



July 30, 2020

Editor, JoVE  
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Dear editorial board of JoVE,

Enclosed is my revised manuscript: A method for assessing intertidal populations of the invasive European green crab, by Marissa D. McMahan. I have reviewed and agree with the contents of the manuscript. Below is my response to each of the many thoughtful points raised by the editor and reviewers, which I feel have greatly strengthened the manuscript.

Editorial Comments:

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammatical errors.

**I have thoroughly proof read the revised manuscript.**

Please include at least 6 keywords/phrases.

**I have added additional key words (Lines 13-14).**

Protocol Language:

1) Please ensure that ALL text in the protocol section is written in the imperative voice/tense as if you are telling someone how to do the technique (i.e. “Do this”, “Measure that” etc.) Any text that cannot be written in the imperative tense may be added as a “Note”, however, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Examples NOT in the imperative: 3.1, 3.2, 4.1, 4.2, etc.

**I have addressed this issue throughout the protocol text.**

2) Please remove the materials list and merge it into the table of materials.

**Done.**

3) Split long steps (e.g., 2.2, 2.3) into 2 or more steps. Some of your longer steps could be split into a step and a note.

**Done (see steps 4.5-4.12 for example).**

Protocol Numbering: Add a one-line space between each protocol step.

**Done.**

Discussion: JoVE articles are focused on the methods and the protocol, thus the discussion should be similarly focused. Please ensure that the discussion covers the following in detail and in paragraph form (3-6 paragraphs): 1) modifications and troubleshooting, 2) limitations of the technique, 3) significance with respect to existing methods, 4) future applications and 5) critical steps within the protocol.

**Thank you for this helpful feedback. I have rearranged the discussion to focus more on the above mentioned areas (Lines 257-308).**

References:

1) Please move the in-text http weblinks (line104) into the reference list, and use superscripted citations. **Done.**

2) Remove references to unpublished work (line 174, 248) **Done.**

3) Please spell out journal names. **Done.**

Table of Materials:

1) Sort the list alphabetically. **Done.**

**I thank the editor for this helpful feedback.**

Reviewer #1:

Manuscript Summary:

The manuscript describes a standard methodology for sampling crabs in the intertidal zone. It concentrates in sampling the invasive green crab but it could be used for sampling crabs in general. I believe it is a straight forward protocol that is well explained and provides all the information needed to conduct the sampling protocol. The gather data can be useful for monitoring the conservation status of a particular site but also can be used for management decisions related with invasive species. The authors put particular emphasis explaining that it is essential to have longterm data in order to be able to forecast an manage invasive species, I concur.

Major Concerns:

NO major concerns

Minor Concerns:

Since you are proposing people from different states and countries to use the same protocol why don't propose a webpage or repository where people can upload their findings? I think this could be particularly interesting for citizens that want to participate in scientific endeavours but are not familiarized with statistical analysis or with other uses of the compiled data.

**This is a great idea, and inspired me to create an intertidal green crab monitoring platform on Anecdata. This platform allows users to enter data post-survey, or enter directly into the app while in the field. I've referenced this platform several times in the text (e.g., Lines 78-81, 93-96, 262-263 etc.). Thank you for this suggestion, as I think the Anecdata platform will greatly expand participation.**

Reviewer #2:

Manuscript Summary:

I understand the purpose of this submitted manuscript to be to offer a suggested a standardized protocol for field sampling abundance of European green crabs within the northeastern US. I recognize that the author is trying to make the protocol appropriate and adaptable for use by a wide range of groups, and a fairly broad geography. This is a worthy goal.

Major Concerns:

I unfortunately find the techniques as described, are neither generalizable nor specific enough to satisfy the goals. The generality with which some steps are described to facilitate ease of sampling (e.g. delineating the low intertidal zone) reduces their utility for research purposes to the extent that they are not sufficiently informative. By contrast, some instructions are described with unnecessary specificity (e.g. GPS coordinate units, and see below comment about consistency). That is, it is not made clear what details of the protocol are actually necessary for the protocol to generate interpretable data. This may make this protocol very well suited for a particular program or collaborative effort, but that does not, to my mind, mean the protocol/method rises to the level of inclusion as a standard evaluated by peers and usable/interpretable for scientists beyond the scope of that program, i.e., it does not seem like a good fit for a methods publication.

**I understand these concerns and have made changes to the protocol to address them. See below for a detailed account of the edits, modifications, and additions that have been made.**

P1, Line 66-67: The scale at which monitoring occurs can be flexible, but should always be consistent from year to year.

This direction needs more specificity, and also is arguably unnecessary. Is "scale" referring to geographic scale, temporal scale? The "scale" of a single transect is fairly well standardized, so does this mean number of transects per site? Or "temporal scale," i.e., duration of monitoring season and frequency of sampling efforts? So, it is not clear to me

which aspects of the sampling are desired to be implemented consistently year to year. Either way, and perhaps more importantly, I disagree with the notion that there is any necessity that either of those scales needs to be implemented consistently from year to year for any reason inherent to the protocol itself. Consistency is primarily an advantage if the goal is inter-annual comparison within a site or group, but analytical approaches and data selection can always be adapted to account for inconsistent sampling. This paper states analysis is flexible and doesn't identify specific research questions that would require inter-annual consistency that I can find. If this consistency is desired by the authors to meet certain research questions or analytical goals, these should be stated explicitly. It seems any research goals or questions would arise within each "group" doing the sampling, and thus they may be best able to determine where consistency in implementation is most important.

**Thank you for pointing this out. I have removed this text, and added a section to the protocol entitled "Timing of survey work" that explains the target season (May-November) and target tides (zero of negative) (Lines 84-89). I agreed that the geographic and temporal scale of surveys should be determined by each individual user, depending on the questions they are trying to answer.**

Minor Concerns:

\* The language in site delineation section (section 2) is difficult to interpret. I fully acknowledge this type of description is difficult to communicate in words only, and is why video is a suitable application! Nevertheless, I find myself unable to evaluate how suitable the protocol is toward meeting the goal of being adaptable across sites because I can't be sure I am clear on how authors intend a site to be delineated. (a diagram may have been helpful). I believe the tape is stretched along the beach profile or perpendicular to the shoreline/waterline rather than "vertically" - which to me means away from the center of the earth. If a third of the true vertical distance is the desired benchmark for the low zone, a transit is a much more appropriate tool. My concern here would be that uneven beach profiles could lead to a widely variable zonation being included within the "low" zone confounded across sites, though maybe that would not be the case.

**I appreciate these comments, as the tidal height description as previously written was inadequate. I have revised this section to hopefully be clearer (Lines 109-118), and have added 2 diagrams (Figs 1 & 2) to help visualize the process. I understand that this zonation technique may lead to variations in tidal height within the targeted section of shoreline; however, I have also found that other techniques, such as tidal height corrections performed over the course of an entire tidal cycle, or using specialized equipment that measures tidal elevation, prevents many users from conducting the surveys. In an attempt to address your concerns about tidal height, I have also added instructions to avoid sampling elevated boulders/ledges that may be more representative of the mid-intertidal zone (Lines 148-151). Finally, I have included tidal height delineation as a potential limitation of the protocol in the discussion (Lines 272-274).**

P2 Line 116: for what horizontal distance along the shoreline should this zone be delineated?

**I have added instructions and a diagram to address this (Lines 99-101, 116-118, Fig 2).**

What is the definition of a site? That is, if groups want to do multiple sites, does it matter how far apart they are from one another?

**I have added a description of this as a special consideration in the discussion section (Lines 274-278).**

What counts as a "Movable" rock? Gravel?

**I have added an explanation of this (Lines 99-100, 142-143).**

Consider using "haphazard" instead of "random," which has a specific statistical definition not met by this technique, when describing the placement of the quadrat.

**Thank you for bringing this to my attention, as it was a holdover from an earlier version of the protocol where quadrat were randomly sampled along a transect. Haphazard is absolutely the term needed to describe this quadrat placement since there are habitat requirements (Lines 139 & 188).**

Is there a minimum distance quadrats should be separated from each other to be considered independent?

**I have added instruction for this (min 1 m, max 10 m; Lines 189-192).**

Temperature and salinity are described as optional, but without providing standards or protocols for their measurement or measurement tools.

**I have added details for this section, and equipment is listed in materials list (Lines 132-137).**

Use of a color index sheet provided by the participant may leave room for variability and false accuracy of data collected, as printers vary substantially. Will actual chips, or standardized printed guides be available to samplers wanting to use this protocol?

**This is an important point. I don't have a way (currently) to make standardized printed guides or provide chips, but I have added that investigators should source the paint chips specified in the protocol if they are going to use it (Lines 178-180). This has proven fairly easy for those of us using the protocol in Maine.**

Reference for Young and Eliot listed in references (18) appears to be a different article than intended. Color plates used in Appendix of this manuscript are originally published in: Alan M Young, James A Elliott, Joseph M Incatasciato, Mae L Taylor, Seasonal catch, size, color, and assessment of trapping variables for the European green crab *Carcinus maenas* (Brachyura: Portunoidea: Carcinidae), a nonindigenous species in Massachusetts, USA,

Journal of Crustacean Biology, Volume 37, Issue 5, September 2017, Pages 556-570,  
<https://doi.org/10.1093/jcbiol/rux068>

**Thank you for pointing this out, I'm not sure how I mixed up these references, but I've addressed it in the text.**

Reviewer #3:

Manuscript Summary:

This manuscript outlines a sampling protocol designed to examine spatiotemporal dynamics of green crab (*Carcinus maenas*) populations in rocky intertidal habitats in New England and Atlantic Canada. The protocol is designed to be accessible, both in terms of equipment and complexity. In particular, this design is meant to be accessible to citizen scientists and students in grades as low as 3rd and 4th grade. The design is also meant to be flexible could be applied to other intertidal species. The methods involve the determination of the sampling area (low to high intertidal), the deployment of 1m<sup>2</sup> quadrats (10 times ideally), the collection of crabs within the quadrats, and recording data on provided datasheets. The author presents data from 2019 surveys and 2018-2019 data collected by 3rd and 4th graders to illustrate the ability of the method to detect changes in *Carcinus* populations across space and time.

Major Concerns:

Overall, I think that this is clearly written and could be highly useful for the specific purpose outlined in this manuscript. However, I do have several issues with it. I will admit though that some of these issues may stem from the unique format (I am not entirely sure what content will be clearly included in the video other than the highlighted text) and the exact intent the technique will be utilized. The feedback I am providing assumes that if you were to give this publication to a 3rd grade class, could they perform this survey in a satisfactory fashion.

While a great deal of effort is given to examining and guiding the reader in identifying *Carcinus* color and shell characteristics (and it is well done), at no point to you actually tell the reader how to identify *Carcinus*. Granted, you highlight that this technique can be applied to a range of organisms, but this is a major omission. An image highlighting key features (the 5-3-5 spine pattern primarily) or at the absolute least a link to a page with identification information (perhaps both if the outside source includes other local crab species) is needed.

**Thank you for pointing this out, and what a great example of tunnel vision on my part! I've added a description in the Introduction of the 5-3-5 spine pattern (Lines 58-61), and I've also developed an Intertidal Crab Field Guide that is now included as Appendix 1. The guide has ventral and dorsal images of each crab species and each sex, as well as some distinguishing characteristics.**

Similarly, some guidance on identification of crab sex is needed. While you do include sex

identification for the crabs in the color guide, but do not indicate what characteristics indicate they are male or female. A dedicated section for this would be needed.

**I have added a protocol step addressing this (Lines 167-170), and also have a male and female image of each species on the Intertidal Crab Field Guide (Appendix 1).**

The measuring technique must also be clarified. When working with citizen scientists on crab measurements, I always stated they should measure from the widest part of the crab, usually tip to tip of the terminal carapace teeth. Just "width" can be misconstrued and in my experience, people find a way to confuse themselves.

**I have added clarification on the measuring technique on Lines 163-165.**

On line 177-179 there needs to be more clarification of the movement along the shore. I would set some predetermined distance along the shoreline for each quadrat, with some understanding that there may not be enough habitat or room to always utilize this approach. For example, in our trap sampling we space out every 10 m but perhaps 1m or 5m would be sufficient for this approach. Otherwise I just image people sampling in a very small area or tossing huge distances.

**I have added a description of the survey area (100 m, Lines 116-118), separation of the quadrats (min 1 m max 10 m apart, Lines 189-192), and a diagram (Figure 2).**

I understand that this is a method journal, and so I may be off the standards expected. However, I would like more information from your catch data figures (even error bars for standard error) to support your conclusions that you are actually detecting changes in crab abundance, size, sex ratio. This may be true, but it is equally true that it could the variation in your catch per quadrat would overwhelm any such differences. If this is the case, then that raises the issue of if your sampling efforts are sufficient to detect anything other than the presence of *Carcinus* (which is still a positive thing). I know you are not claiming statistical significance, but you allude to trends that I do not think the data fully supports in its current presentation.

**Thank you for pointing out that additional information would be useful in evaluating the sampling design. I have added  $\pm 1$  SE bars to Figure 3 to show that variance does not overwhelm the density trends, and I have included results from statistically comparing cumulative size frequency among populations using K-S tests (Lines 212-215). Standard error bars are not appropriate for the sex ratio or cumulative size frequency figures, as these are percent values.**

Minor Concerns:

One detail that came up often in my own surveys is the status of female *Carcinus*. Listing the option for ovigerous females is good, but I would also consider an option for juvenile (sexually immature) females. We determined this was a useful piece of information to include as while size is related to maturity you might see changes in size at sexual maturity over time. I would recommend changing the "Ovigerous" category to "Female status" or similar with the options of NA (for males), O (ovigerous), A (Adult) and J (Juvenile).

**I appreciate this comment, but am hesitant to design a protocol for use with citizen scientist/students that has them categorizing juvenile vs. adult crabs. I think the CW measurements are a more reliable measure. Other than the presence of extruded eggs, I'm not sure how users would externally determine if a crab is juvenile or adult (apart from size, which is already recorded...)?**

Similarly, when males of some species of Hemigrapsus become parasitized the exhibit feminized abdominal shapes, which can complicate sex ID for citizen scientists. While you do not need to get into that level of detail for all possible species that could be sampled using this protocol, it is worth mentioning somewhere that all aspects of the datasheet should be reevaluated depending on the species targeted.

**This is a very important observation and I have added a comment about this in discussion (Lines 264-267).**

Not a concern per se, but when you discuss how warming ocean temperatures can increase Carcinus abundance, I would also include that it can lead to Northern expansion off ranges. Ties in well with the idea of this low impact survey technique.

**This is an excellent point, and I have mentioned it in the introduction (Line 51).**

You might consider making the collection of water temperature and salinity a separate step. It may be optional, but it gets kind of buried in the section it is in.

**Thank you for this observation, I have made this a separate step and added more detail (Lines 132-137).**

What was the 2018 school catch data? You mention an increase in Hemigrapsus data in 2019 but never state what the previous year showed.

**I have added the 2018 catch data in the results (Line 222) and in Figure 8.**

On line 147, you refer to the quadrat deployment as random. It is not. Haphazard is the accurate term since there is a level of bias in the deployment (both in aiming for likely locations meeting criteria and just the way people throw things). I know that may be splitting hairs a bit but if you want to have actual random deployment you can (lay out transect perpendicular to shoreline, random number sheet, deploy quadrat at specific point).

**Thank you for bringing this to my attention. That language is a holdover from an earlier version of the protocol that was random, but haphazard ended up being a better fit for this work since there are habitat requirements. I have changed random to haphazard in the text.**

I really enjoyed the paint swatch color names. Not an issue I just wanted you to know.

**This made me laugh, so thank you for including. We can all use a little more laughter these days! I also enjoy the names, and my interns and I have a lot of fun using the**



**names rather than the assigned numbers. Flaming roasted pepper is so much more fun than 'red'!**

**I appreciate the opportunity to revise this manuscript to incorporate the many helpful suggestions provided by the reviewers.**

Sincerely,

A handwritten signature in dark ink, appearing to read 'Marissa McMahan', written in a cursive style.

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