



Brood-grooming behavior of American lobsters *Homarus americanus* in conditions of ocean warming and acidification

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ABSTRACT: The relationship between adverse environmental conditions and grooming behavior is an unresolved mechanism whereby a changing climate may impact reproductive success in animals that brood their eggs. Although important to embryo survival and development in decapod crustaceans, brood grooming by ovigerous females may be impacted by energetically demanding conditions associated with climate change, which may contribute to lethal and sublethal outcomes for brood health and survival. Despite its potential importance to reproduction, brood-grooming behavior has not been empirically described in the American lobster *Homarus americanus* H. Milne Edwards, 1837, a commercially important marine decapod. The relationship between brood-grooming behavior, temperature, and pH was explored at different points in the embryogenesis of American lobsters. For a period of 5 mo, egg-bearing females were exposed to different combinations of ecologically relevant conditions of temperature and pH, including those reflecting ocean warming (+4°C), ocean acidification (−0.5 pH), and the combination of warming and acidification. Fecundity, embryo development, and female grooming behavior were assessed at multiple time points. The proportion of time that lobsters spent fanning, but not probing, their broods increased with advancing embryo development. Neither egg loss, nor any measured brood-grooming behaviors, varied significantly with temperature or pH in this experiment. American lobster reproduction appears well suited to tolerate future conditions of ocean acidification and warming based on the ability to maintain stable brood grooming and brood mortality levels under a range of conditions.

KEY WORDS: Active parental care · Climate change · Crustacea · Decapod · Reproductive biology

1. INTRODUCTION

Among brooding crustaceans, grooming of retained offspring is a highly conserved behavior that serves to regulate the conditions experienced by developing embryos (Fernández et al. 2020). While brooding is an effective reproductive strategy to facilitate prolonged embryo development in stressful environments, high densities within crustacean egg masses can pose a challenge to embryo health (Vogt 2013, Fernández et al. 2020). Oxygen limitation caused by respiring embryos can produce critical delays in development (Fernández & Brante 2003). Furthermore, metabolic activity increases over the course of embryogenesis,

exacerbating the limitations of high-density brooding (Baeza & Fernández 2002). Egg masses in brooding crustaceans also serve as a platform for parasites, disease, and fouling, which are often culprits of high embryo mortality (Kuris 1991). Brood-grooming behavior moderates the limitations of brooding and reduces embryo mortality by increasing water flow over the egg mass, removing harmful parasites, and synchronizing hatching (Bauer 1979, Forward et al. 1987, Kuris & Wickham 1987).

The relationship between environmental parameters and grooming behavior is an unresolved mechanism by which the changing climate may impact reproductive success in brooding crustaceans. For example, in

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high temperature conditions, the metabolic rates of embryos increase, which in turn drives greater oxygen demand in the egg mass and increased ventilation activity (Dick et al. 1998, Baeza & Fernández 2002, Brante et al. 2003, Tarutis et al. 2005). Despite the advantages to reproductive success, brood-grooming behavior can be costly to females. The maternal energetic demand associated with grooming behavior is high and increases during embryogenesis (Naylor et al. 1999, Fernández & Brante 2003). Under adverse environmental conditions, the capacity of females to respond behaviorally to changes in embryo oxygen demand may be limited, as there are likely trade-offs between expending energy for maternal care and other behavioral and physiological functions. Although limited research is available to explore trade-offs in brood-grooming behavior specifically, grooming is considered a 'secondary behavior' for several crustacean species and only occurs when more critical behaviors (i.e. those associated with feeding, mating, and fighting) are not required (VanMaurik & Wortham 2014, Wortham & Jedlicka 2019). For example, Arundell et al. (2014) observed that *Crangonyx pseudogracilis* females responded to predator cues by reducing brood-grooming behavior, whereas *Gammarus duebeni* females reduced overall brood duration in the presence of predators. Given that species vary in their responses to environmental change, the effect of environmental stressors on brood-grooming behavior, and ultimately reproductive success, will likely be specific to the species, stressor type, and stressor intensity.

Two environmental stressors of relevance for marine crustaceans are ocean acidification and ocean warming. The absorption of anthropogenic atmospheric CO₂ by the ocean and subsequent change in ocean carbonate chemistry has resulted in a global decline in ocean pH by an average of 0.1 pH units since the onset of industrialization (Hoegh-Guldberg et al. 2014). Surface temperatures for the global ocean are projected to rise approximately 1–4°C relative to pre-industrial conditions (Bindoff et al. 2019), with 0.25°C of warming already observed between 1971 and 2010 (Levitus et al. 2009, Rhein et al. 2013). The consequences of ocean acidification and warming on crustaceans will likely depend on their interactive effects as well as species and life-stage specific responses (Byrne 2011, Whiteley 2011, Faulkner et al. 2014).

Although prior work suggests that brood-grooming behavior may be sensitive to warming (Dick et al. 1998, Tarutis et al. 2005), it is not yet clear whether crustacean brood-grooming behavior varies with carbonate chemistry, alone or in combination with temperature. The impacts of acidification on brood

grooming specifically will depend on the mechanism by which females sense the ventilation needs of the brood (Tierney & Atema 1988) and the effect of acidification on embryo metabolism. Reports on freshwater crayfish suggest a species-specific effect of pH on general body grooming in the presence of chemical stimuli (Tierney & Atema 1986). Brood grooming may be a useful tool to mitigate stressful conditions experienced by embryos, which have limited stress-tolerance mechanisms. However, the energy required to mount a maternal physiological response to stress may limit the amount of energy a female can invest in brood grooming (Arundell et al. 2014, Gallardo et al. 2019, Prestholdt et al. 2022). A better understanding of how brood-grooming behavior will be impacted by climate change-related stressors is essential to predict future reproductive success in ecologically and commercially important crustacean species.

The American lobster *Homarus americanus* H. Milne Edwards, 1837 represents one such commercially important crustacean for which understanding reproductive dynamics is key to fishery management. This species supports one of the largest fisheries in the USA, with an ex-vessel value of over US \$464 million in 2023 (Maine Department of Marine Resources 2024). Female lobsters typically extrude their eggs in the fall, up to a year after mating, and brood their egg masses for a period of 9–12 mo before their eggs hatch in the spring and summer months. A single lobster can produce between several thousand and tens of thousands of eggs depending on her size (Campbell & Pezzack 1986, Estrella & Cadrin 1995), but estimated survival to settlement is as low as 1% (Chassé & Miller 2010). Brood grooming has been reported for American lobsters, particularly in response to the presence of parasites and for synchronization of hatching (Talbot et al. 1984, Aiken et al. 1985). However, variability among individuals and patterns associated with environmental conditions or embryo development are not known. There is evidence to suggest that American lobsters will be sensitive to acidification and warming (Waller et al. 2017, Menu-Courey et al. 2019, Niemisto et al. 2021); indeed, lobster populations have already shifted northward as a result of changing temperature regimes in the North Atlantic (Le Bris et al. 2018).

Declining landings of American lobster in southern New England (SNE) have been linked to climate change, specifically rising temperatures (Le Bris et al. 2018, Mazur et al. 2020). Two potential contributors in the collapse of the SNE lobster stock are rising rates of epizootic shell disease (ESD) and declining rates of fecundity associated with a reduction in size at maturity, both of which are correlated with ocean warming and

reported in SNE (Castro & Somers 2012, Goldstein et al. 2022). Relevant to this study, the severity and mortality rates of ESD are higher in ovigerous females because, during brooding, they are unable to remove the infected carapace by molting (Castro et al. 2006, Hoenig et al. 2017, Groner et al. 2018). In the Gulf of Maine and Georges Bank, maintaining the spawning stock biomass (SSB) is a management priority to protect the long-term sustainability of the American lobster fishery in response to climate change. For example, a change in the minimum legal harvest size was recently approved by the Atlantic States Marine Fisheries Commission as a measure to protect the SSB (Atlantic States Marine Fisheries Commission 2023). Although modifications to fecundity, hatching success, and embryo and larval conditions could affect recruitment dynamics and subsequent management of the lobster fishery, the relationship between brood care and reproductive success is not well understood for American lobsters. Describing patterns of brood grooming in this species could shed light on both the reproductive activities of American lobsters and the lesser-understood impacts of climate change with implications for successful management of the fishery.

The aim of this study was to understand whether brood-grooming behavior by crustaceans may be sensitive to ocean warming and acidification. Specifically, the objectives were to characterize brood-grooming behavior in American lobsters at different points during embryogenesis and to evaluate how brood grooming and reproductive success vary in conditions associated with future ocean warming and acidification. We hypothesized that American lobsters would engage in brood-grooming behavior, and that these behaviors would increase in duration during embryogenesis. Additionally, we hypothesized that brood-grooming behavior would vary with temperature and carbonate chemistry conditions. Finally, we hypothesized that reproductive success, determined based on egg mortality, would vary with temperature and carbonate chemistry.

2. MATERIALS AND METHODS

2.1. Experimental design

2.1.1. Collection and acclimation of animals

Ovigerous American lobsters were collected off the coasts of Maine and Massachusetts from Management Zone E (Boothbay area) and Cape Cod Bay, respectively. Collections were made during October

and November of 2020 by personnel from the Maine Department of Marine Resources (Special Licenses #2019-11-02, #2020-37-01) and the Massachusetts Division of Marine Fisheries on commercial fishing vessels as a part of their sea-sampling programs. Lobsters were shipped on ice packs and kelp overnight to the Virginia Institute of Marine Science. The animals were acclimated for at least 8 wk at $9.73 \pm 2.90^\circ\text{C}$ and 30.40 ± 1.14 psu (mean \pm SD) in an aerated 550 l recirculating seawater aquarium. Seawater was prepared with Crystal Sea Marine Mix Salt and deionized water. Dissolved oxygen and pH were maintained at 8.81 ± 0.78 mg l⁻¹ and 7.70 ± 0.18 pH units (National Bureau of Standards [NBS] scale), respectively, during acclimation and monitored daily using a YSI handheld meter. During acclimation, lobsters were fed every 2 wk with thawed frozen fish and squid. Lobsters were banded with cable ties at the start of the acclimation period, and bands were maintained throughout the experimental period.

2.1.2. Treatment preparation

Following the acclimation period, lobsters were transferred to 100 l aquaria (2 individuals per aquarium, 24 individuals total) and randomly assigned to 1 of 4 long-term treatments; each tank held 1 lobster sourced from Maine and 1 lobster sourced from Massachusetts. The experiment was devised as a full factorial design, with combinations of 2 temperature and 2 pH levels. Treatment levels were chosen to represent ecologically relevant conditions that lobsters may experience during embryogenesis, as well as anticipated future conditions. Specifically, pH treatments were selected to reflect the IPCC RCP8.5 scenario, which projects decreases in pH by up to approximately 0.4 pH units by 2100 (Ciais et al. 2013, Gledhill et al. 2015). Average pH values were 7.94 in the high ('control') pH treatments and 7.52 in the low pH treatments (see Table 1 for full treatment conditions). Our temperature treatments were selected as an approximate end-of-century ocean warming scenario based on recent rates of warming determined for the Gulf of Maine (Brickman et al. 2021). An offset of 4°C separated the low ('control') temperature from the elevated temperature treatments to approximate end-of-century warming for the Gulf of Maine, based on recent rates of warming for the region (Brickman et al. 2021). Technical limitations in the experimental aquaria prevented a perfect replication of present-day temperature in the Gulf of Maine, particularly wintertime temperature minimums. Instead, the actual

Table 1. Water conditions for each treatment group during the 5 mo experimental period (mean \pm SE). Temperature and total alkalinity were directly measured. pH_{total} was calculated from continuous electrode measurements. $p\text{CO}_2$ and saturation state of calcite (Ω_{calcite}) were calculated. Temperature reflects the range of temperature values experienced across the entire 5 mo duration of the experiment. For carbonate chemistry parameters, values reflect the mean \pm SE of conditions experienced during the entirety of the experiment

Temperature treatment	pH treatment	Temperature ($^{\circ}\text{C}$)	pH_{total}	Total alkalinity ($\mu\text{mol kg}^{-1}$)	$p\text{CO}_2$ (μatm)	Ω_{calcite}	Dissolved oxygen (mg l^{-1})	Salinity (ppt)
Control	Control	10.51 \pm 0.07–14.49 \pm 0.22	7.97 \pm 0.01	1861 \pm 9	411 \pm 6	1.46 \pm 0.02	9.37 \pm 0.03	32.75 \pm 0.03
Elevated	Control	14.64 \pm 0.05–18.06 \pm 0.09	7.91 \pm 0.01	1865 \pm 10	497 \pm 11	1.53 \pm 0.02	8.21 \pm 0.04	33.11 \pm 0.03
Control	Low	10.98 \pm 0.06–12.65 \pm 0.07	7.54 \pm 0.01	1830 \pm 8	1188 \pm 16	0.60 \pm 0.01	9.05 \pm 0.39	32.40 \pm 0.02
Elevated	Low	13.91 \pm 0.04–16.83 \pm 0.16	7.50 \pm 0.01	1847 \pm 8	1307 \pm 14	0.62 \pm 0.01	7.62 \pm 0.05	33.02 \pm 0.04

temperature in the aquaria varied monthly to approximately reflect seasonal variation and encompass water temperature experienced during embryogenesis (Gulf of Maine Research Institute Ocean Data Products Team 2024), with average temperatures ranging from 10.36 $^{\circ}\text{C}$ (February) to 14.01 $^{\circ}\text{C}$ (May) in the low temperature treatments and 14.02 $^{\circ}\text{C}$ (February) to 17.31 $^{\circ}\text{C}$ (May) in the elevated temperature treatments (Table 1). Dissolved oxygen was maintained above 80% saturation, with the assumption that brood-grooming behavior would not vary significantly above that level (McLeese 1964). Three replicate tanks were prepared at each treatment combination of pH and temperature, such that 6 lobsters (3 sourced from Maine, 3 sourced from Massachusetts) were held under each treatment combination.

For preparation and maintenance of the long-term treatments, the salinity of filtered water was adjusted before filling the 100 l experimental aquaria where temperature and pH treatment conditions were maintained. First, water pumped from the York River in Gloucester Point, Virginia, was filtered to 20 μm and temporarily stored and aerated in a 2000 l mixing tank. Deionized water and a brine solution prepared with Crystal Sea Marine Mix Salt (80 psu) were mixed with the filtered water to achieve a final salinity of \sim 32 psu. The salinity-adjusted water was further filtered to 1 μm as experimental aquaria were filled. Tetra Bio-Bag filter cartridges were seeded with API Quick Start and added to the aquaria 1 wk prior to the addition of animals to establish a biofilm of nitrifying bacteria. The filter cartridges were removed shortly before animals were added to the aquaria.

Temperature and pH levels were established and measured in each aquarium independently. Glass electrodes (GF Signet) in each aquarium measured temperature and pH on a per minute basis to document treatment conditions and inform the automated control system regulating temperature and pH. The glass electrodes were calibrated weekly

using pH 7 and pH 4 NBS buffers. Each aquarium was fitted with a water jacket connected to a recirculating water bath. Hot and cold heat exchangers in the water bath were used to dynamically adjust bath temperature to achieve aquarium temperature setpoints. Dosing of either CO_2 from compressed cylinders or CO_2 -free air prepared with a CAS4 CO_2 adsorber (Altec AIR) into the water in each aquarium was dynamically adjusted to meet the set target levels based on aquarium pH as measured by the glass electrodes. Nitrate, nitrite, and ammonia concentrations were monitored weekly using an aquarium water test kit (API Saltwater Master Test Kit).

The 2 ovigerous lobsters in each experimental aquarium were individually isolated in 25 l plastic containers (\sim 24.1 \times 44.6 \times 24.0 cm) topped with 7 mm mesh. The individuals were physically, but not visually or chemically, isolated. Each plastic container held a \sim 5 cm (2 in) layer of aquarium substrate (Super Naturals). Once transferred to the experimental aquaria, animals were acclimated in their containers at control temperature and pH levels (\sim 12 $^{\circ}\text{C}$, \sim 7.9 pH) for 4 d. Temperature and pH levels were gradually adjusted at a rate of 0.5 $^{\circ}\text{C}$ and 0.03 pH units per hour until the target treatment levels were reached. Ovigerous lobsters were fed thawed frozen squid once per week, and 75% water changes were conducted weekly on the day after feeding. Animals remained in the experimental aquaria under treatment conditions for 5 mo, or until their entire brood hatched.

2.1.3. Carbonate chemistry analysis

To characterize the carbonate chemistry of the experimental treatments, weekly assessments of pH and total alkalinity in each experimental aquarium were conducted following best practices for ocean acidification research (Dickson et al. 2007). To validate aquarium electrode measurements, pH_{total} was mea-

sured independently from water samples using spectrophotometry and m-Cresol indicator dye (Aldrich, Cat. #211761, Batch #MKBR3556V) (Dickson et al. 2007). Measured pH values were compared with electrode measurements to calculate a weekly offset value between the continuous electrode measurements and weekly water samples. Each calculated offset was applied to the respective week of continuous electrode measurements across the time-series for that experimental aquarium. Total alkalinity (TA) was measured from water samples collected from each aquarium using open-cell potentiometric titration (Dickson et al. 2007). Titration accuracy was determined using certified reference material (A. Dickson Laboratory; 19.19 ± 7.37 mmol kg deviation from reference material; mean \pm SD). Carbonate chemistry parameters (i.e. $p\text{CO}_2$, saturation state of calcite) were then calculated for each aquarium from measurements of temperature, salinity, pH_{total} from adjusted electrode measurements, and TA using the 'seacarb' package in R (Gattuso et al. 2021) with constants K_1 and K_2 from Lueker et al. (2000) and K_f from Perez & Fraga (1987).

2.2. Lobster behavior

2.2.1. Brood-grooming behavior

To assess brood-grooming behavior, ovigerous lobsters were recorded using either a GoPro Hero 5 or Olympus Tough 3 digital camera once monthly during January, February, and March 2021. The opaque aquaria prevented the observation of brood-grooming behavior in real time. Videos were recorded for approximately 30 min during the day when minimal disturbance in the aquarium room was expected. During preliminary trials, brood-grooming behavior was observed for the vast majority of lobsters within a 30 min period when observed during the day. Previous work indicates that the diurnal nature of maternal care is, in part, species specific (Ruiz-Tagle et al. 2002, Baeza et al. 2019) and has not been evaluated in American lobsters. Nighttime brood-grooming behavior is certainly possible for American lobsters, but it was not examined in this experiment. Video cameras were placed in the aquaria outside of and adjacent to the plastic container housing an individual lobster. Videos were blinded by assigning a random number to each video and trimming identifying information from the first few seconds of each video. Each blinded video was scored by 2 people who were not aware of treatment group or lobster identification. Time stamps for the initiation and cessation of each behavior type and condition were

recorded during the portion of each video where the abdomen and tail of the animal were visible from the side. The duration of the behaviors and conditions as well as the percentage of time spent engaging in each behavior and condition were calculated. There was no relationship between the number of seconds an animal spent visible to the camera and the proportion of time that animal engaged in one of the defined behaviors (Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m744p083_supp.pdf). Videos where the animal's side view was not visible to the camera were excluded from statistical analysis.

Two behaviors were identified as related to maternal care based on literature for other decapod crustaceans and preliminary observation of the study animals: pleopod fanning and pereopod probing (Baeza & Fernández 2002, Baeza et al. 2016, 2019). Because both behaviors occurred only during periods when an animal's tail was extended, 2 additional conditions were also categorized: tail extended and tail curled. Definitions for maternal care-related behaviors are as follows:

- Pleopod fanning: Lobsters extended their pleopods backwards and then forwards in a continuous rhythmic motion, shaking, or jostling the egg mass. The fanning motion varied in speed, so that the pleopods were extended either slowly or rapidly depending on the fanning event. Rapid fanning typically occurred in short periods of several seconds, whereas slower fanning occurred over a range of time from a few seconds to several minutes.
- Pereopod probing: Lobsters used 1 of their 5 pereopods, or walking legs, to poke or probe at the embryo mass. This behavior occurred before, after, or between pleopod fanning events.
- Tail extended: The tail was extended so that the pleopods and egg mass were visible.
- Tail curled: The tail was curled so that the pleopods and egg mass were obscured and not visible.

2.2.2. Embryo development

To determine whether brood-grooming behavior varied over embryogenesis, eggs from each brood were photographed to assess embryo development over the experimental period. On a monthly basis, 18–30 eggs were removed from the center of each brood using forceps and scissors. Three eggs from each brood were examined using an Olympus SZ stereomicroscope. The presence of a heartbeat was observed to confirm vitality of the embryo. Eggs were later photographed on their side with the eye spot fac-

ing up using a Leica microscope camera with a 4.5× zoom. The total area of the egg, total area of the yolk, largest diameter, and smallest diameter of the eye spot were measured using ImageJ software (Schneider et al. 2012). A scale bar, also photographed with a 4.5× zoom using the Leica microscope camera, was used as a reference to convert pixel number to length in μm . The Perkins eye index (PEI) was calculated as the average of the largest and smallest diameter of the eye spot (Perkins 1972). PEI is a metric of embryo development in American lobsters, which ranges from 70–570 μm , where 570 μm represents the approximate eye spot size at which hatching occurs (Perkins 1972).

2.3. Reproductive success

2.3.1. Female condition and fecundity

Female condition was assessed at the conclusion of the acclimation period to assess differences among treatments at the start of the experiment. The presence of missing limbs, damage to the carapace, v-notching, and shell disease was assessed for each animal. The carapace length (CL) of each lobster was measured to the nearest 1 mm with Vernier calipers, and the live body weight of each lobster was measured to the nearest 0.01 lb (~45.4 g) with a digital hanging scale and converted to kg. The egg mass of each lobster was photographed using an Olympus digital camera. Each month during the experiment, female lobsters were again evaluated for the presence of shell disease or injury, the egg mass was photographed alongside a ruler, and the depth of the brood at each pleopod was measured to the nearest 0.01 mm using calipers. If shell disease was visible, the disease intensity was characterized as 'high' (>30% of the carapace shows symptoms), 'moderate' (>10% of carapace shows symptoms), or 'low' (<10% of carapace shows symptoms) according to the shell disease index (New York Sea Grant & Connecticut Sea Grant 2000, Landers 2005, Smolowitz et al. 2005). Damage to the fifth walking legs was recorded so that videos of those individuals could be excluded from analysis of probing behavior. Photographs and depth measurements were used to estimate the volume of each egg mass following Currie et al. (2010). The volume of the entire egg mass was calculated using the volume of a cylinder halved, where height was the average depth of the brood measured from each pleopod and length was the length of the egg mass. Egg loss at each time point was determined based on the change in egg mass volume:

$$\text{Egg Loss} = \frac{\text{Egg Mass Volume}_f - \text{Egg Mass Volume}_i}{n} \quad (1)$$

where n = days between measurements, f = final, i = initial and

$$\text{Egg Mass Volume} = \frac{1}{2}\pi r^2 h \quad (2)$$

where r = depth of the egg mass and h = height of the egg mass.

2.3.2. Hatching success

As embryo development progressed, experimental aquaria were monitored daily for the presence of hatching larvae. Once hatching started, newly hatched larvae were collected using hand nets and enumerated daily until hatching ceased. Water pump covers were checked daily for the presence of larvae, which were also removed and counted. Hatching success was calculated as the total number of larvae collected from each adult over the hatching period divided by the estimated egg mass volume at the last recorded time point (see Section 2.3.1). In instances where there was overlap in the hatching period of 2 ovigerous lobsters sharing an experimental aquarium, hatching success was not determined, as larvae could not be assigned to a particular adult.

2.4. Statistical analysis

All statistical analyses were conducted with the statistical program R (R version 4.3.2, RStudio version 2023.09.1-494). One-way ANOVAs were used to determine whether female or brood condition varied with assigned treatment groups at the start the experimental period. In these comparisons, initial CL, weight, fecundity, and PEI were response variables. The assumptions of normality and homogeneity of residuals were tested using Shapiro-Wilk and Levene tests, respectively. Data that did not meet the assumption of normality were square root or log₁₀ transformed.

To evaluate the relationship between brood-grooming behavior, embryo development, and our treatment conditions, generalized linear mixed models (GLMMs) with a binomial distribution were employed. In these comparisons, dependent variables were the proportions of time spent pleopod fanning, pereopod probing, or with the tail extended. Videos were excluded from statistical analysis if they contained lobsters whose brood had hatched or contained a lobster that did not spend time with her side visible to the camera

(Table S1). Across the entire length of the experiment, 49 videos of lobster behavior met the criteria to be included in analysis: 20 videos in January, 17 videos in February, and 12 videos in March. Data from only those females with both of their fifth walking legs intact were included in the analysis of the proportion of time spent pereopod probing because the effects of treatment could not be separated from the effects of appendage damage on time spent probing. Five females had 1 or more of their fifth walking legs damaged, so videos from those 5 individuals were removed from analysis of probing behavior. A subset of 42 videos met the criteria for probing behavior. PEI, temperature, and pH were fixed effects used to evaluate the effects of embryo development and treatment on brood-grooming behavior. Female identification number, sampling time point, tank identification number, source population, and video identity were included as random effects in the full model. Source population was not independent from the date of capture and length of acclimation in this experiment and did not appear to explain variation in the response variables in this study (Fig. S2). Further, there is no substantial evidence indicating that lobsters sourced from the Boothbay Harbor and Cape Cod areas are distinct populations. For that reason, source population was selected as a blocking factor, rather than a fixed factor. Akaike's information criterion (AIC) model selection was used to identify the best fit model (Akaike 1973; Table S2). The selected model for all 3 behavioral metrics included only video identity as a random effect. The interaction between temperature and pH was not significant, and therefore was not included in the selected models. Models that did not include temperature and pH were excluded from model selection to retain our ability to test the hypothesis that behavior would vary with treatment condition. An additional analysis of probing behavior was conducted to examine the relationship between experimental treatments, embryo development, and whether a lobster would engage in probing behavior. A GLMM was employed with the presence of probing behavior as a binary response variable. The selected model for this metric included source population and measurement time point as random effects, and interactions were included among fixed effects.

To understand drivers of reproductive success in our experiment, a Pearson's correlation coefficient test was used to evaluate whether egg mass volume and CL were correlated. The assumptions of normality and homogeneity of residuals were tested using Shapiro-Wilk and Levene tests, respectively. Egg mass volume data were square root transformed to meet the assumption of normality. To test the hypothesis that egg

loss would vary with experimental treatment, a GLMM with a binomial distribution was used. The proportion of the initial egg mass remaining at the last measured time point was evaluated as the dependent variable. Temperature and pH treatment were included as fixed effects, with female identification number, tank identification number, source population, and CL treated as random effects. AIC model selection was used to identify the best fit model, which included female identification number as a random effect, and temperature and pH, but not their interaction, as fixed effects. Models that did not include temperature and pH were excluded from model selection to retain our ability to test the hypothesis that egg loss would vary with treatment condition. To better understand the relationship between lobsters' ability to probe the egg mass and reproductive success, unpaired 2-sample *t*-tests were used to evaluate whether egg mass volume or egg loss differed significantly among lobsters with and without missing fifth walking legs. Data were square root transformed to meet the assumptions of normality.

Hatching success could not be statistically evaluated in this experiment. Not all broods hatched by the end of the experiment, and of broods that did hatch, overlapping hatching between broods in a single tank and prolonged hatching periods of several weeks made enumerating the total number of larvae from a single brood difficult in some instances.

3. RESULTS

3.1. Experimental conditions

Four unique combinations of 2 temperature and 2 pH levels were successfully sustained in the experimental aquaria over the 5 mo exposure period. No overlap in temperature or pH was observed within 1 SD between our elevated and control temperature treatments or low and control pH treatments, respectively (Table 1). During the exposure period, average salinity and dissolved oxygen concentration were maintained at 32.82 psu and 8.56 mg l⁻¹ (Table 1).

3.2. Lobster behavior

3.2.1. Brood-grooming behavior

Over the measurement periods, lobsters spent 34 ± 26% of the time with their tails extended, 18 ± 21% of the time fanning their broods, and 14 ± 17% of the time probing the brood with the pereopods (mean ± SD).

Individual lobsters varied substantially in their time dedicated to brood-grooming behaviors (Fig. S3). Probing behavior was only observed for the fifth walking legs. In all videos that met the criteria for analysis, pleopod fanning and tail extension were observed, regardless of measurement time point. All lobsters in the analyses spent time pleopod fanning and with their tails extended at each of the measured time points. Of the 24 lobsters in this experiment, 20 were observed probing the brood with their pereopods during at least 1 of the measured time points. Of these lobsters, 50% were observed pereopod probing during 2 or more of the measured time points. Inter-rater variability for all behavioral metrics averaged 12.6%.

The proportion of time a female spent with her tail extended varied significantly with embryo developmental stage (as PEI), but not with temperature or pH (Table 2). In all treatments, lobsters significantly increased the proportion of time spent with their tails extended as PEI increased (Fig. 1). The proportion of time a female spent fanning her brood varied significantly with PEI, but not temperature or pH (Table 2). Females brooding eggs with a greater PEI value spent significantly more time fanning their broods than females brooding eggs with a smaller PEI value (Fig. 2). The proportion of time that a female spent probing the brood did not significantly vary with any of the measured fixed effects (Table 2, Fig. 3). Time spent probing was correlated with female fecundity (i.e. egg mass volume; Fig. S4), but the relationship was driven by a high proportion of videos in which no probing was observed. With zeros removed, the correlation was no longer significant (Table S3). The narrow range of fecundity and low incidence of probing in this experiment did not allow us to evaluate this relationship statistically. The presence of probing in a video was not significantly related to temperature, pH, PEI, or their interactions (Table S4).

3.2.2. Embryo development and PEI

PEI, a proxy for embryo development, varied among lobsters at the start of the

experimental observation period, ranging from 144 ± 9 to $405 \pm 24 \mu\text{m}$ (mean \pm SD), but was not significantly different among treatments at the start of the experiment (Table S5). Over the course of the experiment, PEI increased from $381 \pm 16 \mu\text{m}$ in January to $389 \pm 16 \mu\text{m}$ in March across all broods and was similar among treatments, with overlap in SD at each time point (Table 3).

3.3. Reproductive success

3.3.1. Female condition and fecundity

The experimental lobsters ranged in CL from 79.1 to 90.7 mm, with an average CL of 83.56 mm (Table 4). CL

Table 2. Generalized linear mixed models with associated estimates and standard errors, comparing brood-grooming behavior and egg loss for American lobsters held at different temperature and pH levels. Brood-grooming behavior was examined using 3 response variables: the proportion of time that a lobster spent with its tail extended, fanning the egg mass, and probing the egg mass. For all behavior response variables, a random identifier associated with each video was included as a random effect, and estimates are in log odds. For egg loss, the proportion of the initial brood that was remaining at the point of hatching was the response variable. Parameters with a statistically significant effect ($p < 0.05$) on the response variable are shown in **bold**. PEI: Perkins eye index

Proportion of time spent extended				
Time spent extended ~ Temperature + pH + PEI + (1 Random ID)				
	Estimate	SE	z	Pr(> z)
Model	-0.672	0.332	-2.026	0.043
Temperature	-0.618	0.386	-1.602	0.109
pH	0.211	0.380	0.556	0.579
PEI	0.494	0.195	2.531	0.011
Proportion of time spent fanning				
Time spent fanning ~ Temperature + pH + PEI + (1 Random ID)				
	Estimate	SE	z	Pr(> z)
Model	-1.586	0.283	-5.600	<0.001
Temperature	-0.485	0.330	-1.472	0.141
pH	-0.050	0.325	-0.155	0.876
PEI	0.683	0.167	4.096	<0.001
Proportion of time spent probing				
Time spent probing ~ Temperature + pH + PEI + (1 Random ID)				
	Estimate	SE	z	Pr(> z)
Model	-4.478	1.119	-4.00	<0.001
Temperature	0.611	1.251	0.488	0.625
pH	0.274	1.223	0.224	0.823
PEI	-0.287	0.634	-0.453	0.651
Proportion of egg mass volume remaining at hatch				
Remaining egg mass volume ~ Temperature + pH + Temperature \times pH + (1 Lobster ID) + (1 Carapace length)				
	Estimate	SE	z	Pr(> z)
Model	-1.2563	0.3128	-4.016	<0.001
Temperature	-0.4040	0.4763	-0.848	0.396
pH	0.1673	0.4957	0.337	0.736
Temperature \times pH	0.0873	0.6774	0.129	0.898

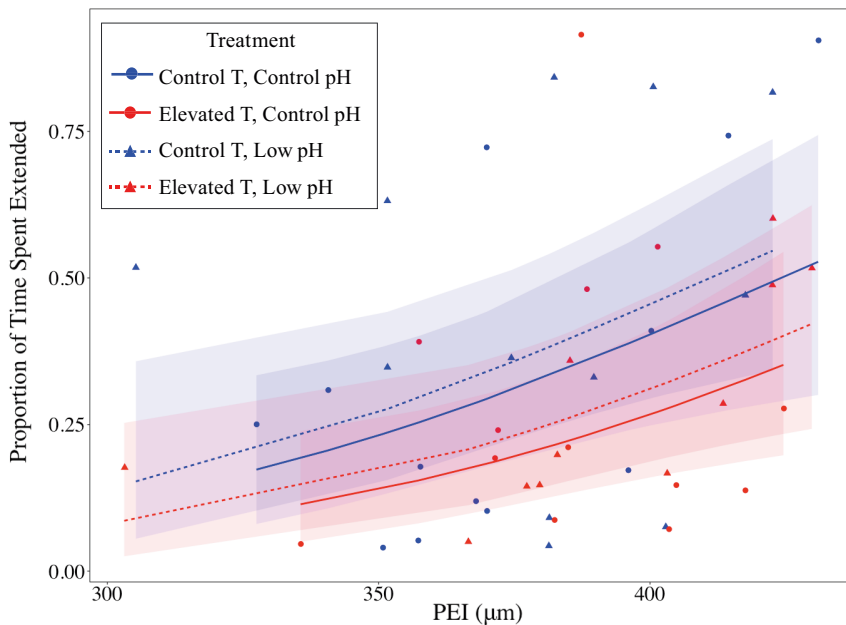


Fig. 1. Proportion of time that an American lobster spent with its tail extended as a function of treatment and Perkins eye index (PEI). Points represent raw data, solid and dashed lines represent model predictions, and shaded regions represent 95% confidence intervals. Color corresponds to temperature (T). Shape and line type represent pH. See Table 1 for treatment conditions

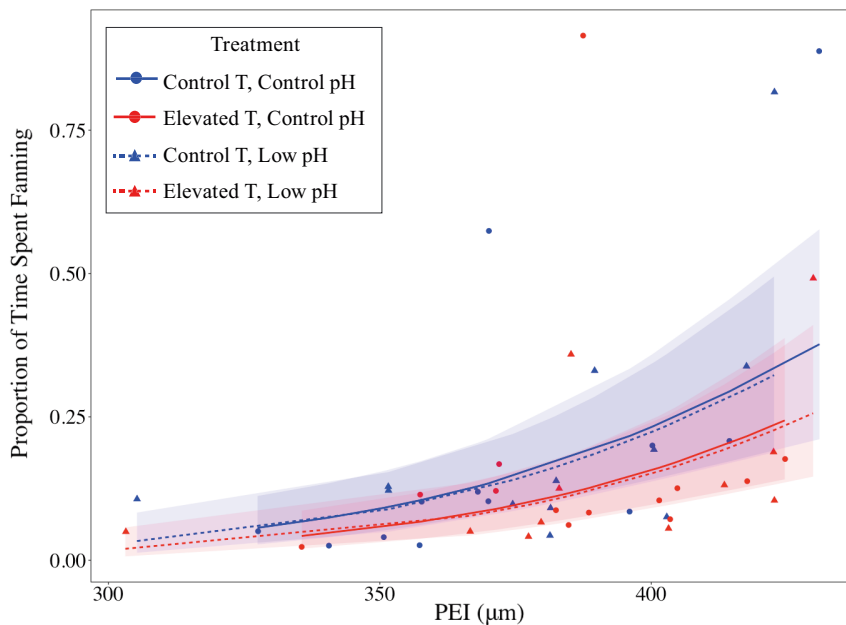


Fig. 2. Proportion of time that an American lobster spent engaged in fanning behavior as a function of treatment and Perkins eye index (PEI). Other details as in Fig. 1

was not significantly different among treatment groups at the start of the experiment (ANOVA, $p = 0.325$; Table S5). At the start of the experiment, no individuals were observed with ESD. However, 7 lobsters were

visibly infected by the end of the 5 mo experiment. Of the infected individuals, 2 were identified as having a 'high' or 'moderate' amount of shell disease (>30 and >10% of carapace shows symptoms, respectively), and 5 were identified as having a 'low' amount of shell disease (<10% of carapace shows symptoms) according to the shell disease index (New York Sea Grant & Connecticut Sea Grant 2000, Landers 2005, Smolowitz et al. 2005). Six of the 7 lobsters displaying symptoms of ESD had been sourced from Massachusetts waters. Additionally, 5 individuals were identified as having a damaged or missing fifth pereopod. Individuals with irregular fifth pereopods were not included in the analysis of pereopod probing behavior.

Egg mass volume, a proxy for fecundity, ranged from 1344 to 21 896 cm^3 , with an average of $6257 \pm 4648 \text{ cm}^3$ (mean \pm SD). Fecundity was not significantly correlated with CL (Pearson correlation coefficient = 0.38, $p = 0.077$; Table S3). Egg mortality was high over the course of the experiment, with 3.2–61.2% of the initial brood remaining at the end of the experiment. Egg loss was defined as the proportion of a brood's egg mass volume at the start of the experiment that was remaining at the initiation of hatching or the end of the experiment, whichever occurred first. Egg loss was high over the time course of the experiment, but it was not significantly different among treatment groups (Table 2, Fig. 4). Neither initial egg mass volume nor egg loss varied significantly with damage to the fifth walking legs (Table S6).

3.3.2. Hatching success

Hatching success, defined as the proportion of the egg mass volume present in the brood at the last measurement point before hatching began that successfully hatched into larvae, was evaluated for 13 of the 24 female lobsters. Hatching success ranged from 0.06 to 2.18 larvae

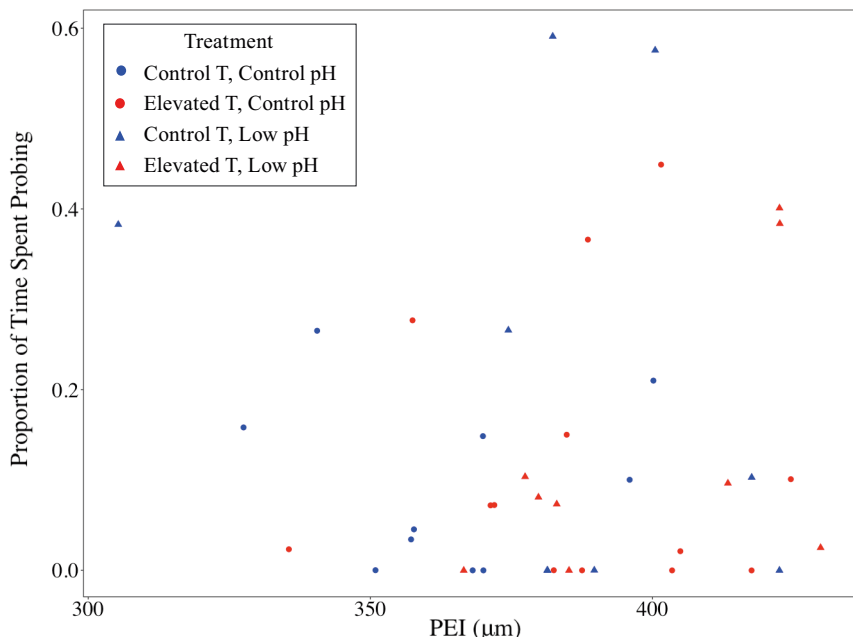


Fig. 3. Proportion of time that an American lobster spent engaged in probing behavior as a function of treatment and Perkins eye index (PEI). Points represent raw data. Color corresponds to temperature (T). Shape corresponds to pH. No significant relationships were observed between time probing, PEI, and pH/temperature. See Table 1 for treatment conditions

Table 3. Summary statistics for egg broods of American lobsters, averaged by treatment combination and measurement time point. All values are mean ± SD. PEI: Perkins eye index

Temperature treatment	pH treatment	Brood PEI (μm)	Egg mass volume (cm ³)	No. of lobsters
Time Point 1 – January 2021				
Control	Control	370 ± 42	5301 ± 3505	6
Elevated	Control	384 ± 16	5301 ± 4215	4
Elevated	Low	382 ± 49	7881 ± 7225	5
Control	Low	380 ± 45	5321 ± 3303	5
Time Point 2 – February 2021				
Control	Control	409 ± 65	1294 ± 708	5
Elevated	Control	382 ± 27	1570 ± 723	5
Control	Low	386 ± 29	1261 ± 637	4
Elevated	Low	389 ± 30	1707 ± 1275	3
Time Point 3 – March 2021				
Control	Control	369 ± 1	1423 ± 343	2
Elevated	Control	397 ± 31	1018 ± 509	4
Control	Low	372 ± 18	1075 ± 343	3
Elevated	Low	404 ± 19	1159 ± 631	3

hatched per cm³ of egg mass volume among broods. On average, 0.84 ± 0.67 larvae hatched per cm³ of egg mass volume (mean ± SD). In the remaining lobsters, hatching success could not be evaluated due to overlap in hatching within a single tank, lack of hatching within the experimental period, or a prolonged hatch-

ing duration that exceeded the experimental period.

4. DISCUSSION

Maternal care is a critical component of reproduction in brooding crustaceans, and measurements of brood-grooming behavior can provide insight into reproductive dynamics and success under future ocean change. In this study, brood-grooming behavior was documented at different points in embryo development, demonstrating that ovigerous American lobsters significantly increased the proportion of time that they dedicated to grooming and fanning their broods later in embryogenesis. Contrary to the stated hypotheses, discernable effects of elevated temperature or reduced pH conditions were not observed on either brood-grooming behavior or egg loss. These results point to the potential resilience of maternal care to future ocean warming and acidification in the American lobster.

4.1. Lobsters engage in brood-grooming behavior over the course of embryogenesis

In many brooding crustaceans, grooming of the egg mass underlies successful embryo development and survival through hatching. This study presents the first empirical characterization of brood-grooming behavior in the American lobster, both at multiple points in embryo development and in response to abiotic environmental parameters. Lobsters in this study were expected to participate in brood-grooming behavior, given anecdotal reports of brood grooming in this species (Herrick 1895, Talbot et al. 1984, Aiken et al. 1985). Indeed, ovigerous lobsters were observed fanning the egg mass with the pleopods, probing the egg mass with the pereopods, and alternating between extending and curling their tails, presumably to aerate their clutch (Figs. 1–3).

Table 4. Summary statistics for female American lobsters, averaged across treatments. Carapace length was measured only at the start of the experiment, as molting does not occur during embryo development. Weight was measured at the start of the experiment. All values are reported as mean \pm SD

Temperature treatment	pH treatment	Carapace length (mm)	Initial weight (kg)	Initial egg mass volume (cm ³)
Control	Control	85.0 \pm 5.5	0.53 \pm 0.10	5266 \pm 2666
Elevated	Control	84.2 \pm 3.7	0.48 \pm 0.05	5364 \pm 3680
Control	Low	82.5 \pm 3.5	0.48 \pm 0.05	5710 \pm 3992
Elevated	Low	83.8 \pm 4.0	0.50 \pm 0.06	8504 \pm 7217

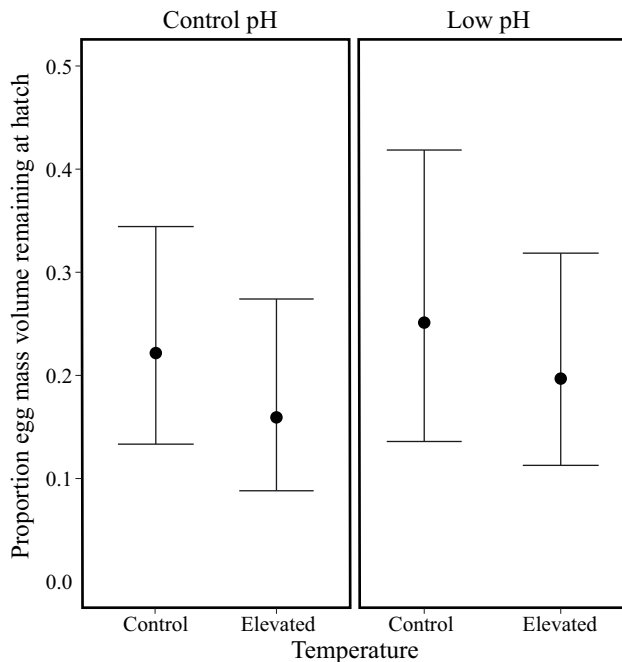


Fig. 4. Proportion of the initial American lobster egg mass volume (proxy for total number of eggs) that was remaining at the point of hatching or the end of the experimental period, whichever came first. Points represent model predictions, and error bars represent 95% confidence intervals

As in other crustacean species, the observed increase in pleopod fanning behavior with embryo development in American lobsters is likely linked to oxygen availability in the egg mass (Fernández et al. 2002). Here, the proportion of time that lobsters spent with their tails extended and fanning the egg mass increased significantly with advancing embryo development (Figs. 1 & 2). These results are in line with substantial evidence that ovigerous crustaceans increase ventilation when brooding late-stage embryos (i.e. Baeza & Fernández 2002, Fernández et al. 2002, Brante et al. 2003, Reinsel et al. 2014). Ventilation of the egg mass relieves oxygen limitation by enhancing

oxygen delivery in the presence of these late-stage embryos (Fernández et al. 2002) and ultimately facilitates more synchronized embryo development (Bauer 1979, Dick et al. 1998).

Most, but not all, of the lobsters in this study engaged in pereopod probing, another behavior considered important to embryo survival and development (Förster & Baeza 2001, Silva et al. 2003). The function of probing behavior in embryogenesis appears to be independent of oxygen availability in

American lobsters, as the proportion of time that a lobster spent probing the brood did not vary significantly with embryo development (Fig. 3). Pereopods are considered key appendages for grooming in decapod crustaceans and are rich in chemosensory receptors (Bauer 1981, 2020), but the low specificity in removing foreign objects from an egg mass via probing may limit our ability to interpret pereopod probing behavior (Förster & Baeza 2001). In American lobsters, for example, some ovigerous females remove damaged eggs with the pereopods to limit infestation by the nemertean egg predator *Pseudocarcinonemertes homari* (Aiken et al. 1985). However, not all females participate in the behavior, and of those that do, some groom to the point of fully stripping the egg mass (Aiken et al. 1985). Our ability to interpret the relationship between pereopod probing and either environmental condition or embryo development stage may be limited due to a relatively smaller sample size. This study was limited by the ability to obtain ovigerous females without damage to one or more walking legs, and lobsters with missing or damaged pereopods were removed from our analysis of probing behavior to avoid possible confounding effects. Missing appendages are not uncommon in wild-caught American lobsters, and differences in brood-grooming behavior among American lobster females with and without missing appendages remains to be characterized (Pickering & Quijon 2010). In the lithodid crab *Lithodes maja*, setae removal on the fifth pereopod led to an increase in general fouling of the gills and, in one ovigerous female tested, some embryo mortality (Pohle 1989). Higher embryo mortality was also observed in the anomuran crab *Petrolisthes violaceus*, following ablation of the fifth pereopods (Förster & Baeza 2001). Future work should investigate how pereopod amputation or damage impacts probing behavior and embryo mortality. In this study, initial fecundity or egg loss in lobsters with damage to the fifth pereopods were not significantly different than in lob-

sters with intact fifth pereopods. Here, results demonstrate that brood-grooming behavior in the American lobster resembles other decapod crustaceans, with similar patterns throughout embryogenesis, underscoring the long-standing role of the American lobster as a model for animal behavior, particularly with respect to chemosensory mediated behavior (Atema & Steinbach 2007, Derby & Weissburg 2014).

4.2. Warming and acidification did not mediate brood-grooming behavior

Identifying the underlying environmental drivers of brood-grooming behavior in American lobsters can shed light on the extent to which future ocean warming may impair reproductive success. Contrary to the stated hypotheses, an increase in the amount of time that lobsters in this experiment spent fanning their brood was not observed in the elevated temperature treatments. In other crustacean species, the frequency of and total time spent fanning are responsive to environmental parameters, including elevated temperature (Dick et al. 1998, Brante et al. 2003) and low oxygen concentration (Gallardo et al. 2019). In the natural environment, oxygen concentration and temperature are negatively correlated. Our observation that fanning behavior was consistent across temperatures may point to oxygen availability, rather than temperature itself, as the driver of fanning behavior in American lobster. In laboratory environments, brooding crustaceans respond to low environmental pO_2 conditions by increasing fanning of the egg mass, regardless of embryo developmental stage (Fernández et al. 2002). Oxygen demand may not have been a limiting factor in our treatments, either due to high oxygen availability in general or relatively low oxygen demand by the broods, regardless of experimental temperature. Experimental tanks were manipulated to maintain high oxygen concentrations independent of temperature (Table 1) to isolate the effects of temperature and pH on our response variables. If dissolved oxygen concentration alone drives changes in fanning behavior, consistently high oxygen levels among treatments could explain why pleopod fanning did not vary significantly with temperature in our experiment. Alternatively, the threshold of oxygen concentration over which lobsters change grooming behavior may be lower than the difference produced by oxygen consumption rates of embryos across the experimental temperature range. Although measurements of respiration are not currently available for American lobster

embryos, embryos of the related Norway lobster maintain stable oxygen consumption rates between 14 and 18°C (Styf et al. 2013). Measurements of oxygen demand and availability within American lobster broods would further a mechanistic understanding of the stability of brood-grooming behavior with rising temperature.

Ocean acidification has the capacity to alter the behavior of marine organisms, including those behaviors related to foraging, feeding, and predator avoidance, through changes to metabolic activity and chemosensory disruption (reviewed by Ashur et al. 2017, Draper & Weissburg 2019). No significant change was observed in any behavior response variables under low pH conditions, alone or in combination with elevated temperature. Brooding crustaceans dynamically adjust fanning behavior in response to chemical cues produced by developing embryos (Tankersley et al. 2002), which increase in concentration over development (Forward et al. 1987, Ziegler & Forward 2007, Darnell & Rittschof 2010). As a chemosensory-mediated process, brood-grooming behavior may be sensitive to pH. Indeed, fanning (Roggatz et al. 2016) and general body grooming (Tierney & Atema 1986) significantly decreased under low pH conditions in the shore crab *Carcinus maenas* and freshwater crayfish *Orconectes virilis*, respectively. Additionally, ocean acidification conditions have been shown to alter the oxygen consumption rates of some crustacean embryos (Carter et al. 2013), which should drive changes in oxygen availability and therefore fanning behavior (Fernández et al. 2002). As such, brood-grooming behavior was expected to vary with pH, due to either changes to overall metabolic activity or chemical cue regulation in the form of altered cue structure or receptor binding (Tierney & Atema 1986, Roggatz et al. 2016). These results highlight the importance of species- and context-specific investigations of behavior under ocean acidification conditions. For example, natural concentrations of the cue(s) that induces fanning behavior may be produced in sufficient quantities to overcome pH-mediated changes in bioavailability. Alternatively, the acid dissociation constant of the grooming cue used by American lobsters may be low enough to be unaffected by the 0.5 pH reduction used in this study, as was the case for a mud crab *Panopeus herbstii* predator cue (Draper & Weissburg 2022). Although it is not possible to distinguish between aspects of cue reception and oxygen availability in driving pleopod fanning in this study, brood-grooming behavior by American lobsters appears resilient to reduction in pH of up to 0.5 units from representative present-day levels.

Under stressful environmental conditions, the metabolic costs of grooming may induce a trade-off between brood ventilatory needs and maintaining physiological homeostasis in ovigerous females. For example, the conditions of ocean warming and acidification used in this study induce stress in American lobsters, as evidenced by significant reductions in Arrhenius break point temperatures (Harrington et al. 2020) and significantly greater rates of oxygen consumption (Klymasz-Swartz et al. 2019) in adult and sub-adult lobsters. It is possible that changes in oxygen availability and demand in the brood were responsive to treatments in this study, but that the costs to increasing ventilation were too great. Such trade-offs have been explored in response to other environmental stressors, such as the presence of predator cues, which cause a reduction in brood-grooming behavior by female amphipods (*Crangonyx pseudogracilis*) (Arundell et al. 2014). Although female oxygen consumption rates were not measured in this study, such data would provide insight into the metabolic costs and physiological stress induced by our treatments. Outside the laboratory environment, some ovigerous females also must contend with the energetically taxing processes of migration to offshore regions to facilitate optimal conditions for larval hatching (Campbell & Pezzack 1986, Goldstein & Watson 2015), and molting shortly after their brood hatches (Hughes & Matthiessen 1962, Penkoff & Thurberg 1982, Nelson et al. 1988). The tradeoffs between brood care and other behavioral and physiological processes may be size-specific. In this study, ovigerous lobsters were near the minimum harvest size for individuals of their respective regions (82.55 mm CL). As both size at maturity and minimum size for commercial harvest shift, understanding the relationship between female size and reproductive investment and success will be critical for management of the species under future climate change conditions. In this study, it appears that females prioritized brood-grooming behavior, even under conditions of environmental stress, which resulted in consistent brood-grooming behavior across temperature and pH treatments. However, the metabolic costs associated with molting, migrating, and co-occurring environmental stressors may limit that capacity in the natural environment.

4.3. Reproductive success was resilient to warming and acidification

Future success of American lobster populations depends on the successful development and hatching of

embryos in conditions of ocean warming and acidification, and their survival and recruitment into the population. In this study, egg loss and hatching success were used as proxies for reproductive success. Results indicate that egg loss in this species may be resilient to warming and acidification, although the importance of a more thorough examination of embryo health under both natural and laboratory conditions should be noted. Temperature and pH did not significantly change the proportion of eggs surviving to hatching. Overall, rates of egg loss were high, with an average of 23% of egg mass volume remaining following the 5 mo experiment. Low levels of egg retention are common in laboratory settings (30–100% egg loss), particularly at high temperatures (Talbot et al. 1984, Waddy & Aiken 1995), and egg loss can be highly variable in the environment (Goldstein et al. 2022). There is also anecdotal evidence to suggest that elevated temperature, particularly in wintertime months, can contribute to reduced egg retention (Waddy & Aiken 1995). High rates of egg loss in laboratory environments may indicate some degree of stress, for either females or embryos themselves. With regards to temperature, low fecundity in American lobsters at the southern edge of their range has been associated with high temperatures, but the relative importance of co-varying stressors has not been determined (Waddy & Aiken 1995). Impacts of ocean warming and acidification on reproductive success are not consistently observed in crustacean species (Styf et al. 2013, Long et al. 2023), but the embryo stage may be sensitive to acidification in some species. For example, hatching success is significantly reduced at low pH in the red king crab *Paralithodes camtschaticus* and the Tanner crab *Chionoecetes bairdi* (Long et al. 2013, Swiney et al. 2016). Hatching occurred earlier in this study than what is typically observed in the natural environment, likely due to the temperature conditions maintained in the laboratory (Hughes & Matthiessen 1962, Aiken & Waddy 1986). While we did not statistically evaluate hatching success in this experiment, values were highly variable and appeared similar among our treatment groups (Figs. S5 & S6).

The relationship between reproductive success and ESD is important for the maintenance of the spawning stock biomass, and warming and acidification are expected to increase disease prevalence. In this study, the emergence of ESD was observed in almost 30% of the lobsters, despite their having no symptoms of ESD at the start of the experiment. The rate of ESD progression increases significantly between 12 and 18°C (Barris et al. 2018), which encompasses much

of the temperature range used in this experiment. Although the size of individual lesions was not routinely measured in this study, no clear relationship between treatment and the emergence of ESD symptoms was apparent; individuals with ESD were reported in all treatment groups. The relationship between ESD and grooming, including brood grooming, has not been documented. However, ESD lesions commonly emerge or are most severe in areas of the carapace that are difficult to groom, suggesting that general body grooming may reduce the emergence or severity of ESD (Cobb & Castro 2006). Because ESD symptoms were not observed until late in embryogenesis, it is unlikely that the presence of ESD affected brood-grooming behavior and differences in egg quality among lobsters with and without ESD. ESD may compromise reproductive success by spurring ovigerous lobsters to molt before their brood hatches, resulting in full brood mortality (Stevens 2009); however, none of the females in this study molted before their brood hatched. For infected ovigerous females that do not molt, ESD does not impact potential or realized fecundity (Goldstein et al. 2022), which aligns with results in this study that initial fecundity and egg loss did not appear to differ between females with and without ESD (Fig. S7). ESD prevalence increases from north to south with increasing water temperatures in the Gulf of Maine, likely due to the combination of higher temperatures supporting increased growth rates of ESD bacteria and increased temperatures leading to decreased size at maturity and subsequently longer intermolt periods for smaller, now mature lobsters (Glenn & Pugh 2006). Six of the 7 individuals with symptoms of ESD by the end of the experiment were sourced from Massachusetts. Although this study was not intended to examine the relationship between temperature, pH, and ESD in ovigerous lobsters, the results indicate that females may sustain fecundity, hatching, and brood grooming during moderate ESD infection under conditions of ocean warming and acidification. The long-term fate of females with ESD after hatching is uncertain and could have important consequences for reproductive dynamics.

4.4. Future directions and impacts

By employing ecologically relevant treatment conditions, potential effects of projected future temperature and pH conditions on brood-grooming behavior were isolated in a laboratory environment. However, the experimental temperatures may limit a broad

interpretation of these results, due to difficulty in reproducing present-day winter minima conditions experienced by brooding lobsters in the Gulf of Maine. Below 10°C, embryo development enters a period of stasis, or diapause, with little to no growth (Helluy & Beltz 1991). In this experiment, control temperature treatments were akin to spring and summertime conditions in the Gulf of Maine. These laboratory conditions may have accelerated embryo development and supported hatching of larvae between February and May (Figs. S5 & S6). Future work examining reproductive behavior in American lobsters should characterize how the frequency and duration of brood-grooming behavior vary over development during winter-time conditions. Evidence suggests that populations experiencing different temperature regimes vary in their investment in maternal care (Brante et al. 2003). Although brood-grooming behavior was stable over the range of temperature and pH explored in this study, behavior is often a first line of defense when facing environmental stress (Schreck et al. 1997).

By demonstrating that American lobsters engage in brood-grooming behavior throughout embryogenesis, these results add to the complex tapestry of potential drivers and indicators of reproductive success in this commercially important crustacean. Our findings suggest that brood-grooming behavior and egg loss in American lobsters are resilient to ecologically relevant conditions of elevated temperature and reduced pH. This positive forecast for American lobsters stands in contrast to observations in other crustacean species, highlighting the need for species-specific investigations of behavior and reproduction under climate change conditions. As warming and acidification continue across the range of American lobsters, a mechanistic understanding of how temperature, pH, and other co-occurring environmental factors influence all aspects of reproduction will be essential for predicting and managing the long-term success of the American lobster fishery.

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