

## HEMOLYMPH-BASED MOLT PREDICTION IN THE EUROPEAN GREEN CRAB, *CARCINUS MAENAS*: POTENTIAL TOOLS FOR A SOFT-SHELL INDUSTRY

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**ABSTRACT** As invasive European green crabs (*Carcinus maenas* Linnaeus) continue to impact Gulf of Maine ecosystems and fisheries, harvesters and coastal resource managers are exploring strategies to mitigate the effects of this species. One option is to establish and promote a soft-shell crab fishery for this species; however, it can be difficult to accurately and consistently identify premolt crabs for harvest using external morphological cues alone. This study evaluated the application of several hemolymph indices as diagnostic tools for determining the molt stage in *C. maenas*. Captured crabs were tagged, measured, and held in individual compartments in a floating enclosure for up to 51 days. Hemolymph samples were collected twice per week from individual crabs ( $n = 126$ ) and evaluated for hemolymph color, total protein (refractive index, RI), and calcium concentration. The resulting data suggest a positive relationship between these metrics: darker hemolymph samples had higher protein and calcium concentrations; crabs with darker hemolymph and higher initial RI were more likely to molt within 3 wk; and initial RI  $\geq 9.5$  resulted in a  $>50\%$  chance of molting within 3 wk. In addition to improving our understanding of the biological underpinnings of molting in brachyuran crabs, these results provide practical information for creating an accessible molt assay tool (i.e., hemolymph color) that can be used by harvesters to further facilitate the development of a soft-shell crab market.

**KEY WORDS:** *Carcinus maenas*, crustacean hemolymph, molt prediction, ecdysis

### INTRODUCTION

Invasive species disrupt natural ecosystems in which they have established populations, often leading to negative ecological and economic impacts (reviewed in Grosholz 2024). For example, invasive species may prey on native species, outcompete those species for resources such as food and shelter, introduce pathogens, alter community composition and dynamics, and reduce native biodiversity (Preisler et al. 2009, Gallardo et al. 2016, Griffen et al. 2021). Conversely, some nonnative species contribute to increases in regional biodiversity (Sax & Gaines 2003, Thomsen et al. 2014), improve ecosystem functions such as erosion control (e.g., Japanese sedge, *Carex kobomugi* Ohwi, Charbonneau et al. 2017), or provide new fishery opportunities (Carey et al. 2011, Fischer et al. 2024). For example, although the smallmouth bass (*Micropterus dolomieu* Lacepède) poses numerous threats to native species in its invaded range, it also supports a vast recreational fishery in over 20 countries (Carey et al. 2011). Invasive blue crabs (*Callinectes sapidus* Rathbun) have also led to the creation of new fisheries and products in the western Mediterranean Sea (Marchessaux et al. 2023, Arena et al. 2024).

One of the most prevalent and destructive marine invasive species in the world is the European green crab (*Carcinus maenas* Linnaeus, herein green crab; Klassen & Locke 2007, Frederich & Lancaster 2024) which has been introduced to every continent except Antarctica (Young & Elliot 2020). Since its introduction to the east coast of the United States in the early 1800s (Say 1817), it has become the predominant crab species in northern New England salt marshes (Edgell & Hollander 2011, Goldstein et al. 2017, Raposa et al. 2019,

Frederich & Lancaster 2024). As its population expands, the green crab has been shown to compromise the physical integrity of salt marshes and submerged aquatic vegetation through burrowing and grazing (Aman & Wilson-Grimes 2016, Carman & Grunden 2019, Monteiro et al. 2021) and prey on a wide variety of benthic marine organisms (Rangeley & Thomas 1987, Mascaró & Seed 2001, Beal et al. 2020). In coastal Maine, the green crab has contributed to sharp declines in softshell clams (*Mya arenaria* Linnaeus; Bryan et al. 2015) and also preys on northern quahogs (*Mercenaria mercenaria* Linnaeus; Glude 1955), juvenile sea scallops (*Placopecten magellanicus* Gmelin; Matheson & McKenzie 2014), blue mussels (*Mytilus edulis* Linnaeus; DeGraaf & Tyrrell 2004), and eastern oysters (*Crassostrea virginica* Gmelin; Miron et al. 2005). In some estuarine systems, green crabs may curtail catch of American lobsters (*Homarus americanus* H. Milne Edwards), interact aggressively with lobsters, and limit foraging activity and shelter use of small juvenile lobsters (Rosson et al. 2006, Haarr & Rochette 2012, Goldstein et al. 2017, Rayner & McGaw 2019). Although the economic impacts of green crabs in some areas may be negligible (Grosholz et al. 2011), green crab predation on commercial fisheries species in the United States as a whole is estimated to cost millions of dollars per year (Lafferty & Kuris 1996, Pimentel et al. 2001).

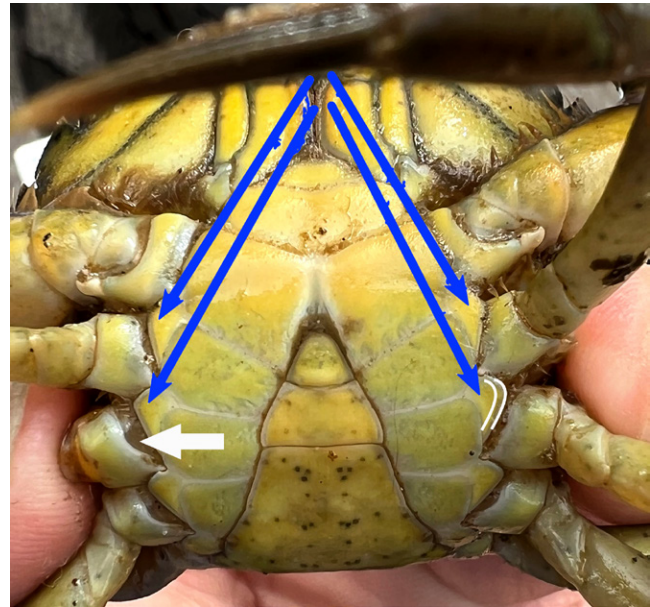
Minimizing the harm of green crabs on coastal systems requires active mitigation efforts along with a comprehensive understanding of green crab movements and habitat use (Zarrella-Smith et al. 2022). In the northeastern United States, there are currently few mitigation efforts in place due to the pervasiveness of the invasion and a lack of a formal regional mitigation action plan for green crabs. Without formal mitigation programs, researchers and harvesters in New England and Atlantic Canada have begun to investigate the development

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DOI: 10.2983/035.044.0114

and feasibility of a green crab fishery which could introduce a new seafood product, provide an alternative source of income for harvesters, and potentially reduce green crab populations (Poirier et al. 2016, Bradt & McMahan 2022, Fischer et al. 2024, Tummon Flynn et al. 2024). Developing such an initiative requires local participation and interest from harvesters and evidence that the fishing effort will be financially beneficial to participants (St-Hilaire et al. 2016, Fischer et al. 2024). Local harvesters and coastal resource managers in northern New England have expressed interest in developing such a market, stemming from growing concerns over the impacts of green crabs on existing fisheries (Fischer et al. 2024). Although local harvesters have become proficient at capturing large numbers of green crabs, the success of this emerging fishery may be limited by a lack of established markets, consumer demand, and price points (e.g., in Canada; McNive et al. 2013, Walter 2021). Extracting green crab meat for human consumption is time-consuming with comparatively low meat yields, so recipes that use the whole crab may prove more viable (McNive et al. 2013). Steering the market toward a soft-shell product—similar to soft-shell blue crab in the mid-Atlantic and southern United States (Kennedy et al. 2007) and soft-shell green crab in Italy (i.e., Venetian “moeche”; Cataudella et al. 2015)—could increase the overall market and value of this product (Walter 2021). In Italy, in the traditional practice of “molechicoltura,” green crabs that exhibit morphological characteristics of premolt status are sorted and held in enclosures until they molt; postmolt, they are removed from the water to prevent the hardening of their carapace and sold as soft-shell product (Cataudella et al. 2015).

A new soft-shell green crab market is predicated on the ability to identify large numbers of premolt crabs and hold them through successful ecdysis (i.e., molt). This is facilitated by having a solid understanding of key biological data including local seasonal trends in green crab molting and the ability to distinguish subtle morphological cues that indicate premolt status (e.g., white and gray outlines on episternites, dull carapace coloration; Poirier et al. 2016, Bradt & McMahan 2022; see Fig. 1). The ability to efficiently distinguish these subtle cues requires extensive training and practice which can be time-consuming and therefore may be a barrier to some harvesters aiming to enter this market. In Italy, the specialized skill of identifying premolt crabs for moeche production has been passed down through generations over the past several hundred years, but relatively few harvesters still practice this technique (Cataudella et al. 2015).

In this study, several possible hemolymph-based indicators of molting (hemolymph color, total protein, and calcium concentration) were assessed to provide supplemental diagnostic tools for more definitively identifying premolt status in green crabs. Most body calcium in crustaceans is found as calcium carbonate in the exoskeleton; before and after molting, the uptake, movement, and storage of calcium ions ( $\text{Ca}^{2+}$ ) and bicarbonate ions ( $\text{HCO}_3^-$ ) are crucial for exoskeleton formation and postmolt hardening (Graf 1978, Smith & Chang 2007, Middlemiss et al. 2016). Although green crabs mostly rely on externally available  $\text{Ca}^{2+}$  for postmolt hardening (reviewed in Chang & Mykles 2011), calcium is stored internally to some degree, including in the hemolymph and hepatopancreas (Robertson 1937, Greenaway 1976, 1985, Roer 1980). Similarly, major blood proteins such as hemocyanin fluctuate with the physiological state of crustaceans including over the molting cycle (Lorenzon et al.



**Figure 1.** Traditional morphological indicators of premolt condition in *Carcinus maenas* include graying outlines and halo on episternites (blue arrows; Poirier et al. 2016). The bottom white arrow indicates the location of the arthroal membrane where the insulin needle was inserted to collect hemolymph samples. Figure adapted from Bradt and McMahan (2022).

2011, Li & Cheng 2012, Gianazza et al. 2021). Hemolymph refractive index (RI) is an indirect measure of total protein concentration that has been widely used as a nondestructive index of nutritional condition in other decapods (lobster: Gutzler & Butler 2017, Butler et al. 2022; crab: Matveev & McGaw 2022). Additionally, the color of fresh hemolymph samples from green crabs (personal observation) and other crustaceans (e.g., southern rock lobster, *Jasus edwardsii* (Hutton); Musgrove 2001) has been found to vary from clear to beige to orange. Therefore, as hemolymph color, total protein, and calcium concentration fluctuate with the biological condition of the crab and are relatively simple and nondestructive to obtain, it was hypothesized that these hemolymph indices could be used to help determine the molt stage of green crabs.

## MATERIALS AND METHODS

### Study Site and Set-Up

A crab holding system (i.e., “crab condo”; similar to a design used in other studies, e.g., McMahan et al., unpublished data) was constructed by dividing a PVC-coated wire mesh holding tray (76 × 76 × 8 cm, 24-mm mesh) into 36 equal compartments (13 × 13 cm) each lined with 7 mm plastic mesh to separate crabs from one another and prevent cannibalistic interactions. This system was deployed off a floating dock (~15 cm below the surface; frequently shaded) at Wells Harbor, Wells, ME (43° 19′ 12.82″ N, 70° 33′ 48.11″ W) near the inlet to the Webhannet River estuary. Macroalgae was placed on the top of the condo for added shade and protection. The Webhannet estuary is a well-mixed, back-barrier estuary dominated by strong tidal currents (Ward 2004, Dionne et al. 2006). Throughout the study (June 3–September 2, 2021; May 19–September 22, 2022),

water temperature ranged 7.9–25.1°C (mean 16.8°C ± 3.1 SD) and salinity ranged 19.6–32.3 (mean 30.0 ± 1.5 SD). Both parameters were measured continuously every 15 min with a YSI EXO2 multiparameter sonde (YSI Inc., Yellow Springs, OH) as part of the NERRS System Wide Monitoring Program (NOAA NERRS 2024).

### Specimen Collection

Green crabs were collected from the Webhannet estuary using baited green crab barrel traps as detailed in Raposa et al. (2019). Green crabs were inspected visually for external signs of premolt status including white and gray outlines on episternites and dull carapace coloration (Poirier et al. 2016, Bradt & McMahan 2022; see Fig. 1). Crabs exhibiting these visual indicators of premolt status were added to individual compartments within the condo tray. Due to temporal differences in molting seasons, male crabs ( $n = 111$ ) were monitored May through mid-July, and female crabs ( $n = 122$ ) were typically monitored mid-July through September. As crabs were removed throughout the experiment (see below), empty cells were refilled with more candidate premolt crabs. Crabs were not fed during the monitoring period to avoid confounding effects of diet and hemolymph RI. A cable tie with a unique color banding was secured around the upper section of the cheliped of each crab to identify individuals. Carapace width (mm), wet weight (g), sex, and number of limbs missing were recorded for each crab added to the condo tray. Crabs averaged 51.7 ± 6.4 mm carapace width (range 38.0–69.0 mm) and 40.2 ± 16.0 g (range 15.1–103.1 g).

### Monitoring

Crabs were monitored twice per week (every ~3–4 days) until they molted, died, or escaped, but not for more than 51 days each. At each monitoring event, the condo was removed from the water and covered in a seawater-soaked towel to prevent crab desiccation. The status of each crab was recorded as either alive, dead, newly molted, in the process of molting, or missing. Any dead crabs were discarded. For each live crab, the following procedures were used (twice per week unless otherwise stated).

### Refractive Index

A hemolymph sample (0.1–0.3 mL) was collected using a 29-gauge insulin needle attached to a 1 mL syringe (BH Supplies, Jackson, NJ) inserted through the arthroal membrane between the leg and carapace (see Fig. 1). Hemolymph RI was measured using an IFT40 temperature compensated

handheld industrial analog refractometer (Reichert Inc., Depew, NY) which was calibrated regularly throughout the study. In June–midJuly 2021, RI was measured weekly; beginning July 19, 2021, all crabs were measured twice per week.

### Hemolymph Color

In 2021, it was observed that hemolymph samples ranged greatly in color; consequently, it was hypothesized that there was a correlation between RI and color. In 2022, each hemolymph sample collected for RI was also observed against a white background and compared against a color scale to be assigned a color category: 1 (clear), 2 (cloudy), 3 (light), 4 (medium), 5 (orange) (Fig. 2). To create this color scale, a separate set of crabs were trapped from the Webhannet estuary on Feb 23 and Mar 8, 2022, and hemolymph samples were taken from a random subset of males ( $n = 38$ ) and females ( $n = 22$ ). Hemolymph samples were photographed against a white background under white light and the photographs were used to create a “color swatch” for each hemolymph sample in Affinity Photo 1.8.3.641 [Serif (Europe) Ltd., Nottingham, UK]. These swatches were organized by hue and divided into categories to create the color scale shown in Figure 2.

### Calcium Concentration

Hemolymph calcium concentration was measured for a random subset of crabs between June 21 and July 26, 2022 ( $n = 128$  samples). A minimum of 0.15 mL of hemolymph was collected (see “Refractive Index” for collection methods) and immediately stored in a 2.0 mL Eppendorf tube on ice. Calcium concentration was measured for each sample using a Hanna Instruments H1758 Marine Calcium Checker (Hanna Instruments, Smithfield, RI). The Hanna Calcium Checker is a single-parameter colorimeter using a modification of the Zincon method to monitor relative hemolymph calcium concentrations. Similar methods have been used to measure ionized serum calcium in humans (Corns 1987) and other biological fluids (Hilario et al. 1990).

### Statistical Analyses

### Correlations Between Hemolymph Metrics

A total of 96 crabs were included in the full dataset for analysis (excluding any crabs that died or escaped). Using the hemolymph samples from these crabs throughout the study ( $n = 649$  samples; ≤10 samples taken per individual crab), an ANOVA and follow-up Tukey HSD posthoc test were used to test whether there was a correlation between hemolymph color

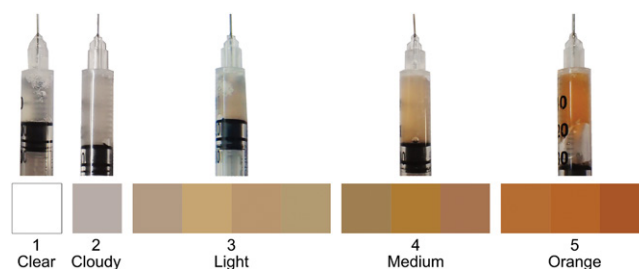


Figure 2. Color swatch scale used as a reference for quantifying the color of raw hemolymph samples (bottom). Examples of fresh hemolymph samples immediately after extraction from the crab are categorized as clear (1), cloudy (2), light (3), medium (4), and orange (5) (top).

category (1–5) and hemolymph RI. Test assumptions were verified by inspection of residuals. Using the random subset of hemolymph samples measured for calcium ( $n = 127$ ), a linear model determined whether there was a correlation between hemolymph RI and calcium concentration (ppm); and a second ANOVA was used to test for relationships between hemolymph color category (1–5) and calcium concentration (ppm).

#### Molt Stage Prediction

Several analyses were conducted to determine whether hemolymph color and/or RI could be used to predict the likelihood of a crab molting within 3 wk. After a crab begins to display visual premolt indicators (demonstrated in Fig. 1), it is typically considered to be within 3 wk of molting (McMahan 2020, Bradt & McMahan 2022); therefore, 3 wk was selected as a cut-off for the molt prediction analyses. For these analyses, the dataset was restricted to crabs held in the condo for  $\leq 3$  wk (crabs that died or escaped were excluded; crabs held  $> 3$  wk were excluded as “indeterminate” outcomes;  $n = 54$  crabs). Using this subset of crabs, a Welch two-sample  $t$ -test compared the initial hemolymph RI values for crabs that molted ( $n = 19$ ) and crabs that did not molt ( $n = 35$ ). Additionally, a binomial logistic regression model determined the hemolymph RI threshold above which crabs are  $> 50\%$  likely to molt within 3 wk. Crabs were binned based on initial hemolymph RI (1 RI unit steps). For each bin, the average hemolymph color value (1–5) and proportion of those crabs that molted within 3 wk were calculated. A binomial logistic regression was conducted on these proportions. In these analyses, “initial hemolymph” refers to the first hemolymph sample collected from each crab before being added to the condo at the start of its trial.

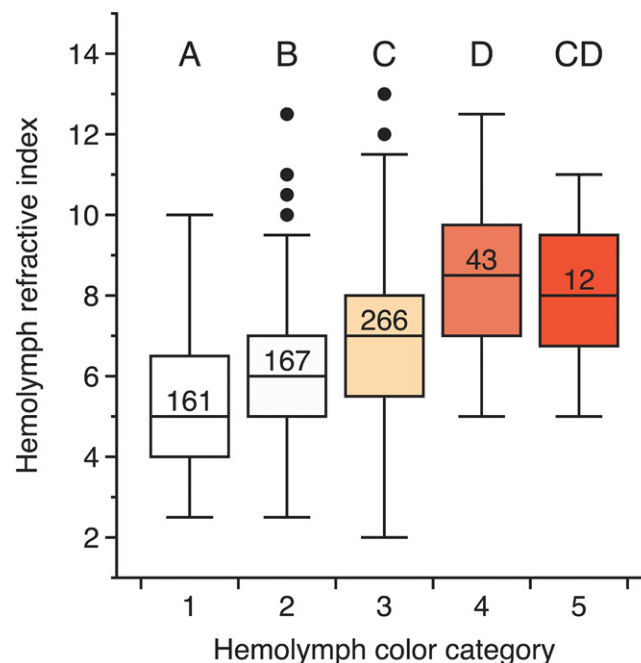
All analyses were conducted in R 4.2.2 (R Core Team 2021) and RStudio 1.4.1717 (Posit Software PBC, Boston, MA).

#### RESULTS

Hemolymph RI differed significantly between hemolymph color categories (on a scale of 1–5; ANOVA,  $F_{4,644} = 34.88$ ,  $P < 0.001$ ). Color categories 1 through 4 were significantly different from one another in terms of RI ( $1 < 2 < 3 < 4$ ; Tukey HSD); color 5 was not significantly different from colors 3 or 4 possibly due to the comparatively small sample size for color 5 ( $n = 12$ ; Fig. 3).

Calcium concentration (ppm) was significantly and positively correlated with hemolymph RI (linear regression;  $F_{1,125} = 19.40$ ,  $n = 127$ ,  $r^2 = 0.13$ , slope = 10.2,  $P < 0.0001$ ; Fig. 4A). Calcium concentration also differed significantly between hemolymph color categories (ANOVA;  $F_{4,122} = 7.00$ ,  $P < 0.0001$ ; Fig. 4B). Posthoc multiple comparisons revealed that color category 1 was associated with significantly lower calcium concentrations than colors 2 through 4; color 5 was not significantly different from any other color (1–4), likely due to the small sample size for color 5 ( $n = 2$ ; Fig. 4B).

When the dataset was restricted to only crabs held for  $\leq 3$  wk ( $n = 54$ ), 19 crabs molted within this timeframe and 35 did not. The initial hemolymph RI was significantly higher for crabs that molted within 3 wk ( $n = 19$ ; mean RI = 10.5) compared with crabs that did not ( $n = 35$ ; mean RI = 7.6) (ANOVA;  $F_{1,52} = 41.42$ ,  $P < 0.0001$ ; Fig. 5A). Logistic regression of this dataset indicated a significant positive relationship between initial hemolymph RI and the probability of a given crab molting within 3 wk (chi-square = 13.94,  $df = 1$ ,  $P < 0.001$ ; Fig. 5B). Examination of the ogive coefficients (function formula  $P = 1/1 + e^{a+\beta*RI}$ ; coefficient estimates  $a = 9.9871$ ,  $\beta = -1.0487$ ) produced by the logistic regression indicated that the probability of molting within 3 wk of condo intake exceeded 50% for crabs with an initial RI  $\geq 9.5$  (Fig. 5B). Additionally, crabs with darker hemolymph showed a higher probability of molting within this timeframe (Fig. 5B).



**Figure 3.** Refractive indices (RI) of hemolymph samples of varying colors on a scale of 1–5. Numbers inside boxes indicate the sample size for each color category; whiskers represent the extent of data within 1.5 IQR; boxes represent the upper and lower quartiles; the horizontal bar inside each box represents the median; outliers are shown by dots; letters indicate homogeneous subsets as determined by the Tukey HSD posthoc test.

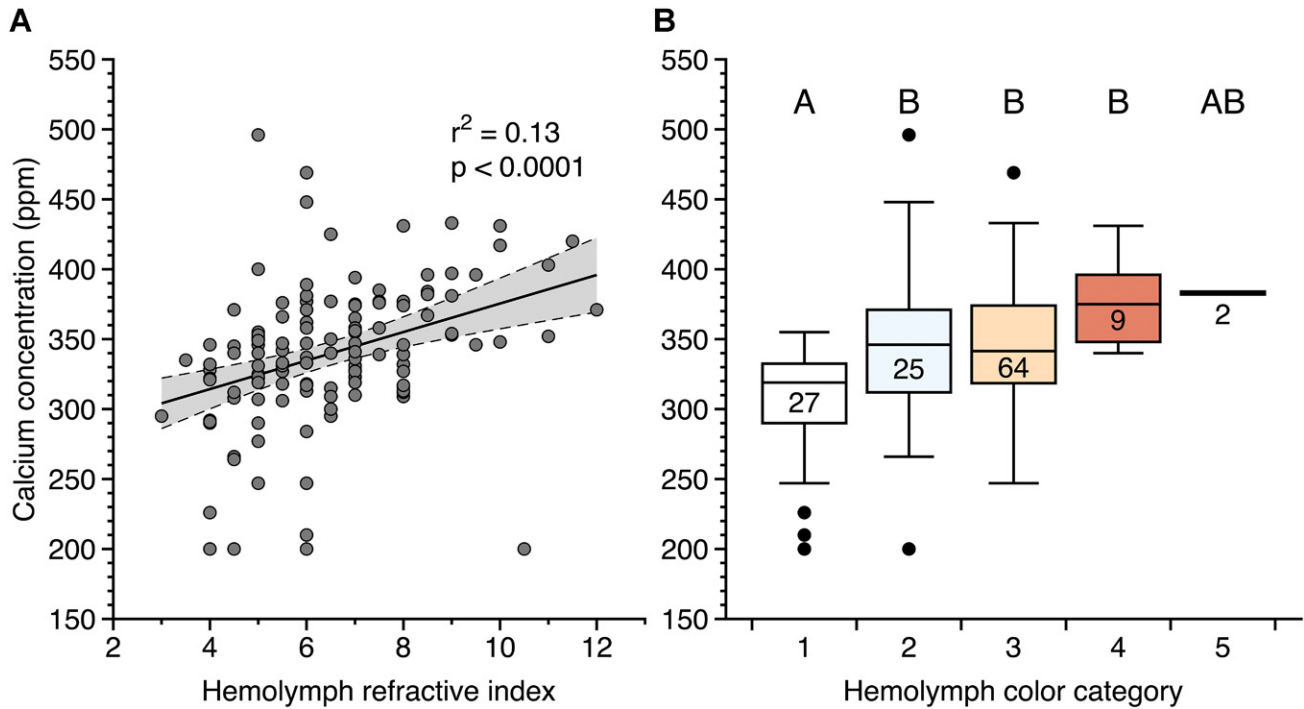


Figure 4. Correlation between calcium concentration (ppm) and (A) hemolymph RI, including a line of best fit and 95% confidence interval (gray shaded region;  $n = 127$ ); and (B) hemolymph color on a scale of 1–5. Numbers inside boxes indicate sample sizes for each color category; whiskers represent the extent of data within 1.5 IQR; boxes represent the upper and lower quartiles; the horizontal bar inside each box represents the median; outliers are shown by dots; letters indicate homogeneous subsets as determined by the Tukey HSD posthoc test.

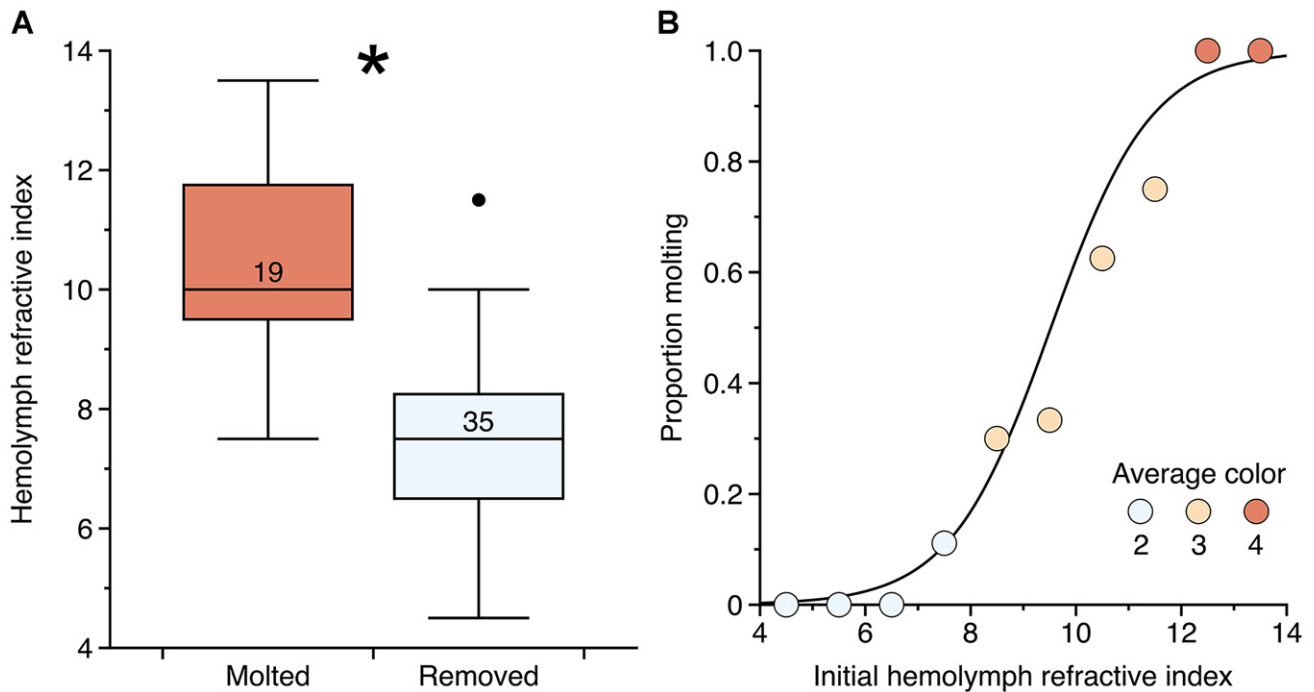


Figure 5. Hemolymph refractive index (RI) as a predictor of molt status (whether a green crab molts within 3 wk). (A) Initial hemolymph RI for crabs that either molted within 3 wk or were removed (did not molt within 3 wk). Numbers inside boxes indicate sample size; whiskers represent the extent of data within 1.5 IQR; boxes represent the upper and lower quartiles; the horizontal bar inside each box represents the median; outliers are shown by dots; asterisk indicates a significant difference between groups. (B) Logistic regression model showing the relationship between initial hemolymph RI and the probability of a crab molting within 3 wk. Dots represent the proportion of crabs within 1 RI unit bins that molted within 3 wk; dots are colored based on the average hemolymph color value for that bin; the line shows the logistic regression fit.

## DISCUSSION

The results from this study suggest that hemolymph color and RI can be useful predictors of molt status in the European green crab, *Carcinus maenas*. Crabs that molted within 3 wk of condo intake had significantly higher hemolymph RI and darker hemolymph at the start of their trial compared with crabs that did not molt within this timeframe. The results from the logistic regression suggest that crabs with initial hemolymph RI  $\geq 9.5$  are  $>50\%$  likely to molt within 3 wk of condo intake. This study also found that hemolymph color, RI, and calcium concentration were correlated with one another; as hemolymph darkens from clear to orange, RI and calcium concentration increase, likely due to the movement of protein and calcium between biological compartments in preparation for molt.

Previous studies have demonstrated that hemolymph protein concentration and RI vary with the molt cycle and are closely correlated with one another; this has been shown in green crabs (Busselen 1970, Truchot 1978, Terwilliger et al. 1999) as well as a wide diversity of other arthropods (e.g., cockroaches: Duhamel & Kunkel 1978; Dungeness crab *Cancer magister* Dana: Terwilliger et al. 1999; black tiger shrimp *Penaeus monodon* Fabricius: Berry et al. 2019). In particular, several studies have demonstrated that hemolymph protein in green crabs declines sharply after ecdysis, then increases through the intermolt phase, reaching a maximum level during the premolt stage (Busselen 1970, Truchot 1978). The results from the present study build on this existing knowledge by determining an RI threshold above which green crabs are more likely to molt within a set period of time, and by demonstrating a correlation between hemolymph RI, color, and calcium concentration within this species.

A study by Musgrove (2001) evaluated the relationship between hemolymph chemistry and pigment in the southern rock lobster, *Jasus edwardsii* (Hutton), and found that pigment—which ranged from light blue-gray to beige to deep orange—correlated with RI and molt stage. Hemolymph protein and calcium were at minimum concentrations immediately postmolt, increased through intermolt, and peaked in the early premolt stage (Musgrove 2001). The results from the present study corroborate these trends in the green crab and support the suggestion that hemolymph protein and color may be useful indicators of molting in aquaculture settings (Musgrove 2001).

These diagnostic tools—hemolymph color and RI—may be practical supplements to the use of external morphological cues already used by harvesters and researchers interested in producing soft-shell green crab products. If a crab is suspected to be premolt based on external morphological cues (i.e., due to the presence of graying outlines and/or halo on episternites; Poirier et al. 2016, Bradt & McMahan 2022), its molt probability can be further confirmed by determining the color and/or RI of a hemolymph sample. Although these biochemical markers do require obtaining a hemolymph sample, they may be particularly advantageous when morphological cues are unclear and further information is needed to confirm the molt status of a crab. Although all crabs used in the present study were suspected to be premolt based on external visual cues, the majority of crabs did not molt within 3 wk of condo intake, demonstrating the difficulty of determining the molt stage with this method alone. Hemolymph chemistry has been used to determine molt status in other crustacean species. The molt

stage of blue crabs (*Callinectes sapidus* Rathbun) is often distinguished based on the coloration along the margin of the swim paddle which transitions from blue to white to red as the crab approaches molt (Smith & Chang 2007). In a study by Lycett et al. (2018), ecdysteroid levels in blue crab hemolymph samples were used to determine finer-scale molt stage classification than could be determined by swim paddle coloration alone.

The trends shown in the present study are likely complicated by a number of environmental and physiological factors that influence crab hemolymph chemistry and color such as crab nutritional condition (Uglow 1969, Busselen 1970, Duhamel & Kunkel 1978; Berry et al. 2019), reproductive state (Ruddell et al. 2003), and salinity or calcium concentration of the surrounding seawater (Robertson 1937, Greenaway 1976). Fortunately, the salinity of the surrounding seawater did not vary greatly throughout this study over all tidal cycles ( $30 \pm 1.5$  SD). Although crabs were not actively fed in this study, they could occasionally access food that floated into the enclosure (e.g., shrimp, plant matter, other organic debris), affecting the nutritional condition of the crab and therefore the hemolymph RI (Uglow 1969, Busselen 1970, Duhamel & Kunkel 1978, Berry et al. 2019). The few crabs that extruded eggs during this study were excluded from the data set. It is possible that some of the female crabs were preovigerous and had not yet extruded their egg clutch. If so, egg development could have affected the hemolymph chemistry and/or color of some of the female crabs in this study, as has been demonstrated in other species (e.g., *Cancer pagurus*, Ruddell et al. 2003).

The present study aims to develop a supplemental diagnostic tool for determining if a green crab is premolt. With this goal in mind, the data presented here characterize the hemolymph of green crabs already suspected to be premolt based on subtle external cues and therefore may not represent the full range of hemolymph RI, calcium, and color exhibited throughout the green crab molt cycle. Although not a formal part of the present study design, hemolymph samples were collected opportunistically from a subset of postmolt green crabs ( $\leq 4$  days postmolt) which revealed RI ranging from 2 to 7 (mean  $5.3 \pm 1.2$  SD;  $n = 16$ ) and color ranging from clear to cloudy to light (color scale 1–3; mean  $2.2 \pm 1.0$  SD;  $n = 5$ ). Further research would be needed to fully characterize green crab hemolymph RI, color, and calcium across the full molt cycle.

It is well known that green crabs can drastically and negatively impact local ecosystems and fisheries in the areas they invade (reviewed in Frederich & Lancaster 2024). Development and expansion of a soft-shell market for green crabs in their invaded range are currently being explored to mitigate their impacts and capitalize on a rampant invasive population (Walter 2021, Bradt & McMahan 2022). In the past 5 y, an increasing number of local restaurants and organizations in Maine, New Hampshire, and Massachusetts have been slowly expanding consumer interest and a few harvesters are supplying soft-shell green crabs for this new market [G. Bradt (New Hampshire Sea Grant), M. McMahan (Manomet), & M. Masi (shell + claw), personal communication 2024]. The proportion of green crabs that successfully molt in holding is highly variable and dependent on environmental conditions as well as the level of expertise in premolt sorting; successful molting rates reported from local researchers and harvesters ranged from 24%–81% (Bradt et al., unpublished data). As mentioned previously, accurately determining molt status in green crabs with external morphological cues alone can be

challenging and requires extensive practice which can be a barrier to some harvesters interested in capitalizing on this resource. Having access to additional methods of determining molt status in green crabs such as hemolymph RI and color could potentially improve the sorting of premolt crabs that will successfully molt thereby improving molt rates and soft-shell crab production.

#### ACKNOWLEDGMENTS

We are grateful for the help and support from research interns and volunteers including Emma Spies, Gordon Shannon, Hannah Craft (NOAA Hollings Scholar), Anya St. Martin, Billy Bachelder, and Emily Burke. We also thank the Wells Harbor Master, Mike Yorke, for his support of this project. Three commercial harvesters (Mike Masi, Chris Jamison,

and Billy Bachelder) helped to pilot many aspects of this work and provided constructive feedback. This project was supported through a grant from NOAA's Saltonstall-Kennedy Fisheries Program (NA20NMF42701546). This research was conducted under Maine DMR Special Licenses 2021-18-02 and 2022-52-01.

#### AUTHOR CONTRIBUTIONS

Conceptualization, experimental design, data collection, writing: L. Crane. Experimental design, data collection, data analysis, writing: E. Dombrowski. Data analysis, writing: B. Gutzler. Conceptualization, experimental design, writing: S. Jury. Conceptualization, experimental design, data collection, writing: G. Bradt. Conceptualization, funding support, experimental design, writing: J. Goldstein.

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