

COMMUNITY SCIENCE IN THE DAMARISCOTTA RIVER ESTUARY: AN EDUCATIONAL EXPLORATION OF SHELLFISH ECOLOGY & HARVESTER LOCAL KNOWLEDGE



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**Shellfish Ecological Survey:
A Method for Assessing Shellfish Ecology in Muddy and Rocky Intertidal Habitats**



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SUMMARY:

Updated information on the abundance, distribution, and diversity of shellfish species is incredibly important to effective stewardship of the shellfish resource. This protocol was developed to document population data on commercially important shellfish species in Maine. Surveys are intended to be performed in areas that are important to shellfish harvesters, either as active or historic harvesting grounds, and will ideally be conducted on a consistent annual basis in order to help inform shellfish management decisions. This survey also collects data on the shellfish ecosystem, including information on worms, crabs, and other species who share habitats with shellfish.

INTRODUCTION:

Maine's intertidal shellfish populations support coastal livelihoods and have historically been Maine's second or third most valuable commercial marine fishery ¹. In 2020 alone, 6.5 million pounds of softshell clams were landed with a value of \$15.7 million in Maine, making it the second highest earning fishery in the state ². Regardless, the shellfish fishery is changing and facing new challenges. Warming waters, increases in predator populations, and decreasing waterfront access are factors that are affecting the shellfish resource and fishery ³⁻⁵. Therefore, improved knowledge about the state of the shellfish resource in the Damariscotta River Estuary and potential challenges facing the industry is essential for sustainable use and stewardship.

This protocol seeks to combine local ecological knowledge, held by shellfish harvesters, with environmental data on the spatiotemporal population dynamics of Maine's commercially important shellfish species. These environmental and local ecological knowledge data collection methods are meant to promote long-term monitoring by high school students, led by University of Darling Marine Researchers in partnership with high school educators. This protocol is designed to be integrated into high school coursework and can occur in either the fall or the spring semesters of the school year.

Species of commercial importance that are included in this protocol are soft-shell clams (*Mya arenaria*), quahogs (*Mercenaria mercenaria*), American oysters (*Crassostrea virginia*), European oysters (*Ostrea edulis*), blue mussels (*Mytilus edulis*), razor clams (*Ensis directus*), and surf clams (*Spisula solidissima*). Worm species—including blood worms (*Glycera dibranchiata*) and sand worms (*Nereis virens*)—as well as crab species—including green crabs (*Carcinus maenas*), jonah crabs (*Cancer borealis*), and rock crabs (*Cancer irroratus*)—are also included in this protocol in an effort to take an ecological approach by quantifying the abundance of other intertidal inhabitants that influence and interact with shellfish species. Although all of these species are included in the protocol, the survey can be adapted to focus on particular species of interest or additional intertidal species.

This protocol is designed to target locations that are of current or historic importance to shellfish harvesters and show signs of being shellfish habitat (e.g. clam holes or shells) ⁶. The protocol documents important habitat information, including sediment type, percent algal cover, percent rock surface, and siphon hole count in order to understand the relationship between these habitat characteristics and shellfish distribution, abundance, and diversity. The scale at which monitoring occurs can be flexible, but should always be consistent from year to year.

PROTOCOL:

1. Materials:

- 40-50 m fiberglass transect tape
- 1 PVC 0.25m² quadrat (0.5mx0.5m, made of ½" PVC)
- Clam rake
- 1 Mesh sieve (for spat samples, 0.25" aperture)
- Vernier calipers
- Gallon plastic bags (for spat samples)
- Clip board
- Pencils and permanent markers
- Datasheets (see Appendix 4-5)
- Field Guides (see Appendix 2)
- 1-2 wooden stakes (for marking transect areas)
- Waterproof gloves

- **Optional*
 - Jet sled (to transport spat samples)
 - Thermometer (if collecting environmental data)
 - Refractometer (if collecting environmental data)

2. Identifying Shellfish Areas

2.1. *Local Ecological Knowledge Surveys*

Shellfish harvesters and Shellfish Committees possess a deep knowledge of the location, species, and abundance of current and historic shellfish populations. Therefore, asking harvesters where they dig and what species they harvest in those areas is an essential first step to this survey protocol. Local ecological knowledge surveys (Appendix 1) should be conducted with harvesters on an annual basis to document important context information and determine the locations of surveys within monitoring sites. This way, field survey participants can ensure that shellfish data is being collected in areas important to harvesters and the shellfish resource.

2.2. *Walk-Over Surveys*

If for any reason input from shellfish harvesters or the Shellfish Committee is unable to be obtained, a short walk-over survey may be conducted at monitoring sites. This can be done by selectively digging at the site and looking for signs of shellfish species, including shells and siphon holes, or identifying areas with sediments known to be preferred by the shellfish species of interest (e.g. sand, mud, and gravel mixture areas for soft-shell clams). Mark these areas on a printed map of the site, using the maps from the local ecological knowledge surveys (Appendix 1).

3. Determining Tidal Zones

Information on tidal zones boundaries for monitoring sites is helpful for both identifying shellfish habitat areas and exploring relationships between shellfish population abundance and tidal zone distribution. Therefore, although not required, it is helpful to determine tidal zones for each monitoring site.

Tidal delineation can be done three ways:

- 3.1. Using wooden stakes, mark the high tide mark and the low tide marks on or as close as possible to a 0.0 ft tide. Then visually divide the flat by thirds to identify the high, mid, and low tidal zones.
- 3.2. Use a GPS or cell phone to mark waypoints/pins every 10-25 m along the high and low tide lines in the site on or as close as possible to a 0.0 ft tide. Then divide the map into three zones in QGIS/GIS to identify the tidal zones.
- 3.3. Use aerial photography that was taken on, or as close as possible, to a 0.0 ft low tide to estimate tidal zones by dividing the flat area into low, mid, and high tidal zones.

4. Conducting the Survey

Shellfish in the Damariscotta River Estuary live in diverse habitats that range from soft mud and sand to large, seaweed-covered rocks. Because these habitats are so diverse, the shellfish survey is divided into two distinct approaches: a mudflat survey and a rocky survey.

Mudflat Habitat Survey: If shellfish harvesters or the walk-over survey indicate that areas below the rock line where the mudflat begins are the most important, and are the locations where they actively harvest commercially important shellfish species, then a mudflat survey should be conducted. This survey covers the mid tidal mudflat area just below the rock line of a shore or cove and extends as far down as the subtidal line that is only accessible on negative tides. In the Damariscotta, this survey will generally target quahogs, soft-shell clams, and razor clams. The sediment can be sandy, muddy, gravelly, and may contain small to medium-sized rocks.

Rocky Habitat Survey: If shellfish harvesters of the walk-over survey indicate that areas within the rock line above the mudflats are the most important, and are the locations where they actively harvest commercially important shellfish species, then a rocky survey should be conducted. This survey covers the mid-tidal rocky area to the high tide mark. In the Damariscotta, this survey will generally target oysters and quahogs. These areas should have at least 50% rock surface and/or 50% algae cover.

5. **Mudflat Habitat Survey Protocol**

5.1. Before arriving at the site, fill out the datasheet with tide information, moon phase, participants, etc.

5.1.1. *If collecting spat:* Pre-label gallon plastic bags using a permanent marker with the transect and plot number prior to arriving at the site. These bags will be used to collect that spat surface sample for each plot.



5.2. Arrive at the site at least one hour before low tide. Give yourself enough time to park and walk into the site. Locate your survey area using a site map (paper or digital) and other indicators that this is the targeted shellfish area, such as siphon holes or sediment type.

5.3. Prior to beginning the survey, assign roles to each team member. The number of team members determines the number of roles:

5.3.1. *If there are four:* **Digger**= Uses the clam rake to dig out each plot, **Sorter**= Combs through mud—given to them from the digger—for shellfish, **Measurer**= measures each specimen from the sorter, **Recorder**= Records all species and measurements in the datasheet.

5.3.2. *If there are three:* **Digger**= Uses the clam rake to dig out each plot, **Sorter/Measurer**= Combs through mud—given to them from the digger—for shellfish and measures each specimen, **Recorder**= Records all species and measurements in the datasheet.

5.3.2.1. For groups of four or three, the role of the digger should rotate after each plot. This is to ensure that no one gets too tired and the searching is thorough each time.

5.3.3. *If there are two:* **Digger/Sorter**= Uses the clam rake to dig out each plot and combs through mud for shellfish, **Measurer/Recorder**= Measures and records all species and measurements in the datasheet.

5.4. Begin by measuring out a 25 m transect within your survey area that is parallel to the low water line. Mark one end of your transect with a wooden stake and then walk out the transect tape until you have reached 25 m. This

will delineate the boundaries of the shellfish area in which you will be surveying.

- 5.5.** Start the survey at the end of the 25 m transect. Randomly toss the 0.25 m² quadrat within the predefined shellfish area. This is Plot 1. If the plot does not contain siphon holes, throw the quadrat again. Press the quadrat firmly into the sediment or use your hands to outline the plot in the sediment. Remove the quadrat. Record the algae species (if none=NA), the algal cover to the nearest 25% (25%, 50%, 75%, 100%), the sediment type, % rock surface, the number of siphon holes, and any other species visible within the plot.

- 5.5.1.** Siphon holes are usually about the size of the circumference of a pencil. Worm holes are commonly seen near siphon holes, but should not be confused with or counted as siphon holes. These holes will either be smaller, closer to the size of a pencil tip, or a similar size but surrounded by a mound of sand.



- 5.6.** Next, if sampling for spat (clams < 10mm), use your hands to remove the top 1-2" of mud from one-half of the plot. Place the sample into your pre-labeled gallon size bag. Samples from all plots will be processed after completing the full survey. The samples can either be:

- 5.6.1.** Sieved at the site using a 5-gallon bucket, sieve, and seawater. Use the jet sled to carry samples down to the waterline, place the sediment in the screen frame and sieve with water. Identify and measure all softshell clam spat.
- 5.6.2.** Transported off the flat using a jet sled and sieved at another location with running seawater. Pour the samples into the screen frame and sieve with water. Identify and measure all softshell clam spat. Note: A collection permit is required to remove shellfish from the flats. All spat samples must be returned to the flats from which they were collected.
- 5.6.3. NOTE:** Species other than softshell clams will be present in the samples. Use a hand lens to distinguish between species.

- 5.7.** Now prepare to dig out your plot and look for shellfish. Prior to digging the plot, the digger will clean the sediment away from one edge of the plot to make it easier to dig.
- 5.8.** Next, the digger will use the clam rake to dig the plot edge-to-edge, down to a depth of at least 8" or until no more clams are found. They will use the rake to cut the mud into thin slices (~1") and use their hands to remove the sediment from the plot, placing it to the side of the plot for the sorter.
 - 5.8.1.** Think of the plot like a grid and dig slice by slice, making sure to not take overly large sections. Make sure that the rake goes as far down as possible by alternating between carefully wiggling it and pushing it down.
 - 5.8.2.** If the slice you are digging contains, or is near a siphon hole, move the entry site of the rake so that you are not digging directly into the hole. Take even greater care pushing the rake into sections like these to try to minimize the number of clams punctured by the rake.
 - 5.8.3.** If you are digging in an area where you think there are clams, it is sometimes easier and safer to use your hands than to use the rake.
- 5.9.** The sorter will search through the sediment and will identify all living shellfish specimens. They will set the shellfish specimens into the sieve to be measured. If there is a measurer, measure the shellfish as you dig. If there is not, measure all shellfish after the plot is complete. See Appendix 3 for guidelines on how to measure shellfish.
- 5.10.** The recorder will mark the presence of any worms (Appendix 2C) or crabs (Appendix 2c) in the datasheet and will record the carapace width of any crabs found . The recorder will also mark down all shellfish species and their measurements. They will need length measurements for soft-shell clams, length and height measurements for oysters, and length and hinge measurements for quahogs (see: Appendix 3).
 - 5.10.1.** When you find a worm, even if you have found many before, you still need to take the time to distinguish if it is a bloodworm or a sandworm (Appendix 2C). If you find just a piece of a worm, odds are likely that you have already counted another piece of it, so this piece should not be counted. After you count a worm, throw it far away to make sure that it doesn't get recounted.

5.11. Once the plot is completely dug out and all specimens are recorded and measured, return all specimens to the plot, covering them up with mud.

5.11.1. A completely dug out plot will be dug as far down as possible, or until clams are no longer being found. You will typically either hit clay or water will start pooling at the bottom.



5.12. Randomly toss the 0.25 m² quadrat again for Plot 2, so that you are progressing through the predefined shellfish area. Repeat the digging, sorting, and measuring process. Continue sampling until a total of 5 plots are completed.

5.12.1. As a reminder, for groups of four or three, the role of the digger should rotate after each plot. This is to ensure that no one gets too tired and the searching is thorough each time.

6. **Rocky Habitat Survey Protocol**

6.1. Before arriving at the site, fill out the datasheet with tide information, moon phase, participants, etc.

6.2. Arrive at the site at least one hour before low tide. Give yourself enough time to park and walk into the site.

6.3. Locate your survey area using a site map (paper or digital) and other indicators that this is the targeted shellfish area, such as rock surface or algal cover.



6.4. Prior to beginning the survey, assign roles to each team member. It is important that each team member sticks with their role so that the survey is consistent. The number of team members determines the number of roles:

6.4.1. *If there are four:* **Searcher**= Uses hands (with gloves) to search under algae, rock, and in mud for specimens, **Measurer 1 and Measurer 2**= Measures each specimen, **Recorder**= Records all species and measurements in the datasheet.

6.4.2. *If there are three:* **Searcher**= Uses hands (with gloves) to search under algae, rock, and in mud for specimens, **Measurer**= Measures each specimen, **Recorder**= Records all species and measurements in the datasheet.

6.4.3. *If there are two:* **Searcher**= Uses hands (with gloves) to search under algae, rock, and in mud for specimens, **Measurer/Recorder**= Measures each specimen and records all species and measurements in the datasheet.

6.5. Begin by measuring out a 25 m transect within your survey area that is parallel to the low water line. Mark one end of your transect with a wooden stake and then walk out the transect tape until you have reached 25 m. This will delineate the boundaries of the shellfish area in which you will be surveying.

6.6. Start the survey at the end of the 25 m transect. Randomly toss the 0.25 m² quadrat within the predefined shellfish area. This is Plot 1. If the plot does not contain at least 50% rock surface and/or 50% algae cover, throw the quadrat again. Record the algae species (if none=NA), the algal cover to the nearest 25% (25%, 50%, 75%, 100%), the sediment type, % rock surface, the

number of siphon holes, and any other species visible within the plot.

- 6.6.1.** Again, siphon holes are usually about the size of the circumference of a pencil. Worm holes are commonly seen near siphon holes, but should not be confused with or counted as siphon holes. These holes will either be smaller, closer to the size of a pencil tip, or a similar size but surrounded by a mound of sand.



- 6.7.** The searcher will then explore within the quadrat, section by section, looking under algae and rocks for any shellfish specimens. They will feel through the top layer of sediment for any unattached oysters or quahogs. If there are distinct siphon holes, they will spot dig where the hole is to try to find the soft-shell clam. They will give any unattached shellfish specimens to the measurer(s). They will point out any attached shellfish specimens that need to be measured. See Appendix 3 for guidelines on how to measure shellfish.
- 6.7.1.** Think of the plot as if it is split into quarters and thoroughly search through one section at a time. Clear away as much seaweed as possible and check along the edges and corner before moving onto the next quarter section.
- 6.8.** The recorder will mark the presence of any worms (Appendix 2C) or crabs (Appendix 2E) in the datasheet and will record the carapace width of any crabs found. The recorder will also mark down all shellfish species and their measurements. They will need length measurements for soft-shell clams, length and height measurements for oysters, and length and hinge measurements for quahogs (Appendix 3).
- 6.8.1.** Again, when you find a worm, even if you have found many before, you still need to take the time to distinguish if it is a bloodworm or a sandworm (Appendix 2C). If you find just a piece of a worm, odds are likely that you have already counted another piece of it, so this piece should not be counted. After you count a worm, throw it far away to make sure that it doesn't get recounted.

- 6.9. Once the plot is completely searched and all specimens are recorded and measured, return all specimens to the plot, covering them up with sediment or algae.
- 6.10. Randomly toss the 0.25 m² quadrat again for Plot 2, so that you are progressing through the predefined shellfish area. Repeat the searching and measuring process. Continue sampling until a total of 5 plots are completed.

7. Data Entry & Analysis:

- Scan and upload paper data sheets to the shared Google drive folder.
 - Scanning can be done through the notes app on an iPhone.
- Enter data into the Shellfish Ecological Survey Excel sheet.
 - Enter site information first,
 - Enter worm and crab information next,
 - Enter shellfish information (plot and surface sample spat data) last.
 - Make sure that each worm, crab, and shellfish found gets its own line in the spreadsheet.

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The protocol below is courtesy of Dr. Marissa McMahan and Manomet. Green crabs are voracious predators that affect many important bivalve shellfish species^{8,9}. Therefore, learning about the spatial distribution and abundance of green crabs in the Damariscotta River Estuary offers insights into how, where, and to two degree these predators may be affecting shellfish populations.

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



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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.

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-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 green crab abundance and increasing ocean temperature^{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. 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. The scale at which monitoring occurs can be flexible, but should always be consistent from year to year. For example, this protocol was developed in Maine and is conducted on a monthly basis from April-November each year. Sampling does not occur from December-March because intertidal crab populations become scarce in the winter months, and accessing the shoreline can be dangerous in cold, icy conditions. Investigators in southern New England will likely have a wider seasonal sampling window due to more mild winter conditions, and investigators in Atlantic Canada will likely have a narrower sampling window. Some users, such as school groups, may be confined to sampling once or twice per year, in which case they should always sample during the same time periods, and at the same sites, so data can be compared across sites and years.

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. The survey can be adapted to include other species of interest that may occur in the areas where it is utilized (e.g., mud crabs may be a species of interest in southern New England).

PROTOCOL:

1. Materials:

- 40 m fiberglass transect tape
- 1 m² quadrat (made from 1/2" PVC)
- Vernier calipers
- Clip board
- Pencils
- Data sheets (see Appendix 7)
- 5 gal bucket
- Waterproof gloves

***Optional:**

- Color protocol (Appendix 6)
- 1/2" Rebar
- Thermometer
- Refractometer

2. Site selection and description

- 2.1)** Locate a wave-sheltered rocky intertidal site with cobble and algal canopy habitat. The first site visit should be scheduled during an average low tide (i.e., tidal height as close to 0.0 m as possible), as determined from the NOAA Tide Predictions website (https://tidesandcurrents.noaa.gov/tide_predictions.html?gid=1401), and should be set aside specifically for site description activities as there will likely not be time for conducting an actual survey on the same day. Plan to arrive at the survey site at least 1 hr before low tide.
- 2.2)** Determine the area of the shoreline where the survey will be conducted by running a transect tape vertically from the low intertidal zone (i.e., the splash zone on an average 0 m low tide) to the high intertidal zone (i.e., the black microalgal zone that is typically dry at high tide) as close to the scheduled low tide time as possible. Divide the resulting distance into three equal sections (high, middle, and low). This is a relatively simple method of determining tidal range that can be deployed by a wide range of users (i.e., it does not require specialized equipment or rigorous methodology). The survey is conducted in the low section and parallel to the shoreline. Establish permanent markers delineating the low intertidal zone parallel to the shoreline using rebar or natural permanent landmarks such as immovable boulders, ledge, dock pilings, etc. The use of permanent markers allows the investigator to avoid having to delineate the shoreline using a transect tape on every visit.
- 2.3)** 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 written site coordinates in degrees, minutes, and seconds (DMS) format (e.g., 43°48'14.2"N, 69°44'50.5"W) on a site description data sheet. The site

description data sheet should also include directions to the site, instructions for parking and accessing the shoreline, and a description of how the low intertidal is delineated (i.e., natural landmarks, permanent markers, etc.). Any other special considerations unique to the site should also be noted.

3. Conducting survey

- 3.1) Prior to arriving at the survey site, the following information should be recorded on the Intertidal Survey data sheet (see Appendix 1 for example): site name, sampling date, participants, time and height of low tide at the location/date you will be sampling (determined using the NOAA Tide Predictions website, or an app such as Tides), and lunar phase (determined using a lunar calendar such as www.moongiant.com).
- 3.2) Surveys occur within a two hour window of time straddling one hour before and one hour after mean low water, as this is the window in which the low intertidal zone is exposed. Plan to arrive at the survey site early enough to park, get to the survey area, unpack, and start the survey 1 hr prior to low tide. Upon arriving at the shoreline area where you will be sampling, unpack and setup gear including the 1 m² quadrat, clipboard with Intertidal Survey data sheet and color protocol (if using), calipers, and a small bucket. Water temperature and salinity measurements are optional, but should be taken prior to the start of the survey using a thermometer and refractometer in the water directly adjacent to the survey area.
- 3.3) To begin the survey, randomly toss 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 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%). Rocky intertidal habitat is often patchy and can contain areas of sand, mud, ledge, or other habitats where green crabs are not found. To avoid skewing density estimates by sampling unsuitable habitat, only quadrats with greater than 50% movable rock, or greater than 50% algal canopy, are sampled.
- 3.4) 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 you found them. Collect all of the crabs you find and store them in a bucket until the entire quadrat has been searched.
- 3.5) For each crab, identify and record the species (to save space, species codes are used on the Intertidal Survey sheet (Appendix 1) and measure the carapace width (CW) to the nearest 1 mm using Vernier calipers. Additional characteristics recorded for all crab species include sex, number of claws, number of legs, shell condition (i.e., hard- or soft-shell as determined by whether the shell resists (hard) or gives (soft) when finger pressure is applied), and the presence (i.e., ovigerous) or absence of extruded eggs for females. Sex and shell condition are

often difficult to determine for crabs measuring less than 10 mm. To avoid this common measurement error, 'NA' (not available) is recorded for these categories for crabs ≤ 10 mm.

- 3.6)** Color is an optional characteristic that can be recorded for green crabs, but not other crab species, using the color protocol developed by Young and Elliot¹⁷ (Appendix 2). Investigators should print a copy of this protocol and bring it with them if they choose to collect color data. The pre-molt shell condition is also an optional characteristic that can be recorded for green crabs if the investigator is comfortable identifying external pre-molt indicators (see Appendix 3 for example). Pre-molt green crabs are within 3 weeks of molting and are of particular interest to the emerging soft-shell green crab fishery^{19,20,McMahan unpublished data}. Color and pre-molt condition are not determined for green crabs ≤ 10 mm ('NA' is recorded).
- 3.7)** Return all crabs to the habitat within the quadrat once all measurements and characteristics have been recorded.
- 3.8)** Continue randomly tossing the quadrat within the predefined low intertidal area until you have sampled a total of 10 m². Continually move forward along the low intertidal area of shoreline to ensure that resampling does not occur.

4. Data management and analysis

- 4.1)** Post-survey, all raw data sheets should be checked for errors and legibility, copied, scanned, and archived. The copy is used for data entry into an excel spreadsheet (see Appendix 4 for example) and then also archived. Scanned data sheets are stored electronically.
- 4.2)** Data analysis can be simple or complex, depending on the needs of the investigator. Density is perhaps one of the easiest and most useful calculations. Site density is calculated by dividing the total number of crabs by the total number of quadrats sampled. Other useful metrics to calculate include, but are not limited to, sex ratio, cumulative size frequency, injury rate, shell condition ratio, and overall species encounter rates (e.g., % of native vs. invasive crabs).

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