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A method for assessing intertidal populations of the invasive European green crab

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TITLE:

Assessing Intertidal Populations of the Invasive European Green Crab

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KEYWORDS:

European green crab, Carcinus maenas, invasive species, rocky intertidal, ecological survey, population monitoring

SUMMARY:

Understanding spatiotemporal patterns in green crab population dynamics is essential for predicting and managing the ecological and economic impacts of this harmful invasive species. This protocol was developed in an effort to create a standardized method for assessing green crab populations in the rocky intertidal zone of the Northwest Atlantic.

ABSTRACT:

Invasive species have caused major disruptions to ecosystems worldwide. The European green crab invaded North America in the 1800s and is considered one of the world's 100 worst invaders by the IUCN. Observations of spatiotemporal green crab population dynamics are essential for predicting and managing the ecological and economic impacts of this harmful invasive species. This protocol was developed in an effort to create a standardized method for assessing green crab population dynamics in the rocky intertidal zone of New England and Atlantic Canada. The protocol was designed to be accessible to multiple users including researchers, educators, students, and citizen scientists. Although it was designed for surveying crab populations, this protocol is easy to adapt and could be used for any number of intertidal species. The resulting data collected using this protocol has a wide range of uses, including to inform ecological research, conservation efforts, mitigation strategies, and fishery development, as well as for educational outreach purposes.

INTRODUCTION:

Biological invasions can potentially disrupt species interactions and ecological processes, and may have far reaching ecological[1,2,3] and economic consequences[4]. The ability to successfully predict, mitigate, and adapt to invasions strongly depends on characterizing spatiotemporal population dynamics[5]. While a range of tools exist (e.g., population genetics, stable isotopes) and are emerging (e.g., eDNA) for tracking invasive species, traditional in situ monitoring

techniques continue to be widely utilized for assessing invasive species distribution and abundance.

The European green crab (*Carcinus maenas*) is an invasive species that was first detected in North America in 1817 and has successfully invaded ecosystems worldwide[6,7]. Green crabs have a multitude of negative impacts on local ecosystems, including reducing native bivalve populations through predation[8,9] , competing with native crustaceans for food and shelter[10,11,12] and destruction of eel grass habitat and subsequent changes to fish community structure[12,13,14]. Compounding these issues is the link between increasing temperature and increasing green crab abundance and/or range expansion[15,16], which has had severe ecological and socio-economic consequences in areas such as the Gulf of Maine, where warming is occurring faster than 99% of the world's other oceans[17].

On the eastern seaboard of North America, green crabs range from Virginia to Newfoundland. They are most commonly found on wave-protected shorelines, estuaries, and embayments in depths ranging from the high tide level to 5-6 m[18]. Their presence in the intertidal zone makes them an ideal marine species for shoreline surveys. The most distinguishing characteristic used to identify green crabs is the pattern of five spines or 'teeth' on each side of the eyes and three spines between the eyes (see **Appendix 1**). Their carapace (dorsal side) is typically a mottled dark green and brown, but ventral color patterns can vary greatly (see **Appendix 2**).

There are many organizations, researchers, citizen scientist groups, and educators currently conducting green crab population monitoring. However, the lack of a standardized protocol makes it difficult to compare datasets and to ultimately understand green crab populations on both a local and regional scale. This protocol is designed to quantify spatiotemporal population dynamics of green crabs in the rocky intertidal zone in New England and Atlantic Canada. Ideally, the development of a standardized, inexpensive, and easily adaptable survey will promote long-term monitoring efforts by a wide range of users, including researchers, citizen scientists, educators, and students.

Although green crabs are the target species of interest in this protocol, data is also collected for native Jonah and rock crabs (*Cancer borealis and Cancer irroratus*), as well as the invasive Asian shore crab (*Hemigrapsus sanguineus*). These are crab species commonly found in the rocky intertidal zone in northern New England, and trends in their population distribution and abundance have ecological and economic significance. An Intertidal Crab Field Guide was developed alongside this protocol to aid in crab identification (Appendix 1) specific to northern New England. A data entry and storage platform called "Intertidal Green Crab Project" was also developed for this protocol using Anecdata[19]. Anecdata is a free online citizen science platform that provides web-based and mobile solutions for gathering and accessing observations, and provides a user-friendly platform to easily collect, manage, and share data.

PROTOCOL:

1) Timing of survey work

1.1) Conduct surveys between May-November, during the height of productivity in the intertidal zone.

1.2) Schedule surveys around negative or zero tides (generally new and full moon cycles) to allow for adequate time in the low intertidal zone (i.e., at least 2 hours).

2. Pre-survey preparation

2.1) Locate and print all field guides and data collection sheets (see Appendices) prior to conducting the survey if that is the preferred method. If using the Anecdata app for field guides and data collection, visit the Anecdata website and join the Intertidal Green Crab Project[19]. The data collection sheet and Anecdata collection categories are identical.

3. Site selection and description

3.1) Locate a wave-sheltered rocky intertidal site with cobble (i.e., not stable, rolled by wave action) and algal canopy habitat. Ensure that there is at least 100 m of shoreline to accommodate the planned sampling.

3.2) Record the location of the study site using a global positioning system (GPS) unit, or a device such as a smart phone that has GPS capability (e.g., many compass apps are free to download or are already pre-programmed on smart phones). Record site coordinates on the Intertidal Survey Data Sheet (Appendix 3) or directly into the Intertidal Green Crab Project on Anecdata.

3.3) At the predicted low tide time (determined from the NOAA Tide Predications website or an app such as Tides) run a 50 m transect tape vertically from the low intertidal zone (i.e., the splash zone) to the high intertidal zone (i.e., the black microalgal zone that is typically dry at high tide). Divide the resulting distance into three equal sections: high, middle, and low (**Figure 1**). The low intertidal section, parallel to the shoreline, is the target sampling area.

3.4) Within the low intertidal zone, measure a distance of 100 m parallel to the shoreline (**Figure 2**) and establish permanent markers delineating this zone using rebar or natural permanent landmarks such as immovable boulders, ledge, dock pilings, etc.

4. Conducting survey

4.1) Prior to arriving at the survey site, record the following information on the Intertidal Survey Data Sheet (**Appendix 3**) or directly into the Intertidal Green Crab Project on Anecdata: site name, sampling date, participants, time and height of low tide at the location/date to be sampled (determined using the NOAA Tide Predications website, or an app such as Tides), and lunar phase (determined using a lunar calendar such as www.moongiant.com).

4.2) Upon arriving at the survey site, locate the 100 m section of low intertidal shoreline where the survey will be conducted, unpack gear, and organize data sheets and field guides.

4.3) Optionally, measure water temperature using a waterproof digital thermometer in the shallow water adjacent to the sampling area.

4.3.1) Measure salinity by placing several drops of water collected adjacent to the sampling area onto the refraction prism of a salinity refractometer.

4.3.2) Record water temperature in °C and salinity in parts per thousand (ppt) on the Intertidal Survey Data Sheet or directly into the Intertidal Green Crab Project on the Anecdata app.

4.4) Begin the survey by haphazardly tossing the 1 m^2 quadrat within the predefined low intertidal zone area that runs parallel to the shoreline (a transect tape is not needed to conduct the survey because the sample area has already been defined). Record a visual estimate of the percent of both moveable rock (i.e., cobble/gravel that you can look underneath) and algae canopy cover (e.g. *Ascophyllum* or *Fucus spp.*) within the quadrat to the nearest quarter percent (i.e., 0, 25, 50, 75, or 100%). A rocky intertidal habitat is often patchy and can contain areas of sand, mud, ledge, or other habitats where green crabs are not found.

4.4.1) To avoid skewing density estimates by sampling unsuitable habitat, only sample quadrats with greater than 50% movable rock, or greater than 50% algal canopy. Also avoid sampling areas where boulders or ledges are noticeably elevated above the profile of the shoreline within the low intertidal zone, as this habitat may be more representative of the mid intertidal zone.

4.5) Within each quadrat, lift moveable rocks or cobble and carefully move aside algae to look for crabs. Be sure to replace all rocks and algae as they are found. Collect all of the crabs found and store them in a bucket until the entire quadrat has been searched.

4.6) Identify the species of each crab using the Intertidal Crab Field Guide (**Appendix 1**, or source on Anecdata project platform) and record using the species codes listed on the Intertidal Survey Data Sheet (**Appendix 3**) or on the Intertidal Green Crab Project on the Anecdata app.

4.7) Measure the carapace width (CW) of each crab across the widest part of the carapace, spanning from tip to tip of the terminal spines, to the nearest 1 mm using Vernier calipers.

4.8) Use the abdomen (or 'apron') on the ventral side of the crab to determine sex. Male crabs tend to have a narrow, pointed abdomen and female crabs tend to have a wider, beehive shaped abdomen (**Appendix 1**). Only record sex for crabs ≥ 10 mm CW.

4.9) For all crabs, record number of claws, number of legs, shell condition (i.e., hard- or soft-shell as determined by whether the carapace resists (hard) or gives (soft) when finger pressure is applied), and the presence (i.e., ovigerous) or absence of extruded eggs for females.

4.10) Optionally, record the color for green crabs, but not other crab species, using the color protocol developed by Young and Elliot[20] (**Appendix 2**). This protocol should only be used if the actual paint chips can be sourced and brought into the field, as printed versions can vary substantially. Identify pre-molt shell condition for green crabs using external pre-molt indicators (**Appendix 4**, or source on Anecdata project platform). Pre-molt green crabs are within 3 weeks of molting and are of particular interest to the emerging soft-shell green crab fishery[21,22].

4.11) Return all crabs to the habitat within the quadrat once all measurements and characteristics have been recorded.

4.12) Continue haphazardly tossing the quadrat within the predefined low intertidal area until a total of 10 m^2 is sampled. Continually move forward along the low intertidal area of shoreline and ensure that quadrats are separated by a minimum of 1 m so that resampling does not occur and a maximum of 10 m so that the survey area does not exceed 100 m.

5. **Data management and analysis**

5.1) If using data sheets, check all raw data sheets for errors and legibility post-survey, photocopy, scan, and archive. Use the photocopy for data entry into an excel spreadsheet (see **Appendix 5** for example) or into the Intertidal Green Crab Project on Anecdata[19]. Store scanned data sheets electronically.

5.2) Conduct data analyses appropriate to the design of the study. Useful population metrics include crab density (total number of crabs divided by total number of quadrats sampled), sex ratio, cumulative size frequency, injury rate, shell condition ratio, and overall species encounter rates (e.g., % of native vs. invasive crabs).

REPRESENTATIVE RESULTS:

In 2019, this protocol was used to conduct monthly intertidal green crab surveys at three locations from May-November (Sandy Point, Yarmouth, ME (43°46'17.92"N, 70° 8'45.52"W), Robinhood Cove, Georgetown, ME (43°48'13.80"N, 69°44'50.97"W), and New Meadows River, West Bath, ME (43°51'17.84"N, 69°51'55.20"W)), and at one location from May-August (Damariscotta River, Walpole, ME (43°56'9.42"N, 69°34'52.75")). The data collected indicated wide variations in spatial and temporal green crab population density (**Figure 3**) and sex ratio (**Figure 4**), as well as significant differences in cumulative size frequency among sites (e.g., DMC crabs were significantly smaller and SP crabs were significantly larger than the other populations sampled (Kolmogorov-Smirnov test, $p < 0.05$ for all comparisons), **Figure 5**). Shell condition changed seasonally within each site, with peak pre-molt and soft-shell phases for males in the spring and for females in the spring and fall (**Figure 6**).

The protocol was also used in 2018 and 2019 by Georgetown Central School 3rd and 4th grade students who surveyed the same site in Georgetown, ME each year in October. They observed a shift in the population from being dominated by 5-15 mm CW sized crabs in 2018 to 15-30 mm CW sized crabs in 2019 (**Figure 7**). They also observed an increase in the percent occurrence of the invasive Asian shore crab from 3.5% in 2018 to 9% in 2019 (**Figure 8**).

FIGURE AND TABLE LEGENDS:

Figure 1: Tidal height zonation. Diagram of tidal height zonation along the shoreline. Surveys are conducted in the low intertidal zone (i.e., the lower third of the intertidal zone that runs parallel to the shoreline).

Figure 2: Defining survey area. Image of a survey site in Yarmouth, Maine with tidal zonation displayed in yellow and survey area in green.

Figure 3: Green crab density. Average monthly density (#/m^2) of green crabs at Damariscotta River, New Meadows, Robinhood Cove, and Sandy Point intertidal survey sites. Error bars represent ± 1 standard error.

Figure 4: Green crab sex ratio. Sex ratio by month for green crabs at Damariscotta River, New Meadows River, Robinhood Cove, and Sandy Point intertidal survey sites. Sex ratio is expressed as the percent of the population that is male. The green "0" appearing in July indicates 0% male crabs and 100% female crabs at Robinhood Cove in that month. Blue "NA" indicates no data collected at Damariscotta River from September-November.

Figure 5: Green crab size frequency. Cumulative size frequency of green crabs at Damariscotta River, New Meadows River, Robinhood Cove, and Sandy Point intertidal monitoring sites for all months combined (May-November for Damariscotta River, New Meadows River, Robinhood Cove, and May-August for Damariscotta River).

Figure 6: Shell condition ratio. Monthly shell condition ratio of A) female and B) male green crabs at New Meadows River intertidal monitoring site.

Figure 7: Student-collected size frequency. Size frequency of green crabs surveyed by Georgetown Central School 3rd and 4th grade students in 2018 and 2019.

Figure 8: Species occurrence. Percent occurrence of crab species surveyed by Georgetown Central School 3rd and 4th grade students in A) 2018 and B) 2019.

DISCUSSION:

This protocol describes a survey method for assessing spatial and temporal trends of crab populations in the rocky intertidal zone that is accessible to multiple users including researchers, educators, students, and citizen scientists. The benefits of this protocol include the following: it does not require specialized or expensive equipment, the methodology is

approachable for a wide range of skill levels (e.g., 3rd and 4th grade students have successfully used it), and it can easily be adapted to suit the needs of the investigator. In addition, the optional use of Anecdata allows for observations to be recorded directly into a data storage platform and accessed by a broad array of users. While the species of interest here is the invasive European green crab, this protocol could be used for any number of intertidal species. However, it is important to carefully consider unique characteristics and behavior of each species targeted when generalizing this protocol.

Many citizen scientist and education initiatives that conduct shoreline surveys for green crabs do not utilize a standard unit of measurement (e.g., quadrat) or target a specific area of the shoreline (e.g., low intertidal zone), making spatial and temporal comparisons difficult. This protocol utilizes simple techniques to target a standardized area along the shoreline. However, this is also a limitation of the protocol in that more rigorous methods or specialized equipment would yield more exact tidal height measurements within the survey area. The survey area is defined as 100 m of shoreline in the low intertidal, but the site should further be defined as a section of shoreline delimited by natural or manmade boundaries that are easy to distinguish, or by a minimum boundary distance of 300 m from the survey area. This is an important delineation if investigators are considering surveying multiple sites in close proximity.

The protocol also includes collecting detailed measurements that can be useful for researchers and managers. It is important to note that not all measurements will be appropriate for all users, such as identifying pre-molt green crabs, which takes a considerable amount of training. Furthermore, sex identification is only recommended for crabs ≥ 10 mm CW, as the sex of smaller juveniles can be difficult to determine. Although it is not part of the protocol, it is also recommended that citizen scientists, students, educators, and others who may not be familiar with the target species utilize a digital camera to document uncertainties and allow for troubleshooting outside of the survey timeframe or to upload to the Anecdata platform for review.

As ocean temperatures continue to warm[23,24] and become more favorable for green crab populations in the Gulf of Maine and Atlantic Canada[15,16], assessing their distribution and abundance will be critical for timely mitigation and adaptation strategies. For example, recent efforts to develop a soft-shell green crab fishery that would allow coastal communities to benefit from green crabs have relied on population monitoring data to determine when and where to target pre-molt and soft-shell crabs[21,22]. Bounty programs and other removal efforts may also benefit from distribution and abundance data that can aid in determining how best to allocate time and resources. Similarly, municipal shellfish management programs often conduct yearly conservation activities such as replenishing local mudflats with hatchery raised clam seed and local trends of green crab distribution and abundance could inform best practices for these conservation measures.

Finally, this protocol has proven to be an effective outreach and education tool for students and citizen scientists. In addition to the 3rd and 4th grade students who have been using the protocol for several years, it has also been used with undergraduate students from the University of

Southern Maine, and community service volunteers from Idexx Laboratories. Furthermore, an adapted version of this protocol is now being used by the Gulf of Maine Research Institute's Vital Signs Program, which partners with Maine students and citizen scientists to monitor invasive species. Ultimately, the data collected using this protocol has a wide range of uses, including to inform ecological research, conservation efforts, mitigation strategies, and fishery development, as well as for educational outreach purposes.

ACKNOWLEDGMENTS:

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DISCLOSURES:

The author has nothing to disclose.

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threat of climate change, sustainable development, and efforts to eradicate poverty [Masson-Delmotte, V. et al. (eds.)]. World Meteorological Organization, Geneva, Switzerland, 32 (2018).

Figure 1

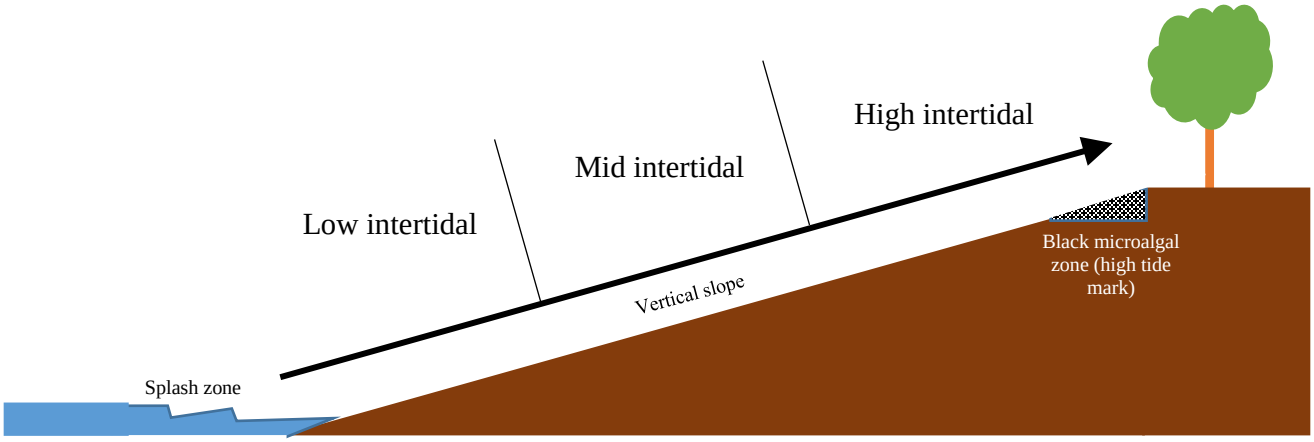

Figure 2

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Figure 3

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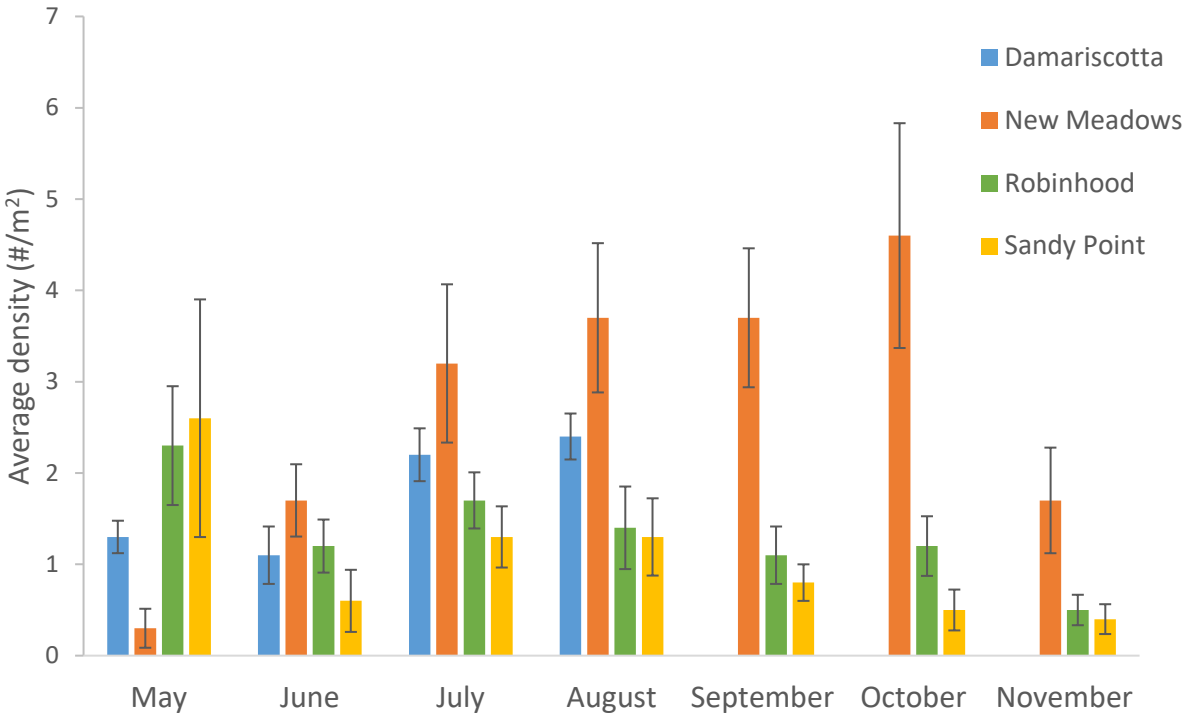


Figure 4

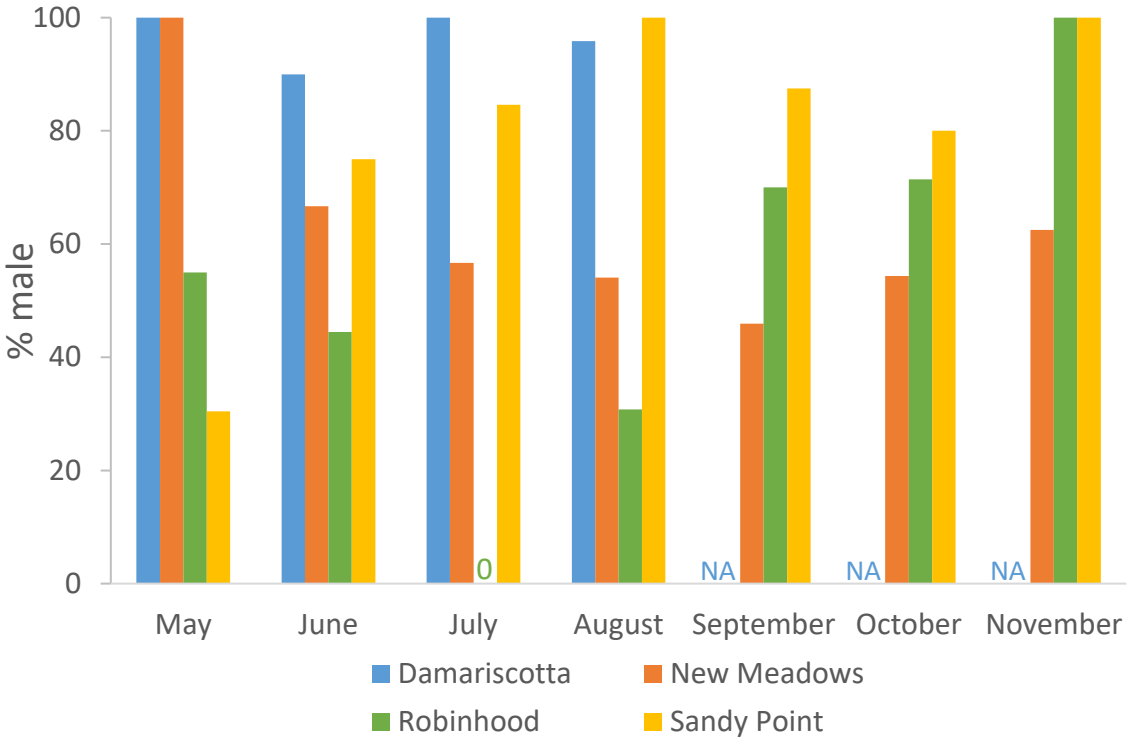
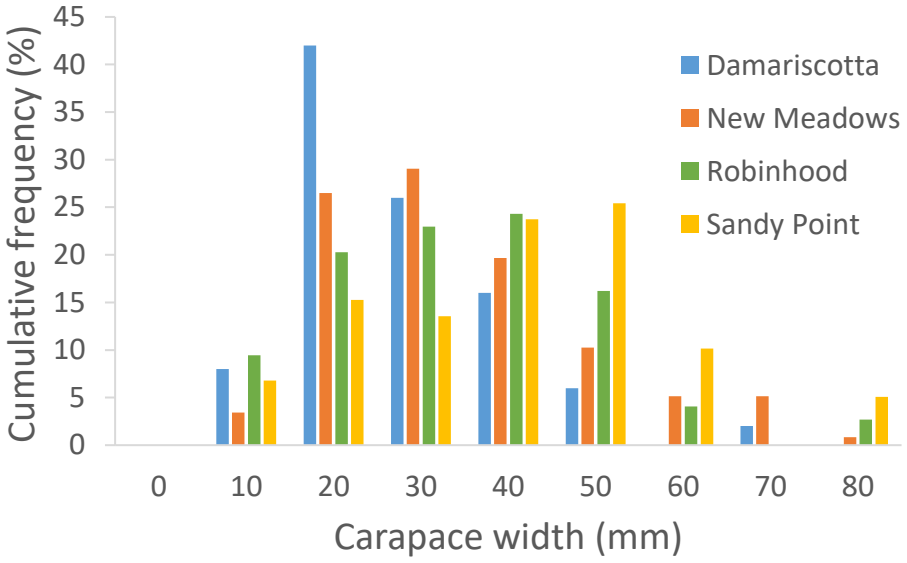


Figure 5



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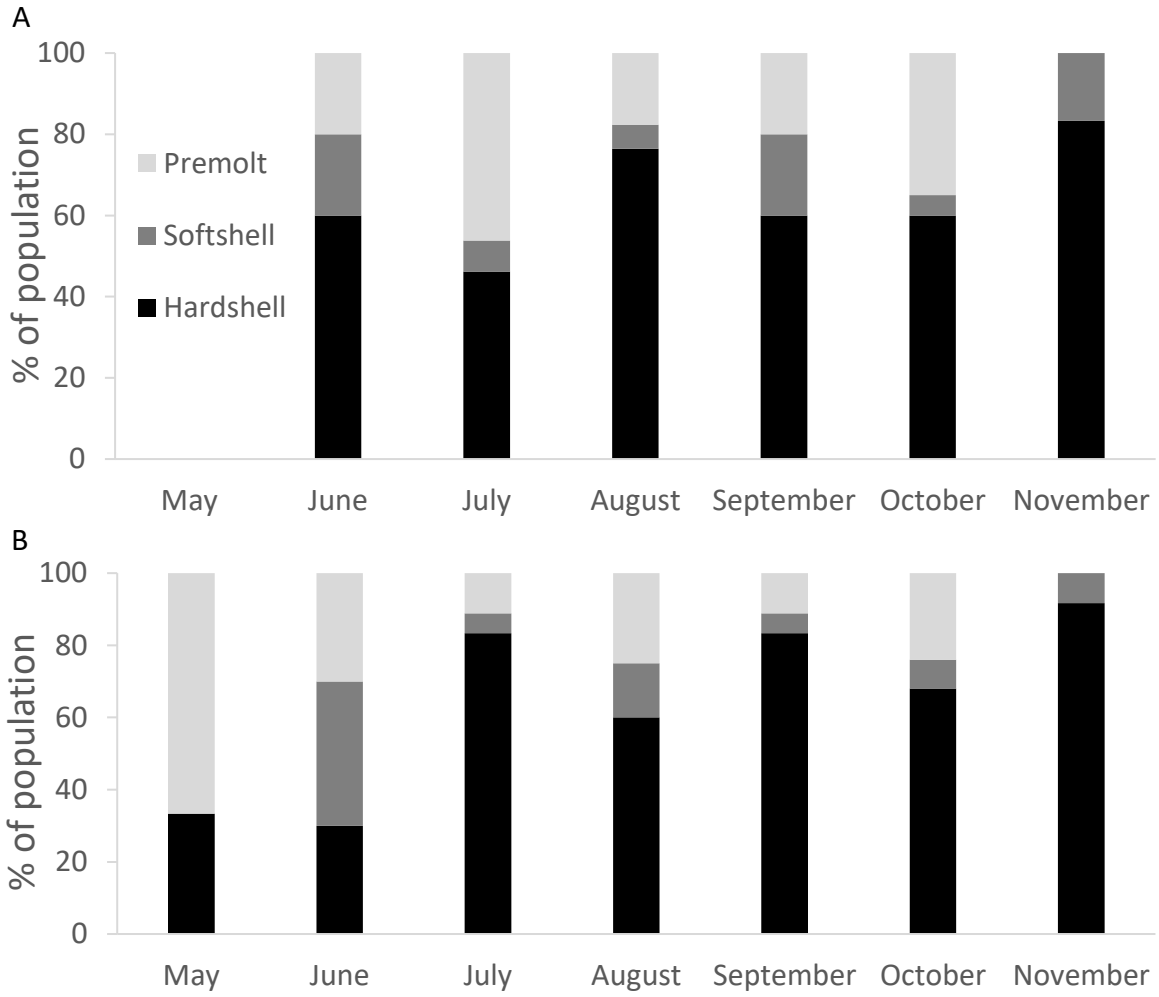
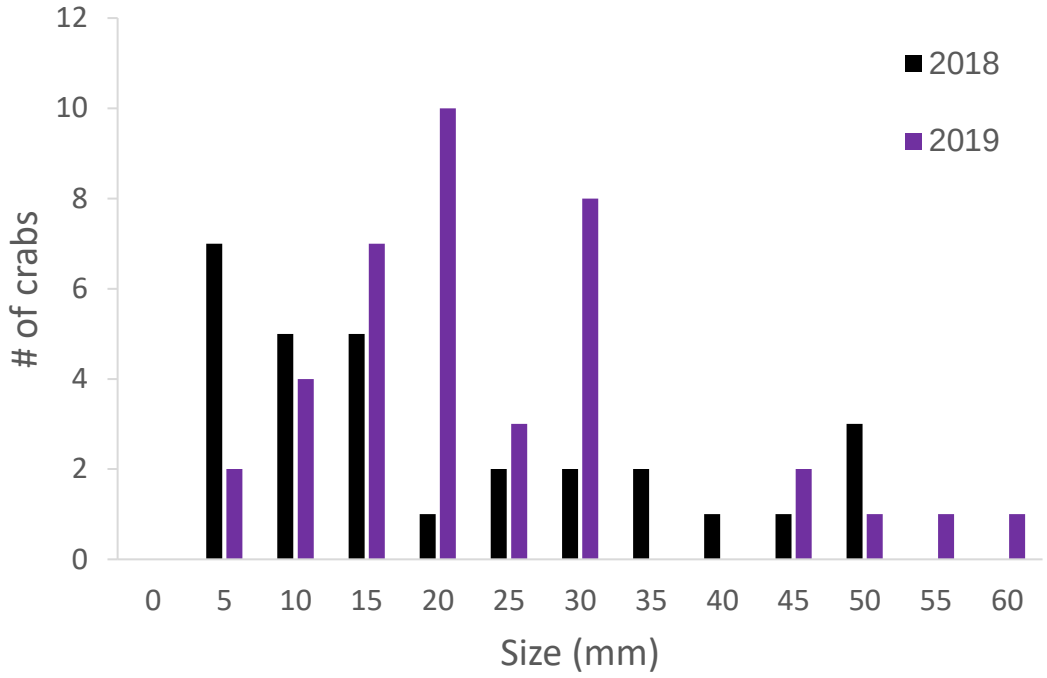


Figure 7



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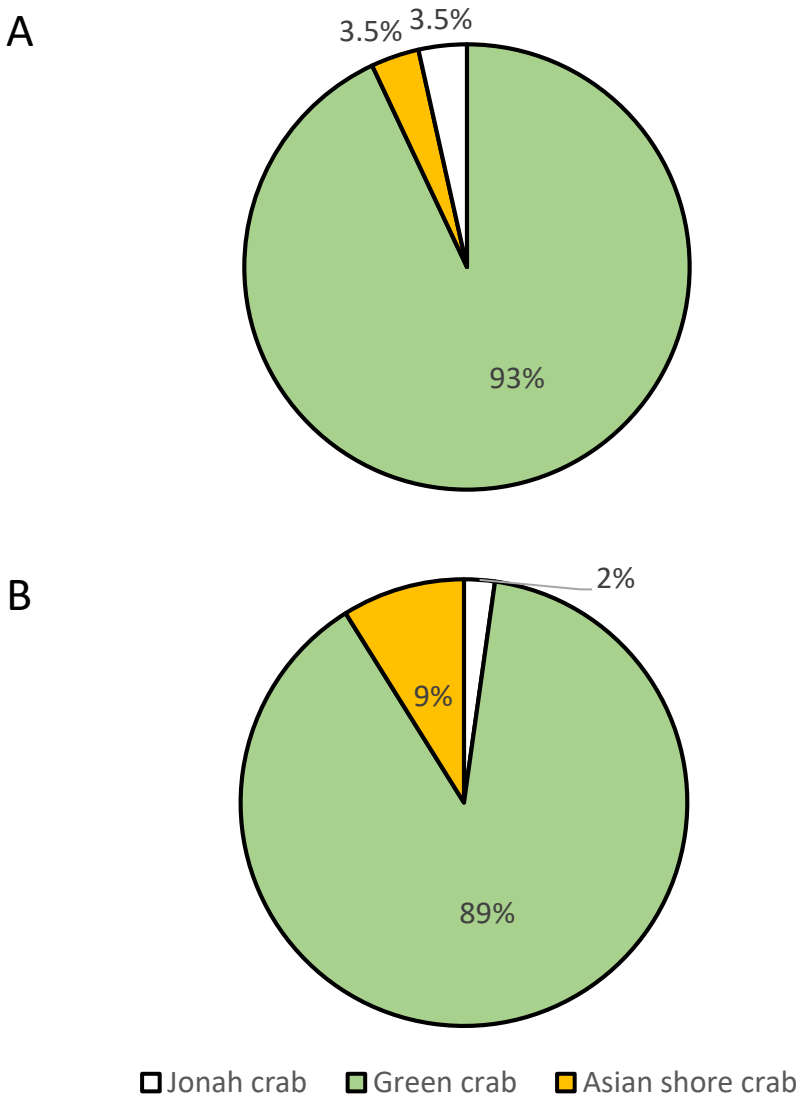

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
1 m^2 PVC quadrat (1/2" PVC)	Any hardware/home improvement store		PVC can be sourced at any hardware/home improvement store and cut into 1m lengths to form quadrat (4 1/2" PVC elbows will also be needed to connect 1 m lengths into square)
1/2" rebar	Home depot	5152	*optional (for marking low intertidal area)
40 m Fiberglass Transect Tape	Grainger	3LJX1	
5 gal bucket	Home depot	05GLHD2	
Ade Advanced Optics Salinity Refractometer	Amazon		*optional
Clip board	Any office supply store or Amazon		
Uei Waterproof Digital Thermometer	Amazon		*optional
Vernier calipers	Bel-Art		Many companies make calipers, however our preferred brand is Bel-Art which can be sourced on Amazon

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.

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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 traight 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 endevaours 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 1m2 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 ± 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,

Marissa McMahan, Ph.D
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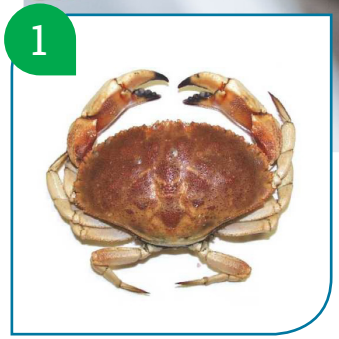
Brunswick, ME 04011
(207) 837-5987
mmcmahan@manomet.org

A Field Guide to Intertidal NE Crabs

Native and invasive crab species commonly found in the intertidal zone in northern New England

1. JONAH CRAB
2. ROCK CRAB
3. GREEN CRAB
4. ASIAN SHORE CRAB



PURPOSE

The purpose of this field guide is to help identify the native and invasive crab species most commonly found in the intertidal zone in northern New England. This guide is also a tool for conducting intertidal green crab quadrat surveys. The protocol for these surveys can be found at manomet.org/project/fisheries/.

OVERVIEW

The two native crab species that can be found in the intertidal zone are the rock crab (Cancer irroratus) and the Jonah crab (Cancer borealis).

The two invasive crab species commonly found in the intertidal zone are the European green crab (Carcinus maenas) and the Asian shore crab (Hemigrapsus sanguineus).

Common identifying features:

- Green crab: 5 spines or 'teeth' on either side of eyes, 3 spines/teeth in between eyes.
- Asian shore crab: 3 spines on either side of eyes and distinct banding on legs
- Jonah crab: bumby, non-distinct spines
- Rock crab: smooth, pointed spines

Sex distinction: broadly, male crabs have a narrow, pointed, triangular abdomen or 'apron' and female crabs have a wider more beehive shaped abdomen. Distinctions within species shown below.

COMPILED BY: Marissa McMahan (mmcmahan@manomet.org)

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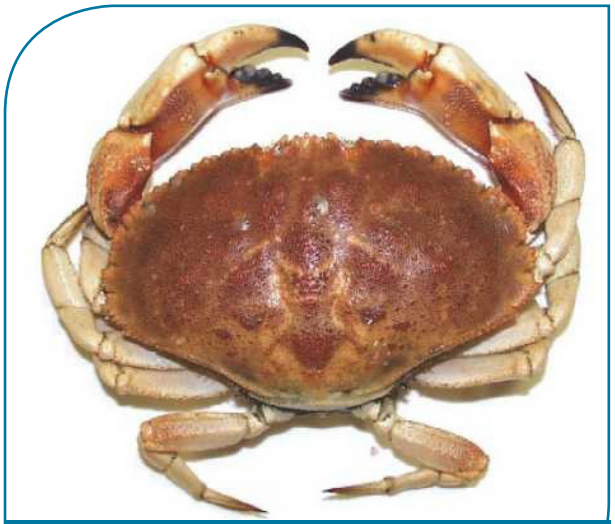
A Field Guide to Intertidal NE Crabs

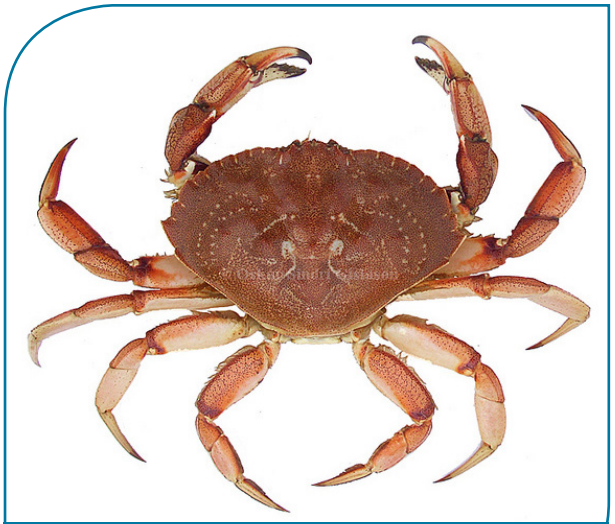
Native Crabs



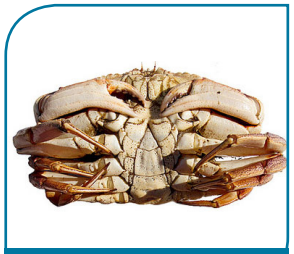
CANCER BOREALIS (JONAH CRAB)
Bumpy spines.

MALE

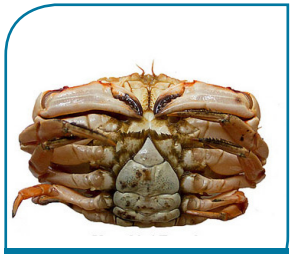
FEMALE



CANCER IRRORATUS (ROCK CRAB)
Smooth spines.



MALE



FEMALE

Invasive Crabs

CARCINUS MAENAS (GREEN CRAB)
Five spines on each side of eyes.

MALE

FEMALE

HEMIGRAPSUS SANGUINEUS (ASIAN SHORE CRAB)
Three spines on each side of eyes. Distinct banding on legs.

MALE

FEMALE

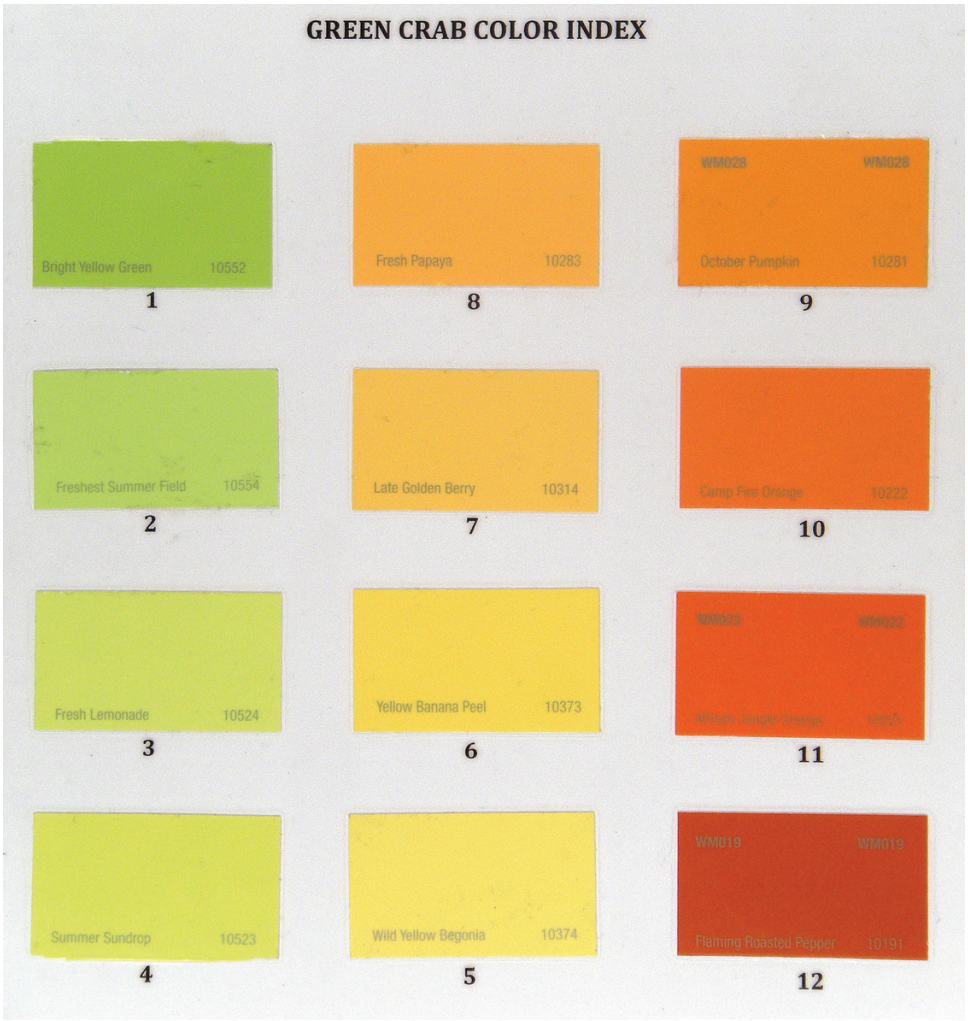


Figure 2. Color index used to quantify ventral coloration in *Carcinus maenas*. Color chips are sections of paint swatches. Color of third maxillipeds was matched to a color chip to determine the appropriate color index (CI) value.

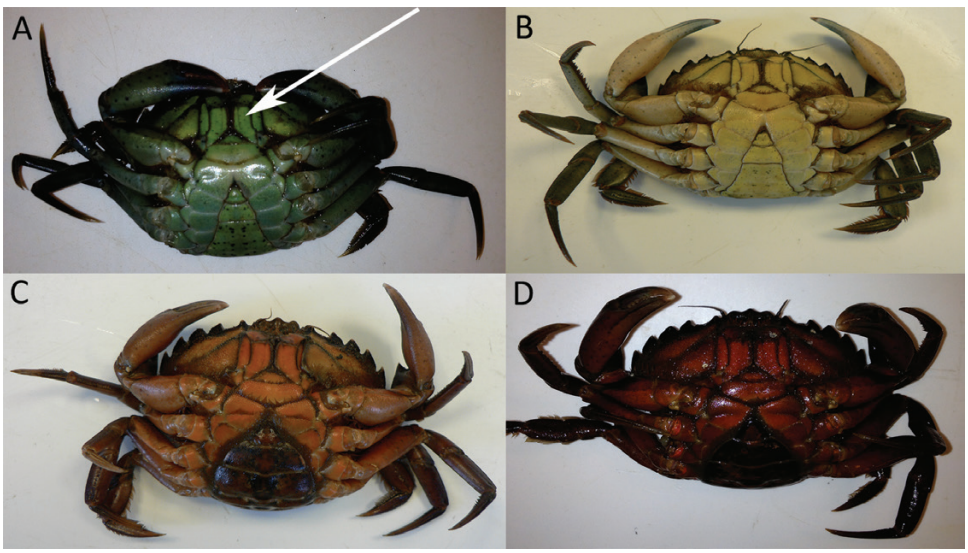


Figure 3. Examples of *Carcinus maenas* ventral coloration. Arrow points to third maxillipeds that were used to determine color index (CI) values. A, green male (CI = 1); B, yellow male (CI = 6); C, orange female (CI = 9); D, red female (CI = 12).

Site:	**GPS lat/long (deg min sec):**	
Date:	**Participants:**	
Low Tide Time (24 hr):	**Low Tide Height (m):**	**Lunar Phase (% illumination):**
***Water Temp (°C):**	***Salinity (ppt):**	**Start-Stop Time:**

*Optional

Quad #	**% Movable Rock** (nearest 25%)	**% Algal Canopy** (nearest 25%)	***Species Code (Cm,Cb,Ci, Hs)**	**CW (mm)**	**Sex (M,F,NA)**	**# Claws (0, 1, 2)**	**# Legs (1-8)**	***Shell Condition (H,S,†P)**	**Ovigerous Y = Yes N = No**	**Color (1-12)**	**Notes**
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
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					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
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					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		

*Species code: Cm = Carcinus maenas (green crab), Cb = Cancer borealis (Jonah crab), Ci = Cancer irroratus (rock crab), Hs = Hemigrapsus sanguineus (Asian shore crab)
Shell condition: H = hardshell (shell does not give when finger pressure applied), S = softshell (shell gives when finger pressure applied), †P = *optional* pre-molt shell condition characteristic (only used for green crabs)

Quad #	% Movable Rock (nearest 25%)	% Algal Canopy (nearest 25%)	*Species Code (Cm,Cb,Ci, Hs)	CW (mm)	Sex (M,F,NA)	# Claws (0, 1, 2)	# Legs (1-8)	*Shell Condition (H,S,†P)	Ovigerous Y = Yes N = No	Color (1-12)	Notes
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
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					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		
					M F NA	0 1 2		H S †P	Y N		

Notes: _____

A Field Guide to Pre-molt Green Crabs

PURPOSE

The purpose of this field guide is to help identify pre-molt European green crabs (*Carcinus maenas*) in New England. The key to a successful soft-shell crab fishery is identifying pre-molt crabs and holding them until they molt.

SIGNS

Subtle changes in color along the margins of the ventral episternites (platelets) indicate that the crab is likely to molt in the next several weeks (see 2). Males are more likely to molt in synchrony in the spring (May-July). Imminent-molt crabs (likely to molt within 1-3 days) lose the shadowy line on the margins of the ventral plates, their color fades and becomes more opaque, and they become lethargic. The abdomen also becomes brittle on the posterior end where it wraps around and meets the carapace. Imminent-molt crabs must be checked daily for molting. Once a crab molts, it must be removed from the water and chilled within 6-18 hours, otherwise the new shell will begin to harden.

SIZE MATTERS

For soft-shell crabs, the target size is 45-65 mm. Large-old male crabs seldom molt. Medium males are the best because they are still actively molting and they are worth more money than small males.

THE ITALIAN CONNECTION

The Venetian crab (*C. aestuarii*) sets the standard for molting signs in the European green crab (*C. maenas*) of New England.

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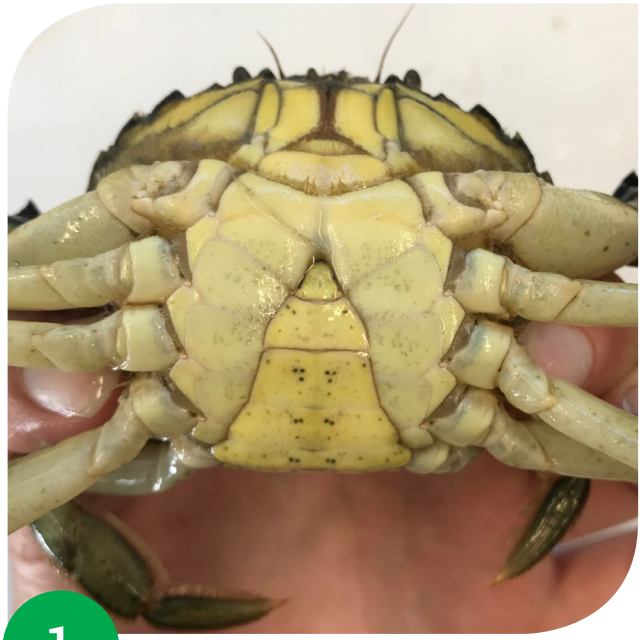
MOLTING SEQUENCE TERMINOLOGY

Most trapped crabs are in the "intermolt" phase. They are not good for the soft-shell fishery and can be discarded. "Pre-molt" crabs are of interest for holding until they become "imminent" molts and then finally molted "soft-shell" crabs. The challenge is to figure out which of these 4 types you are holding in your hand.



1

"Intermolt"

"Intermolt" crabs are hardshelled, in-between molts and not good for the softshell fishery. The key is to identify and select out the "pre-molts" (see 2) from a large catch of "intermolts." (*C. maenas*).

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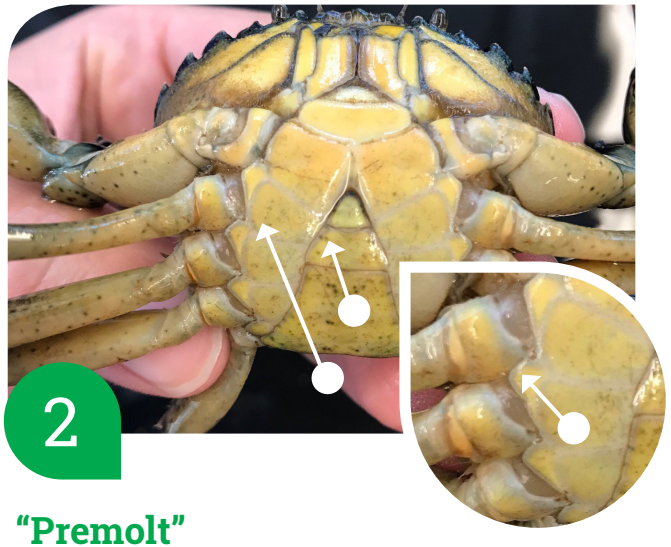
1 "INTERMOLT" CRAB
no signs of molt, or molted recently and hard-shell again. Not of interest.

2 "PRE-MOLT" CRAB
signs of molt in the next **1-3 weeks.**

3 "IMMINENT-MOLT" CRAB
molting in the next **1-3 days**.

4 "SOFT-SHELL" CRAB
just molted in the **last 24 hrs**, market product.



2

"Premolt"

"Pre-molt" crabs are 1-3 weeks from molting. These crabs have a thin white line and blue-green shadow on the margins of their adominal platelets (note arrows). This sign is best observed in the shade (not sun). These crabs should be kept aside and monitored daily for signs of "imminent" molt—see 3 on reverse). (*C. aestuarii*).

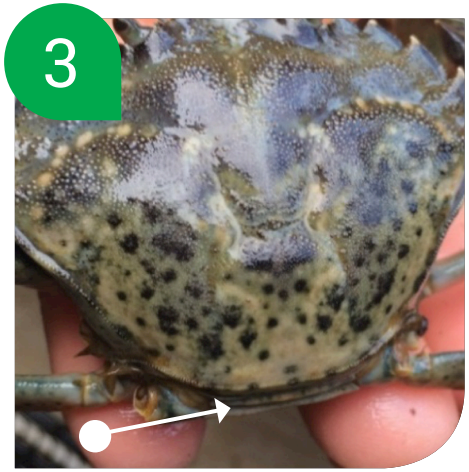
A Field Guide to Pre-molt Green Crabs

"Imminent"

The carapace of crabs likely to molt in the next 1-3 days looks cloudy and washed out on the back (dorsal side). Where the abdomen meets the carapace, the crab is soft to the touch (see arrows). (*C. maenas*).

Note the pale, washed out look of the abdomen and platelets of an "imminent" molt crab. The shadowy white line on the ventral side of "pre-molts" shown in 2 is faded or lost. Often "imminent molt" crabs are lethargic. (*C. aestuarii*).

3



MOLTING

Note loss of color in the old shell, and the significantly more vivid color of the "new" crab.

4

"Soft-shell"

Desired product! Crabs that are soft-shell (molted in the last 12-24 hours) are obvious because (1) they are soft to the touch, and (2) typically bright green or yellow-green. They should be removed from water and put on ice immediately to prevent shell hardening.

MORE PRE-MOLT EXAMPLES

OTHER THINGS TO KNOW

FEMALE
The abdomen is much rounder and broader on a female than a male.

MALE
Note the pointed, spear-shaped abdomen of the male.

FEMALE WITH EGGS
Eggs are held under the abdomen. Females can carry up to 165,000 eggs at one time.

Supplemental File (Figures, Permissions, etc.)

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Appendix 5_Excel data entry template.xlsx

Site	GPS	Date	Participants	Low Tide Time (24 hr)	Low Tide Height (m)	Lunar Phase (%)

Water temp (℃)	Salinity (ppt)	Survey Start Time	Survey End Time	Quad #	% Movable rock

% Algal canopy	Species Code	CW (mm)	Sex	# Claws	# legs	Shell condition	Ovigerous

Color (1-12) Notes