



Review of USB FFPW PhD thesis

First name(s), surname, titles of the PhD student: Marie Eugenie Sancho Santos, M.Sc.	First name(s), surname, titles of supervisor: Prof. Dipl.-Ing. Tomáš Randák, Ph.D.
Title of PhD thesis: Psychoactive compounds in aquatic environment and their effects on fish	

REVIEWER:

Surname: Brodin	Institution: Swedish University of Agricultural Sciences, Sweden
Name: Tomas	
Titles: Prof.	E-mail: tomas.brodin@slu.se
Please describe your professional relationship to the PhD student: No relationship	Please describe your field of expertise: Aquatic ecology, ecological effects of pharmaceuticals in the environment

QUESTIONNAIRE

Originality, scientific importance, perspectives and impacts of results presented in the PhD thesis for basic and/or applied research

Evaluate competitiveness of the PhD thesis in the international context and compare its level with the current state of the art in the field (**extent ¼ – ½ page**):

The thesis "Psychoactive compounds in aquatic environment and their effects on fish" by Marie Eugenie Sancho Santos, M.Sc. focus on a very timely and understudied topic. It contains 3 published research-papers and 1 commentary paper – all in very good journals. This thesis adds new and important information regarding the potential for ecological effects of pharmaceuticals/drugs in the environment and represents a very comprehensive and novel set of studies containing a number of highly interesting results and conclusions. State-of-the-art methods are used throughout.

This thesis presents a strong body of evidence for potential effects of behaviourally modifying pharmaceuticals/drugs on fish behaviour and physiology. These results are presented in a clear and concise way and are original, some even novel, and of high scientific importance. All in all, the research presented here is well in level with the current state of the art in the field and has even pushed the boundaries forward in some aspects.



Elaboration of the PhD thesis, objectives of the work and deliverables

Evaluate the overall level of elaboration of the PhD thesis (structuring of the main text, comprehensibility, logicity of the chapters and their ordering) and the originality of the selected approaches to solve the objectives; evaluate publications and whether the results described correspond to objectives of the PhD thesis (extent ¼ – ½ page):

The thesis reads well, and follows a logical flow with a clear structure that makes it easy to read. All chapters are in a logical and intuitive order and both the introduction and the methods sections are well composed. The introduction provides the reader with a nice and comprehensive overview of the field. All experiments and samplings were conducted using appropriate methods and the analyses were made with appropriate and highly advanced analytical protocols. The thesis follows the guidelines and the included publications are all published in the Q1 of the journals in the field.

References are relevant, extensive and cover the addressed field, and the abstract provides a clear and to-the-point version of the results and methodology.

All publications provide new and important knowledge regarding the potential effect of behaviourally modifying pharmaceuticals/drugs in the environment by using a combination of state-of-the-art analytical techniques and behavioural experiments. The thesis also contains novel ideas, e.g. studying potential withdrawal in fish, which strengthen the impact of the findings.

OVERALL COMMENTARY ON THE PhD THESIS

Please write in the box specific comments concerning the PhD thesis in extent of 1-2 pages:

Three publications are included in this thesis;

Environmental concentration of methamphetamine induces pathological changes in brown trout (*Salmo trutta fario*).

2 citations, Impact factor 7.09 (20210713). Marie Eugenie Sancho Santos was responsible for 60% of this paper. The aim of this study was to expose brown trout (*Salmo trutta fario*) to environmental (1 µg L⁻¹) and higher (50 µg L⁻¹) concentrations of methamphetamine for 35 days with a four-day depuration phase to assess the possible negative effects on fish health. Degenerative liver and heart alterations, similar to those described in mammals, were observed at both concentrations. The parent compound and a metabolite (amphetamine) were detected in fish tissues in both concentration groups, in the order of kidney > liver > brain > muscle > plasma. Bioconcentration factors ranged from 0.13 to 80. A therapeutic plasma concentration was reached for both compounds in the high-concentration treatment.



Methamphetamine pollution elicits addiction in wild fish.

0 citations, Impact factor 3.01 (2020). Marie Eugenie Sancho Santos was responsible for 5% of this paper. In this publication the authors show that methamphetamine, considered an important global health threat, causes addiction and behavior alteration of brown trout *Salmo trutta* at environmentally relevant concentrations (1 $\mu\text{g/L}$). Altered movement behavior and preference for methamphetamine during withdrawal were linked to drug residues in fish brain tissues and accompanied by brain metabolome changes. The paper is very well written and present much needed information about how methamphetamine creates addiction, behavioural changes, and even withdrawal in fish, I predict that this publication will be highly cited.

Traces of tramadol in water impact behaviour in a native European fish.

1 citation, Impact factor 6.29 (20210713). Marie Eugenie Sancho Santos was responsible for 40% of this paper. This publication investigate how European chubs (*Squalius cephalus*) are affected by exposure to 1 $\mu\text{g/L}$ of tramadol in water for 42 days with a subsequent 14 days of depuration. The results showed that chubs exposed to this analgesic underwent changes in their behaviour compared to the control group. The behavioural outcome was also influenced by the concentration of tramadol in individual brain tissue. More specifically, experimental fish displayed anxiolytic-like effects, characterized by less bold and less social behaviour. After the depuration phase, the social alteration remained whereas the boldness effect disappeared. Additionally, the amplitude of behavioural changes was correlated with the concentration of the substance in brain.

Comment on “Diluted concentrations of methamphetamine in surface water induce behavior disorder, transgenerational toxicity, and ecosystem-level consequences of fish” by Wang et al.

This is a comment and not a regular research paper. The publication form in itself can be very important and useful to clarify or rectify other authors research.

0 citations, Impact factor 11.24 (20210713). Marie Eugenie Sancho Santos was responsible for 80% of this paper. In this publication the authors points out several mistakes and inaccuracies in the study by Wang et al. reporting ecological effects of methamphetamine on fish.

Some specific comments;

The language in the second half of this section (Introduction 1.1) is awkward and I suggest changes below:

Emerging substances have certain characteristics that raise suspicions about their possible harm to the environment: persistence, bioaccumulation and toxicity (Ebele et al., 2017). Although the majority of the pharmaceuticals and drugs of abuse do not have physicochemical characteristics to be considered as persistent, they are continuously released into the environment. Therefore, they behave as if they were as such due to a “pseudo-persistence”



phenomenon (Ebele et al., 2017; Rosi-Marshall et al., 2015), resulting in the exposure of aquatic fauna to a mixture of substances during their entire life (Fent et al., 2006). In the same line, the uptake from the water of these substances cause their bioconcentration in tissues, where they can produce effects due to the similarity in receptors with the target organisms (Cervený et al., 2021; Grabicova et al., 2014). In some cases, their bioaccumulation via food web has been also reported (Grabicova et al., 2017; Lagesson et al., 2016). Because the presence and concentration of these compounds in water is variable, the toxic effects can also differ due to the dose-time exposure, the species, and/or the developmental stage. Furthermore, the mixture of components in the environment produces additive, synergistic, and/or antagonistic actions between each singular substance (Ebele et al., 2017).

1. Change " In the same line, the uptake from the water of these substances cause their bioconcentration in tissues, where they can produce effects due to the similarity in receptors with the target organisms (Cervený et al., 2021; Grabicova et al., 2014).“ To: “Also, the uptake of these substances from water leads to bioconcentration in tissues of exposed organisms, which in turn can lead to additive effects of substances acting on the same (or similar) drug targets (Cervený et al., 2021; Grabicova et al., 2014).“
2. Change „In some cases, their bioaccumulation via food web has been also reported (Grabicova et al., 2017; Lagesson et al., 2016).“ To: “In some cases, their bioaccumulation through a food web has also been reported (Grabicova et al., 2017; Lagesson et al., 2016).“

Introduction 1.2:

1. Change: „For instance, a 13.2% of adult Americans took some antidepressant medication in the last 30 days during the period 2015-2018 (Brody and Gu, 2020).“
 - This sentence makes no sense. As it reads now it states that they only looked at the 30 last days during that period (2015-2018) and that can not be correct. Re-write it.
2. Change: „The use of antidepressants in OECD countries is 200% higher from 2000 to 2017 (OECD, 2019a).“ To: „The use of antidepressants in OECD countries has increased with 200% between 2000 and 2017 (OECD, 2019a).“




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FINAL RECOMMENDATION

- PhD thesis can be recommended for defence
- PhD thesis can be recommended for defence with reservations
- PhD thesis cannot be recommended for defence

2021-07-13 Umeå, Sweden
Date and place

Tomas Brodin 
Name and signature