

Effects of hypoxia on lateralization in three coastal species of fish

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Abstract

Coastal fish are daily subjected to several environmental changes mainly due to tidal rhythms. Hypoxic conditions are widespread in many coastal habitats and their occurrences have been increasing over the past years thanks to human activities. Our study used a simple turning preferences test (detour test) to assess the effects of hypoxia in fish behavioral lateralization using three commonly diffused coastal species as a model (*Cymatogaster aggregata*, *Gasterosteus aculeatus* and *Leptocottus armatus*). We found that hypoxia exposure disrupts behavioral lateralization at population level in *Leptocottus armatus*. In the population used in our experiment individuals showed a distinct preference for right turns in the detour test. After exposure to hypoxia (20% [O₂] for 2 hours) individuals showed equal probability to turn right or left. Given the value for survival of behavioral lateralization (e.g. enhanced of cognitive and escaping ability) this loss in behavioral lateralization could have severe costs for individual fitness in hypoxic environments. No effect was found in the other two species, which did not show behavioral lateralization on the population level in the control treatment.

1. Introduction

Oxygen concentration is one of the main factors affecting fish ecology, behavior and physiology. Depending on the life strategy some fish can actively search for optimal oxygen concentration conditions by migrating or by moving on the water column (Pihl et al. 1991) whereas others are naturally exposed to hypoxia (lack of oxygen), especially in intertidal zones (e.g. benthic fish), stratified waters and big schools. Since the 1960s hypoxia areas in the coastal oceans have significantly spread mostly due to an increase of the primary production in the ecosystems and in addition to coastal eutrophication generated by runoff waters rich in fertilizers. The accumulation of organic particles matter results in an increase of bacterial activity and oxygen consumption occurring at the seafloor resulting in a depletion of oxygen levels. More than 400 hypoxia systems have been reported so far (Diaz et al. 2008) which are mostly related to human activities. These hypoxic systems cover an area of more than 245 000 km² (Diaz et al. 2008). Consequently by increasing the occurrence of hypoxia events along coastal areas, anthropogenic activities could substantially modify the life of fish. In a conservational perspective it is relevant to understand the consequences of the phenomenon and to assess the necessary measures to avoid its occurrence.

Several studies have shown the negative effects of hypoxia on fish. During suboptimal oxygen level reproductive output is reduced (Wu et al. 2003; Thomas et al. 2006; Thomas et al. 2007), growth is limited (Chabot and Dutil 1999), swimming performance is decreasing (Jones 1971) and escape responses are impaired in number and accuracy (Lefrançois et al. 2005). At brain level exposure to hypoxia conditions enhanced several modifications in physiology and metabolism (e.g. Nilsson et al. 1993; Van Ginneken et

al. 1996). However little is known about how these changes affect brain functioning and consequently behavioral output. For all we know the only indirect evidence is a study that affirms hypoxia affected negatively responsiveness and directionality of “c-bend” during escape responses in golden grew mullets (Lefrançois et al. 2005). These effects could be due to an interference with the nervous system functioning.

Lateralization, a behavioral manifestation of functional brain asymmetry, is a widespread phenomenon among almost all vertebrate taxa going from reptiles to mammals (Vallortigara and Bisazza 2002). In fish it is diffused in a large number of species (Bisazza et al. 2000) and it is suspected to increase cognitive ability (Sovrano et al. 2005; Dadda and Bisazza 2006), schooling ability (Bisazza and Dadda 2005), spatial orientation (Sovrano et al. 2005) and escape performances (Dadda et al. 2010). It is supposed that the occurrence of lateralization in fish is subjected to evolutionary pressures. Consequently any modification of lateralization naturally observed in populations could have an effect on survival of –individuals. Lateralization could be assessed in fish with simple tasks, such as detour (Bisazza et. al. 1997) or mirror test (Sovrano et al. 1999). This makes lateralization a powerful tool to explore modification of fish brain functioning facing different environmental conditions. However this behavior has barely been used for that scope. Recently Domenici and colleagues (2012) showed that high CO₂ concentration, an environmental factor strongly related to human activities, acts on fish brain functioning reducing the degrees of lateralization at individual levels in larval coral fish species. Additionally a study made by Jutfelt and colleagues (2013) confirmed the effect of severe CO₂ concentration level on lateralization in fish using three-spined sticklebacks as a model.

The aim of this study was to assess the effect of hypoxia on lateralization in fish. Our hypothesis was that lateralized fish population might be affected by hypoxia and consequently loose on their behavioral lateralization. We used as a model three species of fish: shiner perch (*Cymatogaster aggregata*), three-spined stickleback (*Gasterosteus aculeatus*) and staghorn sculpins (*Leptocottus armatus*). All of these three coastal fish species are naturally subjects to daily fluctuations in oxygen concentrations and therefore represent ideal models to study the effect of hypoxia on lateralization in an ecological perspective.

2. Materials and Methods

2.1 Fish species and collection methods

Shiner perch and staghorn sculpin were sampled by beach seining at the Jackson beach (48°31'11''N, 123°00'40''W) on San Juan Island, Washington, US. Three-spined sticklebacks were sampled by dip-netting at Roche Harbor (San Juan Island, Washington, US). After transporting the fish to Friday Harbor Laboratories, they were kept in flow through seawater tanks (Length: 130 cm, width: 60 cm and depth=26,5 cm) under natural light conditions and at a temperature of 11,64 ±0,52 C°. All tested fish had been acclimatized to laboratory conditions for at least 24hrs before experimental trials.

2.2 Experimental protocol: progressive hypoxia treatment

Shiner perch and three-spined sticklebacks were exposed to progressive hypoxia. The experimental scheme consisted of decreasing the oxygen concentration in a stepwise process from normoxia to 20% of DO concentration. The oxygen concentration was measured and regulated with an oxygen probe (Oxyguard Galvanic oxygen probe) which was connected to an oxygen concentration regulator (PR electronic 5714 programmable LED indicator see Figure 1.). The oxygen levels were measured approximately every 10 minutes during the decreasing period (in the experimental tank and the column see Figure 1.) in order to control the stepwise fashion decrease. The experimental set up is described in Figure 1. At 20% of oxygen concentration, the fish were kept for two hours before being brought back to normoxia (stepwise in 120 min) and tested in a detour test to assess their behavioral lateralization. At 20% of DO concentration, the oxygen levels were recorded every 20 minutes over two hours period in the experimental tank and the column. The hypoxia treatment was divided into three experimental groups, one group of fish was tested in the detour test directly after hypoxia exposure (from now on referred as “hypoxia 0hrs”), one group was tested 24 hours after hypoxia exposure (from now on referred as “hypoxia 24hrs”) and the third group was tested 48hrs after hypoxia exposure (from now on referred as “hypoxia 48hrs”). A control group at normoxia (left undisturbed for 4hrs) was performed under the same experimental conditions as for the hypoxia treatment and then tested in the detour test for behavioral lateralization. In order to avoid an increase in temperature in the experimental tank, a thermobath was connected to the experimental set up to keep the flowing water at a constant temperature during the hypoxia treatment (see Figure 1). Before each treatment the water in the column and in the experimental tank was

changed. The water depth in the experimental tank was kept at 25 cm during the experiment.

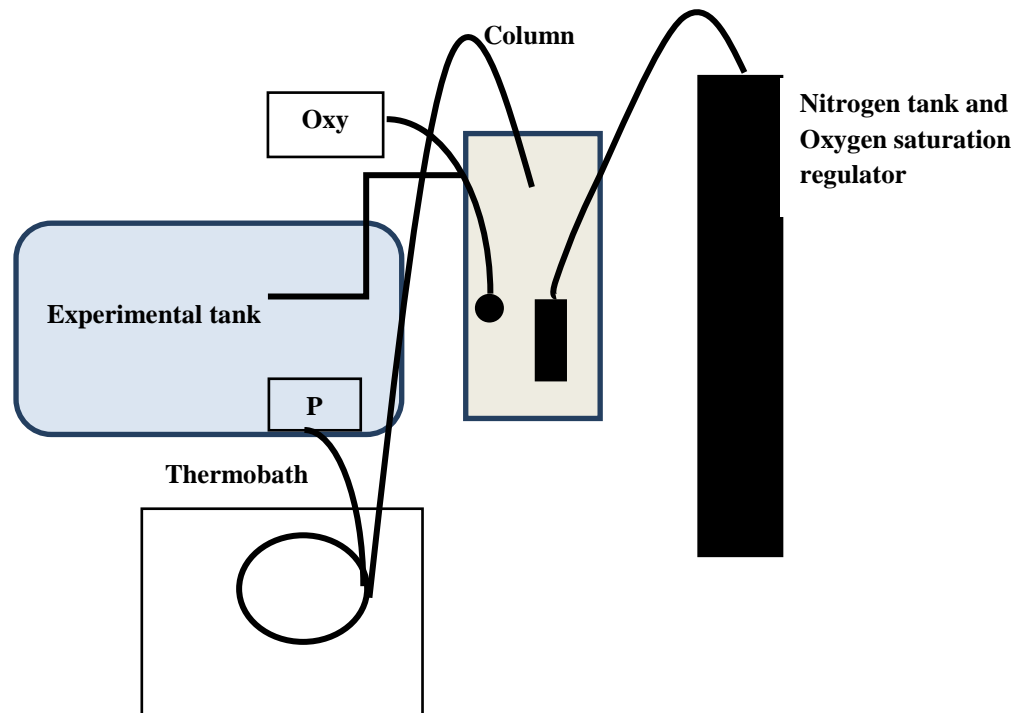


Figure 1. Experimental set-up for the hypoxia treatment. Nitrogen coming from a tank was bubbled into a column (Height:55 cm and width: 24cm). The oxygen level in the column was measured and regulated by an oxygen saturation regulator (represented as Oxy in the figure) which was controlling the flow of nitrogen added to the water in the column. The column was connected to the experimental tank (Height: 45 cm and width: 50 cm) where the tested fish were placed, creating a flow through of nitrogen rich water. A pump (P) was installed to recirculate the water between the experimental tank and the column. In order to keep the water in the experimental set-up at a constant temperature, the pump was connected to a thermobath.

2.3 Experimental Protocol: Acute hypoxia treatment

Staghorn sculpins are supposed to be more resistant to hypoxia condition, for that reason they were exposed them to acute hypoxia treatment. The experimental set up was the same as for the progressive hypoxia treatment (see Figure 1.). However the sculpins were subjected to a more rapid decrease in DO concentration up to 20% of oxygen dissolved in the water (in a period of 20 mins). During the abrupt decrease, oxygen levels in the experimental tank and column were measured approximately every 2 mins.

As for the other fish, sculpins were also exposed for 2hrs to 20% of DO concentration.

During this period the oxygen levels were measured every 10 minutes. A control group of 42 sculpins was kept under the same conditions as for the hypoxia treatment (during 2hrs 20 mins). After the two hours of treatment the subjects were tested for their behavioral lateralization directly under 20% of DO concentrations.

2.4 Experimental Protocol: Lateralization test

The standard detour test used in this project to evaluate individual behavioral lateralization was described by Bisazza et al. (1997). The experimental apparatus consisted of a tank with a central runaway. At each ends of the runaway there was a barrier perpendicular to the runaway (Figure 2.). Fish were individually inserted in one side of the apparatus. After two minutes of acclimatization the fish was gently pushed using a pair of fish net at the starting point of the runaway. The fish swam along the runaway until it faced the barrier and turned left or right. Run was repeated ten times on each fish and direction of turns was recorded. Two detour apparatus were designed according to the size of the fish species tested. For shiner perch and staghorn sculpin the runaway was 75 cm in length and 10 cm in width and the barrier was placed 6 cm away from the runaway end. For three-spined stickleback runaway was 35 cm in length and 5 cm in width and the barrier was placed 3 away from the runaway end. All experiments were performed in natural day light conditions.

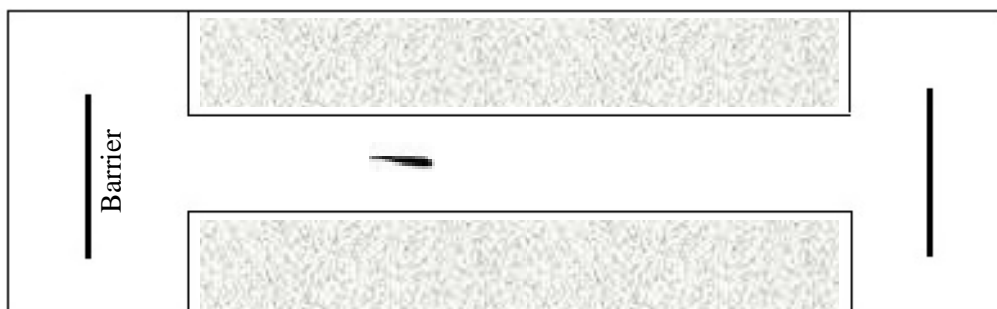


Figure 2. Representation of double T-maze apparatus.

2.5 Data and statistical analysis.

In order to investigate a possible turning preference at the population level we calculated the Relative Lateralization Index (L_R) (Bisazza et al. 1997), using the following formula:

$$\frac{[(\text{Turn to the right} - \text{Turn to the Left})]}{[(\text{Turn to the right} + \text{Turn to the Left})]} * 100.$$

In this kind of analysis fish were attributed values that go from -100 to +100, indicating complete preference for left and right turning, respectively. We used also Absolute Laterality Index ($L_A = |L_R|$) to assess the degree of individual lateralization.

We tested normality of our data L_R and L_A by using a Shapiro-Wilk test. We used one-sample t-test and one sample Wilcoxon signed rank test against zero (mean of a non-lateralized population) for assessing lateralization at population level. We used independent sample t-test, Kruskal-Wallis ANOVA and Wilcoxon rank sum test to assay the presences of differences between the experimental group in L_R and L_A .

3. Results

3.1 Shiner perch

118 shiner perches were tested overall (control=30, hypoxia 0hrs=30, hypoxia 24hrs=30, hypoxia 48hrs=28). Standard length of experimental subject was not different between the four experimental groups (SL(mm)=mean±sd: control=47.13±3.51; 0hrs=46.90±3.41; 24hrs=48.67±3.90; 48hrs=46.75±9.44; $F(114,3)=0.746$, $p=0.527$).

Control group L_R Index was not significantly different from zero (Wilcoxon Signed Rank Test, $p=0.063$) (Figure 3a.). L_R Index did not differ between the four experimental

groups (Kruskal-Wallis test, $p=0.583$) (Figure 3.; Figure 4a.). L_A Index did not differ between the four experimental groups (Kruskal-Wallis test, $p=0.573$) (Figure 4b.).

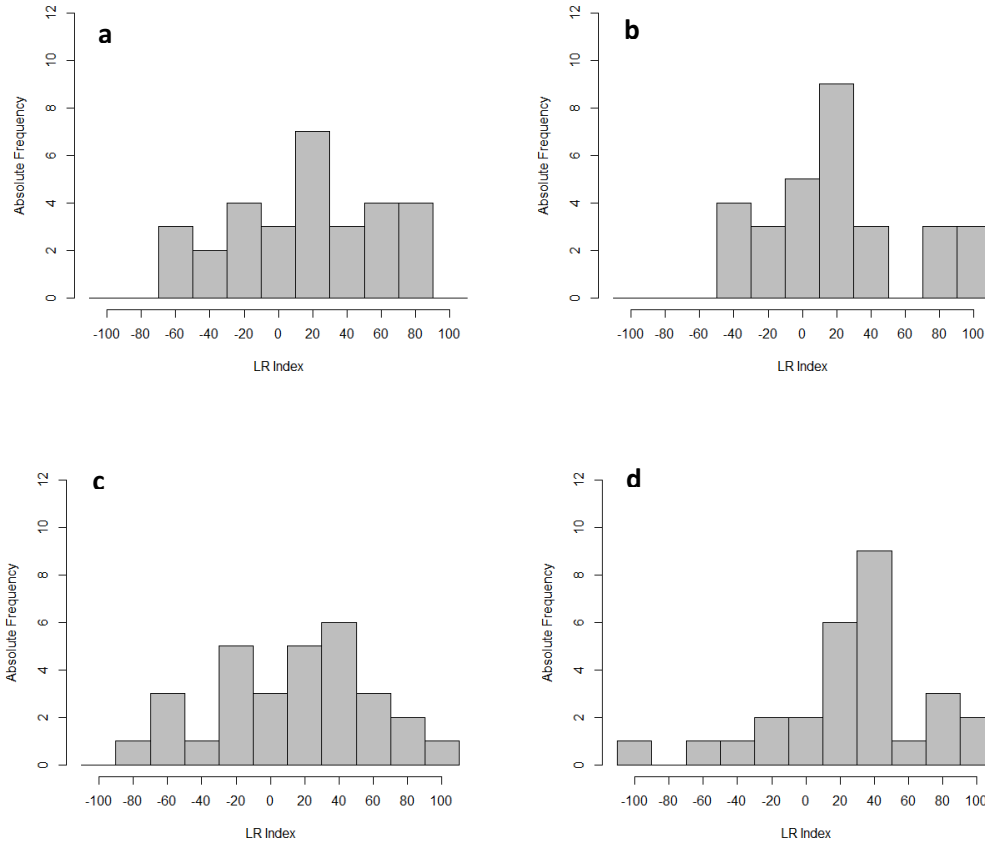


Figure 3. Distribution of frequency for L_R Index in the four experimental groups of shiner perch. a) Control group; b) hypoxia 0hrs group; c) hypoxia 24hrs group; d) hypoxia 48hrs group.

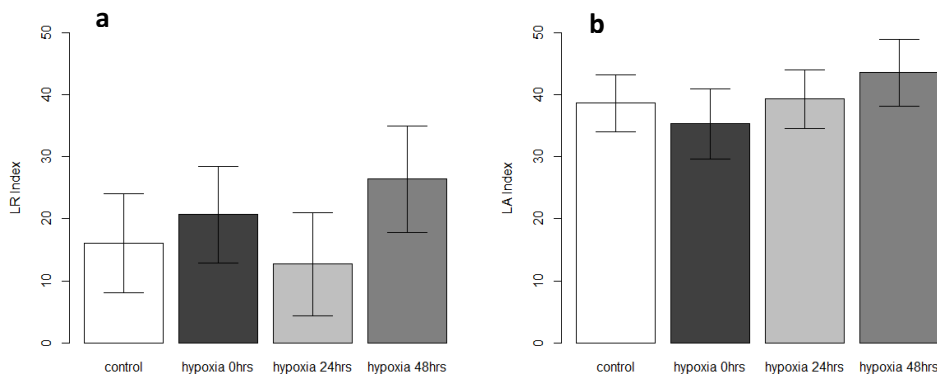


Figure 4. a) LR Index (mean \pm s.e.) in control group (white bar), hypoxia 0hrs group (black bar), hypoxia 24hrs (light grey bar), hypoxia 48hrs group (dark grey bar) of shiner perch; b) LA Index in control group (white bar), hypoxia 0hrs group (black bar), hypoxia 24hrs (light grey bar), hypoxia 48hrs group (dark grey bar) of shiner perch.

3.2 Three-spined stickleback

154 three-spined sticklebacks were tested overall (control=40; hypoxia 0hrs=40; hypoxia 24hrs=40; hypoxia 48hrs=34). Standard length of experimental subject was not different between the four experimental groups (SL(mm): control=23.65±4.03; 0hrs=25.05±4.57; 24hrs=24.80±4.52; 48hrs=22.91±3.40; F(150,3)=2.122, p=0.100).

Control group L_R Index was not significantly different from zero (Wilcoxon Signed Rank Test, p=0.544) (Figure 5a.). L_R Index did not differ between the four experimental groups (Kruskal-Wallis test, p=0.223) (Figure 5.). L_A Index did not differ between the four experimental groups (Kruskal-Wallis test, p=0.733) (Figure 6a.).

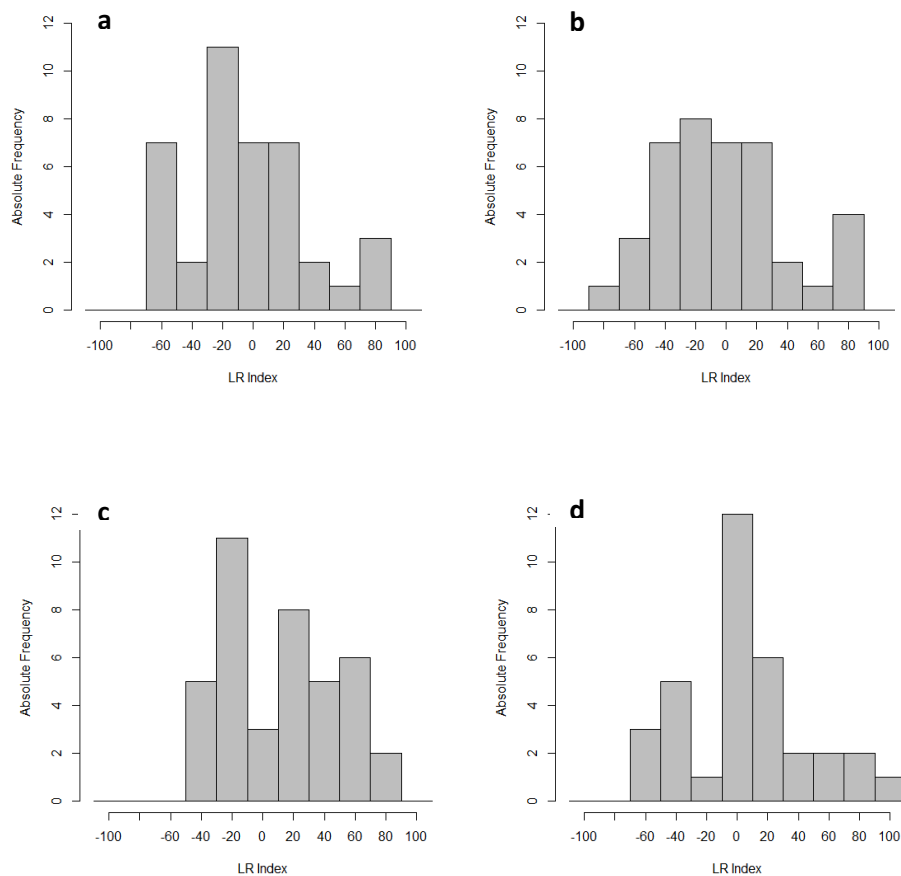


Figure 5. Distribution of frequency for LR Index in the four experimental groups of three-spined stickleback. a) Control group; b) hypoxia 0hrs group; c) hypoxia 24hrs group; d) hypoxia 48hrs group.

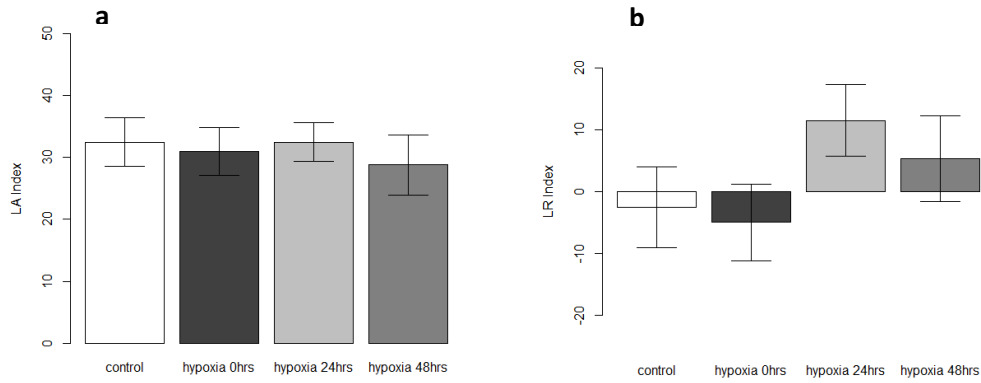


Figure 6. a) LA Index (mean±s.e.) in control group (white bar), hypoxia 0hrs group (black bar), hypoxia 24hrs (light grey bar), hypoxia 48hrs group (dark grey bar) of shiner perch; b) LA Index in control group (white bar), hypoxia 0hrs group (black bar), hypoxia 24hrs (light grey bar), hypoxia 48hrs group (dark grey bar) of three-spined stickleback.

3.3 Staghorn sculpin

84 staghorn sculpins were tested overall (control=42; hypoxia=42). Control group L_R was significantly different from zero ($t(41)=5.439$, $p<0.001$) (Figure 7a.). L_R Index was significantly different between the two experimental groups ($t(82)=2.165$, $p=0.033$) (Figure 7). The hypoxia treated group L_R was not significantly different from zero ($t(41)=1.808$, $p=0.078$) (Figure 7b.). L_A Index did not differ between the two experimental groups (Wilcoxon rank sum test, $p=0.556$) (Figure 8b.).

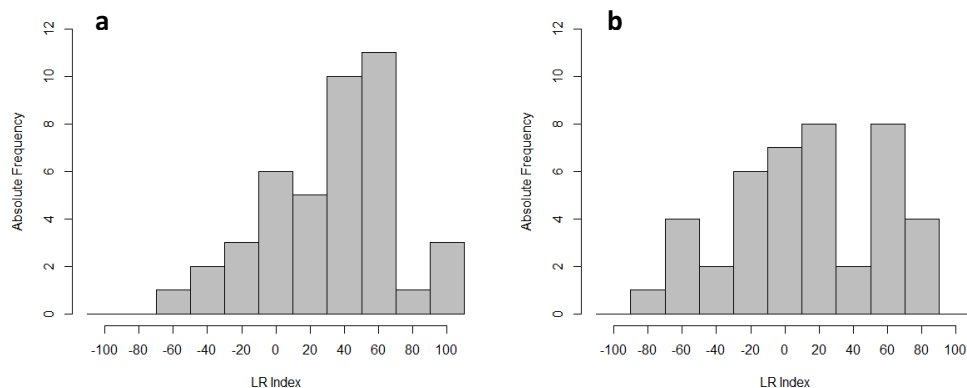


Figure 7. Distribution of frequency for L_R Index in the four experimental groups of staghorn sculpin. a) Control group; b) hypoxia group.

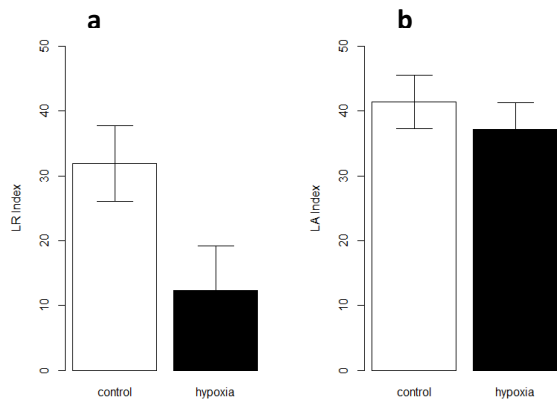


Figure 8. a) L_R Index (mean \pm s.e.) in control group (white bar), hypoxia group (black bar); b) L_A Index in control group (white bar), hypoxia group (black bar) in staghorn sculpin.

4. Discussion

The aim of this study was to investigate the effects of hypoxia on behavioral lateralization in fish, a manifestation of brain functional asymmetry. For that purpose we compared the tendency to turn left or right in a classical detour test (Bisazza et al. 1997) between subjects exposed to hypoxia and control conditions in three coastal fish species (shiner perch, three-spined stickleback and staghorn sculpin). Our results showed that hypoxia exposure affected fish's brain functioning by disrupting behavioral lateralization at population level in staghorn sculpin.

Staghorn sculpin control group was right lateralized at population level: most of the individuals exhibited a distinct preference for right turns when facing a straight barrier. Staghorn sculpin exposed to hypoxia (20% of $[O_2]$ for 2 hours) showed a significant reduction in behavioral lateralization at population level compared to the control group: individuals exhibited equal probability to turn left or right when facing the barrier.

Our experiments allowed us only to speculate about the mechanisms involved in the effects of hypoxia on behavioral lateralization. Since lateralization in fish is thought to reflect an asymmetry in hemispheric brain specialization, hypoxia should inhibit this phenomenon. Brain tissues are the most oxygen requiring tissues and it is well known that hypoxia exposure enhanced several changes on those. Most of fish species are thought to rapidly lose ion homeostasis in their brain resulting in an increase in extracellular $[K^+]$ reflecting a sudden depolarization (Nilsson et al. 1993). On the other hand some fish species show brain protection against hypoxia as an increase or redistribution of inhibitory neurotransmitters in brain that suppress brain functioning and limit O_2 requirements (Hylland and Nilsson 1999; Mulvey and Renshaw 2009). Although there are some evidences that staghorn sculpin brain owns some defensive strategy to hypoxia (Mandic et al. 2013) we do not know the exact response in this species. However both of these two described mechanisms could disrupt brain asymmetry: in the first case by the dysfunctioning of the brain following a lost in ion homeostasis; in the latter case by the suppression of brain functioning caused by inhibitory neurotransmitters.

While physiological processes deriving from hypoxic condition have been studied for years to determine how a lack of oxygen kills or disturbs fish brain, the direct functional output of brain suffering of sub-lethal hypoxia exposure have barely been investigated. Our results showed for the first time that brain damages given by sub-lethal hypoxia could affect fish's brain functional asymmetry. This could have consequences on the fitness of the fish by reducing survival. The major advantage of a functional asymmetric brain is that it achieves a higher degree of hemispheric specialization that results in a greater efficiency in computing specific functions (Levy 1977). In fish, predator

detection, escape response, prey catching and social interactions are supposed to be controlled mainly by specific neural circuits in one of the two brain hemispheres (e.g. Sovrano et al. 1999; Cantalupo et al. 2005). We hypothesize a disadvantage in processing information under these circumstances in our staghorn sculpins exposed to hypoxia because, after the loss of asymmetric brain functioning, they are not using mainly their specialized hemisphere anymore to compute specific processes. Regarding the specific directionality of lateralization at population level, little is known about its ecological meaning. The existence of developmental mechanisms favoring the presence of same direction lateralized individuals in the same environment at the same time (Dadda and Bisazza 2012) supports the hypothesis that it may be an adaptive mechanism. For a predator, for example, it could be matching its prey lateralization patterns in order to enhance attack success (Vallortigara and Rogers 2005). This hypothesis reveals other possible fitness consequences for staghorn sculpins that lost directionality of their lateralization following hypoxia treatment, especially if the effects continue to persist over time after reestablished normoxia conditions. During low tide in pole environment staghorn sculpins are naturally subjected to hypoxia condition. For the reasons mentioned above, in this situation predation, escape response and social interactions could be negatively affected by the lack of hemispheric specialization deriving from hypoxia. It is not less important that the increasing occurrence of hypoxia conditions along sea coasts thanks to human activities could be a relevant factor in reducing species survival even though it does not cause directly individual death.

In the other two species used (shiner perch and three-spined stickleback) control groups were not lateralized at population level and they did not show significant effects due to

hypoxia exposure. Both hypoxia treated and control fish had equality probability to turn right or left when they faced a barrier.

Overall only one out of the three species used in this study showed an effect due to hypoxia treatment on their behavioral lateralization. This contradictory results could hardly been explained with our study. The three fish species differ between each other in many characteristics that could affect the output of our experiments. At first we need to consider that different species could have different susceptibility to hypoxia and thus maybe our treatment was not enough severe to see an effect in shiner perch and three-spined stickleback. However this was not the case for hypoxia treatment made on staghorn sculpins. Furthermore previous studies reported the presences in staghorn sculpins of several adaptations to hypoxia condition (Wagner 1990) and this discredits this hypothesis. More intrigued is the possibility that the three species differ in the metabolic response to hypoxia at brain level. Another possibility is that since shiner perch and three-spined stickleback were not lateralized at population level it is impossible to see any reduction of lateralization as we found in staghorn sculpin. At last we cannot exclude the differential response to the treatment was given by the differences in the experimental protocols. Shiner perch and three-spined stickleback were subjected to progressive hypoxia treatment with a gradually recovering to normoxia. Both the slower decreasing of [O₂] and recovering could reducing the detectable effects of hypoxia on brain functioning. Whereas for the staghorn sculpin we performed a more severe hypoxia treatment which could explain that we found a significant effect of hypoxia on their behavioral lateralization.

Nevertheless this study scope needs more investigations in the future focusing on other fish species, hypoxia-tolerant and not hypoxia tolerant, facing different DO

concentrations. Furthermore in non-lateralized fish populations it would be interesting to focus on extreme lateralized individuals to observe if they would lose their behavioral lateralization when facing severe hypoxic conditions.

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