The Effect of Synthetic Estrogen on Foraging and Predator Avoidance in the Common Roach (*Rutilus rutilus*)

Gustaf Boström

2012

Miljövetenskap

Examensarbete för kandidatexamen 15 hp

Lunds universitet

The Effect of Synthetic Estrogen on Foraging and Predator Avoidance in the Common Roach (*Rutilus rutilus*)

http://www.loddean-fiske.se/fiskarter/m%C3%B6rt

Gustaf Boström

2012-05-23

Supervisor:
Per Hallgren
Aquatic Ecology
Lund University

Abstract

Synthetic estrogens that originate mainly from oral contraceptive pills reach our waters through sewage effluent and potentially affect organisms in the natural environment. This study focused on the effects of the synthetic estrogen 17-α-ethinylestradiol (EE2) on the foraging and predator avoidance behaviors of the planktivorous fish roach (*Rutilus rutilus*), a common fish in Swedish lakes and rivers. To assess the effects of EE2, roach were exposed in aquariums to environmentally relevant water concentrations of EE2 at 0, 0.5, 5 and 50 ng/l for 3 weeks. They were then taken out for foraging trials where their foraging on the plankton Daphnia magna was quantified. Foraging trials were made both with and without predator cues from pike (*Esox lucius*) to assess predator avoidance behavior in the roach. Results showed that foraging of roach on *D. magna* significantly increased at the highest concentration of EE2, compared to control, and this may be due to increased metabolic rate from the synthetic estrogen, or a higher energy demand due to induced detoxification mechanisms in the roach. No significant effect of the EE2 on predator avoidance behavior could however be discerned. Increased foraging of planktivorous fish on zooplankton could potentially have an effect on ecosystem balance in lakes and rivers.

Introduction

There is a widespread and growing concern about the ecological effects from pharmaceuticals and personal care products that spread into the natural environment through sewage systems. Due to the fact that pharmaceuticals are designed to have a physiological effect they have a great potential to affect non-target organisms as they end up in aquatic ecosystems (Naturvårdsverket 2008). Besides intended effects of the pharmaceutical there is the issue of toxicity of the compounds even when organisms lack the receptors for the specific pharmaceutical (Walker et al 2006). To study these effects is interesting and essential to assessing the ecological impacts of pharmaceuticals in the environment. An environmentally troublesome group of compounds are endocrine disrupting chemicals (EDC) that mimic gonadal steroid hormones and thereby have various effects on reproduction, behaviour and physiologic functions (Walker et al 2006).

This study focuses on the effects of the specific EDC synthetic estrogen, 17-α-ethinylestradiol (EE2), on the foraging and predator avoidance behaviour in the fish common roach (*Rutilus rutilus*). EE2 is the active compound in most oral contraceptive pills and post-menopausal hormone treatments. Estrogens have of course existed in nature long before mankind but the concern regards the increasing emission of man-made synthetic estrogens, of which EE2 plays a big role, and their potential adverse effects (Jobling et al 2006). Because of this it is of interest to study adverse effects of EE2 at environmentally relevant concentrations to see which effects it possibly already exhibits in nature. Naturvårdsverket (2008) (swedish EPA) surveyed concentrations of EE2 in sewage effluents and found it to be 3 ng/l and the corresponding figure for drinking water in Sweden to be 0.4 ng/l. Purdom et al (1994) found EE2 at concentrations as low as 0.1-0.5 ng/l to have effects in inducing the egg yolk precursor protein vitellogenin in male trout *(Salmo trutta)*. EE2 has, in many studies, been proven to have effects on the endocrine system such as reduced gamete production and reproduction of fish (Jobling et al 2002, Bell 2001) but behavioural effects not associated with reproduction are not studied to the same extent. Current knowledge is unclear whether EE2 increases or decreases the foraging and growth rate of fish and differing results indicate that this is species specific. Leal et al (2008) showed that foraging and growth rate of the Sea Bass (*Dicentrarchus labrax)* decreased when they were exposed to 17-β-estradiol (E2). However, Bell (2003) found the opposite result that foraging and growth rate of the threespined stickleback (*Gasterosteus aculeatus*) increased when it was exposed to EE2.

Another less studied aspect of synthetic estrogens is the effects they have on predator avoidance. Bell (2003) found that exposure to EE2 increased “risky behaviour” when foraging of the threespined stickleback and argued that this might be either due to a generally increased activity induced by estrogenic compounds, and therefore a higher risk of encounter with predators, or it might be due to a higher demand for food because of the higher growth rate induced by ethinyl estradiol.

This study examined how the foraging behaviour of roach on the planktonic crustacean *Daphnia magna* is affected by exposure to 0, 0.5, 5 and 50 ng/l of EE2. The effect of different concentrations of EE2 on predator avoidance was also examined by performing the foraging experiments both with and without predator cues for each fish.

Material and Methods

120 Juvenile roach of between 45-50 mm were caught through netting in Krankesjön, a shallow, eutrophic lake (Brodersen et al 2008) situated east of Lund in the south of Sweden at the coordinates (55°42´N, 13°28´E) (Hargeby 2003). No sex differentiation was made between the fish as they have no obvious outer sexual traits. The roach were put in tanks of 50 litres filled with regular tapwater from Lund municipality and they were left to acclimatize for 7 days. During this time the exposure aquariums were set up and roach were then put in to be exposed to different concentrations of EE2 for 21 days.

Setup of exposure aquariums

2-litre plastic containers were filled with 2 litres of water each with concentrations of EE2 at 0 (solvent control), 0.5, 5 and 50 ng/l, with 6 replicates for a total of 24 aquariums. 2 roach (4.4-4.9 cm) were then put in each aquarium and oxygenation was set up through gently bubbling air through the water. The roach were fed with Brine Shrimp eggs (*Artemia salina)* and *D. magna* twice each day. Water and EE2 was changed twice each week on Mondays and Thursdays. EE2 is readily degradable with a half-life of approximately 33 ± 13 hours (Ministry of Environment Province of British Columbia 2009) which means that concentrations need to be maintained through changing the water and adding new EE2 continuously.

The fish exhibited signs of fungus or parasite infection which was seen as white dots on scales and/or red markings on fins. The pathogen was likely the highly fatal ectoparasite *Ichthyophthirius multifiliis* because white spots, which are a common symptom of this infection (Scholz 1999), could be seen on the fish. This is one of the most common diseases for fish kept in aquariums (Encyclopædia Britannica 2011). The fish were possibly also infected with fungi or bacteria as

secondary infection is common (Scholz 1999) and fin rot could be seen on some fish as red parts at the base of fins. The affected fish were taken out of the aquarias but mortality was quite high so more fish were put in continuously to get sufficient data from the foraging trials. All fish were kept in the exposure aquarias for 21 days before foraging trials were carried out.

Pilot experiment

To determine the abundance of *D. magnia* to be used in the foraging experiments 4 pre-experiments were done before the foraging experiments began. The arenas for these were prepared by setting up four white plastic buckets with a diameter of 23 cm and a height of 25 cm. These were then filled with water to a depth of approximately 10 cm and 1 unexposed roach of similar length as the exposed roach was put in each arena and let to acclimatize for 4 hours.

After the 4 hours *D. magna* were added to the arenas to determine the foraging rate of the roaches. 10-20-30-40 *D. magna* respectively were added to the 4 arenas. Two experiments were done where the roach were left to forage for 5 minutes and two experiments for 10 minutes. After 5/10 minutes had passed, the fish were taken out of the aquariums and the remaining *D.magna* were counted to determine how the feeding rate varied with *D. magna* abundance. It was decided that 30 *D. magna* were to be used in the foraging experiments as the roach ate the most at that density. The roach ate quite little even in 10 minutes, maybe because of the parasite infection. Because of this it was decided that the time would be extended to 20 minutes for the foraging experiments.

Foraging experiment

After 22 days of exposure to EE2 the foraging experiments were started and for these the same 4 arenas as for the pre-experiment were used with the addition of a refuge in the form of a fake plant (fig 2). On one side of the foraging arenas a hole covered with a net was made and outside of this hole a smaller bucket was attached with the net being the divider. In the smaller bucket a pike was put for the experiments of foraging under predator threat as the roach get both visual and olfactory cues of their natural predator pike *(Esox lucius)* through the net.

One day before each day of experiments one fish was taken out from the exposure aquariums with a hand net, weighed (Precisa junior 3100 CD Floating Range) and then put into one of the four arenas to acclimatize to the next morning.

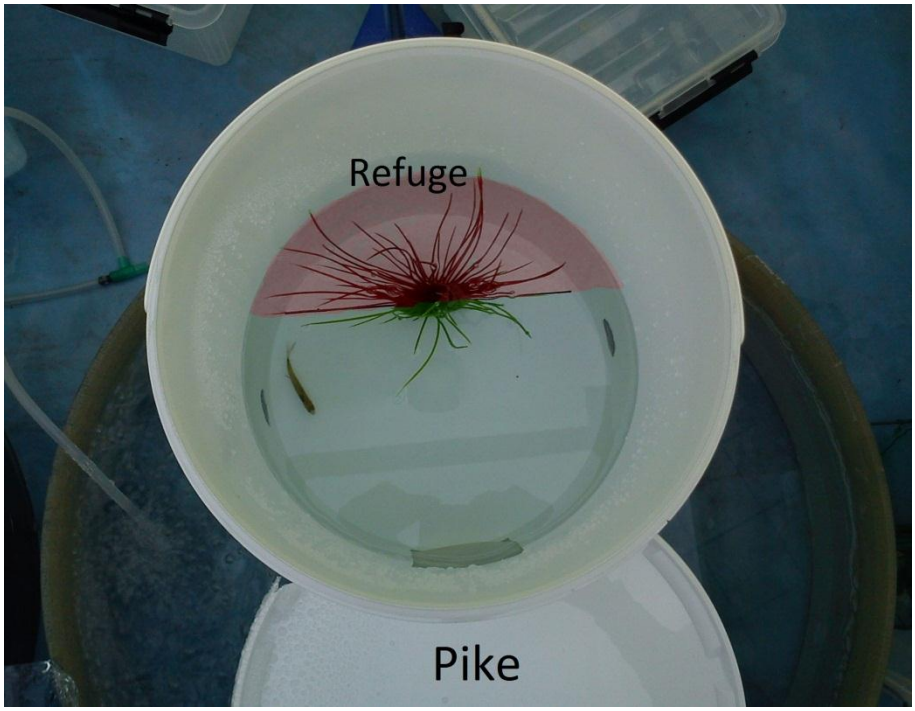

Figure 1. ***Arenas for foraging experiment. The area marked with red in the figure was considered to be refuge in the predator avoidance experiment.***

For each fish one foraging experiment was done in the presence and one in the absence of olfactory and visual cues from a Pike. One experiment was done at 09.00 and one at 16.30 with every fish. The order that the fish from the different treatments were put into the arenas and whether they were exposed to pike cues in the morning or afternoon was randomized. To assess foraging 30 *D. magna* were put in each arena and the roach were left to forage for 20 minutes. After this time the fish were taken out of the arenas with a net and the number of remaining *D. magna* was counted and percentage consumed *D. magna* was calculated.

For analysis of behaviour in the absence or presence of a predator all arenas were filmed during the 20 minute foraging experiment with a “Kodak playsport Zx3” digital video camera. The films were analysed through measuring the time that the roach spent in refuge versus open water through ocular inspection in the

program VLC media player. The fish were considered to be in refuge when any part of the fish was in the red area (fig. 2) and the judgment was done with a resolution of 1 second. Time spent in the refuge was calculated as percentage of the 20 minutes total foraging.

Data compilation and statistical analysis

The mean foraging rate and time spent in refuge was calculated for each replicate and treatment. The effects of EE2 exposure and predator presence were analysed with MANOVA using EE2 and predator as fixed factors and percent foraging and percent time spent in refuge as dependent variables. Percentages were transformed by arc-sin transformation to fit the assumptions for ANOVA. Since there was no effect from the predator treatment data were merged for one test, to see the overall trend for the effect of EE2 treatment. The mean for all roaches' weight was 1.51 ± 0.22 g and there was no significant difference in weight between EE2 treatments ($p=0.442$) which tells us that any observed foraging difference is not due to size differences. All analysis was done with IBM SPSS Statistics 20.

Results

EE2 treatment without predator had a significant positive impact on the roaches' foraging (MANOVA $F=4.052$, $p=0.022$) whereas predator presence had no significant impact (MANOVA $F=0.997$, $P=0.331$). Roach exposed to 50 ng/l EE2 with no predator present foraged significantly more than roach from the control treatment (fig 3) ($p=0.050$, Dunnet post hoc). If the means of each treatment are plotted against the concentration of EE2 an interesting pattern shows where effects on foraging with and without predator have diverging trends indicating a trade-off between foraging and avoiding the predator (fig. 3).

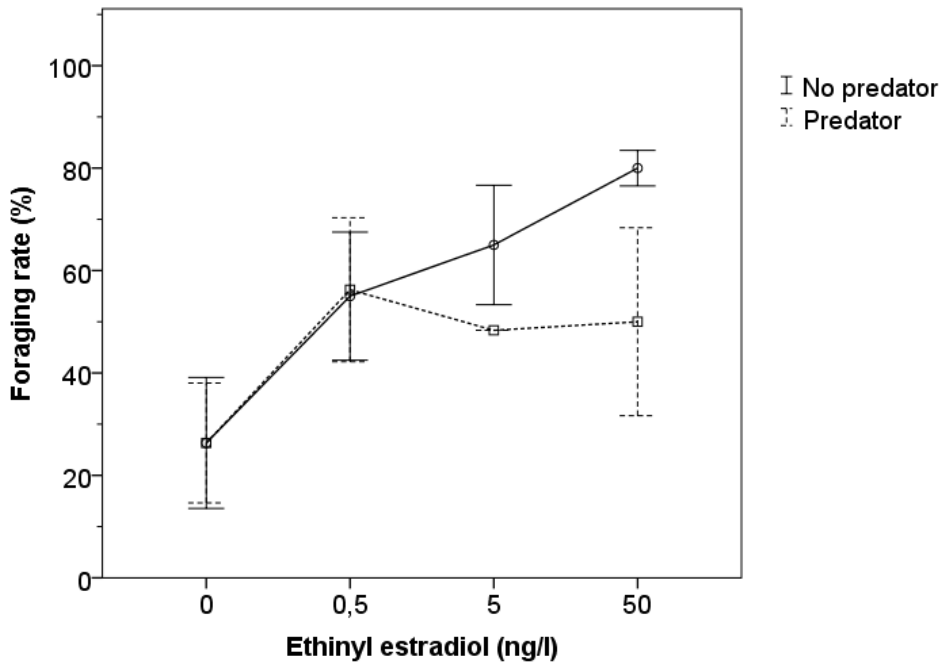

Figure 3. Roach foraging rate as percent (mean±SE) consumed *D. magna* with predator (dashed) and without predator (line) at concentrations of 0, 0.5, 5, 50 ng/l of ethinyl estradiol (EE2).

When foraging trials with and without a predator present were merged a significant increase could still be seen from concentrations 0 and 50 ng/l (p=0.019) with a clear and near-significant positive trend for the intermediate concentrations (fig 4).

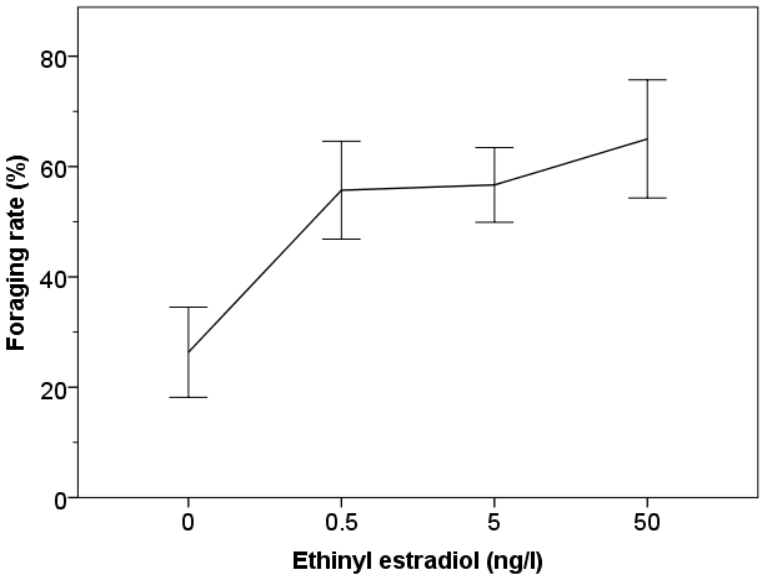

Fig 4. Foraging rates (means ± SE) of merged data from trials both with and without predator present, at concentrations of 0, 0.5, 5, 50 ng/l of ethinyl estradiol (EE2) show a trend of increasing foraging in higher concentrations of EE2 .

There was a significant effect of EE2 and predator on the time spent in refuge (MANOVA F=2.772, p=0.070) (Fig. 5). The post-hoc revealed that roach in the

treatment of 0.5 ng/l was spending more time outside the refuge (p=0.003, Dunnet post hoc) but there is no discernible overall trend as the foraging goes back up at 5 and 50 ng/l.

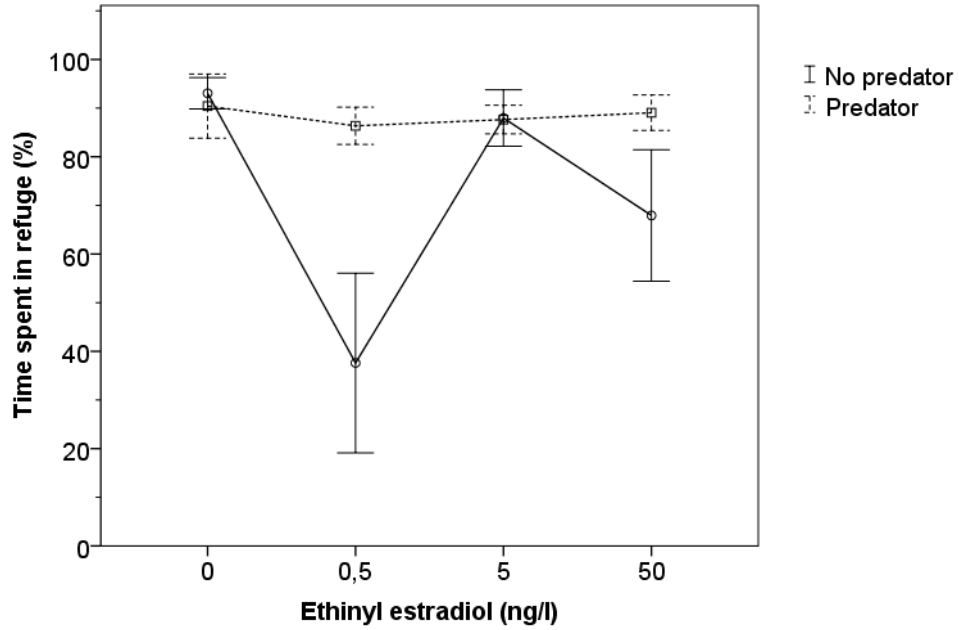

Figure 5. Time spent in refuge (means ± SE) as a percentage of the 20 minute trials with predator (dashed) and without predator (line) at concentrations of 0, 0.5, 5, 50 ng/l of ethinyl estradiol (EE2).

Discussion and conclusion

In this study we quantified the effect of EE2 and predation on the foraging and risk taking behaviour of roach. We found that EE2 did affect the foraging behaviour but that there was no interactive effect of EE2 and predation. Foraging without a predator present increased with increased concentrations of EE2. This increase in feeding behaviour might be due to an increased energy demand due to detoxification mechanisms in the roach which is seen by many species exposed to xenobiotics (Walker et al 2006). A study on primates by Edelman et al (2010) showed that oral contraceptive pills (where EE2 is the active substance) increased the metabolic rate in the exposed individuals. If the study by Edelman et al (2010) is applicable this would be another possible explanation to why foraging increased, as metabolic rate is correlated with energy demand. One common side-effect of oral contraceptives, mainly with high doses of EE2, in humans is also increased appetite and weight gain as EE2 effects insulin levels and metabolism of lipids and carbohydrates (Godsland et al 1990).

No earlier published studies could be found on EE2 affecting foraging for the roach, although effects have been seen on other species such as the sea bass (Leal et al 2008) and the threespined stickleback (Bell 2003). However, the previous studies by Leal et al (2008) on sea bass as well as studies by Per Hallgren (unpublished) on roach have both shown a decrease in foraging at higher concentrations of EE2. The inconsistency in results might be accredited to species difference in the case of the study by Leal et al (2008). In the case of Hallgren´s study on roach there was a difference in method as the roach, in Hallgren's earlier study, was reared from egg to one year old in the estrogen concentrations whereas the fish in this experiment were caught in a lake at approximately one year of age and then exposed for EE2 for three weeks. Fish that were reared under EE2 exposure might have had negatively affected growth and development due to toxic effects at an early age and therefore impaired foraging behaviour.

Another plausible explanation is that roach exposed to higher concentrations of EE2 throughout their ontogeny had a slower growth rate than controls since they have a higher energy demand for detoxification but have the same access to food due to the controlled environment of the experimental setup.

This would lead to exposed roach being smaller at the time of foraging trials which might lead to a difference in foraging behavior at higher concentrations of EE2, for example through lower energy demand or difficulty handling bigger prey.

With the more acute exposure exhibited in the present experiment, the effects would rather be due to activation of detoxification mechanisms and short-term effects on chemical receptors in the roach and not due to effects on ontogeny, long term differences in development or reformed coping mechanisms.

In this study it seems like foraging increases in a higher concentration of EE2. However, when exposed to a predator there is a trend of lower increase of feeding rate at higher concentrations of EE2, possibly due to a trade-off between increased feeding behaviour and predator avoidance. Even though there is no significant difference between treatments it is interesting to speculate on what the difference in trend is due to, for foraging with predator and without predator. For the trials with a predator present there seems to be a trade-off between foraging

more and avoiding the predator which makes the increasing trend level off after an initial increase. Foraging increases due to increased EE2 exposure but as it comes to a certain point no further increase is benefiting under the threat of a predator as foraging is coupled with the risk of being eaten oneself while searching for or handling prey. If the increased foraging rate is due to an increased energy demand for detoxification and the roach under predation threat are not able to acquire this extra energy this could lead to a decreased growth rate.

Predator avoidance, i.e. time spent in refuge in absence versus presence of pike, showed no obvious trends and variance was high, contrary to Martin et al (2010) who found that juvenile roach respond strongly to pike and clearly choose refuge if exposed to both visual and olfactory cues from pike. A big difference can be seen between controls and 0.5 ng/l where fish exposed to 0.5 ng/l spend significantly less time behind refuge. However this effect is not observed at all in higher concentrations of EE2. It is likely that the low mean time spent in refuge seen in 0.5 ng/l treatments is due to high individual variation between fishes coupled with few data due to mortality. The large variance may be due to *Ichthyophthirius multifiliis* infection which symptoms include loss of appetite and lowered activity (Francis-Floyd and Reed 2002) as well as abnormal hiding behavior (Encyclopædia Britannica 2011), which of course all potentially could affect the outcome of both the foraging and the predator avoidance experiment. Some fish used in the experiments had signs of parasite infection such as white dots on the body or slightly reddish fins. This fact, paired with few data due to the high mortality in the aquariums could perhaps affect the outcome of this study.

In future experiments sex identification and subsequently separating effects on males and females might be interesting. As EE2 is a sex hormone that males and females have different quantities of receptors for and natural concentrations of, I hypothesize that there could very well be a difference in behavioural effects on males and females. With this difference put aside the results of foraging and predator avoidance trials might be more easily made evident. On the other hand, with an experimental setup like in the present study we get a net effect on the population that is more alike to what would happen in nature. Also, experiments should aim to eradicate pathogens such as parasites and fungus to keep the

roach in good condition for the experiments. This could possibly be done with the use of pesticides such as formalin, malachite green (Scholz 1999, Francis-Floyd and Reed 2002), salt treatment, heat treatment (Francis-Floyd and Reed 2002), Nifurpirinol or a combination. Although this would bring in other toxins and thus another factor to the experiment, the effect would be the same for all treatments and therefore work from an experimental design point of view. To avoid using pesticides but still keep the roach in better condition, semi-natural mesocosms including other species that naturally cohabitate with roach could be used for the exposure aquariums. With a more diverse environment for the roach they would probably fare better, as all present pathogens would have to compete and be preyed upon by other species, and thus kept in check. This would of course also make it harder to tie the effects that possibly could be seen on foraging or predator avoidance directly to exposure to EE2 as there are several other factors coming into play with more species and a more complex experimental environment. On the other hand these prerequisites would be more similar to what occurs in the natural environment and therefore might be more relevant from an environmental science point of view.

A higher foraging rate of roach on the *D. magna* could have implications on the natural environment regarding trophic cascades in lakes or other water bodies. If roach forage more on the zooplankton community the zooplankton will decrease in abundance and as zooplankton such as Daphnia forage on phytoplankton these will be released from grazing and thus grow in abundance. This could possibly contribute to the algal blooms that already commonly occur. A multi-species experiment such as that would be interesting as studying the effects of EE2 on organisms and interactions in the food webs are important to assess what effects our effluents have. A ban of EE2 is neither likely nor desirable but a more effective and specialized purification of estrogens in sewage treatment works might have to be implemented to avoid a negative impact on nearby waters.

To conclude, roach exposed to environmentally relevant levels of EE2 seem to increase their foraging rate on *D. magna* which could possibly have implications for growth rate of the roach and potentially have indirect effects on the plankton communities in lakes and therefore ecosystem balance.

References

Leal E, Sánchez E, Muriach B, Cerdá-Reverter JM (2008) Sex steroid-induced inhibition of food intake in sea bass (*Dicentrarchus labrax*). *J Comp Physiol B* 179:77-86.

Bell AM (2001). Effects of an endocrine disrupter on courtship and aggressive behaviour of male three-spined stickleback,Gasterosteus aculeatus. *Animal Behaviour. 62:*775-780.

Bell AM (2004). An endocrine disrupter increases growth and risky behavior in threespined stickleback (*Gasterosteus aculeatus*). *Hormones and Behaviour* 45:108-114.

Bowen JM (2011). ich. *Encyclopædia Britannica*. http://www.britannica.com/EBchecked/topic/1301664/ich Gathered 2011-05-23

Edelman A, Jensen J.T, Bulechowsky M, Cameron J.(2010) Combined oral contraceptives and body weight: do oral contraceptives cause weight gain? A primate model. *Human Reproduction*.

Francis-Floyd R and Reed P (2002) Ichthyophthirius Multifiliis (White Spot) Infections in Fish http://www.twra.biz/FishDiseasesParasites_TWRA/files/WhiteSpotDisease.pdf Gathered 2011-05-21

Godsland I. F., Crook D, Simpson R, Proudler T, Felton C, Lees B, Anyaoku V, Devenport M, Wynn V (1990). The Effects of Different Formulations of Oral Contraceptive Agents on Lipid and Carbohydrate Metabolism. *New England Journal of Medicine* 323:1375-1381.

Hargeby A (2003) Lake Krankesjön, Limnology dep. Lund University http://www.limnol.lu.se/kranke/page/background.asp Gathered 2011-04-20

Jobling S, Williams R, Johnson A, Taylor A, Gross-Sorokin M, Nolan M, Tyler C. R, van Earle R. Santos E, Brighty G (2006) Predicted Exposures to Steroid Estrogens in U.K. Rivers Correlate with Widespread Sexual Disruption in Wild Fish Population. *Environmental Health Perspectives.* 114:32-39.

Jobling S, Beresford N, Nolan M, Rodgers-Gray T, Brighty GC, Sumpter JP, Tyler CR (2002). Altered Sexual Maturation and Gamete Production in Wild Roach (Rutilus rutilus) Living in Rivers That Receive Treated Sewage Effluents. *Biology of reproduction.* 66:272-281.

Ministry of Environment Province of British Bolumbia (2009) Water Quality Guidelines for Pharmaceutically-active-Compounds (PhACs): 17α-ethinylestradiol (EE2) Technical Appendix. http://www.env.gov.bc.ca/wat/wq/BCguidelines/PhACs-EE2/PhACs-EE2-tech.pdf Gathered: 2011-05-21.

Naturvårdsverket (2008) Avloppsreningsverkens förmåga att ta hand om läkemedelsrester och andra farliga ämnen, rapport 5794 (Bilaga 1). Gathered: 13 April 2011 http://www.naturvardsverket.se/Documents/publikationer/620-5794-7.pdf

Scholz T (1999). Parasites in cultured and feral fish. *Vet Parasitol*. 84(3-4):317-35.

Purdom CE, Hardiman PA, Bye VVJ, Eno NCC, Tyler CR, Sumpter JP (1994) Estrogenic Effects of Effluents from Sewage Treatment Works**.** *Chemistry and Ecology***.** 8:275-285

Walker CH, Hopkin SP, Sibly RM, Peakall DB (2006). Principles of Ecotoxicology. Boca Raton, Florida, US: CRC Press. p1-315.

LUNDS UNIVERSITET

Miljövetenskaplig utbildning

Centrum för klimat- och miljöforskning

Ekologihuset

22362 Lund