Jaydev Upponi <em@editorialmanager.com>

AttachmentsJan 4 (2 days ago)

to me

CC: alison.hamlin@jove.com

Dear Mr. Chalupnicki,

Your manuscript JoVE54316R1 "Alternative Method of Removing Otoliths from Sturgeon" has been peer-reviewed and the following comments need to be addressed. Please keep JoVE's formatting requirements and the editorial comments from previous revisions in mind as you revise the manuscript to address peer review comments. Please maintain these overall manuscript changes, e.g., if formatting or other changes were made, commercial language was removed, etc.

Please track the changes in your word processor (e.g., Microsoft Word) or change the text color to identify all of the manuscript edits. When you have revised your submission, please also upload a separate document listing all of changes that address each of the editorial and peer review comments individually with the revised manuscript. Please provide either (1) a description of how the comment was addressed within the manuscript or (2) a rebuttal describing why the comment was not addressed if you feel it was incorrect or out of the scope of this work for publication in JoVE.

Your revision is due by Jan 25, 2016. Please note that due to the high volume of JoVE submissions, failure to meet this deadline will result in publication delays.

To submit a revision, go to the JoVE Submission Site and log in as an author. You will find your submission under the heading 'Submission Needing Revision'.

Sincerely,

Jaydev Upponi, Ph.D.

Science Editor

JoVE

1 Alewife Center, Suite 200, Cambridge, MA 02140

tel: 617-674-1888

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Editorial comments:

NOTE: Please download this version of the Microsoft word document (File name: 54316\_R1\_111615) for any subsequent changes.

Please keep the editorial comments from your previous revisions in mind as you revise your manuscript to address peer review comments. For instance, if formatting or other changes were made, commercial language was removed, etc., please maintain these overall manuscript changes.

•We recommend adding an additional section showing one of the uses for this extraction, although this is not required. The entire protocol is currently ~0.6 pgs long.

•Additional detail is needed:

-2.6 - What is the cartilage "gently rinse"d in?

**Reworded sentence**

-2.6 - Ends abruptly. "Place otoliths in an open 25ml scintillation vial to dry for 24 hours for any further analysis that ."

**Fixed sentence**

•References appear to be listed in alphabetical order instead of the order they appear in the manuscript. References also do not have any DOI numbers provided.

**Corrected reference order**

•If your figures and tables are original and not published previously, please ignore this comment. For figures and tables that have been published before, please include phrases such as “Re-print with permission from (reference#)” or “Modified from..” etc. And please send a copy of the re-print permission for JoVE’s record keeping purposes.

**These are orginals**

•\* JoVE reference format requires that DOIs are included, when available, for all references listed in the article. This is helpful for readers to locate the included references and obtain more information. Please note that often DOIs are not listed with PubMed abstracts and as such, may not be properly included when citing directly from PubMed. In these cases, please manually include DOIs in reference information.

•NOTE: Please copyedit the entire manuscript for any grammatical errors you may find. This editing should be performed by a native English speaker (or professional copyediting services) and is essential for clarity of the protocol. Please thoroughly review the language and grammar of your article text prior to resubmission. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

•NOTE: Please include a line-by-line response letter to the editorial and reviewer comments along with the resubmission.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

The authors provide a detailed account of an alternative methodology for removing otoliths from sturgeon in the laboratory. The step-by-step process is detailed effectively in multiple figures. I believe the authors could strengthen the rationale for the work by highlighting the applicability of their in press work (otolith shape) to other sturgeons and mentioning some of the other potential uses for intact sturgeon otoliths. Overall, a good, concise, paper that should facilitate high reproducibility of the technique.

Major Concerns:

1. I believe the authors should attempt to strengthen their case for the applicability of this technique. Specifically, the otolith shape analysis seems to hold promise but is only briefly mentioned. This could have wider applicability to European sturgeons for example or sturgeons that occur sympatrically in general. I also think that there is some promise for detailing past habitat use based on microchemical constituents in sturgeon otoliths (see papers by Arai on Russian sturgeon for example). Such information could be invaluable given the opportunistic nature of otolith collection and the critically endangered status of many sturgeons worldwide, but would require intact otoliths!

Minor Concerns:

1. Can the authors indicate the specific otolith removed throughout the manuscript (e.g. saggital)?

**I have only viewed one pair of otoliths in the sturgeon carcasses, probably the saggital, but the purpose is the method getting to them, not specifically the scientific name of the otolith in question.**

2. Lines 82-84: What species of sturgeon was used for this work and how big (e.g., length) were they? I know the authors are aiming for generality, but I think this is still pertinent information to include given potential differences in otolith shape among species. I have seen saggital otoliths from sturgeon that look very different from the one shown in figure 2e.

**I would like to keep this general but I added language to correspond to all US species, the species of sturgeon used for extraction is referenced in figure 2e.**

3. Line 99: What type of electric saw was used to make the lateral incision that bisected the head of the sturgeon? Adding this information would give readers an idea of the specific tool applied for the job.

**Added electric bone saw**

4. Line 103-104: Please clarify what the red boxes denote in figure 2b. There are two red boxes that appear to be showing two different things. The red box on the right seems to be showing intact brain matter ready for removal and the red box on the right seems to show brain matter removed and exposure of semi-circular canal. Is this correct?

**Yes this is correct, and corrected.**

5. Line 114 is incomplete (ends in "that").

**Fixed sentence**

6. Were the sturgeon used in this protocol frozen or thawed? They look thawed in the figures and if so, this may be a helpful point to make. Attempting to remove otoliths from frozen sturgeon I imagine is not very feasible.

**These were not frozen fish**

7. Is it possible for the authors to include scale bars on all of their figures? This would be particularly helpful given otoliths from sturgeon tend to be small relative to the size of the fish.

**Unfortunately this is not possible. Carcasses were disposed of so there is no way to get an accurate scale on the photographs.**

Additional Comments to Authors:

N/A

Reviewer #2:

Manuscript Summary:

As I am unfamiliar with this journal's format, I won't comment much on the layout. However, I did find the writing style to be a bit odd and informal. There were a few sloppy formatting issues, such as ., and spaces between text and full stops. Please pay attention to these issues as they make the authors appear to be under-prepared and are annoying to reviewers who spend quite a bit of time on these reviews. Some of the subject matter was overly described for an audience of researchers in the field, while other sections were under-researched. The method of extraction is not terribly novel, as this method can be used on any species (and is, especially for small juveniles), though without a saw. Overall, the methods will likely be useful for sturgeon researchers, but the utility is somewhat limited due to the equipment (saw) needed, and the fact that most sturgeon are protected and therefore only mortalities sampled; however, this is addressed in the discussion. It is also debatable whether the method will result in fewer broken otoliths because of the use of the saw. Specific comments follow:

Line 38. Fish biologists know that otoliths are the ear bones, and this does not need to be specified.

**True but what about everyone else that is not a fish biologist that does not know this?**

Page 69. I believe the authors are referring to the first marginal pectoral fin ray, commonly called the fin spine.

**Reworded sentence**

Lines 70-72. This information isn't very relevant in this paragraph, and it's generally not acceptable to cite a manuscript in review.

**This shows current work regarding sturgeon otolith morphology, I have cited manuscripts in review before in past publications.**

Line 74. This reference is a technical manual, therefore it is inappropriate to use this as a reference for "the most common otolith extraction method." There are hundreds of papers on these methods, please find some of the seminal research papers to use for this statement (and see if it holds true).

**Reworded sentence**

Line 75. This method is not primarily used for striped bass. Otolith extraction is done for thousands of species.

**Reworded sentence**

Line 87. Commonly, this would be described as "Length was measured in a curved length using a measuring tape from the snout..."

**This is total length, reworded sentence**

Line 99. What kind of saw?

**Electric bone saw**

Lines 117-118. The otolith placement is exactly the same for every fish despite species or size.

Line 167. Personally, I have used a hack saw to split the head in a mid-line sagital cut from the dorsal side. I am unfamiliar with other researchers' methods, but I do not believe that the usual transverse cut would work, as sturgeon morphology is odd. The researchers should contact other sturgeon researchers to ask how they extract otoliths.

**Correct and that was already noted as not being applicable for sturgeon**

Major Concerns:

I have no major concerns.

Minor Concerns:

Realizing that this is a methods-oriented journal, I still recommend that the authors do more literature review on otolith extraction and use before resubmitting. Sturgeon are notoriously difficult to age using otoliths (hence most authors using the first marginal pectoral fin ray), so the authors should make more of a case of the utility of otolith use in these species.

Several of the pictures are of low quality, especially the otolith extraction images. Please resubmit higher-resolution images if available. The authors should also note which otolith they are extracting: the lapillus is usually the largest otolith in sturgeon while the sagittus is usually the largest otolith in other species.

Additional Comments to Authors:

N/A

Reviewer #3:

Manuscript Summary:

General comments:

I thought the paper was poorly written and organized at all levels (within sentence, within paragraph, within section). Further, the claims that this is new and relevant are not supported in the text. I'd suggest reading Chapter 15 of the 3rd edition of Fisheries Techniques which discusses otolith extraction techniques, including "the bottom-up approach" (which references Schneidervin and Hubert 1986). Much of the "results" probably belong in the discussion. The Journal of Fish and Wildlife Management Guide for Authors includes this statement, which probably holds true in most circumstances: "Careless preparation of manuscripts implies careless research and thought and may lead to negative critiques."

DISCLAIMER: I have not viewed any products of the Journal of Visualized Experiments, though I did read about the journal scope, etc. I reviewed this for technical scientific merit as though it would end up as a more traditional peer-reviewed scientific article; perhaps it is ok to write as casually as we speak at this stage for this journal. My review is extensive, yet incomplete due to the careless preparation of the manuscript and my refusal to rewrite it entirely.

Line 38: The word very is included in this document 11 times starting here. It is not normal to include excessive adverbs that serve no real purpose in scientific writing.

**This is an opinion and I understand the point but I don’t think it’s a valid change that is needed.**

Line 39: The second sentence is awkward and in the middle of two sentences that should probably be neighbors. The second sentence also applies to the method discussed in this paper. Shouldn't transverse vertical be sagittal or frontal?

**No, transverse refers to the midline from top to bottom, not front to back**

Line 41: Why is Family capitalized? I'm not sure that sturgeon have a skull so much as they have dorsal skull bones/boney-plates. If they have a skull, most of it is cartilage…

**Used lower case f on family. Not true, skull is a general term and is the correct terminology**

Line 43: Easier? They were tried? How was easier measured? Was it quicker? Take less horsepower? They state methods, yet only mention one. Again with the "methods" in the next sentence.

**Changed sentence**

Lines 45-48: Statements are made about time and success, neither of which are quantified.

**This is a general statement on the applicability of this method**

Line 52: Sentence should be rewritten due to awkwardness, wordiness, etc. Sturgeon (A) populations throughout the world have been declining for over a century due to impacts including habitat loss, population fragmentation, and overfishing such that many populations are protected by state and federal laws.

**Reworded sentence**

Lines 56-58: The first half of the sentence doesn't make sense and doesn't marry well with "standard accepted metrics", whatever that means. Further, I'm not sure the authors understand the material and I don't know if most have been studied...a citation might clear that up. Perhaps "Population characteristics (e.g., growth, recruitment) of most sturgeon populations have been studied to better understand basic biology and life history characteristics (Absent 1999)."

**Reworded sentence**

Lines 58-60: Awful sentence. Due/because, "can be tricky", "necessity of no sacrifice or harm to individuals", what about endangered stocks? Age isn't mentioned before now, nor is why managers would want to collect structures, which would be an awfully convenient lead into the next paragraph.

**Reworded sentence**

Line 62: I have no idea what natal population determination means, but Campana didn't discuss it.

**Reworded and removed natal population determination**

Line 64: If the authors read Campana, they would not have used "determine age" or "verification".

**This statement makes sense and is found in Campana, disregarded**

Line 69: Excessive concludeds and cite who actually concluded that, because I know that all those papers didn't.

**These all are cited for their conclusions made.**

Lines 70-72: Poorly written, out of place.

**No, this shows current work on otolith shape analysis**

Lines 72-74: Most common or only?

**Yes most common, there are several other methods**

Line 76: very brittle and would tend to break...how about small and brittle? Small and delicate?

**Changed to small and delicate.**

Line 79: sturgeon by entering

**Corrected**

Line 87: Cut "Before any incisions" and "using a tape"

**Removed**

Line 88: It's a rostrum, not a snout; nobody measures total length on a heterocercal-tailed critter, they measure fork length—in fact, nobody measures total length unless the fish doesn't have a forked tail; perhaps add "using a metric meter stick" at the end since that what is recommended later.

**Reworded**

Line 90: Tare scale and weigh sturgeon to the nearest gram. "Look over carcass for any external tags or marks and record the body condition"…little of this is clear, direct, and useful. Perhaps "Evaluate the carcass for marks and tags and "whatever record body condition means". Seriously, what does record the body condition mean? Condition in fisheries could most directly mean a measure of plumpness, which progressed from K factor to Fulton's K to relative weight (Wr), but I'm guessing the wording is actually a poor choice of words and actually means something more along the lines of evaluating for DELTs (deformities, erosions, lesions, and tumors), but who knows given the lack of detail and accuracy.

**Removed body condition**

Line 94: "at the base of the skull at the first dorsal scute"…this is an awful description…perhaps "separate the head from the body using a fillet knife by making a transverse cut between the base of the skull and first dorsal scute"

**Fixed sentence**

Line 99: an electric saw is about as specific and useful as "a fish". Reciprocating saw? Dremmel? Fillet knife? Meat saw? Circular saw? Electric chain saw?

**Corrected to electric bone saw**

Line 100: cut "into two halves" and "using hands"

**Removed the words**

Line 101: replace "to expose the brain cavity" with "by hand"

**Replaced**

Line 106: This is not cartilage. There is a membranous sac that contains the three otoliths within each semicircular canal. Fix throughout (107, 108,…).

**Yes, but also known as semicircular canals**

Line 113: 5s…why?; rinse with what?

**Removed rinsed**

Line 114: "or any further analysis that ." huh?

**Removed**

Line 117: "the placement" perhaps location is more accurate

**Changed**

Lines 118-119: Awkward sentence; easiest???, compared to what? Was something else tried?

**Reworded**

Lines 119-120: What kind of saw, preferred compared to what, "expose the brain matter" is off-point and unnecessary. Expose the semi-circular canals.

**Reworded**

Line 120: "However, attention to bisecting the midline of the skull is" tough to read. This also highlights the fact that this method has the same concerns as the method that this is supposed to be an improvement of.

**No, ease of entering the brain cavity from the ventral side to remove the otoliths is the highlight**

Lines 122-123: not brain cavity, not brain matter; canals; damage, not harm

**Brain cavity**

Lines 123-124: finding, then found is awkward

**Disagree and not changed**

Line 125: base of the brain cavity is not helpfully descriptive

**Changed**

Lines 125-126: "of a jelly consistency" is ridiculous and inaccurate; it is not cartilage, it's a membranous sac with a serosanguineous fluid that is nothing like jelly

**Reworded**

Lines 128-132: not results; all awkward sentences, mostly useless; authors fail to show actual useful purpose for sturgeon otoliths; WHO IS USING THEM AND FOR WHAT?, NOT WHO HAS LOOKED AT THEM AND DECIDED THAT FIN RAYS WERE BETTER?; outcome success sentence is without purpose; age determination and growth rate sentence suggests that the authors aren't familiar with age estimation and the limitations of using otoliths for growth calculations. Suggest critically reading Campana 2001. When would shape analysis ever be useful? Hopefully, one could ID the critter that the otoliths were extracted from, instead of needing to evaluate the shape of an internal calcified structure to do so… If there is some circumstance that the authors envision where a pile of otoliths are found without other identifying marks and only the shape can be used to identify species…they should make the case somewhere.

**This is the format of the Journal!!**

Line 137: Is E actually showing the use of a non-electrical saw to split the head?

**This is showing the bisected cut using a bone saw but is being opened with a fillet knife for the photo**

Lines 145-171: The discussion is an awkward and unorganized mess. The first sentence could not be worse. The second sentence demonstrates that this method has the same concerns as the method this is supposedly better than. This goes on and on. There is no single sentence that should be retained.

**Again this is the journal format which is different than a standard journal format.**

Outline, outline, outline.

Currently, this is the outline:

P1. Alternative protocol done did got done.

Don't screw up

Gotta cut deep

Becomes simple and most otoliths found

Method works for other fish

P2. Better for lab than field

Not for field sampling

Sturgeon are protected

P3. Permitting is hard.

Get extra info

P4. This technique is awesome

Dorsal don't work

Access to areas for other reasons (not true)

Method may work for other fish

Equipment list: go with fillet knife, as it's not a filleting knife; scalpel; forceps

Major Concerns:

N/A

Minor Concerns:

N/A

Additional Comments to Authors:

N/A

Reviewer #4:

Manuscript Summary:

The authors pull together a nice description of how to extract otoliths from sturgeon in the laboratory. The images are straightforward and clearly referenced in the manuscript. The protocol looks sound, but there are a few edits that would help focus the direction of the paper before it is published.

Major Concerns:

The Introduction does not seem to fit the objective exactly. There was a substantial focus on aspects of sacrificing fish and the value of otoliths versus other structures. That information, while important, seems moot for this paper that is aimed at demonstrating the technique to remove otoliths from sturgeon. Seems to me that a focus on proper extraction would be better. Otolith extraction from sturgeon has been problematic and this approach seems a viable alternative if time allows. The emphasis should be on that.

Minor Concerns:

A list of what sturgeon species have been successfully extracted using this technique might be good. Otolith extraction from some of the smaller sturgeon species has been particularly difficult. If this technique helps get otoliths from these species that is a definite value to know.

Which set of otoliths are being extracted (saggital presumably, but it is not clear).

Protocol

1.1 - The lab prep in length and weight is fine, but not entirely needed. Also, different programs measure different lengths (e.g., total, fork, or standard) so maybe a revision to state measure length as appropriate would be in order.

**Added total length**

2.2 - an electric saw is referenced here and in other locations but no description of the type of saw and blade are described. That information seems critical to make the appropriate cut without damaging the otoliths.

**Electric bone saw**

2.6 - last sentence in this step needs some edits.

**Fixed**

Line 150 - what percent of otoliths can be recovered intact? A high percentage was mentioned, but not quantified

**No quantification but just a general statement**

Line 156 - I believe "contusive" should be "conducive"?

**Fixed**

Line 168 - sentence is not clear on intent.

**Trying to make point that this technique can be used for other areas of fish brain research not just otolith removal**

Additional Comments to Authors:

N/A

Rviewer #5:

Manuscript Summary:

The manuscript presents a fairly simple summary of otolith extraction from sturgeon that may be applicable to other "hard-headed" fish. The manuscript does not document an experimental method and as such I believe the submission is of limited value to readers/viewers of JoVE.

Major Concerns:

Not suitable for publication in a journal focused on "...experimental approaches in biological, medical, chemical and physical research." The subject matter in the manuscript does not represent an experimental approach but rather a simple method.

Minor Concerns:

The text is rough in some spots and would benefit from some editing if it did proceed to publication.

Additional Comments to Authors:

N/A