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The effects of ocean acidification on the olfactory system of fish.

How can the sensory and behavioural effects be mitigated ?

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Abstract

High atmospheric carbon emissions from burning fossil fuels are responsible for rapid climate change. After reaching the ocean, the atmospheric carbon undergoes a chain of reactions and ultimately alters the chemistry of the ocean. The current trend is that pH levels are slowly decreasing resulting in a more acidic ocean. Research has focused on the effects of ocean acidification on marine calcifiers, as they will be directly impacted through the dissolution of their carbonate shells or skeleton. However, fish seem to be threatened by the disruption of their olfactory system. Olfaction is essential to many fish species as it allows them to navigate, find food, find mates for reproduction, find their habitats and avoid predators. Therefore, any impairment of the olfactory system could have serious consequences on an individual's fitness and survival and have cascading effects at the ecosystem level. This essay discussed and reviewed the relevant literature focusing on answering the following research question: What are the mechanisms and the effects of ocean acidification on the olfactory system of fish and how can these effects be mitigated? This was done by answering the following three sub-questions: (a) What are the physiological mechanisms of ocean acidification on the olfaction of fish? (b) What are the sensory and behavioural effects caused by ocean acidification ? (c) How can fish mitigate the effects of ocean acidification on their olfactory system? Several studies show discrepancies in their results with experiments revealing that ocean acidification will have negligible effects on fish olfaction, whereas others point out strong detrimental effects including sensory and behavioural impairments. If the latter occurs, adaptation and resilience will be essential for fish to survive in future ocean acidification. Future studies (laboratory and mesocosm) should develop experimental approaches that scale up from: single to multiple environmental drivers (ocean warming), single species to communities and ecosystems and measure the species acclimation capacities for adaptation to future conditions.

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1| Introduction

a. Ocean acidification

Increased anthropogenic CO_2 emissions from the combustion of fossil fuels are altering the chemistry of the ocean through a process called ocean acidification (IPCC, 2022). Henry's law predicts that an increase in atmospheric CO_2 will result in an increase in the amount of CO_2 absorbed by the ocean, leading to a shift in the ocean's carbonate chemistry (Zeebe *et al.*, 2001; Shrivastava *et al.*, 2019). This phenomenon starts when released atmospheric CO_2 gets in contact with seawater and then forms carbonic acid (H_2CO_3) that later dissociates into bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions after losing hydrogen ions (H^+) (*Eq. 1*). This chemical reaction lowers the pH of the seawater resulting in a more acidic environment compared to initial pH levels. Currently, atmospheric CO_2 levels have almost doubled compared to pre-industrial levels, reaching 414 ppm. Future predictions reveal that this value could increase up to 1000 ppm by the end of this century resulting in a pH of approximately 0.3 lower than current levels (Cohen-Rengifo *et al.*, 2022). Marine calcifying organisms will be directly impacted by a lower pH, as there will be a high availability of H^+ in the ocean, this will affect the balance of the equation (Eq. 1) and capture all carbonate back into carbonic acid which then will become unavailable for marine calcifiers to form their skeletons and shells (Westphal, Ries and Doo, 2022). In the Southern Ocean for example, ocean acidification will likely affect species such as bryozoans, and bivalves by reducing calcification, growth at the larval stage and limited larval dispersal. All of the aforementioned species are part of an ecosystem that will suffer from the impacts of ocean acidification leading to an altered ecosystem structure and function, carbon export and biogeochemical cycling (Figuerola *et al.*, 2021). However, the effects of ocean acidification on higher trophic levels are less studied although species like fish could also be affected by ocean acidification through disruption of their olfactory system. The paragraph will explain what the olfactory system of fish is and why it is important.

$$CO_{2(g)} \rightleftharpoons CO_{2(aq)} + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

Eq. 1, (Leung, Zhang and Connell, 2022)

b. Olfaction in fish (teleosts)

Fish (teleosts) have a sense of smell; olfaction wish is the main sense that is based on chemo sensation (Laberge and Hara, 2001; Poncelet and Shimeld, 2020). Consequently, fish are equipped with chemosensory receptors to detect small molecules of water-soluble chemicals (Olivares and Schmachtenberg, 2019). The anatomy of the olfactory system of fish is made of different types of chemosensory receptor neurons integrated in a paired olfactory organ. These neurons make a first synapse in the olfactory bulb and from there the olfactory information is relayed to higher processing centres in the telencephalon (Fig. 1) (Olivares and Schmachtenberg, 2019). There are four main chemicals, often called 'odorants', that fish detect through olfaction: amino acids, gonadal steroids, bile acids and prostaglandins. Odorants such as bile acids facilitate migration to spawning sites, steroids and prostaglandins excreted from urine trigger reproductive behaviours and injured individuals release alarm pheromones (Villamayor *et al.*, 2021). Although many fish species use olfaction, certain species called '**macrosmatic**' (zebrafish, white catfish) have a greater number of olfactory lamellae and show higher responses to olfactory stimulation. Conversely, certain species called '**microsmatic**' (guppy, three-spined stickleback) have a lower number of lamellae and show a lower response to olfaction cues compared to visual cues for example (Atta, 2013; Santacà, Dadda and Bisazza, 2021). Olfaction mediates key behaviours such as finding a mate for reproduction, detecting food, avoiding predators, recognising conspecifics, finding habitats and migration. These behaviours can have a critical impact on the survival and fitness of an individual. Research on Sea lampreys (*Petromyzon marinus*) has found that they use chemical cues and pheromones to find productive spawning habitats, synchronize spawning behaviour and avoid predators (Buchinger *et al.*, 2015). Deprived or altered olfaction would prevent sea lampreys from completing their breeding migration and decrease their chances of survival. Consequently, the chemistry of the ocean and freshwater systems is essential for many species of fish to complete their life cycle and should remain adequately stable depending on the habitat of the fish (highly variable versus stable environments).

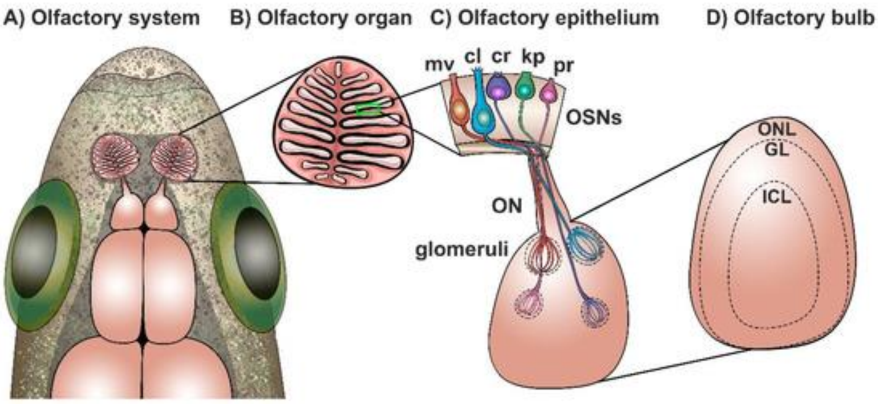

Fig. 1 Anatomical and morphological organization of the olfactory system of a zebrafish (*Danio rerio*). (**A**) Dorsal side of the olfactory system that is made of all the other components(B,C,D). (**B**) Olfactory organ with (**C**) olfactory epithelium arranged in lamellae and made of different olfactory sensory neurons (**OSNs**) including microvillous (**mv**); ciliated (**cl**); crypt (**cr**); kappe (**kp**); and pear (**pr**). OSNs extend their axons to the olfactory bulb via the olfactory nerve (**ON**) to form discrete **glomeruli**. (**D**) Olfactory bulb organization in three laminae: olfactory nerve layer (**ONL**); glomerular layer (**GL**); and intracellular layer (**ICL**) (Calvo-Ochoa and Byrd-Jacobs, 2019).

c. The link between ocean acidification and olfaction

Aquatic environments fluctuate in their pH levels compared to terrestrial environments. Therefore, to cope with this, fish can regulate their internal pH levels using acid-base homeostasis (Yan and Hwang, 2019). This process is dependent on the habitat of the fish: freshwater versus saltwater. In essence, in a freshwater system, the blood of a fish is hyper-osmotic; the individual loses ions across its gills and gains water. In this case, ion loss is actively compensated by branchial ion-uptake and renal ion-reabsorption while the kidney excretes the water load. In a saltwater system, the blood of a fish is hypo-osmotic, individuals gain ions and lose water. This is compensated by intestinal ion-uptake to drive water uptake and active branchial ion-excretion (Damsgaard *et al.*, 2020). Although acid-base homeostasis helps fish regulate pH, ocean acidification predicted at the end of the century is likely to exceed the acid-base homeostasis capabilities of certain species of fish and considerably alter the chemistry composition of freshwater and saltwater systems.

Fish rely heavily on chemical cues for olfaction; therefore, ocean acidification could significantly affect their fitness and survival. To find out how ocean acidification can alter the olfaction of fish, research has focused on using both laboratory and mesocosm experiments. Scientists studied the mechanisms, responses and adaptations of various fish species under different acidification scenarios to give an insight into 2100. However, it is still under discussion how these proposed mechanisms operate and how they differ between species and habitats. More research is required to understand and improve the current knowledge of the effect of ocean acidification on fish olfaction. Therefore, in this essay, I will discuss and review the relevant literature focusing on answering the following research question: What are the mechanisms and the effects of ocean acidification on the olfactory system of fish and how can these effects be mitigated? To do this I will answer the following three sub-questions: (a) What are the physiological mechanisms and the effects of ocean acidification on the olfaction of fish ? (b) What are the sensory and behavioural effects caused by ocean acidification ? (c) How can fish mitigate the effects of ocean acidification on their olfactory system?

2| Ocean acidification impairs olfaction.

a. Physiological, neurological and molecular mechanisms

There is still ongoing research on the number of mechanisms that will be altered by predicted ocean acidification and what effects will fish species experience around the world. First, studies showed that the effect of elevated CO_2 on fish was exclusively explained by changes in the brain neurotransmitter function (Porteus *et al.*, 2018). More specifically, ocean acidification impaired the gamma-aminobutyric acid type A ($GABA_A$) receptors of fish. These receptors are ligand-gated ion channels and are the major inhibitory mechanism **in the central nervous system** of vertebrates (Hamilton, Holcombe and Tresguerres, 2014). The $GABA_A$ gated channel has conductance for Cl^- and HCO_3^- ions. In normal conditions, channel opening results in Cl^- and HCO_3^- inflow causing membrane **hyperpolarization** and **inhibited** neural activity (Nilsson *et al.*, 2012). However, in elevated CO_2 conditions, fish regulate their acid-base balance to avoid acidosis by accumulating HCO^{3-} with compensatory reductions in Cl^- ions. This process can lead to ion-regulatory adjustments in blood and tissues that affect transmembrane gradients for Cl^- and HCO_3^- ions in some neurons. Accordingly, $GABA_A$ receptors become **depolarizing** and **excitatory** (Fig. 2). This mechanism was tested by several studies using $GABA_A$ receptor antagonist; gabazine that closes the $GABA_A$ receptor (Chivers *et al.*, 2014; Hamilton, Holcombe and Tresguerres, 2014; Heuer *et al.*, 2016). It is, therefore, expected that the effects of ocean acidification on fish would be reversed when exposed to gabazine. This has been found in a study using three-spined sticklebacks (*Gasterosteus aculeatus*). In acidified water conditions, sticklebacks (microsmatic**)** displayed a loss of lateralization, however, when sticklebacks were exposed to a gabazine treatment the behavioural and sensory impairments were reversed (Lai, Jutfelt and Nilsson, 2015). Similarly, scientists tested whether fish species that live in a naturally high CO_2 environment also suffer physiological changes when exposed to low CO_2 conditions. Results showed that fish in the low CO_2 treatment displayed higher activity levels compared to the control. When exposed to gabazine the activity levels were successfully restored, supporting once more the implication of the $GABA_A$ neurotransmitter in the mechanism responsible for fish sensory and behavioural impairments (Regan *et al.*, 2016).

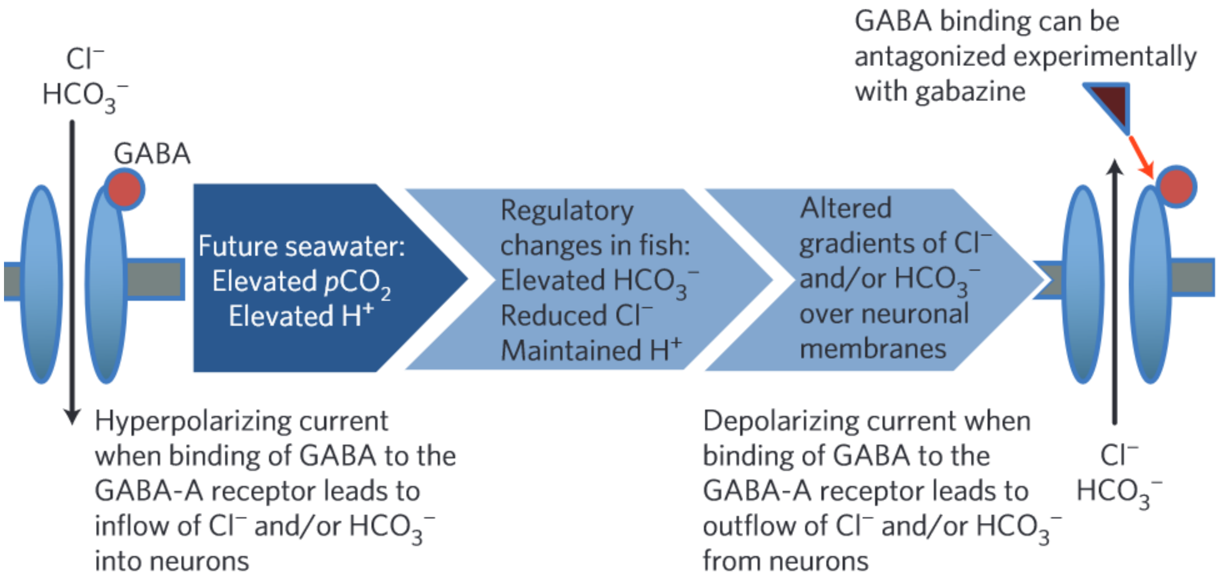

Fig 2. Proposed response of $GABA_A$ receptor function of fish to ocean acidification in the central nervous system. This GABA-gated ion channel becomes depolarizing instead of hyperpolarizing. (Nilsson *et al.*, 2012).

Nevertheless, recent studies show that there is more than the neurological mechanism mentioned above, involved in the impairment of fish olfaction in acidified water conditions. A physiological and molecular mechanism could be operating outside the central processing of sensory information and affect directly the olfactory system (Porteus *et al.*, 2018). Acidic water conditions could directly affect the olfactory system of fish provided that the olfactory epithelium is directly in close contact with the surrounding water pH fluctuations (Fig. 1). This entails that the cells of the olfactory epithelium are directly exposed to environmental changes. To test this, electrophysiology measurements and transcriptomics were used on European sea bass (*Dicentrarchus labrax*). Sea bass juveniles were exposed to both current (450 µatm; control) and predicted end-of-the-century levels of CO_2 (1000 µatm; elevated CO_2) and their response to a predator's smell was quantified. Electrophysiological recordings from peripheral sensory neurons of the olfactory system were used to isolate the peripheral olfactory response from the central brain processes. Overall, the study proposed the following physiological and molecular mechanism: ocean acidification can have a direct effect on the sensitivity of olfactory reception to various odorants probably due to reduced affinity of odorant-receptor binding in the olfactory epithelium. The electrophysiological data showed that less impulses are sent to the olfactory bulb in response to most odorants (regardless of concentration). Ultimately, this can lead to a decrease in **synaptic plasticity** and a decrease in **the activity of olfactory bulb synapses**. This decrease in synaptic plasticity is also shown through the **downregulation of gene expression** involved in synaptic plasticity and maintaining the excitability of both peripheral olfactory receptor neurons and central olfactory bulb neurons (Fig. 4) (Porteus *et al.*, 2018).

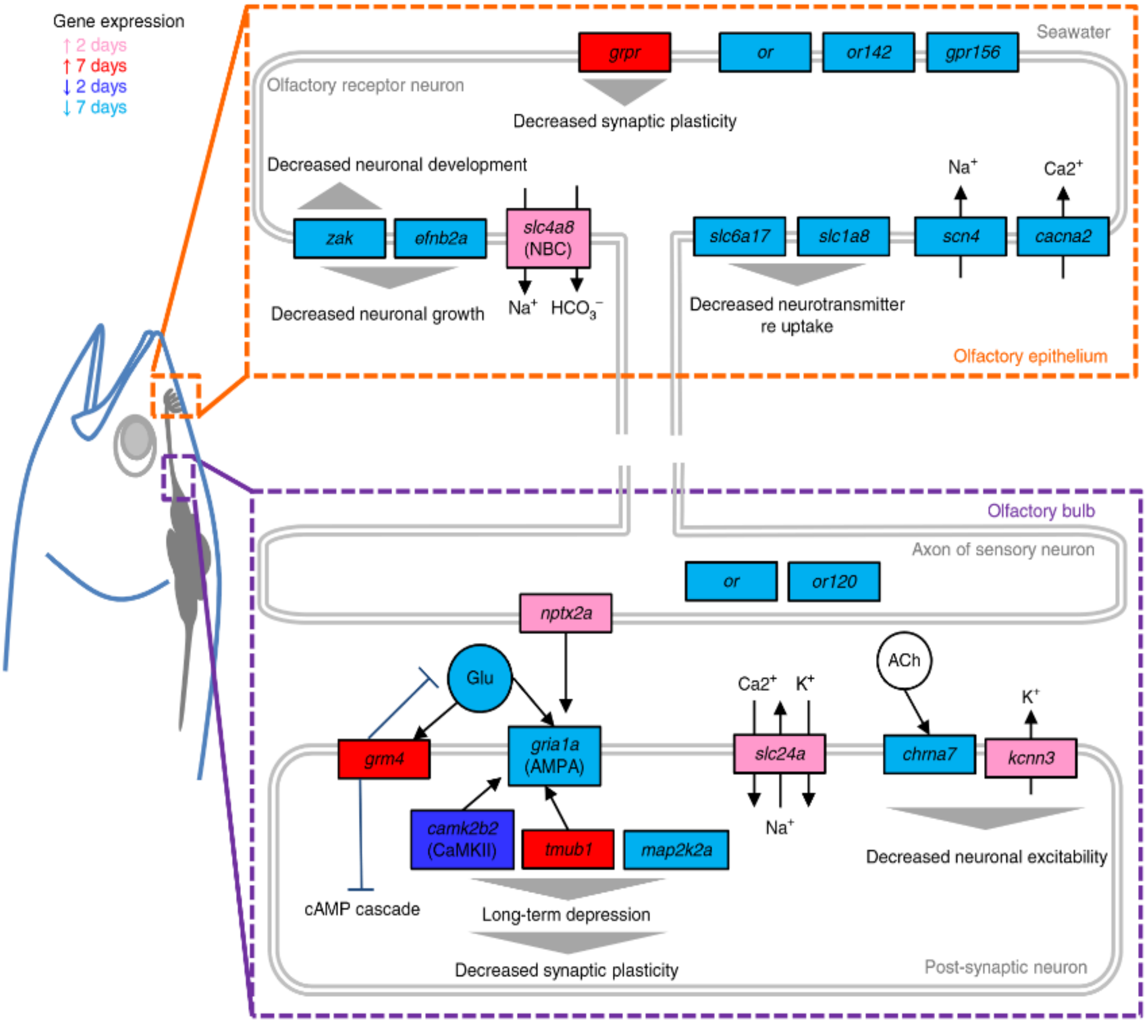

Fig. 4 Differential regulation of genes in the olfactory epithelium and olfactory lobe of European sea bass exposed to control and high CO_2. Genes involved in neuronal growth (efnb2a) and development (zak) were significantly downregulated in the olfactory epithelium. Additionally, genes encoding ion channels (scn4, cacna2, chrna7 and kcnn3) were also downregulated in both the olfactory epithelium and the bulb. Moreover, olfactory receptor genes were downregulated in both the olfactory epithelium and the bulb, indicating no compensatory mechanism for loss of olfactory function and changes in the wiring of the olfactory system in juvenile sea bass. Arrows represent direct pathways of activation, and T bars represent direct pathways of repression. Note that the axons of the olfactory sensory neurons in the epithelium synapse with neurons in the olfactory bulb. Colours represent the amount of time individuals were exposed to the treatments. Pink and blue represent high CO_2 and dark and light blue control treatment (Porteus *et al.*, 2018).

The neurotransmitter mechanism mentioned previously, is based on the extracellular acid–base regulatory changes in gradients of HCO_3^- and Cl^- ions in response to ocean acidification. Consequently, these changes interfere with the normal functioning of the $GABA_A$ receptor, causing excitation rather than inhibition of the nervous system. However, not all fish are good acid–base regulators, and some do not regulate extracellular pH in acidified water conditions (Brauner *et al.*, 2004). The physiological and molecular mechanism proposed is independent of any changes in blood acid–base chemistry but rather is dependent on the external water changes in CO_2 and H^+. Therefore, all fish species exposed to ocean acidification could suffer the direct impairment of peripheral olfactory sensitivity, whereas the central brain impairment of sensory behaviour will be relevant to species that are good acid-base regulators (Porteus *et al.*, 2018). To summarise, the impairment of sensory and behavioural response could be induced by two **complementary** physiological mechanisms acting on the olfactory system and $GABA_A$ receptor function in the brain.

b. Altered neurosensory and behavioural responses

Now that we understand the physiological mechanisms affected by ocean acidification, we can discuss the sensory and behavioural effects experienced by fish species in various experimental set-ups. Fish will undergo sensory impairments as aforementioned including sensing less information through their olfactory receptors and less information being transmitted to higher brain centres (Porteus *et al.*, 2018). This will lead to reversed olfactory preferences (Lai, Jutfelt and Nilsson, 2015). For example, a study on the success of larvae settlement of clownfish (*Amphiron percula*) reared in control treatment (pH: 8.15) and later exposed to acidified water conditions (pH: 7.8 or pH: 7.6) showed that acidification disrupted their olfactory cue preferences. In acidified treatments, clownfish larvae could not discriminate between cues that are beneficial for locating suitable adult habitat and cues that lead to unsuitable settlement sites (Munday *et al.*, 2009). Another study observed that these larvae were attracted to the smell of predators instead of avoiding it. These impacts on the olfaction of fish could have consequences at the individual, population and ecosystem levels (Dixson, Munday and Jones, 2010). Furthermore, it appears that olfaction is not the only sense that ocean acidification will affect (physiology and behaviour); for instance, a slower retinal response was observed in acidified conditions of spiny damselfish (*Acanthochromis polyacanthus*) (Chung *et al.*, 2014). Similarly, an absence of avoidance behaviour from juvenile clownfish to potentially predatory reef sounds (clicks and chirps of crustaceans and fish) was detected in acidified conditions. This is linked to the slower growth of carbonate otoliths (inner ear of fish) due to altered water chemistry from ocean acidification (Simpson *et al.*, 2011). Overall, olfaction, vision and hearing could all be disturbed by ocean acidification and significantly modify the behaviour of fish and their ecosystems.

Behavioural impairments will include loss of behavioural lateralization (left-right preference), loss of learning, and increased boldness and activity (Lai, Jutfelt and Nilsson, 2015). For instance, sand smelt larvae (*Atherina presbyter*) exposed to either control (pH: 8.10) or acidified conditions (pH: 7.61) for 21 days showed a significant decrease in individual lateralization when exposed to acidified conditions. Lateralization is an advantage in anti-predator responses, as prey can be right or left biased, preventing the predator from predicting prey movements. Additionally, lateralized individuals show better performance in multitasking, such as foraging, while being alert to predators. Therefore, temperate species in coastal ecosystems could suffer detrimental effects from ocean acidification that could lead to increased mortality (Lopes *et al.*, 2016). Furthermore, predator learning of pre-settlement damselfish (*Pomacentrus amboinensis*) exposed to acidified conditions revealed that they failed to learn to respond to their common predator, the Dottyback, (Pseudochromis fuscus). If ocean acidification affects the cognitive abilities of fish species by preventing them from learning, this could have important ecological consequences for species' survival in a constantly changing environment (Ferrari *et al.*, 2012). Although it will depend on the initial conditions of the environment of the fish, some systems such as coastal areas experience already variation in water chemistry compared to open ocean systems. Therefore, individuals in coastal areas might be more used to changing conditions and adapt better in the future. Finally, field experiments showed that juvenile damselfish were more active and ventured further from a shelter than juveniles exposed to current-day acidification. This riskier behaviour was associated with increased mortality (5-9 times higher) from predation compared to control fish (Munday *et al.*, 2010). This increase in activity and anti-predatory boldness will make prey significantly more vulnerable to predators and shift the dynamics of the ecosystem. Overall, ocean acidification will likely result in less olfactory information processed by the brain and less olfactory cues detected by individuals this will in turn reverse the natural behaviour of many fish species and decrease their survival (Table 1, appendix). However, recent studies contradict the findings mentioned in this paragraph and highlight flaws in these studies.

c. Discussion of methodological bias of impact studies

More recent studies disagree with the apparent strong behavioural effects that will result from ocean acidification mentioned in the previous paragraph. A multi-species, multi-year, and multi-life-stage experiment on the sensory and behavioural impairments of coral reef fishes under end-of-century ocean acidification found no consistent detrimental effects on the avoidance of predator chemical cues, activity levels or behavioural lateralization. In spite of the authors dedication to replicate the previous studies conditions by matching species, life stages, locations and seasons, the results were opposite. The authors discuss that the small sample sizes and other methodological and analytical biases might be responsible for the discrepancies between these results and previous studies (Clark *et al.*, 2020). They conclude that after testing 900 wild and captive individuals of 6 different species across 3 years that ocean acidification will not considerably alter the olfaction and behaviour of coral reef fish. Similarly, a meta-analysis of 91 studies explored the consistency and robustness of scientific evidence over the past decade regarding the direct effects of ocean acidification on fish behaviour and found evidence for a 'decline effect'. Large effects found in initial studies such as Munday et al., 2009 have disappeared in subsequent studies over the last ten years. (Clements *et al.*, 2022). The authors point out biases in those previous studies from paragraph 2.b.

Firstly, most studies have focused on coral reef fish species showing strong effects when exposed to treatments, whereas less studies have used cold-water fish that experience higher temporal variability in temperate regions. Therefore, cold-water species may be less sensitive to changes in acidification following the 'Ocean Variability Hypothesis' and should be studied as much as tropical fish species. Secondly, several studies have used larval fish that are typically considered to be more sensitive to environmental perturbations compared to juveniles or adults. This could also increase unreasonable effect sizes. Similarly, many authors do not provide background information on the levels of acidification historically experienced by the experimental species and use extremely low pH values in their experiment to identify thresholds to create models. Sensitivity thresholds for different types of odorants depend evidently on the species, but also on the type of assay, behavioural versus electrophysiological (Olivares and Schmachtenberg, 2019). The time of exposure to acidified water treatments varies widely between studies ranging from a few hours to days. Thirdly, the authors highlight that several studies with large effect sizes tend to be characterized by low sample sizes ($n<30$) published in high-impact journals with a high influence on ocean acidification research in terms of citations (Fig. 5). These small sample sizes are more prone to statistical errors; Type I and mostly Type II errors. Moreover, most studies used controlled conditions with a laboratory set-up and that allows very little comparison with the set-up of the natural world. To gain a better understanding of what impacts ocean acidification will have on wild fish it would be important to increasingly use mesocosm experiments. Additionally, using other abiotic factors that will act alongside ocean acidification such as ocean warming would provide very valuable insight in the effects fish will face in 2100. Overall, research needs to develop experimental approaches in ocean acidification that scale up from: single to multiple environmental drivers (Ocean warming), single species to communities and ecosystems and measure the species acclimation capacities for adaptation to future conditions (Stark *et al.*, 2019).

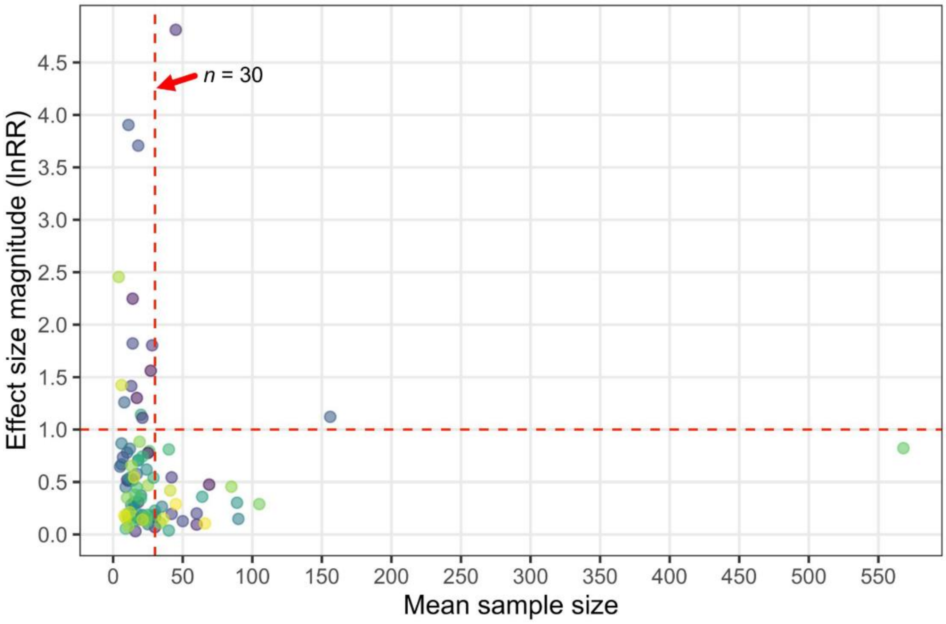

Fig. 5 Studies with large effect sizes have low sample sizes (Clements *et al.*, 2022). Colours follow a gradient according to year of publication online (blue represents older publications with green publication being more recent until yellow which represents very recent publications).

3| Mitigation of behavioural impairments

a. Mitigation of the effects of ocean acidification

This essay discussed the physiological mechanisms that will be affected by ocean acidification and the effects that can possibly rise from impaired olfaction on fish behaviour. Research showed contradicting results as to the direction (negative or neutral) of the effect of ocean acidification on fish behaviour. It appears that either fish will not be affected by ocean acidification through their olfaction system, or they will be severely impacted. I believe that there is certainly the potential for neurosensory and behavioural impairments as important physiological elements seem to be involved such as $GABA_A$ receptors and the higher brain centres. Additionally, it is important to remember that even in a scenario where ocean acidification would have negligible effects on the olfactory system of fish, it is not the only changing factor that fish will have to endure. Ocean warming and extreme weather events for instance are other factors from climate change that will most likely have detrimental effects on the physiology and behaviour of many fish species. Therefore, the additive effect of multiple elements might have the potential to disrupt many sensory and behavioural responses of fish species. This will most likely have consequences at the individual, population, community and ecosystem level. In essence, the Precautionary principle states that if something has the potential to cause harm then protection should be offered until there is complete scientific evidence proving otherwise. Therefore, I believe that although there are discrepancies in the results, we should try to protect fish species by reducing the future predictions of ocean acidification and ocean warming by reducing fossil fuels for example. Although this is a difficult process as many stakeholders are involved, in the meantime we should focus on increasing our understanding and evidence of the effects of ocean acidification on the olfactory system of fish and find out the adaptive potential of different species. In order to elucidate what impacts ocean acidification will have on fish olfaction, future research should incorporate several components including; larger sample sizes, semi-natural experimental set-up, reasonable pH levels (predicted at the end of this century), use a variety of fish species from temperate and tropical habitats and monitor effects of ocean acidification using long-term experiments and find out more about the resilience and adaptation potential of fish to ocean acidification.

b. Transgenerational adaptation and plasticity of fish

Recent studies have started to investigate the adaptation potential and resilience of fish species to ocean acidification using a combination of laboratory and mesocosm experiments. Adaptation has been shown in sand smelt. Larvae exposed to acidified conditions for 7 days showed a more random structure when compared to the aggregative structure exhibited by control larvae. However, this random distribution was reversed after 21 days of exposure to treatment, which suggests that group cohesion disturbance is not permanent. This suggests that sand smelt larvae will take longer to acquire shoaling behaviours under acidified conditions but will eventually restore their group cohesion (Lopes *et al.*, 2016). Additionally, a mesocosm approach revealed that ecological complexity buffered the impacts of ocean acidification on marine consumers (fish and crustaceans) (Goldenberg *et al.*, 2018). Consumers in acidified treatment were less attracted to either olfactory or visual food cues (simple level of complexity) (Fig. 6a). However, when both olfactory and visual cues were present consumers restored their attraction to food cues in the acidified treatment. Consequently, the hunting success of consumers was not affected by the acidified treatment (Fig. 6b). In the most complex level, resource availability was increased by elevated CO_2 conditions and thus, consumer assemblages revealed higher biomass. This was likely explained by the use of CO_2 as a nutrient that was transferred to secondary and tertiary producers (Fig. 6d). Increasing trophic diversity can indeed provide stability to food webs and enhances ecosystem services. Although isolated sensory cues were disturbed by ocean acidification potentially leading to a population decline, consumers restored their performance through compensatory responses at the organismal level. These responses may occur through two mechanisms based on the **cognitive flexibility** of animals. Firstly, the authors suggest that the impaired sensory system could be replaced by a functioning one (olfaction with vision) called '**sensory redundancy'**. Secondly, two impaired sensory systems complement each other '**sensory complementation'**. In the experiment shown in Figure 6, impaired vision and olfaction complemented each other. Neuroplasticity and learning probably play a role in the buffering of the negative effects of ocean acidification. The authors also point out that the compensatory processes may only occur if individuals are offered choices (resources and habitat) under long-term selective pressure (survival and competition) in the wild. These criteria are found near CO_2 vents and might have favoured the development of behavioural strategies to maintain survival in increasingly difficult tasks such as hunting. The authors conclude that by using the complexity that characterizes ecological niches, **behavioural plasticity** could improve the fitness of individuals through climate change (ocean acidification) and allow for genetic adaptation to occur. However, they also highlight that ecosystems as a whole will still likely experience losses in species and functional diversity (Goldenberg *et al.*, 2018).

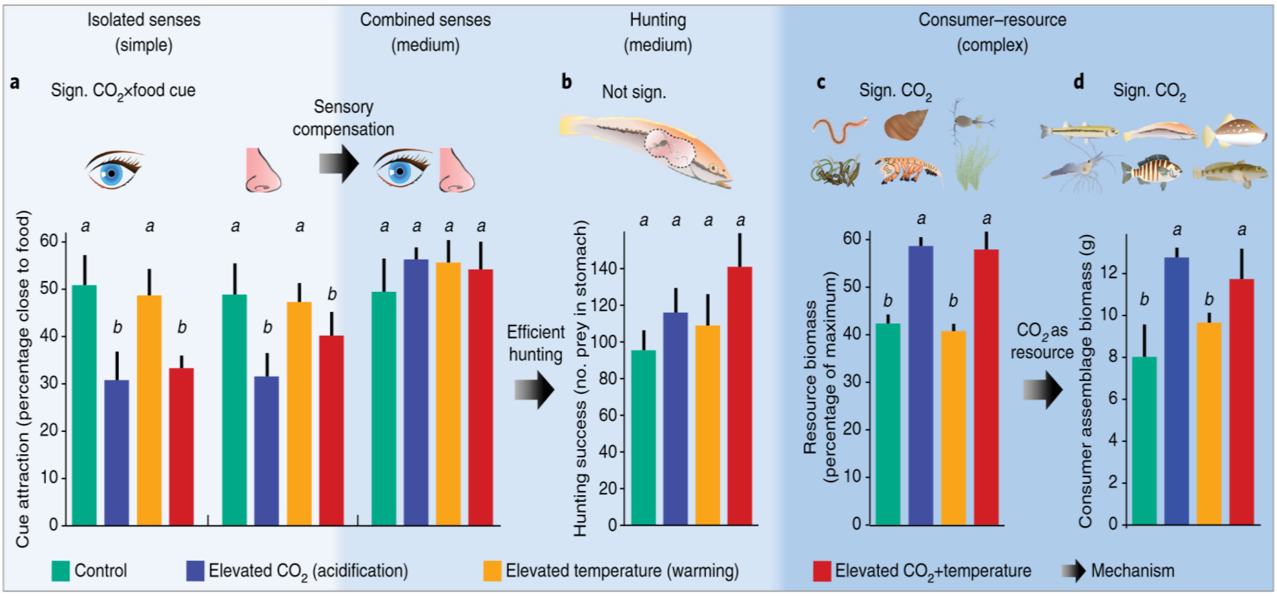

Fig. 6 Mesocosm study illustrating how the negative effects of ocean acidification on consumers can be buffered and reversed through ecological complexity. (a) sensing of visual, olfactory and combined visual-olfactory food cues. (b) Invertebrate prey captured during foraging. (c,d) availability of resources with (c) overall performance of consumers and (d) estimated as biomass after long-term exposure (Goldenberg *et al.*, 2018).

It is difficult to predict how fish species will acclimate and adapt to an increasingly acidic marine environment. Studies suggest that one generation doesn't seem enough for complete adaptation to occur and highlight that phenotypic modification at the peripheral and central nervous system will likely be required. This makes it difficult to assess the amount of time for adaptation on different levels to occur (Porteus *et al.*, 2018). However, another study that investigated the transgenerational long-term consequences of ocean acidification on the olfactory epithelium of seabass showed different results. Two generations of the European sea bass (*Dicentrarchus labrax*) were exposed to end-of-century predicted pH levels (Ph: 7.6), with parents (F1) exposed for four years and their offspring (F2) for 18 months. Transgenerational exposure to ocean acidification can induce adjustments of the transcriptomic profile in the olfactory epithelium that include **plastic responses** related to ion balance and transport, neuronal activity and plasticity, energy metabolism and innate immunity (Fig. 7). This transgenerational plasticity may be treated as an acclimation to prevent more severe physiological disruption at the whole organism level (Cohen-Rengifo *et al.*, 2022). Increasing the number of generations might show stronger results. However, it is difficult to assess the adaptation potential as many studies use short-term exposure which shows more of a physiological plasticity response to change, it will most likely require many generations. Overall, fish show potential to adapt to ocean acidification and could do so through transgenerational processes.

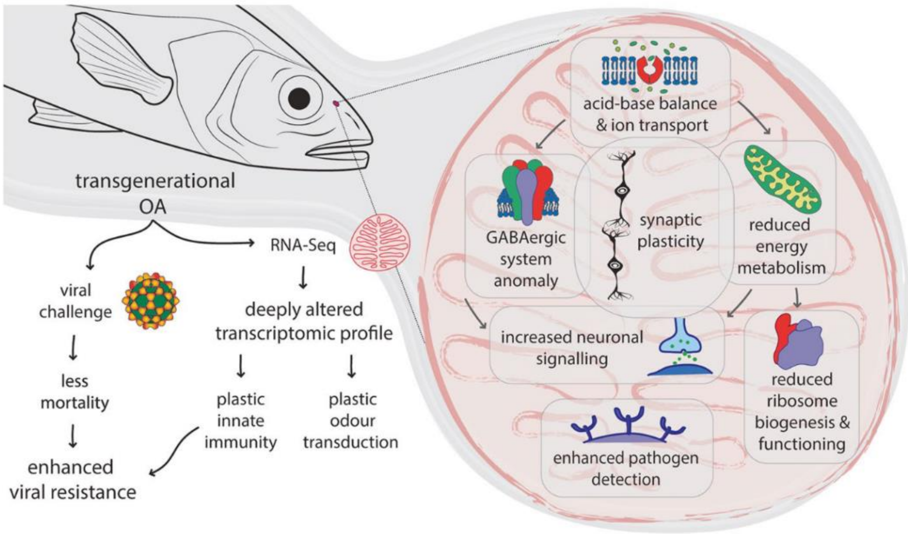

Fig. 7 Viral challenge and the RNA sequencing analysis of transgenerational ocean acidification on F2 of European sea bass (Cohen-Rengifo *et al.*, 2022).

c. Conclusion

In conclusion, a combination of physiological, neurological and molecular mechanisms acting together on the olfactory system of fish will be disrupted by current and future ocean acidification. Olfaction is a key sensory system that mediates key behaviours such as finding prey, reproduction, habitats and conspecifics. Altered water chemistry through ocean acidification will likely translate into an impaired sensory and behavioural response for several species of fish. This could entail reduced fitness and survival for many species of fish with cascading effects at the population, community and ecosystem levels. Although studies have shown highly contracting evidence supporting either negative or neutral effects of the olfactory system of fish it is important to keep in mind that ocean acidification will not be the only environmental change experienced by fish and that many methodological biases have been found in several studies. Therefore, it is of utmost importance that research develops experimental approaches in ocean acidification research that standardize their methods, scale up from: single to multiple environmental drivers (ocean warming), single species to communities and ecosystems and measure the acclimation to species capacities for adaptation to future conditions. Species spend different part of their life cycles in different environment (pelagic/coastal) and these environments will be affected differently by ocean acidification. Therefore, future studies should also try to assess what the overall effect this will have on the life cycle of fish. Despite the fact that it is difficult to predict how fish species will acclimate and adapt to an increasingly acidic marine environment. Recent publications show promising results for plastic behavioural responses to ocean acidification buying time for genetic adaptation to occur. Overall, future ocean acidification (slow long-term event) combined with ocean warming/extreme weather events (punctual rapid events) will create an increasingly challenging environment for entire ecosystems to thrive.

4| References

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Appendix

Table 1. Effects of acidification/alkalinization on olfactory/chemosensory abilities and its consequences in fish (Leduc *et al.*, 2013).

species	type of cue	behavioural response	treatment/ environmental change and range	consequence
freshwater				
Pimephales promelas	food stimulus	food searching	acidification (H_2SO_4)	
			pH 6.5	neutral
			pH 6.0	negative
Poecilia sphenops	food stimulus	food searching	acidification (H_2SO_4)	
			pH 6.0	neutral
			pH 5.0	negative
Salmo salar	L-serine	odour discrimination	acidification (HNO_3 and H_2SO_4)	
			pH 5.1	negative
	alanine	—	pH 5.1	positive
Onchorynchus mykiss	L-serine	electro-physiological response	acidification (H_2SO_4) metal dilution (Al^{-})	
			pH 4.7	negative
			pH 4.7, 20 μmol l^{-1} Al^{-}	negative
Orconectes virilis[a]	amino acids mixture	food searching	acidification (H_2SO_4)	
Procambarus acutus[a]			pH 6.8	neutral
			pH 5.8	neutral
			pH 4.5	negative
			pH 3.5	negative
Cambarus bartoni[a]	food stimulus	food searching	acidification (H_2SO_4)[b]	
			pH 7.5	neutral
			pH 4.5	negative
			pH 7.5	intermediate
S. salar	testosterone, ovulated female urine	electro-physiological response	acidification (H_2SO_4)	
			pH 6.5 – 3.5	increasingly negative
			alkalinization (NaOH)	
			pH 8.5 – 9.5	increasingly negative
Pimephales promelas, *Phoxinus neogaeus*	conspecific skin extract,	alarm response	acidification (H_2SO_4) pH 6.0	negative
	hypoxanthine-3-*N*-oxide	—	—	negative
Lepomis gibbosus	conspecific skin extract,	alarm response	acidification (H_2SO_4) pH 6.0	negative
	congener skin extract,	—	—	negative
	hypoxanthine-3-*N*-oxide	—	—	negative

O. mykiss	conspecific skin extract	alarm response	acidification (H_2SO_4) pH 6.0	negative
Salvelinus fontinalis	conspecific skin extract	—	stream acidification[c] pH $\sim$ 6.1	negative
S. salar	conspecific skin extract	alarm response	acidic rain[c] pH $\sim$ 6.2	negative
O. mykiss	—	survival to predator	acidification (H_2SO_4) pH 6.0	negative
S. salar	conspecific skin extract	alarm response	stream acidification[c] pH $\sim$ 5.8–6.1	negative
S. salar	conspecific skin extract, lemon odour	acquired predator recognition	stream acidification[c]	
		conditioning	pH $\sim$ 6.1	negative
		1 day PC	—	negative
O. mykiss	conspecific skin extract, predator odour	acquired predator recognition	acidification (H_2SO_4)	
		conditioning	pH 6.0	negative
		2 days PC	—	negative
		7 days PC	—	negative
S. salar	conspecific skin extract	alarm response	stream acidification[c]	
		RTE	pH $\sim$ 6.1	negative
		—	pH $\sim$ 7.3	neutral
marine				
Gasterosteus aculeatus	male odour	mate choice	alkalinization (NaOH)	
			pH 9.5	positive
Pomacentrus moluccensis, *P. amboinensis*, *P. chrysurus*	habitat odour	habitat choice	elevated carbon dioxide 700, 850 ppm CO_2	negative
Cheilodipterus quinquelineatus	habitat odour	habitat choice	elevated carbon dioxide 550–950 ppm CO_2	negative
—	habitat odour	homing response	elevated carbon dioxide[c] 550–950 ppm CO_2	negative
Amphiprion percula	habitat odour, conspecific odour	habitat choice/ homing	acidification, elevated carbon dioxide	
			pH 7.8, 1050 ppm CO_2	negative
Pagurus bernhardus[a]	gastropod shell cues	resources assessment	acidification, elevated carbon dioxide	
			pH 6.8, 12 000 ppm CO_2	negative
Pagurus bernhardus[b]	food odour	food searching	acidification, elevated carbon dioxide	
			pH 6.8, 12 000 μatmCO_2	negative
A. percula	predator odour	avoidance	acidification, elevated carbon dioxide	
			pH 7.8, 1000 ppm CO_2	negative

Plectropomus leopardus	predator odour	antipredator	elevated carbon dioxide 700 – 850 μatm CO_2	negative
A. percula	predator odour	predator discrimination	elevated carbon dioxide 900 μatm CO_2	negative
			900 μatm CO_2, GABA-A receptor antagonist	neutral
A. percula	predator odour	antipredator	elevated carbon dioxide	
			550 ppm CO_2	neutral
			700, 850 ppm CO_2	negative
Pomacentrus wardi	live predators	antipredator	elevated carbon dioxide[c]	
			700 ppm CO_2	neutral
			850 ppm CO_2	negative
P. moluccensis, *P. amboinensis*, *P. nagasakiensis*, *P. chrysurus*	live predator	predator avoidance	elevated carbon dioxide 700 μatm CO_2	negative
Pseudochromis fuscus	live prey	prey selection	elevated carbon dioxide	
			700 μatm CO_2	reversed preferred size selection
P. moluccensis, *P. chrysurus*, *P. amboinensis* *P. nagasakiensis*	conspecific skin extract	antipredator	elevated carbon dioxide 700 – 850 ppm CO_2	negative
P. chrysurus	live predators	survival	elevated carbon dioxide[c] 700 – 850 ppm CO_2	negative
Pseudochromis fuscus	prey skin extract	prey odour discrimination	acidification, elevated carbon dioxide	
			pH 8.0, 630 μatm CO_2	negative
			pH 7.8, 950 μatm CO_2	negative
Pomacentrus amboinensis	conspecific skin extract, predator odour	acquired predator recognition	acidification, elevated carbon dioxide	
			pH 8.0, 700 ppm CO_2	negative
			pH 7.9, 850 ppm CO_2	negative