Assessing Intertidal Populations of the Invasive European Green Crab

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Introduction

Biological invasions can potentially disrupt species interactions and ecological processes, and may have far reaching ecological^{1,2,3} and economic consequences⁴. The ability to successfully predict, mitigate, and adapt to invasions strongly depends on characterizing spatiotemporal population dynamics⁵. While a range of tools exist (e.g., population genetics, stable isotopes) and are emerging (e.g., eDNA) for tracking invasive species, traditional in

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.

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

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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 oceans¹⁷.

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 m¹⁸. 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 Anecdata¹⁹. Anecdata is a free online citizen science platform that provides webbased 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

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

 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 Project¹⁹. The data collection sheet and Anecdata collection categories are identical.

3. Site selection and description

- 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.
- 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. 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.
- 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

 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).

- 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.
- Optionally, measure water temperature using a waterproof digital thermometer in the shallow water adjacent to the sampling area.
 - Measure salinity by placing several drops of water collected adjacent to the sampling area onto the refraction prism of a salinity refractometer.
 - 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. Begin the survey by haphazardly tossing the 1 m² 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.
 - 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.

- 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.
- 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.
- 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.
- 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.
- 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.
- Optionally, record the color for green crabs, but not other crab species, using the color protocol developed by Young and Elliot²⁰ (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}.

- 11. Return all crabs to the habitat within the quadrat once all measurements and characteristics have been recorded.
- 12. Continue haphazardly tossing the quadrat within the predefined low intertidal area until a total of 10 m² 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

- 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 Anecdata¹⁹. Store scanned data sheets electronically.
- 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., Damariscotta crabs were significantly smaller and Sandy Point 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**).

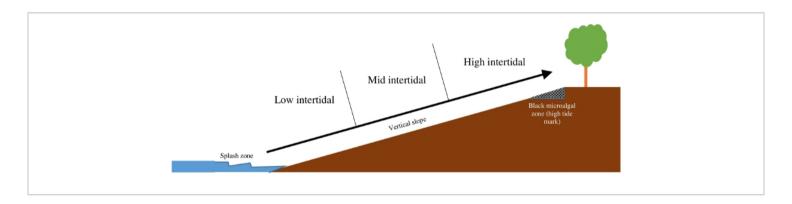


Figure 1: Tidal height zonation. Diagram of tidal height zonation along the shoreline. Surveys are conducted in the low intertidal zone (i.e., the lower third of the intertidal zone that runs parallel to the shoreline). Please click here to view a larger version of this figure.



Figure 2: Defining survey area. Image of a survey site in Yarmouth, Maine with tidal zonation displayed in yellow and survey area in green. Please click here to view a larger version of this figure.

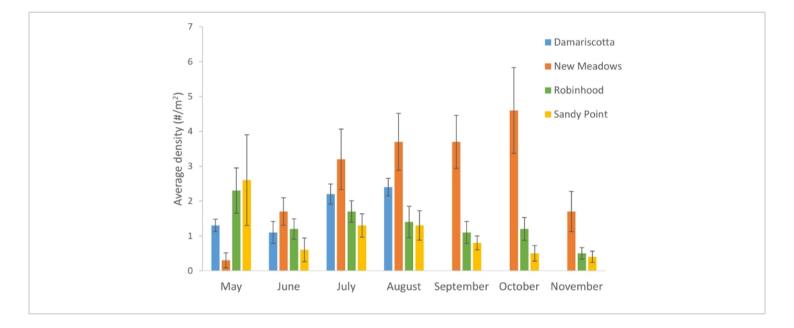


Figure 3: Green crab density. Average monthly density (#/ m^2) of green crabs at Damariscotta River, New Meadows,

Robinhood Cove, and Sandy Point intertidal survey sites. Error bars represent ± 1 standard error. Please click here to view a larger version of this figure.

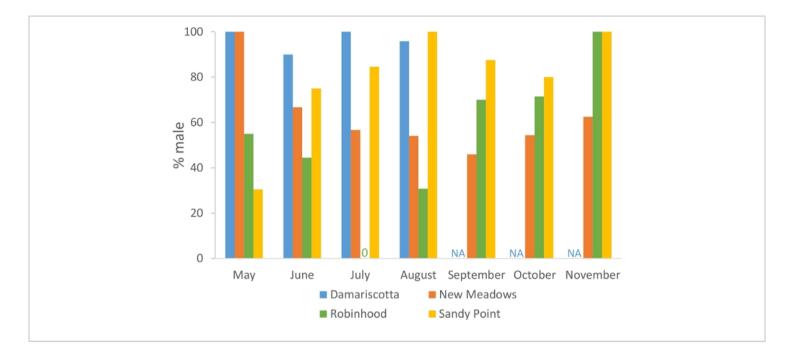


Figure 4: Green crab sex ratio. Sex ratio by month for green crabs at Damariscotta River, New Meadows River, Robinhood Cove, and Sandy Point intertidal survey sites. Sex ratio is expressed as the percent of the population that is male. The green "0" appearing in July indicates 0% male crabs and 100%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

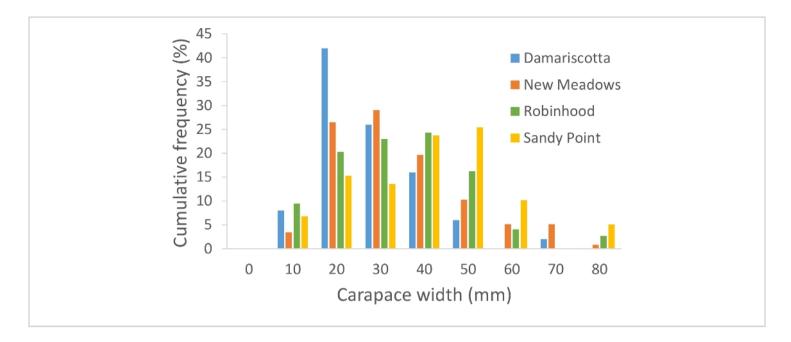


Figure 5: Green crab size frequency. Cumulative size frequency of green crabs at Damariscotta River, New Meadows River, Robinhood Cove, and Sandy Point intertidal monitoring sites for all months combined (May-November for Damariscotta River, New Meadows River, Robinhood Cove, and May-August for Damariscotta River). Please click here to view a larger version of this figure.

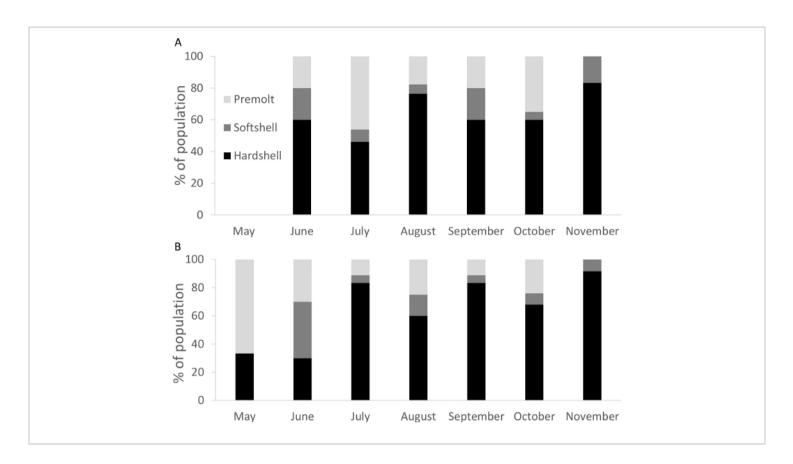


Figure 6: Shell condition ratio. Monthly shell condition ratio of A) female and B) male green crabs at New Meadows River intertidal monitoring site. Please click here to view a larger version of this figure.

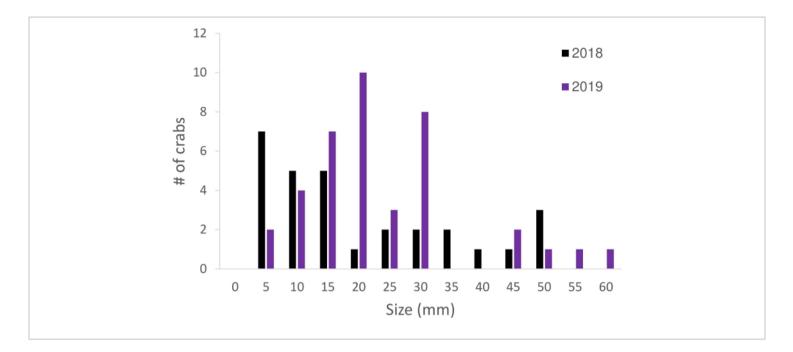


Figure 7: Student-collected size frequency. Size frequency of green crabs surveyed by Georgetown Central School 3rd and 4th grade students in 2018 and 2019. Please click here to view a larger version of this figure.

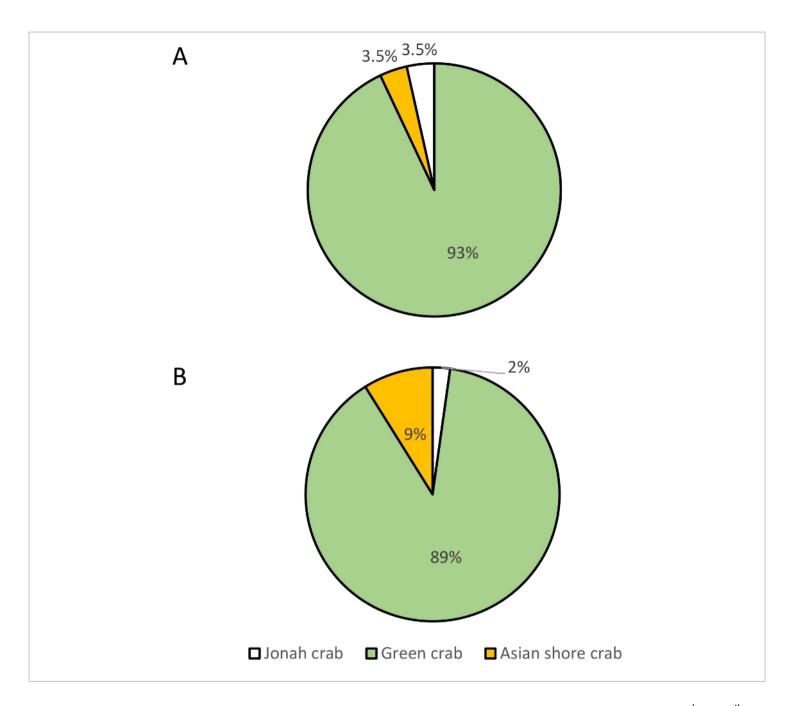


Figure 8: Species occurrence. Percent occurrence of crab species surveyed by Georgetown Central School 3rd and 4th grade students in A) 2018 and B) 2019. Please click here to view a larger version of this figure.

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., 3^{rd} and 4^{th} 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 \geq 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.

Disclosures

The author has nothing to disclose.

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