Physiol Genomics 57: 146–147, 2025.
First published January 18, 2025; doi:10.1152/physiolgenomics.00181.2024

american physiological society | PHYSIOLOGICAL GENOMICS

EDITORIAL FOCUS

Off the shelf: Regulation of fish blood pH through ionocyte membrane remodelling and protein translocation without additional synthesis

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Ocean acidification (OA), together with warming and deoxygenation, forms the "deadly trio," a combination that reduces ecosystems' resilience, makes marine biodiversity vulnerable, and is considered a main contributor to global mass extinctions in the Earth's history (1). As the ocean's surface water absorbs the increasingly elevated atmospheric CO_2, the pH has steadily decreased from 8.11 in 1985 to 8.05 in 2021. This trend is expected to continue and is projected to decline between 0.15 and 0.5 pH units by 2,100 with a negative impact on the physiology of marine organisms, the food webs, and the whole ecosystem (2).

However, OA's effects on fish are less clear and even controversial. Early studies reported exacerbated impacts of OA, which either could not be replicated or used unrealistically high levels of P_{CO_2} and drops in pH (3). More recent studies using environmentally relevant OA conditions reported only slight or no osmoregulatory, metabolic, sensory, or behavioral responses in fish, and the mechanisms involved are still to be clarified or under debate (4).

Fishes can live in waters with a wide range of environmental pH. Freshwater and migratory species inhabit or move across waters ranging from pH 3 to 10, and many coastal and estuarine species live in waters ranging between 7.0 and 8.6, depending on daily changes in photosynthesis, salinity, and upwelling phenomena. Fish generally cope with acidity disturbances by buffering the extra- and intracellular media and excreting or absorbing H^+ and HCO_3^- across the gills and, to a lesser extent, through the kidneys and intestine, maintaining blood pH between 7.7 and 8.0 (5). The movements of H^+ and HCO_3^- across the gill are mediated by a set of membrane exchangers and co-transporters in specialized epithelial cells—the ionocytes—and primed by ATP-driven ionic gradients created by the Na^+/K^+ pump (Na^+/K^+-ATPase, NKA). Experimentally induced acid-base disturbances in blood pH can be compensated by increasing the activity or the amount of ion transporters (through changes in mRNA and protein expression), which include Na^+/H^+ and Cl^-/HCO_3^- exchangers (of the NHE and SLC26 families, respectively), Na^+/HCO_3^- co-transporters (NBCs) and the H^+ (V-type H^+-ATPase, VHA) and Na^+ (NKA) pumps (6). However, the dispersion of the studies across species, the inconsistency in levels of experimental hypercapnia, exposure, methodologies, and parameters analyzed limit the consensus about mechanisms and chronology of the physiological response.

In this journal, Kwan et al. (7) used transcriptomic, proteomic, and cellular approaches to establish that the splitnose rockfish (*Sebastes diploproa*) when exposed to environmentally relevant P_{CO_2} for 72 h (pH ~7.5, 1,600 μatm P_{CO_2}), can maintain their blood pH around 7.75–7.85, similar to fish in control conditions (pH ~7.9, 570 μatm P_{CO_2}) without significant alterations of gill mRNA coding for proteins involved in blood acid-base regulation, NKA and NHE3 protein abundance or ionocyte density. Instead, the ionocyte apical plasma membrane surface area increased, the apical microvilli extended, and cytoplasmic NKA and NHE3 proteins were recruited to the plasma membrane (7), to counteract the blood increase in P_{CO_2} with a rise in HCO_3^- (8). A similar lack of transcriptomic or protein abundance response to hypercapnia while fully regulating blood pH was observed in European sea bass (*Dicentrarchus labrax*) and red drum (*Sciaenops ocellatus*) exposed to ~pH 7.8, 1,000 μatm P_{CO_2} 2 to 3 wk (9 and refs therein, 10). Interestingly, a doubling of cell membrane surface area was observed in European sea bass exposed to 10,000 μatm P_{CO_2} for 130 min and was interpreted as a mechanism to rapidly increase acid excretion during sudden blood acidosis, possibly through post-translational modifications resulting in increased ion transporter activity (9). However, how this would explain the increased membrane surface area is not clear.

The study by Kwan et al. (7) shows that the solution to the puzzle is the translocation to the plasma membrane of pre-existing cytoplasmic proteins without changing total protein. Interestingly, neither the amount of membrane protein for the proton pump VHA nor the number of VHA-containing cells were modified, suggesting steady-state concentrations are either sufficient or not involved in the response to this level of acidification. However, Kwan et al. (7) have not measured the actual activity rates of either ATPase, which could allow for evaluating their contribution and estimating the metabolic costs of the initial functional response at the gill.

Examination of the several studies on ecologically relevant hypercapnia suggests the response mounts from rising the buffering capacity by increasing the branchial uptake of HCO_3^- as an initial response to low and mild P_{CO_2}, buffering blood pH paralleled by increasing H^+ extrusion using the

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Submitted 25 November 2024 / Revised 23 December 2024 / Accepted 1 January 2025

established capacity, followed by the recruitment of proteins from intracellular stores to the membranes. Transcriptional responses may occur at higher or more prolonged P_{CO_2} exposure. The model proposed by Kwan et al. (7) supports this notion and adds novel information on structural changes at the apical membrane, with widening and the extension of microvilli, already during acute environmentally relevant hypercapnia, that further change to include the recruited proteins during chronic environmentally relevant hypercapnia. Although the difference between ecologically relevant and extreme hypercapnia is relatively straightforward, the definition of "chronic" in the context of these studies still needs to be agreed upon and used consistently. Whether differential transcriptional responses occur beyond the 72-h exposure in the rockfish remains to be determined.

Altogether, Kwan et al. (7) and other studies indicate that the level of response (molecular and cellular) to hypercapnia depends on the magnitude and duration of exposure and likely differs among species according to their habitat-related physiological adaptations and level of phenotypic plasticity. Furthermore, hypercapnia occurs in conjunction with increased ocean temperatures and, in some cases, hypoxia, requiring more complex physiological responses (2). Holistic approaches and high-resolution techniques will be crucial in multifactorial environmentally-relevant experiments to disentangle the effects of these stressors, especially since large disruptions may cause obvious impacts, and small changes may be rapidly resolved and/or difficult to perceive.

GRANTS

This work received financial support from the Science and Technology Foundation (FCT), Portugal (Project IDs: UIDB/04326/2020, DOI: 10.54499/UIDB/04326/2020; UIDP/04326/2020, DOI: 10.54499/UIDP/04326/2020; LA/P/0101/2020, DOI: 10.54499/LA/P/0101/2020).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

P.M.G. and A.V.M.C drafted manuscript; edited and revised manuscript; and approved final version of manuscript.

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