Review: Biopsy-based normalisation of gill fluke-infected European catfish (*Silurus glanis* L. 1758) stocks for laboratory-based experiments (#87749)

General comments

This is an interesting article which outlines the use of a gill biopsy for determining monogenean parasite load for subsequent allocation of infected fish in experimental applications. Once catfish are challenged through co-habitation, the authors then take a snip of the gill tissue using scissors and then count the parasites in the tissue. That count is then used to estimate the total parasite load of the fish (based on correlation). Based on the estimate, fish with equivalent 'total' parasite loads were assigned to experimental populations. This present a useful indicator of parasite infection intensity, and with suitable validation, has the potential to be applied to other gill parasite-host systems.

The paper is currently unsuitable for publication and requires a major revision. The key problem with the article is that not enough information is given in the methodology to understand what was done. It is evident once the reader reaches the Stastical analyses (ln 199-200) and looks at the Results section (ln 236) and Figures, that a lot more work and analyses were done that are not described in the Methods. Moreover, evidence of these data are not presented in the supplementary data, and thus it was confusing to interpret what the authors actually did.

For example, under ln 138 **Examination and characterization of parasites** it is not clear that the authors removed all parasites from all gill arches to standardise the biopsy and demonstrate that it is a true representation of total parasite count (from the same fish). However, Figures 4B and 5 present total parasite counts (from all gill arches). The estimation of total parasite load must be validated for this method to be able to be used for experiments routinely. The authors needed to have conducted total parasite counts on the fish to ensure that the estimate was a true proxy for total parasite load (i.e., predicted and actual infection intensity). The fact that this was done (see Results ln 236-253) needs to be carefully and clearly described in the Methods section.

The authors have unfortunately missed two important references for this paper that use monogenean parasite intensity estimates to real counts. These papers, and others on monogenean spatial distribution should be incorporated into the Introduction and Discussion.

- Forwood et al. 2013 Validation of a rapid counting method for assessing treatment efficacy against *Lepidotrema* bidyana infecting silver perch *Bidyanus bidyanus* Diseases of Aquatic Organisms 105:253-257.
- Forwood et al. 2012 Host impact of monogenean *Lepidotrema bidyana* infection and intensity estimates for onsite monitoring Diseases of Aquatic Organisms 100:51-57

When preparing the revised version that authors should pay attention to the introduction which contains sections that could be reduced or omitted to focus on the key question that the paper is asking. For example, Ln 91-96 could be removed, whereas 97-112 establish the context for the work.

Consider the use of the term "gill monogeneans" as opposed to the colloquial name of 'fluke' which typically refers to trematodes. If you choose to keep the word fluke, then it needs to be identified as a common name typically used in the industry.

The authors make a bold claim in the Abstract that the results can be adapted to other cultured fish species. However, parasites can exhibit microhabitat specificity and occupy specific regions of the gills and in different intensities. For example, Roubal et al. 1983 (see Figure 163-165) showed parasite distribution on different gill arches and different gill parasites in snapper – it is plausible that a replicated biopsy may miss some parasite species entirely. Thus, while this can be suggested as a method for other systems, it must be stressed that it must be validated for each model and a clear understanding of the spatial distribution of parasites across microhabitats should be ascertained.

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The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved:

ln 35 'balanced distribution' – balanced isn't the appropriate word and could be removed

Ln 47-48 We believe that our results can be adapted to other cultured fish species and their gill flukes, to fine-tune sensitive experimental designs **obtain cleaner data** – is there a word missing?

I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.

Specific comments and suggested corrections by section

Abstract

Ln 32 – what is the correct species authority? Siwak or Sivak? Please check as listed as Sivak in WoRMS. Please edit sentence to : '…monogenean gill parasite, *Thaparoleidus vistulensis* **(**Sivak, 1932) Lim, 1996

Ln 38 Was the biopsy test subsequently validated?

Ln 47 This is a bold statement – do you mean similar gill monogeneans? Perhaps of the same genera? What evidence is there that the biopsy model would apply to other gill monogeneans that are perhaps not as small in size?

Introduction

Ln 66-90 There is a lot of text on monogeneans, host-specificity and life cycles that could be edited/written more concisely. The key message to emphasize for this study is their site specificity, and that their distribution on a host (i.e., preferred sites of attachment) may not be uniform. Given their small size and that they can occur in high abundance (making it very time consuming to count them) this experiment sought to identify a method that would provide accurate estimates on parasite infection intensity for in vivo treatment/selection experiments.

Ln 73 Orders and Families are never italicised, only genera and species

Ln 75 *Thaparocleidus* (should be in italics throughout the document)

Ln 75 the way this sentence is written suggests that there are only three describe species in the genus, however, there are 160 recognised children – see WoRMS - World Register of Marine Species - Thaparocleidus Jain, 1952.

Rewrite: "Within the family, three species of *Thaparocleidus*, namely…x, x, and x can cause specific infections on European catfish"

Ln 77 Please check the correct taxonomic citation for the species WoRMS - World Register of Marine Species - Thaparocleidus vistulensis (Sivak, 1932) Lim, 1996

Ln 79 species are **considered** extremely host-specific

Ln 93-96 The sentence is not clear to me – be specific about which molecular mechanisms cannot be explored from field samples – as many can!

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Ln 100 be specific with respect to in vitro methods for monogeneans here, are there are established methods for other fish parasites e.g. protozoa

Ln 106 do you mean experiments on selecting for disease resistance require a similar parasitic load? "For our experiments on selection for disease resistance, we require a consistent challenge whereby several groups of European catfish are infected with a similar parasitic load'.

116 Include an animal ethics statement

123 physical debris removal – do you mean fish waste or other debris as well? Presumably scum could contain parasite eggs?

169 Was the floating cage also removed when the donor fish were removed?

172 Was the biopsy weighed to ensure equivalent/standard amount of gill was examined for fish of different sizes?

Figure: In the Figure the section taken for the biopsy is shown as an oval– is this accurate? This would be hard to achieve with scissors.

Results

Ln 219 But differences in burden are not unexpected given that individuals were replaced with healthy ones during the experiment?

Discussion

Ln 272 Removal of heavily infected individuals and replacement is less desirable from an animal ethics perspective – are there potential alternatives with respect to maintaining moderate infection levels that are less pathogenic to host fish?

Ln 278 specify that this statement applies to the model parasite species

Ln 290-293 Delete, this does not make sense – risk assessments are not applied to assess infection levels. A more appropriate comparison here are the methods developed by Forwood.

Ln 294 Please write in paragraphs.

Ln 324-328 Compare to Roubal et al. 1983 and Sharp et al. 2003 New Zealand Journal of Marine and FW Research 37:273-282 who have reported uneven distribution of monogeneans across the four gill arches.