Variation in the behavioral tolerance of congeneric marine snails to low-pH exposure

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ABSTRACT: The ocean is acidifying, with multiple consequences for coastal organisms. However, species may differ in their sensitivities. Some taxa may find future conditions mildly stressful or even advantageous, while others will persist only through acclimatization or genetic adaptation. An open question is whether such variation in the sensitivity of species to ocean acidification relates to their evolutionary histories of exposure to reduced pH. Here, we explored this unknown through an investigation of differences in pH tolerance of 2 shoreline gastropods, the black turban snail *Tegula funebralis* and its congener, the brown turban snail *T. brunnea*. These species occupy distinct vertical distributions on rocky shores, each subject to different extremes in low pH. We assayed the extent to which low pH degrades the flight behavior of each species in response to the predatory sea star *Pisaster ochraceus*. Across a range of pH, the tidepool-inhabiting *T. funebralis* exhibited less behavioral disruption than *T. brunnea* at any given pH value but also experienced impairment at a pH characteristic of the present-day minimum in its habitat (7.1 pH). The latter pattern suggests *T. funebralis* operates near what may be a fixed tolerance limit to low pH. *T. funebralis* also exhibited hints of elevated intraspecific variation in its behaviors, which could imply scope for selection to act. Deeper-dwelling *T. brunnea*, in contrast, showed little sensitivity to present-day pH minima found where it lives (7.6 pH) and displayed less variation upon which selection might operate. These results provide a initial framework for investigations into the capacity of species of differing evolutionary histories to cope with future ocean acidification.

KEY WORDS: Climate change · Comparative study · Antipredator behavior · Ocean acidification · Tolerance thresholds · Tidepool · Invertebrates

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1. INTRODUCTION

The ocean is changing at an unprecedented rate, threatening organisms and the ecology of marine systems (Kroeker et al. 2010, Gaylord et al. 2015, Nagelkerken & Connell 2015). Among other perturbations, human-produced CO_2 that enters the ocean reduces seawater pH and alters concentrations of important ions in a process called ocean acidification (OA; Caldeira & Wickett 2003). OA can impair organism survival, growth, calcification, reproduction, and acid–base regulation in many marine taxa (Ries et al.

2009, Kroeker et al. 2010). Reduced pH is additionally known to disrupt the behavior of some species, with implications for ecological interactions (Briffa et al. 2012, Leduc et al. 2013, Clements & Hunt 2015, Wang & Wang 2020, but see Clark et al. [2020] and Clements et al. [2022] for criticism of some of this work in vertebrates). In marine snails, low pH attenuates anti-predator flight responses, increasing mortality while simultaneously altering foraging rates and indirect interactions in the food web (Watson et al. 2017, Jellison & Gaylord 2019, Froehlich & Lord 2020, Jellison et al. 2022).

A major unknown in OA research is which species will be most sensitive to shifts in ocean chemistry. One measure of a species′ sensitivity (and other measures are possible) is the extent to which the species is intolerant of emerging environmental changes and will need to rely on plastic acclimatization or genetic adaptation to cope with future conditions. An obvious first expectation might be that taxa with multigenerational exposure to low pH would be better equipped to deal with heightened acidification, and thus be less sensitive. However, alternative expectations, in some cases equally plausible, could also be advanced. Consider the analogy of thermal stress. Species that have evolved to inhabit hotter locations often exhibit greater rather than lesser sensitivity to future warming compared with congeners from cooler environments; a standard explanation is that the former operate closer to their acute, potentially immutable, physiological limits. Whether a similar trend holds for pH exposure remains an open question, in that we do not yet know whether species that evolved to live in locations subject to lower pH will exhibit greater or lesser sensitivity to predicted future OA conditions (Hofmann et al. 2014).

In this work, we take a first step towards exploring how closely related species with distinct long-term histories of pH experience respond to reduced pH. We focused on 2 shoreline gastropods, the congeners *Tegula funebralis* (the black turban snail) and *T. brunnea* (the brown turban snail). *T. funebralis* lives in the mid-intertidal zone of rocky coasts and often inhabits tidepools that become separated from oceanic waters. These transiently isolated water volumes undergo strong chemical modification due to photosynthesis, calcification, and respiration of tidepool residents, which causes dramatic diel shifts in seawater chemistry. Consequently, *T. funebralis* experiences widely fluctuating pH conditions with minima as low as 7.1 during nighttime low tides. *T. brunnea*, in contrast, occupies the low intertidal to subtidal zone of rocky coasts. Although pH also varies in this lower elevational band due to upwelling and biological activity, this species is unlikely to encounter pH values below 7.6 in the near future, as waters in its habitat freely exchange with those offshore so experience less dramatic chemical fluctuations (Hofmann et al. 2011, Feely et al. 2016, Chan et al. 2017).

Turban snails experience a range of negative impacts from OA (Jellison et al. 2016, Barclay et al. 2019), and we focus here on behavioral impairments that occur when seawater pH is low. Under such conditions, *T. funebralis* that encounter a cue from the keystone predator *Pisaster ochraceus* (the ochre sea star)

fail to flee the water. This degraded flight response leaves the snails more susceptible to consumption (Jellison et al. 2016, 2022, Jellison & Gaylord 2019). In contrast, snails in typical seawater conditions crawl quickly out of tidepools after encountering scent from sea stars, placing them in relative safety (Bullock 1953, Gravem & Morgan 2019). Considerably less is known about *T. brunnea* in the context of reduced seawater pH, but there is reason to anticipate it might react differently than *T. funebralis* given its limited historical exposure to low pH. We tested this possibility, asking whether these 2 snail congeners exhibit differential behavioral sensitivity to reduced pH. Our aim was to improve understanding of the extent to which species with alternative exposure histories will be forced to rely more strongly, or less so, on plasticity and adaptational responses to OA in coming decades. To test such relative sensitivity, we exposed individuals from each species to a range of pH levels spanning 8.0–6.4 (total scale; Zeebe & Wolf-Gladrow 2001) and measured their responses to a cue from *P. ochraceus*.

2. MATERIALS AND METHODS

2.1. Study species

Black turban snails *Tegula funebralis* are significant grazers of the mid-intertidal zone and within tidepools throughout their range (Nielsen 2001, Morgan et al. 2016), which extends from Vancouver Island, Canada, to Baja California, Mexico. Brown turban snails *T. brunnea* are important low-intertidal and subtidal grazers and are found on the coast of the USA from Cape Arago, Oregon, to the Channel Islands, California. Adult individuals of each of these species were sourced from Horseshoe Cove, in Bodega Bay, California, within the Bodega Marine Reserve (BMR) located adjacent to the Bodega Marine Laboratory (BML), where all experiments were conducted. Snails were collected in December 2014, June 2015, and August 2017 for use in 3 separate experimental trials that were then merged for the current study. All snails brought into BML were placed immediately into a flow-through seawater aquarium and were fed ad libitum for 14 d until experiments began. The sizes of individuals were standardized to ensure they were similar across species and trials (first trial *T. funebralis* diameter: 20.3 ± 0.3 mm, *T. brunnea* diameter: 20.7 ± 0.5 mm; second trial *T. funebralis* diameter: 19.7 ± 0.3 mm, *T. brunnea* diameter: 30.3 ± 0.3 mm; third trial *T. funebralis* diameter: 19.0 ± 1.8 mm, *T. brunnea* diameter: 19.3 ± 1.6 mm).

2.2. Habitat pH

Seawater conditions within rock pools and in adjacent shoreline environments were characterized using a DuraFET-style sensor and discrete bottle samples, respectively (see Chan et al. 2017, Silbiger & Sorte 2018 for characterization of geographic variation in seawater conditions within these environments). Further details can be found in Jellison et al. (2016) and Gaylord et al. (2018), but in brief, the DuraFET sensor was deployed in the low intertidal zone within the BMR and recorded pH every 10 min (total scale; Zeebe & Wolf-Gladrow 2001). The device was attached to the rock within a protective housing at +0 m mean lower low water, and the active element of the sensor was encased by a copper cage to minimize biofouling. Per standard protocols, the sensor was calibrated in the laboratory before and after deployment using a Tris buffer (2 amino-2-hydroxymethyl 1, 3-propanediol in synthetic seawater, certified reference material, A. Dickson, Scripps Institution of Oceanography) while immersed in a 25°C water bath. For tidepool measurements, A YSI ProPlus sensor was used to determine *in situ* pH, temperature, salinity, and dissolved oxygen hourly during a low tide over 4 consecutive nights. Measurements were collected from 12 rock pools in the mid to high intertidal zone within the BMR. These YSI pH measurements were validated by means of discrete bottle samples that were taken from each pool during the first and last sampling periods. Bottle samples were analyzed for pH and total alkalinity, respectively, via a JAZ spectrophotometer (Ocean Optics) and using Gran titration (Riebesell et al. 2010).

2.3. Experimental overview

Once acclimated to laboratory tanks fed by a common seawater supply drawn from subtidal waters immediately adjacent to BML, individuals of both species were exposed, in three 6 d trials, to each of 3–15 reduced-pH exposure regimes spanning a pH range of 8.0–6.4 on the total scale (Zeebe & Wolf-Gladrow 2001). This exposure period was implemented, as prior work has shown that pH-induced behavioral modifications in invertebrates is most evident following several days of exposure to low-pH seawater (Watson et al. 2013). Following the 6 d exposure periods, the snails were assayed for effects of low pH on their antipredator flight responses, initiated by exposure to dissolved cue

from the ochre sea star *Pisaster ochraceus*. Because the 3 trials were conducted alongside other research efforts, it was necessary to use slightly different sampling schedules, levels of replication, and pH treatment levels in each; implications of these differences are discussed below (and see Appendix Text A1 for further details). Also note that the match of experimental conditions to those characterizing the habitats of the 2 species differed for each in important ways; we elaborate on this subject in Section 4.

2.4. Seawater treatments

Throughout the pH exposure phase (3–15 levels of pH for 6 d) in each of the 3 trials, sets of snails of each species were each held separately in 13 l treatment containers (first trial, n = 1 snail container^{-1}; second trial, n = 2; third trial, n = 3). Seawater pH was manipulated via equimolar addition of sodium bicarbonate and hydrochloric acid, a method that increases dissolved inorganic carbon without changing alkalinity, thus duplicating changes induced by bubbling CO_2 into seawater (Hurd et al. 2009, Schulz et al. 2009, Riebesell et al. 2010, Jellison & Gaylord 2019). Water in each container was replaced daily, ensuring stable pH treatments, maintained via chemical modification throughout the experiment. Containers were held within a flow-through water table to maintain consistent temperature throughout all trials.

Values of pH, temperature, salinity, and dissolved oxygen were measured daily in each container before and after water changes using a YSI ProPlus sensor (Table 1). Discrete water samples were also collected from individual containers across the suite of treatment levels each day for analysis of pH (total scale; Zeebe & Wolf-Gladrow 2001) via a JAZ spectrophotometer (Ocean Optics). These latter samples were then used to re-calibrate the YSI to the total scale (Zeebe & Wolf-Gladrow 2001). Additional water samples were also acquired from containers for analysis of total alkalinity via Gran titration (Riebesell et al. 2010).

2.5. Behavioral assay

After the 6 d pH exposure period for each trial, snail responses to a cue from the predatory sea star *P. ochraceus* were examined. The antipredator responses were assayed by noting the fraction of photographs within a sequence (1 photograph approxi-

Table 1. Seawater parameters for each treatment level averaged over the 6 d exposure period for each trial of the experiment with *Tegula funebralis* and *T. brunnea*. Alk: alkalinity; sw: seawater; SAL: salinity; DO: dissolved oxygen

Trial	Treatment level	pH_T	±SE	Alk ($\mu mol\ kg^{-1}$ sw)	±SE	Sal (ppt)	±SE	Temp (°C)	±SE	DO (%)	±SE
1	1	7.93	0.01	2187	6	32.3	0.04	15.1	0.0	98.5	0.3
	2	7.19	0.01	2190	6	32.3	0.04	15.1	0.0	97.8	0.2
	3	6.34	0.01	2195	6	32.3	0.04	15.1	0.0	97.7	0.2
2	1	7.72	0.02	2219	3	33.7	0.01	13.1	0.1	97.8	1.0
	2	7.66	0.02	2216	4	33.7	0.01	13.0	0.2	97.9	0.9
	3	7.60	0.02	2226	3	33.7	0.01	13.1	0.2	97.2	0.6
	4	7.49	0.02	2234	6	33.7	0.01	13.1	0.2	98.5	0.5
	5	7.36	0.02	2233	4	33.7	0.01	13.0	0.1	96.9	0.6
	6	7.24	0.02	2239	4	33.7	0.01	13.0	0.2	97.4	0.3
	7	7.12	0.02	2235	5	33.7	0.01	13.0	0.2	96.7	0.3
	8	7.02	0.01	2239	5	33.7	0.01	13.0	0.2	97.2	0.3
	9	6.93	0.01	2249	7	33.7	0.01	12.9	0.2	97.0	0.3
	10	6.84	0.01	2245	6	33.7	0.01	13.0	0.2	96.6	0.2
	11	6.74	0.01	2236	5	33.7	0.01	13.0	0.2	96.8	0.3
	12	6.65	0.01	2241	6	33.7	0.02	13.1	0.2	96.2	0.5
	13	6.55	0.01	2252	8	33.7	0.01	13.1	0.2	96.8	0.2
	14	6.45	0.01	2257	8	33.7	0.01	13.0	0.1	96.8	0.3
	15	6.34	0.01	2257	10	33.7	0.01	13.1	0.2	96.9	0.2
3	1	7.72	0.03	2230	2	34.1	0.03	14.2	0.2	98.6	0.7
	2	7.45	0.03	2226	6	34.1	0.02	14.2	0.2	98.9	1.3
	3	7.16	0.03	2234	8	34.1	0.02	14.2	0.2	98.0	0.7
	4	6.86	0.01	2177	57	34.1	0.02	14.3	0.2	98.2	0.8
	5	6.58	0.01	2226	18	34.1	0.02	14.3	0.2	98.1	0.7

mately every 3 min) that showed snails above the waterline after they experienced seastar cue (first trial, n = 16 min sequence; second trial, n = 15; third trial, n = 16). This water-exiting behavior places snails in a spatial refuge that eliminates the threat of predation. During the behavioral assessment, each snail container was filled with 2 l of seawater adjusted to the same pH used during the preceding exposure period. Snails were placed in the center of the container and 1 l of either cue or no-cue treatment water was added by gently pouring it into the container. Cue water was obtained by placing 10 sea stars into an oxygenated and well-mixed 50 l tank filled with ambient seawater for approximately 2 h before the behavioral assay. The pH of the cue and no-cue water was set via chemical modification to match that of the corresponding snail container.

2.6. Statistical analysis

To determine differences in how avoidance behaviors of the congeneric snails changed with reductions in pH, a generalized linear mixed effects model (binomial distribution, logit link function, 'lme4' package [Bates et al. 2015] in the statistical software R v.4.1.2 [R Development Core Team 2011]) was used with Species, pH, Cue, pH × Cue and Species × pH as independent variables. Snail and Container were included as random intercepts to account for repeated measures on an individual, and Trial was included as a random effect. Scaled residuals were inspected to verify assumptions for the generalized linear mixed effects model using the 'DHARMa' package (Hartig 2022) in R v.4.1.2.

An examination was also made of potential differences in the level of intraspecific variation in antipredator response occurring across the 2 species. The focus here was on levels of variation in the behavioral response within a pH band centered on the present-day pH minima of each species' habitat. The absolute values of the residuals from the statistical model were selected from a pH range that spanned 0.2 units below to 0.2 units above the habitat minimum for each species (*T. funebralis* pH range: 6.9–7.3; *T. brunnea* pH range: 7.4–7.8). Residuals were calculated as the difference between the proportion of time an individual snail spent out of the water and the predicted proportion from the generalized linear model at that pH level.
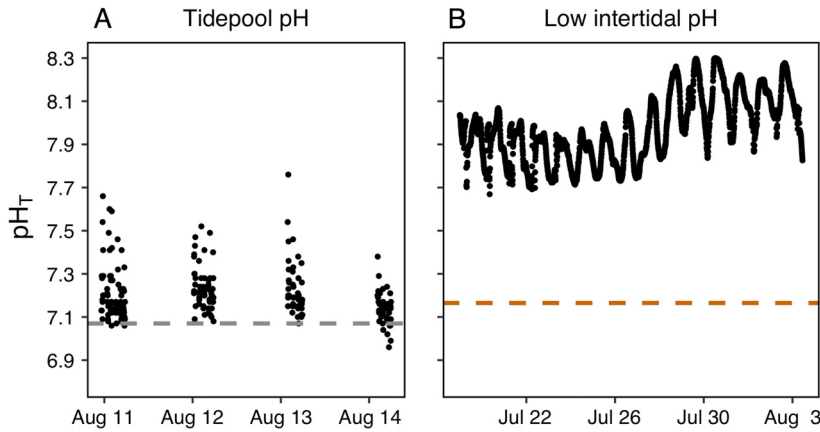
A Tidepool pH

B Low intertidal pH

Fig. 1. Representative records of pH (total scale) in habitats occupied by the congeneric snails *Tegula funebralis* and *T. brunnea*. (A) Values of pH in tidepools where *T. funebralis* often lives can decline to below 7.1 during nighttime low tides when the pools are isolated from oceanic waters (values shown are from discrete bottle samples; not depicted are pH maxima during daytime low tides when positive excursions occur due to carbon uptake by photosynthetic organisms). (B) Low-intertidal environments inhabited by *T. brunnea* where pH minima are more modest (minima pH of 7.6) due to the ready exchange of water with the surrounding ocean. The latter records, collected as autonomously sampled time series, were acquired within the Bodega Marine Reserve during July and August 2016. Dashed horizontal lines: pH at which the efficacy of antipredator responses declines to 50% of maximum for (A) *T. funebralis* (grey dashed line) and (B) *T. brunnea* (brown dashed line) based on a generalized linear mixed effects model applied to data of this study
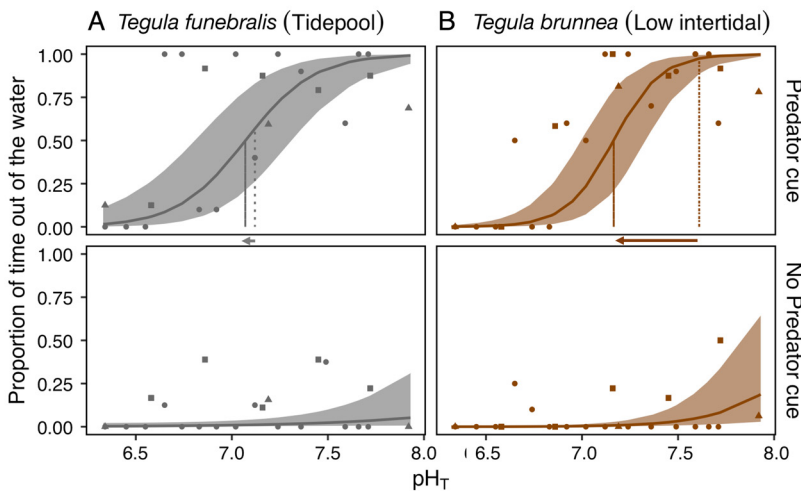

Fig. 2. Behavioral responses induced by low pH in (A) *Tegula funebralis* (grey) and (B) *T. brunnea* (brown) with predator cue (top panel) and without predator cue (bottom panel). When exposed at ambient pH to predator cue (top panels, ambient pH at right side of *x*-axes), both species exited the water. In contrast, under low pH (left side of *x*-axes), both species demonstrated attenuated antipredator responses. Dotted lines: predicted antipredator behavior at the present-day pH minimum for each species (*T. funebralis*: 7.1; *T. brunnea:* 7.6); vertical solid lines: pH at which the efficacies of the antipredator responses decline to 50% of baseline. Horizontal arrows below the top panel: change in habitat pH from the current pH minima to the pH necessary to see such a 50% decline in efficacy. Points: average data from individual containers; symbols: experimental trial (squares: trial 1; circles: trial 2; triangles: trial 3). Solid curved lines: expected trends based on a generalized linear mixed effects model; shaded regions: 95% CI

3. RESULTS

3.1. Habitat pH conditions

Tegula funebralis and *T. brunnea* occupy distinct vertical distributions: the mid-intertidal zone and tidepools for the former versus the low-intertidal to subtidal zones for the latter. Measurements of pH within these habitats during July and August of 2016 revealed differences in the low-pH conditions that occur in these 2 locations. In tidepools often occupied by *T. funebralis*, pH levels dropped as low as 7.1 during nighttime low tides when respiratory carbon accumulated in isolated tidepool waters (Fig. 1A). For *T. brunnea*, which lives in the subtidal to low-intertidal zone, pH values reached only as low as 7.6, typically during summer upwelling (Fig. 1B).

3.2. Tolerance thresholds

Tegula congeners demonstrated differences in their behavioral tolerance to low-pH exposure (Fig. 2, Table A1 in the Appendix). Three patterns are apparent. First, *T. funebralis* experienced behavioral impairment even at the present-day pH minimum of its habitat (pH 7.1, total scale). Although such extremely low pH values occur only episodically in the environments in which *T. funebralis* lives, this finding suggests at least transiently elevated susceptibility to predation by sea stars in this species. *T. brunnea*, in contrast, exhibited negligible behavioral impairment under its present-day pH minimum (pH 7.6). The latter trend arose largely because of the less extreme pH conditions characteristic of this species' habitat. Second, if a 50% failure rate in anti-predator behavior is viewed as an important threshold, then *T. funebralis* is likely to cross this threshold with a smaller additional decrease in pH than *T. brunnea*. In the former species, an additional 0.1 decline in pH (down to 7.0)

would pull it below the 50% failure rate, while *T. brunnea* could withstand a decline of 0.4 in minimum habitat pH before this happens (down to 7.2). Third, although pH values in habitats occupied by *T. brunnea* would have to decrease further to generate widespread behavioral disruption, the pH response curve is steeper for *T. brunnea* compared to *T. funebralis*, suggesting the former would experience a greater change in response performance as pH declines.

3.3. Variability differences

Intraspecific variation in traits is the fodder for natural selection when those traits are heritable. Thus, an important consideration is how much variation might be available for selection to operate on, focusing especially on present-day conditions surrounding the habitat pH minimum for each species. In the 2 *Tegula* congeners of this study, individual variation in anti-predatory responses of snails was higher in *T. funebralis* compared to *T. brunnea*. Specifically, the average absolute deviation in the behavioral failure rate away from predicted levels was 0.36 for *T. funebralis* in the band of pH values within ±0.2 units of the habitat minimum (Fig. 3). This deviation value for *T. funebralis* was 3 times the analogous number for *T. brunnea*, which was 0.12 (across the whole range of
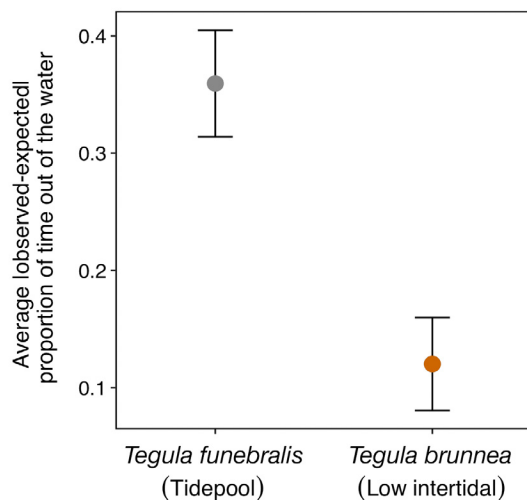

Fig. 3. Phenotypic variation at the minimum habitat pH for *Tegula funebralis* compared to *T. brunnea*. Points: average absolute value of the residuals from the generalized linear model within a 0.4-unit wide pH band surrounding the habitat minimum, computed for each species (*T. funebralis* pH range: 6.9–7.3; *T. brunnea* pH range: 7.4–7.8). Residuals were calculated as the difference between the proportion of time an individual snail spent out of the water and the predicted proportion from the generalized linear model at that pH level (*T. funebralis*, n = 21; *T. brunnea*, n = 22). Error bars: ±SE

experimental pH the average absolute deviation away from predicted values was 0.28 ± 0.03 for *T. funebralis* and 0.22 ± 0.03 for *T. brunnea*).

4. DISCUSSION

OA is likely to alter behaviorally mediated species interactions, and the severity of these effects will be modified by the sensitivity of the interacting taxa (Briffa et al. 2012, Kroeker et al. 2014). Here, we considered sensitivity through the lens of a species needing to rely on acclimatization or genetic adaptation. Using 2 rocky shore congeners, we demonstrated that subtidal snails *Tegula brunnea* are less tolerant in their antipredator behavior to any particular value of low pH than their intertidal counterparts, *T. funebralis*. These results parallel previous findings for temperature tolerance differences in heart function and survival for these 2 species (Stenseng et al. 2005, Somero 2010). At the same time, if behavioral responses of the 2 species are considered relative to present-day pH conditions within the species' respective habitats (*T. funebralis* pH minimum: ~7.1; *T. brunnea* pH minimum: ~7.6), the subtidal snail *T. brunnea* appears more tolerant to emerging changes in seawater chemistry than its intertidal counterpart. That is, pH conditions for *T. brunnea* can decline from the current minimum of its preferred habitat by 4 times as much as that for *T. funebralis* before the same extent of behavioral impairment occurs (a decline in minimum habitat pH from 7.6–7.2 for *T. brunnea* versus a decline from 7.1–7.0 for *T. funebralis*). These combined results suggest an understanding of both exposure history and habitat context will be paramount in anticipating whether and how much marine organisms will need to rely on plastic responses or genetic adaptation to cope with future pH stress.

It is tempting to attribute the differences in absolute pH sensitivity across the *Tegula* congeners to differences in the characteristic pH extremes of their preferred habitats. Since *T. funebralis* can live in mid-intertidal rock pools where extreme pH variation occurs when the pools are isolated at low tide, it is possible that this species has evolved to be adapted physiologically to a broader range of pH. Such a perspective would be consistent with other studies that have found different sensitivities in congeners originating from environments with distinct exposure histories to low pH (Kurihara et al. 2004, Frieder et al. 2014, Zhang et al. 2014, MacLeod & Poulin 2015, Hamilton et al. 2017). However, other studies demonstrate analogously large differences in pH tolerance

between sympatric species, which makes broad conclusions limited (Ferrari et al. 2011a,b, Couturier et al. 2013, Frieder 2014, Hardy & Byrne 2014, Hall et al. 2015). Additionally, it is difficult to attribute differences in pH tolerance to exposure history by comparing only 2 related species. Other environmental conditions and life history characteristics associated with habitat pH could differ between these congeners, making it challenging to ascertain causal relationships. We also note that the static pH exposures we employed to examine consequences of intertidal seawater conditions are incomplete. The lowest pH levels occurring in intertidal rock pools arise during nighttime low tides that last a few hours, after which the tide climbs and pH levels rise to match oceanic waters. These cyclic pH conditions were not duplicated in our treatments. That said, Jellison et al. (2016) implemented temporally varying pH conditions that more closely resemble those present in tidepools and documented behavioral responses in *T. funebralis* that were similar to those manifested by this snail under static pH exposures.

Our study did not strive to isolate the physiological origin of the pH responses and their differences across species. However, some clues may exist in other works. Previous studies on marine fish and a few invertebrates have implicated altered ion channel function and disrupted neuronal processes as a mechanism for OA-induced impairments in behavior (Briffa et al. 2012, Nilsson et al. 2012, Watson et al. 2013, Tresguerres & Hamilton 2017, Heuer et al. 2019, Williams et al. 2019, Thomas et al. 2021). Although intense discussion of some of these investigations continues (Clark et al. 2020, Clements et al. 2022), this prior research suggests that marine vertebrates increase bicarbonate and decrease chloride ion levels under low-pH conditions to buffer against changes within internal fluids (Nilsson et al. 2012, Hamilton et al. 2014, Thomas et al. 2021). Resultant changes to internal chemistry would then help combat acidosis. At the same time, the underlying shifts in ion balance might also interfere with proper functioning of γ-aminobutyric acid type A (GABA$_A$) receptors (Nilsson et al. 2012, Hamilton et al. 2014, Heuer et al. 2019, Zlatkin & Heuer 2019, Thomas et al. 2021). These models for behavioral impairment have garnered appreciable support in the literature, although we also note that they remain incompletely tested, with indications that other processes such as chemical alterations to the biochemical structure of cue molecules should also be considered, especially when focusing on a breadth of marine groups including invertebrates (Roggatz et al. 2016, Tresguerres &

Hamilton 2017, Porteus et al. 2021, Schirrmacher et al. 2021).

Even without complete knowledge regarding physiological drivers of behavioral impairment, insights into the potential impacts of future acidification are possible. As noted, although our results revealed that subtidal snails are more sensitive in an absolute sense to pH stress than their intertidal counterparts, they are less susceptible in the context of present-day conditions. Thus, it may be that changes to the chemistry of the ocean might first alter the predation vulnerability of intertidal snails. In particular, *T. funebralis* demonstrates a 50% reduction in the efficacy of its anti-predator behavior at pH levels just below those it encounters currently (Fig.1) (Truchot & Duhamel-Jouve 1980, Jellison et al. 2016, Kwiatkowski et al. 2016, Silbiger & Sorte 2018). Similar results have been seen among intertidal and subtidal congeners of *Tegula* in their tolerance to heat stress (Tomanek & Somero 2000, Stenseng et al. 2005, Somero 2010). Such results highlight the importance of evaluating tolerance limit assays within the context of current and predicted habitat conditions (Cheng et al. 2017).

Future implications of pH declines for *T. brunnea*, in terms of its vulnerability to predation, should be inferred only with caution. We used an index of behavioral impairment in our study that, while directly applicable to *T. funebralis* and easily operationalized for both species, has limited direct relevance to the life history of *T. brunnea*. Unlike *T. funebralis*, which has ready access to spatial refuge above the waterline, most *T. brunnea* live at sufficient depth that little opportunity for fleeing the water exists. Additional work is therefore warranted to explore the sphere of behavioral responses *T. brunnea* might actuate when encountering a broader array of predators, and the way in which changes in pH could alter such reactions.

In our study, *T. funebralis* demonstrated impaired behavior near present-day field conditions (Fig. 2) and also substantial intraspecific variation compared to *T. brunnea* at those pH conditions (Fig. 3). These trends hint at the potential for selection to operate in this species; such capacity is less apparent for *T. brunnea*. However, it remains important to determine whether the variable behavioral responses in *T. funebralis* under low pH derive mostly from phenotypic plasticity or whether heritable standing genetic variation also contributes (see Gleason & Burton 2013 for an example of local adaptation to temperature stress in this species). Careful assessment of the strength of selection due to low pH is also required.

In the case of *T. brunnea*, additional information is likewise required regarding plasticity, while the lower level of intraspecific variation in this species' pH sensitivity suggests that longer-term changes due to OA could be harder to combat through adaptation and might therefore eventually have a greater impact in this taxon than in *T. funebralis* (although we again emphasize the caveat that a water-exiting flight response has uncertain bearing in this species). We additionally note that the minor differences in experimental protocol across the 3 trials of our study (i.e. in the timing of sampling, the number of pH treatment levels, and the quantity of replication; Appendix Text A1) warrant consideration. Although we accounted statistically for these differences via inclusion of a trial-level random effect in our model, trial-specific factors could, in theory, have operated.

In summary, we documented divergent responses across congener species in their pH-induced behavioral disruption, which implies differences in the degree to which plastic responses or genetic adaptation will be required to cope with future OA. The tidepool-inhabiting *T. funebralis* has a multigenerational history of encountering challenging pH conditions and thus may already be selected to deal with variable and low-pH minima. Indeed, *T. funebralis* demonstrates increased capacity to withstand present-day acidification levels compared to its subtidal congener. However, *T. funebralis* may actually be more vulnerable to future acidification given that its antipredator behavior is impaired at values just slightly below the present-day pH minimum of its habitat. At the same time, even though this species operates close to its apparent physiological threshold, signifying a reduced scope for plastic or adaptational change, it also demonstrates increased intraspecific variation near its minimum habitat pH compared to *T. brunnea*. Thus, *T. funebralis* may actually have an expanded capacity for selection to improve low-pH tolerance across generations. Further work is necessary to elucidate mechanisms, population-level variation, and heritability of pH-induced behavioral vulnerability, as well as the potential of long-term plastic acclimatization; however, these results provide a beginning framework for wider insight into the capacity of marine species to surmount looming thresholds of pH sensitivity.

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Appendix. Generalized linear mixed effects model results and a description of protocol differences across trials

Table A1. Summary results for a generalized linear mixed effects model testing the effects of Species (*T. funebralis* or *T. brunnea*), Cue, and pH on the anti-predator behavior of snails. The lowest pH level was subtracted from all pH levels to adjust the intercept for interpretation (lowest pH level interpreted as intercept). In addition, the intercept is 'no cue' and *T. funebralis*. Trial, Snail and Container were included as random effects and estimates are in log odds. Thus, invlogit(Intercept) is the expected proportion of time out of the water for *T. funebralis* snails exposed to no cue, and invlogit(Intercept+Cue) is the expected proportion of time out of the water for *T. funebralis* snails exposed to cue at the lowest pH level tested. Note that invlogit(y)=exp(y) / [1 + exp(y)]. **Bold** indicates parameters with a statistically significant effect ($p < 0.05$)

Response variable	Predictor	Estimate	±SE	Wald Z	p
Proportion of time out of the water	**Intercept**	**−6.00**	**1.09**	**−5.49**	**<0.001**
	pH	1.93	1.04	1.85	0.06
	Species	**−2.69**	**1.23**	**−2.18**	**<0.001**
	Cue	**1.77**	**0.50**	**3.58**	**<0.001**
	pH × Species	**2.56**	**1.32**	**1.94**	**0.05**
	pH × Cue	**3.71**	**0.61**	**6.11**	**<0.001**

Text A1. Protocol differences across trials

As noted in the main text, because the 3 trials of the study were conducted alongside other research efforts, it was necessary to use slightly different protocols across the 3 trials. Despite these differences, the character of the data sets and the questions they were designed to address were the same. Thus, the following specific details are included here primarily for completeness. In the first, second, and third trials, there were 1, 2, or 3 snails container^{-1} respectively. In the first and third trials, no-cue and cue water assays were run consecutively on each container, and water changes were conducted between the assays. In the first trial, 3 pH levels were utilized, and there were 4 containers per pH level by species combination (n = 4 snails per pH × Species × Cue combination). In the second trial, 15 pH levels were used, and there were 2 containers per pH level by species combination (n = 2 snails per pH × Species × Cue combination). In the third trial, 5 pH levels were used, and there were 2 containers per pH by species combination (n = 6 snails per pH × Species × Cue combination). In the first trial, exiting behavior was noted within a 16 min sequence every 2 min. In the second trial, exiting behavior was analyzed from a 15 min sequence (1 photograph every 3 min) and in the third trial, snail behavior was determined from a 16 min sequence (1 photograph every 4 min).