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Viewpoint

# Hydrostatic pressure and the experimental toxicology of marine fishes: The elephant in the room

Benjamin Lemaire

*Institut des Sciences de la Vie, Université Catholique de Louvain, Louvain-la-Neuve 1348, Belgium*

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ABSTRACT

Hydrostatic pressure (HP) increases linearly with depth in aquatic environments, so that many fish species routinely experience moderate-to-high HP levels (i.e., from a few to dozens of MPa). Biological effects of this thermodynamic variable are evidenced by a reduced functionality of many biomolecular systems, even in barotolerant and barophilic species. It is likely that environmentally-relevant HP levels (i.e., above atmospheric) could also modulate the responsiveness to and toxic effects of pollutants in fish. Still, only a few laboratories have investigated this possibility. The already-published ecobarotoxicological studies have brought strong support to the notion that HP can indeed modulate pollutant response in shallow-water and deep-sea animals. A careful reassessment of toxicity responses is therefore required. To quantify the exact influence of HP in marine fish toxicology, a research framework is proposed that should ensure the collection of meaningful data for risk assessment, using standard toxicity testing and mechanistic approaches.

## 1. Introduction

Hydrostatic pressure (HP) increases by about 0.1 MPa for each ten-meter depth in the water column (Saunders and Fofonoff, 1976), so that many aquatic organisms routinely experience high HP (HHP; from a few to dozens of MPa). In favor of this, a recent analysis of about 12.000 marine fish species notably revealed that the vast majority live at depths corresponding to several dozens to thousands of meters (Priede and Froese, 2013). Marine organisms of the hadal zone experience HP levels in the range of 60–110 MPa (Jamieson et al., 2010), while the freshwater biota of the deep Baikal Lake has to cope with HP levels up to 16 MPa (Somero, 1992). Biological effects are to be suspected from exposure to HP levels above atmospheric (i.e., > 0.1 MPa); HP is one of the few parameters governing free energy changes. This thermodynamic factor is a key determinant of the vertical distribution of marine biota and of speciation events in the deep-sea (Somero, 1990, 1992; Morita, 1999).

Biochemical systems and processes can be strongly impacted upon exposure to HP levels above atmospheric. At the biomolecular level, reactions with negative (or positive) volume change are favored (or repressed) with increasing HP. For rate processes, the equilibrium volume change ($\Delta V$) is replaced by the activation volume ($\Delta V^{\neq}$) (Somero, 1990; Pradillon and Gaill, 2007). According to the transition state theory, $\Delta V^{\neq}$ is interpreted as the volume change associated with the transition from a ground-state complex to the activated state. $\Delta V^{\neq}$ is the sole factor governing reaction velocity under conditions of saturating substrate concentrations; it can be derived from experimental measurements of the HP-dependence of $V_{max}$ (Low and Somero, 1975).

HP increases can promote the (de-)stabilization of non-covalent interactions that are key to the static and dynamic behavior of biomolecules (e.g., ligand binding, protein-protein and protein-lipid interactions) (Silva et al., 2014). Increasing HP can therefore lead to a state of inactivation for biochemical systems and processes having large positive $\Delta V$ or $\Delta V^{\neq}$. The above rules are to be understood in the molecular context of solute-solvent systems; interacting water molecules are part of $\Delta V$ or $\Delta V^{\neq}$ calculations.

### 1.1. Adaptation to HHP comes with a cost in deep sea fishes

It follows that achieving "life at low volume change" (Somero, 1990) is a necessary adaptation of the molecular arsenals of deep-living aquatic organisms. Studies with deep-sea fish enzymes notably showed limited impacts of HHP on $K_m$ values of cofactors (i.e., binding affinity), which is in sharp contrast with shallow-water fish homologs. This evolutive trait seems to be acquired at the expense of $k_{cat}$ (i.e., catalytic efficiency) (e.g., Somero, 1990; Brindley et al., 2008).

The case of lactate dehydrogenase (LDH) is particularly good to illustrate this. LDH has long been a model of choice to investigate the hyperbaric behavior of deep-sea fish cytosolic enzymes subjected to large volume changes during catalysis. Binding of ligand to LDH indeed implies a substantial increase of system volume, due to the

*E-mail address:* benjamin.lemaire@live.be.

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displacement of densely-packed water molecules around the ligand itself and conformational changes of the enzyme upon binding. Deep-sea fish LDH show limited perturbations of $K_m$ with increasing HP (Somero, 1990; Brindley et al., 2008). Maintenance of $K_m$ at values close to in vivo concentrations of substrates and cofactors appears key to allow a fine tuning of reaction velocity under deep-sea conditions (Somero et al., 1983). As explained below, amino acid substitutions are involved in this phenomenon (Somero, 1990; Brindley et al., 2008; Nishiguchi et al., 2010). This molecular adaptation of LDH to HP is however associated with a loss of catalytic efficiency (i.e., lower $k_{cat}$ values) (Somero, 1990; Brindley et al., 2008). The exact role of each substitution in this effect remains to be solved (i.e., in vitro mutagenesis studies are needed). Importantly, not all deep-sea fish enzymes show higher stability of $K_m$ values with HP compared to shallow-water fishes. Some marine fish enzymes, like pyruvate kinase, may already be preadapted to function at HHP (Siebenaller and Somero, 1982).

HHP can also greatly perturb the cascade leading to the activation of membrane-bound G protein-coupled receptors in shallow-water fishes, and corresponding molecular events in deep-sea species also are affected but to a lesser extent (e.g., Siebenaller, 2003). HHP effects on these signaling systems could be explained by the same molecular mechanisms as for isolated enzymes such as LDH (i.e., effects on ligand binding, protein-protein interactions). Additionally, perturbations of protein-lipid interactions could be involved in this case (i.e., membrane-bound systems).

HHP decreases the ability of an agonist to stimulate guanosine triphosphate hydrolase activity in a shallow-water as well as a deep-sea *Sebastolobus* (i.e., reduced agonist efficacy). Still, basal hydrolase activity increases with HP in both species. This leads to low intracellular signal amplification as well as early signal termination in the two *Sebastolobus*; similar effects in human are associated with negative health outcomes (Siebenaller and Garrett, 2002).

Molecular biology and biochemistry data support the fascinating view that a few amino acid substitutions, along with changes of internal milieu composition, are sufficient for deep-sea fish proteins to resist the HHP-induced inactivation typical of shallow-water homologs (e.g., Somero, 2003; Brindley et al., 2008, Nishiguchi et al., 2010; Lemaire et al., in press). Amino acid side chains indeed govern the formation of non-covalent interactions and protein cavities and, in concert with the internal milieu, define their solvation state; small changes in the amino acid sequence of proteins can significantly modify system volume. Further, the increased incorporation of mono- and poly-unsaturated fatty acids in cell membranes of deep-sea fish appears key to ensure resistance to HHP-induced membrane-ordering effect (Macdonald, 1984; Somero, 1992). This homeoviscous adaptation accounts for the lower repression of membrane-bound proteins with increasing HP, compared to shallow-water homologs (e.g., Gibbs and Somero, 1990).

### 1.2. An emerging picture of HP influence in marine fish toxicology

HP levels encountered by most marine fishes could somehow influence their responsiveness to the many pollutants that accumulate in their environment. Further, responses of biomarkers of exposure might well be significantly impacted by the recent history of HP exposure of the individuals analyzed in biomonitoring studies. Both shallow-water and deep-sea fishes can thus be affected; the magnitude of effect would depend on the breadth of molecular adaptation of the species considered and the history of exposure to HP levels above atmospheric of the individual under study.

Very few groups, however, have analyzed the possibility that HP could influence metabolism, responsiveness to and toxic effects of pollutants in marine organisms such as fishes. This is perhaps because experimental marine toxicologists have not challenged the technical difficulties associated with HP studies or lack the theoretical background needed to appreciate the potential influence of HP on their models.

Kopecka-Pilarczyk & Coimbra have analyzed responses of biomarkers of exposure to biologically-relevant HP levels in marine fishes, however in the absence of pollutants. In their first study, *Pagellus bogaraveo* juveniles showed reduced basal hepatic EROD activity (a phase 1 detoxification proxy) and increased hepatic protein carbonyl content (an oxidative stress proxy) compared to 0.1 MPa controls, following 14-day exposure of the animals to 0.5 MPa (i.e., 50 m depth) (Kopecka-Pilarczyk and Coimbra, 2010a). In their second study, silver *Anguilla anguilla* showed increased hepatic levels of oxidized proteins compared to 0.1 MPa controls, following 7-day exposure to 5 MPa (i.e., 500 m depth) (Kopecka-Pilarczyk and Coimbra, 2010b). Together, these data suggest that molecular systems detoxifying persistent organic chemicals (e.g., the aryl hydrocarbon receptor pathway) and perhaps also enzymatic antioxidant systems targeting reactive oxygen species (ROS) might be pressure-dependently repressed in marine fish.

In the laboratory, studies were designed to investigate the capacity of deep-sea fish to cope with pollutants at HHP. Precision-cut liver slices (PCLS) of shallow and deep-sea fishes were used in co-exposure experiments with a model polycyclic aromatic hydrocarbon (PAH) and HP levels representative of both surface and deep-sea ecosystems. The short-term co-exposure studies with PCLS of a coastal (*Dicentrarchus labrax*) and a deep-sea (*Coryphaenoides rupestris*) fish revealed a reduced transcriptional response of the phase 1 Cytochrome P450 1A (CYP1A) with increasing HP; this effect was less pronounced in the deep-sea fish (Lemaire et al., 2012, 2016). Non-monotonic changes in the expression of antioxidant genes were also noted with increasing HP in both species. PCLS studies with *D. labrax* further suggested an increased basal ROS production at HHP in shallow-water fish liver (Lemaire et al., 2016). These data support the view that the responsiveness of PAH detoxifying systems (here, the aryl hydrocarbon receptor signaling pathway, which mediates CYP1A induction; Hahn, 1998) could be pressure-dependently repressed in marine fishes, including in deep-sea species. Further, there could be imbalances between pro- and anti-oxidant forces in marine fish cells at HHP.

## 2. Methodological considerations

The field of “ecobarotoxicology”, as quoted by Vevers et al. (2010), aims at quantifying the exact influence of HP on the metabolism, responsiveness to and toxic effects of pollutants in aquatic organisms. Although only a handful of studies have been published in the field, a variety of conceptual approaches has already been employed. There are concerns that some of the toxicity data collected so far might have limited value in terms of species extrapolation of risk.

### 2.1. Different experimental approaches yield information of different value

The work of Kopecka-Pilarczyk and Coimbra (2010a, 2010b) cannot be considered as “true ecobarotoxicology” in the sense that no co-exposure scenario (i.e., pollutant stress applied at HP levels above 0.1 MPa) was tested. Nevertheless, their observations raised awareness on the possible impact of HP on the pollutant responsiveness of marine fishes. Studies with PCLS, however, were based on co-exposure scenarios. The pressure-dependence of the PAH response was analyzed at various HP levels in this case, revealing that the transcriptional responsiveness of the deep-sea fish PAH detoxifying system had adapted to some extent to HHP (Lemaire et al., 2012, 2016).

Very few additional ecobarotoxicological studies have been published. These are conceptually different from the ones cited above. Vevers et al. (2010) used a coastal polychaete as a surrogate for deep-sea relatives in 6 h or 48 h crude oil exposure studies at HHP levels up to 30 MPa, while Auguste et al. (2016) used hydrothermal vent shrimps in 72 h exposure studies with copper at a single HHP level (i.e., 30 MPa). Although the two studies are based on co-exposure scenarios with model invertebrates, there are concerns regarding the appropriateness of the experimental designs applied in both cases.