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Compromised development of flatfish (*Solea senegalensis)* larvae under ocean warming and acidification

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The continuous CO_2 uptake by the oceans is changing the seawater chemistry and is estimated to lead to a drop of 0.4-0.5 units in seawater pH by the end of the century [1]. Concomitantly, the oceans are also becoming warmer, and the global sea surface temperature is expected to increase about 4°C by 2100 [2]. Early life stages are expected to be the most vulnerable to climate changes as they lack of well-developed gills with specialized ion-regulatory mechanisms to regulate and maintain their internal ionic environment [3-5]. Nevertheless the knowledge of their capacity to cope under such conditions remains poorly understood.

The effect of ocean climate change on the development of *Solea senegalensis* early life stages was evaluated by exposing eggs and larvae to ocean warming (+4ºC) and acidification (pCO_2 ~1600µatm). The identification and observation of the skeletal deformities and the morphometric analysis of otoliths were made with an optical stereomicroscopy (Leica S6D). Here we show that future conditions caused deleterious effects on early ontogeny of a teleost flatfish, *Solea senegalensis*, anatomy (Fig. 1, 2).

The percentage of all skeletal abnormalities increased by 31.4% ($p<0.001$) under the future warming and acidification scenario. Skeletal deformities consisted mainly of vertebral abnormalities, such as fusions (Fig.1, C) and body malformations (Fig.1, A-F). From a general perspective, caudal vertebra was the most impacted region (Fig.1, D-F), followed by the cranium (Fig.1, D, E), caudal fin (Fig.1, A, C, F) and abdominal vertebra (Fig.1, B, F).

Although fish skeleton is predominantly composed by calcium phosphate (in the form of hydroxyapatite and cartilaginous material [6]) the additional buffering of tissue pH with bicarbonate and non-bicarbonate ions caused by acidified conditions may interfere with larval skeletal development. pCO_2 was responsible for the greater incidence of severe skeletal abnormalities in flatfish larvae. Under present-day conditions, less than 1.9% of the larvae presented severe vertebral curvatures such as kyphosis or lordosis. Moreover, no kyphotic larvae were observed under such conditions. On the other hand, all types of anomalies significantly increased with future environmental predictions, especially with ocean acidification ($p<0.05$). Skeletal deformities founded may affect larval swimming behavior, feeding efficiency and the larval capacity to maintain their position in a current [7]. In addition to skeletal abnormalities, *S. senegalensis* larvae will also be affected by changes in otolith size (Fig. 2) under future climate change scenario. Sole larvae experienced a 109.3% increase in otolith area (normalized to fish length) with rising temperature and CO_2 ($p<0.001$), from 1063.6 ± 398.8 mm^2 to 2226.2 ± 187.0 mm^2 (Fig. 2). We argue that larvae with greater skeletal deformities and larger otoliths may face major ecophysiological challenges that might translate into substantial declines in fish adult populations, putting in jeopardy species fitness under this predicted climate change scenario.

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[1] K. Caldeira, *et al*., *Nature*, **425**:365, 2003.
[2] S. Solomon, *et al*., *IPCC Climate Change* 2007. Cambridge Univ. Press, 2007.
[3] H. Baumann, *et al*., *Nature Climate Change*, **2**, 38-41, 2011.
[4] A. Ishimatsu, *et al*., *Mar. Ecol. Prog. Ser*., **373**, 295-302, 2008.
[5] A.Y. Frommel, *et al*., *Nature Climate Change*, **2,** 42-46, 2011.
[6] L. Gil-Martens, *et al*., *Aquaculture*, **261**, 80-88, 2006.
[7] M. D. Powell, *et al*., *Dis. Aquat. Org.*, **85**, 59-66, 2009.

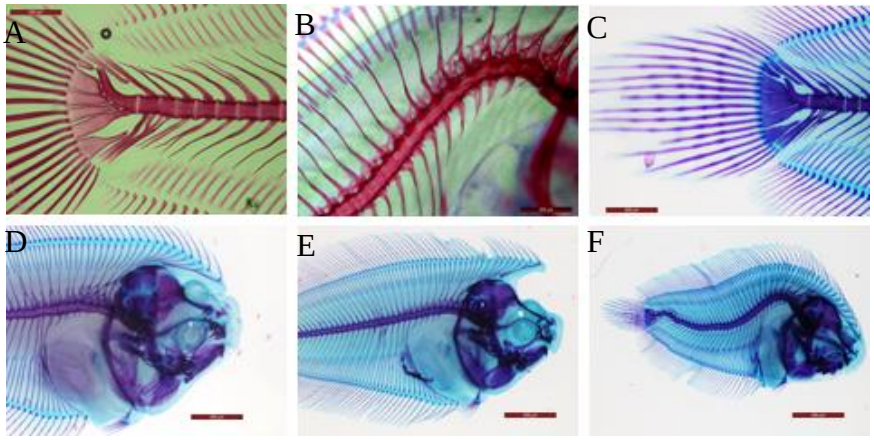

Figure 1. *Solea senegalensis* larvae skeletal deformities at 30 dph (days post-hatching) under the effect of ocean warming and acidification. Images from A to F were obtained using optical stereomicroscopy. Caudal fin complex anomalies such as modified neural and hemal spine, hypural and fin rays (A); kyphosis (B); vertebra fusion and compression, deformed spines, arches (C); vertebra fusion and compression, deformed spines, arches and parapophysis (D); cranium abnormality, ocular migration anomaly (D, E); lordosis and kyphosis (F).

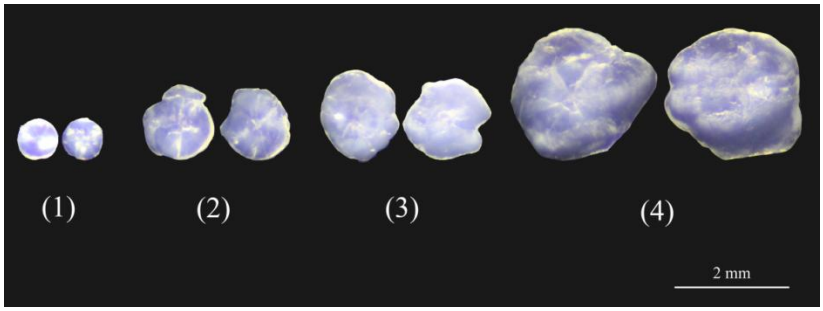

Figure 2. Effect of ocean warming and acidification on otoliths of 30 dph *Solea senegalensis* larvae. Otolith images were obtained using optical stereomicroscopy. (1) 18ºC, the mean sea surface temperature for the western coast of Portugal in summer and normocapnia (pCO_2 ~400µatm, pH=8.0); (2) 18ºC and hypercapnia (pCO_2 ~1600µatm, ΔpH=0.5); (3) 22ºC (+4ºC above the average summer sea surface temperature) and normocapnia; (4) 22ºC and hypercapnia.

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