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# Proteomic responses of European flounder to temperature and hypoxia as interacting stressors: Differential sensitivities of populations

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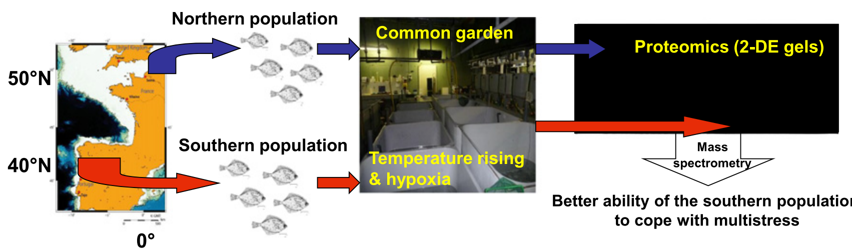
## HIGHLIGHTS

- Differential sensitivities of fish populations to temperature rising and hypoxia
- Specific regulation of crucial enzymes (ATP-synthase, G6PDH) in the populations
- Probable higher resilience of southern vs northern populations to climate changes

## GRAPHICAL ABSTRACT

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## ABSTRACT

In the context of global change, ectotherms are increasingly impacted by abiotic perturbations. Along the distribution area of a species, the populations at low latitudes are particularly exposed to temperature increase and hypoxic events. In this study, we have compared the proteomic responses in the liver of European flounder populations, by using 2-D electrophoresis. One southern peripheral population from Portugal vs two northern core populations from France, were reared in a common garden experiment. Most of the proteomic differences were observed between the two experimental conditions, a cold vs a warm and hypoxic conditions. Consistent differentiations between populations were observed in accumulation of proteins involved in the bioenergetics- and methionine-metabolisms, fatty acids transport, and amino-acid catabolism. The specific regulation of crucial enzymes like ATP-synthase and G6PDH, in the liver of the southern population, could be related to a possible local adaptation. This southern peripheral population is spatially distant from northern core populations and has experienced dissimilar ecological conditions; thus it may contain genotypes that confer resilience to climate changes.



## 1. Introduction

Estuaries are highly variable ecosystems, where living organisms must cope with environmental changes at the scale of a day (tidal cycle), seasons and even decades in the current context of global changes (Scavia et al., 2002; Roessig et al., 2005). These coastal zones are particularly exposed to the impact of global warming as well as to eutrophication-related decrease in oxygen availability (Breitburg et al., 2009; Rabalais et al., 2009; Zhang et al., 2010; Gillanders et al., 2011); thus numerous coastal systems are periodically submitted to temperature rising and hypoxia. Faced with these combinations of stressors, fish populations have to adjust to the new conditions by means of phenotypic plasticity, and/or adapt by means of genetic changes (evolution

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by natural selection) (Crozier and Hutchings, 2014; Gunderson et al., 2016). Recent studies have suggested that thermal- and hypoxia- tolerance might be functionally associated in families of *Salmo salar* (Anttila et al., 2013; McBryan et al., 2013).

The European flounder (*Platichthys flesus*) is a catadromous flatfish widely distributed in the North-East Atlantic, from the polar circle to Portugal. In spring, young juveniles are recruited in estuarine nurseries where they stay for at least two years, before migration to the mouth of estuaries for reproduction (Dando, 2011). *P. flesus* is considered as a pertinent fish model to explore the acclimation/adaptation of populations facing stressors in coastal systems over Europe (Hemmer-Hansen et al., 2007; Larsen et al., 2013; Dupuy et al., 2015; Lavergne et al., 2015).

In the present study, common garden experiments were conducted in an experimental structure to compare the responses of field collected flounder juveniles from three estuaries to different thermal regimes and hypoxia. This approach produced new insights in differential energetic metabolism and contrasted capacities of fish populations to cope with stressors (Whitehead and Crawford, 2006; Baumann and Conover, 2011).

Proteomic tools are widely used in aquaculture, studies in experimental conditions or environmental diagnostic purposes, but so far very few studies have dealt with marine populations directly sampled in the field. Yet, proteomic tools can be useful to enlighten metabolic differences between natural populations. For example, a recent study on the great scallop *Pecten maximus* showed a marked contrast in the cytoskeletal proteins content between populations along the European coast (Artigaud et al., 2014). Another study on fish used proteomics to investigate variation in protein content of two organs in three natural populations of European Hake (Gonzalez et al., 2010). In recent studies from our group, three natural populations of European flounder from both sides of the English Channel were compared, showing significant differences in their energetic- and glutathione- metabolisms (Galland et al., 2013; Dupuy et al., 2015).

In a changing world, multi-stressor experiments must incorporate naturalistic physicochemical variation into their designs to make ecologically relevant inferences about physiological responses to global changes (Gunderson et al., 2016). Thus, in the present study, to improve our understanding of the metabolism responses of the European flounder to multistress, juveniles were collected in three natural populations (two French northern core populations sampled in the Canche and Vilaine estuaries, and one Portuguese southern peripheral population collected in the Lima estuary). These three populations were shown to display a significant genetic differentiation (Calvès et al., 2013). Immediately after the catch, the fish were transported in the laboratory. Our objectives were: (1) to qualify the liver physiological adjustment occurring in flounders subjected to a realistic combination of temperature rising and hypoxia, (2) to determine possible differences in the responses of populations to multi-stress. Thus fish from the three estuaries were acclimated for 2 months in the laboratory and subjected to either a "cold condition" or a "warm and hypoxic condition". Proteins were then extracted from the liver and the proteomic responses of populations were compared using 2-DE based mass spectrometry.

## 2. Materials and methods

### 2.1. Fish sampling

Juvenile flounders were sampled by a beam trawl in October 2013. Between 100 and 120 juveniles of the year (0+ cohort: 8 cm < total fish length < 15 cm) were collected per estuary, in France (Canche and Vilaine estuaries) and in Portugal (Lima estuary) (Fig. 1). The Canche is considered as a relatively pristine estuary, as it displays very low levels of contaminants in comparison with large and heavily polluted systems (Amara et al., 2007; Amara et al., 2009).

The Vilaine is a moderately polluted estuary (Laroche et al., 2013), characterized by possible summer hypoxic events, mainly caused by eutrophication and by a dam located in the upper part of the estuary that favors water stratification. The Lima estuary is located only two hundred kilometers north of the southern latitudinal limit of *P. flesus*, the Mondego estuary in Portugal (Calvès et al., 2013).


**Fig. 1.** Sampling locations, with the additional location of the Mondego estuary, where is found the southernmost known population of European flounder along the Atlantic coast.

### 2.2. Experimental challenge

Fish were acclimated in the rearing facilities of the IFREMER center in Plouzané (France), at the local temperature and salinity of the sea in the Bay of Brest (9 °C, 35 PSU) for 60 days, and fed ad libitum with commercial pellets. Then, fish were challenged in two experimental conditions designed to mimic contrasted estuarine environments: the first one was a low water temperature (10 °C) and normoxic environment (above 90% $O_2$ saturation), which may be regularly encountered in winter by fish in French estuaries, but not by the Lima southern fish population, 10 °C being rarely observed in Portuguese estuaries (Martinho et al., 2007). The second condition was characterized by a high temperature (22 °C) and a moderate hypoxia (40% $O_2$ saturation); this rather high temperature has sometimes been observed in summer in flounder habitats over the English channel (Attrill and Power, 2004), whereas higher temperatures (until 25 °C) were frequently detected in summer, in Portuguese estuaries (Martinho et al., 2007).

Thus, in the present study, after the acclimation period, fish were marked and randomly assigned to a "cold" tank or a "warm and hypoxic" tank. Both tanks (water volume: 1.2 $m^3$) contained 150 fish (50 fish from each population). The cold tank temperature was set to 10 °C, and dissolved oxygen was maintained over 90% during the 60 days of the experiment (Fig. 2). Fish weight and total length were measured every 15 days during the experiment.

In the warm and hypoxic condition (Fig. 2), temperature was raised by 1 °C per day, until it reached 22 °C. The 53rd day (T53), dissolved oxygen level was lowered to 40% in 24 h. This multistress was maintained for six days. At T60, 30 fish from each estuary and each condition (total n = 120) were sacrificed by cervical dislocation. Fish total length and weight were measured, and livers were immediately collected, flash-frozen in liquid nitrogen and stored at −80 °C until further analysis.

### 2.3. Biometric comparison

Hepato-somatic index (HSI) is the ratio between the liver weight and the total weight of each individual. It was calculated to evaluate