Round #1

by Francois-Xavier Dechaume-Moncharmont, 2020-07-07 14:49
Manuscript: https://www.biorxiv.org/content/10.1101/436295v3

Biologging: assessment of spawning activity in fish based on accelerometer data

The present manuscript is a methodological study assessing the potential use of accelerometer for estimating spawning activity in a fish species. Despite the limited sample size, the idea is original, appealing and, if its reliability is confirmed, possibly very useful in field works. It could therefore be a valuable contribution to the field and it could be recommended by PCI-Ecology provided some clarifications and developments.

In its present form, one of the major weaknesses of the manuscript lies in the lack of argumentation about the chosen equations and metrics: how were they calibrated? How appropriate were they to estimate the energy consumption? Two referees expressed serious concerns about the calibration of the measures. I understand that it will not be possible to perform additional experiments. Yet, complementary statistical analysis may be presented. At the very least, if relevant information is not accessible any more, these caveats and limitations should be extensively discussed in the MS as a warning for the reader. The referees also pointed out that utmost care must be taken to justify the measurement as a proxy for energy expenditure. I strongly advise the authors to carefully take into account their suggestions.

Finally, I share one referee's opinion about the structure of the manuscript. The end of the Introduction section is too abrupt. By contrast, a great deal of information presented in the Material and Methods section (p.8) concern hypotheses and predictions and should be moved at the end of the Introduction section. Similarly, some details about the statistical analysis (around line 270) should be moved down to the Statistics section. This latter section should be carefully edited. In its present form, it contains many details which are more related with experimental procedure than statistical analysis.

Dear François-Xavier,

Thank you and the three reviewers for your work on this manuscript. You will see below that we tried to address all the reviewers' comments, in most cases by implementing their suggestions, occasionally by justifying why we preferred not to implement them, yet upgrading the manuscript to justify our choice. In the present document, we recall each of the reviewers' comments and answer it below, in blue.

We acknowledge the weaknesses of our approach, especially the limits of using a bioenergetic model fitted for another species (American shad, not Allis shad), at another phase of its cycle (migration, not reproduction) and not bearing the tags we imposed to our fish. As we cannot perform additional calibration experiments on our biological system, we left the analysis as it was, but allocate a substantial part of the discussion to the methodological limits of our approach.

As you and one of the reviewers proposed, we pulled the predictions from the 'methods' section up to the introduction, and removed the non-statistical methods from the section devoted to statistical analyses. However, contrary to your suggestion, we preferred to keep the description of the mixed model used to analyze the data from Leonard et al. (1999) before the Statistics section, because the output of this analysis is directly plugged in equation 1, which precedes the Statistics section.

We hope the revised manuscript will be suitable for recommendation, but are willing to consider further revision if required.

Yours,

Cédric Tentelier, on the behalf of all co-authors.

Reviews

Reviewed by Aidan Jonathan Mark Hewison, 2020-06-22 17:13

The dynamics of spawning acts by a semelparous fish and its associated energetic expenses
This manuscript presents empirical data issued from accelerometers born by female Allis shad to detect spawning events and infer associated energetic expenditure. The authors seek to link behaviour to energy expenditure and loss of body condition over the reproductive season. The empirical data indicated that female shad exhausted their energetic stock faster than their egg stock. The authors conclude that this approach using accelerometers is promising for monitoring behaviour-dependent energy expenditure in the wild.
This is something of a pilot study that investigates how high technology sensors can be employed in the wild to measure important physiological parameters such as energy expenditure and infer behaviours that are otherwise impossible to observe. As such, the sample size is small (N = 8), partly due to protocol failure that was beyond the control of the authors, and the monitoring quite short (1 month). However, the feasibility of this kind of study will be of interest to behavioural ecologists across a wide range of cryptic species. Furthermore, the monitoring of energy expenditure, the raw currency of evolution, is an exciting and promising technological advance. The manuscript is globally well-written, although there is scope for minor language improvement (particularly the abstract, see below), the analyses appear sound, and there is a lot of novel information here (although I am no specialist of the literature on this system).

Below I list the some points that the authors might like to consider:

Major comments:
1. Lines 120-21: it is a bit of a shame to just put this at the end of the introduction, it reads like a throw away; while earlier the authors nicely set up the context around optimality of coincidence between gamete exhaustion and energetic exhaustion (Lines 72-78). Why not translate this into a hypothesis about what to expect in terms of mismatch, for example, in relation to water temperature, as they appear to do in the Abstract (although this needs to be more explicit, see below).

We rewrote the introduction, where this hypothesis is reformulated (line 143): " In particular, we tested the predictions that shad were more active and spent more energy during the night than during the day, and that the individuals that better managed their energy expenditure (higher night time energy expenditure and lower daytime energy expenditure) died with fewer residual eggs."

2. Hypotheses (end of Introduction): indeed, following on from the previous comment, it would be nice to have explicit hypotheses rather than just description at this point in the manuscript. What are the expectations for the link between temperature and spawning schedule? And how should this translate into energy expenditure? Much of the required contextual information for this actually appears in the first part of the methods, describing the study system and the predictions, which is unusual to say the least. I would much prefer to see much of this information in the Introduction leading to clear predictions. Please try to re-work the text to improve this aspect, providing enough contextual information on the study system to generate predictions in the Introduction, while keeping accessory detail (e.g. life cycle) in the Methods.

We rearranged the introduction and methods sections, in order to give the predictions in the introduction, as well as the necessary information on shad. We left more general considerations on shad in the first part of the methods.

3. Calibration: The use of accelerometer data to estimate roundness is an interesting and novel application which the authors have calibrated against a traditional measure of body condition. However, I do feel that behavioural calibration for Tail Beat Frequency would also be useful, but there is no information provided. What information is available about the reliability of estimating tail beat frequency with accelerometer data? For spawning events, the authors did indeed do a calibration experiment, which is convincing, although it was based on a single couple. Could there be any inter-individual heterogeneity (see point 5) in the signal which might lead to faulty inference? This might be worth discussing.

Using the two fish on which we calibrated the acceleration pattern corresponding to the spawning act, we checked the correspondence between the TBF based on acceleration data and visual observation of swimming. However, we did not bother to run a proper calibration test (correlating visual and accelerometer-based measures) because the measure of TBF with accelerometers is quite common, and the wave pattern in our data was very clear. We now illustrate the TBF pattern in an additional panel of Fig. 1. We also indicate (p 13, l 286) "Acceleration on the sway axis is commonly used to measure TBF on fishes of different species and sizes (Kawabe et al. 2003; Tsuda et al. 2006; Broell and Taggart 2015; Brownscombe et al. 2018), and preliminary data on the two captive individuals mentioned above showed that our acceleration-based measurement of TBF corresponded to the visual measurement."

4. Analysis: eg Line 325: what is considered night vs. day changes very fast across the spring. If activity is linked to daylight cycles, then it might be worthwhile considering a moving window for what is actually night-time determined by the times of sunrise and sunset. This could provide better precision when contrasting behaviour and energy expenditure across the 24h cycle.

In fact, the experiment was performed in June, when the timing of sunrise and sunset does not change very much (not more than 15 minutes throughout the spawning season) compared to other periods of the year.

5. Inter-individual heterogeneity: while I appreciate that the sample size is small, it would still be nice to discuss the inter-individual variability that the authors observed. For example, Line 545 there is a total energetic expenditure that varies by a factor of 2, which is considerable. Could this be linked to reproductive phenology? behaviour (eg swimming depth)? or reproductive allocation (eg spawning events)? This discussion (eg following on from line 566) would be of course a little speculative given the sample size, but an interesting perspective.

As mentioned in other reviewer's comments (with which we agree), our estimation of total energetic expenditure may suffer several biases and we do not want to linger on the biological meaning of this estimate (we discuss them from a more methodological perspective in the second part of the discussion). Moreover, with such a small sample size, it is true that any discussion on the link between individual variables would be very speculative, something reviewers usually urge authors to avoid. We nevertheless present it as an interesting perspective (p 28, l 589): " Although our small sample size precludes conclusive inferences, the continuous measurement of spawning activity and energy expenditure at the individual level opens the door to the quantification and decomposition of interindividual variation in the schedule of reproductive behaviour and energy management. Moreover, coupling telemetric data on behaviour with additional fitness indicators such as offspring production (egg sampling and genetic parentage analysis) could inform on the strength of natural and sexual selection acting on these phenological strategies in the field (Tentelier et al. 2016). "

Minor comments:
1. Title: “costs” not “expenses” (also Line 589)

Done.

2. Abstract – this is the first time I have seen the expression “gametic budget”, and it is not immediately clear what that means. Consider modifying.

With "gametic budget", we meant the distribution of gamete across time (and possibly mates). We changed it to "gamete stock".

3. Line 45: synchronised among individuals? This needs to be clearer throughout.

We added: "(both individually and inter-individually)"

4. Line 45-47: this sentence is not clear; perhaps “When daytime temperature was higher, they remained at greater depths, and spent more energy on nights when they swam at faster pace”.

The sentence you propose seems to imply a relationship between daytime temperature and the depth at which the shad remained, but our analysis just shows that temperature was higher during daytime than nighttime and that shad remained deeper during daytime. We changed to a simpler form: "They experienced warmer temperature, remained deeper, swan more slowly and spent less energy during daytime than night time.".

5. Line 53-54: this is not easy to understand, can you help the reader interpret this prediction?

We changed the sentence to " Water warming will increase the rate of energy expenditure, which might increase the risk that shad die with a large stock of unspent eggs. "

6. Line 77: “steep” is a bit strange here, “pronounced”?

Done.

7. Lines 78-80: not explicit enough ‘eg “social factors” is very vague, what do you mean by that? This sentence reads like an add-on which needs a mini-paragraph in itself.

We transformed the sentence into a mini-paragraph (lines 80-88): " Beside energy expenditure, the temporal distribution of breeding events within a season may be linked to ecological, pheromonal or behavioural cues that synchronize breeding activity (Gochfeld 1980; Ims 1990; Fürtbauer et al. 2011). Here, internal and external stimulations probably interact, with the hormone-driven reproductive cycle and energetic condition determining whether an individual is ready to breed at a given time, and social or ecological factors triggering the actual breeding behaviour (Jovani and Grimm 2008; Koizumi and Shimatani 2016). At the ultimate level, reproductive synchrony affects intraspecific competition for resources (including mates; Emlen and Oring 1977) and the risk of predation on breeders or their offspring (Ims 1990). "

8. Line 84: not “supervision”, “recording”?

Done.

9. lines 96-105: I am no expert here, but I was surprised that the authors did not refer to the use of dynamic body acceleration to index energy expenditure (e.g. in the Introduction,), reviewed in Wilson et al. 2019 J Anim Ecol (https://doi.org/10.1111/1365-2656.13040

We referred to ODBE implicitly at line 102, but we now refer to it in a clearer way, citing Wilson et al. 2020.

10. Line 109: add “pattern”.

Done.

11. Line 117: “vertical plane” (and throughout the text)

Done throughout the text (9 occurrences).

12. Line 118 (and throughout): “thinning” is a bit strange, I would suggest “weight loss” or “loss of body condition”, but I acknowledge its not quite the same thing.

Indeed, the measure we get is related to the shape of the fish, not its weight. And body condition seems vague, as it can be measured through visual assessment (like the score used for cattle) or through a ratio involving mass and body size (as the Fulton coefficient of condition we used). We thought "thinning" would capture the idea of getting thinner, but apparently it doesn't. "Slimming" may be a better way to translate the French "amincissement" we have in mind, so we replaced "thinning" by "slimming".

13. Line 132: although I understand the link, capital breeding is not necessarily associated with fasting. It is strictly related to the fact that energy for offsetting increased needs during reproduction are sourced from stored reserves, rather than from current intake (see Jonsson 1997 Oikos 78).

Thank you for the precision and the reference. We agree that capital breeding does not necessarily involve fasting during reproduction (if the animal eats but fuels reproduction with energy stored beforehand), but it seems to us that species which fast during reproduction must be capital breeders, because they have no income. Hence, we keep this sentence as it is.

14. Line 149: replace “with a regular delay” by “by a consistent time elapse”

We replaced by "by a consistent time lapse".

15. L163: “deviate” not “depart”

Done.

16. Line 181-3: not clear

This sentence has been modified and moved to the introduction (line 143): " In particular, we tested the predictions that shad were more active and spent more energy during the night than during the day, and that the individuals that better managed their energy expenditure (higher night time energy expenditure and lower daytime energy expenditure) died with fewer residual eggs."

17. Line 292: do you mean “compared with” ?

Yes, we replaced "paralleled" by "compared".

18. Line 319: this is fine, but be careful to be clear throughout the text that the synchronisation you wish to look for is “among” not “within” individuals.

We added "with each other" at the end of this sentence. Throughout the text, we now indicate clearly that synchronization is measure among individuals.

19. Line 341: it’s a bit odd to come back to predictions at this stage of the manuscript. These aims/predictions should be clearer earlier (see above).

According to your comments, we moved the predictions from the methods section to the last part of the introduction (lines 131-156)

20. Fig 2: you do not have a measure of “longevity”. I presume this is supposed to be the number of days between capture and death, which may be related to longevity, but not necessarily. Please modify label.

In the legend, we indicated that what we call "longevity" is the number of days between capture and death. We have now changed the label to "days.alive".

21. Results: while the relationships are not strong, this is likely due to a lack of statistical power; it is notable however that the number of spawning acts seems to be negatively correlated with all the size/condition parameters, which is surprising to me, who knows nothing about this particular model. Worth discussing (eg Line 515)?

We have quite a lot of things to discuss, and we do not want to use space to discuss non-significant trends, especially if we do not have specific predictions. We nevertheless added (line 537) " Larger females, which have more eggs and more energy, did not perform more spawning acts (a nonsignificant tendency towards negative correlation was observed)."

22. Line 418: “total energy expenditure” not “energy expenditure cumulated”

Done.

23. Line 421: “representing” not “making”

Done.

24. Fig 4 legend: spell out what TBF is in the legend

We replaced "TBF" with "tail beat frequency".

25. Line 426-7: unless I have misunderstood something, this seems to be wrong. The authors say “the predicted weight loss was on average 1.5 times less than the actual loss”, but the Fig 4 indicates that real weight loss was much higher than predicted, by up to 4 fold! Also, it would be more usual to turn the thing around, to say “observed weight loss was XX times more than predicted…”

Indeed, if predicted loss was less than the actual loss (as we wrote), then observed loss was more than predicted (as you propose), so we see nothing wrong. But your misunderstanding probably comes from the way we expressed is, so we now adopt your wording (line 440): " the observed mass loss was on average 1.5 times more than predicted"

26. Line 439: “experienced” not “underwent”

Done.

27. Lines 437-445: I would like to see the effect sizes for each of these also in the text, as they are not easy to judge in the Fig 5.

The effect sizes were already given in the text, as R^2_m and R^2_c in parentheses. But we now give the values estimated by the models.

28. Fig 6B: this figure is the wrong way round, it would be more logical to show a decrease in condition over time from initial release until death

Do you mean we should turn the x-axis with lower values on the right and higher values on the left? The purpose of this plot is not to show that body condition decreases across the spawning season (this is obvious) but to show the positive link between two indicators of body condition. This would therefore seem very strange to swap the x-axis, because the positive correlation between theta and Fulton's index would be illustrated by lines going from upper left to lower right, which usually illustrate a negative correlation. We therefore prefer to keep the plot as it is.

29. Line 468-73: it would be nice to have the full table of AIC values for all tested models as a Table in the Supplementary materials.

We don't think the absolute value of the AIC is worth showing and discussing, because AIC is just used to compare models. We therefore prefer reporting in the text the ΔAIC of all tested models with the best one than making the reader open a supplementary file to find out the absolute AIC values, which are useless.

30. Lines 473-5: I do not really understand what this result means (or indeed why the period of the day is included in the model). Does this mean that the fish get fatter at night then thinner again during the day? Is body shape expected to vary at this temporal scale, within a 24h cycle?

Neither did we understand this result. If the value of theta was a perfect indicator of the fish's body shape, it would mean that the fish get thinner during the day and rounder during the night but we had no idea why shad would "inflate" at night. However, the third (anonymous) reviewer had a great idea to explain this: swim bladder inflation! The fish stay closer to the surface at night, so their swim bladder is more inflated than during the day, when they stay deeper. Indeed, including depth in the model of theta vs. time of the season shows a significant negative relationship between depth and theta. We now mention this in the discussion (line 696): " On top of slimming, θ was linked to depth, and increased during some periods when the fish stayed closer to the surface, in particular during nights. This was probably due to the inflation of the swim bladder, which represents around 8% of the volume of the fish, and inflates as the fish stays closer to the surface (Alexander 1966). Although here, swim bladder inflation introduced additional variation in our monitoring of fish slimming, it could also be the process of interest in some field studies, for example to untangle the contribution of swim bladder inflation and active swimming in the vertical migration of pelagic fishes (e.g. Pelster 2015). "

31. Line 489: delete “individual”

Done.

32. Line 493: give effect size, by how much?

We now indicate this in the results (line 408): " probability of a spawning act estimated 0.18 at 18°C and 0.23 at 19°C"

33. Line 494: “too high to allow shad to spawn all their eggs” is not clear, please clarify (females could not spawn all eggs prior to death due to energetic exhaustion? Or something like that).

We rephrased as: "may have been so high that shad died from energy exhaustion before having spawned all their eggs"

34. Line 494: “novel”, not “original”

Done.

35. Line 496: for what? Be explicit as to the use of this approach

We are now clearer (line 517): "…a promising method to monitor changes in animal condition in the field"

36. Line 529: “are reminiscent” not “remind”

Done.

37. Line 540: “end” not “final”

Done.

38. Line 541: “occurred in” not “struck”

We though that invoking a death that struck shad would add a dramatic dimension to the text, but we replaced "struck" by "occurred in".

39. Line 543 “with” not “to”

Done.

40. Line 557: “by” not “of”

Done.

41. Line 591: “fish have to conserve protein” not “fishes have to spare proteins”

Done.

42. Line 607: “wiped away” – this is not clear.

We rephrased as: " make untagged shad drift downstream"

43. Line 633: while this is true, it is difficult to conclude that the two are not related; from Fig 6B, it seems that there is some degree of consistency in the two measures with the exception of the individual represented by the green line which has a much steeper slope than the others

Indeed, but given the small sample size, we prefer not to discuss whether the lack of effect is due to one particular individual. The limit of the method we point at in this sentence also gives room for improvement.

Mark Hewison

Reviewed by Loïc Teulier, 2020-07-03 08:28

This study deals with a really great idea to improve the protocols of non-invasive technics, to accurately estimate the energy expenditure of fish in the wild. Throughout the introduction, the authors explain the central role of studying energy allocation in the ecological context of spawning periods, using a highly relevant model, the anadromous Allis shad, which is a semelparous fish. This latter point is of interest, because the cost of reproduction corresponds almost to the total energy expenditure of the spawning period. And as a capital breeder, no food supply is expected. As stated line 81-82, the energy budget estimation of wild fish is really challenging. And with this study, the authors give some new tools for measuring some proxies. For example, using the accelerometers for evaluating the roundness of fishes is really interesting, but as highlighted by the authors, this method needs further investigation to be improved.

I however have some concerns about the other methods, which are described below. - The first concern is about the choice of external tags. Intuitively, I would think that this kind of device would negatively impact the locomotion, and the cost of transport, through an increase of drag force, for example, which is intensively commented in the literature in diving birds (Ropert-Coudert et al. 2000) or in fish (reviewed in Cooke et al. 2012). The authors referred to Breine et al. 2017 for the tagging protocol, and therefore, fish body mass should be higher than 900 g to be in accordance with the limit of 2% cited in Breine. Unfortunately, the morphological characteristics of fish (body mass, body length, width and height) are missing, but seem highly relevant to estimate the effect of tags on fish used in this experiment. I would suggest to add a table with these characteristics.

We did not measure width and height (although we should have done this, to get the actual shape). Instead of adding a table with mass and length, we just give the minimum, median and maximum at the beginning of the results (line 386): " The minimal, median and maximal fork length and body mass of the eight females were 470, 510 and 550 mm, and 1320, 1635 and 1810 g."

The choice of external tagging was driven by several constrains. First, previous studies involving internal tagging showed that shad either die or permanently drift downstream (mentioned in the discussion, line 641). External tagging allowed fast manipulation and short anesthesia, and a good recovery. Second, we had to implant both a radio tag and an accelerometer, the size and shape of which would fit neither in the stomach (too shrunk) nor in the body cavity (full of eggs) where it may have prevented egg expulsion and impacted spawning behaviour. Third, internal implantation would have not allowed the 'theta as body condition' part.

As they did for characterizing the spawning act with the accelerometers, I wonder why the authors did not perform any preliminary test (i.e. in swim tunnel, in vivo respirometry) to validate their tagging protocol. A comparison among tagged, sham and control Allis shad would have been great to disentangle the consequences of these external tags in the fish performance. Indeed, some studies show that kind of results: Jepsen et al. (2015) described a list of possible effects of external tag, especially on swimming performance. Tagged fish get exhausted earlier than control ones, which could mean a higher energetic cost of locomotion with external tags. But Breine et al. explained that the effect of such external tag was still unknown in 2017.

We don't have a swim tunnel, but yes, this is something we should do if we want to develop telemetry for the estimation of energy expenditure. We now mention this in the discussion, in a part devoted to the limits of the method (see below).

Line 613: “tagging did not seem to impair swimming activity either, as TBF was not lower in the three days following tagging than in the remaining of the season.” Could the authors clarify this sentence: is it the “spawning” season? Therefore, I did not catch the argument that the TBF was not lower in the 3 days after tagging. It would have been great to compare fish locomotion parameters before and after tagging, instead.

We revised this part of the discussion to temper our conclusion. The point on TBF being different just after tagging refers to Føre et al. (2020) who showed that salmon were more active in the 3-4 days after tagging than later. We had read it wrong, and our increased TBF just after tagging may indicate post-tagging stress. To answer these comments, we changed the part of the discussion devoted to the impact of tagging and presence of tags: " However, shad may have suffered post-tagging stress, as indicated by the higher tail beat frequency (TBF) during the three days following tagging that during the remainder of the spawning season, consistent with the increased activity of Atlantic salmon during the three days after tagging (Føre et al. 2020). On the other hand, a higher TBF just after tagging may be due to the fish finishing their upstream migration, before settling near spawning grounds (unpublished radio tracking data). This moderate negative impact suggests that external tagging under the dorsal fin, provided fish are continually kept immerged and rapidly handled, is a suitable tagging technique for Allis shad (Jepsen et al. 2015; Breine et al. 2017). Although the swimming behaviour did not seem to be strongly impaired, the additional weight or drag force caused by the tag may have increased the energy required to perform this behaviour (Jepsen et al. 2015). In this case, the energy expenditure of tagged fish computed from equations derived from swim tunnel experiments performed on untagged fish would be underestimated. To develop the telemetric measurement of realistic energy expenditure, attention should be paid to both designing tags that do not impose too much additional energetic costs on individuals and estimating these additional costs."

For estimating the energy expenditure (EE) from the Tail Beat Frequency, the authors referred to equations from Leonard et al. (1999) and Castro-santos & Letcher (2010), which were fitted for adult American shad, during migration. In addition, these fish are iteroparous, which is an important point, when dealing with EE. The authors discuss about that very thoroughly in the “methodological considerations” (line 568 and beyond). I therefore wonder about the accuracy of the equations the authors used for EE calculations and if they are correct for Allis fish. Indeed, figure 4 B shows a huge difference between the modelled and the observed “weight” loss. Estimations of energy expenditure from biologgers need to be cautiously taken, due to the several parameters which are playing major roles. For instance, considering the fact that American shad were migrating and that Allis shad were spawning, swimming gaits are likely different and are sustained by two different muscle types, such as red and white fibers. As they are relying to two different metabolic pathways, red and white muscle would need separate equations to estimate EE (Gleiss et al., 2011). Again, preliminary studies in experimental conditions (swim tunnel + respirometry + accelerometers + video) would have been great to validate the EE calculation.

In line with my last comment, concerning the metabolic fuels: even if carbohydrates only represent a small part of energetic substrates for long-term exercise, glycogen and lactate are commonly used for burst swimming (Moyes and West, 1995; Weber and Haman, 1996), which appears closely related to spawning effort. I do not understand why the authors assumed that shad get 69% of their energy from lipids and 31% of energy from proteins (lines 280-284). They cited the study of Leonard and McCormick (1999), which reports the energy use pattern of migrating American shad. Here, the main goal is about estimating the energy expenditure during spawning period, which is unlikely relying on the same pattern of swimming effort. Indeed, line 586, the authors wrote about recovery after sprint, citing Kieffer 2000.

We agree with these two comments. The paper would have been much more solid if we had done a swim tunnel experiment with tagged Allis shad, but we haven't and we won't be able to do it for the revision of the paper. The question is then whether we should throw away all the analyses involving the estimation of energy expenses or keep them to illustrate the method. We chose the second option and devoted a substantial part of the discussion to the limits of the method we chose (lines 599-637), including your comments and the references you provided.

I also have minor interrogations, Please find them below: - How to discriminate females from males? Is there any specific sexual character? - A picture of the equipped fish could be useful, in addition to the figure 1. - Roundness estimation is a great idea but is the position of the accelerometer accurate enough to generate good data? But I totally agree with the authors, if this new method could be fine-tuned, it will be of great interest for field eco-physiological studies.

Females tend to reproduce one year later than males and are therefore larger, although the distributions of fork length overlap. We gently press the abdomen of every individual with a fork length > 450 mm and check whether milt is emitted. As we catch the fish close to the onset of spawning, males should already be spermiating, so large fish with no sperm are assumed to be females. We added (line 204): " gently press the abdomen to check the absence of sperm emission".

We added a picture of an equipped fish (in the tagging cradle) in figure 1.

We already acknowledged (line 684) that the position of the accelerometer on the fish may generate variability in the estimation of roundness. In particular, this probably generate interindividual variability (because the exact position differs across individuals) so mixed models with random individual effect must be used when analysing the effect of behavioural or ecological covariates on shifts in the theta angle. The question of accuracy is what we try to address by testing the four predictions related to the hypothesis that this measurement is reliable (lines 148-156).

Figure 5.D needed? I did not understand the message, because this graph shows average cumulated EE per 8h. It does not take into account the duration (in contrast with the Fig. 4A) and did not give any further information. It would have been great to get the same graph for each spawning event. For example, what the EE for fish #1 (grey plot), which got 7 or 8 spawning acts the 11/06 (Fig. 3A).

The idea here is to see how the diel rhythm of activity and temperature fluctuations translate into energy expenditure. During the day water gets warmer but shad stay deeper, where water is fresher, and are less active. Given that temperature and activity (TBF) affect energy, it is worth looking whether activity rhythm actually translates into rhythm of energy expenditure.

To link energy expenditure during a given period with the number of spawning events in that period, the reader can look at figures 3A and 4A, which respectively show the number of spawning events and the estimated EE as a function of time.

I am puzzled about using body weight instead of body mass.

Yes, we used both terms interchangeably as in common language, which is unfortunate in a paper dealing with acceleration. We have now replaced "weight" by "mass" throughout the text.

Reviewed by an anonymous reviewer.

1. Reviewer comments:

__P01L33__ depletion of gametic stock, so that individuals get exhausted just after their last egg is laid.
[comment]: "...after their last egg is laid and fertilized."
Corrected.

__P09L202__ before to weigh it
[comment]: "before weighing it"
Corrected.

__P16L369__ , hence tag retrieval.
[comment]: "..., hence making tag retrieval impossible."
Corrected.

__P22L463__ Moreover, the θ angle globally decreased over time for all individuals (LRT c2=516.9; p=0.001; R2m=0.29; R2c=0.78;Fig. 6.B), with a slope of -0.21 degree per eight hours, although it increased during some 8-hours periods.
[comment]: How much is the angle theta effected by the change in depth? Given that the swim bladder can make up much of the volume of a fish and therefore effect the body shape I would expect the depth to account for some change in this angle.
Thank you very much for this great idea, which would actually explain the puzzling increase in theta during night time. Indeed, shad stay closer to the surface at night, and we hadn't thought about the swim bladder. Moreover, the sensitivity of theta to swim bladder inflation illustrates how our approach could be used to study other things than slimming. We now refer to it:

In methods (line 356): " Depth was added in this model, to account for the possibly positive effect of swim bladder inflation (hence negative effect of depth) on body roundness."

In results (line 479): " The θ angle globally decreased over time for all individuals and was negatively related to depth, resulting in occasional increases in θ during 8-hour periods when shad stayed closer to the surface (full model R2m=0.28; R2c=0.79; Fig. 6.B). The effect of time corresponded to a slope of -0.63 degree per day (LRT χ2=518.4; p<0.001) and the effect of depth corresponded to a slope of -0.017 degree per cm (LRT χ2=9.6; p=0.002). "

In discussion (line 696): "On top of slimming, θ was linked to depth, and increased during some periods when the fish stayed closer to the surface, in particular during nights. This was probably due to the inflation of the swim bladder, which represents around 8% of the volume of the fish, and inflates as the fish stays closer to the surface (Alexander 1966). Although here, swim bladder inflation introduced additional variation in our monitoring of fish slimming, it could also be the process of interest in some field studies, for example to untangle the contribution of swim bladder inflation and active swimming in the vertical migration of pelagic fishes (e.g. Pelster 2015). This illustrates the potentially broad application of our acceleration-based approached, which, once refined, could be used to detect the many ecologically or behaviourally relevant changes in animal shape beyond slimming due to energy consumption…"

__P23L522__ Interestingly, while data collected at the population level indicate that spawning activity increases with temperature (Paumier et al. 2019), our data collected at the individual level showed that temperature increased the probability that a female performed some acts during the night, but not the number of acts it performed.
[comment]: A somewhat long, and unclear sentence. It is not clear what "number of acts" is performed, or not performed during the nights.
For clarity, we now refer to the components of the zero-inflated regression corresponding to each part: " the probability that a female performed some acts during the night (the zero-inflation component of the zero-inflated regression), but not the number of acts it performed (the count component of the zero-inflated regression)".

__P28L601__ ,due additional weight
[comment]: "..., due to additional weight"

Corrected.

__P28L613__ Tagging did not seem to impair swimming activity either
[comment]: This is not a clear argument to me given that the fish could just be compensating for the injury, additional weight or drag. From the previous observations for analyzing the spawning events a baseline TBF could be retrieved (optically through tracking etc.) in order to compare to the telemetry data.
In the preliminary observations performed for the calibration of spawning events, the fish were also tagged so they can't be used as control. We have no observation of untagged fish swimming. The best test would certainly be to make tagged and untagged fish swim in a swim tunnel with a respirometer, and test whether swimming at the same pace costs more energy to the tagged fish. We now acknowledge this by adding (line 658) " Although the swimming behaviour did not seem to be impaired, the additional weight or drag force caused by the tag may have increased the energy required to perform this behaviour (Jepsen et al. 2015). In this case, the energy expenditure of tagged fish computed from equations derived from swim tunnel experiments performed on untagged fish would be underestimated."

2. Note to Authors:The manuscript titled "The dynamics of spawning acts by a semelparous fish and its associated energetic expenses" is well written and clearly structured. The question is interesting and the approach well executed and thoroughly applied. Further, the methodology applied in this study incorporates novel applications of established hardware which greatly improves the understanding of the study species and specifically their behaviour, while being minimally invasive. Beside a few comments and requests I would consider this manuscript well suited for acceptance

Thank you for your relevant comments and suggestions on this manuscript. We have tried to implement them in order to improve the paper.