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LIFE HISTORY TRAITS CONFERRING LARVAL RESISTANCE AGAINST OCEAN ACIDIFICATION: THE CASE OF BROODING OYSTERS OF THE GENUS *OSTREA*

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ABSTRACT As oceans and many estuaries become more acidic, identifying adaptable or nonadaptable species (''winners'' or ''losers'') will enable better predictions of community and ecosystem function alterations due to climate change. Marine bivalves are frequently subjects of ocean acidification (OA) research because of their perceived vulnerability, which also threatens loss of their valuable ecosystem services. Studies indicate that larvae of many broadcast spawning oyster and mussel species are physiologically sensitive to alterations in carbonate chemistry. Running counter to this trend are recent investigations of brooding oyster species (genus *Ostrea*) that suggest their offspring may be considerably more resistant to OA stress. Although the precise mechanism conferring OA resistance to *Ostrea* larvae is unknown, a strong candidate appears to be exaptation of traits developing embryos that require to cope with adverse carbonate conditions they typically encounter in the brood chamber. New and previously reported data on *Ostrea* brood chamber conditions are discussed in the context of OA. Novel technical and experimental approaches are offered to address current knowledge gaps in future studies.

KEY WORDS: *Ostrea,* brooding, ocean acidification, life history

INTRODUCTION

Identifying ''winners and losers'' in the face of ocean acidification (OA) is important to predict how community composition and ecosystem function will respond to climate change (Fabricius et al. 2011, Inoue et al. 2013, Kroeker et al. 2013, Busch & McElhany 2016). To date, most OA bivalve studies have focused on broadcast spawning species, likely because this happens to be the predominant reproductive strategy among marine bivalves and includes many commercially valuable species. For example, broadcast spawners represent 62% of all oyster species (WoRMS Editorial Board 2018) and include *Crassostrea* spp., which dominate most commercial markets globally. Brooders have received much less attention. Nevertheless, several studies have demonstrated that the embryos, veligers, and larvae (hereinafter collectively referred to as larvae) of brooding oyster species (genus: *Ostrea*) (Chaparro et al. 2009b, Cole et al. 2016, Waldbusser et al. 2016) may be temporarily resistant to acidic (low pH, reduced Ωar, and high CO_2) conditions and may represent ''winners'' (negligibly or positively impacted). In addition, the larvae of other non-oyster brooding species, including *Calyptraeid* limpet species (Noisette et al. 2014, Maboloc & Chan 2017) and *Sepia* cephalopod species (Gutowska & Melzner 2009), also appear to be relatively more resistant to OA stress than those of broadcast spawning species, which typically display high sensitivity to acidic conditions (Lucey et al. 2015) and may be ''losers'' in the future.

Among brooding species, one hypothesized driver of larval resistance to OA is the occurrence of fluctuating environmental conditions in the brood chamber. The conditions in the marine invertebrate brood chamber may rapidly acidify after mothers isolate their pallial fluid from the surrounding environment in response to acute drops in salinity or other environmental fluctuations, resulting in the accumulation of CO_2 and other acidic byproducts of metabolism (Chaparro et al. 2009a, 2009b, Montory et al. 2009). Consequently, larvae may have evolved traits to cope with acidic conditions found in the brood chamber that can be exapted for developing under OA stress (Waldbusser et al. 2016). Another school of thought is that direct maternal protection from adverse water conditions helps brooded larvae cope with acidified environments (Lucey et al. 2015). Whether the driver of OA resistance is provided by exapted larval traits or by maternal protection, these mechanisms reflect the classic nature versus nurture debate about the modes animals use to cope with environmental stress, a subject discussed elsewhere within the OA literature (e.g., Applebaum et al. 2014). As ecophysiology of brooding has received relatively little attention, more studies are needed to elucidate the role brooding or parental care in conferring larval resistance against OA.

To begin filling this gap, several exploratory physiological studies were conducted on brooding *Ostrea edulis* adults and their larvae. Specifically, pH and O_2 from the brood chamber of early brooding (1–3 days postfertilization) *O. edulis* were measured. In addition, following methods of Waldbusser et al. (2016), a developmental assay of larvae extracted from the brood chamber and reared under static conditions was conducted to explore the physiological role of brooding for this species. Methodological details are reported with the intention that this material and diagrammatical descriptions of relevant *Ostrea* anatomy (Fig. 1) can aid future discussion and exploration of the biogeochemistry of marine invertebrate brood chambers. Using a synthetic approach, these data were discussed along with previous studies on other *Ostrea* species and fundamental knowledge gaps surrounding the ecophysiology of brooding within the context of OA were

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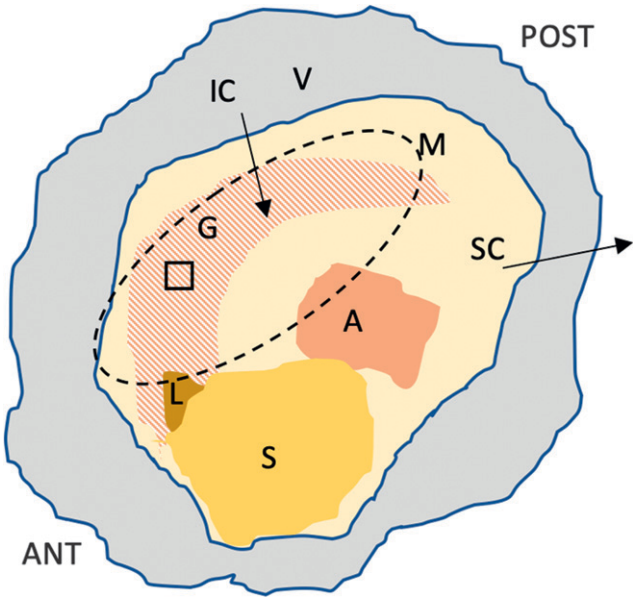

Figure 1. Diagrammatic view of *Ostrea edulis* anatomy and study details: anterior region (ANT), posterior region (POST), adductor muscle (A), infrabranchial cavity (IC) with inhalant flow direction (arrow), gill (G), labial palps (L), mantle tissue (M), stomach and gonad (S), suprabranchial cavity (SC) with exhalent flow direction (arrow), left valve (V), brood chamber (outlined with dashed line), and probe location within brood chamber above gills (open square).

Figure 2. A brooding *Ostrea edulis* adult from studies. A white mass of extruded eggs can be seen to the right of the hinge against the black floor of the conditioning tank.

highlighted. Addressing these gaps is important to characterize the adaptive potential of brooders to OA and will greatly improve general understanding of brooding ecophysiology.

MATERIALS AND METHODS

Adult *Ostrea edulis* ($n = 30$) were obtained from the Damariscotta River Estuary (DRE), ME, in June of 2016 and 2017. Oysters were placed in a conditioning tank under flow through conditions at the Darling Marine Science Center hatchery. Seawater flowing to conditioning tanks was unfiltered, untreated, and largely reflected ambient conditions found in DRE (upper DRE 2015 to 2018, pH_{total} mean 7.90, total range: 7.02–8.40; http://maine.loboviz.com/). For brood chamber investigations, a small hole (6.5 mm) was drilled in the right valve above the brood chamber (Fig. 1) and a small piece of Tygon tubing (length: 15 mm, OD: 6.46, ID: 3.71) was inserted into the shell. The tube was held in place with a small amount of super glue (Loctite, Westlake, OH) on the outer part of the shell followed by a thin layer of polymer clay (Sculpey, Polyform Products Company, Elk Grove Village, IL) around the entire tube to prevent water exchange to and from the chamber. Clay covering the tube opening was pierced with a syringe needle at the initiation of the monitoring trial to permit passage of the microelectrode.

Brooding oysters were identified by the extrusion of some fertilized eggs from the brood (Fig. 2), a phenomenon previously observed (e.g., Orton 1933) but not characterized in detail in the literature. Presently, it is unknown why these eggs are extruded or if they develop normally after their release. Once identified as brooding, the brood chamber of these animals was examined for either O_2 or pH over time. The brood chambers were monitored for 3 to more than 48 h depending on experimental objectives. One brooder was sacrificed to obtain the full brood of embryos and reared in 300 mL of 0.2 µm filtered seawater in biological oxygen demand bottles that were sampled daily following the methods described by Waldbusser et al. (2016).

At the initiation of the brood chamber monitoring trials, oysters were transferred to a temperature controlled room set at 18°C and placed into an aquarium filled with 1 µm filtered seawater (salinity = 30 ± 1). A small air stone was used to maintain oxygen saturation and circulate water within the aquarium. A pH microelectrode (500 µm, Unisense A/S, Denmark) or an oxygen microelectrode (500 µm, Unisense A/S) mounted to a micromanipulator was used to measure the ambient water conditions before being lowered through the tube to the brood chamber. The microelectrode was withdrawn periodically to determine drift in ambient conditions over time. Valve gape was measured and related to brood chamber conditions of a single brooding female by pairing time lapse photography (1 photo min^{-1}; Hero 3 GoPro, San Mateo, CA) with pH sensor data. Valve gape among images was analyzed and determined using ImageJ v 1.51.

Data Analysis

To examine whether brood chamber pH of brooding females was significantly reduced relative to ambient pH, Student's *t*-tests were performed for each of the experiments ($n = 3$). To test for a significant positive association between brood chamber pH and ambient pH among experiments, measurements of *Ostrea edulis* from this study ($n = 3$) and those reported in the

literature for *Ostrea angasi* ($n = 1$, Cole et al. 2016) and *Ostrea chilensis* ($n = 1$, Chaparro et al. 2009b) were pooled for a one-sided Kendall tau rank correlation test. The nonparametric Kendall robust line-fit method (Sokal & Rohlf 1995) was used to estimate a linear slope among these ($n = 5$) data points. The Kendall test was selected because the small sample size ($n = 5$) was deemed too small to effectively test whether the assumptions of linear regression were met. Furthermore, this nonparametric method is more robust than linear regression against the influence of outliers and leverage.

The temporal association and likely cause–effect relationship between valve gape and brood chamber pH of a single *Ostrea edulis* mother ($n = 1$) monitored at 1 min intervals for more than 1 h were examined by time lag analysis. Because both time series were nonstationary (i.e., the averages shifted over time), which reduces the utility of auto- and cross-correlation function analysis, the raw data were transformed to become stationary by taking their numerical derivatives. For the time delay with peak cross-correlation, the strength and statistical significance of this association were estimated by a nonparametric Kendall tau rank correlation test. To avoid overestimating this relationship because of repeated, temporally auto-correlated measurements, untransformed gape and pH were first binned into fully statistically independent time-bins and then averaged. Time-bin size was determined by identifying the smallest time lag with no significant temporal autocorrelation ($P > 0.05$) in either transformed time series.

Fluctuations in oxygen concentration in the brood chamber of a single brooding *Ostrea edulis* female ($n = 1$) measured for over 57 h were analyzed by decomposing the time series into smoothed low- and high-frequency components. The low-frequency component was operationally defined as the 3-h running median value, and the high-frequency component as the 15-min running median of the residual. Intervals with a sustained high-frequency component of 1 µmol/L or greater were used to identify exchange events between the brood chamber and the ambient water.

Developmental rates of oyster larvae extracted from brooding chambers were compared with values cited in the literature through multiple linear regression analysis. Assumptions of linear regression were validated before analysis. Linearity was inspected visually with plots. Normality and heteroscedasticity were checked by inspection of data and Shapiro–Wilk test and Levene's test, respectively.

RESULTS

Chamber Responses to Ambient Conditions

The pH within the brood chamber of brooding *Ostrea edulis* open and ventilating females was consistently lower than that of ambient seawater. Individual *t*-tests found that the mean brood chamber pH for each animal examined was significantly lower (P value < 0.001) than the ambient level (difference range = 0.26–0.30 units; mean difference = 0.29, SD = 0.03 units). Furthermore, pH of the brood chamber was strongly positively correlated with ambient seawater pH (Fig. 3, $n = 3$, $r^2 = 0.99$). To better test the statistical significance of this correlation, previously published data from the literature were included in an expanded analysis. Cole et al. (2016) previously determined that the brood chamber pH of *Ostrea angasi* was significantly lower than the overlying water pH. Chaparro et al. (2009b) reported that the mean pH of the *Ostrea chilensis* brood chamber was also lower than ambient seawater pH, although the authors did not find this to be statistically significant. For the pooled data set (Fig. 3), brood chamber and ambient pH were strongly positively correlated ($r^2 = 0.90$) and significantly positively rank-correlated (Kendall tau = 0.80, one-sided $P < 0.05$). The linear slope, as estimated nonparametrically by the median slope among all possible pairs of data points (the Kendall robust line fit method, Sokal & Rohlf 1995), was 1.05, which is essentially identical to unity (Fig. 3). The mean offset of the line regressed with all available data ($n = 5$) was 0.252 units below ambient pH with 95% bootstrapped confidence interval of 0.172–0.304 units.

The oxygen concentration in the brood chamber of a brooding *Ostrea edulis* female ($n = 1$) was monitored for over 58 h. The mean (±SD) oxygen concentration of the overlying water before and after the experiment was 239.1 ± 0.4 and 229.1 ± 0.5 µmol O_2 L^{-1}, respectively. Over the monitoring period, brood chamber oxygen concentration declined from around 200 µmol O_2 L^{-1} to around zero with numerous intermittent primarily positive spikes in brood chamber oxygen content, presumably from exchanges with the overlying water (Fig. 4). The mean concentration was 32.95 µmol O_2 L^{-1} (±45.97 µmol L^{-1} SD), but 50% of the measurements were below the median of 10.9 µmol O_2 L^{-1}. The number and frequency of distinct positive spikes in measured oxygen concentration was estimated by identifying unique time intervals when the smoothed high-frequency component of the signal exceeded 1 µmol O_2 L^{-1}. There were 60 such

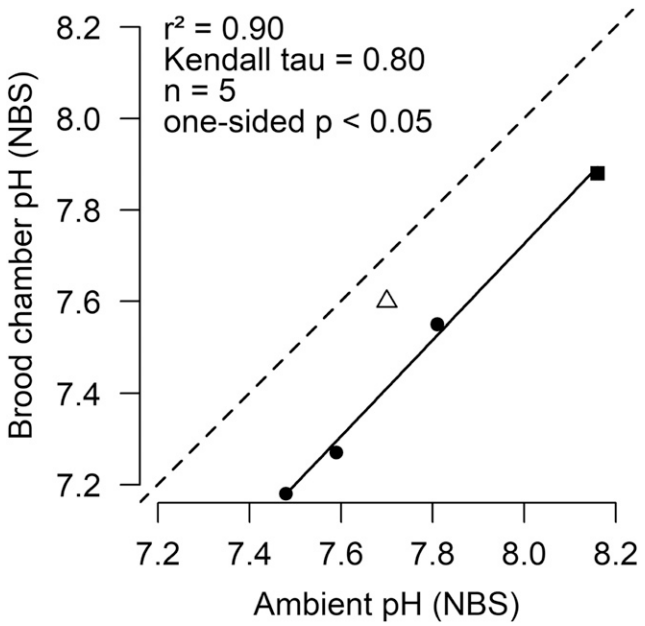

Figure 3. Comparison of ambient seawater pH and mean brood chamber pH of brooding *Ostrea edulis* (this study, solid circles), *Ostrea angasi* (Cole et al. 2016, solid square), and *Ostrea chilensis* (Chaparro et al. 2009b, empty triangle) adults. Solid symbols depict brood chamber measurements that were significantly lower in pH than ambient seawater conditions, whereas the empty symbol was similar in pH to overlaying water. The solid trend line (Kendall robust line-fit method) suggests an offset of approximately 0.25 pH units below ambient conditions (dashed 1:1 line).

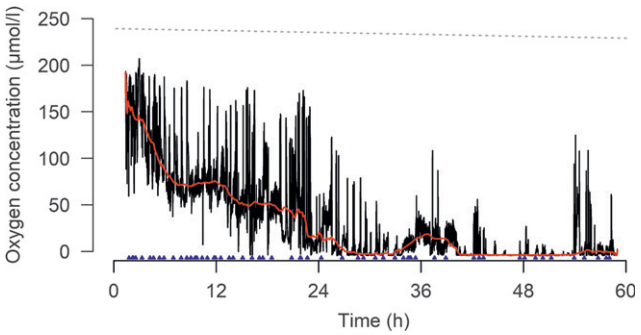

Figure 4. Oxygen content in the brood chamber of a single female brooding *Ostrea edulis* mother observed to be actively pumping over more than 2 days. Dashed gray line denotes oxygen content of the overlying water. Red line indicates 3-h running median value. Blue triangles indicate distinct spikes in oxygen concentration.

events in total (mean 1.04 events h^{-1}), between 0 and 5 occurring within a single hour.

Influence of Valve Position on Brood Chamber pH

Image analysis on another female revealed acute declines in brood chamber pH (0.015 pH units/min) following valve closures (Fig. 5). A more detailed time series analysis revealed a correlation between valve gape and brood chamber pH even when valves remained ajar and the brooding mother appeared to be ventilating (Fig. 6). This relationship was strongest with a delay of 2 min between changes in valve gape and changes in brood chamber pH. Repeated measurements of pH (but not valve gape) were estimated to be significantly auto-correlated up to a lag time of 1 min and, thus, not fully statistically independent ($P < 0.05$). Consequently, the data were first pooled into 2-min bins and then compared with 2-min lag time (Fig. 6). A positive correlation ($r^2 = 0.17$) and significantly positive rank correlation ($n = 39$, Kendall tau = 0.29, $P < 0.01$) were detected.

Development Assay of Extracted Ostrea edulis *Larvae*

Techniques developed in Waldbusser et al. (2016) using *Ostrea lurida* appeared applicable to *Ostrea edulis* as a brood ($n = 1$) could be extracted and reared outside of their maternal

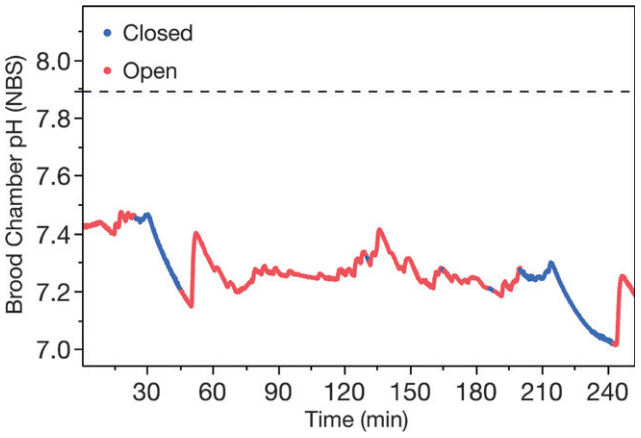

Figure 5. Influence of valve position (open/closed) on variation in brood chamber pH of a single ($n = 1$) brooding *Ostrea edulis* over time. The solid black line represents average pH of the overlying water.

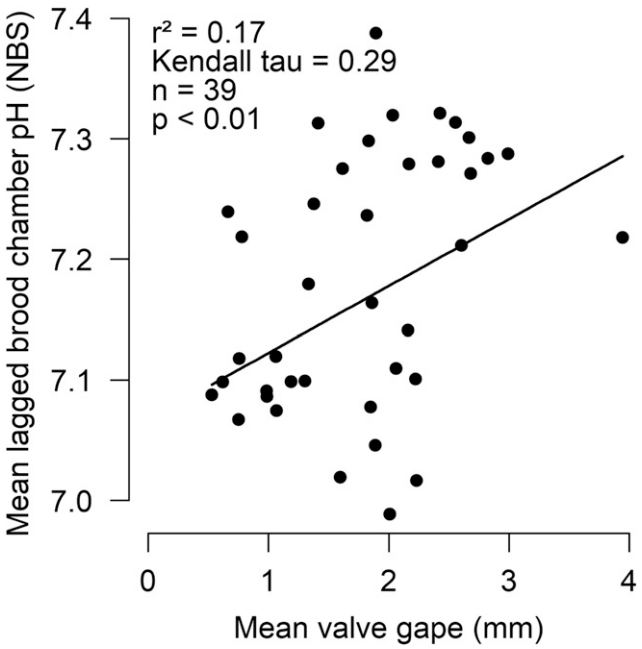

Figure 6. Brood chamber pH of a single ($n = 1$) brooding *Ostrea edulis* mother over valve gape. Data are 2-min averages of brood chamber pH (initially taken every second) and valve gape made through time lapse photography made at a similar interval and lagged by 2 min. The trend line (Kendall robust line-fit method) indicates a weak but significant rank correlation.

brood chamber past the prodissoconch II (PDII) stage. Furthermore, multiple linear regression analysis indicated that these larvae grew at a similar rate (larval source × time, $F_{3,34} = 54.127$, P value 0.95) as those reared in brooding chambers as reported in the literature for *O. edulis* (Fig. 7).

DISCUSSION

Results from this investigation and previous studies are discussed to address some basic questions around the environmental, maternal, and larval drivers of conditions found in the brood chamber of *Ostrea* spp. In addition, these data are discussed in the context of how brood chamber conditions may have led to the development of either larval traits (nature) or maternal behaviors (nurture) that confer resistance to larvae against the negative impacts of OA during their development.

How Does Overlying Water (Nature) Influence the Conditions within the Brood Chamber?

If brooders protect their young from adverse environmental conditions (e.g., low seawater pH), it is important to determine if the brood chamber is isolated or exposed to these conditions while mothers are open and actively pumping seawater. Data collected in this study indicated the pH of the *Ostrea edulis* brood chamber was significantly correlated with ambient conditions (Fig. 3). It appeared, that within the range of pH values tested in this study (7.81–7.48), the brood chamber was passive and apparently did not represent chemical refuge under relatively low pH (i.e., 7.48).

The passivity of the brood chamber to ambient seawater must be recognized as some *Ostrea* spp. are native to upwelling regions

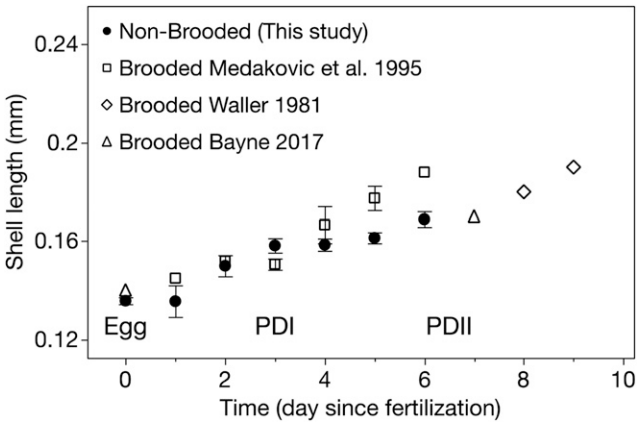

Figure 7. Development of extracted (nonbrooded; this study) and brooded *Ostrea edulis* embryos over time from previous studies.

that deliver CO_2-rich (Barton et al. 2012) and oxygen-depleted seawater into estuaries (Roegner et al. 2011) throughout spring–summer when *Ostrea* spawn (e.g., *Ostrea lurida*) (Hopkins 1936). Previous studies have suggested species that are native and adapted to naturally hypercapnic environments are also likely to be affected by OA conditions (Hall-Spencer et al. 2008, Cigliano et al. 2010, Lucey et al. 2015). Arguably, some of the most compelling evidence of OA resistance among larval marine invertebrates was among *O. lurida* larvae held in OA-simulated water (Waldbusser et al. 2016). Although authors attributed much of this resistance to slow developmental rates of larvae and maternal effects on brood chamber pH, the infiltration of corrosive water into the brood chamber cannot be ruled out as contributing to this species remarkable OA resistance potential.

How Does Maternal Physiology and Behavior Influence Brood Chamber Conditions (Nature) and Are There Signs of Maternal Carbonate System Buffering (Nurture) in the Brood Chamber?

The observed average brood chamber pH among brooders that were open and ventilating was 0.29 units lower than ambient levels, presumably because of maternal respiration. The brood itself also likely contributed to pH reductions in the brood chamber, but previous studies have found their effect to be negligible at early larval development stages (see Chaparro et al. 2009a). When combined with the other brood chamber measurements of *Ostrea* spp. (i.e., Cole et al. 2016), a consistent pattern of 0.25 unit reduction in brood chamber pH relative to ambient levels was clear (Fig. 3). In contrast to this trend, Chaparro et al. (2009b) observed that the brood chamber pH of *Ostrea chilensis* was similar to the ambient seawater. This datum was included in the analysis and displayed in Figure 3 to illustrate that maternal respiration effects on brood chamber carbonate chemistry may be species specific.

As the work presented here represents an exploratory investigation of common brood chamber conditions during brooding, more studies are needed to confirm findings and to determine how brood chamber conditions vary within and among species. Until that time, these findings suggest some promising areas for further study. For example, reduced pH within the brood chamber suggests mixing of respired water and incoming seawater, which defies the conventional thought that flow of water from infrabranchial area, behind the gill lamellae, to the suprabranchial area is unidirectional (Fig. 1) (Yonge 1926, Nelson 1960, Newell & Langdon 1996, Bayne 2017).

Larval exposure to pH conditions consistently lower than ambient conditions in the brood chamber is meaningful within the context of OA as this chronic exposure may have promoted developmental traits that prepare the young for future ocean conditions. For example, when brooding mothers are held in present day, upper DRE surface seawater (average pH = 7.90), data presented here suggest the pH in the brood chamber (estimated pH = 7.65) would exceed the "business-as-usual" (i.e., no action taken) scenario expected for 2100 (7.85; IPCC Fifth Assessment Report 2014). Future work should more closely examine brood chamber–ambient condition connectivity across a greater dynamic range of conditions than those used in this study. In addition, to better understand the limits of exapted developmental traits, more measurements are necessary to fully constrain the carbonate chemistry (e.g., dissolved inorganic carbon or total alkalinity) within the brood chamber for accurate descriptions of mineral thermodynamics in the chamber.

It is important to note that only one carbonate system parameter was measured in this study, but previous investigations on the pallial and calcifying fluids of bivalves illustrate the importance of constraining the full carbonate chemistry for estimating mineral thermodynamics. Crenshaw (1972) observed the extrapallial fluid of several bivalve species to be approximately greater than 0.5 units below seawater (7.91), but he also observed DIC values 1.5–2.1 times higher than that of the overlying seawater. As a result, despite the low pH values (7.41), the extrapallial fluid remained supersaturated ($\Omega arg = 1.89$ for *Crassostrea virginica*; calculated using CO_2Sys v. 2.1) with respect to aragonite. Presumably, the DIC added to this fluid was derived from maternally respired CO_2. In contrast to these findings, other studies observed continuous increase in Ca^{2+} concentration in the pallial fluid of brooding and nonbrooding *Ostrea chilensis* after brood chamber isolation (e.g., ~0.22 mg L^{-1} after 12 h), indicating that the pallial fluid was undersaturated with respect to calcite, but also partially buffered after 5 μmol L^{-1} CO_3 was liberated (recalculated from Chaparro et al. 2009b) (or linked to Ca-carbonic anhydrase pumps). It should be noted that any buffering from maternal shell (calcite) sources would commence only after dissolution of larval shells (aragonite) had occurred. Despite conflicting accounts, maternal alterations in brood chamber carbonate chemistry is an intriguing new avenue worth pursuing and require detailed, multiparameter measurements. In general, maternal effects have received less attention within the OA community, but several studies have shown them to play an active and important role in the survival of the young under OA stress (Sunday et al. 2011, Parker et al. 2012, Griffith & Gobler 2017).

In addition to indirect effects of maternal respiration and shell dissolution, the mother may exert direct influence over brood chamber chemistry by controlling fluid exchange between the chamber and surrounding environment. In this study, oxygen content of the *Ostrea edulis* brood chamber was highly variable over 58 h of monitoring and reached near zero values when a brooding female fed/pumped infrequently (Fig. 4). Food was not provided to the brooding mother at any time, which may have reduced the animal's filtration rate (Riisgård & Larson 2014) and, subsequently, ventilation of the brood

chamber. The brood chamber was also highly responsive to acute changes in valve position. Indeed, the pH within the brood chamber was observed to drop precipitously (~0.015 pH units min^{-1}) after valve closure (Fig. 5), indicating quick response time of this fluid to maternal respiration when isolated from ambient conditions. In general, a weak ($R^2 = 0.17$) but significant relationship was observed between brood chamber pH and valve gape when mothers were open (Fig. 6). Chaparro et al. (2009a) also observed the pH within *Ostrea chilensis* to decline after the first hour of brood chamber isolation but at a much slower rate (~0.001 pH units min^{-1} or approximately 10 times slower than *O. edulis*). The drivers behind brood chamber response time differences between these species were not clear but could be addressed with side-by-side comparisons under controlled experimental conditions. Also, capturing more than one carbonate chemistry parameter during future examinations will also help determine drivers of pH variability within the brood chamber. The fact that the brood chamber was acidified quickly by mothers is another source of evolutionary pressure on larvae to develop and retain traits to develop in hypercapnic conditions. Exploration of relationships between environmentally mediated evolutionary larval resistance to brood chamber isolation/OA among brooding species would be worth investigating.

The brood itself, which consists of tens to hundreds of thousands of developing larvae (Hopkins 1936, Walne 1964, Chaparro et al. 2008), may also influence brood chamber conditions. Previous studies observed early developmental larval stages of *Ostrea* spp. to have little to no direct effect of the brood chamber pH, oxygen, or ammonium concentration (Chaparro et al. 2008, Chaparro et al. 2009a, 2009b). More recently, Segura et al. (2015) demonstrated that as larvae mature and their oxygen demand increases, late-stage larvae can greatly reduce the oxygen availability within the brood chamber, contributing to brood chamber acidification. Similar relationships between chamber oxygen depletion and development of brooded young have been observed among other marine invertebrate species (see review by Pechenick 1999). Future studies may want to examine how broods of various sizes contribute to brood chamber hypercapnia, developmental stress, and OA resistance among brooding species.

How Does Maternal Care of Larvae (Nurture) Influence Larval Exposure to Low pH Water?

Embryos of *Ostrea chilensis* are free living in the brood chamber, sometimes temporarily attached to mucus strings and transported along the gills until reaching the palps where they are cleaned of mucus and shunted posteriorly to the maternal countercurrent. It has been hypothesized that circulation of brooded larvae by means of the female countercurrent increases the access of larvae to oxygenated areas of the brood chamber (i.e., posterior, inhalant opening) (Chaparro et al. 1993, Mardones-Toledo et al. 2015). Indeed, older larvae may rapidly deplete the maternal palps of oxygen when mothers isolate their brood chamber (Segura et al. 2015); therefore, circulation and other maternal care behaviors may be increasingly important as larva develop. Although mothers do not increase feeding or pumping rate to compensate for the growing demands of broods as they develop (Chaparro & Thompson 1998), these and other parental behaviors (e.g., mucus coating and larval circulation) require further exploration to better understand maternal care under OA stress. Such studies seem timely given previously observed carryover effects among other marine brooders under salinity stress (Segura et al. 2014), but also the recent translife cycle and transgenerational studies illustrating adults exposed to OA conditions can confer negative (Dupont et al. 2012, Griffith & Gobler 2017) or positive carryover effects on larvae (Parker et al. 2012, Parker et al. 2015, Thor & Dupont 2015).

The length of time the brood spends within the brood chamber before their release (i.e., brooding period), pelagic period, and total larval life stage duration varies markedly for *Ostrea* spp. (Table 1). For example, *Ostrea puelchana* may only brood the young for 3–9 days (Castro & Le Pennec 1988);

TABLE 1.

Reproductive and developmental traits of *Crassostrea* and *Ostrea* oyster species.

Species	Reproductive strategy	Egg size (μm)	Larval developmental mode	Development to PDI (days)	Brooding period (days)	Pelagic period (days)	Source
Crassostrea virginica	Broadcast	45	Planktotrophic	<1	n/a	17	Carriker (1996) Langdon and Newell (1996)
Crassostrea gigas	Broadcast	52	Planktotrophic	<1	n/a	24.5	Strathmann (1987)
Ostrea puelchana	Brooding	110–130	Lecithotrophic?	?	3–9	?	Pascual and Zampatti (1995), Castaños et al. (2005), Castro and Le Pennec (1988)
Ostrea angasi	Brooding	?	Lecithotrophic?	?	15–22	14–21	O'Sullivan (1980), Dix (1976), Hickman and O'Meley (1988)
Ostrea edulis	Brooding	150	Lecithotrophic?	?	7–10	12–20	Walne (1964, 1965)
Ostrea lurida	Brooding	100–105	Opportunistic lecithotrophic	5–7	10–14	10	Buroker (1985), Gray (unpublished data)
Ostrea chilensis	Brooding	220–323	Opportunistic lecithotrophic	21–28	21–56	20–30	Buroker (1985), Toro and Chaparro (1990)
Ostrea equestris	Brooding	?	Lecithotrophic?	?	?	<0.01–2	Buroker (1985)

Question marks either indicate unknown information or hypothesized larval developmental mode.

Ostrea lurida, *Ostrea edulis*, and *Ostrea angasi* will brood for 1–2 wk (Hopkins 1936, Walne 1964. Dix 1976); and *Ostrea chilensis* will brood to for nearly the full larval developmental period of 8 wk (Toro & Chaparro 1990). Lucey et al. (2015) suggested marine species with longer brooding periods and greater parental care exhibit greater stress tolerance than congeners with similar life history strategies but invest less care in their young. The relationship between the length of maternal care and exaptation of traits to cope with hypercapnic stress in *Ostrea* may be complicated by the fact that the brood chamber may also contain increasingly adverse conditions as the young develop. Furthermore, it is not clear if brooding periods are affected by OA or increasing water temperatures. Brooding females may release late-stage larvae early when faced with the onset of stress (Segura et al. 2014). Releasing the young presumably relieves mothers of the physiological burden of brooding the young but dramatically increases the predation threat to the offspring. Could OA indirectly increase larval mortality through early release and longer exposure to predation pressure?

How Resistant Are Larvae (Nature) to OA Stress and What Is the Mechanism Underlying Their Resistance?

Previous OA experiments on *Ostrea* larvae have frequently used late-stage, umbonated larvae or larvae released from the brood chamber (Hettinger et al. 2012, Hettinger et al. 2013, Buckham 2015, Cole et al. 2016) that are well past the highly sensitive, unshelled developmental stages (e.g., embryos; Barton et al. 2012, Waldbusser et al. 2013, Barton et al. 2015). Experimentation on *Ostrea* larvae extracted and reared outside of the brood chamber represents a viable means to tease apart natural OA resistance from maternal benefits of brooding. Although early failures (e.g., Horst 1886) were followed by later successes (e.g., Hori 1933, Walne 1979, DiSalvo et al. 1983), Waldbusser et al. (2016) was the first study to rear *Ostrea lurida* embryos through PDII stage for experimental purposes. To explore the nonbrooded larval developmental rate of another *Ostrea* species, here *Ostrea edulis* larvae were extracted and reared from the blastula stage to PDII (Fig. 7). The developmental rate of extracted *O. edulis* larvae was similar to brooded larvae from previous studies under optimal laboratory conditions (Fig. 7).

Extracted *Ostrea lurida* embryos developed normally from the embryonic stage to PDII larvae in seawater undersaturated with respect to Ωar (Waldbusser et al. 2016). These results contrast with previous larval OA studies conducted on broadcast spawning species, all of which were highly sensitive to Ωar (Waldbusser et al. 2015a, 2015b). Waldbusser et al. (2016) suggested that the kinetics of calcification and the relatively slow time to first shell development of *Ostrea* compared with *Crassostrea* and *Mytilus* larvae (24 h versus 6 h, respectively) result in low susceptibility to low Ωar conditions for *Ostrea* spp. (see Waldbusser et al. 2013, 2016 for greater detail). In short, the relatively slow developmental time of *Ostrea* places a lower energetic demand on larvae as they deposit their initial shell, especially under OA conditions. Among other genera, slow-growing brooding corals have also been found to be more resistant to acidification stress than fast-growing broadcast spawning species (Comeau et al. 2014, Comeau et al. 2017). Although there are encouraging signs that *O. lurida* larval development may be more resistant to OA stress than some broadcast spawning species, others have observed OA to exert sublethal impacts on pelagic larval stages of *O. lurida* as well as carryover effects from these impacts into the juvenile life stage (Gaylord et al. 2011, Hettinger et al. 2012, Hettinger et al. 2013). Therefore, although larvae are highly sensitive to environmental perturbations, it is also important to look beyond this life stage so as not to neglect ecologically important impacts of OA. Long-term studies that expose brooded larvae and extracted larvae to simulated OA would greatly improve understanding on how truly resistant these species are to OA stress.

Other Traits of Brooders

Aside from developmental rates, *Ostrea* larvae may possess other traits that provide resistance to OA stress that broadcast spawning species lack. Specifically, the larger egg size and nonfeeding larval developmental mode (lecithotrophy) of brooders is markedly different from many broadcast spawners congeners (Table 1). Possible evolutionary drivers of these differences include environmental conditions (Thorson 1950, Mileikovsky 1971), constraints on adult morphology (Levin & Bridges 1995), and correlations between egg size and larval developmental mode (Jaeckle 1995). Larval developmental mode theory suggests broadcast-spawned larvae that hatch from smaller eggs with less energy (less yolk) must develop quickly and begin feeding earlier to fuel later development (Hart 1995); conversely, brooded larvae develop slowly, fueled exclusively or partially by the large egg yolks (Hart & Strathmann 1995). Positive correlations between egg volume and energy content has been established for numerous invertebrate species (Jaeckle 1995), which suggests the larger energy density of the eggs of brooders and other lecithotrophic species may reduce the energetic impact of OA on development. Although this precise mechanism may not be applicable to *Ostrea* larval resistance (see Waldbusser et al. 2016), Dupont et al. (2010) observed that the lecithotrophic sea star *Crossaster papposus* that hatch from large eggs (>0.8 mm) have growth rates 2× higher under OA conditions than under present day conditions. Similarly, the authors suggested their findings were supported from the successful development of brooded lecithotrophic larvae of the cephalopod *Sepia officinalis* under low pH conditions (Gutowska & Melzner 2009). Planktotrophic larvae may be able to compensate for energetic impacts of OA if food is abundant (Thomsen et al. 2013) and assuming their feeding rates (Gray et al. 2017), selectivity (Vargas et al. 2013), or digestive processes (Stumpp et al. 2013) are not already disrupted. Collectively, these studies suggest the greater vulnerability of planktotrophic broadcast-spawned larvae under OA because of a greater dependence on food conditions than lecithotrophic larvae typical of brooding species.

Exaptation versus Adaptation and Acclimation

From the outset, this study questioned whether brooding prepares the young to cope with the acidic conditions anticipated in the future. It is exceedingly difficult to answer this question conclusively. Several potential trait-based mechanisms capable of conferring fitness to brooded young under OA stress were presented. The present study focused on the exaptation of brooding traits, but perhaps did not pay enough attention to the evolutionary adaptation or physiological acclimation potential

of brooders as these are important subjects to be addressed when attempting to determine "winners and losers." Among some cultured populations, studies indicate oyster and other bivalves may quickly adapt to OA after only a few generations of selective breeding (Parker et al. 2011). Bivalve adaptation to climate change may be rapid for some natural populations, but it will depend on numerous complex factors, such as population genetic variance, gene flow/dispersal between populations, demographics, generation time, local environmental pressures, etc., all of which cannot be accounted for during laboratory studies and may only be estimated through complex modeling efforts (Harley et al. 2006, Hoffmann & Sgrò 2011). Acclimation, on the other hand, to high CO_2 conditions has already been observed in bivalves native to naturally hypercapnic sites (e.g., upwelling regions), indicating some populations may have the ability to adapt physiologically to long-term alterations in carbonate chemistry (Michaelidis et al. 2005, Thomsen et al. 2010), but acclimation may come at the expense of growth or other important functions (Thomsen & Melzner 2010). Generally, many coastal and estuarine systems possess dynamic conditions that include natural fluctuations in carbonate chemistry (Waldbusser & Salisbury 2014, Wallace et al. 2014). Marine bivalves are equipped with some molecular machinery (e.g., proton pumps/exchangers) and enzymes (e.g., carbonic anhydrase) necessary to combat CO_2/pH stress at the cellular level (Melzner et al. 2009). Much more information is needed on the energy requirements of bivalves and other calcifiers to operate these physiological tools to maintain homeostasis under OA stress at the individual level. Collectively, exaptation, adaptation, and acclimation all represent viable means of surviving environmental alteration. More research is required to understand the capacity of each strategy for marine invertebrates under the extreme conditions of climate change (e.g., Collins & Bell 2004, Sammarco & Strychar 2009, Strychar & Sammarco 2009, Lohbeck et al. 2012, Seebacher et al. 2015).

Looking Forward

Although evidence is mounting that some brooding species represent potential "winners" under "business-as-usual" climate scenarios, the mechanisms and extent to which brooding provides this resistance is still poorly understood largely because these species have received relatively less attention than broadcast spawners. Dupont et al. (2010) notes that "*it is essential to include different life history strategies in any global prediction of impact of OA on species and ecosystems*." Data from this study support this sentiment and may incentivize others to examine brooders and their associated life history traits to better understand their adaptation potential to OA. In this study, data from several exploratory studies were presented, but more studies are warranted. Within the context of OA, future studies should continue to explore environmental and maternal drivers of brood chamber chemical variability to resolve the conditions larvae typically have encountered over evolutionary time. Such analysis should include brood chamber carbonate chemistry to evaluate $CaCO_3$ dissolution as declines in chamber pH may be offset by increases in DIC, TA, and/or other maternal sources buffering pallial fluid (e.g., carbonic anhydrase). The influence of other environmental factors, such as temperature, on maternal respiration rates should be considered as well because of their well-documented effects on adult *Ostrea* metabolic activity (Walne 1972, Hutchinson & Hawkins 1992, Haure et al. 1998, Rödstroem 1999, Gray & Langdon 2017), which may exacerbate these maternal impacts on brood chamber conditions. Second, evaluating brood chamber conditions will always require detailed and precise examinations, especially considering the small scales and volumes these measurements would require and the quick response times of the brood chamber to maternal behavior. Third, this manuscript highlighted several attributes of brooding (brood chamber isolation, maternal respiration, and prolonged retention periods) that may have inadvertently promoted larvae to develop traits that confer resistance to OA (Table 2). It should be noted that maternal behaviors, brooding characteristics, and larval traits (e.g., slow development times) should themselves be tested under OA and elevated temperatures to examine the response of the traits to these stressors. It is recommended that future work should study brooded larvae under simulated OA conditions throughout the full larval life cycle (embryo through settlement) to generate the most ecologically relevant data. Indeed, OA resistance observed at early larval stages can be short lived (Dupont et al. 2010) or driven by ontogeny such that brooded larvae may become more sensitive to OA after leaving encapsulating structures (Melzner et al. 2009). Finally, comparisons between brooded and extracted larvae may represent an insightful experimental approach to explore many of the questions posed in this study including, but not limited to, under which conditions such as OA that the brood chamber represents an essential refugia for developing larvae.

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TABLE 2.

Factor and effects on brood chamber conditions.

Factor	pH	O_2
Maternal effects		
Respiration	↓	↓
Valve closure	↓	↓
Valve position	↑↓	↑↓
Chamber buffering	⇡	⇡
Brood isolation	↑↓	↑↓
Brood effects		
Respiration	⇣	⇣
Waste generation	⇣	⇣
Environmental effects		
Upwelling	⇣	⇣
Hypoxia	⇣	⇣

Solid arrows are based on data generated in this study and among other brooding marine invertebrates; broken arrows are hypothesized effects. Downward facing arrows are associated with promotion of larval traits (nature) to resist adverse alteration in brood chamber conditions that may also be exapted to confer resistance against OA; upward facing arrows indicate maternal behavior traits associated with protecting the young (nurture) from adverse environmental condition that may lend these to help larvae cope with OA in the future.

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