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# SHELL GAME: IMPROVING THE SHELL QUALITY AND VALUE OF MAINE'S

# MOST VALUABLE FISHERY

by

Abigale Shaughnessy

A Thesis Submitted in Partial Fulfillment of the Requirements for a Degree with Honors (Marine Biology)

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Advisory Committee:

Richard Wahle, Research Professor of Marine Science, Advisor Curtis Brown, Marine Biologist, Ready Seafood Co. Paul Rawson, Assistant Professor of Marine Science Joshua Stoll, Assistant Research Professor of Marine Policy Mark Haggerty, Honors Preceptor, Honor College

# ABSTRACT

The American lobster is Maine's most valuable export commodity. Hard-shell lobster commands the highest price because they survive shipping to overseas markets, but much of Maine's summer production comes as perishable, low-value, softshell lobster. Lobster processors would like to know whether they can enhance profitability by holding over low-quality lobsters to increase their hardness instead of the standard process of receiving and shipping lobsters in under 48 hours. In a collaborative experiment with Ready Seafood Co. (Portland, Maine), we conducted week-long trials to evaluate the joint effects of temperature and feeding on shell hardness and hemolymph protein levels, an indicator of lobster health. Lobster were hand graded into four shell hardness categories used in the trade. Hemolymph total protein levels were quantified on the Brix percentage scale with a refractometer. Over the duration of 7-day trials, an average of 33.5% of lobster improved in grade and the average survival rate was 85%. Hemolymph protein levels improved significantly with higher shell grade but declined over the duration of the trials. We found no significant effect of temperature or feeding treatment on shell grade, survival, or hemolymph protein levels. We estimate that with an 85% survival rate and a 33.5% improvement in shell grade over 7 days, at the highest observed market price differential between A and B grade lobster, Ready Seafood Co. would lose over \$10,000. The company would realize a profit, however, if it improved survival rate by a few percent, and with a survival rate of 100%, would gain more than \$72,000 in profits. Therefore, by holding over lobster there is much potential to enhance the value of Maine's lobster industry.

# TABLE OF CONTENTS

TABLE OF FIGURES	iv
INTRODUCTION	1
METHODS	5
Laboratory Animals	5
Testing	5
Experimental Design	6
Grading	8
Field Survey of Lobster Hemolymph Protein Levels	8
Field Survey of Lobster Hemolymph Protein Levels	8
Field Survey of Lobster Hemolymph Protein Levels Economic Analysis RESULTS	
Field Survey of Lobster Hemolymph Protein Levels Economic Analysis RESULTS Economic Analysis	
Field Survey of Lobster Hemolymph Protein Levels Economic Analysis RESULTS Economic Analysis DISCUSSION	
Field Survey of Lobster Hemolymph Protein Levels Economic Analysis RESULTS Economic Analysis DISCUSSION LITERATURE CITED	

# TABLE OF FIGURES

TABLE 1	8
FIGURE 1	7
FIGURE 2	11
FIGURE 3	12
FIGURE 4	13
FIGURE 5	14
FIGURE 6	1

# INTRODUCTION

The American lobster, *Homarus americanus*, is the State of Maine's largest export commodity (State Exports of Maine 2017). The lobster fishery is also the most valuable marine fishery in both the United States and Canada and landings have continually increased in the last two to three decades (D'Agaro et al 2014). Ready Seafood Company is a wholesale lobster company based in Portland, Maine. Ready Seafood exports live lobster to markets across the globe. In recent years, Asian market demand for live lobster has greatly increased. Chinese markets favor red lobster because the color red is associated with good luck, happiness, and prosperity (Simon et al 2016). Companies, such as Ready Seafood, are working to meet demand brought on by this increasing market. Asian markets favor the sale of live lobster; therefore, successful export of live lobster is essential to the industry (Simon et al 2016).

Shipping exposes live lobster to several weakening factors such as air, hypoxia, temperature changes, and handling (Lorenzon et al 2007). To survive the shipment process, a lobster must be resilient enough to handle these stressful conditions. Not all lobster can survive the shipment process, in fact, newly molted lobster are more likely to experience mortality in situations of high temperature, low salinity, and low oxygen compared to hard-shell lobster (McLeese 1956). When companies like Ready Seafood receive lobster from lobstermen, lobsters are sorted into four shell hardness grades. Only the lobsters with the hardest shells are durable enough to survive the journey to Asian markets. During the height of Maine's summer lobstering season, much of the catch consists of low grade, softshell lobster that do not ship well and are destined for local

markets. However, hard-shell lobster yields the highest profit for companies like Ready Seafood because they survive shipping to overseas markets. This study seeks to improve shell hardness in lobster through different temperature and feeding treatments in an attempt to increase the economic gain of lobster exports.

As with most wholesalers, Ready Seafood buys lobsters from fishermen, places them in cold water ( $\sim$ 7°C) to purge their waste, transfers them to even colder water ( $\sim$ 4° C) to slow their metabolism, and then ships them within 48 hours of receiving them. This study asks whether it might be more profitable to hold over lobster for 7-days to harden their shells and make them more fit for overseas shipping.

Lobsters are poikilotherms, meaning they do not regulate their own body temperatures. Temperature is therefore an extremely important parameter for lobster and they are capable of detecting minute changes in temperature, made apparent through their cardiac activity in changes of just 0.5 °C (Worden 2006). Because temperature is so important, we decided to investigate whether holding temperature impacts shell hardness. Temperature is known to impact many aspects of a lobster's life. Metabolism, growth, reproduction, and early development can all be influenced by temperature (Green et al 2014). In particular, warm temperatures have been found to increase growth by shortening the intermolt period. This occurs due to increased temperatures speeding up metabolic processes like the mobilization of energy reserves needed for molting (Green et al 2014). This study elevates temperatures in attempt to speed up the hardening of lobster shells.

Diet was an additional parameter tested in this study as diets have been shown to have significant effects on the strength of the shell of new shell lobsters. Shell hardness is

improved through vitamins and minerals that help enhance lobster health and growth (Donahue & Bayer 1998). Growth occurs through the molting of the shell and hardening of the new shell until it is ready to molt again. Successful molts occur through accumulation of metabolic reserves gained through diet (Ciaramella et al. 2014). In this study, some lobsters were not fed, while others were fed one crushed mussel to increase their metabolic reserves and vitamins in an attempt to harden their shells over a 7-day trial.

The physiological condition in crustaceans is influenced by the amount of nutrient reserves accumulated that allow for normal function and growth (Ciaramella et al. 2014). The Brix index is a method used in the lobster industry to evaluate lobster health. The term "degrees Brix" honors the nineteenth-century German scientist Adolf Brix (Roll 1984). The refractive index of hemolymph, which is measured using the Brix scale, is a measure of the concentration of all dissolved solids in hemolymph, mainly proteins. Hemolymph proteins consist of hemocyanin, a copper based respiratory protein that binds with oxygen. Lipid and protein levels in the hemolymph strongly affect the refractive index and are therefore useful indicators of lobster nutritional status and health (Leavitt & Bayer 1977; Ciaramella et al. 2014; Simon et al 2016; Clark et al 2017). Hemolymph protein and lipid levels can be influenced by environmental factors such as salinity. temperatures, food availability, season, life stage, and stress (Ciaramella et al. 2014). A small drop of hemolymph collected with a syringe provides a non-lethal, simple way to measure lobster health that lobster dealers can use to make informed decisions regarding shipping and processing (Clark et al 2017).

Hemolymph carries oxygen and nutrients that sustain metabolic demands of tissues. Hemolymph contains two lipids, triglycerides and cholesterol. Triglycerides commonly serve as metabolic reserves for an organism. Cholesterol is a major structural component for cell membranes and is a steroid that synthesizes hormones. Hormones play a key role in the lobster molt cycle. Thus, triglycerides and cholesterol are key players in determining lobster molt success and, therefore, the lipid concentration in hemolymph can aid in assessing overall health and growth of crustaceans (Ciaramella et al. 2014). A variety of hemolymph-borne proteins influence the molt, shell formation, and metabolism (Ciaramella et al. 2014). Therefore, we use the Brix Index as an indicator for lobster health and shell quality in this study.

## **METHODS**

#### Laboratory Animals

We obtained lobsters for use in laboratory experiments from Ready Seafood Company, Portland, ME. Each week, 48 B-grade American lobster, *Homarus americanus*, were inspected for damage to the exoskeleton, legs, or antennae. We packaged animals into two separate boxes containing 24 animals per box. A piece of newspaper soaked in seawater was placed on top of the animals along with two gel ice packs to improve survival during transport. The animals were transported by car the 96 km from Ready Seafood, Portland to the University of Maine's Darling Marine Center, in Walpole, ME. At the laboratory, animals were taken out of packaging and randomly assigned an identification number, a cell number, and a treatment. The animal's sex, hemolymph protein level, size, and claw and eye response were recorded. The sex of the animal was determined using the anatomical differences in the first pair of pleopods.

## Testing

Hemolymph protein levels were used to test the overall health and shell hardness of the lobster. Hemolymph protein was measured with a refractometer on a Brix percentage scale. A syringe was used to draw two mL of hemolymph from the abdomen, directly below the final set of walking legs. A drop of hemolymph was placed on the refractometer lens and the hemolymph protein level was recorded. The carapace length of the lobster was recorded using calipers and measured to the nearest tenth of a mm. To test the vitality of the lobster, behavioral tests were performed. If the lobster took a defensive stance, claws raised, when handled, it was marked positive for claw response. If the

lobster retracted its eye when poked, it was marked positive for eye response. Testing occurred at the start and end of each trial, day 1 and 7, respectively.

# Experimental Design

We used a two-factor experimental design with feeding and temperature treatments as independent variables. Lobster were held in captivity in the flowing seawater system at the University of Maine's Darling Marine Center. They were held in stacked trays, with six individual chambers per tray (Figure 1). Lobster were exposed to one of four treatment combinations: heated and fed, heated and unfed, ambient water temperature from the Damariscotta River and fed, or ambient water temperature from the Damariscotta River and unfed. We ran 7 experimental trials, however, trial 1 is excluded from all data analysis due to water flow issues and trial 2 is excluded from the improvement in shell grade analysis because at the point in the lobstering season these samples were collected, the industry did not differentiate between C, B, or A grades and hard-shell lobster. There was no way to test for grade improvement in the shells of trial 2 lobster.

Water flowed from one chamber to the next and cascaded to the trays below through an outlet at one end of the tray, and finally into a floor drain. We set up two stacks of four trays. The bottom two trays in each temperature treatment contained the treatments with food. The feeding treatment consisted of one crushed mussel provided at the beginning of the trial. The top two trays in each temperature treatment were not fed. Because ambient sea water flowed through the system, temperature varied over the course of the study; however, two aquarium heaters were used to elevate the temperature of the heated treatment relative to the ambient treatment. HoboTemp data loggers were

used to record temperature hourly both in the heated and ambient treatments. Each cascade system was also provided with two aerators per treatment to maintain oxygen levels as water flowed throughout the system. The water flow rate was set to approximately 2.4 L per min. We ran seven experimental trials, each with a new set of lobsters. The air supply was interrupted during the first trial which was consequently removed from the analysis.

We used a full two-factor Analysis of Variance to test the significance of the effects of temperature (warm vs ambient), feeding (fed vs not-fed) and their interaction on change in lobster survival, shell grade, and hemolymph protein levels. For lobster survival the replication trials served as the level of replication (n = 24; 4 treatment combinations x 6 trials). For the analysis of shell grade and hemolymph protein, individual lobsters were the level of replication (n = 144; 6 lobster x 4 treatment combinations x 6 trials).



Figure 1: Two stacks of experimental cascading seawater trays housing 24 lobsters per stack for a total of 48 lobsters used in 7-day trials. The top tray served as a cover.

#### Grading

To test for shell quality improvement, all lobster were graded according to industry guidelines. At the start of the experiment, all lobster were B-grade lobster. All graded lobster were confirmed by a professional grader from Ready Seafood Company. At the end of each trial, experimental lobster were graded blind, meaning the treatment of the lobster was unknown to graders. To test the grade, the lobster's carapace was squeezed between the thumb and fingers. Lobster were graded on the scale C, B, A, Hard-shell (Table 1). When squeezed, the carapace of a C-grade lobster will depress easily with very little resistance. The carapace of a B-grade lobster will have some resistance. An A-grade lobster will not depress under the squeeze test, but when the claw is squeezed, the soft membrane at the joint will bulge outward. A Hard-shell lobster will have no give in the shell and no bulge at the joint.

Table 1: Lobster shell hardness grading scale used by industry members.

Grade	Description
С	Soft
В	Firmer, but soft
А	Firm
Hard-Shell	Hard

#### Field Survey of Lobster Hemolymph Protein Levels

To compare hemolymph protein levels of our experimental lobsters in captivity to the freshest lobsters sampled in the wild we conducted a field survey with a commercial harvester during a day of fishing off the coast of Cape, Elizabeth, ME, on August 10, 2017. Brix hemolymph data were collected from wild lobster at the time they came out of the water. For each lobster collected, we recorded the sex, size, hemolymph protein level, and shell grade as well as the depth substrate type, and surface temperature where it was caught. Brix hemolymph protein levels of wild caught lobsters were compared to those of lobsters in the lab experiments.

A single factor Analysis of Variance was used to evaluate the difference in hemolymph protein levels among shell grades of wild lobster. A t-Test was used to compare the average blood protein level of B-grade lobster in the wild and the average blood protein level of B-grade lobster at the start of each experiment.

#### Economic Analysis

Our economic analysis evaluated the profitability of holding lobsters under different assumptions of survival, percentage with improved shell grade over the period, and price differential between grades. We obtained weekly summer prices per pound for A and B grade lobster from June 4 to September 2, 2017. We used the greatest price difference from June 4 to September 2, 2017, to create our scenarios.

We start with the assumption Ready Seafood facilities can hold 100,000 lbs. of lobster. We calculate it would require \$12,400 to move that much lobster in and out of the holding facility. To break this down to a daily cost, we assume 10,000 lbs. of lobster can be moved per day, a process requiring 6 workers for 5 hours at \$14 per hour per worker, for a daily cost of \$420, plus a daily trucking cost of \$200, for a total daily cost of \$620. To move each daily batch of 10,000 lbs. of lobster into the facility on day one and out on day 10 would double the cost from \$620 to \$1,240. Thus, for the 10-day process of moving 100,000 lbs. of lobster in and out of the facility would cost \$12,400.

The highest price differential between A and B grade lobster occurred during the week of August 27. Although Ready Seafood Co. would need to hold lobster for 10 as opposed to the 7 days for our experiment, we assumed the same average survival rate of 85.07% and the same percentage improvement to A-grade of 33.52%. To calculate the net profit, the survival rate was used to determine the number of lobster that can be sold after holding for a 10-day period. Using this number, the percent improvement to A-grade lobster was used to determine the number of lobster that could be sold for the price of an A-grade. The total value of the remaining B-grade and new A-grade lobsters was calculated. Labor costs were then subtracted from this value and compared to the value of the original group of B-grade lobsters. We then evaluated expected profits assuming an 87% and 100% survival rate.

## RESULTS

The average temperature difference between heated and ambient treatments was 0.66 °C across trials 2 through 7. Trial 2 had the largest temperature difference at 0.9 °C. Trial 6 had the smallest at 0.3 °C. Trial 1 was not included in the analysis because the air supply was interrupted during the experiment.

Overall, between 30 and 40% of the experimental lobsters advanced from shell B to A grade. The average percent of lobster improving from B-grade to A-grade across all trials was 33.52%. Our experimental warming and feeding treatments had no statistically significant effect on the change in shell grade, however (Figure 2).



Figure 2: Average percent of lobsters ( $\pm 1$ SE) improving in shell grade over the weeklong trial for each treatment combination. Temperature and feeding effects were not statistically significant (ANOVA results: Temperature: F<sub>1,12</sub> = 0.41, p = 0.53; Feeding: F<sub>1,12</sub> = 0.61, p = 0.45; Interaction: F<sub>1,12</sub> = 0.51, p = 0.51).

Survival among treatments over the course of the six 7-day trials ranged from 80 to 90%, averaging 85.07%. Temperature and feeding treatments had no statistically significant effect on lobster survival (Figure 3).



Figure 3: Average (±1SE) percent of lobsters surviving week-long trials for each treatment combination (N= 6 trials). Temperature and feeding effects were not statistically significant (ANOVA results: Temperature:  $F_{1,20}$ = 1.45, p = 0.24; Feeding:  $F_{1,20}$ = 0.13, p = 0.72; Interaction:  $F_{1,20}$ = 0.52, p = 0.48).

At the start of each trial, the mean Brix hemolymph protein level was 5.5. Protein levels in lobster hemolymph declined in five of the six week-long trials (Figure 4). Even lobster found to improve in shell grade had a decrease in hemolymph protein levels. Temperature and feeding treatments had no statistically significant effect on hemolymph protein levels.



Figure 4: Change in average ( $\pm$ 1SE) initial and final hemolymph protein levels of lobsters in each week-long trial. Temperature and feeding effects were not statistically significant (ANOVA results: Temperature: F<sub>1,241</sub>= 0.6984, p = 0.4041; Feeding: F<sub>1,241</sub>= 2.4215, p = 0.1210; Interaction: F<sub>1,241</sub>= 1.3372, p = 0.2487).

In our field survey of wild-caught lobster we found Brix hemolymph protein levels to be significantly higher in hard-shell lobster than those with lower shell grades (Figure 5). Furthermore, hemolymph protein levels of wild-caught B-grade lobster were significantly higher than those of captive B-grade lobster at the onset of our experiment (t = 2.68; p = 0.0044). Wild B-grade lobster had an average hemolymph protein of 5.8 (n = 67), whereas the average hemolymph protein level for lobsters at the onset of the experiment was 5.5 (n = 245).



# Figure 5

: Average ( $\pm 1$ SE) hemolymph protein levels according to grade of wild lobster caught off Cape Elizabeth, ME, on August 10, 2017. Hemolymph protein levels significantly increased with shell grade (ANOVA: F = 130.97, p < 0.0001).

# Economic Analysis

Profitability of holding lobster for shell hardening is strongly linked to survival rate and the price differential between shell grades. At \$7.27 per pound for A-grade lobster and \$4.73 per pound for B-grade lobster, Ready Seafood would make \$473,000 by selling 100,000 lbs. of B-grade lobster. Assuming a survival rate of 85.07% and an improvement in shell grade of 33.52%, Ready Seafood Company would lose \$10,589 by keeping lobster in a holding facility for 10 days (Figure 6). If the survival rate is 87%, however, the company would earn only \$182, and at a survival rate of 100% the company would gain \$72,741.



Figure 6: The profit or loss realized from holding 100,000 lbs. of B-grade lobster for 10 days for shell hardening given survival rates of 85.07, 87, and 100%, assuming 33.52% of the lobster improved from B to A grade, and the price per pound for A- and B-grade lobster was \$7.27 and \$4.73, respectively. Under these assumptions the break-even threshold occurs at a survival rate of about 87%.

## DISCUSSION

In this experiment, we found that temperature and feeding effects did not significantly affect shell grade improvement or survival. About one-third of the lobster improved from a B- to A-grade shell and about 85% of the lobsters survived regardless of treatment. This does not mean, however, that temperature and feeding have no effect on shell grade improvement or survival. The average temperature difference between the heated and ambient experiments was only 0.66 °C. The experiment was limited due to the inability to hold constant temperatures in the treatments. Our ability to achieve much of a temperature difference was limited by the need to keep high enough flow rates to keep the lobsters in fresh, well oxygenated seawater. It is likely that this temperature difference was not large enough to see a difference in shell grade improvement in the separate treatments. As seen in several studies, higher temperatures increase growth rates by reducing the intermolt period (Green et al 2014). The difference between the two temperature treatments may have been insufficient to produce a statistically significant difference in response with the 197 lobsters divided among the four treatments in this study.

Additionally, feeding regime can have a strong influence on lobster growth. For example, Bordner & Conklin (1981) found that lobster fed 7 days a week were an average of 31% heavier after 90 days than those fed only 5 days a week. In our experiment, lobsters were given only one crushed mussel to last the entirety of the week. Diet has also been reported to have measurable effects on improving shell hardness of American lobster (Donahue & Bayer 1998). In that case, cod racks yielded the most

improvement. These results suggest that the potential exists to use feeding regime to increase the percentage of lobster improving in shell grade. While brine shrimp or cod racks are possible food sources, the cost effectiveness of these feeding regimes needs to be considered.

It is also possible that the lobster just needed additional time to improve shell hardness. Time appeared to have the greatest effect on shell hardening, and within the limited time available in a lobster dealer facility the question remains whether manipulating temperature or diet will hasten the hardening process enough to make a difference. The trade-off of holding lobster for longer time is the greater cost of housing and risk of mortality.

We observed a survival rate of about 85% regardless of temperature or feeding treatment. This does not mean, however, that temperature and feeding do not have any effect on survival. As mentioned above, the temperature differential between the heated and ambient treatments was minimal. It is possible that with controlled temperature, or with a larger temperature differential, the survival of lobster may have been affected. Moreover, temperature throughout the trials was not constant. Temperatures that are too high can adversely affect the physiological condition of lobster (Lorenzon et al 2007). At certain points throughout our trials, particularly late in the season, temperatures exceeded 18°C and could have become stressful. In a laboratory-based thermal performance study, Worden (2006) found lobsters to prefer temperatures between 13 and 20°C but will move to avoid higher temperatures. They also found temperatures of 25 to 30°C to significantly degrade cardiac performance. If the temperatures in our study had been better controlled,

we could ensure that temperatures do not reach the lethal level and survival rates would likely improve.

In stressful conditions, such as handling, transport, and elevated temperatures, lobster require more energy. Lipids are the major energy reserve for lobster (Clark et al 2017) and they can replenish them through diet. Feeding lobster more frequently throughout the holding period will provide lobster with more energy to better handle the stressors that occur and to improve survival rates.

The experimental setup itself may have caused complications in lobster survival rate. The system was a flow through system in which the water that entered at the top of the cascade system traveled cell by cell down through the bottom. The waste of each lobster was thus carried with the cascading water through each cell. In addition, the death of a lobster in an upper cell could have affected the health of a lobster further down the cascade system due to the contaminated water flow. If each lobster had his/her own self-contained system, there is potential that survival rates could have improved because there would be no contamination of the water flowing through due to other lobsters.

Over the course of the week-long trial, hemolymph protein levels tended to decline in all trials excluding trial 5. Trial 5 did not differ in temperature, setup, or flow rate compared to all other trials, so it is difficult to conclude the reasoning for this. We found a significant difference in the hemolymph protein levels between wild caught and captive lobsters at the same shell hardness stage. From this, we can infer that handling and travel adversely affect hemolymph protein levels. Hemolymph levels are sensitive to changes in salinity, temperature, food availability, season, life stage, and stress (Ciaramella et al. 2014). It is possible that handling and time out of water before the

experiment induced a drop in lobster hemolymph protein levels. For example, if a lobster is injured, the hemolymph protein level will be much lower than an uninjured lobster (Clark et al 2017). It is possible that using the syringe to test for hemolymph protein levels at the beginning of the week caused harm to the lobsters and lowered their overall hemolymph protein levels at the conclusion of the experiment. There is also potential that the conditions the lobster were held in caused stress to the lobster, therefore reducing their overall health. No significant effect of feeding and temperature treatment was found on lobster hemolymph protein levels. In future studies, higher hemolymph protein levels may be maintained with an increase in feeding frequency of the lobster, as opposed to a single feeding for the entirety of the week. Additionally, as mentioned above, with an increased temperature difference or an increased sample size a larger effect may have been seen on hemolymph protein levels.

Hemolymph protein levels are known to increase prior to molt and rapidly decrease post molt (Ciaramella, Battison, & Horney, 2014). As the lobster shell hardens during the intermolt, hemolymph protein levels increase and severely decrease with the soft shell that is apparent post molt. The field data provide a good baseline for average hemolymph protein levels typical for each grade. We see that hemolymph protein level and shell grade have a direct relationship meaning as hemolymph protein levels increase lobster shells tend to be harder.

Throughout the season, price commanded by A-grade and B-grade lobster varies. If a shell hardening procedure were to be implemented at Ready Seafood Company, it would cost the company \$12,400 to handle 100,000 pounds of lobster over a ten-day period. The survival rate in our experiment was relatively low, likely due to the stressful

temperatures. If survival rates could be improved by just 2% (to reach 87%), Ready Seafood Company could gain a profit of about \$180. If survival could be increased to 100%, at an improvement rate of 33.5%, Ready Seafood could potentially make a \$72,700 profit per 100,000 pounds of lobster. The company has the potential to further increase profits if the shell improvement rate is increased as well. It is important to note, however, that for this scenario, the highest price differential between A and B grade lobster was used. Throughout the season, prices vary greatly. It may be beneficial for a company to only hold over lobster at times of peak prices, otherwise, holding lobster may be less profitable.

While holding lobster for hardening has the potential to increase profit for lobster dealers, more experiments are warranted to determine the optimum temperature and feeding regime that will maximize the rate of shell hardening. While we were unable to hold lobster at a constant temperature in the present experiment, future studies should aim to maintain multiple constant temperature treatments under well oxygenated, uncontaminated flow. This will help identify the conditions minimizing hardening time and maximizing survival, and in turn, profits.

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# AUTHOR'S BIOGRAPHY

Abigale L. Shaughnessy was born in Hartford, Connecticut on July 24, 1996. She was raised in Enfield, Connecticut and graduated from Enrico Fermi High School in 2014. Majoring in marine biology, Abigale has a minor in fisheries science. She is a site leader for the service organization Alternative Breaks, a member of Alpha Omicron Pi, Order of Omega, Phi Beta Kappa, Marine Science Club, and Best Buddies.

Upon graduation, Abigale plans to work as an intern at Allied Whale and Bar Harbor Whale Watch Company. She will work at several internships for a year before returning to work on an advanced degree in marine biology.