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COMMENTARY

Acidification can directly affect olfaction in marine organisms

Cosima S. Porteus[1,2,*], Christina C. Roggatz[3], Zelia Velez[4], Jörg D. Hardege[5] and Peter C. Hubbard[4]

ABSTRACT

In the past decade, many studies have investigated the effects of low pH/high CO_2 as a proxy for ocean acidification on olfactory-mediated behaviours of marine organisms. The effects of ocean acidification on the behaviour of fish vary from very large to none at all, and most of the maladaptive behaviours observed have been attributed to changes in acid–base regulation, leading to changes in ion distribution over neural membranes, and consequently affecting the functioning of gamma-aminobutyric acid-mediated (GABAergic) neurotransmission. Here, we highlight a possible additional mechanism by which ocean acidification might directly affect olfaction in marine fish and invertebrates. We propose that a decrease in pH can directly affect the protonation, and thereby, 3D conformation and charge distribution of odorants and/or their receptors in the olfactory organs of aquatic animals. This can sometimes enhance signalling, but most of the time the affinity of odorants for their receptors is reduced in high CO_2/low pH; therefore, the activity of olfactory receptor neurons decreases as measured using electrophysiology. The reduced signal reception would translate into reduced activation of the olfactory bulb neurons, which are responsible for processing olfactory information in the brain. Over longer exposures of days to weeks, changes in gene expression in the olfactory receptors and olfactory bulb neurons cause these neurons to become less active, exacerbating the problem. A change in olfactory system functioning leads to inappropriate behavioural responses to odorants. We discuss gaps in the literature and suggest some changes to experimental design in order to improve our understanding of the underlying mechanisms and their effects on the associated behaviours to resolve some current controversy in the field regarding the extent of the effects of ocean acidification on marine fish.

KEY WORDS: Invertebrates, Fish, Acidification, Carbon dioxide, Behaviour, Electrophysiology

Introduction

For aquatic animals, the sense of smell is vital; it plays important roles in feeding and reproduction, among other processes. Olfaction has been linked to kin recognition, mate selection and predator avoidance. Moreover, similar principles apply to how most aquatic organisms smell their environment (Ache and Young, 2005); therefore, a change in the quality or effectiveness of this sense could affect not only individuals, but populations, species and ecosystems in both fresh water and seawater. Importantly, chemical signals (hereafter referred to as odorants) differ from visual, mechanical, acoustic and electrical signals in that they persevere in the environment beyond the moment of production or release. Odorants can persist over long time scales or distances and can be important in communication (Atema, 1995), especially when other sensory modalities are unreliable (e.g. reduced visibility).

Although the organs used to detect odorants differ widely between fish and invertebrates, they all contain the same basic organization as that of humans and other vertebrates. As such, this Commentary focuses on the effects of ocean acidification on the olfactory systems of marine fish and invertebrates, as effects have been shown experimentally in both these groups. Just like other animals, marine fish and invertebrates sense odorants using odorant receptors located in sensory neurons in their olfactory epithelia (e.g. snout in fish and antennae in crustaceans; for detailed reviews, see Ache and Derby, 1985; Hansen and Zielinski, 2005; Zeiske et al., 1992). Odorants in the water bind to receptors in the olfactory epithelium (i.e. G-protein-coupled receptors in fish, ionotropic receptors in crustaceans; Buck and Axel, 1991; Derby et al., 2016; Ngai et al., 1993), causing these neurons to depolarize. The sensory neurons send information to the brain via their axons, which form the olfactory nerve. The olfactory neurons synapse with other neurons in the olfactory bulb, in the part of the forebrain where olfactory information is processed. From this first stage of olfactory processing, the input is relayed to higher centres within the brain (Hamdani and Døving, 2007; Olivares and Schmachtenberg, 2019; Zeiske et al., 1992) and produces a response (e.g. changes in behaviour).

Ocean acidification is the reduction in average pH of the ocean over an extended period and is caused primarily by the uptake of CO_2 from the atmosphere. Over a decade ago, it was shown that the acidification of seawater with CO_2 can alter the behaviour of coral-reef fish larvae (Dixson et al., 2010; Munday et al., 2010). These findings have since spurred a flurry of studies indicating that the behaviour of some, but not all, marine species and/or stages are affected by ocean acidification (Allan et al., 2013; Andrade et al., 2018; Domenici et al., 2012; Hamilton et al., 2013; Jutfelt et al., 2013; Jutfelt and Hedgärde, 2014; Kwan et al., 2017; Nadler et al., 2016; Watson et al., 2014). Indeed, some recent attempts at replicating the initial studies have sparked a lot of controversy (Clark et al., 2020a,b; Munday et al., 2020; Williamson et al., 2020). Behaviour measurements are generally more subjective in nature, thus, here we focus on mechanistic studies that provide more direct evidence.

The mechanism underlying the behavioural changes observed was suggested to be a disruption in the distribution of H^+ and HCO_3^- ions over neuronal membranes, resulting in a switch of gamma-aminobutyric acid-mediated (GABAergic) innervation from hyperpolarizing (i.e. inhibitory) to depolarising (i.e. excitatory) – the 'GABA$_A$ receptor theory' (Nilsson et al., 2012). However, in our previous work, we proposed an additional

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[1]Cell and Systems Biology, University of Toronto, 25 Harbour St, Toronto, ON, M5S 3G5, Canada. [2]Department of Biological Sciences, University of Toronto Scarborough, 1265 Military Trail, Toronto, Ontario, M1C 1A4, Canada. [3]Energy and Environment Institute, University of Hull, Cottingham Road, Hull, HU6 7RX, United Kingdom. [4]Centro de Ciências do Mar do Algarve, Universidade do Algarve, Campus de Gambelas, 8005-139, Faro, Portugal. [5]Department of Biological and Marine Sciences, University of Hull, Cottingham Road, Hull, HU6 7RX, United Kingdom.

*Author for correspondence (cosima.porteus@utoronto.ca)

C.S.P., 0000-0003-4730-4960; C.C.R., 0000-0002-8566-3078; J.D.H., 0000-0002-7815-3969; P.C.H., 0000-0002-3007-4647

mechanism by which ocean acidification could affect the behaviour of both fish (Porteus et al., 2018; Velez et al., 2019; Ou et al., 2015) and crabs (Roggatz et al., 2016), through direct effects on the olfactory system caused by changes in water chemistry. Reversible chemical processes outside the organisms may alter receptor–ligand interactions through changes in the protonation state of the ligands and/or receptors (discussed below), resulting in altered nerve responses. Ocean acidification therefore has the potential to affect the interface between the animal and its environment. This Commentary focuses on mechanisms that occur during olfactory signal reception, by which ocean acidification can affect olfaction prior to, and therefore independently of, acid–base changes that take place inside the animal, such as alterations of ion gradients across the $\mathrm{GABA_A}$ receptors (Schunter et al., 2019). Acutely altered olfactory responses can accompany the mid- to long-term changes through alterations in morphology and gene expression as well as altered ion gradients over GABAergic neurons, leading to long-lasting effects on the behaviour of individual organisms (discussed below), which can result in effects on population density and structure. We begin our Commentary by presenting evidence for the direct effect of ocean acidification on the olfactory sensitivity of marine animals and the odorants involved, followed by evidence that these acute effects do not show any signs of acclimation/acclimatization over longer exposure times. We end with a discussion of how our model fits into the wider context, and provide suggestions for areas of focus in future studies.

Direct effects of ocean acidification

Many, but not all, studies have shown effects of ocean acidification on olfactory-mediated behaviour in marine animals. Documented disturbances include feeding, resource/shell assessment, prey and predator detection and recognition, environmental recognition and foraging behaviour (see Draper and Weissburg, 2019 for a recent review). Most of the initial studies were carried out on larvae reared in water with a high partial pressure of CO_2 (P_{CO_2}) as a proxy for ocean acidification (Dixson et al., 2010; Munday et al., 2009) and adults exposed for different periods to acidified water before testing (Dixson et al., 2015; de la Haye et al., 2011, 2012). The length of high P_{CO_2} exposure varies greatly in these studies, but it is usually longer than 4 days, because previous studies indicated that treatment for this period was sufficient to affect olfactory-mediated behaviours (Munday et al., 2010). Thus, little information is available on how behaviour changes with time during more acute exposure to ocean acidification conditions. However, a study carried out on deep-sea hermit crabs showed that, under low pH conditions, crabs have lower antennular flicking rates (the equivalent of 'sniffing') and the CO_2 effects become apparent immediately after exposure to high CO_2; however, the effects were not statistically significant until 7 days of exposure (Kim et al., 2016). A different study shows acute disruption of the ability of intertidal hermit crabs to locate a food source under low pH conditions (Roggatz et al., 2019b). Thus, effects of high P_{CO_2} and/or low pH may occur within 4 days of exposure, and electrophysiological measurements reveal that olfaction is affected even earlier than behavioural effects have been assessed (discussed below). Moreover, behavioural responses to odorants depend heavily on detection and context [i.e. involvement of higher centres within the central nervous system (CNS) and/or physiological status]. For example, hungry fish are less likely to respond to conspecific alarm cues than recently fed fish (Brown and Smith, 1996; Chivers et al., 2000; Giaquinto and Gilson, 2001). Thus, behavioural experiments measure the end result of a complex, multi-step process. We propose that ocean acidification affects the olfactory system directly, by altering the olfactory sensitivity to some odorants. To test this, olfactory sensitivity must be assessed independently of behaviour.

Assessing olfactory sensitivity using electrophysiology

To assess olfactory sensitivity in marine fish, the method of choice is multi-unit recording from the olfactory nerve (Hubbard and Velez, 2020). This is a measure of nervous activity in the olfactory nerve in the form of action potentials passing down the axons from the olfactory receptor neurons (the cell bodies of which are in the olfactory epithelium) to the appropriate glomeruli in the olfactory bulb (Hamdani and Døving, 2007; Olivares and Schmachtenberg, 2019). The higher the potency of an odorant and its concentration at the olfactory epithelium, the higher the frequency of action potentials and the activity recorded in the olfactory nerve. Using this technique (Hubbard and Velez, 2020), we have shown that olfactory sensitivity to most (but not all) ecologically relevant odorants – including amino acids, bile acids, bile, intestinal fluid and alarm cues – is reduced in high P_{CO_2} seawater, and that this effect is immediate and reversible (Porteus et al., 2018; Velez et al., 2019) (Fig. 1). This suggests that any effect on olfactory sensitivity will occur within seconds and does not depend on longer-term changes in acid–base status that underlie the $\mathrm{GABA_A}$ receptor theory (Nilsson et al., 2012).

The advantage of multi-unit recording from the nerve is that it assesses a purely sensory signal, prior to any neural processing in the olfactory bulb. As such, by constructing concentration–response curves, the thresholds of detection (the lowest concentration that the olfactory system can detect) can be estimated. This reveals that seemingly small reductions in the amplitude of neuronal responses may reflect significant impairment of the olfactory detection of some odorants. This holds true particularly when the odorant concentration is low, as seen in amino acids where environmental background concentrations are often in the nanomolar to micromolar range (10^{-9}–10^{-6} mol l^{-1}), close to their thresholds of detection (Hara, 1994; Kasumyan, 2004), resulting in a very low signal to noise ratio. In two marine teleosts, acute exposure to acidified seawater over the olfactory epithelium decreases the amplitude of the neuronal response to most odorants tested, and increases their detection threshold (Porteus et al., 2018; Velez et al., 2019), suggesting a direct effect of pH on the odorants and/or their receptors. Therefore, our proposed mechanism affects food search and predator avoidance in a simple way; in this case, animals would need to be closer to an odorant source or exposed to higher odorant concentrations in order to detect it (Porteus et al., 2018).

Effects of acidification on odorants and their receptors

The absorption of CO_2 by our oceans and the resulting shift in carbonate chemistry leads to an increased concentration of protons. These can react with chemical groups commonly found in marine odorants, such as amine, imidazole, thiol, hydroxyl or carboxyl groups. A change in their protonation state can alter the odorants' properties (e.g. charge, structure and 3D conformation; Fig. 2) and interaction(s) with other molecules. It is currently unrealistic to estimate what proportion of marine odorants are potentially affected, because our knowledge of the chemical structures of the entirety of odorants used by fish and marine invertebrates is still too scarce. This protonation process is reversible and happens rapidly, consistent with electrophysiological findings (see above).

Although a chemical message can either be transported over long distances or conveyed upon touch, all chemicals detected by an aquatic animal are exposed to the surrounding water. This means that

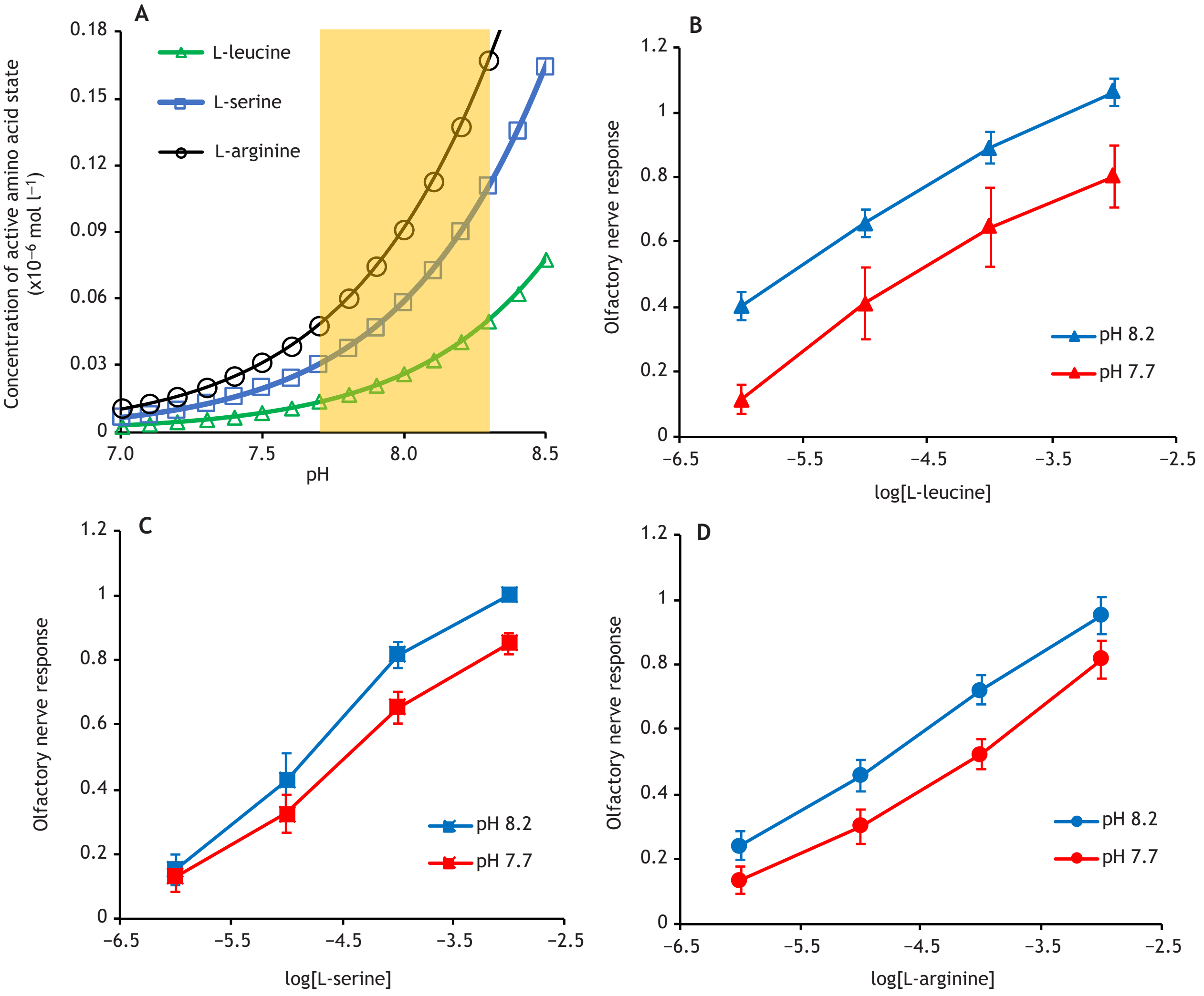

Fig. 1. The effect of pH on olfactory sensitivity to amino acids in fish. (A) Amino acids contain ionisable chemical groups; their protonation state depends on the pH of the solution (seawater in this case). Here, we calculate how the proportion of the active amino acid form (L-leucine, L-serine and L-arginine) decreases because of protonation as the seawater pH falls from 8.2 to 7.7 (yellow area). (B–D) Amino acids are potent odorants for fish; we show that the olfactory sensitivity of seabream (*Sparus aurata*) is reduced as the pH of the seawater falls by the same range from 8.2 (normal seawater) to 7.7 ('end of the century' climate change scenario) for L-leucine (B), L-serine (C) and L-arginine (D) concentrations (in mol l^{-1}). Data are redrawn from Velez et al. (2019).

a reduced pH can directly affect these chemical signals. Odorants, such as amino acids, peptides, nucleotides and their derivatives, contain functional chemical groups with nitrogen, phosphate or sulphur. Depending on the surrounding pH, these functional groups can be protonated (addition of a proton, H$^+$) or deprotonated (removal of a proton, H$^+$), a process that happens in nano- or micro-seconds. Protonation or deprotonation can change an odorant from its biologically active form (able to bind an olfactory receptor and elicit a response) to an inactive form (less likely to bind the same olfactory receptor and unable to elicit a response).

It has been recently shown that pH changes of as little as 0.4 units can alter peptide cues reversibly and lead to a loss of function of the odorant (Roggatz et al., 2016). The peptides investigated by Roggatz and colleagues are known to mimic odorants that induce brood-care behaviours, such as egg ventilation and pumping in crustaceans (Reinsel et al., 2014; Roggatz et al., 2016), as well as larval settlement of oysters (Browne et al., 1998) and barnacles (Browne and Zimmer, 2001). Protonation at one or more groups of these peptides affects their chemical characteristics, such as their three-dimensional structure and charge distribution (Roggatz et al., 2016) (Fig. 2). These are key characteristics for successful odorant–receptor interactions (Sheinerman et al., 2000); hence, they are crucial for the reception of the chemical signal and its message.

Amino acids, such as L-serine, L-leucine, L-arginine, L-glutamine and L-glutamate, which are commonly assumed to play a role as feeding cues (Hara, 1994; Velez et al., 2007), can also be protonated at their amine headgroup (Fig. 2). A reduction from pH 8.1 to 7.7 can lead to reduced amounts of the active form (Velez et al., 2019) (Fig. 1). However, the reduced abundance of the active forms is, in most cases, only partly responsible for the reduced signal reception (Velez et al., 2019); thus, other components in the olfactory process may also be acutely affected (Rong et al., 2018). Therefore, it is possible that protonation changes in the receptors may also be important.

In order to detect odorants, the receptor binding sites are exposed to the surrounding environment. Receptors are proteins and, as such, consist of amino acid chains that are susceptible to pH changes, as hypothesised previously (Tierney and Atema, 1988). Protonation or deprotonation can change residues present in the binding domain itself, or residues that are crucial for the conformation of the binding domain or the functioning of the receptor, resulting in altered

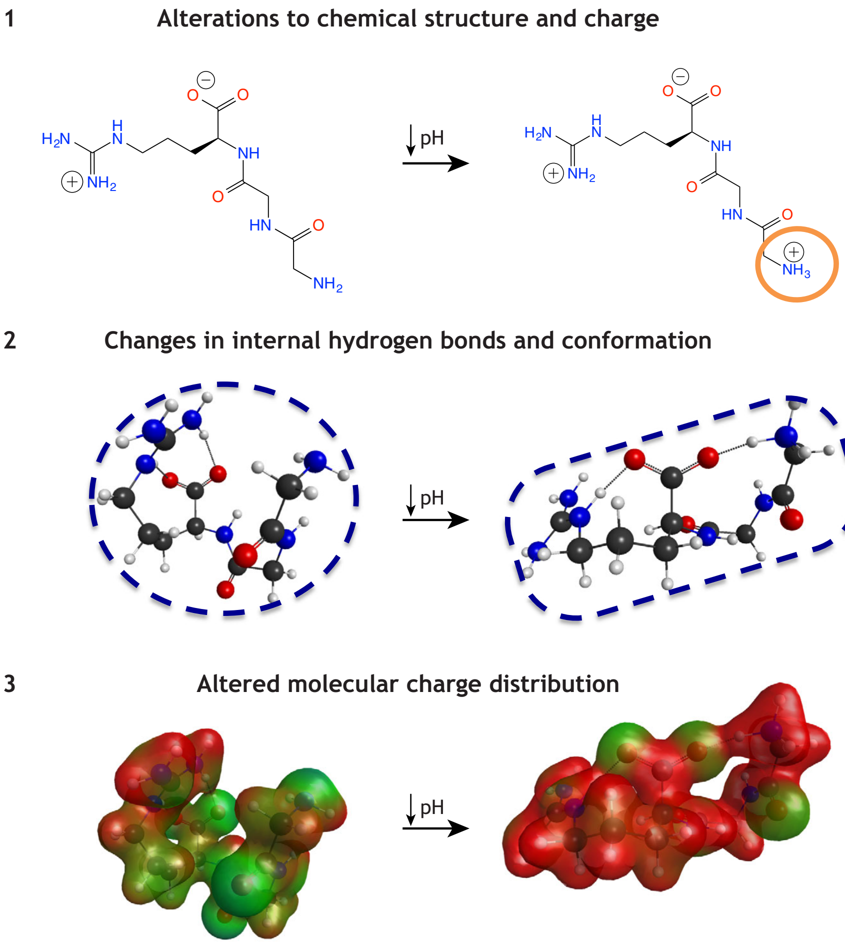

Fig. 2. Changes in pH can affect the protonation, 3D conformation and charge distribution of odorants. The effects of pH change on the chemical properties of glycyl-glycyl-L-arginine (GGR), a tripeptide mimicking brood care and settlement odorants in crustaceans and molluscs, are shown (adapted from Roggatz et al., 2016). Protonation at the glycine amino group under lower pH conditions (orange circle) (1) increases the overall charge of GGR, (2) affects the conformation through a change in hydrogen bonding and (3) alters the charge distribution around the molecule. Charge distribution is essential for successful binding to a receptor and is indicated as follows: red, positive; green, neutral.

ligand–receptor kinetics (D'Souza and Strange, 1995; Gillard and Chatelain, 2006) or even receptor inactivation (Tombaugh and Sapolsky, 1990; Traynelis and Cull-Candy, 1991). It has been suggested that receptor–ligand complexes have evolved under characteristic pH levels to minimise proton uptake/release during binding (Petukh et al., 2013). Externally forced shifts to these conditions, for example, due to environmental acidification, may therefore have significant impacts. Odorants and their receptor-binding domains are key for an organism to receive a signal or cue, and their sensitivity to changing pH indicates that there may be a significant impact of ocean acidification on the reception of information prior to any neural processing.

So far, the biological systems and odorants covered by our research have revealed cases where acidification causes a reduction in the associated function or observed behaviour. However, protonation of the odorant and/or receptor could also enhance signalling function if the protonated odorant/receptor is the active form (Roggatz et al., 2019a; Schirrmacher et al., 2021). Which protonation state acts as the active odorant could be organism specific and depend, for example, on the natural conditions to which the organism is adapted. These aspects of our proposed mechanism warrant further investigation.

Direct effects are linked to longer-term responses

Although the effects of high P_{CO_2}/low pH on olfactory sensitivity in fish are rapid and reversible, even after several weeks of exposure to acidified water, seabream (*Sparus aurata*) retain a reduced olfactory sensitivity to most odorants just like acutely exposed fish (Velez et al., 2019). That is, in the longer term, these fish are unable to compensate for the immediate reduction in odorant–receptor affinity and the excitability of the receptor neurons remains unaltered after 4 weeks of exposure compared with acute exposure. However, this does not necessarily mean that acidification cannot evoke some long-term changes in the olfactory system at the molecular and/or morphological and functional levels.

In fish, high CO_2/low pH exposures have been linked to changes in gene expression in the olfactory pathway, indicating that marine animals may respond, to some extent, to the decrease in olfactory sensitivity. However, the changes in gene expression are sometimes apparently maladaptive. For example, sea bass (*Dicentrarchus labrax*) exposed to 1000 µatm P_{CO_2} for 1 week decrease the expression of olfactory receptors in the olfactory rosette compared with control (400 µatm P_{CO_2}) fish (Porteus et al., 2018). Moreover, seabream exposed to 700 and 2000 µatm P_{CO_2} for 2 weeks show changes in gene expression in the olfactory epithelium consistent with both a reduction in olfactory transduction (Rong et al., 2018) and a decrease in olfactory sensitivity shown in other studies (Velez et al., 2019). Additional changes associated with high CO_2/low pH exposures have been observed in the olfactory bulb of fish. For example, coho salmon (*Oncorhynchus kisutch*) exposed to 1600 and 2700 µatm P_{CO_2} for 2 weeks show changes in the expression of genes associated with neurotransmitter signalling (GABA and glutamate), neural differentiation, melatonin production and neural energy production (Williams et al., 2019). Juvenile European sea bass exposed to 1000 µatm for 1 week show decreases in the expression of genes associated with glutamate signalling, cell excitability and patterning of the olfactory bulb (Porteus et al., 2018). Interestingly, in both these studies, fish exposed to high CO_2/low pH had an increase in the expression of neural *pentraxin 1* in the olfactory bulb; this gene encodes a protein associated with negative regulation of excitatory synapse density and synaptic plasticity (Figueiro-Silva et al., 2015). Therefore, changes in gene expression are in fact hindering the functioning of the olfactory system under acidified conditions. The apparent lack of compensatory gene expression is puzzling, as we expected changes to occur during long-term exposures to maintain neuronal signalling; perhaps the decrease in sensitivity is insufficient to stimulate this. These changes in gene expression are an additional effect to the potential pH-induced change of the existing receptors and might even further alter olfactory-driven behaviour. However, we cannot fully understand these changes in gene expression without knowing in which cells they occur. This could be addressed by functional studies (e.g. electrophysiology) of the olfactory bulb of fish in acidified water; this is clearly an area for future work. In crustaceans, there are currently no studies on possible effects of ocean acidification on the gene expression of the olfactory system.

In addition to changes in gene expression, the morphology of the olfactory epithelium can be affected by high P_{CO_2}. The olfactory epithelium of seabream exposed to high P_{CO_2} for 4 weeks shows an increase in the area of non-sensory epithelium (but not the sensory epithelium where olfactory receptors are found), higher densities of mucus cells (indicating increased mucus production, which is known to reduce the ability of odorants to bind to their receptors), and a shift towards a more neutral pH of the mucus they produce, which might positively affect the binding of odorants to their receptors (Velez et al., 2019). The likely effects of these changes are conflicting, and therefore the overall effect – if any – on olfactory sensitivity is unknown.

Proposed mechanism

Here, we suggest a mechanism for how ocean acidification can affect the behaviour of marine animals in response to various odorants, which is an alternative to, but not exclusive of, the GABA-receptor theory. First, an increase in CO_2 leads to a reduction in pH that affects the chemical structure, conformation or charge distribution of odorants (Fig. 2) (Roggatz et al., 2016; Velez et al., 2019), altering the binding of odorants to their receptors (Fig. 3). In a similar way, the charge distribution and conformation of the receptors themselves is likely to be affected by a decrease in pH, further altering the binding of odorants to their receptors (Fig. 3) (Tierney and Atema, 1988). This usually translates into either a decrease in the response amplitude and/or an increase in the detection threshold of the olfactory neurons, as measured using electrophysiology (Porteus et al., 2018; Velez et al., 2019). That is, under high P_{CO_2}, a higher concentration of odorant is needed to evoke action potentials, or a lower frequency of action potentials are produced in response to certain odorants (Fig. 1). Therefore, animals would need to be closer to an odorant source or exposed to higher concentrations in order to detect it. Although not often encountered, for some odorants the protonated odorant is the active form, and this could explain why some marine animals become attracted to odorants that under current pH/P_{CO_2} conditions are avoided. Furthermore, the nerve responses to different odorants are affected to differing degrees. Most odours are made up of mixtures of odorants, such as amino acids or bile acids, thus the receptor sensitivity to some odorants might be affected more than that to others. This could potentially change the qualitative perception of an odour mixture (e.g. predator smell) (Porteus et al., 2018), making the odour mixture more novel and more likely to elicit exploratory behaviour. This would help to explain the attraction to some predator or undesirable odour mixtures that has been reported previously (Munday et al., 2009; Munday et al., 2010).

Gene expression studies indicate that both the sensory neurons in the olfactory epithelium and those in the olfactory bulb downregulate genes involved in maintaining excitability upon exposure to high CO_2 (Porteus et al., 2018), and a reduction in olfactory transduction has been shown in black seabream (Rong et al., 2018), therefore making these neurons even less sensitive to certain odorants. In the olfactory bulb, the patterns of gene expression observed after 1 week of exposure to high CO_2 in sea bass are consistent with a negative regulation of excitatory synapse density in this region of the brain (Porteus et al., 2018). We propose that these changes in odorant detection and gene expression are consistent with a decreased behavioural response to odorants in fish (Porteus et al., 2018) and crabs (Roggatz et al., 2016; Fig. 3) and possibly a reduction in the capacity to learn about odorants (Chivers et al., 2014).

Wider effects and knowledge gaps

Anthropogenic changes in water chemistry (temperature and CO_2) have been linked to sensory disruption in many aquatic organisms

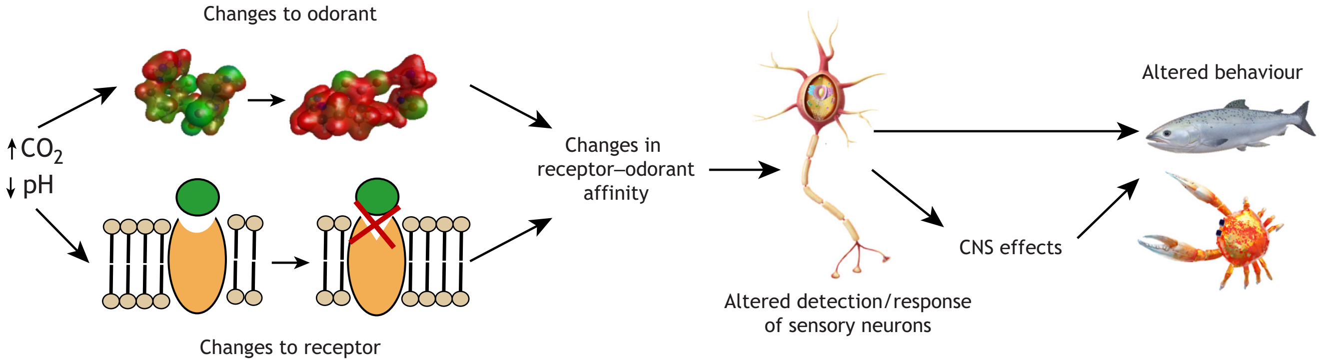

Fig. 3. Proposed mechanism for how ocean acidification can affect the olfactory system directly through changes to odorant molecules and/or receptors. This mechanism is independent of changes in acid–base balance and GABA function previously proposed (Nilsson et al., 2012; Schunter et al., 2019). We propose that direct effects on odorants and their receptors cause a change in the relevant binding affinity, causing altered detection and response of olfactory neurons, which is correlated with impaired behaviour of fish and invertebrates. These responses have also been linked to longer term effects on gene expression associated with various olfactory system functions in the central nervous system (CNS).

(reviewed by Draper and Weissburg, 2019). Most of these previous experiments rely on subjective assessments of behaviour, and more emphasis should be placed on measuring these responses at multiple levels of biological organization to provide a better understanding of the effects of ocean acidification on individual animals. Moreover, direct, causal and relevant ecological endpoints of olfactory changes are still largely missing. Under elevated CO_2, feeding rates in calcifying invertebrates are changing (Clements and Darrow, 2018). In addition, changes in algal grazing in damselfish (*Parma alboscapularis*) may lead to alterations in benthic algal (Ferreira et al., 2018; Randazzo Eisemann et al., 2019) and fish communities (Cattano et al., 2020), but direct links to changes in sensory activity are often missing. Thus, changes in odorant-mediated organismal interactions may lead to fluctuations in communities and populations, and alter inter- and intra-specific species relationships; however, to date, these effects are hypothesized rather than proven experimentally, highlighting the need for research in this area.

The costs associated with decreased olfactory abilities include physiological, behavioural and unquantified energetic costs incurred by maintaining or increasing signal production while coping with physiological stress (Rivest et al., 2019), as described for *Carcinus maenas* (Reid et al., 1997). However, most of these costs have not been proven directly (Nagelkerken et al., 2019). Furthermore, the evolution of chemical signalling systems will be slow and costly if it involves changes to odorant production pathways and active sites in receptors. Receptor plasticity and changes to selection are known to drive odorant–receptor evolution in insects but this remains unproven in aquatic organisms (Rivest et al., 2019). Evidence for adaptation of olfactory systems to acidification stress is lacking, even in studies of CO_2 vent systems, which may be thousands of years old. Olfactory systems may also show plasticity (Schunter et al., 2018) in receptor–ligand interactions in sea-dwelling organisms, as in insects (Ian et al., 2017), possibly due to epigenetic changes (Liew et al., 2018), but few long-term or multigenerational studies exist for aquatic species (Rivest et al., 2019).

There is a need for predictive studies combining models of physico-chemical changes to the aquatic systems with information on odorant bioavailability and bioactivity levels. An example of such a prediction for odorants is illustrated in a recent study on the changes in distribution of tetrodotoxin and saxitoxin (Roggatz et al., 2019a). This analysis shows that pH and temperature changes will result in increased protonation of those two highly potent biotoxins. Geospatial global modelling has been used to predict where this increased toxicity could intensify the incidence of paralytic shellfish poisoning (Roggatz et al., 2019). Although this approach holds great promise, it also requires proof of the direct links between the structural changes of odorants and observed effects, and needs to reflect ecological and local complexities (Rivest et al., 2019). Such predictive tools are essential for decision makers aiming to protect ecosystems, which rely on the stability of functional traits.

To construct an accurate prediction of how ocean acidification will change the marine ecosystem, we need to move away from assessing the impacts in a species-by-species manner and improve our understanding of the community-level responses. This is, of course, highly complex, but we could begin by testing interactions between pairs of species. Because both species in predator–prey interactions may experience sensory impairment (Allan et al., 2013), more studies investigating both sides simultaneously could provide valuable information. Another understudied issue is that of additive and synergistic effects of different environmental stressors, including increased temperature, pollution, hypoxia and habitat degradation. How these stressors may exacerbate or influence each other's effects will complicate an already complex process. Therefore, although it is becoming clear that ocean acidification affects the olfactory sensitivity of marine animals, there are still a number of important areas for future research, including: (1) the capacity for adaptation, (2) associated costs, (3) links to ecological endpoints and (4) responses to multiple stressors.

An additional question that remains unanswered relates to effects on freshwater animals, as pH and P_{CO_2} are much more variable in freshwater environments, not just because of aquatic acidification, but also as a result of acid rain and underlying geology. Although the effects of pH/CO_2 changes on freshwater fish have been well characterized (reviewed by Leduc et al., 2013; also see Ou et al., 2015), the extent to which freshwater animals can compensate for this variation remains unknown. However, that the same, or similar, receptors bind to the same odorants in both freshwater and seawater fish can be inferred from transcriptomic studies of anadromous and catadromous species such as chum salmon and the European eel, respectively (Churcher et al., 2015; Palstra et al., 2015). This indicates that, similarly to marine animals, freshwater animals do not compensate for changes in olfactory sensitivity when experiencing changes in pH/P_{CO_2}, but this is another area that warrants further investigation.

Better experiments on olfactory-driven behaviour

As mentioned above, whether ocean acidification affects the behaviour of coral reef fish remains controversial. One of the problems with the design of experiments to test the effects of ocean acidification on olfactory-driven behaviour is that the odorants involved, and their concentrations in the natural environment, are usually unknown. This is exacerbated by the fact that complex mixtures of odorants are involved, and these may vary between experiments and species. Thus, a possible explanation for studies that find no effect of high P_{CO_2} on the behaviour of reef fish (e.g. Clark et al., 2020a), thereby contradicting previous work (e.g. Dixson et al., 2010; Munday et al., 2010), is that the concentration of chemical stimuli used exceeded the natural threshold concentrations by several orders of magnitude, masking any relevant and observable reduction in olfactory sensitivity caused by low pH and/or high P_{CO_2}. If unidentified odorants are derived from conspecifics, their concentrations cannot be estimated or tightly controlled. Furthermore, the effect of acidification on stability and/or solubility of the odorant may need to be taken into account. For example, alarm cue released from damaged skin may decay faster in acidified seawater (Chivers et al., 2014; Porteus et al., 2018) independently of any effect on the olfactory system. Therefore, we propose that when testing odorant-driven behaviours of aquatic animals, those odorants must be of an environmentally relevant concentration (and not hundreds of times higher) and, ideally, experiments should use a single, identified odorant molecule or a fully identified mixture of molecules with known ratios, so that their concentration can be easily determined and/or controlled. Furthermore, most studies to date use static exposures to control or high/end-of-the-century levels of CO_2, but these experimental designs neglect the fact that – especially in estuarine and coastal areas – significant natural pH/CO_2 fluctuations exist, with organisms experiencing greater 'acute' changes than those that are predicted with climate change (Landschützer et al., 2018). Diel CO_2 cycles may reduce effects on behaviour or amplify these (Schunter et al., 2019). Acute effects on odorant-driven behaviours often described as 'pH-shock' may, as such, represent a more realistic scenario that animals experience.

Conclusions

We suggest that ocean acidification may directly affect olfactory sensitivity of marine organisms by altering odorant–receptor affinity. This could be caused by changes in the conformation of the odorant molecules and/or their receptors as a result of increased protonation and consequent changes in charge distribution, as well as longer-term changes in the olfactory system. This mechanism may act in addition to the GABA$_A$ receptor theory, and it is possible that other yet-unidentified mechanisms exist. However, to what extent either or both mechanisms are responsible for behavioural alterations of marine organisms in acidified water is not yet clear. What is clear is that the effects of ocean acidification on marine life are many and varied, and much more work is necessary to fully understand this complex problem.

Competing interests

The authors declare no competing or financial interests.

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