

Journal of Visualized Experiments

Alternative Method of Removing Otoliths from Sturgeon

--Manuscript Draft--

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Abstract:	<p>Extracting the otoliths (ear bones) from fish that have very thick skulls can be difficult and very time consuming. The common practice of making a transverse vertical incision on the top of the skull with a hand or electrical saw may damage the otolith if not performed correctly. Sturgeons (Acipenseridae) are one family in particular that have a very large and thick skull. A new laboratory method entering the brain cavity from the ventral side of the fish to expose the otoliths was easier than other otolith extraction methods found in the literature. Methods reviewed in the literature are designed for the field and are more efficient at processing large quantities of fish quickly. However, this new technique was designed to be more suited for a laboratory setting when time is not pressing and successful extraction from each specimen is critical. The success of finding and removing otoliths using this technique is very high and does not compromise the structure in any manner. This alternative technique is applicable to other similar fish species for extracting the otoliths.</p>
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United States Department of the Interior

U.S. GEOLOGICAL SURVEY

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January 6, 2016

Dear Editor,

Attached is a revised manuscript entitled "Alternative Method of Removing Otoliths from Sturgeon". This study has detailed an alternative method of removing otoliths from sturgeon from the ventral side of the head. The suggested changes were incorporated to the manuscript. This manuscript is of original data and has not been published or being considered for publication anywhere else. All of the authors have read the manuscript and approve of the data to be considered for publication in the Journal of Visualized Experiments. If you have any question regarding the submittal please contact me.

Sincerely,

Marc Chalupnicki, M.S.
Biological Science Technician
Great Lakes Science Center

TITLE:

Alternative Method of Removing Otoliths from Sturgeon

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KEYWORDS:

Sturgeon, Otoliths, Fish Ear Bones, Extraction, Removal Guide, Acipenseridae

SHORT ABSTRACT:

The goal of the protocol is to show an effective method to extract the otoliths from sturgeon carcasses.

LONG ABSTRACT:

Extracting the otoliths (ear bones) from fish that have very thick skulls can be difficult and very time consuming. The common practice of making a transverse vertical incision on the top of the skull with a hand or electrical saw may damage the otolith if not performed correctly. Sturgeons (Acipenseridae) are one family in particular that have a very large and thick skull. A new laboratory method entering the brain cavity from the ventral side of the fish to expose the otoliths was easier than other otolith extraction methods found in the literature. Methods reviewed in the literature are designed for the field and are more efficient at processing large

quantities of fish quickly. However, this new technique was designed to be more suited for a laboratory setting when time is not pressing and successful extraction from each specimen is critical. The success of finding and removing otoliths using this technique is very high and does not compromise the structure in any manner. This alternative technique is applicable to other similar fish species for extracting the otoliths.

INTRODUCTION:

Sturgeon (Acipenseridae) 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^{1,2}. Management agencies throughout the USA and the world have identified sturgeon as a target species for restoration and recovery¹. Population characteristics (growth, recruitment, diet) of most sturgeon populations have been studied to better understand basic biology and life history characteristics¹. Due to the protected status of most sturgeon populations, age evaluation is difficult in small threatened populations where no sacrifice or harm to individuals is warranted.

The extraction of bony structures for the purpose of age estimation and growth analysis has been a common technique³. The use of fin ray sections to determine age of sturgeon has become a practical nonlethal method, but verification of the technique has been limited and age can be very difficult to decipher in older individuals (>15 years)⁴. Researchers evaluating the use of multiple bony structures in sturgeon for their use in validating the age of Gulf Sturgeon (*Acipenser oxyrinchus desoti*)⁵, Atlantic Sturgeon (*A. o. oxyrinchus*)⁶, White Sturgeon (*A. transmontanus*)⁷, and Lake Sturgeon (*A. fluvescens*)⁸ concluded that the first marginal pectoral fin ray provided the most accurate age but concluded this invasive procedure to be deleterious to larger individuals. Currently, there is the development of using otolith shape metrics to distinguish between North American sturgeon species with a very high rate of success⁹. Details on the process of removing the otolith with a transverse incision at the base of theis a common otolith extraction method found throughout the literature and clearly detailed by Secor et al. (1991)¹⁰. However, this method is difficult to implement on sturgeon that have very thick skulls with otoliths that are small and delicate and would tend to break. A further review of the literature shows no other detailed methodology of the otolith removal process for sturgeon. The objective of this paper was to detail an alternative method of extracting otoliths from sturgeon by entering the brain cavity from the ventral side.

PROTOCOL:

Important Note: Due to the protected status of all species of sturgeon in the United States, permits to acquire and handle fish were obtained prior to laboratory procedure. All specimens were acquired from hatchery sources post mortem due to natural causes and disposed of following procedure.

1. Specimen Preparation:

1.1 Before any incisions lay sturgeon carcass on its side and using a measuring tape to measure total length from the snout to the tip of the tail to the nearest 1mm. [Figure 1a]

1.2 Weigh sturgeon carcass on a tared scale to the nearest 1g. Evaluate carcass for any external tags or marks and record.

2. Otolith Extraction

2.1 Make a vertical incision with a large fillet knife between the base of the skull and the first dorsal scute through the body to separate the head from the body [Figure 1b]. After removing the head, turn the head so the mouth is facing up [Figure 1c].

2.2 Remove soft tissue of the mouth and gills by using the fillet knife to cut along the ventral side of the skull [Figure 1d]. Make an incision with an electric bone saw from the tip of the snout to the base of the skull to completely bisect the head [Figure 1e]. Separate the two halves by hand to expose the brain cavity [Figure 2a].

2.3 Remove and discard brain matter (Red box left) with forceps to view semicircular canals (Red box right) within the brain cavity. [Figure 2b]

2.4 Remove semicircular canal cartilage from brain cavity with forceps [Figure 2c]. Carefully puncture semicircular canal cartilage with the tip of the forceps and remove otolith [Figure 2d]. Remove the otolith (hard bony structure inside the cartilage) with forceps from the semicircular canal cartilage [Figure 2e].

2.5 Repeat steps 2.3-2.4 to remove otolith from the other half of the skull.

2.6 Dip the otoliths in 92% Ethanol for 5 seconds to remove any cartilage. Place otoliths in an open 25ml scintillation vial to dry for 24 hours.

REPRESENTATIVE RESULTS:

The adult size of sturgeon across the world varies greatly but the location of the otoliths is consistent. The use of a sharp fillet knife was found to remove the soft tissue of the mouth easily shown in **Figure 1d**. An electric bone saw is the preferred tool for the lateral incision to expose the brain cavity in **Figure 1e**. However, attention to bisecting the midline of the skull is needed otherwise the otoliths may be crushed during this process. If this process is performed correctly then the brain cavity will be exposed evenly with no damage to the otoliths as shown in **Figure 2a**. The brain matter is easily removed with the use of forceps as in **Figure 2b** and finding the semicircular canal cartilage which contains the otolith is easily found at the base of the brain cavity as shown in **Figure 2c**. The cartilage found in the semicircular canal is easily removed from the otolith with a pair of forceps as viewed in **Figure 2d**.

This technique successfully shows the removal of the sturgeon otolith and is a preliminary step in a more quantitative analysis of the fish's life history. Outcome success can be gauged by the removal of the otolith without breaking the structure. Age determination and growth rates are two metrics that can be evaluated with the use of the removed otoliths. Shape analysis for species differentiation is another measurable metric that can be developed from the otoliths.

FIGURE LEGENDS:

Figure 1: Placement of Sturgeon carcass for otolith removal: A) Sturgeon Carcass Lateral View for Measurement and Weighing, B) Vertical Incision to Separate Sturgeon Head from Body, C) Flip Head to View Mouth and Ventral Side of Head, D) Soft Tissue Removed to Expose Ventral Side of Skull, E) Lateral Incision from Tip of Snout to Base of Skull

Figure 2: Internal examination of the brain cavity to remove otoliths: A) Brain Cavity Exposed after Head is Separated, B) Brain Matter Removed to Expose Semicircular Canals, C) Semicircular Canal Cartilage Removed, D) Otolith Viewed and Removed from Semicircular Canal Cartilage, E) Shortnose Sturgeon Otolith

DISCUSSION:

The overview of an alternative method of extracting otoliths from sturgeon carcasses has been detailed. It is important to note that special attention is needed to the lateral incision placement on the midline of skull in order to bisect the skull evenly to ensure no harm to the otoliths during dissection. If the incision is not deep enough to completely bisect the skull it will be very difficult to expose the brain cavity where the location of the otoliths is clearly visible. , With experience, the overall technique is relatively simple and leads to a high percentage of the otoliths being found intact. This new method gives researches an alternative way to extract otoliths for species other than sturgeon that may be difficult using standard otolith extraction methods.

Limitations to this method are the applicability to a laboratory setting not for field use. The time needed for carcass preparation is not conducive to samples collected in the field in a timely manner. Another concern is the use on sturgeon species that are typically protected under state or federal law due to population declines.

It is very difficult to obtain permits to conduct this type of evaluation at the expense of sacrificing individuals that are needed to keep populations alive. However, when dead specimens are available through natural causes this technique provides a useful way to collect more information than would be available otherwise.

It is important to note the significance of this technique to successfully remove otoliths from sturgeon using this alternative method. Existing techniques removing otoliths from the dorsal region of the head are still useful and beneficial but not applicable for sturgeon. Using this new technique to expose the brain cavity also gives access and applicability to other areas neural function and brain morphology. This new technique may be beneficial to use on other species of fish with very thick skull walls or where there is a limited amount of fish to use for otolith extraction.

ACKNOWLEDGMENTS:

Special thanks to the St. Regis Mohawk Environmental Division, Welaka National Fish Hatchery, US Fish and Wildlife Service Research Unit, Southern Illinois University, US Fish and Wildlife Service Northeast Fisheries Center, Sterling Caviar, University of California-Davis, Garrison Dam

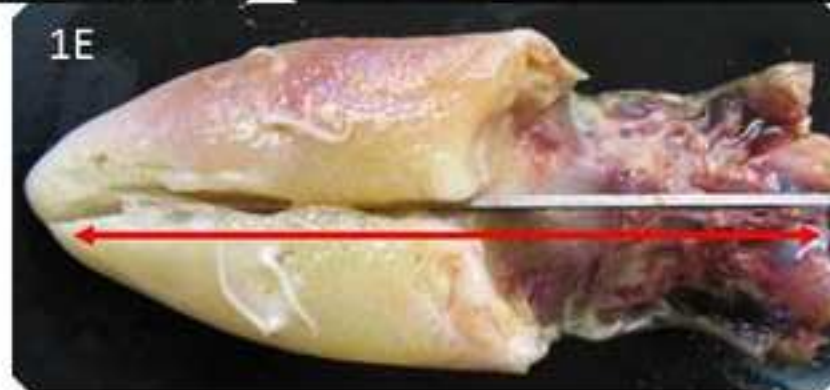
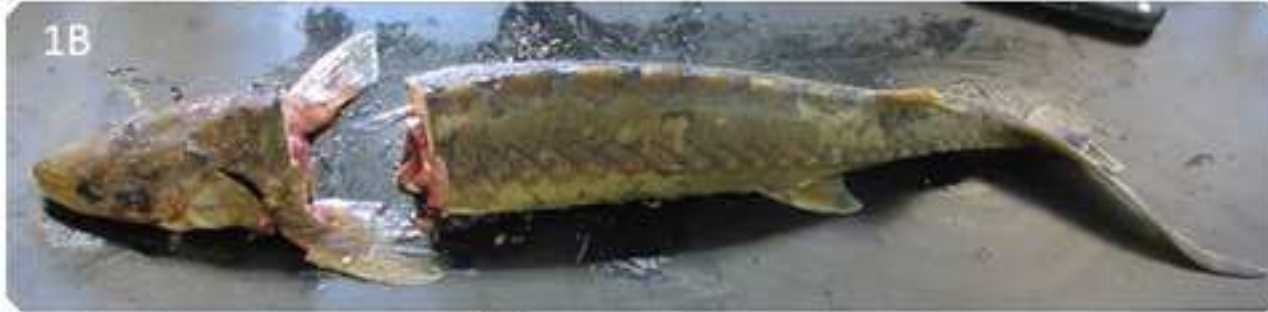
National Fish Hatchery, Bears Bluff National Fish Hatchery, and US Fish and Wildlife Service Panama City Fisheries Resource Office for their help supplying sturgeon for this project. This article is Contribution XXXX of the USGS Great Lakes Science Center.

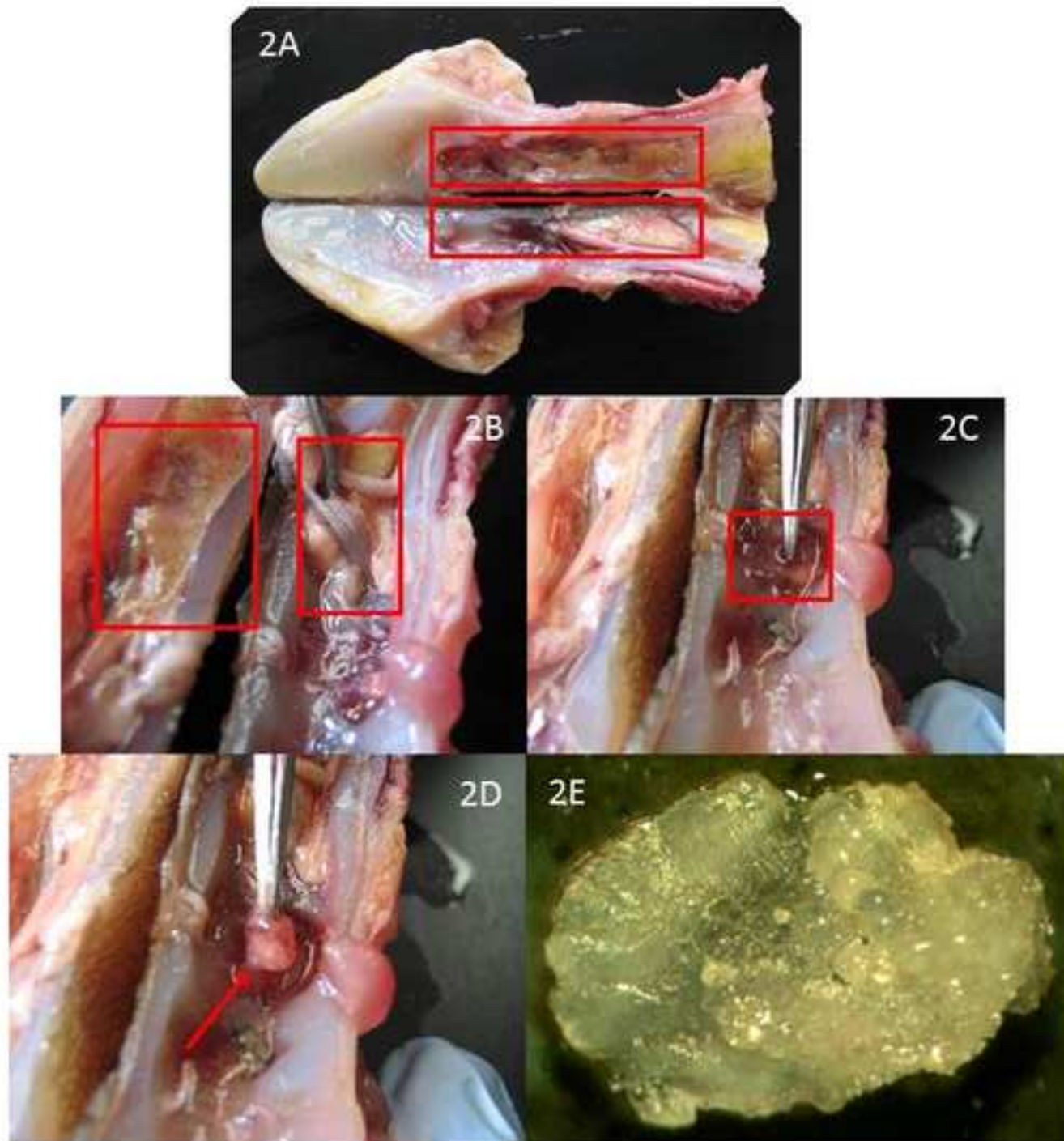
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Jaydev Upponi <em@editorialmanager.com>

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.

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Sincerely,

Jaydev Upponi, Ph.D.

Science Editor

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Editorial comments:

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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

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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 left 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 sagittal 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 (sagittal 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 "conductive"?

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