Recommender

Point 1 “Larger data set”:

At this time, we do not have a larger data set, we agree with the reviewers that this impacts the statistical tests interpretations. In the revised version, we insist on this drawback, see lines 19 to 23, lines 261 to 264 and lines 352–353.

Point 2 “Measurement error”.

We added this sentence within *image acquisition §*: We only test the potential measurement error of total area of the umbrella on a single specimen, taking three pictures after removing it from the glass. The error was lower than 0.05% using ImageJ®.

Vincent Bonhomme

My own comments (Vincent Bonhomme)

56: I grown there, and I would have removed "often"

Done

About the use of Matlab: perhaps you could point to open-source alternatives. eg R + magick package that binds to eponym library?

Being not specialists of R and magick package, we prefer to keep our code in Matlab and to provide it as an appendix. We also have in mind that dealing with a small sample, we write a very simple code, which is not optimal (with nested loops). For treating a larger sample and further researches, we will optimize it and use e.g. some C++ routine, but this is useless for the present paper.

Tab2: You can perhaps remove the N= and n=

Done

174: wonder -> tested, at least past tense everywhere? Also turns like "we continue the study" or "our interest grew" tell your story but can be made more formal.

Done

181+185: "does not make sense" + "bilateral runs test for randomness". I'm not sure to perfectly understand and perhaps this could be made clearer with one more sentence. Overall I think that the balance could be changed a bit towards the method versus the case study you used. With such sample sizes, I'm not sure it is worth discussing so much? I have the feeling this is the main blocking point for Reviewer 1.

Following remark RM4 of Julien Claude, we average the data for each jellyfish. This remark was done for the study of the relative distance, but also holds for the study of the relative size of the gonads. Then, these lines were removed and bilateral runs tests are not yet used in the

revised version. We also removed the boxplots of the ratios $\wp$ and D (Fig. 5 & 6 in the previous version) since the statistical study has been shortened and deals now with the mean individual ratios $\overline{\wp}$ and $\overline{D}$.

Also the method is well described but I think a raw output of the Hough transform on a jellyfish picture would help (eg around lines 91+)

The output of the original Hough transform can be visualized on a picture since it concerns the detection of lines, lines which are characterized by only 2 parameters. In our framework, there is 5 parameters for each ellipse and then the raw output of the Hough transform cannot be easily visualized on a picture, since it is an array with 5-dimensions. We highlight this point in the revised version, see lines around 194–202.

244: "have limits" I have got the same problems myself, segmenting overlapping objects. I'm sot sure it is really limiting, I'd say it's just painful to detect and filter out such overalapping structures. Sometimes I think we could even use them but, given the number of jellyfishes during a bloom, I think the filtering is the way to go.

We have reformulated our sentence, highlighting that our algorithm cannot be directly applied to images e.g. with overlapping ellipses, and mentioned that it has to be optimized, see lines around 321–324.

264: "distinguish the jellyfishes with 4 gonads and the jellyfishes with 5 gonads": if the idea is to discriminant between tetramerous and non-tetramerous why dont just use the number of detected gonads? The test you have made sounds more like a biological investigation in the how and why (as stated in lines 276+) rather than just the non-tetramerous proportion (that seems to have merits by itself) you introduced in Table 1. I think the best option would be to separate what Hough transforms brings here: i) first count the number of gonads, ii) allow to test biological hypotheses as you also got informative descriptors besides the number of gonads.

We have reorganized this part.

We also deleted this problematic §: *Even if the algorithm is optimizable and the sample small, the analysis of the morphometric data permitted to highlight the remarkable characteristics of the tetramerous jellyfishes and the non-tetramerous ones. The ratio $\wp$ rendering the size of the gonads brings out no significant difference between the two groups. The individual variability of the gonads eccentricity S^2 (higher for the jellyfishes with 5 gonads) and the ratio D rendering the distance of gonad center to umbrella center – higher for the jellyfishes with 5 gonads – are discriminant to distinguish the jellyfishes with 4 gonads and the jellyfishes with 5 gonads.*

Reviewer 1

Reviewed by Julien Claude, 2020-04-02 15:43

This paper shows that the Hough transform can be applied to Jellyfish images for extracting some shape parameters relative to gonad and umbrella morphology. It is an interesting paper as the Hough transform is not (yet) a method largely implemented in biology for image detection or for getting morphometric parameters.

RM1: The method requires some input from the user (ie defining ranges for some parameter values); the author should explain how they defined these ranges (and how long it took).

All the ranges are empirically chosen. We explicit that throughout the text

The method has some potential for automatic detection, however I wonder whether it is going faster than manual digitization and application of more traditional morphometric methods such as elliptic Fourier analysis. I do not think it would be necessary to compare both approaches because they are different, but it would be good to know how long it took to extract information from the 19 images to see whether this kind of procedure could be used for very large image dataset.

We have added in lines 250-308 details on the computational time: for each image it takes less than two minutes with a code which is not optimized (since it contains several nested loops).

RM2: In the Matlab algo, you should correct canny ege -> canny edge.

Done

RM3: Since most of the readership here is from the ecology background, it would be good to remind in more details the Hough transform method.

We added sentences to also answer to Vincent Bonhomme (lines 191–196)

RM4: There is a problem of pseudoreplication and misleading information for the relative distance of the gonad to the center. As you have done after, you should average your data for each jelly fish, indeed you test for differences among jellyfishes, not among gonads.

We have followed this remark and averaged the data for the relative distance of the gonad to the center, and also for the relative size of the gonad. For the relative size of the gonad, the Mann & Whitney test leads to the same conclusion as in the first version. For the relative distance of the gonad to the center, the p-value of the unilateral Mann & Whitney done is equal to 0.09729, which does not lead to reject the null hypothesis when taking the classical level of significance of 0.05. However, the p-value is small and suggests a weak evidence around this null hypothesis, rejecting it at a level of significance of 0.1.

I do not see clearly why you introduce the image related to jelly fish bloom, it does not seem to be directly related with the main objective of the paper.

The Figure 5 – jellyfish bloom – was added to show the final purpose of the project which is in three parts:

1– building the algorithm using the Hough transform (this paper)
2– testing the parallax errors due to photo acquisition (future paper)
3– in order to test the algorithm using aerial drone photography (final paper)

The present paper aims to only respond to the first purpose

RM5: Finally, and I think this should be discussed, we have no idea about variation within an individual and the variation introduced by the operator (ie way to take the photography...): what if the orientation of the jelly fish is different under the camera, I guess that ellipses parameters will vary? As the umbrella contract and dilate during the life of the animal, what about the variability of the computed parameters? I think giving an idea of percentage of error measurement is important here because you introduce a new method, we need to now whether there is some precision and repeatability (see paper of Yezerinac et al., 1992, Systematic biology for methods regarding the estimation of error).

Considering its *Bauplan*, the *Aurelia* jellyfish specimen always stretch out in the same planar position.

However: — We only test the potential measurement error of total area of the umbrella on a single specimen, taking three pictures after removing it from the glass. The error was lower than 0.05% using ImageJ®.

This point will be focus for the following step of our project.

This being said, I definitely think that this paper is interesting because it popularize an old methodology not enough well known in ecology. I would recommend to the author to pay attention to individual variation as it might challenge the test performed here (cf the small sample size).

This is a signed recommendation: Julien CLAUDE, Institut des Sciences de l'Evolution de Montpellier. Université de Montpellier

Reviewer 2

Reviewed by anonymous reviewer, 2020-04-21 11:25

I found it surprising that the studied species is not identified..? This should be explained.

We agree this was missing. A paragraph “The model Aurelia” has been added, see p.6 and we also add a part on the studied site, see p. 7

The sample size is extremely low to infer any morphometric difference among groups.

We also agree. However, we do not to have a larger sample at our disposal. The statistical part is then a first step, and all the conclusion have to been relativized. We highlight this in the revised version, see 19 to 23, lines 261 to 264 and lines 352–353.

No detail on the statistical tests applied is provided in the material and methods section. Such details are surprisingly given in the results section... The two sections, material and method and results sections are mixed up.

The sections have been reorganized. In the revised version, the new paragraph “Statistical analysis of some features” is devoted to the introduction of all the studied parameters and we

precise that Mann & Whitney tests are applied (note that bilateral run tests have been removed in the revised version, according to RM4 of Julien Claude).

It is a bit weird that the authors give the formula of the variance L226, as if it was an original statistics... just referring to the variance is fine.

We do not claim it is a new statistics. It is effectively the empirical variance of the observed series of N eccentricities for a given jellyfish. However, we prefer to keep the denomination 'individual variability of the gonad's eccentricity' since in this study, the observations are pseudoreplications and then may not be a statistical sample.

An analysis of measurement error, including the effect of jelly fish positionning and picture acquisition should be performed. This is particularly important as the aim of the present study is to apply this morphometric analysis to unstandardized images – as far as I understood correctly. Even on standardized images, the ME analysis is important to assess its amount relative to the biological signal. This is even more important as the authors are interested in relative variability of the gonads within individuals. For example, testing any paralax effect on the estimation of such variability is needed.

We also answer to that in RM4 and RM5 of the first reviewer