Maine Sea Grant – Program Development Funds
Project Completion Report

Project Title: 'Egg Signals': Embryonic chemical signals and their potential effect on maternal thermal preference and hatch in American lobster, *Homarus americanus*

Award Date: 12/2021
Project Completion: 09/2023
Report Submission: December 29, 2023
Award Amount: $4,400
Grant Recipients Wells National Estuarine Research Reserve (Wells NERR); Saint Joseph's College of Maine (SJCM)

Project Investigators:
Jason Goldstein & Benjamin Gutzler (Wells NERR)
Steven Jury (SJCM)

Awardee Contact Information:
342 Laudholm Farm Road
Wells, ME 04090
phone: (207) 646.1555 x136
email: jgoldstein@wellsnerr.org
web: www.wellsreserve.org

Financial Contact:
Paul Dest, Executive Director, Wells NERR
342 Laudholm Farm Road
Wells, ME 04090
phone: (207) 646.1555 x124
email: pdest@wellsreserve.org

Executive Summary

The overall goal of this ME SG Project Development award was to explore how potential chemo-active compounds produced and released from egg clutches may influence the thermal preferences of both early- and late-stage ovigerous American lobsters (*Homarus americanus*). To explore this question, we piloted a series of lab-based aquarium trials coupled with a suite of biochemical and analytical chemical techniques to assess chemical signals from egg masses and characterize if there is a chemical basis for changes in behavioral thermoregulation of ovigerous females across the egg development period in American lobster.

Sea Grant Strategic Plan Justification

The work of this Project Development award aligns with the Maine Sea Grant's Strategic Plan (2018-2021) for *Safe and Sustainable Seafood* and *Objective 4 to support applied research to improve coastal and marine management and to address population dynamics, monitoring, and biological responses to climate change*.

The results of this ME SG-funded work were fruitful, productive, and engaging with others in the lobster fishery, and we look forward to sharing our results with those important end-users as we continue to learn and address environmental change and impacts to the single most important fishery in North America and Maine, the American lobster.

Project Background & Rationale

Behavioral thermoregulation is a cornerstone of American lobster (*Homarus americanus*) biology and physiological responses to temperature significantly shape the distribution and movement of lobster populations throughout their range including the Gulf of Maine (GoM; Crossin et al. 1998; Jury & Watson 2000, Jury et al. in preparation). However, with the growing body of evidence for ocean climate change in places like the GoM (e.g., Pershing et al. 2015, Le Bris et al. 2018), a number of lobster-related studies (some very recent) suggest substantial changes to lobster spatial and temporal distributions (e.g., Tanaka & Chen 2016).

For ovigerous lobsters, embryonic development involves external egg fertilization as eggs are released and subsequently attached as an egg mass on the ventral tail of the adult female (Aiken et al. 2004). Ovigerous lobsters brood their eggs throughout a protracted 9-11 month embryonic development period and ultimately hatch their eggs presumably in a location suitable for larval release and subsequent survivorship. There are multiple differences in behavior between ovigerous and non-ovigerous females as well as males in terms of escape behavior (Cromarty et al. 1998), maternal aggression (Figler et al. 2003), and seasonal movements (Goldstein & Watson 2015). For example, we know very well that a host of external environmental factors (temperature, osmotic conditions, and diel cycles, among others) modulate and synchronize hatch in many crustaceans including ovigerous lobsters (reviewed in Fritsch et al. 2020). However, egg 'state' (e.g., early- vs. late-stage ovigerous lobsters with embryos in different stages of development) may have a disproportionate effect on behavioral choices, especially those related to thermal preference. Essentially, how and why egg extrusion, egg stage

or egg carrying changes the behavioral state of lobster females is unclear and it is unknown if there is a sensory signal mediating the timing and magnitude of any change.

While much is known about the endocrinology of reproduction and molting in crustaceans (reviewed in Pamuru 2020), there has been relatively little work done in crustaceans on potential pheromone signaling between eggs/embryos and the female carrying the eggs as they develop. The exception to this is the reported release of a suite of peptide-like pheromones involved in pleopod pumping, a maternal behavior that enhances the mechanical release of larvae from eggs by shaking them off the pleopods (Rittschoff & Cohen 2004). It has also been shown in European lobster (*H. gammarus*) eggs that while early-stage eggs (stages I-III) are relatively impermeable, the permeability to salt and water may increase at advanced stages of development (Pandian 1970). Likewise, several other studies have examined a suite of chemical cues and mediators (e.g., small peptides) in a variety of egg-bearing decapod crustaceans including blue crab (*Callinectes sapidus*; Tankersley et al. 2002**)**, Caribbean spiny lobster (*Panulirus argus*; Ziegler & Forward 2007), and the mole crab (*Emerita talpoida;* Ziegler & Forward 2005). However, there have been no studies to-date that have investigated such patterns in *H. americanus* which is surprising.

As such, in this pilot project we are interested in the possibility of a chemical signaling peptide(s) released by developing embryos within egg masses of ovigerous American lobsters that may feedback and influence maternal behaviors that may benefit both the mother and offspring. We expect that as embryos develop, they are releasing increasing amounts of signaling molecules (e.g., low molecular weight peptides) into the water around the egg mass as the embryos develop that can be detected by the ovigerous female carrying her eggs (Reinsel et al. 2014). Furthermore, preliminary data that we have gathered thus far suggest that the mechanical removal of eggs from an egg-bearing female can change behavioral thermoregulation in a laboratory thermal gradient tank (see Fig.1). Furthermore, ovigerous females show seasonal differences in thermal regulation that differ from those in non-berried females of similar size from the same location (Jury et al. in prep).

Based on this background, we set out to address the following three goals:
1) Explore the effects of egg removal on control-stage matched ovigerous females in a behavioral assay chamber to determine changes in activity and temperature preference in early (stage 2-3) vs late (stage 4-5) ovigerous females. Briefly, we will utilize an already assembled fully functioning thermal gradient tank (funded from a separate study) to test pairs of lobsters equipped with HOBO temperature and activity loggers.

2) Determine and characterize the possible presence of amino acids and peptides in water from egg masses vs control (non-ovigerous female water) and from water with crushed eggs from early (stage 2-3) vs. late stage (4-5) ovigerous females. We held ovigerous lobsters in static holding tanks, take water samples, and then use a suite of biochemical and analytical chemistry techniques that we will develop and employ to ascertain these biomolecules.

3) Test the aqueous extracts of crushed eggs on pleopod pumping (i.e., pleonal pumping, Forward (1987) behaviors from early (stage 1-2) vs. late stage (4-5) ovigerous females. We will use time-lapse video to quantify pleopod movements in early vs. late-stage ovigerous female lobsters. *DISCLAIMER: Due to COVID-related issues along with logistical challenges, we were unable to execute and carryout this particular objective but anticipate student participation in re-kindling this study in the coming year or so.*

Project Approach & Result Highlights

Objective-1: Lobster activity runs in a thermal gradient tank

The thermal gradient tank is described fully in a forthcoming manuscript (Jury et al., in prep), but in short, consisted of heated and chilled water circulated at opposite ends of a shallow (25 cm), rectangular trough (3 m x 1.2 m) made of 1.9 cm Azek PVC to establish a long, gradual, thermal gradient. This tank was separated into seven 38 x 120 cm chambers by 2.5 cm closed cell foam insulation to make separate water 'baths.' These pieces were cut and attached into the chamber and cut like 'ribs in a boat' so that a large black polyethylene thin-walled inner tank (3 x 0.6 m by 25 cm depth), Tarter Poly Bunk Feeder 1PBL10) was seated onto the foam frame and held 3 cm above the bottom of the outer tank. The inner tank was divided into two troughs using a piece of the 1.9 cm PVC that was sealed to have two independent raceways for lobsters. These raceways were further separated into 9 sections by foam insulation with a 12.7 cm diameter hole cut in the middle to separate different sections. Each section for lobsters to enter or leave was approximately 28 x 28 cm square (with the exception of smaller end sections that were not accessible to the lobsters). Each section of the outer tank was independently heated or chilled to give a gradient of temperatures from one end of the tank to the other ranging from ~6ºC to ~20ºC (Figure 1).

A total of 6 lobsters were used for this experiment, 3 with early-stage eggs and 3 with late-stage eggs (mean carapace length 88 mm, range 86-95 mm). All lobsters were sourced from New Hampshire Fish and Game Sea Sampling Programs. Each lobster had a HOBO Pendant temperature logger (Onset Inc., Bourne, MA) attached to the carpus ('knuckle') above the claw with a zip tie that logged temperature at 5 min. The thermal gradient was established prior to placing lobsters into the tank as described above. Each lobster was placed randomly into one of the gradient troughs of the inner tank in the morning between 7-9 AM on the first day and kept in the tank, undisturbed, for 48 hrs. Only data from the second 24 hrs. were used for all analyses presented here to reduce potential handling artefacts during the first day. Temperature was monitored continuously by the logger as lobsters moved volitionally through the tank. Each lobster was run through the thermal gradient tank with eggs initially, then eggs were stripped manually into containers of sterile artificial seawater. After 24 hours to recover from the handling, the lobster was run in the thermal gradient tank again.

Two of the three lobsters tested at each egg stage showed clear differences in thermal preference after stripping (Figures 2, 3). However, responses were not consistent in how behavioral preferences shifted. Four of the post-stripped lobsters showed median

preferences of 14-17ºC, consistent with the thermal preferences of non-ovigerous females (Figure 3; Jury et al. in prep). The two other females chose cold water with little movement. The reason for these variable responses is uncertain but may be a product of the underlying behavioral heterogeneity of lobsters in their response to thermal preference that was found in our study with a much larger sample size with intact egg masses (Jury et al. in prep). It was intriguing that ovigerous females did show a change in thermal preference and it is possible that early-stage lobsters showed a different response than late stage but further replication will be needed to make any inferences.

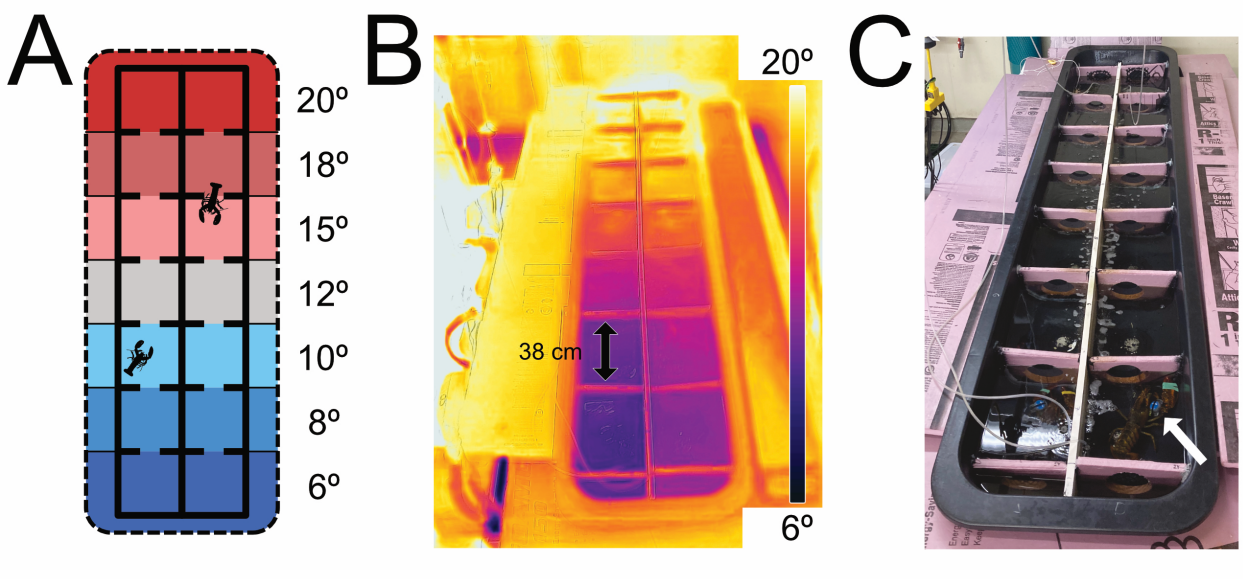

Figure 1. Thermal gradient tank setup. **A:** Notional diagram of the tank and water bath. **B:** Thermal camera image showing temperature gradient present during trials. **C:** Photograph of the tank during a trial. White arrow indicates the position of the HOBO temperature logger on the lobster in the right-hand lane of the tank. (Figure from Jury et al., in prep).

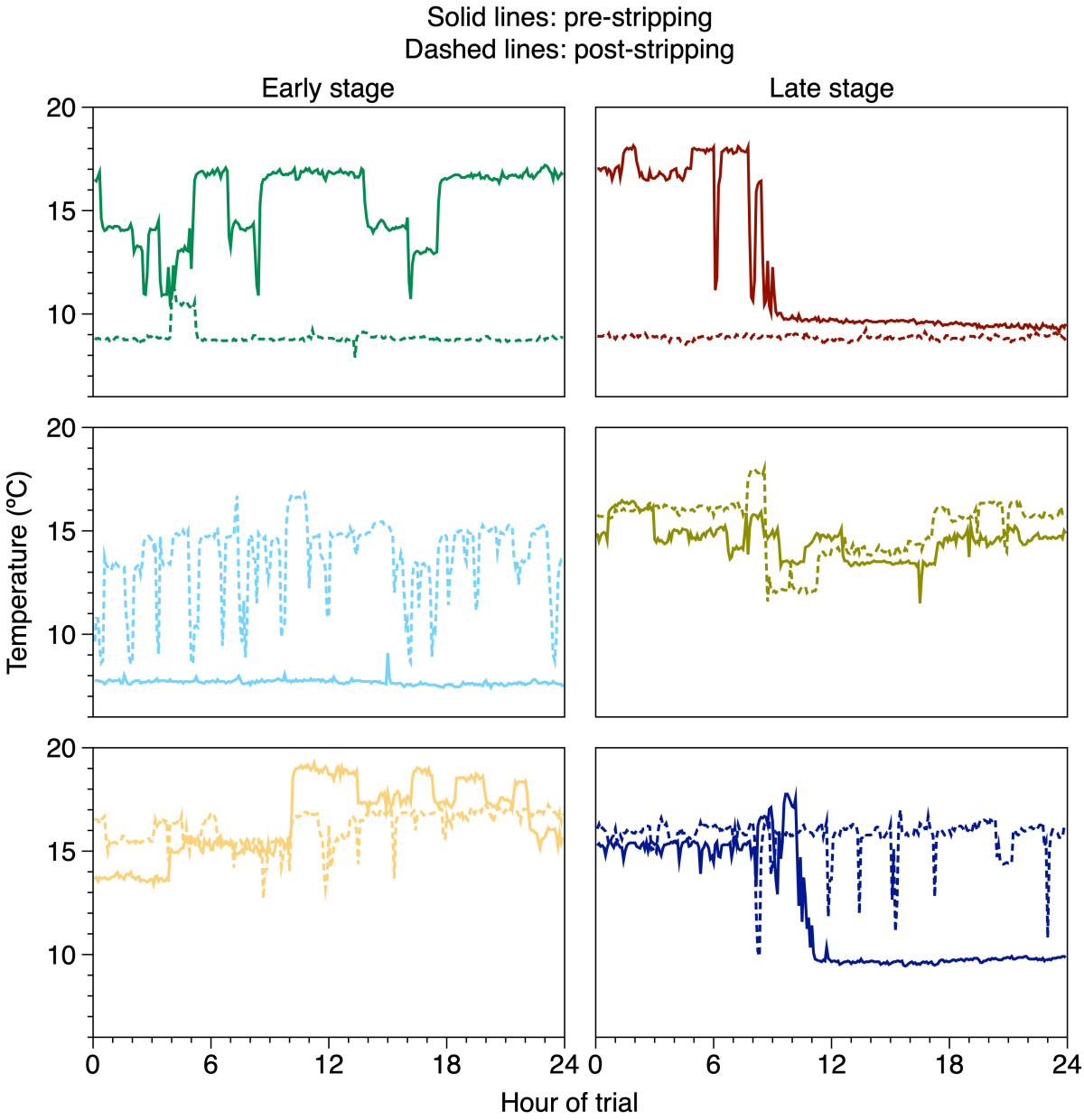

Figure 2. Temperature records of the six lobsters tested, showing their temperature profiles from before and after eggs were stripped. Each panel is a separate lobster.

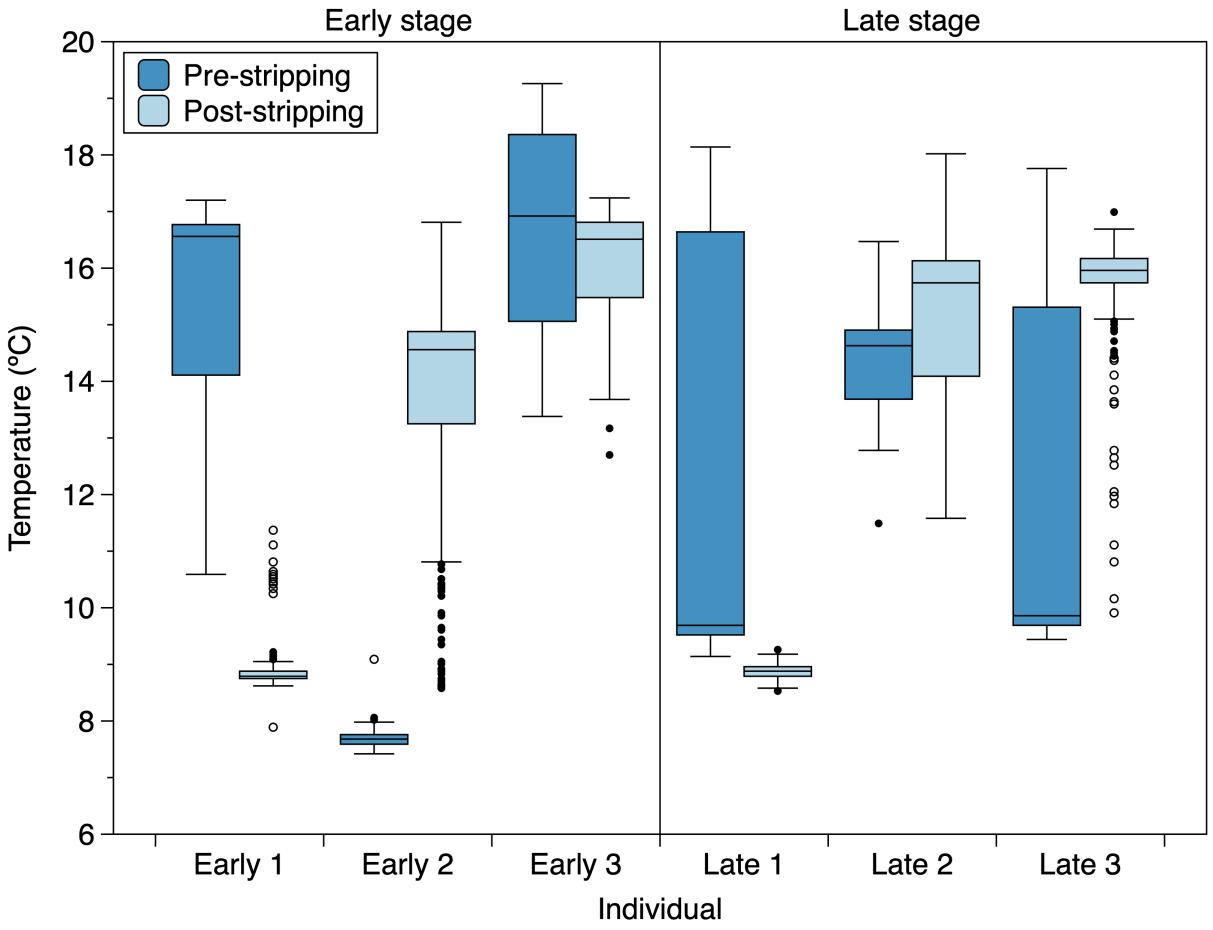

Figure 3. Box plot showing contrasts in preferred temperature before and after eggs were stripped for each of the six lobsters. Lines in boxes represent medians and whiskers represent the extent of data within 1.5 IQR.

<u>Objective-2:</u> Determine if lobster eggs release certain peptides or amino acids that could be used as signals to influence maternal behavioral thermoregulation

Through a series of trials, we developed a low-cost protocol for examining the leachate for lobster eggs that we incubated in small, aerated containers and filtered to run these samples either through gel electrophoresis or high-performance liquid chromatography (HPLC) to begin to determine possible chemical compounds in our extracted egg leachate precipitates. We carried out our trials on both early-stage ovigerous lobsters (n = 3) that we obtained in the summer months as well late-stage ovigerous lobsters (n = 3) that we collected in late spring. We chose these two disparate groups of lobsters to test the hypothesis that egg leachate would be more readily detectable from late-stage eggs.
Below is an outline of the protocol we developed for this method:

- For each egg mass we prepared 1 L of artificial seawater (ASW; 30 psu, Instant Ocean) and made up the following treatments in individual 100 ml beakers.
    a. **Negative Control**: ASW only in 20 ml x 2
    b. **Low egg concentration**: ~250 eggs in 20 ml ASW x 2
    c. **High egg concentration**: ~500 eggs in 20 ml ASW x 2
    d. **Positive Control**: ~500 eggs crushed or broken (using a mortar and pestle) in 20 ml ASW x 2
- All samples were placed into a refrigerator at (~ 7°C) and gently bubbled for 12 hrs (Figure 4).
- After 12 hrs of leaching each treatment was filtered through a 20 ml syringe using a 0.2 µm filter (Acrodisc circular filter, Millipore-Sigma Corp.), to remove the supernatant. 50 ml aliquots were then added to a series of 50 ml pre-labeled scintillation vials and stored in a -80°C ultracold freezer.

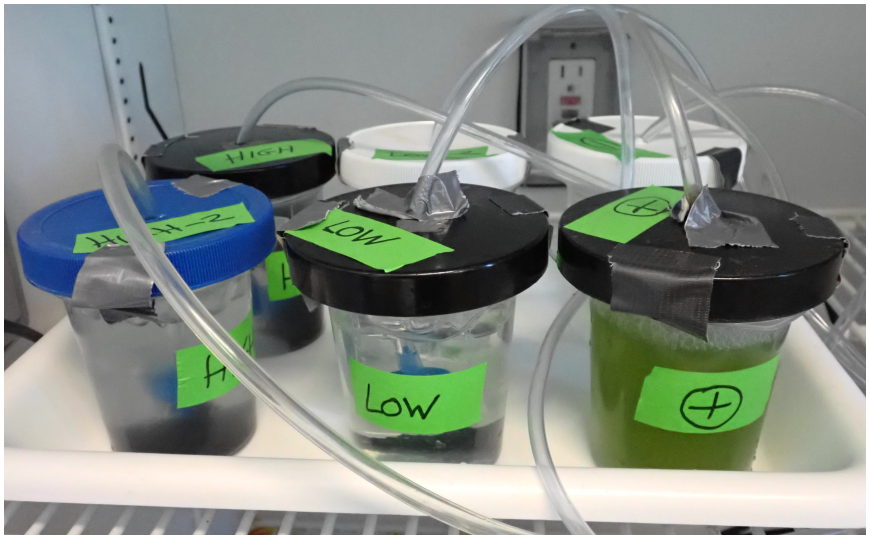

Figure 4. Experimental setup for egg leachate experiments conducted with a total of 6 individual ovigerous lobsters (size range = 75 – 94 mm CL); three lobsters that were of early stage and three that were of late-stage egg development. Egg subsamples were placed in one of three treatments: low or high concentration of eggs as well as a positive control, consisting of crushed eggs, in duplicate; pictured in the back row are two negative controls (i.e., artificial seawater only).

Samples were initially prepped and preliminarily run on an HPLC to determine amino acid and/or peptide profiles, however, the St. Joseph's College chemist identified to develop protocols and run these samples was not available. We were able to identify a third party laboratory (Maine Medical Center Research Institute) to assist in preliminary trials; however, no peptides were found in these samples, possibly due to the high salt content of the samples. Additional HPLC workup of modified protocols were deemed cost prohibitive for this pilot project. We were able to run the samples using gel electrophoresis (Figures 5, 6) to determine the relative size distribution of possible peptides in each sample. While there was some suggestion of protein bands in the 30-50kD range in the leachates relative to the SW controls, few low molecular weight

bands were evident. Clear bands in the approximately 10, 30, and >50 kD are evident in the homogenate suggesting proteins are present in the egg that are not seen in the leachate (Figure 6). Overall, there was little evidence of low molecular weight proteins or amino acids in our samples. Thus, while we believe our egg mass leaching protocol likely was a suitable proxy for potential signaling molecules available to ovigerous females from early- and late-stage eggs, we were not able to identify these compounds. Future behavioral studies or modified biochemical approaches may be helpful in the future as resources allow.

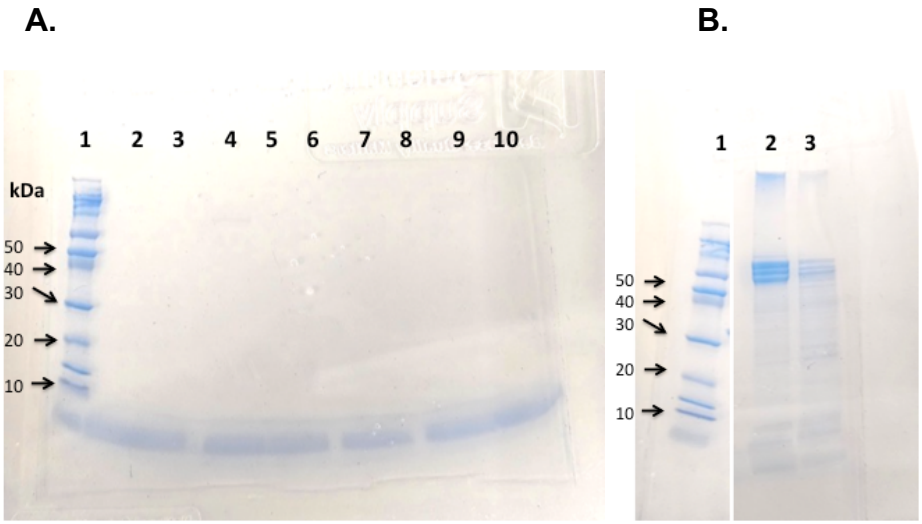

Figure 5. SDS-Page of **A)** lobster egg leachates (n=2, applied in duplicate lanes per sample), negative controls (n=2, seawater - SW) and B) positive control samples (n=2 egg homogenates in SW), stained with Pierce™ PageBlue Protein Staining Solution (ThermoScientific™). Lanes on each gel are as follows: **A)** 1 - Invitrogen Novex Sharp Prestained Protein Standards; 2 – SW1; 3 - blank; 4 – egg leachate 1-1; 5 – egg leachate 1-2; 6 - blank; 7 – SW2; 8 - blank; 9 – egg leachate 2-1; 10 – egg leachate 2-2. **B)** 1 – Invitrogen Novex Sharp Prestained Protein Standards; lane 2 – egg homogenate 1; lane 3 - egg homogenate 2. Overall, there was little evidence of low molecular weight protein in the leachates but some <10kD and approximately 50kD proteins in the homogenates.

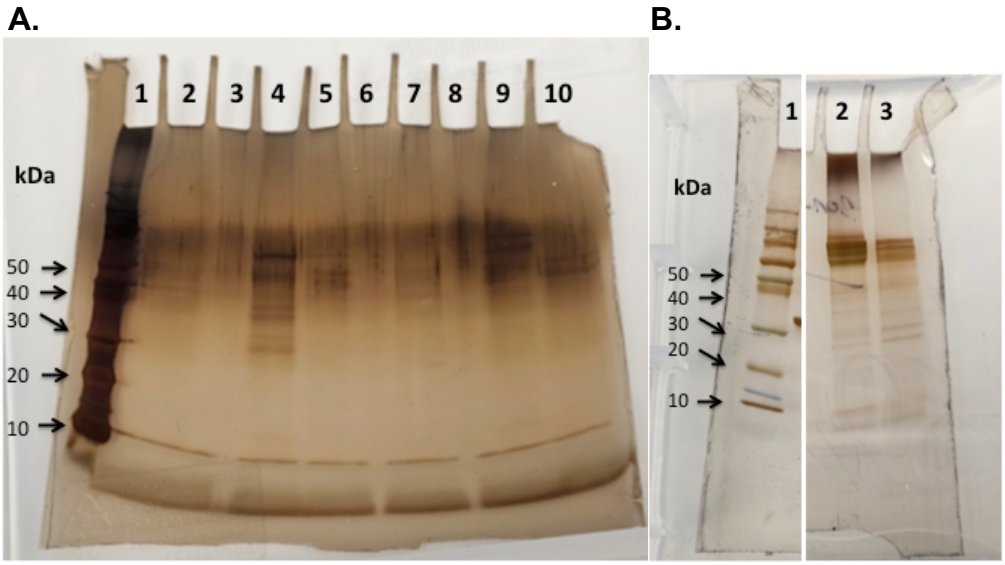

Figure 6. 2: SDS-Page of **A)** egg leachates (n=2, applied in duplicate lanes per sample), negative controls (n=2, seawater - SW) and **B)** positive control samples (n=2, egg homogenates in SW). Gels depicted in Figure 1 were destained overnight and treated with a silver stain kit (Pierce™ Silver Stain Kit, Thermo Scientific™) the following day to enhance the protein bands. Lanes on each gel were as follows (same as in Fig. 4): **A)** 1 - Invitrogen Novex Sharp Prestained Protein Standards; 2 – SW1; 3 - blank; 4 – egg leachate 1-1; 5 – egg leachate 1-2; 6 - blank; 7 – SW2; 8 - blank; 9 – egg leachate 2-1; 10 – egg leachate 2-2. **B)** 1 – Invitrogen Novex Sharp Prestained Protein Standards; lane 2 – egg homogenate 1; lane 3 - egg homogenate 2. While there is some suggestion of protein bands in the 30-50kD range in the leachates relative to the SW controls little low molecular weight bands are evident. Clear bands in the approximately 10, 30, and >50 kD are evident in the homogenate suggesting proteins are present in the egg that are not seen in the leachate.

Anticipated Outcomes & Future Directions

Although most of the results from this pilot study are preliminary in nature, we believe that this work shows a pathway towards an improved and better understanding of reproductive chemical signaling interaction between developing eggs and maternal behavior and a possible role this may play on larval release habitats for American lobster. We suspect these results will allow us to better pinpoint how maternal behavior may further modulate hatching locations and to what degree that is relevant for subsequent bio-physical modeling efforts for lobster stocks in the Gulf of Maine.

The project directly complements work that we are currently engaged in as related to our American Lobster Initiative (ALI) project, supported through the National Sea Grant Program. Our ALI efforts are aimed at better understanding how the distributions of egg-bearing lobsters in the Gulf of Maine may shift as a result of climate change, and how behavioral thermoregulation by ovigerous females may modulate these shifts. Having the ability to enhance that project with this proposed work helps to further our understand of lobster behavior, physiology, and chemical ecology, by helping to provide a mechanistic explanation for the shifts in thermal preference seen between lobsters of different egg stages. These data will ultimately be very applicable to lobster stock

assessment managers and potentially steer future assessment outcomes towards a more informed fishery database for Maine's most important seafood product.

References Cited

Aiken, D.E., S.L. Waddy and S.M. Mercer. 2004. Confirmation of external fertilization in the American lobster, *Homarus americanus*. Journal Crustacean Biology. 24: 474-480.

Cromarty, S.I, J. Mello and G. Kass-Simon. 1998. Comparative analysis of escape behavior in male and gravid and non-gravid, female lobsters. Biological Bulletin. 194: 63-71.

Crossin, G., S.H. Jury, and W.H. Watson III. 1998. Behavioral thermoregulation in the American lobster, *Homarus americanus*. Journal of Experimental Biology. 201: 365-374.

Figler, M.H., H. V.S. Peeke, M. J. Snyder, and E.S. Chang. 2004. Effects of egg removal on maternal aggression, biogenic amines, and stress indicators in ovigerous lobsters (*Homarus americanus*). Marine and Freshwater Behavior and Physiology. 37: 43-54.

Forward, R.B., Jr. 1987. Larval release rhythms of decapod crustaceans: an overview. Bulletin of Marine Science. 41:165-176.

Fritsch, M., J. Olesen, O.S. Moller, and G. Loose. Chapter 5: Hatching. *In*: Developmental Biology & Larval Ecology, Vol. 7 (Anger, K., S. Harzsch, and M. Thiel, Eds.). Oxford University Press, New York. Pp. 143-164.

Goldstein, J.S. and W.H. Watson III. 2015. Seasonal movements of American lobsters in southern Gulf of Maine coastal waters: Patterns, environmental triggers, and implications for larval release. Marine Ecology Progress Series. 524: 197-211.

Jury, S.H., B.C. Gutzler, J.S. Goldstein, J.T. Carloni, W.H. Watson (in prep). Behavioral thermoregulation of ovigerous American lobsters (*Homarus americanus*). Marine Ecology Progress Series.

Jury, S.H. and W.H. Watson III. 2000. Thermosensitivity of the lobster, *Homarus americanus*, as determined by cardiac assay. Biological Bulletin. 199: 257-264.

Le Bris, A., K.E. Mills, R.A. Wahle, Y. Chen, M.A. Alexander, A.J. Allyn, J.G. Schuetz, J.D. Scott, and A.J. Pershing. 2018. Climate vulnerability and resilience in the most valuable North American fishery. Proceedings of the National Academy of Sciences. 115: 1831-1836.

Pamuru, R.R. Endocrinology of Reproduction in Crustaceans. 2019. InTech OpenSource. Available at: doi: 10.5772/intechopen.83018.

Pandian, T.J. 1970. Ecophysiogical studies on the developing embryos of the European lobster *Homarus gammarus*. Marine Biology. 5: 154-167.

Pershing, A.J., M.A. Alexander, C.M. Hernandez, L.A. Kerr, A. Le Bris, K.E. Mills, J.A. Nye, N.R. Record, H.A. Scannell, J.D. Scott, G.D. Sherwood, and A.C. Thomas. 2015. Slow adaptation in the face of rapid warming leads to collapse of the Gulf of Maine cod fishery. Science. 350: 809-812.

Rittschof, D., and J.H. Cohen. 2004. Crustacean peptide and peptide-like pheromones and kairomones. Peptides 25: 1503-1516.

Reinsel, K.A., K. Pagel, M. Kissel, E. Foran, A.S. Clare, and D. Rittschof. 2014. Egg mass ventilation by caridean shrimp: similarities to other decapods and insignt into Pp.1-9.

Rosa, R., R. Calado, A.M. Andrade, L. Narciso, and M.L. Nunes. 2005. Changes in amino acids and lipids during embryogenesis of European lobster, *Homarus gammarus*. Comparative Biochemistry and Physiology B. 140: 241-249.

Tanaka, K., and Y. Chen. 2016. Modeling spatiotemporal variability of the bioclimate envelope of *Homarus americanus* in the coastal waters of Maine and New Hampshire. Fisheries Research. 177: 137–152.

Tankersley, R. A., T.M. Bullock, R.B. Forward Jr., and D. Rittschof. 2002. Larval release behaviors in the blue crab *Callinectes sapidus*: Role of chemical cues. Journal of Experimental Marine Biology and Ecology. 273: 1-14.

Ziegler, T.A., and R.B. Forward. Jr. 2007. Larval release behaviors in the Caribbean spiny lobster, *Panulirus argus*: Role of peptide pheromones. Journal of Chemical Ecology. 33: 1795-1805.

Ziegler, T.A., and R.B. Forward Jr. 2005. Larval release rhythm of the mole carb *Emerita talpoida* (Say). Biological Bulletin. 209: 194-203.