

“Atlantic Sturgeon Research Protocols” by Damon-Randall, and 10 co-authors.

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General Comments:

Overall, my review of this document is very positive. The authors did a good job of presenting many critical aspects of handling and sampling Atlantic sturgeon, and bring to the readers the latest techniques from both the published and grey literature and first hand use (i.e. pers. comm.). I will address the specific issues listed in the Oct 28, 2009 email as I go through the document.

One weakness in this document is the section on anesthesia. More details are needed on the how and when, and specifically to what stage of anesthesia (for the different procedures). I will provide more detailed comments below.

The following came to mind after my first read-through. To further standardize Atlantic sturgeon research/sampling have you considered a summary section listing the “required” standardized-sampling of each Atlantic sturgeon captured, at the end of this document? Maybe a one-page list that can be copied and taken into the field. This could list the minimum information to gather from each fish. Besides the date of capture, location, gear, etc., at least record body size measurements, PIT tag scan (and if none, implant one?), and a fin clip for genetics, and what else?). Have you considered an Appendix with a recommended standardized one page data sheet? On the west coast, when white sturgeon projects come across a green sturgeon, I would want this minimum set of information from each green sturgeon, as they are infrequently caught. But maybe you have already discussed this aspect for the document and found it is not applicable, due to the fish numbers you are encountering, and due to the large number and variety of research projects. Of course the other major issues are: would everyone share such data? (but could this be required if listed under the ESA?), and who or what agency would be the data collator?

After I read through the entire document the use of the term “protocols” in the title (and in the text) did not seem quite right. A “protocol” in my work, and in the medical field (i.e. Animal Use and Care Protocol or a Research Protocol), is a step-by-step list of very specific procedures. What this document provides is more accurately stated in the Background paragraph, and are “guidelines...to help standardize research...”. Only in a couple sections are there what I consider to be a protocol (like page 5, measurements and page 20, laparoscopy). All other sections are guidelines for methodologies that may be altered depending on the specific research project and its objectives, or are techniques that can be considered or need to be further studied and refined. A title that would more accurately reflect the text of the document is appropriate. Some suggestions:

Atlantic Sturgeon Research Protocol Guidelines or,
Atlantic Sturgeon Research Guidelines and Recommendations or,

Atlantic Sturgeon Research Guidelines or,
Atlantic Sturgeon Research Techniques

Also, more clearly describe in the Background section exactly what this document is intended for...a last sentence describing how this document should be used. (After reading the document, I understand it to be guidelines for research, that can help standardize data and sample collection, but the specific techniques to be used will depend on the individual project, its objectives, locations, etc.)

Another suggestion is that each section provides a lot of information but what are the specific points that you really want researchers to consider, regarding the techniques discussed? I am thinking of a summary, or numbered/bulleted list, at the end of each section, just before the “Training Requirements” section.

Specific Comments:

Table of Contents:

I would recommend delete “assigning” (for page 19 heading) and have “..sex identification and determining stage of gametogenesis” (or stage of gonadal development)

For page 23 heading, change “assigning sex” to “sex identification”

List of Figures:

Figure 12 legend (change in text of document also): replace “solid” with “turgid” and “spermatic” with “testicular”, and delete “visible”.

Figure 13 legend (change in text of document also): delete “visible”

Line 41, in between “how research” add “and what type of”

Objectives, page 2, could be more clear and concise, for example:

1) provide Atlantic sturgeon researchers with guidelines for conducting research, to ensure that the safest and most recent and effective techniques are used; and 2) compile the most recent literature review to support the use of these techniques.

Page 3, Identification and measurement

This is a well reviewed and documented section, but I recommend 2 subheadings, one that is the “Background” (all the literature review) and then a 2nd subheading at the end of the section, something like “ required body size and identification measurements”. Under the 2nd heading the lines of text 195 through 210 should be moved and the current last paragraph (lines 217-232) should be moved to the end of the “Background” section, as it is mainly literature review and supports the proposed “required” five minimum measurements.

Line 274, change to “...allow for potential successful hatching. (it is unknown if all eggs will hatch).

Line 349, delete the (chi sq values) as specific p value is not really needed in this type of document.

Line 501 (Electrofishing).

Add at the end of the first sentence: and using this technique induced injuries in juvenile sturgeon (Holliman and Reynolds, 2002). I think this is important to mention.

Holliman, F.M, and J.B. Reynolds. 2002. Electroshock-induced injury in juvenile white sturgeon. North American Journal of Fisheries Management 22: 494-499.

Line 539 (Resuscitation) add after “enhanced”, through the mouth and over the gills... Also refer to a section on the document of how to resuscitate (using pump and freshwater, etc).

Comment: the most effective way to resuscitate is through the mouth, as if the fish was swimming forward. Dragging the fish back and pushing forward is not ideal but if there is no other option the drag back should be very gentle, so not to damage the gill filaments, and besides this retrograde flow while pulling back does not provide as good oxygen exchange as moving forward. The forward motion should be a faster motion than pulling back. The best method is to have a pump and hose of freshwater directed into the mouth. And if the end of the hose is actually placed in the mouth used something soft, like a sponge to keep the metal or hard plastic from hitting the inside of the mouth.

Line 551, change to: “...depending on site of capture, fish size...” (net pens may not be useful in river sections of fast flow, or areas with lots of debris, etc.)

Line 578-579, this last sentence I think could be deleted or re-word as a note of caution, as I do not know of any studies or literature that shows salt treatments are harmful to ripe sturgeon, but we do know the benefits.

Comment: Over the years we have used a salt bath on many ripe female green and white sturgeon with no effects on eggs maturation and spawning. We add salt to the transport tank when we transport ripe males and females from the wild and all the fish have spawned successfully.

Line 663 (Anesthesia).

Comment: this is a critical section, regarding the when and when not to use it, how much, recovery issues, etc. Anesthetics are stressors themselves, and so using it or not will depend on the exact procedure. For instance, I have been approved to incise, collect eggs in a catheter and suture close sturgeon because I showed how the 2-3 minute procedure without anesthesia, would be lengthened to 20 minutes more when using MS-222 (for induction and complete recovery). What folks did not like seeing was the “thrashing” phase when sturgeon are first placed in the anesthetic bath and the potential for trauma.

This section requires more background information and guidelines, as anesthesia is a very important, but risky procedure (in all animals): An Introduction paragraph that reviews anesthesia and describes the anesthesia depth and criteria used for Atlantic sturgeon is important. There are various stages of anesthesia (and within each stage there can be a

light and deep plane). Researchers need to be aware of, and how each stage changes the fishes reaction to touch, equilibrium, respiration and heart rate (see Detar and Mattingly, 2004; Myszkowski et al., 2003; Summerfelt and Smith, 1990).

Detar, J.E., and H.T. Mattingly. 2004. Response of southern redbelly dace to clove oil and MS-222: effects of anesthetic concentration and water temperature. Proc. Ann. Con. SE Assoc. Fish Wildl. Agen. 58: 219-227.

Myszkowski, L., R. Kaminski, and J. Wolnicki. 2003. Response of juvenile tench to the anaesthetic 2-phenoxyethanol. J. Appl. Ichthyol. 19: 142-145.

Summerfelt, R.C., and L.S. Smith. 1990. Anesthesia, surgery, and related techniques. In Methods for fish biology. Edited by C.B. Schreck and P.B. Moyle. AFS, Bethesda, Maryland. pp. 213-272.

Other good information in:

Harms, C.A. 1999. Anesthesia in fish. In: Fowler, M.E. and R.E. Miller (eds). Zoo and Wild Animal Medicine, Current Therapy 4, Philadelphia: W.B. Saunders. pp 158-163.

Harms, C.A. 2003. Fish: In: Fowler, M.E. and R.E. Miller (eds). Zoo and Wild Animal Medicine, 5th Edition, St. Louis, W.B. Saunders. Pp 2-20.

Stetter, M.D. 2001. Fish and amphibian anesthesia. In: Heard, D.J. (ed). Veterinary Clinics of North America: Exotic Animal Practice. Philadelphia: W.B. Saunders. pp 69-82.

Neiffer, D.L. 2007. Boney fish (lungfish, sturgeon, and teleosts). In: West, G., D. Heard, N. Caulkett (eds). Zoo Animal and Wildlife Immobilization and Anesthesia. Ames, IA: Blackwell Publishing. Pp 159-196.

Comment: What I have learned from my years of sturgeon anesthesia, is only use it when you have to. If used, use it at the low doses, to the lowest stage and plane of anesthesia as possible to safely conduct the specific procedure, and for the shortest exposure time. This results in fastest recovery and best long-term survival. If we have to use anesthesia then we typically give the sturgeon an immersion bath until the desired stage of anesthesia is reached and then while conducting the procedure the fish is on freshwater (if the procedure is less than 10 min) so that at completion of sampling the fish is already recovered. For longer procedures, alternate between a maintenance dose and freshwater, so that opercular movement never stop for more than a few seconds, and once we are at about 10 min to completion, then we switch to entirely freshwater.

Line 675 and 684: shouldn't this be "temperatures 5 and 15 C" (from 5-15 implies many different temperatures within that range).

Line 682, delete "stage 4" unless in the re-write this stage is described in more detail, as different schemes use different stage numbers (some have stage 4 as death).

Line 695, delete "...for an extended period of time"... as any stopping of opercular movements is not desired. It is true as stated in Line 697-700 but I would emphasize that if movement stops for more than a few seconds than the fish should be switched to freshwater.

Line 707, what type of electrolyte treatment? And what should be the target concentration?

Line 728, Section Title is not concise. Suggestion:

"Techniques to identify sex and determine stage of gametogenesis (or gonadal development)"

Replace throughout the document "assigning sex" with "sex identification"

Replace throughout the document "degree of sexual maturity" with "stage of gametogenesis or ("gonadal development")" (i.e. Line 736, 740)

Line 740, does the 15 min per fish include the time for anesthesia (induction and recovery)? Due to the time required for this technique, equipment and set-up area needs, is this practical in the field or on a boat? This is eluded to on Line 744, but needs to be more clearly explained, in terms of disadvantages to the technique. Hurvitz et al., 2007, reported only taking 2 minutes to identify gender and stage of gonadal development.

Hurvitz, A., K. Jackson, G. Degani, and B. Levavi-Sivan. 2007. Use of endoscopy for gender and ovarian stage determinations in Russian sturgeon grown in aquaculture. *Aquaculture* 270:158-166.

Line 746, include the current estimated cost for the laparoscopic equipment.

Line 785-786, needs to be more clearly explained, regarding anesthesia use and stage of anesthesia (see earlier comments). Reference to only Matsche and Bakal, 2008 is not really the "best information available". Is this report available on-line? If so, that link should be added to the Reference list and that would strengthen the reference. Some peer reviewed literature should also be included (see earlier comments).

Line 793 and 843, remember if this technique is used, "gently" on the pull back but more forceful on the forward motion.

Line 847, Heading change to: Other methods to identify sex and determine stage of gametogenesis (or gonadal development)"

Line 850, 855, coeliotomy is the British spelling/variant...more common in U.S. is "celiotomy"

Line 851, More accurate would be (3) "blood plasma analyses of steroid levels"

Line 855, change "body" to "abdominal"

Line 858, Re-write sentence “This procedure is limited...” As fish smaller than 10 kg do have visually differentiated gonads...in general, it is easier to identify sex in the larger fish but it can be done on smaller individuals.

Suggest: Identification of sex can be done after differentiation of the ovary and testis and the age and size of when this occurs, varies between sturgeon species. In cultured white sturgeon it is at 1-2 years of age and 1-3 kg in body size, but sexing is not required until the fish are at market size (7-9 kg) (Van Eenennaam et al. 2004)

Van Eenennaam, J.P., F.A. Chapman, and P.L. Jarvis. 2004. Aquaculture. Pp. 277-311 in G.T.O. LeBreton, F.W.H. Beamish and R.S. McKinley (ed). Sturgeons and Paddlefish of North America. Fish and Fisheries Series Vol. 27. Kluwer Academic Publishers, Dordrecht.

Lines 866-869 delete. Add, “To determine the stage of gametogenesis (or gonadal development) you can preserve a tissue sample of the gonad in 10% buffered formalin, process and embed the sample in paraffin, and prepare stained histological sections (Van Eenennaam and Doroshov, 1998).”

Van Eenennaam, J.P., and Doroshov, S.I. 1998. Effects of age and body size on gonadal development in Atlantic sturgeon (*Acipenser oxyrinchus* Mitchill). Journal of Fish Biology 53: 624-637.

Line 886, change to “extremely rare” (because that is the case)

Line 887, delete “likely” (the female would have to be in the process ovulation to collect eggs from the vent)

Lines 888-893, delete, as these sentences refer to spawning (details in Atlantic Sturgeon Manual) and this technique was not supposed to be part of this document.

Line 895, change to: “Blood plasma analysis for steroid levels”

Line 897, add “11-ketotestosterone”

Line 898, add more recent refs: “Fiest et al, 2004 and Wildhaber et al 2006)

Feist, G., J.P. Van Eenennaam, S.I. Doroshov, C.B. Schreck, R.P. Schneider, and M.S. Fitzpatrick. 2004. Early identification of sex in cultured white sturgeon, *Acipenser transmontanus*, using plasma steroid levels. Aquaculture 232: 581-590.

Wildhaber, M.L., D.M. Papoulias, A.J. Delonay, D.E. Tillitt, J.L. Bryan, and M.L. Annis. 2006. Development of methods to determine the reproductive status of pallid sturgeon in the Missouri River. U.S. Geological Survey Final Report to the U.S. Fish and Wildlife Service. 88p. (*Comment: I am pretty sure it is available on-line...contact USGS*).

Lines 900-903, A little unclear. Suggest:
Blood samples need to be kept chilled after collection and centrifuged the same day.
Plasma should then be stored frozen until shipment.

Line 905, Paragraph on Ultrasound. (*Comment: I am biased on this technique as the more I use it, the more accurate it is for sex identification and staging gonad development.*) I would edit this paragraph to provide more detail on its accuracy. Moghim et al (2002) reported overall accuracy of 97% and took only 30 seconds to determine the sex of each sturgeon. Colombo et al (2004) were able to identify 88% of the sturgeon, although only 40% of the post-spawned females were accurately identified, excluding those females, accuracy was 94%.

Line 910, re-write, “Although ultrasound equipment is relatively expensive and the technique requires substantial initial training to interpret the ultrasound images, it is the safest, quickest, and least stressful on the fish, and anesthetic is not required, except during initial training”.

“Other Methods” 5) Meristics and 6) Shape of Urogenital Opening, I would recommend deleting these two methods, as they are not methods with enough documented success to even consider using on Atlantic sturgeon. (If this document was more of a general review of all sturgeon, then they could be included, but the goal of this document is to provide “Techniques that are thoroughly described, accurate and appropriate...”).

Comment: If the shape of the urogenital opening was valid it would have been verified by now. I know myself and numerous other sturgeon folks have tried since this was published and I do not think anyone has had any consistent success. Of course, mature broodstock on a spawning run are different, you can often see differences in the vent but there are also other body meristics that are quite obvious when comparing ripe females and males.

However, one “other method” you should consider adding is the use of a borescope inserted into the genital tract as it has been well published (see Kynard et al., 2002; Kynard and Kieffer, 2002; Wildhaber et al., 2005; Bryan et al, 2007).

Kynard, B., R. Suci, and M. Horgan. 2002. Migrations and habitats of diadromous Danube River sturgeons in Romania, 1998-2000. *J. Appl Ichthy* 18: 529-535.

Kynard, B., and M. Kieffer. 2002. Use of a borescope to determine the sex and egg maturity stage of sturgeons and the effect of borescope use on reproductive structures. *J. Appl Ichthy* 18: 505-508.

Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, M.L. Annis, and J.A. Allert. 2005. Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. *J. Fish Biol.* 67: 114-132.

Bryan, J.L., Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, and M.L. Annis. 2007. Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon. *J. Appl. Ichthyol.* 23: 411-419.

Line 1049, I do not recommend a suture for a PIT tag insertion hole. If done in the correct location the muscle quickly fills in behind the tag. For smaller fish or for extra security the tissue adhesive is fine but not a suture. The tissue irritation from the suture may slow the healing process and lead to PIT tag loss.

Line 1052, after any alcohol sterilization make sure the instruments are air dried or rinsed in a sterile saline solution, as alcohol can irritate and dehydrate tissue.

Line 1228, list the Erickson et al in review paper in the reference list as “in review” or “in press” when appropriate.

Line 1283, “...is strongly encouraged...” These are the type of sampling procedures I was referring to in my 3rd paragraph, 1st page of this review.

Line 1296, insert...”and stage of gonadal development (or gametogenesis)” after “...identify sex...”

Line 1302, That 40 mm incision is large and not typical. If you go through the cited papers you can track how it came from Fox et. al., when they made that large 40 mm incision to insert large telemetry tags.

These days we typically use a 25 mm (up to a 35 mm length incision, for training new folks). You can cite Van Eenennaam et al., 2004.

Line 1314, replace “opposed to some” with “much as” (all sutures irritate, but the degree of irritation varies)

Line 1336, and Line 1351 to Line 1358 (and in between). Why not just use vacutainers from the start? Not sure why you would collect with a needle and syringe and then put the blood in a vacutainer (let alone the extra syringe waste to dispose). Depending on what analyses you will be running they have vacutainers of all types and sizes (smaller ones for the small fish) and it is actually easier to collect blood with vacutainers as they have the small amount of vacuum that pulls the blood in.

Lines 1395 through Line 1404. This is the type of “standardized” information I was talking about in my 3rd paragraph, 1st page of this review. This type of outline of procedures that “should” be done, should be in a separate summary section of this document.

Line 1515, I would change this heading to a more accurate: “Short-term and long-term (cryopreservation) storage of sperm” and divide the section into 2 paragraphs, the first for reviewing short term storage in the fridge and the 2nd paragraph for reviewing cryopreservation.

Line 1555, “invasive techniques”....(delete) what are the invasive techniques on the sturgeon? It is very easy and quick to collect a milt sample and no anesthesia is required. Short term storage is not difficult, however cryopreservation techniques are more involved and require specialized equipment.