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BEHAVIORAL DEFENSES OF SHELLFISH PREY UNDER OCEAN ACIDIFICATION

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ABSTRACT Biological interactions between predators and prey constitute a key component of the ecology and evolution of marine systems, and animal behavior can affect the outcome of predator–prey interactions. It has been recently demonstrated that CO_2-induced ocean acidification can alter the behavior of marine organisms and potentially alter predator–prey dynamics. This study combines both quantitative (meta-analysis) and qualitative approaches to review the effects of ocean acidification on behavioral prey defenses in marine invertebrates. A systematic literature search identified 34 studies that experimentally assessed behavioral defenses under elevated $p\mathrm{CO}_2$ spanning three phyla: crustaceans, echinoderms, and molluscs. A meta-analysis suggested that exposure to elevated seawater $p\mathrm{CO}_2$ can negatively affect behavioral defenses in bivalve molluscs and malacostracan crustaceans. By contrast, defenses of cephalopod molluscs seem to be positively impacted by elevated $p\mathrm{CO}_2$, whereas gastropods and echinoids appear unaffected. A qualitative assessment of studies on combined effects of ocean acidification and warming revealed that combined effects typically differ from ocean acidification–only effects. Based on a qualitative assessment of three studies to date, neurological interference of $\mathrm{GABA_A}$ receptors under elevated $p\mathrm{CO}_2$ may play a major role in ocean acidification effects on prey defense behaviors; however, more research is needed, and other mechanistic underpinnings are also important to consider. Ultimately, the results of this study suggest that behavioral prey defenses in some shellfish taxa may be vulnerable to ocean acidification, that the effects of ocean acidification are often different under warming scenarios than under present-day temperature scenarios, and that $\mathrm{GABA_A}$ interference may be an important mechanism underpinning behavioral responses of shellfish prey under ocean acidification. Despite the importance of shellfish behavioral defenses in the ecology and evolution of marine biological communities, however, research to date has only scraped the surface in understanding ocean acidification effects. Increased research efforts on the effects of multiple stressors, acclimation and adaptation, environmental variability, and complex situational and ecological contexts are needed. Studies of fish behavioral defenses under ocean acidification can help streamline hypotheses and experimental approaches, particularly given the similar effects of elevated $p\mathrm{CO}_2$ on $\mathrm{GABA_A}$ function.

KEY WORDS: cognition, environmental stress, GABA, invertebrates, pH, temperature

INTRODUCTION

Interactions between predators and their prey constitute an integral part of the ecology and evolution of marine organisms, as well as the structure and function of biological communities. In his pioneering work, Connell (1961) documented that the distribution and competitive ability of rocky intertidal barnacles were controlled by the predatory behavior of coexisting whelks. Soon after, Bob Paine demonstrated that the removal of predatory sea stars along the rocky intertidal shores of Washington resulted in less predation on mussels and an extraordinary community transformation (Paine 1966, Paine & Schindler 2002). Since those early studies in rocky intertidal systems, studies have documented the importance of predator–prey interactions in the structure and function of a wealth of marine systems, including pelagic ecosystems (Heithaus et al. 2008), mudflats (Reise 1977, Peterson 1982, Munroe et al. 2015), salt marshes (Silliman & Bertness 2002, Silliman et al. 2005), subtidal reefs (Barkai & McQuaid 1988), and seagrass beds (Heck & Thoman 1981, Orth et al. 1984). Predator–prey interactions are also known to play an important role in marine bioinvasions (Rilov 2016). Given their widespread and influential role in marine systems, understanding predator–prey interactions in the past, present, and future is critical to understanding biodiversity and ecosystem functioning.

Animal behavior is an important component of predator–prey relationships, resulting in a broad literature describing the behavioral responses of prey. For example, personality (Briffa 2013, Belgrad & Griffen 2016), activity and locomotion (Richardson 2001, Sweeny et al. 2013), avoidance behaviors (Cotton et al. 2004), and the use of shelters, burrows, and other refugia (Mima et al. 2003, Hemmi & Merkle 2009, Alcaraz & Arce 2017) can all influence the propensity of an individual or group of organisms to be preyed on. Such alterations in predation potential can have implications for the success and fitness of a given prey species (and the corresponding success and fitness of the predator). It is thus important to consider the behavioral defenses of prey species to predation when assessing predator–prey interactions and associated ecological outcomes.

Behavioral defenses of shellfish prey can be affected by the physical environment. For example, temperature has been documented to affect individual boldness of the hermit crab *Pagurus bernhardus* (Briffa et al. 2013) and the burrowing behavior of the softshell clam *Mya arenaria* (Clements et al. 2017). Similarly, hypoxia has been documented to reduce the burrowing capacity of the infaunal clam *Macoma balthica* (Tallqvist 2001). Consequently, behavioral prey defenses (along with a swath of other behaviors) are vulnerable to a changing marine environment (Wong & Candolin 2015), and behavioral alterations brought about by environmental change in the global ocean have the potential to influence entire marine ecosystems (Nagelkerken & Munday 2016).

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One marine global change stressor that has received much attention over the past 15 y is ocean acidification—the alteration of seawater carbonate chemistry resulting from the absorption of excess atmospheric CO_2 by the oceans (Doney et al. 2009, Hoegh-Guldberg et al. 2014). Research investigating the impacts of ocean acidification on marine life has primarily focused on calcifying marine organisms, in large part because of the anticipated vulnerability of the calcification process to changes in seawater pH (Orr et al. 2005, Ries et al. 2009, Kroeker et al. 2010, 2013). Recent evidence, however, suggests that ocean acidification can also have drastic effects on the behavior of marine organisms (see reviews by Briffa et al. 2012, Clements & Hunt 2015). Thus, although the vulnerability of morphological defenses such as shell has been relatively well studied, less is known of the impacts of ocean acidification on shellfish behavioral defenses (see Fig. 2 in Kroeker et al. 2014).

In this article, the current literature regarding ocean acidification effects on invertebrate defense behaviors was quantitatively and qualitatively reviewed to provide an overview of ocean acidification effects on behavioral prey defenses in marine shellfish. Specifically, a meta-analysis of ocean acidification effects on prey defense behaviors was conducted from studies to date. In addition, available studies were qualitatively reviewed to assess interactive effects of ocean acidification and warming on shellfish behavioral defenses, and to highlight a potential biological mechanism responsible for observed effects ($GABA_A$ neural interference). Knowledge gaps are also discussed, and key considerations for future research are provided.

LITERATURE SEARCH AND DATA COLLECTION

A systematic online keyword search was conducted in Scopus and Google Scholar to obtain relevant studies (peer-reviewed articles and graduate theses) using various combinations of specific keywords: "ocean acidification" or "acidification" or "carbon dioxide" or "CO_2," combined with "behavior" and/or "predator" and/or "defense" and/or "behavioral defense" and/or "predator–prey" and/or "avoidance" and/or "escape." Only original research articles that directly measured prey defense behavior on a species of shellfish (molluscs, arthropods, and echinoderms) were archived for analysis. The reference lists and citations of each relevant article were subsequently checked to obtain any additional articles that may have been missed using the online keyword search.

From each article, qualitative and quantitative attributes were extracted: (1) bibliographical information; (2) study species and associated taxonomic information; (3) life stage of the organism(s) tested; (4) the climatic region where study animals were collected (based on the geographic location of animal collection and/or experimentation stated); (5) seawater conditions (temperature, salinity, pH, and pCO_2 when available), exposure time (i.e., duration of exposure to pCO_2 treatments), the prey defense behavior measured, whether a predator cue was present when the behavior was measured, and the statistical effect and associated functional effect ["function" was defined in relation to avoiding or escaping a predation event; for example, an increase in self-righting time would be interpreted as functionally negative (and *vice versa*), and a decrease in reaction time would be functionally positive (and *vice versa*)]. For pH and pCO_2 conditions, the magnitude of change (i.e., offset) was calculated for each experimental treatment by subtracting the mean of the control from that of each experimental treatment; all treatments considered were static (i.e., experimental pH and pCO_2 conditions were kept stable during experimental exposures). Where possible, unavailable pCO_2 values were estimated in CO2SYS (Pierrot et al. 2006) using the published temperature, salinity, and pH values of the treatments along with a total alkalinity value of 2,345 μatm (WHOI 2012), using the first and second dissociation constants of Mehrbach et al. (1973) refit by Dickson and Millero (1987). For each article relevant to the meta-analysis, the mean, SD, and sample size for each treatment group were recorded; if multiple behaviors or experiments were measured in the same study, each was considered as an individual data point (i.e., multiple data points from the same study were included). The data were collected from either raw data supplied as supplementary information or from published tables and figures in the main article; data from figures were estimated using ImageJ (Schneider et al. 2012).

META-ANALYSIS OF OCEAN ACIDIFICATION EFFECTS ON PREY DEFENSE BEHAVIORS: METHODOLOGY

Deriving Individual Effect Sizes

To derive a quantitative understanding of ocean acidification effects on shellfish behavioral prey defenses, a meta-analysis was conducted. The effect size used was the natural logarithm-transformed response ratio ($\ln RR$), which is defined as

$$\ln RR = \ln\left(\frac{\bar{X}_E}{\bar{X}_C}\right),$$

where $\bar{X}_E$ and $\bar{X}_C$ are the average measured response in the experimental and control treatments, respectively. This effect size metric has a high capacity to detect true effects, is robust to low sample sizes, and quantifies the proportional change in a response variable due to experimental manipulations (Lajeunesse & Forbes 2003). It is important to note that, for some behaviors, an increase in the behavioral measurement corresponds to a negative functional response (but would result in a positive effect size estimate). In such instances, the directionality of the calculated effect size was inverted (i.e., positive values were changed to negative ones, and *vice versa*) to adequately reflect the functional consequence of the behavioral change.

Effect size variance was calculated as

$$v = \frac{(S_E)^2}{n_E\bar{X}_E^2} + \frac{(S_C)^2}{n_C\bar{X}_C^2},$$

where S and n are the SD and sample size, respectively, for a given experimental treatment (denoted by the subscripts C [control] and E [experimental, i.e., elevated pCO_2]); $\bar{X}_E$ and $\bar{X}_C$ are defined as aforementioned. Thus, studies with greater replication and less-variable responses were considered to be more precise estimates of a population effect and, as such, were weighted more heavily when calculating mean effect sizes (Hedges & Olkin 1985). This approach provides a conservative estimate of the mean effect size and increases the confidence that a statistically significant effect size is a true effect.

Determining Drivers of Individual Efect Sizes

Rather than assessing effect sizes between *a priori* defined groups, Akaike's information criterion (AIC) model selection was used to determine parameters that best accounted for the observed variability in effect sizes, and effect sizes were assessed across levels within each contributing parameter identified by AIC. Linear mixed models with additive fixed factors (i.e., interactions were not tested) were built for all possible combinations of predictor variables. Categorical predictor variables included in the models were taxonomy (phylum and class), climatic region (temperate, tropical, or polar; if animals were from the deep sea, they were considered in a separate group), and life stage (larvae, juvenile, or adult); study was included as a random variable to account for nonindependence due to the incorporation of multiple effect sizes from a single study. Before analysis, correlations between effect size and three continuous variables—exposure time, sample size, and $p\mathrm{CO}_2$ offset (experimental–control $p\mathrm{CO}_2$)—were also explored. There was a significant correlation between effect size and $p\mathrm{CO}_2$ offset ($r = -0.66$, $P < 0.001$; Fig. 1), but not exposure time ($r = -0.11$, $P = 0.377$) or sample size ($r = 0.16$, $P = 0.219$); therefore, $p\mathrm{CO}_2$ offset, but not exposure time or sample size, was included in the AIC models as a continuous predictor variable. Predictor variables were not significantly correlated ($r < 0.36$ for all pairwise correlations) with the exception of phylum and class ($r = 0.93$); therefore, models including both phylum and class as predictor variables were excluded from AIC selection. A "null" model (i.e., model with no fixed predictor variables and only a random factor of measurement nested within study) was also included in AIC selection. This resulted in a total of 24 additive models. Model selection was based on AIC and log likelihood values (Burnham & Anderson 2002; lowest AIC and highest log likelihood values indicate the "best" model), and all models with a delta AIC (Δ AIC) ≤ 2 were considered as "best" models (Burnham & Anderson, 2002). The AIC model selection was conducted on all studies, as well as only those with a $p\mathrm{CO}_2$ offset consistent with near-future projections (<1,000 µatm), to determine whether predictors of effect size differed when $p\mathrm{CO}_2$ projections beyond near-future were included versus excluded.

Effect Size Analysis of Predictor-Level Responses

Once the "best" model was defined, the mean lnRR and 95% confidence interval (CI) for each level of the predictor variable(s) that were present in the best model were calculated. To provide conservative estimates of effect size variance, bootstrapped (10,000 replicates), bias-corrected, and accelerated (BCa) 95% CIs were used (Adams et al. 1997). Mean effect sizes were estimated using weighted random effect models, which weight individual effect sizes by the inverse of the effect size variance (Hedges & Olkin 1985). Random effects models were used as opposed to fixed effects models because of inherent differences in conditions between studies (i.e., species and geographic location). To account for nonindependence associated with using multiple data points per study, mean effect sizes were calculated using three-level meta-analytical models (Nakagawa et al. 2015, Noble et al. 2017), including "measure nested within study" as a random variable. Mean lnRR and associated variance (bootstrapped BCa 95% CIs) were only derived for factor levels with $n \geq 3$.

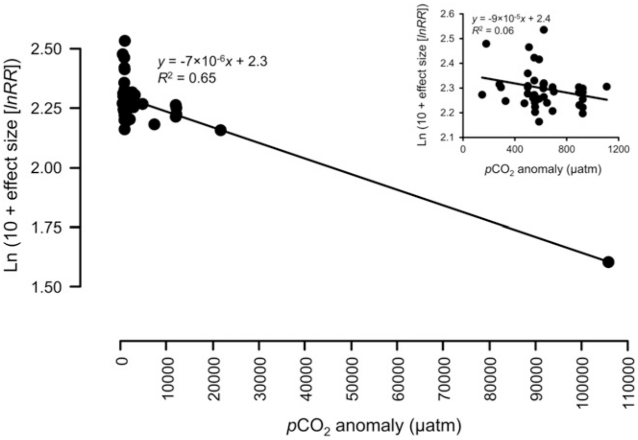

Figure 1. Effect of $p\mathrm{CO}_2$ on effect sizes. Absolute effect sizes as a function of $p\mathrm{CO}_2$ offset (experimental–control $p\mathrm{CO}_2$) with all $p\mathrm{CO}_2$ anomalies (black plot) and only near-future $p\mathrm{CO}_2$ anomalies (gray plots; $p\mathrm{CO}_2$ offset <1,100 based on IPCC 2100 RCP8.5 projection) included.

Statistical significance was determined by assessing whether the BCa 95% CI surrounding a mean effect size overlapped with 0 (Lajeunesse & Forbes 2003). Heterogeneity in effect sizes for each level of the factors identified in the top candidate model(s) was tested using Q-tests of heterogeneity (Lajeunesse & Forbes 2003). Given the significant correlation between effect size and $p\mathrm{CO}_2$ offset for all $p\mathrm{CO}_2$ conditions ($r = -0.66$, $P < 0.001$), but the lack of correlation for near-future only ($r = -0.28$, $P = 0.075$), two separate analyses were conducted: one for all $p\mathrm{CO}_2$ conditions and another for only near-future $p\mathrm{CO}_2$ conditions (i.e., $p\mathrm{CO}_2$ offset <1,000 µatm; upper estimate for average $p\mathrm{CO}_2$ increase based on the IPCC RCP8.5 projection for 2100). A null effect was signified when lnRR = 0, whereas negative and positive effects were defined by negative and positive lnRR values, respectively (Hedges et al. 1999).

Sensitivity Analysis and Publication Bias

The influence of single studies that elicited unusually large effect sizes was investigated using sensitivity analysis. Herein, observations within each level of the factors present in the best AIC model were systematically ranked according to their observed mean effect size; the largest effect size was then removed from the dataset, and the analysis was rerun. The relative contribution of individual studies to the observed mean effect sizes was also considered; however, no single study contributed more than four experiments to the meta-analysis (three studies contained four experiments, whereas many other studies contained three experiments) and, as such, the data were not reanalyzed excluding studies that contributed multiple observations to the overall effect size. To test for publication bias, the number of studies with an lnRR = 0 that would be required to change a significant effect size response to a nonsignificant response was determined using Rosenthal's fail-safe number (Rosenthal 1979). The analysis for a given category was considered robust if Rosenthal's fail-safe number was ≥5 (Kroeker et al. 2010, 2013; Harvey et al. 2013).

Analytical Details

All analyses were conducted in R v. 3.4.1 (R Core Team 2017) with a significance threshold of $\alpha = 0.05$. Effect size analyses were conducted using the *metafor* package (Viechtbauer 2010, 2017), with 95% BCa CIs generated using the "boot" package (Canty & Ripley 2017). Pearson correlations were tested using the *rcorr* function in the *Hmisc* package (Harrell Jr. 2017), AIC model selection analysis was conducted using the

AICcmodavg package (Mazerolle 2017), and R^2 values for linear mixed models were computed using the *MuMIn* package (Bartoń 2018). Before analyses including individual effect sizes (i.e., Pearson correlations and AIC), effect sizes were transformed (ln+10) to achieve normality.

QUALITATIVE ASSESSMENTS OF OCEAN ACIDIFICATION–WARMING EFFECTS, AND THE ROLE OF $GABA_A$: METHODOLOGY

In addition to the meta-analysis of ocean acidification effects on prey defense behaviors, a qualitative review was also used to assess the effects of ocean acidification in the context of warming and to assess the role of $GABA_A$ interference in driving elevated pCO_2 effects on shellfish prey defense behaviors. Qualitative assessments were used for these two topics because of the low number of relevant studies ($n = 7$ for acidification–warming and $n = 3$ for $GABA_A$). These assessments consisted of a vote counting approach, whereby the number of studies lending evidence for or against a certain hypothesis was used to determine the likelihood of a particular outcome. For acidification–warming studies, the percentage of articles that showed statistical difference between the acidification-only and acidification–warming groups was computed. Similarly, for $GABA_A$ studies, the percentage of articles that suggested $GABA_A$ interference may be the driving mechanism of ocean acidification effects on behavior was calculated. It is important to note here that this approach is not quantitatively rigorous and therefore has less inferential power than the meta-analysis in the previous section; however, such an exercise can highlight potential trends and provide directions for future studies in these areas.

AN OVERVIEW OF THE STUDIES

The literature search uncovered 34 studies (peer-reviewed articles and graduate theses), assessing the effects of elevated pCO_2 on shellfish prey defense behaviors (Table 1). Of those, 28 were used in the meta-analysis of ocean acidification effects on defense behaviors (meta-analysis only included studies where the mean, variance, and sample size for each treatment were obtainable). The first study to directly assess ocean acidification effects on prey defense behavior was published by Bibby et al. (2007), who measured predator avoidance behavior in the intertidal gastropod *Littorina littorea*. Therein, snails increased the avoidance behavior in response to predatory crabs as a compensatory defense, as ocean acidification hindered the primary defense of snails—shell thickening. In subsequent years, studies neglected assessments of shellfish behavioral defenses until 2011 when three more studies were published (Fig. 2A). Thereafter, studies of ocean acidification effects on shellfish prey behavior became more popular, peaking in 2014. Since 2011, at least one study assessing ocean acidification effects on shellfish prey defenses has been conducted per annum (Fig. 2A).

The studies obtained in the literature search encompassed three phyla—molluscs ($n = 23$, 68%), arthropods ($n = 5$, 15%), and echinoderms ($n = 7$, 20%) (Fig. 2B; total number of studies >34 and total percentage >100% because of a single study that assessed both a gastropod and an arthropod). Molluscan studies spanned three taxonomic classes and included studies on bivalves ($n = 7$, 20% of all studies), cephalopods ($n = 3$, 9% of all studies), and gastropods ($n = 13$, 37% of all studies) (Fig. 2B). Echinoderm studies and crustacean studies were exclusively restricted to the classes Echinoidea and Malacostraca, respectively. Studies on bivalves, gastropods, and echinoids spanned across the larval, juvenile, and adult life stages of these organisms, whereas studies on juvenile cephalopods were absent, and studies on malacostracan crustaceans have been restricted to adults (Fig. 2B). A total of 19 studies assessed the effect of ocean acidification on the behavioral defenses of adult prey, whereas five and six studies tested such effects in juveniles and larvae, respectively (see Fig. 1B for life stage percentages for each taxonomic class). Importantly, only 15% of the studies ($n = 5$ of 34) included predator cues in their experiments testing for effects of ocean acidification on prey defense behaviors. Thus, most behaviors, although relevant to prey defense, were measured in the absence of predator cues.

Seven studies reported strictly negative (i.e., all experiments finding a significant negative effect of ocean acidification) functional effects of ocean acidification on behavioral prey defenses ($n = 5$ bivalve + 2 gastropod studies), whereas four studies reported strictly positive effects ($n = 2$ cephalopod + 2 gastropod studies) (Fig. 2B). Null responses to ocean acidification were observed in 15 studies; $n = 6$ gastropod + 6 echinoderm + 3 malacostracan studies) (Fig. 2B). Six studies on molluscan taxa reported mixed responses to ocean acidification ($n = 2$ bivalve + 1 cephalopod + 3 gastropod studies) (Fig. 2B).

A META-ANALYSIS OF OCEAN ACIDIFICATION EFFECTS ON SHELLFISH BEHAVIORAL DEFENSES

Meta-Analysis Results

Effects of ocean acidification on shellfish behavioral defenses were predominantly driven by pCO_2 offset, taxa, and life stage. When all pCO_2 conditions were considered, AIC model selection revealed the model including pCO_2 offset + class + life stage as the "best" model (Table 2). This model explained 59% ($R^2 = 0.59$) of the variance in individual effect sizes. A second model including pCO_2 offset + life stage could not be excluded (delta AIC = 1.75) and explained 58% ($R^2 = 0.58$). When only near-future pCO_2 anomalies where considered, a number of candidate models (i.e., those with a delta AIC <2) were evident; these included the model including pCO_2 offset, the model including class, the model including life stage, and the null model. Of these models, the model including class explained the most variation ($R^2 = 0.30$), followed by life stage ($R^2 = 0.23$), the null model ($R^2 = 0.22$), and pCO_2 offset ($R^2 = 0.20$). For both pCO_2 groupings, effect size was negatively related to pCO_2 offset, although the relationship for near-future pCO_2 values was weak and non-significant (Fig. 1). Given that the fixed factors of class and life stage appeared in the best models, the effect size analysis was restricted to testing for ocean acidification effects on prey defense behaviors across classes and life stages. It is important to note, however, that the R^2 values for all pCO_2 conditions were primarily explained by pCO_2 offset and that those for near-future pCO_2 only were relatively low. This suggests that the predictive power of class and life stage was relatively low; nonetheless, they appeared to be more important in explaining the effect size variation than other factors based on AIC model selection.

When all pCO_2 offsets were included, meta-analysis revealed that mean effect sizes of bivalve and malacostracan defenses were significantly lower than zero (Fig. 3A). By contrast, the mean effect size in cephalopods was significantly higher than zero, despite substantial variability (Fig. 3A). Effect sizes in both

TABLE 1.

Summary of experimental studies assessing the effects of elevated pCO_2 on the behavioral prey defenses.

Reference	Species	Climatic region	Life stage	Acclim. time	Behavioral metric	Predator cue?	pH			pCO_2			Functional response
							Control	Exp	Offset	Control	Exp	Offset	
Bivalvia													
Schalkhausser et al. (2013)	*Pecten maximus*	Temperate	Adult	30	Number of claps	No	8.19	7.76	–0.43	395	1,086	691	Null
					Clapping force								Negative
Green et al. (2013)*	*Mercenaria mercenaria*	Temperate	Larvae	0	Percent burrowed	No	n.r	n.r	n.r	n.r	n.r	n.r	Negative
Clements & Hunt (2014*)	*Mya arenaria*	Temperate	Juvenile	0	Percent burrowed	No	Gradient: 6.80–7.80			n.r	n.r	n.r	Negative
Rodríguez-Romero et al. (2014)	*Ruditape s philippinarum*	Tropical	Adult	10	Percent burrowed	No	7.93	7.07	–0.86	392	4,880	4,488	Negative
								6.63	–1.30		21,833	21,441	Negative
								6.14	–1.79		106,029	105,637	Negative
Clements et al. (2016)*	*M. arenaria*	Temperate	Juvenile	0	Percent burrowed	No	Gradient: 6.80–7.60			n.r	n.r	n.r	Negative
Clements et al. (2017)	*M. arenaria*	Temperate	Juvenile	0	Percent burrowed	No	7.26	6.50	–0.76	1,229	8,270	7,041	Negative
Peng et al. (2017)	*Sinonovacula constricta*	Tropical	Adult	7	Burrowing depth	No	8.08	7.74	–0.34	351	825	474	Null
								7.57	–0.51		1,249	898	Negative
								7.22	–0.86		2,859	2,508	Negative
Cephalopoda													
Spady et al. (2014)	*Idiosepius pygmaeus*	Tropical	Adult	5	Mean number line	No	8.13	7.99	–0.14	447	626	179	Positive
					Crosses			7.83	–0.30		956	509	Positive
					Percent high activity								Positive
													Positive
					Percent inking								Positive
													Positive
					Percent defense posture								n/a
					versus jetting								n/a
					Percent display type								n/a
													n/a
Spady et al. (2018)	*Sepioteuthis lessoniana*	Tropical	Adult	28	Distance moved	No	7.99	7.71	–0.28	435	935	500	Positive
					Speed								Positive
					Percent time active								Positive
Zakroff et al. (2018)*	*Doryteuthis pealeii*	Temperate	Larvae	0	Time spent in area	No	8.00	7.88	–0.12	400	550	150	Null
								7.66	–0.34		850	450	Null
								7.54	–0.46		1,300	900	Null
								7.55	–0.45		1,500	1,100	Null
								7.48	–0.52		1700	1,300	Null
								7.37	–0.63		1900	1,500	Negative
								7.40	–0.60		2,200	1800	Negative

continued on next page

TABLE 1.

continued

Reference	Species	Climatic region	Life stage	Acclim. time	Behavioral metric	Predator cue?	pH			pCO_2			Functional response
							Control	Exp	Offset	Control	Exp	Offset	
					Speed	No	8.00	7.70	–0.30	400	1,000	600	Null
								7.58	–0.42		1,600	1,200	Null
								7.40	–0.60		2,200	1800	Null
Gastropoda													
Bibby et al. (2007)	*Littorina littorea*	Temperate	Adult	15	Percent time avoidance	Yes	7.97	6.63	–1.34	507	5,382	4,875	Positive
Fields (2013)	*Conomurex luhuanus*	Tropical	n.r	10	Self-righting time	No	8.16	7.83	–0.33	400	950	550	Positive
					Response latency								Positive
					Speed								Positive
Manriquez et al. (2013)	*Concholepas concholepas*	Tropical	Juvenile	83	Self-righting time	No	8.03	7.80	–0.23	388	716	328	Negative
								7.64	–0.39	388	1,036	648	Negative
Manriquez et al. (2014)	*Concholepas concholepas*	Tropical	Larvae	150	Percent time away from predator cue	Yes	8.05	7.65	–0.40	391	684	293	Null
								7.52	–0.53		945	554	Negative
Schram et al. (2014)	*Nacella concinna*	Polar	Adult	42	Percent to self-right	No	8.11	7.81	–0.30	371	994	623	Null
					Speed								Null
	Margarella antarctica	Polar	Adult	42	Self-righting time								Positive
					Speed								Null
Quíeros et al. (2014)	*Nucella lapillus*	Temperate	Adult	425	Speed	No	8.03	7.93	–0.10	541	689	148	Negative
								7.79	–0.24		2,247	1706	Negative
Watson et al. (2014)	*Gibberulus gibberulus gibbosus*	Tropical	Adult	5	Number of jumps	Yes	8.17	7.85	–0.32	405	961	556	Negative
					Jump distance								Negative
					Percent jumping								Negative
Zhang et al. (2014)	*Reticunassa festiva*	Tropical	Larvae	1	Swimming speed	No	8.05	7.75	–0.30	366	865	499	Null
								7.52	–0.53		1,260	894	Null
	Nassarius conoidalis						8.05	7.75	–0.30	366	865	499	Null
								7.52	–0.53		1,260	894	Null
Jellison et al. (2016)*	*Tegula funebralis*	Tropical	Adult	5	Path length	Gradient: 6.40–8.00				n.r	n.r	n.r	Null
					Path shape								Null
					Percent time out of water								Negative
Manríquez et al. (2016)	*Concholepas concholepas*	Tropical	Juvenile	90	Self-righting time	No	7.85	7.47	–0.38	539	1,463	924	Positive
Domenici et al. (2017)	*Concholepas concholepas*	Tropical	Juvenile	180	Absolute lateralization	No	7.85	7.47	–0.38	539	1,463	924	Null
					Movement duration								Null
					Decision time								Negative
					Route finding								Negative
Watson et al. (2017)	*Conus marmoreus*	Tropical	Adult	14	Distance moved	No	8.19	7.86	–0.33	388	975	587	Positive
					Speed								Positive

continued on next page

TABLE 1.

continued

Reference	Species	Climatic region	Life stage	Acclim. time	Behavioral metric	Predator cue?	pH			pCO$_2$			Functional response
							Control	Exp	Offset	Control	Exp	Offset	
					Time buried								Negative
					Self-righting time								Negative
					Left–right preference								Null
Walsh (2018)	*Tritia obsoleata*	Temperate	Adult	0	Percent burrowed	No	6.98	6.51	–0.47	3,244	9,815	6,571	Null
Echinoidea													
Chan et al. (2011)	*Dendraster excentricus*	Temperate	Larvae	10	Speed	No	n.r	n.r	n.r	380	1,000	620	Null
					Helical pitch								Null
Hatfield-Vaughan (2011)	*Mesocentrotus franciscanus*	Temperate	Juvenile	154	Percent avoidance	Yes	7.96	7.68	–0.28	512	1,053	541	Null
Barry et al. (2014)	*Strongylocentrotus fragilis*	Tropical (deep sea)	Adult	27	Speed	No	7.55	7.14	–0.41	1,132	3,021	1889	Null
Chan et al. (2015)	*Strongylocentrotus droebachiensis*	Temperate	Larvae	14	Speed	No	8.04	7.68	–0.36	398	1,022	624	Null
								7.21	–0.83		3,201	2,803	Null
Chan et al. (2016)	*Amphiura filiformis*	Temperate	Larvae	13	Speed	No	8.05	7.67	–0.38	425	1,126	701	Null
	Strongylocentrotus purpuratus	Temperate	Larvae	29	Speed	No	8.03	7.69	–0.34	458	1,078	620	Null
								7.27	–0.76		2,993	2,535	Null
Malacostraca													
de la Haye et al. (2011)	*Pagurus bernhardus*	Temperate	Adult	5	Latency of shell change	No	8.22	6.85	–1.37	375	12,191	11,816	Negative
					Antennular flicking								Negative
					Movement duration								Negative
Alenius & Munguia (2012)	*Paradella dianae*	Tropical	Adult	21	Percent time swimming	No	8.17	7.60	–0.57	300	1,409	1,109	Null
de la Haye et al. (2012)	*P. bernhardus*	Temperate	Adult	5	Antennular flicking	No	8.21	6.81	–1.40	373	12,061	11,688	Negative
					Movement duration								Negative
Zittier et al. (2013)	*Hyas araneus*	Polar	Adult	12	Self-righting time	No	8.08	7.74	–0.34	351	825	474	Null
								7.57	–0.51		1,249	898	Null
								7.22	–0.86		2,859	2,508	Null
Kim & Barry (2016)	*Pagurus tanneri*	Tropical (deep sea)	n.r	28	Emergence time	No	7.60	7.12	–0.48	1,379	3,596	2,217	Null
Rankin (2017)	*Pelia tumida*	Tropical	Adult	35	Percent decoration	No	7.99	7.75	–0.24	613	894	281	Null
Walsh (2018)	*Corophium concholepas volutator*	Temperate	Adult	0	Percent burrowed	No	6.98	6.51	–0.47	3,244	9,815	6,571	Negative

* Studies not included in the meta-analysis because of lack of data availability. Studies are ordered chronologically within each respective taxonomic class. n.r, not reported; n/a, not applicable; Acclim., acclimation; Exp., experimental; Anom., offset. Acclimation time is reported in days, and pCO$_2$ is reported in µatm.

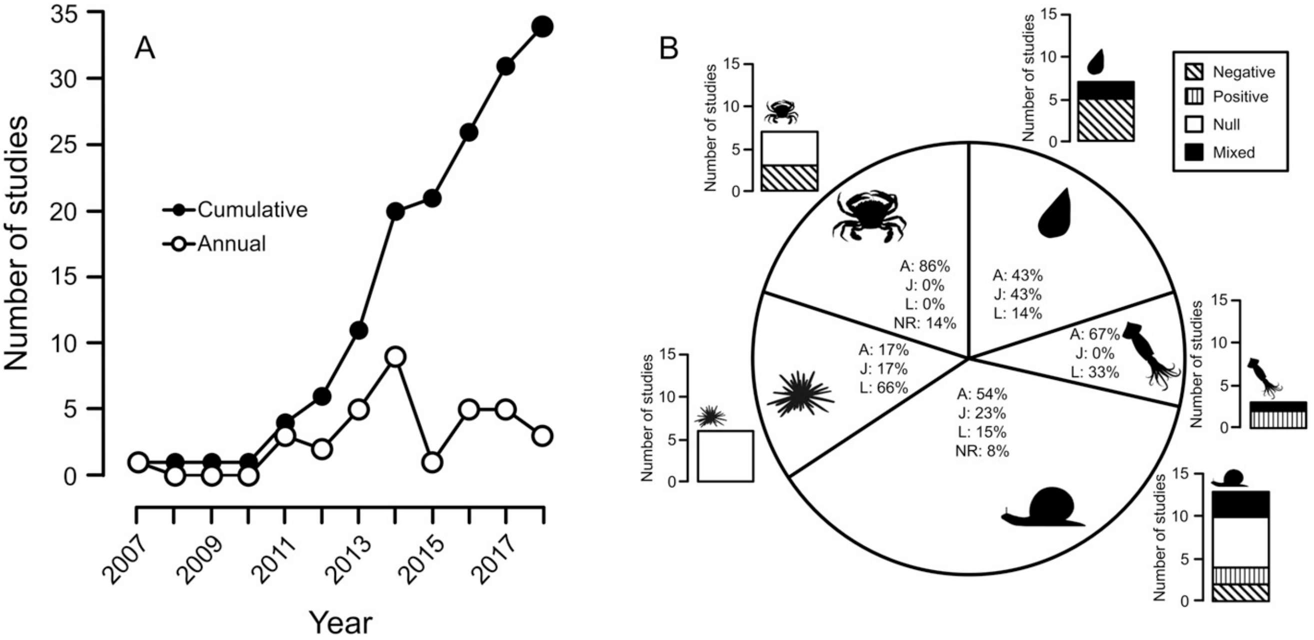

Figure 2. General overview of studies identified in this review. (A) The annual (gray plots) and cumulative (black plots) number of studies assessing the effects of elevated pCO_2 on behavioral defenses of shellfish prey from 2007 to present. Open plots represent the number of studies published during the first third of 2018 (i.e., January–April). (B) Taxonomic spread (pie chart) and statistical effects (bar plots) of the studies. Pie slices represent the percentage of studies that assessed particular taxa (classes: bivalves [black], cephalopods [dark gray], gastropods [gray], echinoids [light gray], and malacostracans [white]). Values within each pie slice denote the percentage of studies within a given taxa that tested one of three life stages: larvae (L), juvenile (J), or adult (A) (NR = life stage not reported). Bar plots represent the number of studies within a given taxa that reported statistically negative (diagonally hatched), positive (vertically hatched), null (horizontally hatched), or mixed (dotted) effects. "Negative," "positive," and "null" were assigned when all reported effects of elevated pCO_2 within a given study were negative, positive, or null; "mixed" was assigned when responses to elevated pCO_2 varied within a given study (contingent on some variable factors such as pCO_2 level, species, or behavioral metric).

gastropods and echinoids were not significantly different from zero (Fig. 3A). Sensitivity analysis revealed that unusually large effect sizes did not influence the statistical outcome of class effect sizes (data not shown). The categorical analysis was robust (Rosenthal's fail-safe >7) for all classes, except for echinoids [Table 3; Rosenthal's fail-safe = 0, which was unsurprising given that all studies on echinoids reported exclusively null statistical effects (Fig. 2B)]. The significant negative effect size for bivalves was retained when only near-future pCO_2 conditions were considered (Fig. 3B). Likewise, the significant positive effect size for cephalopods remained when only near-future conditions were considered, along with the null effect size for gastropods and echinoids (Fig. 3B). In contrast to when all pCO_2 conditions were considered, the effect size for malacostracan crustaceans was not significantly different from zero (Fig. 3B) and the robustness of the malacostracan effect was reduced when only near-future conditions were considered (Table 3; Rosenthal's fail-safe number = 2). Heterogeneity (Q-statistic) for all classes was significant for both pCO_2 groupings (Table 3).

With respect to life stage, the meta-analysis revealed that only juvenile effect sizes were significantly lower than zero (Fig. 4). This was true for all pCO_2 conditions and near-future conditions only. The mean effect size for both larvae and adults was not significantly different from zero for both pCO_2

TABLE 2.

Results of AIC model selection analysis for additive models explaining variance in individual effect sizes.

Model	K	AICc	ΔAIC	AICc Wt	Cum Wt	LL	R^2
All pCO_2							
Class + life stage + pCO_2 offset	10	144.29	0.00	0.40	0.40	–60.03	0.59
Life stage + pCO_2 offset	6	145.88	1.59	0.18	0.59	–66.19	0.58
Near-future pCO_2 only							
Null model	4	104.35	0.00	0.20	0.20	–47.63	0.22
pCO_2 offset	4	104.35	0.00	0.20	0.41	–47.63	0.20
Life stage	5	105.41	1.06	0.12	0.53	–46.87	0.23
Class	7	105.47	1.12	0.12	0.64	–44.09	0.30

Only top models [i.e., lowest AIC and highest log likelihood (LL in table)] are shown.

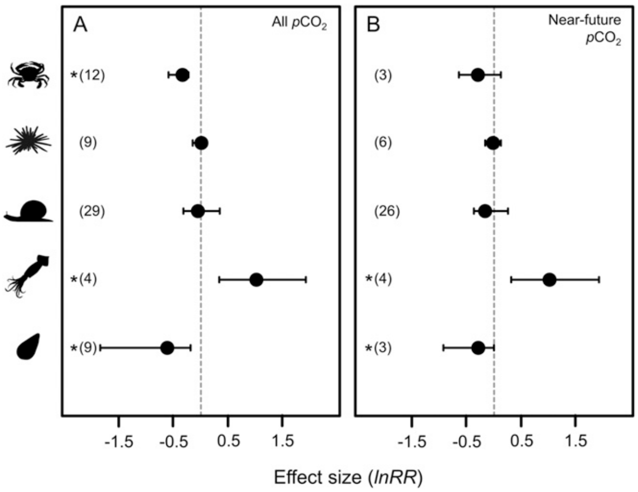

Figure 3. Responses of shellfish behavioral prey defenses to ocean acidification across taxonomic class. Mean effect sizes (ln*RR* ± bootstrapped BCa 95% CIs) for five classes of shellfish: malacostracans (white), echinoids (light gray), gastropods (gray), cephalopods (dark gray), and bivalves (black), with all pCO_2 anomalies included (A) and only near-future pCO_2 anomalies included (B). Values in parentheses indicate the number of observations used to derive the mean effect size for each taxon. Asterisks denote significant responses (i.e., when 95% CI does not overlap with 0). Negative and positive values correspond to negative and positive functional effects, respectively.

groupings (Fig. 4). Heterogeneity (Q-statistic) was significant for all life stages under both pCO_2 groupings (Table 3).

Meta-Analysis Results in a Broader Context

Taxonomic class consistently appeared in the best AIC models as a predictor of effect sizes. Although previous meta-analyses on biological responses to ocean acidification include taxonomy as an explanatory variable, those studies typically select phylum as the taxonomic resolution at which to test differences (Kroeker et al. 2010, 2013, Harvey et al. 2013). The results of the meta-analysis suggest that, at least for calcifying organisms, this level of taxonomy may be too broad and that using finer scales of taxonomic resolution may provide better predications of ocean acidification sensitivity. It is important to note, however, that class alone was unable to explain much of the variation in observed effect sizes herein ($R^2 = 0.27$ for near-future pCO_2), and as such may not be a fine enough taxonomic scale to explain observed differences. Such results highlight the importance of species-specific biology. Furthermore, Q-tests of heterogeneity revealed significant variation across studies within each taxonomic class (Table 3), suggesting that other processes are certainly involved in explaining ocean acidification effects on behavioral defenses of shellfish reported in the experimental studies reviewed here. Such variation could be explained by some variable(s) not included in the analysis (such as the type of behavior, the biological state of the test animals, local adaptation, or mismatches between experimental exposures and natural conditions), and may largely reflect unaccounted experimental artifacts that differed across studies.

When all pCO_2 conditions were considered, a significant negative effect size was observed for the behavioral defenses of malacostracan and bivalve prey, whereas effect sizes for gastropods and echinoderms were not significantly different from zero; in addition, cephalopods exhibited a significant positive effect size. These results are in contrast with previous meta-analyses reporting mostly null effects of elevated pCO_2 on crustacean taxa and sensitivities in molluscs and echinoderms (Kroeker et al. 2010, 2013, Harvey et al. 2013, Whittman & Pörtner 2013). Previous meta-analyses have excluded metrics of behavior for shellfish taxa likely because too few studies were available (see Fig. 2A). The results of this study suggest that impacts of ocean acidification on other biological processes of

TABLE 3.

Results of effect size analysis, including mean effect size, bootstrapped 95% CIs, Q-test results, and Rosenberg fail-safe test results.

	Effect size				Q-test			
	Mean	Upper CI	Lower CI		Df	Q	*P* value	Rosenberg FSN
All pCO_2								
Bivalvia	–0.6075	–0.1862	–1.8144	*	8	263.8	<0.001	445
Cephalopoda	0.9980	1.8897	0.3292	*	3	14.6	0.002	7
Gastropoda	–0.0563	0.3380	–0.3159	—	28	599.6	<0.001	1,281
Echinoidea	0.0063	0.1046	–0.1494	—	8	15.9	0.044	0
Malacostraca	–0.3333	–0.2243	–0.5840	*	11	45.4	<0.001	507
Adult	–0.0572	0.2446	–0.3163	—	40	847.0	<0.001	2,723
Juvenile	–0.6265	–0.4272	–0.8667	*	8	22.3	0.004	265
Larvae	–0.0797	0.0120	–0.2490	—	12	192.6	<0.001	140
Near-future pCO_2								
Bivalvia	–0.2860	–0.0021	–0.9119	*	2	33.8	<0.001	0
Cephalopoda	0.9980	1.8897	0.3076	*	3	14.6	0.002	7
Gastropoda	–0.1263	0.2876	–0.3279	—	25	399.3	<0.001	1,021
Echinoidea	–0.0195	0.1230	–0.1591	—	5	14.7	0.012	0
Malacostraca	–0.2920	0.1218	–0.6340	—	2	6.3	0.043	11
Adult	0.1143	0.5896	–0.1901	—	22	295.9	<0.001	0
Juvenile	–0.5679	–0.3839	–0.8261	*	7	17.2	0.016	194
Larvae	–0.1009	0.0095	–0.2878	—	10	179.9	<0.001	151

Asterisks denote significant effect sizes.

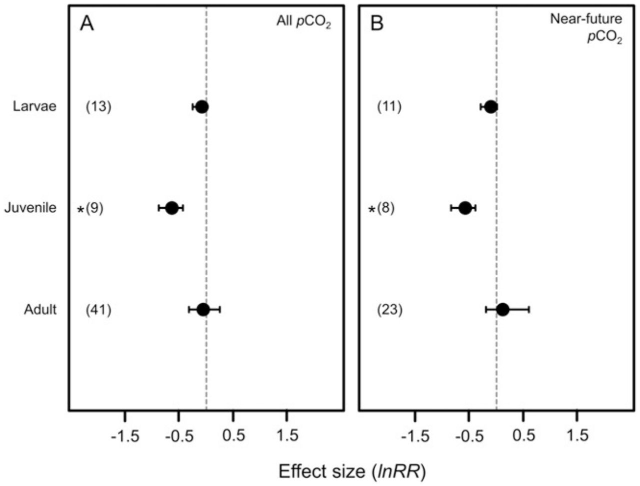

Figure 4. Responses of shellfish behavioral prey defenses to ocean acidification across life stage. Mean effect sizes (ln*RR* ± bootstrapped BCa 95% CIs) for three life stages of shellfish: adults (white), juveniles (gray), and larvae (black), with all pCO$_2$ anomalies included (A) and only near-future pCO$_2$ anomalies included (B). Values in parentheses indicate the number of observations used to derive the mean effect size for each life stage. Asterisks denote significant responses (i.e., when 95% CI does not overlap with 0). Negative and positive values correspond to negative and positive functional effects, respectively.

shellfish such as physiology and survival may not be adequate in defining effects on shellfish behaviors. It is important to note, however, that the negative effect sizes for malacostracans observed in this study were driven by higher-than-projected pCO$_2$ conditions, as significant negative effects on malacostracans became nonsignificant when only near-future pCO$_2$ changes were considered. More confident predictions of shellfish behavioral defenses under near-future pCO$_2$ changes across classes, however, await further investigation, as sample sizes herein were relatively low (i.e., meta-analysis with only near-future pCO$_2$ conditions were not robust; see Rosenthal fail-safe numbers in Table 3).

Life stage was also a fixed factor that consistently appeared in the best models based on AIC model selection. Effect size analysis revealed that juvenile mean effect sizes were significantly lower than zero when all and only near-future pCO$_2$ conditions were considered; larval and adult effect sizes were not significantly different from zero. Previous meta-analyses also highlight the importance of life stage (e.g., Kroeker et al. 2010, 2013, Harvey et al. 2013); however, those previous studies typically report that earlier life stages (i.e., larvae and juveniles) are more susceptible to ocean acidification than later life stages. By contrast, the meta-analysis detected no significant effect on larvae. Although this may suggest that larvae are robust to ocean acidification behaviorally, it is important to note that the number of studies on larvae was low (n = 6) and the nonsignificant effect size may simply reflect a lack of ability to detect an effect.

Although negative effect sizes under elevated pCO$_2$ in this study were largely driven by pCO$_2$ values exceeding near-future (i.e., year 2100) projections, such drastic changes in pCO$_2$ are not uncommon in nearshore coastal and estuarine waters where many of the species assessed herein reside. Diel and seasonal variation in coastal pCO$_2$ conditions can often far exceed near-future projections (Provoost et al. 2010, Duarte et al. 2013, Waldbusser & Salisbury 2014, Wallace et al. 2014). Furthermore, given that ocean acidification studies typically acclimate animals under control and elevated pCO$_2$ conditions for relatively short periods of time (compared with the large timescales across multiple organismal generations in which true ocean acidification will occur; McElhaney 2017), the effect sizes herein are mostly applicable to acute exposures to elevated pCO$_2$ characteristic of nearshore systems. The analysis thus suggests that shellfish behavioral defenses in nearshore coastal systems may already be affected by acute increases in seawater pCO$_2$. Given that the magnitude and temporal duration of pCO$_2$ extremes are expected to increase under ocean acidification (Kwiatkowski & Orr 2018, Pacella et al. 2018), the results herein suggest that shellfish behavioral defenses for some taxa are likely vulnerable to pCO$_2$ variability under current conditions and near-future ocean acidification, particularly when pCO$_2$ conditions are elevated for periods of weeks to months.

Interpretive Caution

It is important to note for this section that this meta-analysis is based on a relatively small number of studies. Given the high degree of heterogeneity observed (i.e., Q-statistics; Table 3), the small number of studies makes definitive conclusions regarding effects difficult. Consequently, the null effects reported here may represent "true" null effects or may be a symptom of low sample sizes (i.e., an inability to detect an effect). Thus, the meta-analysis herein provides a quantitative synthesis of studies to date, and definitive conclusions regarding effects await further research.

A QUALITATIVE ASSESSMENT OF OCEAN ACIDIFICATION EFFECTS IN THE CONTEXT OF WARMING

Studies assessing the combined effects of ocean acidification and warming on shellfish prey defense behaviors were less common than acidification-only studies (n = 7 acidification–warming studies; 21% of all studies), with one reporting on a bivalve, five reporting on gastropods, and one on a malacostracan crustacean (Table 4). A meta-analysis of acidification–warming effects on behavioral prey defenses was not conducted, given the low number of available studies, and thus acidification–warming studies are described qualitatively. All acidification–warming studies used temperature increases consistent with near-future predictions (2°C–5°C), with the exception of Zhang et al. (2014), who used a temperature increase of 14°C (Table 4).

In the single study on bivalves, increased seawater temperature reversed the effect of sediment acidification on the burrowing behavior of juvenile *Mya arenaria* (Clements et al. 2017), whereas the single study on a malacostracan crustacean reported no effect of ocean acidification or warming on the self-righting response of adult *Hyas araneus* (Zittier et al. 2013). In gastropods, effects of ocean acidification and warming varied within and across species. For juvenile *Concholepas concolepas*, there was no effect of ocean acidification or warming on behavioral lateralization (i.e., right–left turning preference), movement time, or self-righting response (i.e., recovery in orientation after displacement); however, ocean acidification and warming acted synergistically, causing a net negative effect on decision time, whereas warming antagonistically reversed the effect of ocean acidification on route-finding ability

TABLE 4.

Summary of experimental studies assessing the combined effects of elevated pCO_2 and temperature on the behavioral prey defenses.

Reference	Species	Climate region	Life stage	Behavioral metric	Acclim. time	pCO_2 offset	Temperature offset	Effect of warming on ocean acidification response
Bivalves ($n = 1$)								
Clements et al. (2017)	*Mya arenaria*	Temperate	Juvenile	% burrowed	0	7,041	2	Antagonistic effect of warming on ocean acidification response
							5	Antagonistic effect of warming on ocean acidification response
Gastropods ($n = 5$)								
Domenici et al. (2017)	*Concholepas concholepas*	Tropical	Juvenile	Absolute lateralization	180	860	3	No effect of warming on ocean acidification response
				Movement duration				No effect of warming on ocean acidification response
				Decision time				Synergistic effect of warming on ocean acidification response (OA effect only under warming)
				Route finding				Antagonistic effect of warming on ocean acidification response
Manríquez et al. (2016)	*Concholepas concholepas*	Tropical	Juvenile	Self-righting time	90	860	4	No effect of warming on ocean acidification response
Quíeros et al. (2014)	*Nucella lapillus*	Temperate	Adult	Speed	425	164	3	Antagonistic effect of warming on ocean acidification response
Schram et al. (2014)	*Nacella concinna*	Polar	Adult	Escape speed	42	545	2	No effect of warming on ocean acidification response
	Margarella antarctica	Polar	Adult	Self-righting time	42	545	2	Warming reversed ocean acidification effect
				Escape speed				Warming reversed ocean acidification effect
Zhang et al. (2014)	*Nassarius festivus*	Tropical	Larvae	Swimming speed	1	499	14	Warming increased swimming speed; no ocean acidification effect
						894		Warming increased swimming speed; no ocean acidification effect
	Nassarius conoidalis	Tropical	Larvae	Swimming speed	1	499	14	Warming increased swimming speed; no ocean acidification effect
						894		Antagonistic effect of warming on ocean acidification response
Malacostracans ($n = 1$)								
Zittier et al. (2013)	*Hyas araneus*	Polar	Adult	Self-righting time	12	461	3	No effect of warming or acidification
						863		No effect of warming or acidification
						2,608		No effect of warming or acidification

Studies are ordered chronologically within each respective taxonomic class. Acclimation time is reported in days; pCO_2 is reported in μatm; temperature is reported in °C.

(Manríquez et al. 2016, Domenici et al. 2017). Under 500 and 900 µatm increases in $p\mathrm{CO}_2$, warming increased swimming speed in *Nassarius festivus*, whereas ocean acidification had no effect (Zhang et al. 2014). By contrast, ocean acidification significantly reduced the swimming speed of *Nassarius conoidalis*, and although warming did not impact the ocean acidification effect under the 500 µatm increase in $p\mathrm{CO}_2$, warming partially alleviated the effect of ocean acidification under an 894 µatm $p\mathrm{CO}_2$ increase (Zhang et al. 2014). Warming was also reported to reverse the ocean acidification effect on speed in adult *Nucella lapillus* (Quierós et al. 2014). For two Antarctic gastropods, warming did not influence the negative effect of ocean acidification on the escape speed of *Nacella concinna* but reversed the effect of ocean acidification on the escape speed and self-righting response in *Margarella antarctica* (Schram et al. 2014).

Although the low number of studies precludes any confident conclusions regarding the combined effects of ocean acidification and warming on shellfish behavioral defenses, it is clear that multiple stressor effects most often differ from single stressor impacts. Of the total number of species ($n = 8$) in the seven studies highlighted earlier, 75% ($n = 6$) exhibited different responses when both ocean acidification and warming were considered in combination when compared with ocean acidification considered alone; the combined effects were variable (Table 4). It is thus clear that the effect of ocean acidification on shellfish behavioral defenses can be influenced by seawater temperature and that future ocean warming is likely to influence the impact of ocean acidification on this behavioral response.

NEUROLOGICAL IMPAIRMENT AS A DRIVING MECHANISM

Recent studies have suggested a potential biological mechanism underpinning behavioral sensitivity to elevated $p\mathrm{CO}_2$: $\mathrm{GABA_A}$ neurotransmitter interference (see Tresguerres & Hamilton 2017 for review). In short, this hypothesis suggests that directional shifts in ionic gradients at $\mathrm{GABA_A}$ neuroreceptors can occur under elevated $p\mathrm{CO}_2$, which can ultimately drive increased neuronal depolarization and neural pathway excitation, resulting in altered animal behavior (Nilsson et al. 2012, Hamilton et al. 2014, Tresguerres & Hamilton 2017). To explore this hypothesis in relation to ocean acidification, studies often dose experimental animals with the $\mathrm{GABA_A}$ inhibitor gabazine. Gabazine effectively closes the $\mathrm{GABA_A}$ neuroreceptor, preventing neuronal depolarization and altered behavior; thus, if $\mathrm{GABA_A}$ interference is driving a behavioral alteration under elevated $p\mathrm{CO}_2$, one would predict that the behavior of animals treated with gabazine would be unaltered by elevated $p\mathrm{CO}_2$, whereas that of animals not treated with gabazine would be altered. Although numerous studies have demonstrated this mechanism in fishes (Nilsson et al. 2012, Chivers et al. 2014, Chung et al. 2014, Hamilton et al. 2014, Lai et al. 2015, Ou et al. 2015, Lopes et al. 2016, Regan et al. 2016), only a handful of studies have tested this hypothesis for shellfish taxa. In this section, these studies were assessed qualitatively (because of a low number of studies as with the aforementioned acidification–warming literature) to highlight the potential role of $\mathrm{GABA_A}$ interference in driven behavioral effects of ocean acidification in invertebrates.

The literature search uncovered three studies that empirically tested the "$\mathrm{GABA_A}$ hypothesis" on prey defenses in shellfish. Watson et al. (2014) first tested this hypothesis in the adult snail *Gibberulus gibberulus gibbosus* from Australia that "jump" away from predators to escape predation. Therein, it was reported that exposure to ocean acidification conditions negatively affected jumping ability in the snails, but that treatment with gabazine restored the jumping behavior under ocean acidification. Since then, this same result has been shown for burrowing responses in temperate bivalves (juvenile *Mya arenaria*; Clements et al. 2017) and malacostracans (adult *Corophium volutator*; Walsh 2018) to CO_2-driven sediment acidification. Walsh (2018) also tested this hypothesis on burrowing behavior for the adult snail *Tritia obsoleta*; however, the burrowing behavior of this species was unaffected by sediment acidification. Furthermore, although the direct effect of $\mathrm{GABA_A}$ neuroreceptor interference was not directly tested on a specific behavior(s), Moya et al. (2016) reported that genes related to $\mathrm{GABA_A}$ neuroreceptor structure and function in the pteropods *Heliconoides inflatus* were upregulated under elevated $p\mathrm{CO}_2$ (along with other genes associated with the nervous system).

These results suggest that $\mathrm{GABA_A}$ interference under elevated $p\mathrm{CO}_2$ may be an important driver in the behavioral responses of marine shellfish. Further investigation into the universality of this biological mechanism for altered behavior in shellfish and other invertebrates under ocean acidification is thus warranted. It is important to note, however, that altered $\mathrm{GABA_A}$ functioning is not the only biological mechanism that can drive ocean acidification effects on prey defense behaviors (and other behaviors) in shellfish. For example, morphological and other physiological effects of elevated $p\mathrm{CO}_2$ have the potential to affect performance and activity behaviors related to prey defense such as locomotion (Watson et al. 2017) and self-righting ability (Manríquez et al. 2013). Ultimately, although $\mathrm{GABA_A}$ interference appears to play a substantial role in ocean acidification effects on shellfish behavioral defenses, further research on this and other mechanisms are needed.

KNOWLEDGE GAPS AND CONSIDERATIONS FOR FUTURE RESEARCH

Multiple Stressors

Although some information pertaining to the interactive effects of ocean acidification and warming on shellfish behavioral defenses was gleaned, the combined effects of ocean acidification and other global change stressors are understudied. Of the 34 studies identified here, only one assessed the effects of ocean acidification in the context of a stressor other than temperature: Zhang et al. (2014) assessed the combined effects of $p\mathrm{CO}_2$, temperature, and salinity on the swimming performance of two gastropods, *Nassarius festivus* and *Nassarius conoidalis*. Therein, it was reported that elevated $p\mathrm{CO}_2$ reduced swimming speed in *N. conoidalis* (but not in *N. festivus*) and that higher temperature and higher salinity increased the swimming speed for both species.

Despite the lack of multistressor studies on shellfish behavioral defenses, it is widely known that responses to ocean acidification can depend on other stressors such as temperature, hypoxia and deoxygenation, salinity, and eutrophication, which can act synergistically or antagonistically with ocean acidification to drive biological responses (Pörtner 2008, Wallace et al. 2014, Breitburg et al. 2015). Food web structure and food supply are also considered to have impacts on biological responses to ocean acidification (Alsterberg et al. 2013, Ramajo et al. 2015, Goldenberg et al. 2018, Sswat et al. 2018). Adding to such complexities, ocean acidification can affect organismal affinity and selection for particular environmental conditions (e.g., temperature and salinity preferences as per Pistevos et al. 2017). Although not assessed in shellfish, studies have assessed ocean acidification effects in the context of some additional stressors on antipredator behaviors in fishes. For example, McMahon et al. (2018) reported that food availability had no influence on ocean acidification–induced effects on antipredator behavior in the juvenile clownfish *Amphprion percula*. By contrast, Miller et al. (2016) found that ocean acidification exposure led to increased surface ventilating behavior under low oxygen in congeneric fishes *Menidia menidia* and *Menidia beryllina*, which could potentially lead to increased vulnerability to aerial predators. Such complex effects highlight the importance of considering multiple global change stressors in predicting how the behavioral prey defenses of marine fauna will be impacted in a future ocean. When it comes to shellfish, more research is necessary, as research has yet to even scrape that surface.

Environmental Variability

As mentioned previously, coastal systems are often characterized by a high degree of spatial and temporal carbonate system variability (Provoost et al. 2010, Duarte et al. 2013, Waldbusser & Salisbury 2014, Wallace et al. 2014), which is likely to increase in the future (Kwiatkowski & Orr 2018, Pacella et al. 2018). From an organismal perspective, such variability is far from benign and can influence biological responses to ocean acidification in a number of potential ways (Helmuth et al. 2014, Waldbusser & Salisbury 2014, Bates et al. 2018). For example, a high degree of abiotic variability may confer tolerance to a wide range of environmental conditions in some coastal organisms, rendering them robust to relatively small baseline changes over long periods of time (i.e., many generations). Consequently, in coastal marine systems, $p\text{CO}_2$ variability is an important consideration in understanding biological responses to ocean acidification (Waldbusser & Salisbury 2014).

In the context of shellfish behavioral defenses, two of the 34 studies identified in this review assessed ocean acidification effects in the context of $p\text{CO}_2$ variability, reporting conflicting results. In a pioneering study, Alenius and Munguia (2012) showed that variable high $p\text{CO}_2$ conditions resulted in negative effects to locomotory behavior in the adult isopod *Paradella dianae*, whereas stable high $p\text{CO}_2$ conditions had no effect. By contrast, Jellison et al. (2016) reported no difference between stable and fluctuating CO_2 conditions on predator avoidance behaviors in the adult snail *Tegula funebralis*. Although the paucity of information regarding the role of $p\text{CO}_2$ variability (along with variability in other environmental parameters) in shaping the responses of shellfish behavioral defenses precludes firm conclusions, the contrasting results observed here underscore the importance and complexity of such variability.

Evolutionary Effects

An increasingly popular topic in contemporary ocean acidification research concerns the role of evolution and plasticity in biological responses to ocean acidification (Sunday et al. 2014, Calosi et al. 2016). Evolutionary forces such as local adaptation (Vargas et al. 2017) and natural selection (Thomsen et al. 2017), along with acclimatory processes such as transgenerational plasticity (Parker et al. 2012, 2015, Borges et al. 2018), can play important roles in shellfish responses to ocean acidification, both in isolation and in the presence of other co-occurring stressors (Gibben et al. 2017, Griffith & Gobler 2017). The literature search revealed no articles testing for evolutionary or acclimatory effects on shellfish behavioral defense responses to ocean acidification. Furthermore, a broader search of the literature suggested that evolutionary studies for invertebrate behaviors (in a broad sense) under ocean acidification remain absent (Ross et al. 2016), with the exception of a recent study reporting that transgenerational acclimation did not alleviate ocean acidification effects on mate guarding in the amphipod *Gammarus locusta* (males guard females from competing mates) (Borges et al. 2018).

Although evolutionary studies of shellfish behavioral defenses are not yet available, studies to this regard in fish may shed some light on potential effects in shellfish. For example, Welch et al. (2014) observed no evidence that transgenerational acclimation affected offspring behavioral responses (behavioral lateralization) to ocean acidification conditions in *Acanthochromis polyacanthus*. In a follow-up study on the same species, Welch and Munday (2017) demonstrated that although acute exposure to elevated $p\text{CO}_2$ elicited heritable behavioral phenotypes in response to predators, prolonged (four week) exposure to elevated $p\text{CO}_2$ negated the observed heritability under acute exposure. Ultimately, the influences of evolution and transgenerational plasticity on behavioral responses of shellfish to ocean acidification deserve attention, particularly given the variable impacts that these processes can have on shellfish responses to ocean acidification (Ross et al. 2016).

Ecological Complexity

The experiments reviewed herein suggest that ocean acidification can potentially influence behavioral defenses in shellfish prey, based primarily on laboratory observations of individual behaviors under control and elevated $p\text{CO}_2$ conditions. Predicting how and whether such changes might manifest themselves under natural conditions is difficult, however, because of the inherent situational and ecological complexity of nature (i.e., the breadth of other things that are occurring in natural systems). For example, individual personalities within a group of organisms can affect group-level responses to predation (Briffa et al. 2008). Similarly, immediate and prior exposures to the risk of predation (e.g., through predator-produced kairomones or through conspecific alarm cues) can influence behavioral prey defenses in shellfish (Hagen et al. 2002, Briffa 2013). Strikingly, of the studies reviewed herein, only 15% ($n = 5$ of 34) included predator cues in their experiments testing for effects of ocean acidification on prey defense behaviors (Bibby et al. 2007, Hatfield-Vaughan 2011, Manríquez et al. 2014, Watson et al. 2014, Jellison et al. 2016). This important situational caveat calls into question the transferability of many of these experiments to natural conditions. Studies including predator cues, however, have still reported significant ocean acidification effects on shellfish defense behaviors, suggesting that behavioral alterations can still affect predator–prey ecology in systems involving shellfish prey (Manríquez et al. 2014, Watson et al. 2014, Jellison et al. 2016).

Other nuances of natural biological communities can likely influence shellfish behavioral defense responses to ocean acidification as well. Recent studies have suggested that although ocean acidification may directly affect individual behaviors of organisms, increased diversity within biological systems may allow for compensatory processes to offset negative direct behavioral effects related to consumer foraging (Goldenberg et al. 2018; although they did note that increases in risky behavior under ocean acidification may make prey more vulnerable to predation). Ultimately, the situational conditions within complex ecological systems may impact shellfish behavioral defenses against predation in a high CO_2 ocean and thus warrant exploration. Furthermore, an understanding of how such behavioral changes influence ecological structure and function is required to understand and predict the ecological consequences of ocean acidification.

Functional Trade-offs

Engaging (or not engaging) in a particular defense behavior may not only affect the vulnerability of an organism to predation but can also result in functional trade-offs with other biological processes. For example, Bridger et al. (2015) reported that bolder male hermit crabs *Pagurus bernhardus* experience lower fecundity than less bold males. Consequently, if ocean acidification results in increased boldness and risk-taking behavior in a given species, not only would vulnerability to predation increase but fecundity may also decrease. Such additive negative effects could have major implications for the ecological functioning of biological communities. These interactions between biological processes may not always be additively negative, however. For example, although reduced burrowing capacity under sediment acidification (Clements & Hunt 2017) conditions can make bivalves more vulnerable to predation (Flynn & Smee 2010), this behavioral response also allows bivalves to avoid other mortality sources such as shell dissolution (Green et al. 2004, 2009). Ultimately, the complex trade-offs between defense behaviors and other biological processes that drive net fitness outcomes and the capacity of organisms to survive and persist under ocean acidification deserve attention.

CONCLUSIONS

This review ultimately suggests that controlled laboratory exposure to elevated $p\mathrm{CO}_2$ can affect prey defense behaviors in marine shellfish. Meta-analysis results suggested that bivalves and malacostracan crustaceans are likely vulnerable to ocean acidification effects on defense behaviors; however, effects on malacostracans may be contingent on the degree of $p\mathrm{CO}_2$ elevation, as negative effect sizes were primarily driven by abnormally high $p\mathrm{CO}_2$ conditions. A major shortcoming of most studies reviewed here, however, is that prey defense behaviors were not tested in the presence of predator cues (kairomones or prey alarm cues)—a component of predator–prey interactions that is necessary for realistic ecological predictions. Although more research is needed, a qualitative review of a handful of studies on the combined effects of ocean acidification and warming suggested that coincident increases in seawater temperature are important to consider when testing for ocean acidification effects in the context of long-term global change and coastal variability; the combined effects of ocean acidification and other stressors, and the effect of temporal and spatial environmental variability, require further research. Although many questions pertaining to ocean acidification effects on shellfish behavioral defenses remain to be tested, studies on fish may help streamline hypotheses and experimental approaches, particularly given the common theme of GABA_A interference across these widely different taxa. Given the importance of prey defense behaviors to the structure and function of marine biological communities, increased research efforts into this topic are warranted and would contribute to a more holistic understanding of the ecological effects of ocean acidification.

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DATA ACCESSIBILITY

Annotated R-script, all raw data files, and a complete dataset are available online from Mendeley Data at https://dx.doi.org/10.17632/vwtfrrtjym.2.

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