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**USING REFLEX ACTIONS TO PREDICT DELAYED POST-HARVEST MORTALITY  
OF AMERICAN LOBSTER (*HOMARUS AMERICANUS*) IN MAINE'S LOBSTER  
SUPPLY CHAIN**

By

Cassandra Leeman

B.S. Eckerd College, 2019

A THESIS

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Science

(in Marine Biology)

The Graduate School

The University of Maine

December 2022

Advisory Committee:

Dr. Damian Brady, Associate Professor of Oceanography, Advisor

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(HOMARUS AMERICANUS) USING REFLEX IMPAIRMENT**

By

Cassandra Leeman

Thesis Advisor: Dr. Damian Brady

An Abstract of the Thesis Presented  
in Partial Fulfillment of the Requirements for the  
Degree of Master of Science  
(in Marine Biology)  
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In live seafood industries, maintaining product quality and survivorship are critical aspects of the supply chain infrastructure. Post-harvest mortality in the American lobster (*Homarus americanus*) fishery can result in a significant loss in revenue for the largest single species fishery in North America. In Maine, the wholesale lobster distribution supply chain directly and indirectly supports state and local economies, providing almost \$1 billion in revenue and dominates the fishery, producing 82% of the total lobster landings in the USA (Donihue, 2018; NOAA, 2021). However, at least 2% of the lobster landed in Maine die before they reach consumers, representing an industry loss of roughly 952 metric tons, or \$14.5 million in value every year (ME DMR, 2022). The lobster supply chain is a network of harvesters, dealers, and distributors that facilitates the transport of live product domestically and internationally. The majority of product loss comes in the form of delayed mortality as a result of stressors within the supply chain. Because of the high volume of lobster transported through the supply chain and its many links, a standard protocol is needed to quickly diagnose whether a high-value live lobster

will survive the trip to the consumer. A reflex action mortality predictor (RAMP) model was developed to reliably predict subsequent mortality days after exposure to the supply chain. RAMP models have been successfully used in commercially important fish and crustacean industries to predict discard mortality, but has never been applied in a post-harvest context or for a *Homarus* species.

To model and predict delayed post-harvest mortality using the RAMP model, a three-part methodology was completed and followed by a pilot field test to demonstrate the feasibility of this method. An initial investigation was conducted to identify which reflexes were stereotypic of a healthy lobster. Subsequently, a holding experiment that monitored the survival after exposure to the supply chain was conducted at lobster dealer holding facilities. Carapace length, sex, shell hardness, injuries, and discrete reflex actions of the experimental lobsters were recorded to build logistic RAMP models. Results suggest that carapace length along with four specific reflex actions and five types of injury are significant predictors of mortality up to five days after arrival to a lobster dealer facility. The reflex actions are: eye motion, pereopod motion, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion. The five injuries are: missing chelae, damaged chela, damaged antenna, damaged carapace, and damaged uropod. Measuring these significant predictors takes no more than 20 seconds per lobster. A final, proof-of-concept investigation was conducted at three transfer points in the supply chain to test the practicality of the RAMP method and to test the differences of predicted post-harvest mortality among these sites.

This model can be an important tool in identifying supply chain stressors that impact lobster quality and inform efforts to improve the efficiency and resiliency of the industry. Developing a RAMP model to predict post-harvest mortality demonstrates the feasibility of using reflex actions as predictors of mortality in a novel context, acting as a foundational method

for future studies. The highly predictive, non-invasive, quick, and cost-effective nature of this method has potential to become a versatile tool for both industry and scientific applications.

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## **LIST OF ABBRIVIATIONS**

AIC – Akaike’s information criterion

AUC – Area under the receiver-operating characteristic curve

CHH – Crustacean hyperglycemic hormone

MLSC – Maine’s lobster supply chain

RAMP – Reflex action mortality predictor

# CHAPTER 1

## INTRODUCTION

### 1.1. Background

The American lobster (*Homarus americanus*) fishery is the most valuable single species fishery in North America, with approximately 80% of the U.S. product harvested from Maine (NOAA, 2022). The lobster fishery in Maine has a long history, and is a model fishery for sustainability and fishery management as populations remain healthy and the market, both supply and demand, continues to grow. Since the 1960's, the value of landed lobster has increased 40-fold (ME DMR, 2022). In Maine, the wholesale lobster distribution supply chain directly and indirectly supports state and local economies, providing nearly \$1 billion in revenue in 2018 (Donihue, 2018a); considering the record breaking \$730 million of landed lobster in 2021 this value has likely grown (ME DMR, 2022). Lobster is able to achieve such a high value partially from the way in which it is delivered to a customer: alive.

Live seafood markets, such as the lobster fishery, are highly valuable, but transporting live animals to a consumer is a taxing experience and can decrease the quality and survivorship of the product. To maintain high-value and minimize quality loss and profits, live seafood industries have adopted strategies and technologies within their infrastructures to minimize supply chain stress, such as cold refrigeration during transport and grading product based on an index of condition. The lobster (*Homarus americanus*) industry is a model live seafood fishery that requires stress reduction infrastructure to maximize profits as it harvests and transports 54.4 million kilograms of lobster annually and is renowned for its value and quality (NOAA, 2022).

Though a profitable industry, transporting high-value live lobsters introduces the particular and costly challenges of maintaining quality and survivorship within the supply chain. The experience of traveling through Maine’s lobster supply chain (MLSC) is essentially unique for each harvested lobster as environmental and handling conditions are discrete. In its most direct form, MLSC begins with a trap set on the seabed. Captured lobsters are then hauled with the trap onto the vessel and placed in a temporary flow-through holding tank until the product is transferred to a holding crate at a wharf. A commercial, often refrigerated, truck arrives at the wharf and transports the catch to a lobster distributor. At the distributor, the lobsters are “graded”, a process of sorting the lobster by size, vitality, and shell condition. The sorting process determines which are shipped live to a consumer or processed at the facility into a value-added product, such as frozen ready-to-eat lobster tails, shucked meat, or lobster cakes.

At each point and transfer along MLSC there are unique sets of stressors that could influence the value and quality of the product. Stressors within MLSC influence the condition of the lobster and in extreme instances can lead to post-harvest mortality. Mortality rates are recorded by lobster dealers after product arrival to the holding facility, a practice that may underestimate true mortality values arising from delayed mortality consequent on MLSC stressors. Maine’s lobster industry has struggled with post-harvest mortality, colloquially known as “shrinkage”, citing it as their third greatest challenge next to labor shortages and profit margins (Donihue, 2018).

Historically, Maine’s lobster fishery held lobsters in impoundments where pathogens (e.g., winter impoundment shell disease and ‘bumper-car’ disease; Cawthorn et al., 1996a; Smolowitz et al., 1992) and crowding led to increased post-harvest mortality rates (D. W. McLeese & Wilder, 1964; Theriault et al., 2008; Tlusty & Preisner, 2005). Globalization,

through technology allowing long distance shipping of live lobster, and diversification of markets led to more streamlined supply chains that reduced post-harvest mortality by-passing the need for impoundment (Leeman et al., 2022). While current estimates are not well known, in personal communications with four of the largest lobster distributors in Maine, the current post-harvest mortality rates of lobsters arriving at the dealer range from 0.5 - 5%, averaging at 2%.

Many possible stressors in MLSC impact the survivability of post-harvest lobsters. Basic physiological principles dictate that mortality occurs when homeostatic mechanisms, either behavioral or physiological, are unable to compensate for a change in environmental conditions (Barton & Iwama, 1991). For crustaceans, size, sex, and shell condition, among other factors can impact vulnerability to stressors (Kruse et al., 1994; Milligan et al., 2009; A. Stoner, 2012). In MLSC, lobsters are exposed to a variety of environmental, mechanical, and/or biological stressors including barotrauma from the rapid change in pressure during hauling (Basti et al., 2010; Lorenzon et al., 2007), thermal stress (Chang et al., 1998; Dove et al., 2005; Giomi et al., 2008; Lorenzon et al., 2007; Spees et al., 2002), handling (DiNardo et al., 2002; Kruse et al., 1994; Lavallée et al., 2000), warm weather, rain, sunlight, and rough seas (Lavallée et al., 2000), low dissolved oxygen (Cheng et al., 2003; Lehtonen & Burnett, 2016; D. W. McLeese, 2011), low salinity (Chang et al., 1998; Harris & Ulmestrand, 2004; D. W. McLeese, 2011), bacterial infections (Basti et al., 2010; Lorenzon et al., 2007), air exposure (Chang et al., 1998; Danford et al., 2001; Lorenzon et al., 2007), and vibrations during transport (Powell et al., 2016).

Physiological and behavioral responses compensate for stress. Initial responses are often behavioral, such as shelter seeking, defensive behaviors, or escaping to a different habitat (Pearson & Olla, 1980). However, if the organism cannot move to a better environment, physiological mechanisms must be engaged to regain homeostasis. They include the rapid



release of crustacean hyperglycemic hormone (CHH) with subsequent hyperglycemia to meet the increase in metabolic demands (Chang, 2005; Patterson et al., 2007; Telford, 1968). The release of CHH induces a cascade of physiological changes, including increased transcription of heat shock proteins (Chang, 2005), decreased pH and increased lactate, calcium, and ammonia (Lorenzon et al., 2007). Chronic or acute stress can decrease immune response and increase the disposition of bacterial infection in *H. americanus* (Lorenzon et al., 1999). Infections of *Photobacterium indicum* have been linked as a result of capture-related stress in Maine's *H. americanus* fishery and is suspected to be responsible for the fatal systemic inflammatory response observed in harvested lobster (Basti et al., 2010).

Current methods to monitor crustacean health include hemolymph assays, vigor assessments, and direct observation (A. Stoner, 2012). Hemolymph chemistry measures different physiological stress markers and the deviations from rest can indicate states of acute or chronic stress. This method is expensive, requires training, special materials, laboratory equipment and space (A. Stoner, 2012). Vigor assessments (the observation of the strength of a lobster's behaviors) has been recorded in past studies as a diagnostic indicator of condition (Basti et al., 2010; Lavallée et al., 2000). The methods to choose which behavioral markers are significant indicators of health have not been well recorded or described. However useful these types of assessments are in understanding lobster health, they are not strong, predictive indicators of delayed post-harvest mortality, hence the need for a novel approach to measuring this mortality in MLSC. (A. Stoner, 2012).

Physiological and behavioral indicators of stress do not necessarily correlate with post-harvest mortality and because the volume and value of lobsters keeps increasing over time, a relatively fast and robust method of identifying future mortality could improve processing

procedures and identify specific links in the supply chain that cause post-harvest mortality. One potential analog to post-harvest mortality is discard mortality, the mortality that occurs from the stress of capture and release in fisheries, based on the absence of selected reflexes (Davis & Ottmar, 2006). Rather than assessing the commonly used physiological stress responses with hemolymph chemistry, the reflex action mortality predictor (RAMP) method uses the absence of specific reflexes, a symptom of the loss of maladaptive behavioral responses, as a direct indication of an individual experiencing extreme stress.

This model determines the relationship between the likelihood of future mortality and the number of reflexes lost after exposure to stress. The RAMP model developed for the snow crab (*Chionoecetes bairdi*) determined that kick, leg retraction, leg flare, chelae closure, eye retraction, and mouth closure reflex actions were predictive indicators of delayed mortality after the stressful interaction of being captured and discarded by a bottom trawl gear fishery (A. W. Stoner et al., 2008). By testing the response of these six reflex actions, the likelihood of mortality was predicted correctly 91% of the time (A. W. Stoner et al., 2008). These results demonstrated a highly effective and inexpensive method for accurately quantifying an often unobservable outcome. The RAMP method is a useful tool in fishery management and it has been applied in several crab and prawn species to identify discard mortality after exposure to fishing stressors with reliable predictive power (M. W. Davis, 2007; Hammond et al., 2013; A. Stoner, 2012, 2012; A. W. Stoner et al., 2008; Walters et al., 2021; Yochum et al., 2015; Kronstadt et al., 2018).

## **1.2. Research Questions**

The RAMP method provides a probability of mortality after an individual is exposed to the combined effect of the system's stressors, remaining independent and reliable over size, sex, reproductive condition, or molt stage (Davis, 2010; A. W. Stoner, 2012). A RAMP model has not been applied to *H. americanus*, but the success of this method applied to other crustacean species suggest a RAMP model would be a powerful tool in predicting delayed mortality and aid in identification of stressors that have the greatest impact to lobster health in MLSC. With an industry worth \$730 million per year in Maine alone, a conservative 2% post-harvest mortality rate caused by stressors within the supply chain equates to \$14.5 million dollars of annual revenue loss (ME DMR, 2022). This work details the development of a RAMP model that predicts post-harvest delayed mortality in MLSC and a trial of this model's ability to quantify mortality trends at different points in the supply chain.

## CHAPTER 2

### METHODS

#### 2.1. Stereotypic Reflex Identification

Initially, we tested eleven reflexes to identify which reflexes healthy lobsters would consistently present in response to stimuli (Table 1). Ten freshly harvested, uninjured lobsters were collected and placed in a chilled cooler and transported to the University of Maine Darling Marine Center's flowing seawater lab within 30 minutes. Prior to placing lobsters in a crate held in a tank, each individual was tagged on their left chelae with labeled zip ties. The flow-through tank was maintained at a mean temperature ( $\pm$ SE) of 13.45°C ( $\pm$  0.03°C), had a volume of 2490 liters and an exchange rate of 7.6 liter minute<sup>-1</sup>. The crate remained closed except when reflexes were tested. The lobsters were held for an acclimation period of seven days before the reflex identification procedure. To test the presence or absence of each response, a precise and consistent handling approach was applied. Each reflex was tested one time per day for three consecutive days. Each lobster was held by the carapace, oriented laterally, and the reflexes were tested in the following order: abdomen turgor, defensive chelae, pleopod motion, 2<sup>nd</sup> antennae motion, 1st antennae motion, eye retraction, pereopod motion, chelate pinch, 3<sup>rd</sup> maxilliped motion, 3<sup>rd</sup> maxilliped retraction, 2<sup>nd</sup> maxilliped motion (Table 1). Reflex scores were evaluated as binary (present or absent) to minimize subjectivity (A. W. Stoner, 2012). Each reflex was tested for a response up to three times (at five second intervals) before it was noted as absent. Each test took ~90 seconds. Finally, reflexes present in every individual were selected as the reliable reflexes to apply in the development of the RAMP model (Table 2).

#### 2.2. Field experiment

After identifying the most reliable reflexes, 975 lobsters were assessed and monitored between the months of June and August of 2021 at two commercial distribution centers to develop the RAMP model. Each sampling session was conducted on single crates (commercial standard of 40.8 kg of lobster per crate, equating to ~75 individuals) separated from the commercial cohort after their arrival to the facility and before their placement in the purge tank. Purge tanks are aerated recirculating tanks that allow lobsters to recover before they are packed and shipped to the consumer (Lorenzon et al., 2007), and conditions were standard for lobster distribution centers; water temperature of ~3 - 5 °C, total ammonia levels <1.0 ppm, and strong, constant aeration. Each crate was held for 5 days because this is the standard maximum amount of time a lobster would remain in the supply chain after arrival at a lobster distribution center. This study was designed to assess lobsters that had been exposed to MLSC regardless of the type of exposure, and as such, the exposure histories of assessed crates were not recorded.

Reflex assessments were conducted following the same precise, systematic approach as the preliminary study before the crate was returned to the holding tank. Reflexes were tested in the following order: abdomen turgor, eye retraction, leg motion, chelate pinch, 3<sup>rd</sup> maxilliped motion, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion. Sex was determined by visual observation of the first pleopod. Molt stage was determined by shell rigidity in the following categories, postmolt stages C<sub>1</sub> and C<sub>2</sub> and intermolt stages C<sub>3</sub> and C<sub>4</sub> or D. Differentiation between stages was conducted with the finger-pressure method as described by Aiken (1980). We did not separate C<sub>4</sub> from D due to the subjectivity of differentiating the two stages without a durometer (Aiken, 1980). In the statistical analysis, shell rigidity was considered as a range from 1 - 4, one for C<sub>1</sub>, two for C<sub>2</sub>, three for C<sub>3</sub>, and four for C<sub>4</sub> or D.

Injuries were recorded if they were new, determined by absence of a sheath, as defined by Durkin et al. (1984), to eliminate data on injuries that happened prior to entering the supply chain. New injuries were noted on a binary scale of present or absent. The 12 types of injuries recorded were: damaged antenna, one damaged chela, two damaged chelae, one missing chela, two missing chelae, damaged carapace, damaged tail, damaged uropods, missing 1 - 4 pereopods, missing 5 - 8 pereopods, damaged rostrum, or other. The length of the carapace was recorded by measuring from the rear edge of the eye socket to the lateral edge of the carapace, parallel to the center line. Individuals were tagged with identifying zip-ties around their left chelae before being placed in a crate. Assessments took approximately 90 seconds per lobster. For the subsequent four days after the initial assessment, each individual was assessed for mortality. If an individual was dead, they were removed from the tank and their identification number and date was recorded.

### **2.3. Reflex impairment, injury indices, and statistical analysis**

The development of the RAMP model followed the method described in Stoner (2008) with an adjustment to accommodate a different injury scoring system. Briefly, the reflex impairment score (RIS<sub>7</sub>) was calculated by adding the number of reflex actions absent, ranging from 0 to 7. This approach weights each reflex action equally and represents the overall condition of the individual (Davis, 2007). Similarly, the injury score (IS<sub>10</sub>) was calculated by adding the number of injuries present, ranging from 0 to 10 (Walters et al., 2022). Due to a low number of “missing two chelae” (n = 9) and “5 - 8 pereopods missing” (n = 2) conditions, we created a new condition: 1 - 8 pereopods missing and 1 - 2 chelae missing for statistical analysis. This scoring approach is different from some past RAMP development procedures that used an injury scoring system that weighed the severity of injuries (Stoner, 2008). We chose the equal

weighting approach used in Walters et al. (2022) and other studies as it is relatively quick and reduces subjectivity allowing for broad application in industry and scientific use. While the reflex actions and injuries were weighted equally, we used the model selection process to identify injuries associated with post-harvest mortality. Mortality was a binary response with (1) representing mortality and survival as (0) over the 5-day confinement period.

During model selection, 11 candidate logistic regression models were developed, with each model consisting of a unique combination of predictor variables using RStudio's "glm" function. We used the following covariates to develop the models: reflex impairment index, injury score index, binary injury score (presence/absence), carapace length, shell rigidity, sex, presence of shell disease, and day of year (DOY). The response variable was binomial (0 = alive, 1 = dead). Reflex actions and injuries were explored as both continuous (RIS<sub>7</sub> and IS<sub>10</sub>) and individual predictor variables in separate analyses. In addition to robust prediction of post-harvest mortality, we considered the speed and simplicity of a test that could be administered at commercial facilities. Therefore, we took the significant individual reflexes and injuries from the backwards stepwise approach to develop a new reflex impairment score (RIS<sub>4</sub>) and injury score (IS<sub>5</sub>).

For each model, a backwards stepwise approach was used using the "dropterm" function in RStudio to determine the most parsimonious model by using the Akaike's Information Criterion (AIC) to determine significance of individual predictors through drop-in-deviance tests (Akaike, 1998; Table 5; RStudio version 2021.09.0 Build 351). Odds ratios were calculated for each parameter in best performing candidate models by exponentiating the parameter estimates. As a measure of discriminatory performance of candidate models, we calculated the area under the receiver-operating characteristic (ROC) curve (AUC), which ranged from 0 to 1, with higher

values indicating greater classification accuracy (1 - sensitivity; Thuiller et al., 2005). Sensitivity is defined as the proportion of true positives correctly predicted (Swets, 1988). Candidate models were developed using maximum likelihood estimators and assessed, using McFadden's pseudo- $R^2$ . McFadden's  $R^2$  was selected as it has been described as a preferred index over other pseudo- $R^2$  analogs (McFadden, 1974; Menard, 2000).

The maximum likelihood estimates of mortality were calculated as:

$$R_{McFadden}^2 = 1 - \frac{\ln(L_{mod})}{\ln(L_0)}$$

Where:

$L_{mod}$  = maximum likelihood from fitted model; and

$L_0$  = maximum likelihood from model with intercept only

The logistic model for mortality was described by the following equation:

$$\text{Log}_e \left( \frac{p}{1-p} \right) = \alpha + \beta'x$$

Where:

$p$  = probability of lobster mortality within the holding period;

$\alpha$  = intercept;

$\beta'$  = model coefficients; and

$x$  = the model matrix of explanatory variables



## **2.4. Predicting mortality in MLSC**

A trial, using this RAMP model, was conducted June to August, 2022 to determine if three specific settings within MLSC significantly impacted survivorship more than others. The lobsters observed in this trial were intercepted at three transfer points in the supply chain: harvesting vessel to wharf buying station, wharf to refrigerated commercial truck, and truck to lobster dealer. Lobsters were selected at random and sampled directly after offloading from the previous point and before loading to the next. Sampling was done in the shade and within a 15-minute window to avoid additional stress. The study was conducted at two wharves and two dealer locations, observing the carapace length, reflex actions, and injuries selected in the RAMP model development as significant predictors of delayed mortality (see 3.3. for further details).

## **2.5. Statistical Analysis for predicting mortality in MLSC**

The best approximating candidate model developed in the RAMP model development study (see 3.3. for further details) was used to predict likelihood of mortality by using RStudio's "glm" and "predict" function. The following covariates were used in the model: eye retraction, pereopod motion, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion, five injury score, and carapace length (mm; see 3.3. for details on score indices). The field experiment data (collected in 2.2.) was used as the training dataset to populate the RAMP model and the data collected at each transfer point were used as the test dataset. The RAMP model was used to predict the likelihood of mortality for each lobster sampled from the test data using the "predict" function. The differences of predicted survivorship between transfer points were assessed using a Welch Two Sample T-test.

Table 1. Reflexes, the “test” to elicit a response, and qualifications for a response to be present or absent.

<b>Order tested</b>	<b>Reflex</b>	<b>Test</b>	<b>Present response</b>	<b>Absent response</b>
1	Abdomen turgor	Lift lobster by the carapace, dorsum up	Abdomen is extended to horizontal position or tail flip occurs	Abdomen hangs limply and without motion
2	Defensive chelae	Lift lobster by the carapace, dorsum up	Claws raise above the horizontal plane	Claws droop below horizontal
3	Pleopod motion	Lift lobster by the carapace, dorsum up	Pleopods are active and in motion	Pleopods are motionless
4	2 <sup>nd</sup> antennae motion	With a blunt probe, manually stimulate the base of the antennae	Antennae move spontaneously or upon stimulation	Antennae are motionless upon stimulation

Table 1 continued

5	1st antennae motion	With a blunt probe, manually stimulate the base of the antennae	Antennae move spontaneously or upon stimulation	Antennae are motionless upon stimulation
6	Eye retraction	Touch eye stalk with blunt probe	Eye stalk retracts strongly in the lateral direction below the carapace hood	Eye stalk retracts weakly or demonstrates low resistance to lifting
7	Pereiopod motion	While lifted by carapace, dorsum up, manually stimulate the pereiopods	Pereiopods move spontaneously when the lobster is lifted or upon stimulation with blunt probe	Pereiopods are motionless upon stimulation
8	Chelate pinch	While lifted by carapace, dorsum up, place a blunt probe in the claw of first walking leg	Chelate apply pressure to blunt probe	Chelate does not apply any pressure to blunt probe

Table 1 continued

9	3 <sup>rd</sup> maxilliped motion	While lifted by carapace, dorsum up, manually stimulate the 3 <sup>rd</sup> maxilliped	3 <sup>rd</sup> maxilliped move spontaneously when lifted or upon stimulation with blunt probe	3 <sup>rd</sup> maxilliped are motionless upon stimulation
10	3 <sup>rd</sup> maxilliped retraction	While lifted by carapace, dorsum up, manually retract the 3 <sup>rd</sup> maxilliped ventrally	3 <sup>rd</sup> maxilliped retract to cover mouth parts	3 <sup>rd</sup> maxilliped hangs limply
11	2 <sup>nd</sup> maxilliped motion	While lifted by carapace, dorsum up, manually stimulate the 2 <sup>nd</sup> maxilliped	2 <sup>nd</sup> maxilliped move spontaneously when lifted or upon stimulation with blunt probe	2 <sup>nd</sup> maxilliped are motionless upon stimulation

## CHAPTER 3

### RESULTS

#### 3.1. Stereotypic Reflex Identification

Abdomen turgor, eye retraction, pereopod motion, chelate pinch, 3<sup>rd</sup> maxilliped motion, 3<sup>rd</sup> maxilliped retraction, 2<sup>nd</sup> maxilliped motion reflexes were selected as stereotypic responses that were included in the development of the RAMP model (Table 2). An individual displayed absence of abdomen turgor, leg motion, and 2<sup>nd</sup> maxilliped motion reflex actions (29, 29, 28 respectively) but the reflexes were included as stereotypical as the lobster was experiencing cannibalistic stress during the time of sampling.

#### 3.2. Field experiment

A total of 975 lobsters from 13 crates were held in a commercial holding tank in order to investigate the relationship between post-harvest mortality and reflex impairment. The tank conditions at both sites were typical of commercial operations within the lobster supply chain, maintaining a mean water temperature ( $\pm$ SE) of 4.37°C ( $\pm$ 0.008°). 21 of the individuals died during the 5 days of holding (Table 3). Mortality was observed across the 5-day holding period: 6 on Day 1, 4 on Day 3, 4 on Day 4, and 7 on Day 5. The sex ratio of the total population was 69% male 31% female, with a nearly identical ratio among those that died: 71% male and 29% female (Table 3). This sex ratio is typical for the American lobster fishery as egg-bearing females are v-notched and returned to the water as part of the fishery's conservation and management efforts (Jury et al., 2019). Of the 13 crates assessed, 4 crates had no mortality, 1 crate with 1 mortality, 2 crates with 2 mortalities, and 4 crates with 3 mortalities, and 1 crate with 4 mortalities.

The mean ( $\pm$  SE) carapace lengths between lobsters that survived and died were 88.62 ( $\pm$  0.13) mm and 90.24 ( $\pm$  0.86) mm, respectively. The group that survived had a smaller mean size than those that died, but the difference was insignificant (Welch Two Sample T-Test,  $p = 0.078$ ; Figure 1).

The greatest difference in frequency of absent reflexes between the lobsters that died in holding and those that survived was pereopod motion with an absence of the reflex 52.4% and 2.2% of each group respectively (Table 4). When just one reflex was lost, 2<sup>nd</sup> maxilliped motion and chelate pinch were most common (Table 4). Damaged antenna was the most frequent single injury, accounting for 61% of all single injuries observed (Table 4).

### **3.3. Logistic regression analysis**

Reflex impairment, injury, and carapace length were the most significant predictors of mortality (Table 5). The 5 most parsimonious models all included these three parameters, with the injuries and reflexes considered as independent categorical parameters or as an aggregated continuous score (e.g., RIS<sub>7</sub>, IS<sub>10</sub>; Table 5). Less parsimonious models (i.e., the sixth through eleventh best candidate models) included additional variables beyond the core three covariates (i.e., injury, reflex actions, and carapace length) such as day of year, shell rigidity, sex, and presence of injury, but the AIC values indicate these models' added complexity without significantly improving predictive power (Table 5).

Developing a RAMP model within the context of a commercial use required exploring multiple models with varying complexity of covariates to identify models with a robust suite of covariates yet condensed to allow for a quick assessment. When using the backwards stepwise approach with categorical reflexes and injuries, the significant independent reflexes or injuries

were aggregated to a new reflex and injury score. The new summed reflex impairment score (RIS<sub>4</sub>) consisted of leg motion, eye retraction, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion. The new summed injury score (IS<sub>5</sub>) consisted of missing claws, 1 damaged chelae, damaged carapace, damaged antenna, and damaged uropods. The reflex scores and injury scores were compared using logistic models to compare the relationship between reflex actions and mortality and injury and mortality (Figure 2). The logistic model including all seven reflex impairment scores (RIS<sub>7</sub>) was only 0.16% more predictive of mortality than the model including only four reflexes (RIS<sub>4</sub>). By contrast, the logistic model including all 10 injuries, IS<sub>10</sub> was 12.65% less predictive of mortality than IS<sub>5</sub> including only five injury categories (Figure 2). In short, little was gained in predictive power by adding more than four key reflexes to the model, and the model became considerably more predictive with only a subset of key injury indicators. Using the abbreviated RIS<sub>4</sub> and IS<sub>5</sub> scoring systems, 96.72% of lobsters sampled had a 0 or 1 RIS<sub>4</sub> score (Table 7) and all uninjured lobsters survived, but 3% (21/704) of the injured lobster died in holding (Table 7).

Indicators of model performance suggest the suite of final models were robust and reasonably predicted the probability of post-harvest mortality. For example, all five top models had AUC scores of 0.95 and McFadden's pseudo-R<sup>2</sup> ranged from 0.41 - 0.5 which are classified as excellent fits (Table 5 & 6; Brotons et al., 2004; McFadden, 1974; Menard, 2000). In the 5 top-performing candidate models, the models that classified reflexes as independent categories, pereopod motion was always the most significant reflex compared to the other reflexes, congruent with the greatest difference in frequency of the absences of that reflex between the surviving group and the post-harvest mortality group (Table 4). Models that classified reflexes as a continuous score had the greatest significance of all included in the model, followed by IS<sub>10</sub>,

IS<sub>5</sub>, and then carapace length (Table 6). In the best approximating model, the odds ratios (OR) demonstrate that for a lost eye retraction, pereopod motion, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion, the individual was 16.87, 24.27, 0.12, 3.59 times more likely to experience mortality during the holding period, respectively (Table 6). For every 1 mm increase in carapace length or one unit increase of IS<sub>5</sub>, there is a 1.16 and 3.97 respective increased likelihood of experiencing mortality during the holding period (Table 6).

Visualization of the fifth most parsimonious model demonstrates the significance of carapace length when predicting post-harvest delayed mortality (Figure 3). Individuals that have either both high or both low injury scores (IS<sub>5</sub>) and reflex impairment (RIS<sub>4</sub>), the likelihood of mortality is concentrated to a small range, independent of size (Figure 3). When RIS<sub>4</sub> and IS<sub>5</sub> are within the range of these injury and reflex scales, carapace length plays a greater role in determining the likelihood of mortality with larger animals having a higher probability of mortality (Figure 3). For example, a lobster at minimum harvestable size (carapace length 83 mm) and with 2 reflexes absent and 2 injuries, has a 10.5% chance of dying. However, 105 mm lobster under the same injury and reflex conditions, experiences a considerably higher 61.6% risk of death.

### **3.4. Predicting mortality in MLSC**

A total of 392 lobsters were observed in MLSC; 252 at the harvesting vessel to wharf transfer point from eight different sampling days, 66 at the wharf to truck point from two different sampling days, and 74 at the truck to dealer point from one sampling day. The mean ( $\pm$  SE) carapace lengths of lobsters that were sampled at the vessel to wharf, wharf to truck, and truck to dealer transfer points were 88.88 ( $\pm$  0.320) mm and 87.86 ( $\pm$  0.484) mm, and 88.09 ( $\pm$



0.424) mm, respectively. Eye retraction, pereopod motion, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion had the greatest frequency of loss of reflex in 22.97%, 28.38%, 25.68%, and 25.68% of the lobsters sampled at the truck to dealer transfer point, respectively (Table 8). Pereopod motion at the vessel to wharf transfer point was absent in 11.11% of the sample size, but 3.03% at the wharf to truck transfer point (Table 8). Injuries increased after each new leg of the supply chain, where the mean IS<sub>5</sub> score ( $\pm$  SE), went from 1.13 ( $\pm$  0.053), 1.46 ( $\pm$  0.081), to 1.85 ( $\pm$  0.12) at the vessel to wharf, wharf to truck, and truck to dealer transfer points, respectively (Table 8). The mean likelihood of mortality ( $\pm$  SE) was highest at the truck to dealer transfer point at 17% ( $\pm$  0.035; Table 8, Figure 4). The predicted mortality at the transfer points of vessel to wharf and wharf to truck were lower at 2% ( $\pm$  0.5%) and 1% ( $\pm$  0.2%), respectively (Table 8, Figure 4). There was a significant difference between the truck to dealer transfer point and both vessel to wharf and wharf to truck points ( $p = 1.64\text{e-}13$ ,  $4.43\text{e-}06$ , respectively), but there was no significant difference between the vessel to wharf and wharf to truck transfer points ( $p = 0.13$ ).

Table 2. Reflex actions and the % presence after being tested on 10 lobsters 3 days in a row (n = 30), \* indicates which reflexes were selected as stereotypic and used for the development of the RAMP model.

<b>Reflex Action</b>	<b>Presence of reflex action (%)</b>
<b>Eye retraction *</b>	100
<b>Chelate pinch *</b>	100
<b>3<sup>rd</sup> Maxilliped motion *</b>	100
<b>3<sup>rd</sup> Maxilliped retraction *</b>	100
<b>Abdomen turgor *</b>	96.7
<b>Pereiopod motion *</b>	96.7
<b>2<sup>nd</sup> Maxilliped motion *</b>	93.3
<b>Pleopod motion</b>	90
<b>1<sup>st</sup> Antennae motion</b>	86.7
<b>Defensive chelae</b>	83.3
<b>2<sup>nd</sup> Antennae motion</b>	83.3

Table 3. Summary statistics of lobsters held during the 5-day holding period reported by sex and survivorship.

	<b>n</b>	<b>Mean carapace length (mm) (SE)</b>	<b>Carapace length range</b>	<b>Mean shell hardness (SE)</b>	<b>Mean RIS<sub>4</sub> (SE)</b>	<b>Mean IS<sub>5</sub> (SE)</b>
<b>female, survived</b>	297	87.66 (0.204)	81 - 110	3.31 (0.056)	0.11 (0.021)	1.08 (0.049)
<b>female, died</b>	6	87.5 (1.478)	83 - 92	2.5 (0.224)	1.833 (0.307)	2.5 (0.563)
<b>male, survived</b>	657	89.05 (0.165)	82 - 115	3.29 (0.035)	0.189 (0.02)	1.033 (0.035)
<b>male, died</b>	15	91.33 (0.934)	85 - 98	2.4 (0.289)	1.2 (0.327)	2.533 (0.256)

Table 4. Frequency (%) of lost reflexes and injuries in dead and surviving groups of *H. americanus*. When just one reflex was absent it was considered the 1st reflex lost. The 2 rightmost columns represent the frequency and percentage of each lost reflex and present injury of all individuals observed.

<b>Reflex</b>	<b>% Of mortalities with reflex lost (n = 21)</b>	<b>% Of alive with reflex lost (n = 954)</b>	<b>1st reflex lost (n = 170)</b>	<b>% Of total losses (n = 386)</b>
Abdomen Turgor	42.86	4.82	14	14.25
Eye Retraction	33.33	1.47	2	5.44
Pereiopod Motion	52.38	2.2	2	8.29
Chelate Pinch	28.57	9.22	57	24.35
3 <sup>rd</sup> Max motion	23.81	4.72	22	12.95

Table 4 continued

3 <sup>rd</sup> Max Retraction	14.29	4.61	13	12.18
2 <sup>nd</sup> Max motion	38.1	8.28	60	22.54
<b>Injury</b>	<b>% Of mortalities with injury present (n = 21)</b>	<b>% Of alive with injury present (n = 954)</b>	<b>1st injury present (n = 365)</b>	<b>% Of total injuries present (n = 1531)</b>
Damaged 1 chela	52.38	19.08	32	12.61
Damaged 2 chelae	0	4.3	11	2.68
Missing 1 chelae	23.81	5.97	8	4.05
Missing 2 chelae	4.76	0.84	1	0.59

Table 4 continued

Damaged antenna	80.95	58.81	224	37.75
Damaged rostrum	23.81	3.04	0	2.22
Damaged carapace	52.38	12.37	13	8.43
Missing 1-4 legs	28.57	17.82	25	11.5
Missing 5-8 legs	0	0.21	0	0.13
Damaged tail	42.86	20.55	41	13.39
Damaged uropods	38.1	7.76	9	5.36
Other	4.76	1.99	1	1.31

Table 5. Model parameters (Model), number of parameters (K), AIC, area under the curve (AUC), and McFadden's  $R^2$  for the best fits of all candidate logistic models explored for prediction of lobster mortality (AT = abdomen turgor, ER = eye retraction, PM = pereopod motion, 3<sup>rd</sup>MR = 3<sup>rd</sup> maxilliped motion, 2<sup>nd</sup>MM = 2<sup>nd</sup> maxilliped motion, CL = carapace length, IS<sub>5</sub> = 5 injury score, IS<sub>10</sub> = 10 injury score, MC = 1 - 2 missing chelea, DC1 = damaged 1 chelae, DC = damaged carapace, DA = damaged antenna, DU = damaged uropods, RIS<sub>7</sub> = 7 reflex impairment score, RIS<sub>4</sub> = 4 reflex impairment score, DR = damaged rostrum, SH = shell hardness, IS<sub>≥1</sub> = injury score  $\geq$  1, DOY = day of year).

<b>Model</b>	<b>K</b>	<b>AIC</b>	<b>AUC</b>	<b>McFadden's R<sup>2</sup></b>
constant + ER + PM + 3 <sup>rd</sup> MR + 2 <sup>nd</sup> MM + CL + IS <sub>5</sub>	8	115.90	0.95	0.50
constant + ER + PM + 3 <sup>rd</sup> MR + 2 <sup>nd</sup> MM + CL + IS <sub>10</sub>	8	118.45	0.95	0.48
constant + ER + PM + 3 <sup>rd</sup> MR + 2 <sup>nd</sup> MM + MC + DC1 + DC + DA + DU + CL	12	123.75	0.95	0.53
constant + RIS <sub>7</sub> + CL + IS <sub>5</sub>	5	125.13	0.95	0.41
constant + RIS <sub>4</sub> + CL + IS <sub>5</sub>	5	127.11	0.95	0.50
constant + RIS <sub>7</sub> + CL + IS <sub>10</sub>	5	131.39	0.94	0.39
constant + RIS <sub>7</sub> + CL + MC + DC1 + DC + DA + DU	9	131.29	0.95	0.43

Table 5 continued

constant + RIS <sub>4</sub> + CL + IS <sub>10</sub>	5	132.47	0.94	0.39
constant + RIS <sub>4</sub> + CL + MC + DC + DR + DU + SH	9	135.44	0.94	0.44
constant + RIS <sub>4</sub> + CL + IS <sub>&gt;=1</sub> + SH	6	148.31	0.93	0.32
constant + RIS <sub>7</sub> + CL + IS <sub>&gt;=1</sub> + SH + Sex + DOY	8	151.43	0.92	0.30



Table 6. Parameter estimates, standard error (SE), *P*-value, and odds ratio (OR) from the five best-approximating candidate logistic regression models explored for prediction of post-harvest lobster mortality (ER = eye retraction, PM = pereopod motion, 3<sup>rd</sup>MR = 3<sup>rd</sup> maxilliped motion, 2<sup>nd</sup>MM = 2<sup>nd</sup> maxilliped motion, CL = carapace length, IS<sub>5</sub> = 5 injury score, IS<sub>10</sub> = 10 injury score, MC = 1 - 2 missing chelea, DC1 = damaged 1 chelae, DC = damaged carapace, DA = damaged antenna, DU = damaged uropods, RIS<sub>7</sub> = 7 reflex impairment score, RIS<sub>4</sub> = 4 reflex impairment score).

<b>Model</b>	<b>Parameters</b>	<b>Estimate</b>	<b>SE</b>	<b><i>P</i>-value</b>	<b>OR</b>
<i>Best approximating model</i>	constant	-20.91	5.65	0.000214	0.00
	ER	2.83	0.99	0.004371	16.87
	PM	3.19	0.73	1.36E-05	24.27
	3 <sup>rd</sup> MR	-2.10	1.20	0.081395	0.12
	2 <sup>nd</sup> MM	1.28	0.61	0.024607	3.59
	CL	0.15	0.06	0.011211	1.16
	IS <sub>5</sub>	1.38	0.27	2.96E-07	3.97

Table 6 continued

<i>Second best approximating model</i>	constant	-21.60	5.67	0.000139	0.00
	ER	2.52	0.91	0.005586	12.43
	PM	3.38	0.71	2.20E-06	29.40
	3 <sup>rd</sup> MR	-2.13	1.14	0.061184	0.12
	2 <sup>nd</sup> MM	1.32	0.61	0.029696	3.73
	CL	0.16	0.06	0.006996	1.18
	IS <sub>10</sub>	0.88	0.17	4.14E-07	2.40
<i>Third best approximating model</i>	constant	-21.05	5.74	0.000247	0.00
	ER	2.81	1.02	0.005962	16.59
	PM	3.21	0.76	2.44E-05	24.66
	3 <sup>rd</sup> MR	-2.05	1.21	0.091362	0.13

Table 6 continued

	2 <sup>nd</sup> MM	1.35	0.63	0.031601	3.86
	MC	1.57	0.70	0.022804	4.80
	DC1	1.37	0.66	0.038471	3.93
	DC	1.41	0.65	0.029607	4.09
	DA	1.22	0.73	0.09386	3.40
	DU	1.26	0.65	0.05531	3.51
	CL	0.15	0.06	0.01147	1.17
<i>Fourth best approximating model</i>	constant	-19.87	5.24	0.000152	0.00
	RIS <sub>7</sub>	0.94	0.15	2.49E-10	2.56
	CL	0.14	0.06	0.011612	1.15
	IS <sub>5</sub>	1.39	0.24	6.94E-09	4.03

Table 6 continued

<i>Fifth best approximating model</i>	constant	-17.38	5.12	0.000685	0.00
	RIS <sub>4</sub>	1.53	0.25	6.51E-10	4.61
	CL	0.12	0.06	0.036709	1.12
	IS <sub>5</sub>	1.35	0.24	2.06E-08	3.84

Table 7. Summary of reflex impairment scores (RIS<sub>4</sub>) of lobsters that were injured or uninjured that survived the holding period and lobsters that were injured or uninjured that died during the holding period. Injured was considered as having at least 1 injury from the IS<sub>5</sub> injury score.

	RIS <sub>4</sub>					Total	% Of total
	0	1	2	3	4		
<b>Injured, Dead</b>	6	5	7	2	1	21	2.15
<b>Injured, Alive</b>	588	80	10	4	1	683	70.05
<b>Uninjured, Dead</b>	0	0	0	0	0	0	0.00
<b>Uninjured, Alive</b>	240	24	4	2	1	271	27.79
<b>Total</b>	834	109	21	8	3	975	
<b>% Of total</b>	85.54	11.18	2.15	0.82	0.31		

Table 8. Frequency (%) of lost eye retraction (ER), pereopod motion (PM), 3<sup>rd</sup> maxilliped retraction (3<sup>rd</sup>MR), and 2<sup>nd</sup> maxilliped motion (2<sup>nd</sup>MM) reflex, mean (SE) carapace length (CL), 5 injury score (IS<sub>5</sub>), and predicted probability of mortality at three transfer points in MLSC.

	<b>Vessel to Wharf (n = 252)</b>	<b>Wharf to Truck (n = 66)</b>	<b>Truck to Dealer (n = 74)</b>
ER	4.37	0.00	22.97
PM	11.11	3.03	28.38
3 <sup>rd</sup> MR	1.98	1.52	25.68
2 <sup>nd</sup> MM	0.79	1.52	25.68
CL	88.88 (0.320)	87.86 (0.484)	88.09 (0.424)
IS <sub>5</sub>	1.13 (0.053)	1.46 (0.081)	1.85 (0.121)
Pred. probability of mortality	0.02 (0.005)	0.01 (0.002)	0.17 (0.035)

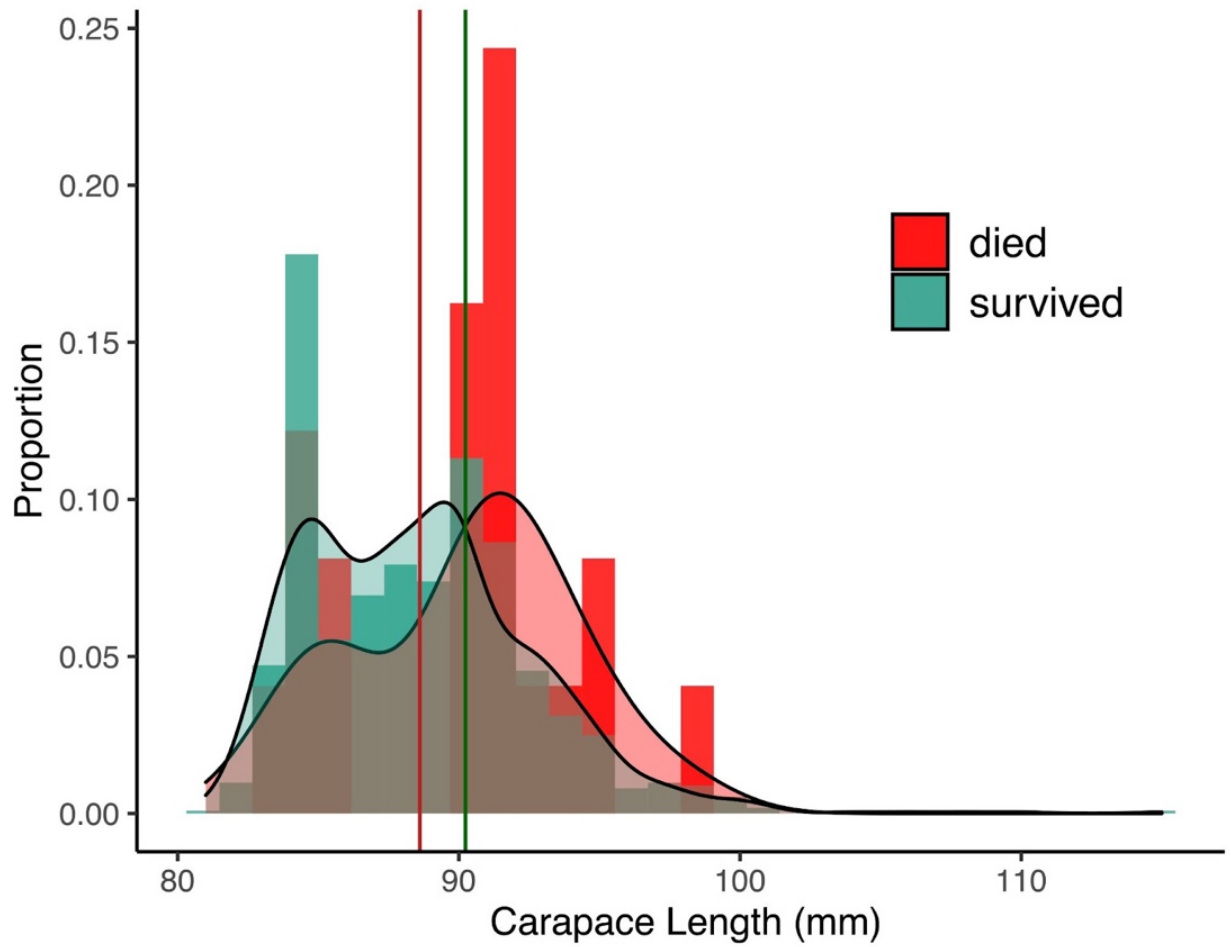


Figure 1. Carapace length distribution of individuals that survived (green) the holding period and of those that died (red). Vertical lines represent the mean length for the two groups.

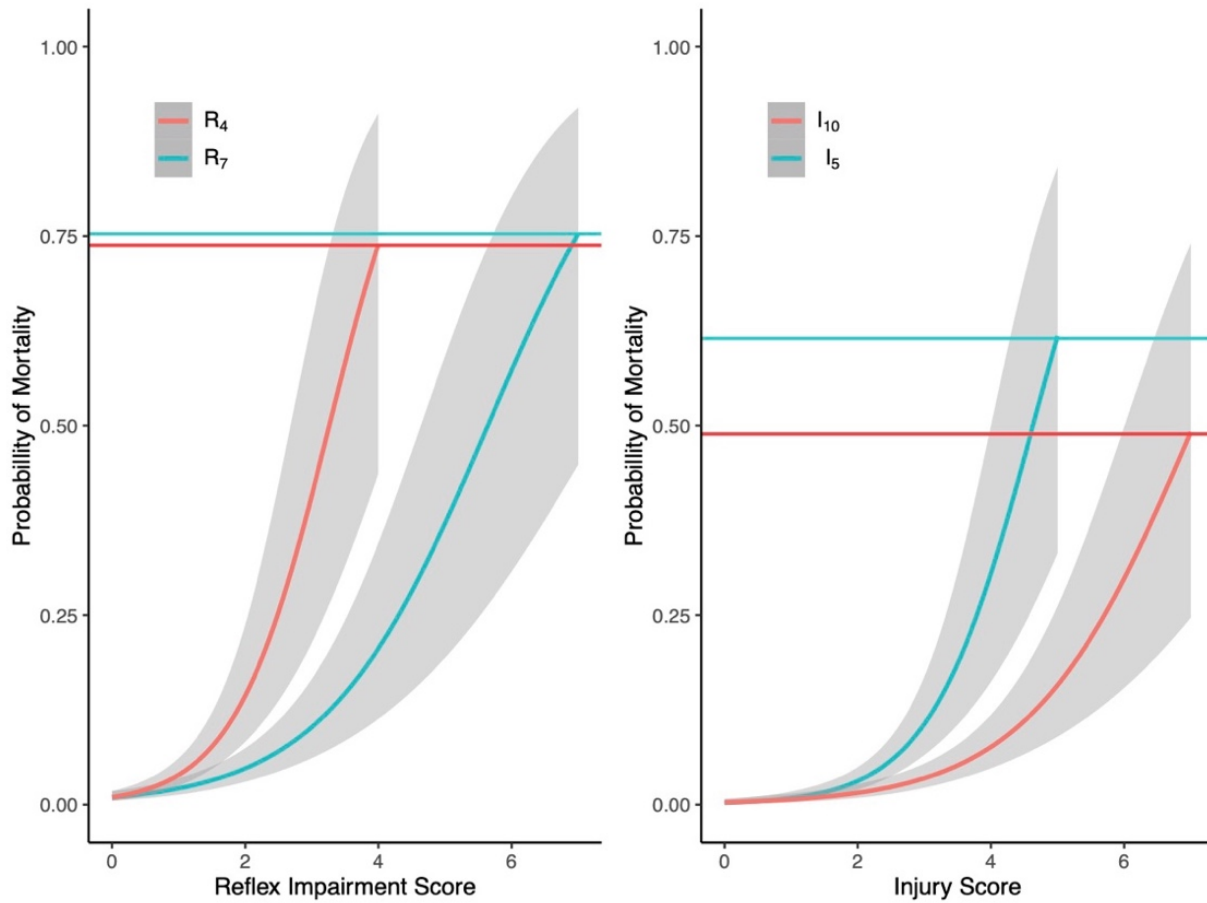


Figure 2. Logistic regression of probability of mortality using the full complement of reflex impairment (RIS<sub>7</sub> & RIS<sub>4</sub>; left) and injury scores (IS<sub>10</sub>; IS<sub>5</sub>; right). Reflex impairment score was calculated as the sum of reflexes lost, and injury score was calculated as a sum of injuries present.



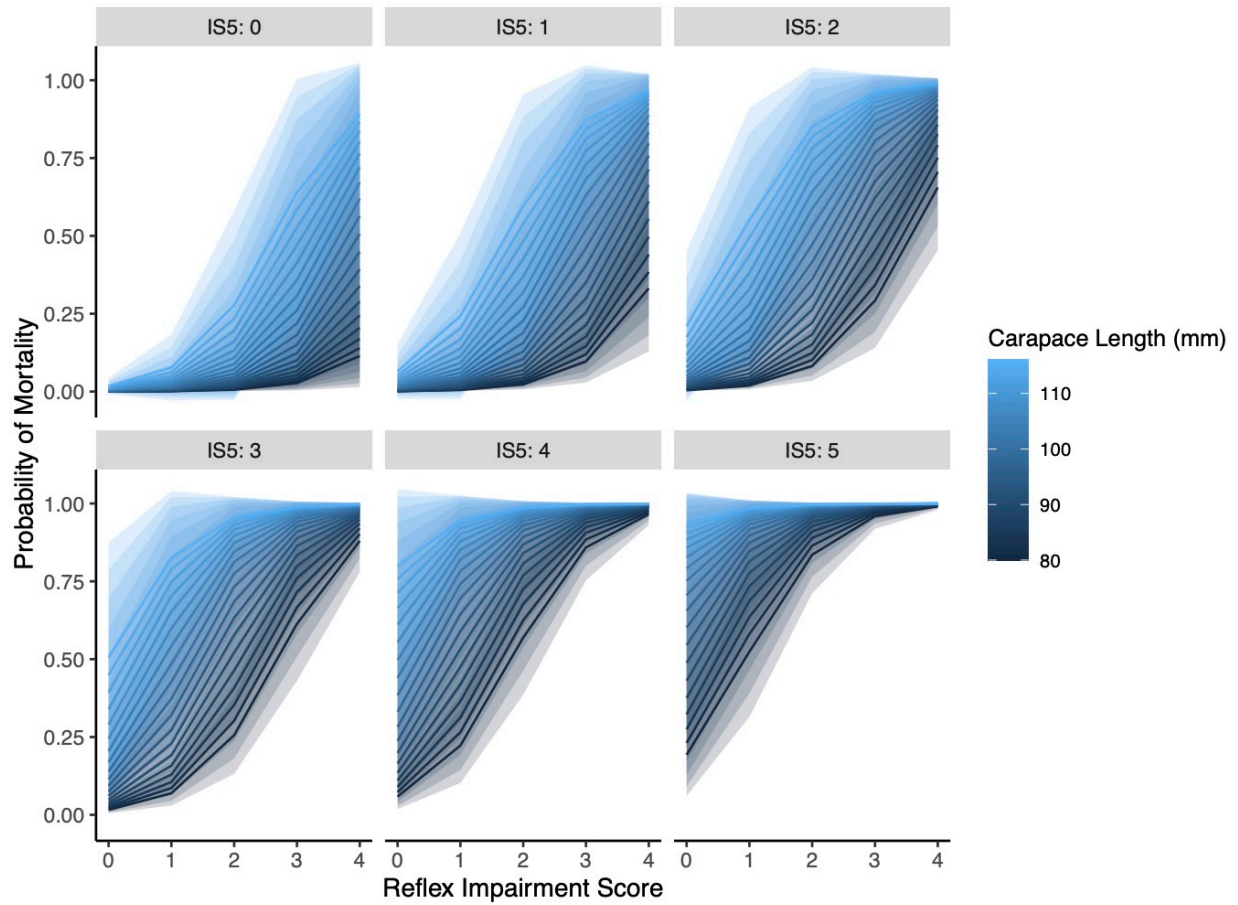


Figure 3. Predicted likelihood of mortality of American lobster, *H. americanus*, as a function of RIS<sub>4</sub>, IS<sub>5</sub>, and carapace length (mm).

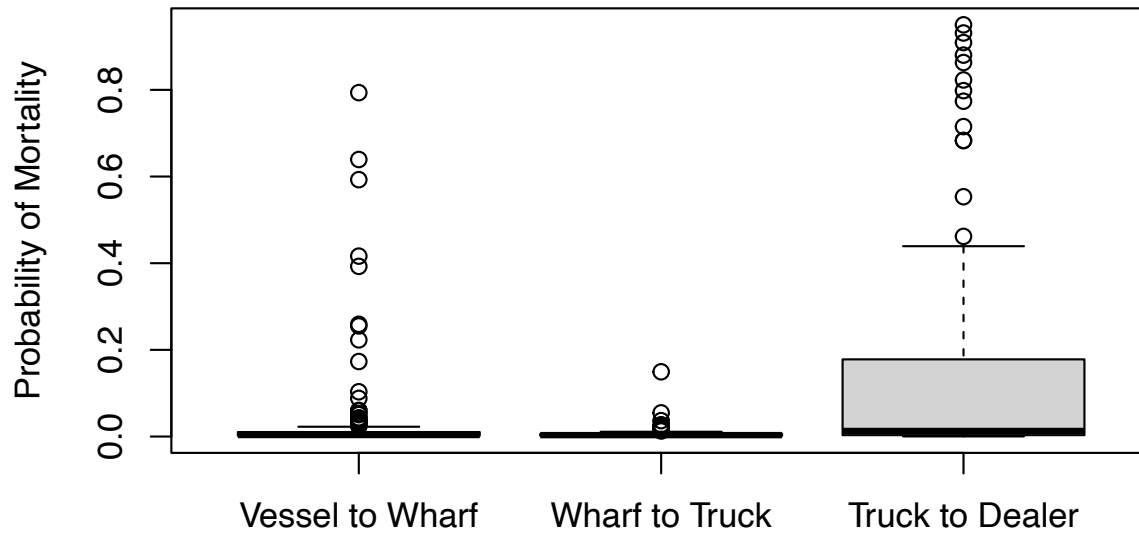


Figure 4. Predicted probability of mortality of lobsters sampled at three different transfer points in MLSC.

## CHAPTER 4

### DISCUSSION

This work presents the first reflex action mortality predictor (RAMP) model for the most valuable single-species fishery in North America and the first application of this method as a predictive diagnostic tool in the supply chain. The RAMP approach is useful because it is non-invasive, non-destructive, quick, efficient, and can be used at any point within the supply chain to attempt to predict future mortality. This development of the RAMP model for the American lobster is the first application in a post-harvest context, with previous RAMP models developed to estimate discard mortality (Davis & Ottmar, 2006; Hammond et al., 2013; Kronstadt et al., 2018; A. W. Stoner et al., 2008; Walters et al., 2022; Yochum et al., 2015) or in aquaculture uses (Turnbull et al., 2021). As demonstrated by the field trial, the RAMP approach has the potential to describe stress events and quantify the impact stressors have on lobster health. This novel approach provides the lobster industry with a new tool for guiding modifications in the supply chain infrastructure that mitigate stressors. Furthermore, this model can substantially increase the supply chain efficiency of this highly valued species by predicting and separating in advance those individuals likely to survive live shipping from those that should be relegated to processed product because they are in a weakened condition. The adaptation of this approach could provide a template for RAMP development in other live seafood supply chains (e.g., king crab (*Paralithodes camtschaticus*), red swamp crawfish (*Procambarus clarkia*), blue crab (*Callinectes sapidus*)).

The five most parsimonious models from the AUC and McFadden's pseudo-R<sup>2</sup> values all consisted of reflex scores, injury scores, and carapace length. Conventionally, a RAMP model predicts the likelihood of mortality based solely on reflex actions as these behaviors integrate the

internal and external physiological stress of the individual (Davis, 2007; Davis & Ottmar, 2006; A. Stoner, 2012). However, partnering reflex and other covariates such as injury scores, salinity, or height of dropping in RAMP models can improve model accuracy without over-extending the duration of the screening process (Kronstadt et al., 2018; A. W. Stoner et al., 2008; Walters et al., 2022). In this study, assessing mortality with carapace length, injury and reflex actions together was a better predictor of mortality than with any of those measures alone (Figures 2 & 3). Importantly, we also found that a subset of the reflex and injury scores provided nearly as good or even better predictive power than the full complement of scores. Once trained, an experienced observer can record the four reflex actions, five injury types, and carapace length in about 20 seconds per lobster. The decision to take the extra time to measure carapace length and assess injury is ultimately an economic one. That is, additional economic analyses that weigh the impact of product loss upon arrival in the live market against the time needed to assess live product before shipping are necessary to recommend the inclusion of these factors. However, this analysis clearly demonstrates this additional information can help make better projections, particularly incorporating carapace length and IS<sub>5</sub>.

Reflex behavior in crustaceans is a universal indicator of internal and external physiological conditions (Davis, 2002; A. W. Stoner et al., 2008). Behavioral markers such as specific reflexes or righting behavior have been used in the past as an assessment to evaluate lobster health (Basti et al., 2010; Lavallée et al., 2000). For example, Lavallée et al. (2000) observed the presence or absence of tail flipping, claws rising, and antennae movement as reflexes that, if present, indicate vigor in the Canadian *H. americanus* supply chain. Basti et al. (2010) used claws rising, abdomen turgor, and tail flipping when held out of water as indicator reflexes of morbidity for harvested *H. americanus* in MLSC. While previous studies have used

behaviors thought to be indicative of vigor, we chose to systematically identify reliable indicators of vigor and then use those to design the RAMP. This process identified seven reflex actions to be stereotypic that were not included in previous attempts, with exception of abdomen turgor. These indicators in turn helped develop a more robust RAMP and identified four of the seven reflexes as significant predictors of post-harvest mortality for *H. americanus*.

The four reflexes most predictive of mortality, pereopod motion, eye retraction, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion, are consistent with other crustacean RAMP studies. For example, in discard mortality studies, Walters et al. (2022) and Stoner et al. (2008, 2012) and Kronstadt et al. (2018) determined that leg retraction or kick, eye retraction, and mouth closure were reliable predictors of mortality in blue crabs (*C. sapidus*), tanner crab (*Chionoecetes bairdi*), snow crab (*Chionoecetes opilio*), spot prawn (*Pandalus platyceros*), and Florida stone crab (*Menippe mercenaria*). In particular, the loss of mouth part reflexes is strongly associated with mortality across all crustacean RAMPs. These reflexes are low energy actions that are required for ventilation of the gills, the final involuntary action before death (Stoner, 2008). Of the four reflexes that were significant predictors of mortality for *H. americanus*, pereopod motion was the most sensitive indicator with the likelihood of mortality 24 times more likely if the reflex was absent (Table 6).

Damaged antennae were the most frequently recorded injury both in lobsters that survived and those that died during holding (58.8% & 81%, respectively). Carapace injury and missing chelae were the second most frequent injuries in those that died (presence of 52.3%) and had the largest difference in frequency compared to the surviving group (40% & 33.3%, respectively; Table 3). The association between damaged chelae or 1 - 2 missing chelae and delayed mortality has been reported in other crustacean fisheries. An investigation of the effect

of injury on mortality rates in the west coast rock lobster (*Jasus lalandii*) concluded that mortality rates increased most significantly when feeding legs were removed (Brouwer et al., 2006). Declawing stone crabs (*Menippe mercenaria*) in Florida's fishery is also strongly associated with delayed mortality, particularly if the injury from declawing extends into the carapace, exposing the body cavity (Gandy et al., 2016; Simonson & Hochberg, 1986). Removal of appendages of blue crab (*Callinectes sapidus*) was associated with increased likelihood of delayed mortality in a simulated gillnet entanglement and discard experiment (Uhlmann et al., 2009). Effects from damaged carapace to mortality have also been observed in other crustacean species. The harbor crab (*Liocarcinus depura*) and spider crab (*Macropodia rostrata*) had an increased likelihood of mortality if their carapace was damaged from interaction with beam trawling gear (Kaiser & Spencer, 1995).

To our knowledge, this is the first RAMP model for crustaceans that includes body size as a predictor. We hypothesize that the risk of mortality in the supply chain is highly body size-dependent because larger lobsters are more vulnerable to respiratory stress. Points in the MLSC creates opportunities for respiratory distress from physical actions from crowding, agonistic behaviors, and from insufficient oxygenation of holding waters. For example, livewells, the flow-through tanks on board lobster harvesting vessels, hold hundreds of lobsters during the day of capture. The activity caused by crowding in the livewell could increase oxygen consumption as demonstrated by McLeese (1964). The demand of oxygen from respiration within the livewell could be higher than the rate of water exchange from the livewell infrastructure, causing respiratory distress. Once lobsters are transferred to a 0.16 m<sup>3</sup> crate, they are temporarily stored at the wharf, where waters can be in shallow, warm, or low-flow (e.g., in a cove) leading to low dissolved oxygen that may cause stress to the high aggregation of lobsters. While all lobsters that

enter the supply chain would experience this potential respiratory distress, larger lobsters would be more susceptible to this stress because they require more oxygen per hour than smaller lobsters even as mass-specific oxygen consumption decreases with size (Bridges & Brand, 1980; Dehnel, 1960; D. C. McLeese, 1964). The size effect of post-harvest mortality has been anecdotally reported in the southern rock lobster (*Jasus edwardsii*) fishery. During a mortality event in a holding facility for *J. edwardsii*, the facility manager noted that larger lobsters (>1 kg) were primarily affected (Day et al., 2022, preprint).

Mortality observed during the 5-day holding period in the present study was 2.15%. The proportion of lobsters that had a high reflex or injury score was small (11 individuals with RIS<sub>4</sub> equal to 3 or 4 and 13 individuals with IS<sub>5</sub> equal to 4 or 5; Table 6). While we observed overall high survivorship, we surmise this sample size was robust enough to have confidence in the models presented. Similarly, a high proportion of individuals with a reflex score of zero was observed in a RAMP study with Dungeness crab (*Cancer magister*; Yochum et al., 2016). This high survivorship is unsurprising as the lobster industry has become highly optimized through decades of handling the high-valued crustacean. Historically, the supply chain included a holding period in lobster impoundments, now recognized to be a potential hotspot for pathogenic infection and delayed mortality (Basti et al., 2010; Cawthorn et al., 1996; Smolowitz et al., 1992; Theriault et al., 2008). Improvements of transportation technology, seasonality of the fishery, and expansion of markets has caused impounding lobsters to be rare and increase survivorship. With the years of improvement, further incremental reduction in mortality will rely on continued quantitative monitoring of lobster health in the supply chain, and disseminating this knowledge to industry members. This work has demonstrated size, behavioral reflex actions, quantity of

injuries, and specific injuries are all key components influencing mortality and these findings can be communicated to industry to continue the sophistication of the supply chain.

The trial study proved the RAMP model can be implemented in the supply chain as a diagnostic tool. While the sampling effort was not robust enough for definitive results that point to problem areas in the supply chain that significantly impact lobster health, it does demonstrate how this model can be used. Two interesting points of information were gained with this low-effort dataset. First, the mean injury score, a predictor of delayed post-harvest mortality, increased after each point in the supply chain. This finding indicates that injuries are accrued along the observed supply chain and suggests that handling at the vessel, wharf, and truck could be a stressor inducing injury to lobsters.

Second, predicted mortality increased significantly upon arrival to a lobster dealer facility. The significantly higher mortality predicted after transport on a truck (17%) demonstrates that this model has the ability to detect stress events that lead to increased mortality. The sample size of that transfer point was low (one sampling day, 74 lobsters); however, the substantial mortality prediction indicates that either the transport stress or the compounded stress from all prior supply chain interactions impacted the lobster's health. The observations made at the truck to dealer transfer point were taken on a notably warm week and industry members noted poor lobster quality during that time period. While the RAMP model can detect stress events, environmental data that captures the exposure history of known stressors, such as temperature, are needed to understand the cause of the stress.

As the total landed value and price per kilogram of lobster continues to rise, the industry is also increasingly strained from bait and fuel costs, and new regulations and proposed rules to



limit the interaction between the industry and endangered North Atlantic right whale. It is therefore more important than ever for the industry to minimize financial losses. The RAMP method has shed light on the significant physical and behavioral characteristics that predict delayed mortality. The inclusion of carapace length to the model further demonstrates the importance of this work, as there is evidence that larger, more valuable, lobsters are more vulnerable to the stressors of MLSC. A techno-economic analysis is recommended to explore the cost-benefits of post-harvest mortality mitigation adaptations to the supply chain, such as onboard chillers or aeration. Policy changes of handling standards within MLSC may also be a successful avenue to improve post-harvest mortality.

The RAMP method addresses the need for an efficient, robust approach to assessing potential post-harvest mortality. Other health assessments include hemolymph chemistry, vigor assessments, and direct observation. While these assessments are very useful in developing a mechanistic understanding of post-harvest mortality, they are limited by the number of lobsters that can be assessed quickly at multiple stages in the supply chain. The traditional RAMP approach can be enhanced by other covariates such as size and injury status to improve model performance (Davis & Ottmar, 2006; Kronstadt et al., 2018). The binary approach to assessing multiple reflexes can also decrease subjectivity such as those present in vitality assessments. While reflex impairment has been a valuable tool in determining discard mortality of commercially targeted crustaceans and fish (Davis & Ottmar, 2006; A. W. Stoner, 2012), the results provide evidence that reflex actions, injury, and carapace length can also be significant explanatory parameters for predicting similarly unobservable mortality later in the supply chain. This RAMP model should continue to be used at particular links in the supply chain to

understand where increased likelihood of mortality occurs aid in future audits of supply chain quality, sustainability, and profitability in North America's most valuable single species fishery.

#### **4.1 Conclusion**

Post-harvest mortality is a chronic major challenge to the live lobster industry. Maine's lobster supply chain (MLSC) is one of the state's most economically important international commercial networks. The delayed post-harvest mortality seen in the industry has a massive impact on the quality of the product and revenue. Given the volume and value of the fishery, a modest increase in mortality rate of less than 1% could mean millions of dollars in lost revenue. The RAMP method robustly and efficiently predicted delayed post-harvest mortality of lobsters that have been subjected to the stress of MLSC, and a preliminary field trial produced promising results for commercial and research use. The RAMP model suggests that four behavioral reflexes: pereopod motion, eye retraction, 3<sup>rd</sup> maxilliped retraction, and 2<sup>nd</sup> maxilliped motion, five injury types: missing chela, damaged chela(e), damaged antenna, damaged carapace, and damaged uropod, and carapace length are significant predictors of mortality. The best models demonstrate the explanatory parameters are comprehensive and the model has excellent classification performance. Of the explanatory variables, pereopod motion was most powerful in predicting mortality with 24-fold increase of likelihood of mortality if the reflex was absent. This is the first crustacean RAMP model that includes carapace length as a significant covariate, hypothesized to be because of respiratory stress within the unique methods of handling in MLSC. This is the first known approach to using the RAMP method in a post-harvest context, which can serve as an example for other live crustacean supply chains to explore. This novel method for assessing lobster health is highly predictive, takes 20 seconds to perform, involves limited subjectivity, and is inexpensive. The RAMP method for *H. americanus* in MLSC is a tool

that the industry can implement at specific links in the supply chain to identify bottlenecks and researchers can use in investigations of lobster physiology and stress.

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# APPENDICES

## APPENDIX A

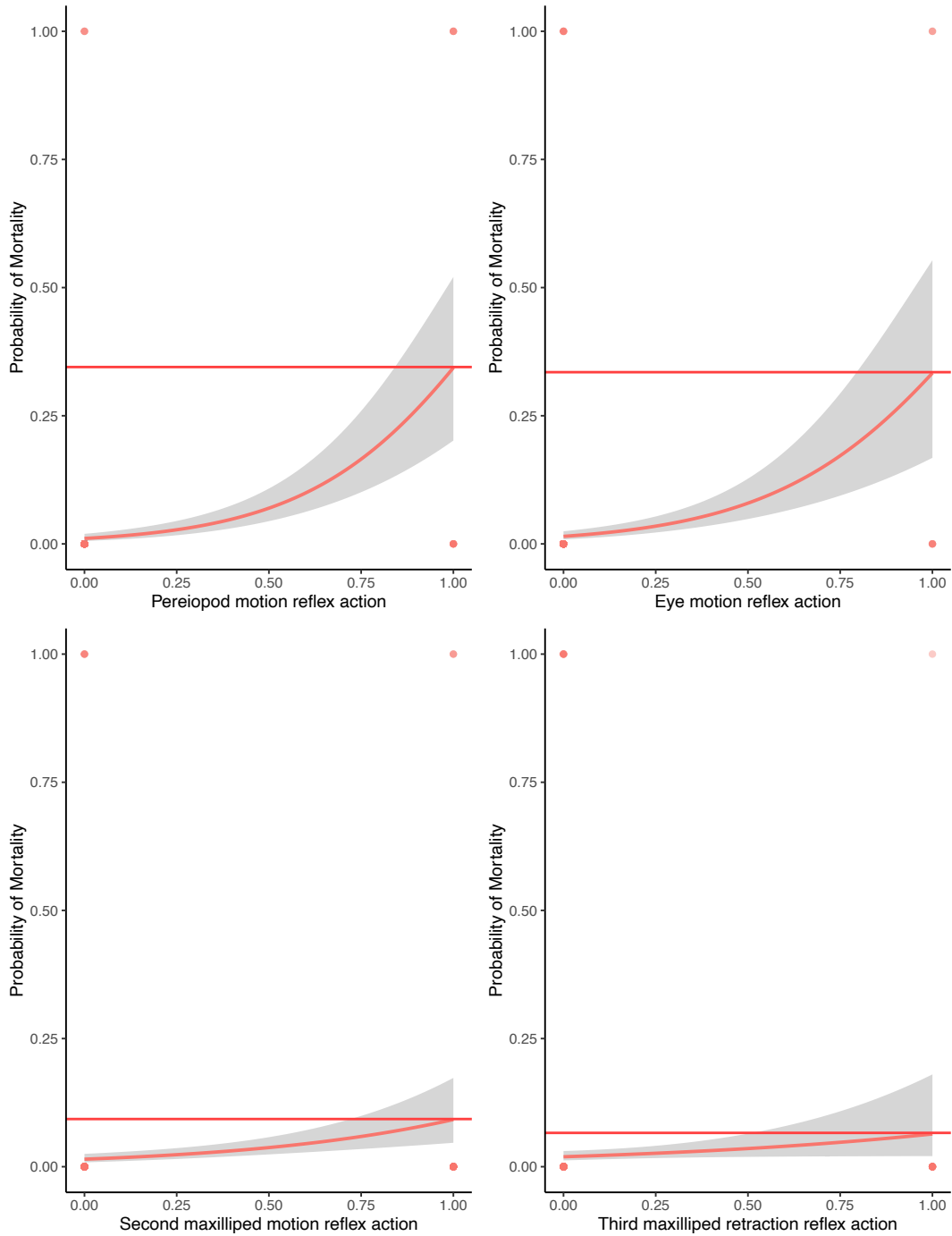


Figure A1. Logistic regression of probability of mortality as a function of individual reflex actions.

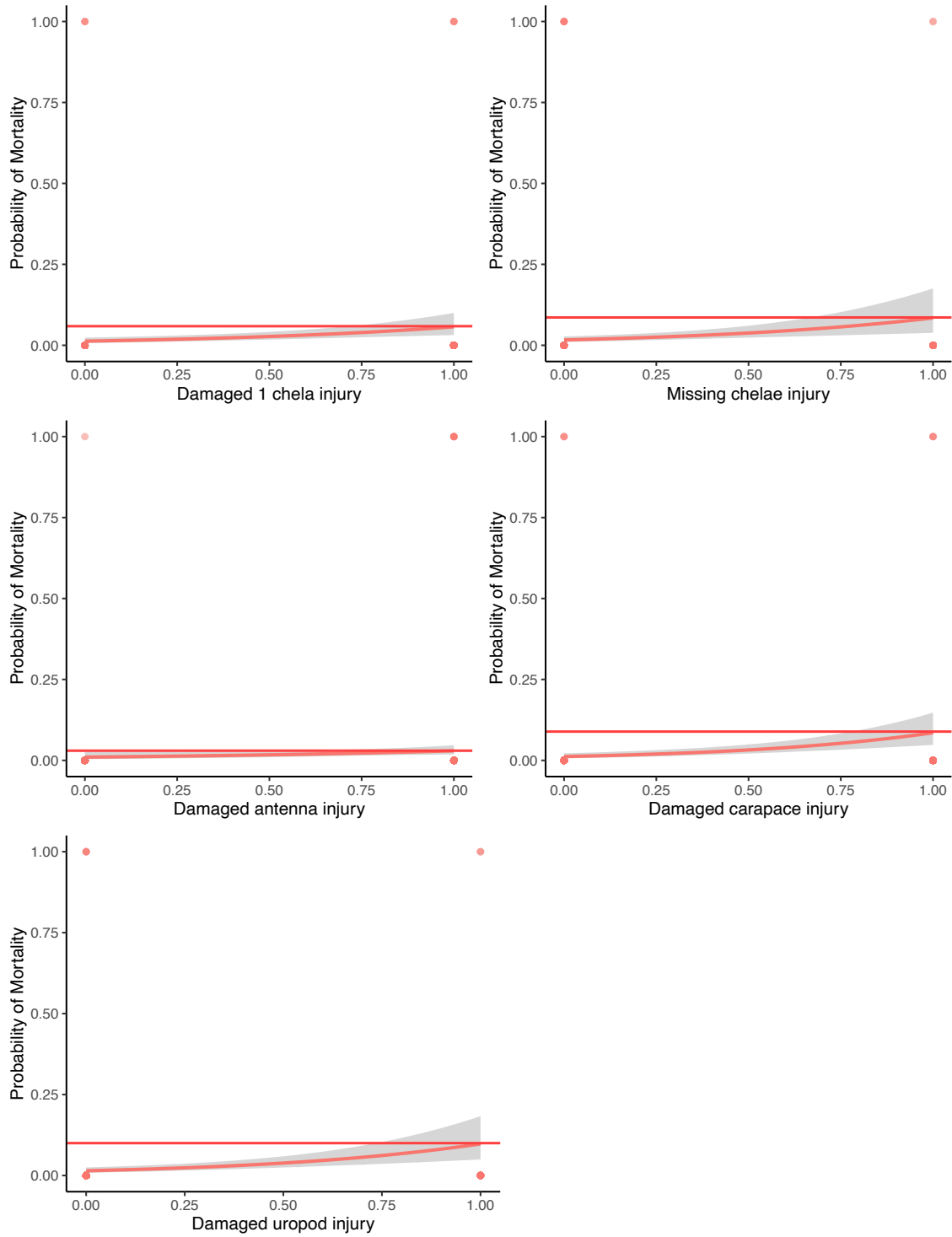


Figure A2. Logistic regression of probability of mortality as a function of individual injuries.

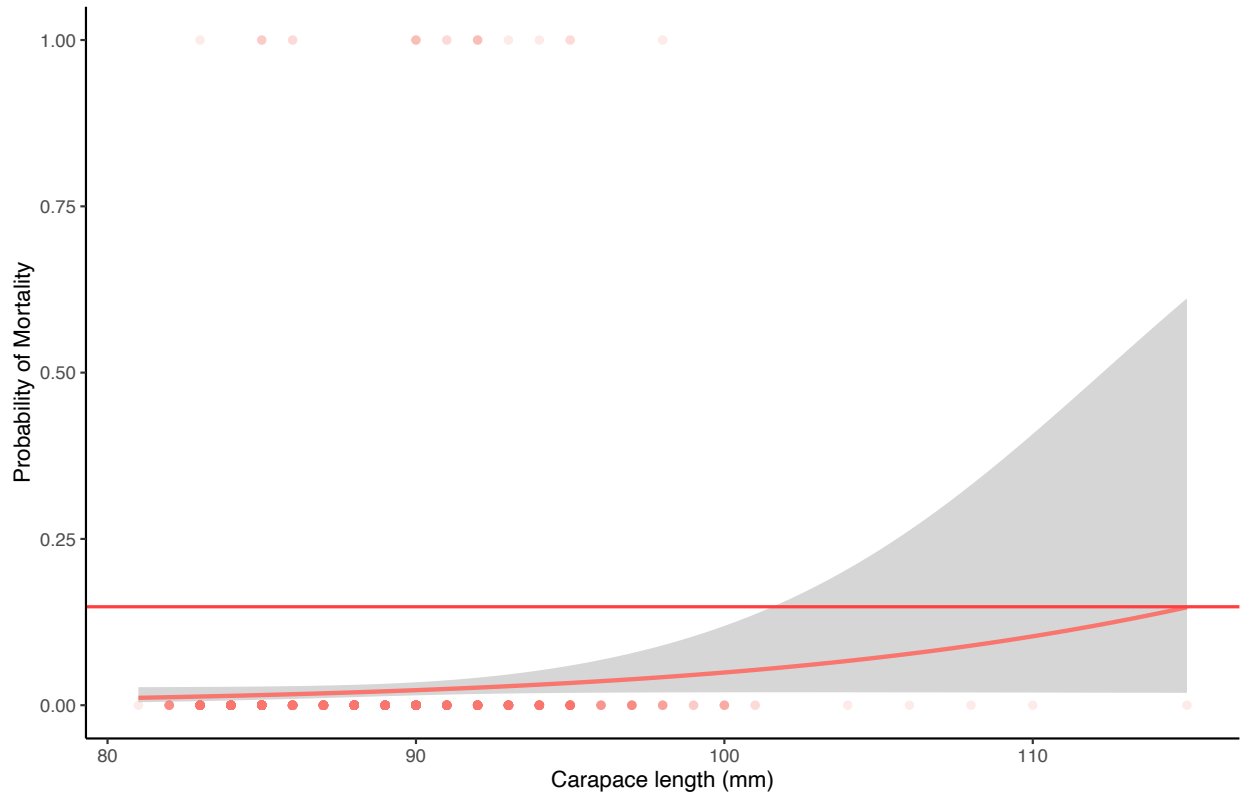


Figure A3. Logistic regression of probability of mortality as a function of carapace length (mm).

Table A1. Parameter estimates, standard error (SE), *P*-value, and odds ratio (OR) from logistic regression models of the individual reflex actions, injuries, and carapace length that were found to be significant predictors of morality when modeled collectively (ER = eye retraction, PM = pereopod motion, 3<sup>rd</sup>MR = 3<sup>rd</sup> maxilliped motion, 2<sup>nd</sup>MM = 2<sup>nd</sup> maxilliped motion, MC = 1 - 2 missing chelea, DC1 = damaged 1 chela, DC = damaged carapace, DA = damaged antenna, DU = damaged uropods, CL = carapace length).

<b>Model</b>	<b>Parameters</b>	<b>Estimate</b>	<b>SE</b>	<b><i>P</i>-value</b>	<b>OR</b>
<i>Pereiopod motion model</i>	constant	-4.5358	0.3179	< 2e-16	0.00
	PM	3.8892	0.4895	1.95e-15	48.86
<i>Eye retraction model</i>	constant	-4.2068	0.2692	< 2e-16	0.00
	ER	3.5137	0.5355	5.33e-11	33.58
<i>Second maxilliped motion model</i>	constant	-4.2093	0.2794	< 2e-16	0.00
	2ndMM	1.9193	0.4645	3.59e-5	6.81
<i>Third maxilliped retraction model</i>	constant	-3.9231	0.2380	< 2e-16	0.00

Table A1 continued

	3rdMR	1.2375	0.6424	1.926	3.45
<i>Damaged 1 chela model</i>	constant	-4.3464	0.3183	< 2e-16	0.00
	DC1	1.5403	0.4446	3.464	4.66
<i>Missing chelae model</i>	constant	-4.0820	0.2604	< 2e-16	0.00
	MC	1.6994	0.4998	0.00067	5.47
<i>Damaged antenna model</i>	constant	-4.5875	0.5025	< 2e-16	0.00
	DA	1.0910	0.5596	0.0512	2.98
<i>Damaged carapace model</i>	constant	-4.4260	0.3181	-13.913	0.00
	DC	2.0533	0.4479	4.55e-6	7.79
<i>Damaged uropod model</i>	constant	-4.2150	0.2794	< 2e-16	0.00
	DU	1.9903	0.4654	1.9e-5	7.32
<i>Carapace length model</i>	constant	-10.999	4.02202	0.00624	0.00
	CL	0.08037	0.04454	0.07115	1.08

## **BIOGRAPHY OF THE AUTHOR**

Cassandra Leeman was born in Maine on February 8, 1997. She was raised in Walpole, Maine and graduated from Lincoln Academy in 2015. She attended the Eckerd College and graduated in 2019 with a Bachelor's degree in Marine Science. She returned to Maine and entered the Marine Biology graduate program at The University of Maine in the Winter of 2020. After receiving her degree, Cassandra hopes to work within fishing communities to participate in meaningful, collaborative research and management that benefits both the environment and harvesters. Cassandra is a candidate for the Master of Science degree in Marine Biology from the University of Maine in December 2022.