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Ocean acidification alters the response of intertidal snails to a key sea star predator

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Organism-level effects of ocean acidification (OA) are well recognized. Less understood are OA's consequences for ecological species interactions. Here, we examine a behaviourally mediated predator–prey interaction within the rocky intertidal zone of the temperate eastern Pacific Ocean, using it as a model system to explore OA's capacity to impair invertebrate anti-predator behaviours more broadly. Our system involves the iconic sea star predator, *Pisaster ochraceus*, that elicits flee responses in numerous gastropod prey. We examine, in particular, the capacity for OA-associated reductions in pH to alter flight behaviours of the black turban snail, *Tegula funebralis*, an often-abundant and well-studied grazer in the system. We assess interactions between these species at 16 discrete levels of pH, quantifying the full functional response of *Tegula* under present and near-future OA conditions. Results demonstrate the disruption of snail anti-predator behaviours at low pH, with decreases in the time individuals spend in refuge locations. We also show that fluctuations in pH, including those typical of rock pools inhabited by snails, do not materially change outcomes, implying little capacity for episodically benign pH conditions to aid behavioural recovery. Together, these findings suggest a strong potential for OA to induce cascading community-level shifts within this long-studied ecosystem.

1. Introduction

Approximately one-third of the carbon dioxide emitted through human activities enters the ocean, driving 'ocean acidification' (OA), a phenomenon characterized by increased aqueous CO_2, reduced seawater pH and decreased availability of carbonate ions [1,2]. Considerable work now indicates that such changes induce physiological or demographic responses in a broad array of marine taxa [3]. Less understood are the wider ecological implications of OA, though, from principles established over decades, it is clear that numerous and varied outcomes are possible [4].

Among the more striking ecological effects of OA that have been documented are those influencing predator–prey interactions. Multiple species of fish, for example, fail to properly process waterborne cue information when confronted with the scent of predators [5,6]. Such impaired behaviours appear to originate from altered neurotransmitter and ion channel function under conditions of reduced seawater pH [7–9]. Fewer studies have explored the potential for analogous effects in invertebrates, although limited evidence suggests that perturbed neuronal function under OA may arise quite generally [10–14].

The capacity for OA to shift interactions between predators and their prey has widespread relevance to many marine ecosystems. This capacity may reverberate especially strongly in intertidal rocky-shore environments where some of ecology's most seminal studies on consumer–resource dynamics have been conducted. For instance, Paine's classic research on keystone predation demonstrated the critical role of the ochre sea star, *Pisaster ochraceus*, in removing competitively dominant mussels that would otherwise exclude a diversity of other macrofauna from rock surfaces [15]. Accompanying research by Paine likewise revealed an

asynchronous relationship between *P. ochraceus* and an important intertidal grazer, *Tegula funebralis* (the black turban snail) [16], one of the most common and familiar herbivores found on emergent substrate and within rock pools in wave-sheltered locations along the west coast of North America [17]. Although *T. funebralis* operates as a less-preferred prey for *P. ochraceus*, and often comprises a minor component of its diet, *Pisaster* can eat as much as a quarter of a given population of black turban snails per year in areas where the species overlap [18].

The *Pisaster–Tegula* interaction has additionally served for many years as a model system for exploring the dramatic flight responses exhibited by numerous intertidal invertebrates when confronted with cues from sea star predators. Multiple taxa of turban snails, top snails, abalone, periwinkles, whelks and limpets react to chemical cues released by sea stars by initiating marked avoidance behaviours [18–21]. In the case of black turban snails in rock pools, they crawl quickly to and up the sides of these pools until they exit the water [18]. Through this behaviour, *Tegula* experiences increased refuge from predation, because sea stars rarely leave the water to forage [22]. Changes in snail behaviour also have the potential to influence densities of algal species consumed by *Tegula*, both because such changes could influence snail foraging activities, and because altered flight responses could increase mortality of snails and thus how many might graze on algae (i.e. behavioural shifts could modify indirect effects of *Pisaster* on basal taxa through either 'non-consumptive' or 'consumptive' pathways [23,24]).

These types of cue-mediated interactions between sea stars and gastropods, and their possible interruption under OA, also interlace with patterns of ocean chemistry in shoreline environments. Even under present-day conditions, organisms along much of the west coast of North America experience, due to oceanographic upwelling processes [25], seawater carbonate chemistry similar to that predicted for open-ocean environments in the coming century (i.e. pH minima of 7.7 or lower; figure 1*a*). Intertidal rock pools—including those inhabited by *T. funebralis* and *P. ochraceus*—routinely encounter further extremes in pH that layer onto upwelling-induced pH depressions, as respiratory carbon from resident organisms accumulates at night in pools that are isolated from the adjacent ocean during low tides [26,27]. In intertidal pools along the coast of California, for instance, pH minima can fall to at least 7.2 during night-time low-tide series (figure 1*c*; [26]), and perhaps down to 6.9 on occasion (Y. Takeshita *et al.* 2015, unpublished data), approaching the lows of 6.5 seen in other systems [30]. Such low-tide series can extend for 5 days or longer, and occur numerous times a year. Trends such as these suggest that rock pools could provide a valuable window into the future for understanding OA-induced changes in predator–prey interactions.

Here, we focus on the flight responses of black turban snails to *P. ochraceus*, using this interaction as a case example for exploring the relevance of OA to coastal invertebrate predator–prey relationships more broadly. We conduct our work not only within the general framework of OA, but also with an eye to current patterns of environmental variability characteristic of intertidal rock pools. To increase the utility of our findings for anticipating future outcomes, we quantify the full behavioural 'response function' of *T. funebralis* to seawater acidification, examining behavioural performance across 16 discrete levels of pH that encompass present-day extremes as well as values expected for rock pools by the
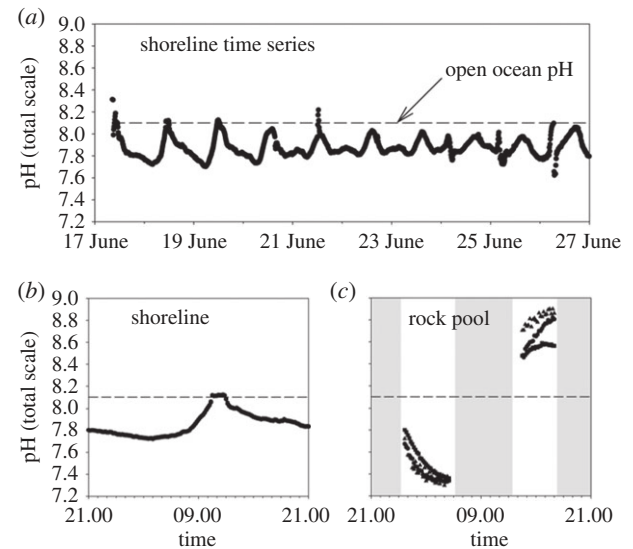
Figure 1. Representative records of pH in shoreline habitats and intertidal rock pools (total scale; [35]) within the Bodega Marine Reserve (BMR) during June 2014. (*a*) Time series of pH in shoreline waters that exchange freely with the surrounding ocean. Note the depression of pH values (solid line), owing to upwelling, relative to the present-day, global-average pH of open-ocean surface waters (dashed line; pH 8.1) [25]. (*b*) Example 24 h record of shoreline pH for which the daily low dropped to 7.7. As is apparent in (*a*), such regional deviations from global-average conditions occur routinely. (*c*) Example 24 h record of fluctuations in pH as amplified within rock pools that are chronically isolated from adjacent oceanic waters during low tides (immersed and emergent periods are shown with shading or no shading, respectively; various symbols depict data from different pools). Minima in pH arise owing to night-time accumulation of respiratory carbon within a confined water body, whereas pH maxima occur during daytime low tides owing to photosynthetic uptake of seawater carbon. Because of monthly and seasonal changes in the timing of low tides, such extreme negative and positive excursions in pH do not always follow one another but can occur independently (e.g. night-time low tides can happen without accompanying ones in the daytime, and vice versa). Note also that the depressed pH levels depicted here are not unusual; even lower values (e.g. down to 6.5 in pools in some systems [30]) have been recorded.

year 2100. We assess the capacity for altered pH to influence three core elements of predator-avoidance behaviours in black turban snails: their ability to locomote in seawater of altered chemistry, their capacity to detect cue and their efficacy in responding to predation risk. Finally, we test whether fluctuations in pH, which occur chronically in the habitats occupied by these species, might provide transient episodes of release from OA stress and thereby offset negative effects of overall reductions in pH.

2. Methods

(a) Study species

Tegula funebralis inhabits the mid-intertidal zone of rocky shores from Vancouver Island, Canada, to Baja California, Mexico. Throughout its range, it typically reaches its highest densities in wave-protected sites where it can be found both on emergent hard substrate and within rock pools. In the latter habitat, it is often one of the most abundant herbivores, consuming microalgae as well as larger seaweeds [16,28]. *P. ochraceus* is a similarly prominent resident of low- and mid-intertidal rocky shores, although it is

more common at wave-exposed sites. Its geographical distribution extends from Alaska to Baja California, Mexico. As a major carnivore in these systems, *Pisaster*, like *Tegula*, has been the subject of many laboratory and field investigations [15,29].

In this study, adult *T. funebralis* and *P. ochraceus* were collected from mid-intertidal pools on the Bodega Marine Reserve (BMR) near Bodega Bay, California, in February and April 2014. Snail and sea star size (mean $\pm$ s.e.) were standardized as much as possible (snail height and diameter $= 19.1 \pm 0.3$ and 21.0 ± 0.2 mm, respectively; sea star diameter $= 143.3 \pm 9.0$ mm). These sizes match those of many individuals involved in predator–prey interactions between these species in the field [24]. Immediately following collection, snails and sea stars were placed in separate flow-through aquaria at Bodega Marine Laboratory (BML) where they were allowed to acclimate to laboratory conditions for two weeks prior to experimentation. During acclimation, snails were fed kelp (*Macrocystis pyrifera*) and sea stars were fed *T. funebralis* individuals *ad libitum*. The latter protocol facilitated 'dietary tagging', in which predators feeding on a particular prey species emit chemical compounds (likely originating from breakdown products of consumed tissue) that operate as especially strong anti-predator cues for that prey [30].

(b) Study overview

Once animals had been acclimated to ambient seawater conditions, anti-*Pisaster* flight responses in black turban snails were assessed under multiple levels of altered seawater pH, in two separate 5 day experiments. Five days is typical of the duration of low-tide series that induce chronic exposure of rock-pool organisms to strongly depressed pH levels. In the first experiment, *T. funebralis* individuals were exposed to waterborne cue emitted by *Pisaster* under conditions of temporally constant pH (constant-pH experiment). In the second experiment, the pH of each treatment was varied semi-diurnally (fluctuating-pH experiment) to mimic temporal changes in pH that occur naturally in intertidal rock pools during many low-tide series, in particular those for which pools become isolated from the adjacent ocean only at night (i.e. approx. 12 low-tide series per year along the northern California coast, each lasting 5 days or longer).

(c) Constant-pH experiment

The altered pH levels used to test OA effects on the response of snails to *Pisaster* cue were established via direct chemical modification of the seawater carbonate system, using an approach that duplicates changes that arise from bubbling CO_2 gas through seawater [31–33]. In the first experiment, 16 discrete pH levels ranging from 8.0 to 6.3 were generated in two procedural steps. Because the pH of the laboratory seawater supply (drawn from local waters adjacent to BML) was prone to fluctuations of order 0.05 pH units per day, a static source tank for the experiments was first established and filled with 400 l of Millipore-filtered seawater. Then, 150 ml of 1 M hydrochloric acid (HCl) was added to this volume to create a low alkalinity (A_T) system that facilitated CO_2 evasion across the air–water interface, thereby reducing the dissolved inorganic carbon (DIC) to levels similar to the current global average for surface waters. After 24 h, 150 ml of 1 M sodium hydroxide (NaOH) was added to the source tank to return the alkalinity to its original value. At this point, the source tank contained seawater with substantially lower DIC and therefore higher pH, consistent with ambient open-ocean conditions. In the second step of the pH adjustment employed to create a series of discrete treatment levels, smaller volumes were extracted from the source tank for use in individual 10 l containers that held snails during experimentation. To achieve a particular pH level in a given snail container, these smaller volumes were manipulated by equimolar addition

of 1 M sodium bicarbonate (NaHCO₃) and 1 M hydrochloric acid (HCl), which increases DIC without changing alkalinity, identical to changes induced by direct entry of CO_2 into seawater [31–33]. The precise amounts of NaHCO₃ and HCl required was determined using the carbonate system software CO2SYS [34].

Once the pH in treatment containers had been established, snails ($n = 32$; one individual per container and two containers per each of the 16 pH levels) were held within them for 5 days, during which complete water changes and associated chemical manipulations were conducted daily. Values of pH (determined on the total scale; [35]), temperature, salinity and dissolved oxygen were recorded prior to and after water changes in each container using a YSI ProPlus sensor. Total pH for three of the containers was also analysed twice daily via a Sunburst SAMI spectrophotometric unit modified for benchtop use. The pH evaluated with the SAMI was furthermore used to calibrate the YSI readings to the total scale [36]. Bottle samples were also acquired from each snail container prior to and after water changes for analysis of total alkalinity. pCO_2 was calculated from daily alkalinity and pH using CO2SYS [34] assuming dissociation constants from [37] as refit by [38], and K_{SO4} from [39].

Following the initial 5 day holding treatments, snail responses to waterborne cue from *P. ochraceus* were examined. Each snail was placed individually into the centre of a 10 l arena filled with 1 l of seawater (depth of 5 cm) that had been adjusted to the same pH as that used during the holding period. One arena from each pH level was then randomly assigned to either a cue or no-cue treatment (one snail per pH level per cue treatment). The cue treatment employed predator-conditioned seawater (cue water) produced by placing five sea stars in a 38 l tank filled with source–tank seawater (1 sea star per 7.6 l) for 1 h prior to the behavioural assay. This density of sea stars was chosen to reproduce cue concentrations that would be expected for a small rock pool containing one sea star predator. The cue water (1 l) was then added to a given snail test arena by decanting it gently into the arena over a period of several seconds. Cue water from the sea star tank, as well as the no-cue water, was manipulated via chemical addition to reach the same pH level as that used during the snail's holding period.

While snails were in the test arenas, they were photographed every 2 min for a total of 28 min per individual. These photographs were then analysed using image analysis (IMAGEJ). Three behavioural responses were quantified, including (i) the *total path length* travelled, an indicator of whether snails maintained an ability to physically crawl about their arenas under altered pH, (ii) *path shape*, an index of cue detection that revealed whether snails exhibited a flee response or a less-directed movement pattern consistent with everyday activities such as searching for food, and (iii) the *proportion of time out of water*, which operated as an index of a fully realized avoidance response, distinguishing between flight behaviours that led to refuge-seeking or not. The path length was measured as the sum of the net distances travelled during each 2 min assessment interval over the 28 min recording period. Path shape was calculated as the total path length divided by the maximum displacement (the latter calculated as the linear distance between the start location and farthest position travelled for a given snail over the 28 min), yielding a relative scale with 1.0 being closest to a straight line (see electronic supplementary material, figure S1). The proportion of time out of water was quantified by noting the fraction of the 2 min assessment intervals a given snail was above the water. This latter assay included only time points subsequent to when the first snail reached the waterline (i.e. the 6 min mark), to ensure that the calculation incorporated only intervals for which snails had the physical opportunity to leave the water. Together, these three metrics enabled tests of hypotheses regarding potential pH effects on locomotory activity, cue detection and the ability of snails to mount a fully realized escape response (figure 2).
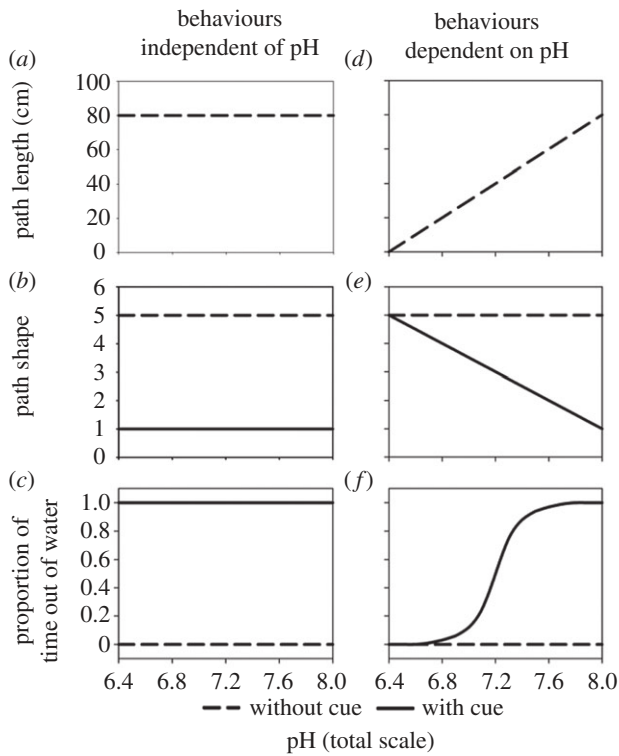
Figure 2. Conceptual representation of expected behavioural outcomes based on the hypothesis that snail behaviours are independent or dependent on pH. Path length, a metric of locomotory capacity (*a*,*d*; top row); path shape, a metric of cue detection (*b*,*e*; middle row) and proportion of time out of water, a metric of whether snails exhibit a fully realized escape response (*c*,*f*; bottom row). Solid and dashed lines indicate potential behaviours in the presence or absence of cue, respectively. Because the locomotory assay does not depend on the presence of cue, only dashed lines appear in panels (*a*) and (*d*).

(d) Fluctuating-pH experiment

The second experiment tested the predator avoidance response of black turban snails that had been held under seawater conditions characterized by daily fluctuations in pH. The goal in this case was to examine whether the natural, but substantial, diurnal variation in carbonate chemistry characteristic of intertidal rock pools might modify any effects of pH on snail behaviour. Such pools become separated from adjacent ocean waters during low tides, and in autumn and summer in our region, the low tides often occur only at night. These conditions make the pools subject to strong biogeochemical changes induced by respiration from resident organisms, driving substantial decreases in pH. These depressed pH levels can persist for 6 h or more. Values of pH then return to ambient levels at high tide when pools again receive water from the adjacent ocean, resulting in swings of as much as 0.75 pH units over the course of a single tidal cycle. Note that a second, daytime low tide may also occur during some tide series; in these cases, strong increases in pH can arise in conjunction with photosynthetic uptake of seawater carbon (cf. figure 1*c*).

To mimic in as simple a manner as possible the natural drops in pH that arise within isolated intertidal pools during night-time low-tide series, snails ($n = 20$, one individual per 10 l container) were subjected for 5 days to one of 10 levels of pH for 18 h each day, followed by a second pH level reduced by 0.75 units that lasted for the remaining 6 h. The high pH levels ranged from 8.0 to 7.2. These values encompass present-day conditions of near-shore waters (i.e. approx. 8.0 to 7.7 regionally) as well as those projected for the next century (i.e. decremented roughly by another half a pH unit). The 6 h low pH levels ranged from 7.2 to 6.5, reflecting the additional 0.75 pH drop that occurs

routinely in rock pools separated from adjacent shoreline waters at night. Two snails were assigned randomly to each of the 10 pH treatments that spanned this range.

Following the 5 day holding period under fluctuating-pH conditions, snails were tested with the cue or no-cue water in individual arenas as in the first experiment, using seawater at a pH corresponding to the lower of the two fluctuating levels. The same three behavioural metrics (path length, path shape and proportion of time out of water) were again quantified using images captured every 2 min for 28 min for each snail individual.

(e) Statistical analysis

The possibility that path lengths of snails were influenced by pH was tested by fitting a general linear model to the distance data (log-transformed response variable to meet model assumptions), with pH as an independent variable. For path shape, a general linear model was employed with pH and cue as independent variables. Path shape values were log-transformed to account for the positive and right-skewed nature of this relative metric. To assess how the presence of cue and pH level influenced the likelihood that snails would exit the water, a generalized linear mixed-effects model (binomial distribution, logit link function, lme4 package in the statistical software, R, v. 3.0.1) was used with snail included as a random effect to account for repeated measures and over-dispersion [40]. The best-fitting model for path shape and proportion of time out of the water was selected using the Akaike information criterion (AIC). The significance of the fixed effects was explored using *t*-values for general linear models and Wald *Z*-tests for the generalized linear-mixed effects models.

3. Results

(a) Water chemistry

During both experiments, seawater conditions in the snail containers were stable and exhibited little temporal variance (figure 3, tables S1 and S2 in electronic supplementary material). Seawater pH differed significantly across pH treatments (electronic supplementary material, table S3) and a pairwise Tukey's test demonstrated that all levels were significantly different from each other ($p < 0.001$), except for the two highest pH levels. During the fluctuating-pH experiment, pH levels across treatments also differed from one another, for both the high and low levels, respectively (electronic supplementary material, table S3). Seawater pH did not vary between cue and no-cue treatments during the constant or fluctuating-pH experiments (electronic supplementary material, table S3). The alkalinity did not vary with pH level or between cue and no-cue treatments in the first experiment or with high or low pH level in the second experiment (electronic supplementary material, table S3).

(b) Constant-pH experiment

Decreased values of pH did not affect overall locomotory activity nor cue detection, as evidenced by the absence of any relationship between pH and either path length or path shape (figure 4*a*,*b*; electronic supplementary material, tables S4 and S5). Had low pH caused physiological declines or locomotory impairment (e.g. through pH-induced metabolic depression or effects on muscle function [41]), one would have anticipated that overall movement, and thus values of the path length metric, would decrease at lower pH (compare figure 2*a*,*d*). No such pattern was apparent (figure 4*a*; note that this assay operated independently of

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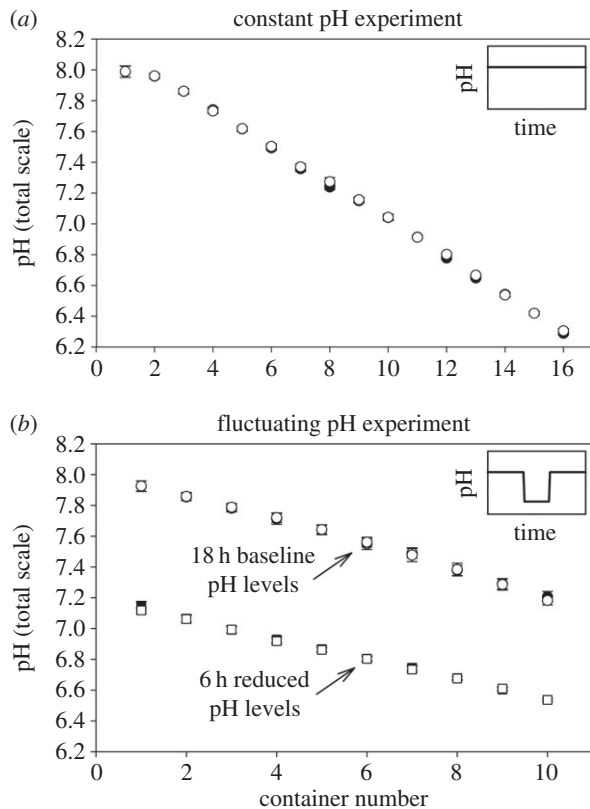rspb.royalsocietypublishing.org Proc. R. Soc. B 283: 20160890

Figure 3. Values of pH (total scale; [35]) for each container averaged over the 5 day holding period for (a) experiment 1 (constant pH) and (b) experiment 2 (fluctuating pH). Insets indicate schematically the temporal character of the pH treatments over a single day. In experiment 2, pH values switched between 18 h at a baseline level (circles) and 6 h at a reduced level (squares), mimicking changes characteristic of rock pools during night-time low-tide series. Cue and no-cue treatments are indicated with solid shapes and empty shapes, respectively, and overlap sufficiently that many data points from a given pH level are obscured. Error bars depict standard error and in most cases are smaller than the symbols.

Figure 4. Effects of seawater pH during experiment 1 (constant pH; left panels) and experiment 2 (fluctuating pH; right panels) on predator avoidance behaviour of *T. funebralis* in the presence (solid circles) and absence (empty circles) of cue from *P. ochraceus*. (a,d) Path length (for which cue is irrelevant); (b,e) path shape (path length divided by maximum displacement) and (c,f) proportion of time spent out of water in the presence (green/solid line) or absence (grey/dashed line) of predator cue. Shading represents 95% confidence intervals.

the presence/absence of predator cue because it was concerned only with the physical ability of snails to move). If snails had failed to detect cue under reduced pH, then one would have expected the path shape metrics from the cue and no-cue treatments to converge at low pH, reflecting the absence under acidified conditions of the typical straightline, directed flight response (compare figure 2b,e). Instead, snails presented with predator-conditioned seawater exhibited a flight response (as indicated by a small numerical value of path shape), regardless of pH, in contrast to the much larger numerical value for this metric in the absence of cue (figure 4b). Greater values of path shape corresponded to a wandering pattern of movement.

In contrast to the results for locomotory ability and cue detection, low pH strongly modified the ability of snails to properly respond to predation risk. This effect manifested as a marked decline in the proportion of time that snails spent in refuge (i.e. out of the water) when predator cue was present (compare figures 4c and 2f). The declining trend with decreased pH is supported by AIC model selection, which suggests an effect of the interaction between pH and cue on predator avoidance (electronic supplementary material, table S6). In particular, the interaction term reflects a difference in the effect of pH on snail refuge-seeking between the cue and no-cue treatments. Reduced pH only
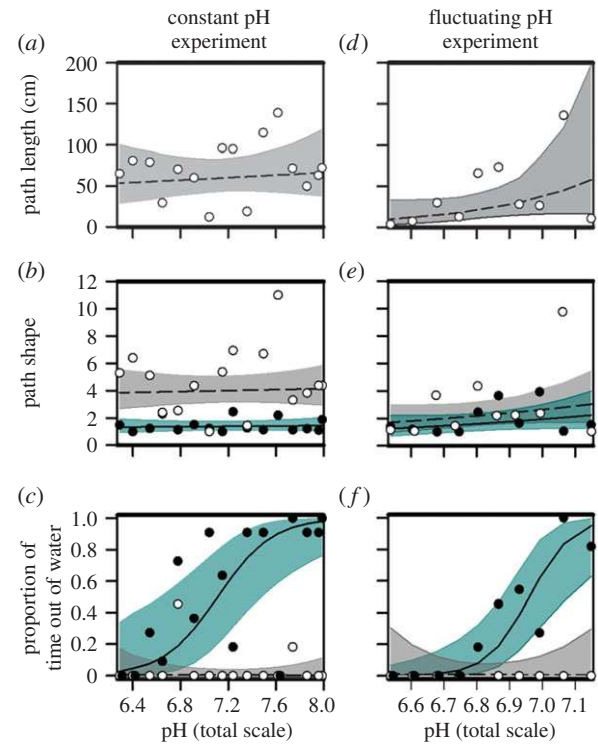
decreased the tendency of snails to exit the water when low pH was accompanied by predator cue (electronic supplementary material, table S4). Model predictions estimate a 50% reduction in refuge-seeking behaviour of snails exposed to predator cue at a pH of 7.1. Reduced pH, however, did not affect snails unexposed to predator cue, which spent almost 90% of their time submerged, regardless of pH.

(c) Fluctuating-pH experiment

Similar results of incompletely realized anti-predator responses arose in snails held under fluctuating-pH conditions. This finding suggests little if any capacity of snails to recover quickly during transient respites of higher pH. Again, pH did not have an appreciable effect on locomotory activity, because there was no significant relationship between pH and path length (figure 4d and electronic supplementary material, tables S4 and S5). However, in this experiment, snails travelled a shorter average distance in the absence of cue, compared with their corresponding behaviour in the constant-pH experiment (compare no-cue lines of figure 4b,e). As a consequence, although decreases in pH still did not alter path shape (figure 4f and electronic supplementary material, table S4), the reduction in overall movement (including wandering activities) under no-cue conditions obscured any differences between the path shape indices for the cue and no-cue treatments. That is, because of the normalized nature of this metric, there was little scope for no-cue treatments to exhibit path shape levels that diverged more than modestly

from 1.0. Snails exposed to predator cue retained their initial flee response at all pH levels, moving directly towards the edge of the container; on the other hand, similar average path shape levels occurred also in the absence of cue simply because of the lower degree of foraging-type movements.

The ability of snails to mount a fully realized escape response in the presence of predator cue was still disrupted under conditions of fluctuating pH. Snails without predator cue spent most of the time below the water line, regardless of pH level, whereas snails subjected to decreased pH that were presented with predator cue spent less time out of the water (figure 4f). As in the constant-pH experiment, AIC model selection suggests effects of the interaction between pH and cue on predator avoidance (electronic supplementary material, table S6). Model predictions estimate a 50% reduction in refuge-seeking behaviour of snails exposed to predator cue at a pH of 7.0.

4. Discussion

Results of this study indicate that the anti-predator behaviours of black turban snails are impaired when individuals experience reductions in seawater pH. The impairment does not accrue from decreased motility, nor from an inability to detect cue, but rather from an incompletely manifested escape response. It remains unclear whether this outcome arises through degradation of a refuge-seeking behavioural pathway, or whether an alternative pathway is activated (e.g. a cowering/hiding response rather than one dependent on flight) [14]. Regardless, because submerged snails remain within the sea stars' preferred habitat and can likely be sensed more readily by them, such declines in the time of emergence could translate into an increased risk of predation. It also appears that periodic rebounds of pH to higher values lasting 18 h each day neither mitigate the initiation of behavioural impairment nor enable recovery of viable escape responses once the impairment has been established (see also [42]). To the extent that T. funebralis serves as a representative model for other intertidal gastropods that react to Pisaster as a generalist predator, these findings imply the potential for disruption of a substantial number of consumptive and non-consumptive links within the food web.

Our use of 16 discrete levels of pH also facilitated evaluation of whether nonlinearities, thresholds and/or tipping points might arise in the behavioural response of snails to pH. The potential for such complex responses of organisms to altered carbonate chemistry have rarely been examined (most studies employ two or three treatment levels in ANOVA-style experimental designs), yet are clearly evident in our study. In particular, we identify a swift transition to behavioural impairment at a pH of around 7.1. Such values of pH occur already in rock pools during night-time low tides (figure 1; [26]). Values of pH below 7.1 can moreover be expected to occur with increasing frequency in the future, as baseline seawater conditions that operate as the 'starting point' for additional alteration by biological processes (like respiration within isolated rock pools) also shift. Model projections, for instance, suggest a 0.3–0.4 drop of global average pH by the year 2100, which can be expected to superimpose on the already-low pH levels characteristic of coastal upwelling zones [25], and it is upon such background changes that further pH declines owing to localized respiration processes will likely layer.

More work is required to understand the nuances of why snails fail to implement a fully realized escape response under reduced pH. Although altered neuronal processes have been implicated in other systems [7], it is also possible that OA stress shifts the physiological state of snails such that they reallocate energy away from metabolically expensive escape responses [43]. This scenario could cause snails to participate in riskier albeit less energetically costly behaviours (e.g. remaining underwater when in close proximity to predators). That said, one might then predict accompanying decreases in other energy-requiring behaviours, such as crawling, in snails exposed to low pH, and we did not see evidence of reductions in overall movement.

This study concerns only low-pH effects on the response of T. funebralis to P. ochraceus. However, parallel behavioural changes under low pH are known in other invertebrates [10–14,44]. These combined results suggest that behavioural impairment under OA may be at least somewhat general across taxa [8,12]. The physiological mechanisms underlying OA-induced behavioural shifts in fish appear to revolve around degraded function of ion channels that involve the neurotransmitter gamma-aminobutyric acid (GABA; [7–9]), and it may be relevant that GABA receptor molecules are conserved across vertebrates and invertebrates [8].

The possibility that many coastal species may exhibit altered responses to predator cues under OA could play out strongly in intertidal systems along the west coast of North America. Avoidance responses to sea stars are similar across a breadth of gastropods, potentially indicating a common biochemical or neurological pathway for sensing [18–22]. If this situation indeed applies—for example, if GABA receptors are involved—a non-trivial subset of the invertebrate food web on eastern Pacific shorelines could be influenced by OA, not only through direct effects of altered carbonate chemistry on growth or survival of specific taxa as is widely recognized [3], but also by altered abilities of many such species to evade predators within the system [45]. Such impaired anti-predator responses could moreover modulate indirect species interactions that emerge from particular predator-dependent prey behaviours (i.e. non-consumptive effects; [23]). For example, prey often reduce their foraging activities upon detection of a predator, with consequent effects on organisms that the prey consume. Although not directly tested in this experiment, OA-induced attenuation of anti-predator responses by snails could potentially lead them to forage under a greater range of conditions (e.g. even in the presence of sea stars), thereby shifting the consumer pressure of black turban snails on their algal food [24]. In particular, if snails increased their grazing rates but did not experience an accompanying elevation in mortality from sea stars, then algal densities might decline; on the other hand, if impaired flight responses incurred greater mortality of snails, then the net result for algae could be positive. Further work is necessary to examine these types of indirect consequences, as well as the possible direct effects of OA on sea stars. Although prior research suggests positive effects of OA on feeding and growth in P. ochraceus [46], there could be other, yet-unanticipated consequences.

An additional, especially critical unknown in the system is the degree to which Tegula and Pisaster might possess the capacity to acclimatize or adapt to long-term changes in carbonate chemistry [47]. Given that snails start to exhibit impaired anti-predator responses near levels of pH that

currently occur in rock pools [26,27], black turban snails might already be living with some pH-associated impairment. Given such considerations, and the possibility that further acclimatization and/or adaptation may be required for the future success of these organisms under conditions of even lower pH, the scatter surrounding the steep–slope portions of the curves in figure 4c,f could be relevant in that it implies that there may be meaningful interindividual variation in behaviour. Whether this variation is heritable remains an important question; likewise, more information is required to assess the potential strength of selection on behavioural traits tied to pH [12,47].

In conclusion, our results indicate that ecologically relevant levels of reduced pH alter essential behaviours in snails that could influence their survival and potentially mediate consumptive and non-consumptive components of trophic interactions. Our results build on other studies that demonstrate analogously impaired sensory-mediated behaviour by marine organisms under reduced pH, suggesting that this class of effect could apply to a variety of taxa. As OA proceeds and modulates existing variability in coastal carbonate chemistry, it could become increasingly important to account for the capacity of OA to alter behaviours that dictate how species interact within the often-complex food webs they inhabit.

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