Comment on "Principles of Sound Ecotoxicology

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The manuscript by Harris et al (2014)[1] details 12 principles of sound ecotoxicology which on the face of it one finds hard to disagree with. However, they singled out for criticism a manuscript we authored in their 12th 'principle', which in their interpretation, we hyped or exaggerated our data as we concentrated on the significant result of the 2nd trial and ignoring the insignificant result of the 1st trial[2]. I believe this to be a misrepresentation of our manuscript and for reasons too lengthy for this correspondence, believe it is potentially damaging for important organisations to incorrectly single out the integrity of science/scientists without correction. Nevertheless, it was puzzling to find this years after publication.

In our paper entitled 'Antidepressants make amphipods see the light'[2], we exposed small intertidal amphipods (*Echinogammarus marinus*) at nominal concentrations of fluoxetine hydrochloride (0.01, 0.1, 1 and 10µg/L) compared with a control. Our underlying rational was based on the fact that serotonin is known to induce phototaxis responses in some crustaceans[3] and that parasites are known to alter serotonin pathways and subsequently behaviour in ways that increase the likelihood of their hosts being eaten[4,5]. Therefore, our hypothesis was fluoxetine (Prozac) would induce phototaxis in these amphipods in a similar way to serotonin and/or serotonin altering parasites. Following 1, 2 and 3 weeks exposure we recorded phototaxis whereby a score (0 or 1) was given the position of the amphipods every 30 seconds in either a light-dark chamber and vertical geotaxis using a measuring cylinder following an established methodology in the field of parasitology[6].

We conducted two trials whereby the first trial indicated some interesting and consistent results between weeks which we felt warranted repeating the experiment but doubling our replicates from 10 to 20 per treatment and doubling the recording time from 5 to 10 minutes. I believe Harris et al[1] misinterpreted our results (Fig 1) because not only was the 2nd trial not an identical repeat of the 1st, we did in fact get some statistically significant results in both trials for geotaxis (trial 1 week 2 & trial 2 week 1).

From Guler and Ford (2010)[2]:

> *"A positive phototaxic response was induced after 3 weeks exposure to fluoxetine in Trial 1 although no significant differences were observed between individual exposure concentrations taking into account Bonferroni corrections (p > 0.0125). Trial 2 of this experiment (using greater number of replicates) found all phototaxis scores greater in the fluoxetine-exposed groups compared to the control. A significant difference in phototaxic score between exposure concentrations after both 1 week (p = 0.026) and 2 week (p = 0.024), just failing to meet statistical significance in week 3 (p = 0.052). For weeks 1–3, pair-wise comparisons only found significant differences (Bonferroni corrected) between control and 0.1 µg/L groups (p < 0.0125) and indicated a non-monotonic concentration response."*

In addition, accepting one should not be over-reliant on p values alone, in week 1 (trial 2) the p values for control vs 0.001 and control vs 10 were p = 0.010 and p = 0.009, respectively; and in 2nd week of exposure (trial 2) the control vs 1 were p = 0.018 (failing the Bonferroni adjustment p < 0.0125).

From Guler and Ford (2010)[2]

> *"All geotaxis scores in fluoxetine-exposed groups were greater than those of the control. The pattern of geotaxis score was consistent between trials and weeks with the greatest score observed in the 0.1 µg/L group again indicating a potential non-monotonic concentration response. Significant differences however were only observed between groups in Trial 1 (Kruskal–Wallis; week 2;* p *= 0.046) and Trial 2 (week 1;* p *= 0.033)."*

In week 2 (trial 1) the p value for control vs 1 and control vs 10 was 0.014 and 0.065 (failing our Bonferroni adjustment). In week 2 (trial 2) the p values for control vs 0.1, control vs 1 and control vs 10 were 0.002, 0.035 and 0.011.

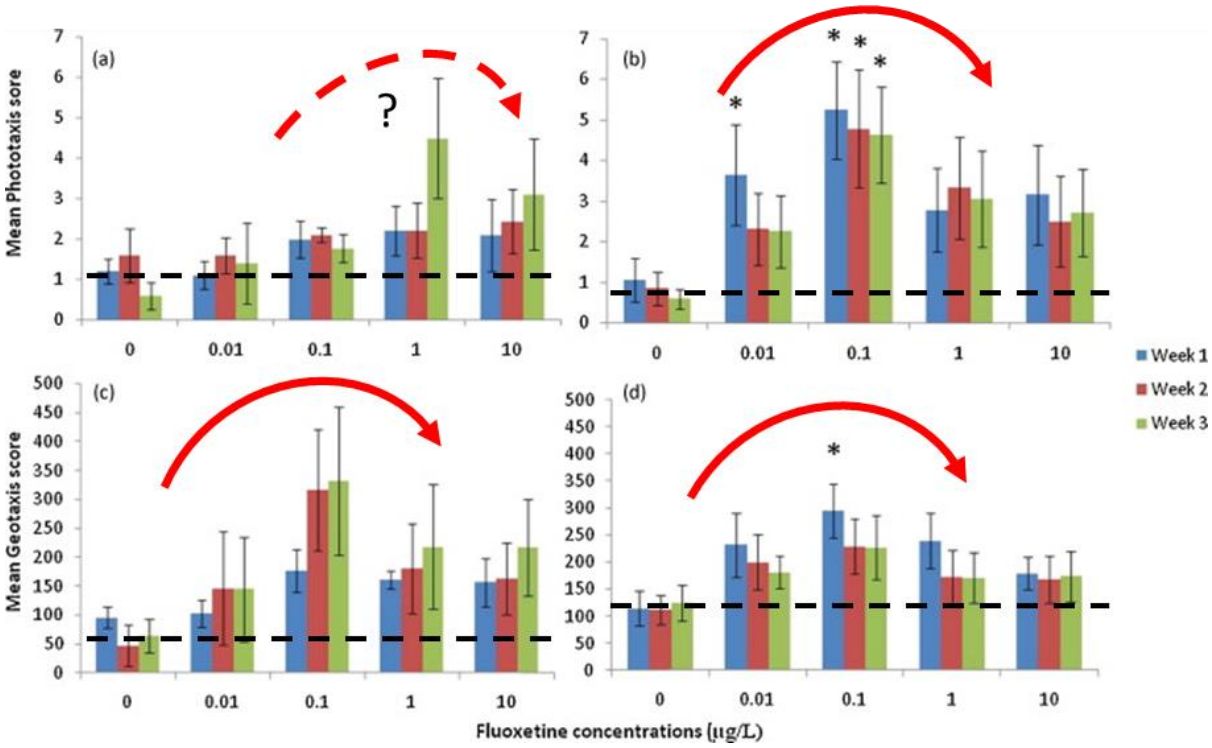

Figure 1: Mean phototaxis and geotaxis score of *E. marinus* exposed to varied concentrations of fluoxetine over a 3-week period. (a) Trial 1 phototaxis behavioural assay, (b) Trial 2 phototaxis behavioural assay, (c) Trial 1 geotaxis behavioural assay, (d) Trial 2 geotaxis behavioural assay. Error bars to one standard deviation. *Significance compared with control determined by Mann–Whitney and Bonferroni correction $p < 0.0125$. (modified to include mean control baseline (dashed black lines) and non-monotonic concentration curves (red lines) and reproduced with permission, Guler and Ford, 2010[2])

Where some have been criticized for omitting 'all trials' we felt it important to include our preliminary trial (1) as supporting evidence for the second trial as we were surprised by the similar non-monotonic concentration curves not only between weeks 1-3, but also between trials 1 and 2 (see Fig 1). Despite the scepticism in these low dose effects[7,8] our results appear to be consistent with those conducted on crustaceans many years prior to our experiment[9,10] and those repeated by ourselves[11], and others afterwards[12]. Excepting some limitations in experimental designs there are an increasing number of

studies highlighting effects of antidepressants at environmentally relevant concentrations in aquatic organisms[13,14]. Whether these effects of antidepressants will, or can be extrapolated to the field will no doubt be challenging[15].

Our paper is not without mistakes or limitations by any means, after publication we realised we incorrectly identified our parasite as an acanthocephalan (known to alter behaviour through serotonin modulation[6, 16]) when in fact it turned out to be an undescribed species of trematode parasite (also known to alter behaviour via perturbations in serotonin[17]; published corrections[11,18]). In revisions of the manuscript we also omitted that we doubled our recording from 5 minutes to 10 minutes between trials 1 and 2.

I also think it is worth highlighting that whilst Harris et al (2014)[6] raises the issue of 'indictment of the peer-review process' we are extremely grateful to the reviewer's anonymous comments used to improve this manuscript. I regret not thanking them in our acknowledgements. The original submitted version of our manuscript had two versions of the statistics, one using a one tailed non-parametric test and the second a two tailed (Kruskal-Wallis) followed by Bonferroni adjusted Mann-Whitney ($p < 0.0125$). We initially thought of using both tests as our phototaxis scores could only go in positive direction (0+). Quite rightly one of the reviewers suggested presenting one set of statistics so we went with the more conservative ones. One very valuable comment from a reviewer was the addition of the word 'conceivably' to our concluding remarks of our abstract "*This study has highlighted the potential for highly prescribed anti-depressant drugs to change the behaviour of an ecologically relevant marine species in ways which could conceivably lead to population level effects*".

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