chemosphere*, impact factor: 5.778)

Kellner, M. & **Olsén, K.H.** 2020. Divergent response to the SSRI citalopram in male and female three-spine stickleback (*Gasterosteus aculeatus*). *Archives of Environmental Contamination and Toxicology*. 79, 478-487. <https://doi.org/10.1007/s00244-020-00776-1>. Open access.

(*Archives of Environmental Contamination and Toxicology*, impact factor: 2.400)

Håkan Olsén participated during short time in another project where we studied the possible effects of the anxiolytic pharmaceutical oxazepam on Atlantic salmon (*Salmo salar*) spawning and behavior. The drug decrease anxiety in humans and has been shown to affect the behavior in perch (*Perca fluviatilis*). Perch became less anguished and their appetite increased (Brodin, T. et al. 2013. *Science*). We were, however, not able to observe any behavior changes in salmon in connection to spawning.

Hellström, G., Brodin, T., Jonsson, M., **Olsén, H.** et al. 2019. Environmentally relevant concentrations of oxazepam do not affect spawning behavior in mature male Atlantic salmon (*Salmo salar*) parr. *Journal of Applied Ichthyology*. <https://doi.org/10.1111/jai.13980>. Open access.

Communication outside the research community

Article published in a Swedish newspaper, Upsala Nya Tidning, February 7, 2021.

Håkan Olsén, *Läkemedel i miljön ett underskattat problem.*