

Dear reviewer

I write you regarding the manuscript -animals-1286622- entitled: Twenty years of research in seabass and seabream welfare during the stunning/slaughtering procedure submitted to Animals.

First, we would like to say thank you for the useful and generous comments aimed at improving the paper. We have addressed all the comments point by point as explained below.

The responses to the concerns raised are below and are color coded as follows: a) Comments from reviewers are shown as text; b) Our responses are shown as text (reviewer 1).

The reviewers' comments were very helpful overall, and we are appreciative of such constructive feedback on our original submission. After addressing the issues raised, we feel the quality of the paper is much improved.

Sincerely
Pedro L. Castro

REVIEWER 1

This is a good topic for review, especially when focused more narrowly on sea bass and sea bream where the information can be brought together cohesively. There are some organizational issues that make it hard for the reader to follow the manuscript and fully grasp the impact of these practices on the welfare of the animals.

Title- the "/" is awkward and the title is therefore awkward. Consider re-phrasing "Twenty years of research in seabass and seabream welfare during slaughter" Or something similar.

We agree with the reviewer comments. The new title is "Twenty years of research in seabass and seabream welfare during slaughter". Line 2-3

Line 8- Simple Summary- The summary focuses on aspects of the pre-slaughter process that are only addressed briefly in the paper. The summary should better encompass all the aspects of the paper.

A new redaction including your suggestion has been added. Line 17-20

The abstract is disorganized and could do a better job describing how the manuscript first covers the ethical grounds for considering fish welfare at slaughter, and then covers preslaughter welfare and then each common method of stunning, then a conclusion.

The abstract has been rewritten.

Introduction

At some point, it would be warranted to explain how the post slaughter flesh quality parameters are related to the welfare of the fish. This may be in the introduction or elsewhere but it is currently not clear how meat quality parameters are related to welfare. If it is related more too whether these methods would be accepted by the industry, that should be explained.

Post slaughter flesh quality parameters relationship with welfare has been included in the section 3.3.

Line 35- The question, what happens to fish after slaughter seems to be out of place here since it is not tied back to welfare (fish are dead after slaughter=no welfare) and if it is meant to tie the post slaughter flesh quality back to welfare parameters pre-slaughter, that is not clear.

The reviewer comment is accurate. The question has been removed.

Line 54 to 59- A more nuanced discussion of whether fish have the anatomic structures capable of pain perception is warranted. The authors seem to argue that fish are not conscious of pain, which then calls the whole manuscript into question as it removes the moral grounds for concern about humane slaughter. A more detailed discussion of fish neuro-anatomy and their early evolutionary split from mammals (as discussed in Lund, 2007) would be warranted here. Line 55 is particularly confusing and should be re-worded. A conclusion that fish, despite their different anatomy from mammals, still are likely conscious of painful stimuli, should be supported in order to move on to the application of the pre-cautionary principle and the grounds for the rest of the manuscript.

A new redaction has been introduced Line 49-57.

Line 63, "regardless of the foregoing" is confusing and unclear what it is referencing.

The sentence has been rewrite.

Line 79- "thoughtfulness..." this is a long title for a section which describes the impact on welfare of various pre-slaughter handling and stunning techniques. Consider rephrasing.

A new title has been included. Line 83.

Line 80- This should be where humane slaughter definition and parameters are introduced. Consider using the AVMA humane slaughter guidelines (or some other that introduces such parameters) and applying those criteria to fish. This citation is related more to the ethical goals whereas the authors are moving on in the manuscript to looking at the application of scientific parameters and their relationship to fish welfare.

We have added the AVMA definition and the parameters involved from the AVMA guidelines for the humane slaughter of animals. Line 84-87.

Line 86-88 is confusing and not a complete sentence.

The paragraph has been rewrite. Line 92-95.

Line 88-90 states that inducing unconsciousness can't cause suffering if it takes a long time. That is not a true statement. Maybe the authors intend to say that it "should not" be painful if it takes a prolonged period in order to be considered humane slaughter.

The reviewer comment is accurate. The paragraph has been rewrite. Line 95-97.

Line 96- Consider starting a new section on pre-slaughter welfare or feed withdrawal and handling.

The new section has been added. Line 105.

Line 100-112- Instead of talking about what methods should be used it would be more informational to the reader to hear about what methods have been used to examine pre-slaughter handling and stress in the species discussed in this paper and how that relates to their welfare and best practices that should be applied to these aspects of the slaughter process.

The results about pre-slaughter handling in these species are included in different sections according to indicators or parameters studied, taking into account effects due to stocking density and crowding time.

Line 113- According to welfare- This is an odd phrase. Consider changing. Also, consider only discussing pre-slaughter welfare here and discussing stunning and killing techniques in the next section.

Remove "According to welfare" has been deleted. We agree with reviewer and divide the text in two sections.

Line 113-134 Consider starting a section on Stunning methods and their impact on welfare. Discussing the basis for whether a method is humane seems repetitive with lines 80-90. Some stunning methods, such as ice slurry are going to limit the behavioral repertoire of the fish based on the fact that they cause paralysis. This renders behavior less useful as an assessment tool for such methods. This should be stated more clearly.

Changes has been introduced:

- The new section has been added. Line 124.

- "Furthermore, a slaughter method could be considered humane if it induces insensibility immediately or, if insensibility is induced gradually, the induction phase should be free of fear or pain [20, 22]" has been removed.

- We agree that fish introduced in ice slurry can move around for a short period. In this situation, behavioural indicators are not enough to evaluate the stunning method. Hence, further information has been added. Line 140-141.

Line 117-119- Confusing. Consider tying the statement to a method instead of discussing "fast" vs "slow" methods.

Further information has been added to clarify the methods commented. Line 126-129.

Line 119 to 121 is lacking a citation and it is still unclear how this relates to welfare. Consider tying this statement to the methods under discussion.

Further information has been added and a new statement was introduced. Line 129-132.

Line 135- Consider changing the title of this section to cover welfare impact of various stunning methods. "convenience" is how easy something is for the industry and it is unclear how this relates to welfare.

The authors agree with the reviewer comments. A new title has been introduced Line 147-148.

Line 134- "slow methods" is imprecise. Explain which methods are being discussed.

The slow term has been removed.

Line 138-148 Consider leaving discussion of anesthetic agents until later and using this paragraph to focus on the impact of temperature on the effectiveness of the stunning process.

The line 141 has been removed.

Line 149-159- Stunning with ice "does not appear to be particularly stressful" is a strong statement. If this is based on behavioral observations then this should be stated, otherwise it should be more clear how we know it is not stressful. It is also unclear how the fact that it is reversible means that it is not stressful. Many other reviews would say that we do not have good evidence to say whether it is stressful or not.

The sentence has been modified. Line 162-165.

Line 160-162- How is this assessment method proposed by Kestin related to the rest of the paragraph since the only metric mentioned is the time to unconsciousness. Were these methods applied in these other studies? Consider a paragraph for sea bass and one for sea bream as it is confusing which sentences apply to each type of fish.

The authors agree with the mentioned remark. The mentioned lines have been removed. The methodology proposed by Kestin et al. is not completely applied in the cited paper. Most cases, only take into account to establish the loss of consciousness the reaction to the external stimuli.

Line 172-178 could be more clear whether addition of these various gases speeds up time to unconsciousness compared to ice alone.

The sentence has been modified. Line 181-182.

Line 179- Remove "as a result"

The mentioned changes have been made.

Line 182- What were the effects on the fish of the clove oil and how was it improved by nano-encapsulation?

-The effects of clove oil as anesthetic are extensively described in the review of Javahery et al. (2012). We did not consider necessary to include this information in the manuscript.

-Regarding the nanoencapsulation improvements, additional information has been added. Line 191-192.

Line 184-191- What was the impact on the fish of electric stunning? did the methods in these studies vary with some being more effective? How does the duration of unconsciousness relate to the time it takes to get to the killing method?

The impact of electrical stunning on fish is described in sections 3.2 and 3.3. In the works working with this technique, after electrical stunning, the fish were introduced into ice slurry dying regardless of the duration of the unconscious state. The variations in the methodology focus on the duration of the exposure, almost all from 1 to 10 seconds, except in one case that lasts up to 2 minutes. In the studies that detail voltage and frequency (in some of them the commercial name of the machine used is issued), they range from 24 to 40 V and from 50 to 133 Hz. Thus, with the higher voltage and frequency, only 1 second is required, corresponding to 2 at 24 V and 50 Hz. The authors do not considered it convenient to detail the characteristics of each methodology since the effects on the parameters studied were similar.

Section 3.2 and 3.3 - Consider whether having these in a separate section is better or whether they should be incorporated into a section on each method. In my opinion, having it separated into methods of assessment makes it harder to come to a conclusion about the welfare impact of each method as its impacts on the fish are separated into multiple areas.

Two approaches are possible: what results do, the different methods analyzed, offer on each of the parameters considered, or what the impact of each method on these parameters is. As the methods are fewer than the parameters, the first approach has been chosen.

Section 3.3- It is unclear how each of these metrics relates to fish welfare.

A new redaction has been added. Line 235-236.

Line 233 QIM needs to be defined.

QIM has been defined in Line 244.

Conclusion

Overall, could do a better job summarizing the best practices for pre-slaughter welfare in these fish, the best stunning methods in these fish, and the impact that would have ethically and in terms of consumer perception as well as on the industry in terms of uptake of these practices based on the impact on flesh quality.

The authors agree with the mentioned remark. The conclusion section has been modified.

The section (line 291-295) on preslaughter welfare should reflect the best practices the authors feel are supported by the information they have reviewed.

The paragraph has been rearranged. Line 301-304.

Line 296-298 Confusing. Relates electrical stunning to a metabolic stress indicator but it is a stunning method.

Rephrasing has been introduced. Line 307-308.