Effect of hypoxia on the behavior and metabolism of the longhorn sculpin (*Myoxocephalus octodecemspinosus)*

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Teleost fish are exposed to varying degrees of environmental low oxygen in their natural habitat and as such make ideal models on which to study the potential coping mechanisms of cells to oxygen deprivation (hypoxia). In an effort to classify the hypoxia tolerance level of longhorn sculpin (*Myoxocephalus octodecemspinosus*), a sub-tidal species found commonly in Frenchman's Bay, behavioral and metabolic responses to varying levels of environmental oxygen were determined and compared to similar responses in already established hypoxia tolerant and intolerant teleost species. Results from this study classify the longhorn sculpin as a moderately tolerant teleost species and establish it as a model system on which to study the cellular mechanisms underpinning natural hypoxia tolerance.

Mammals cannot survive a lack of oxygen for more than a short period of time without sustaining severe organ damage. In contrast, many teleost fish have evolved various traits that enable them to survive exposure to low oxygen in their natural environment. As such, fish make an ideal model system on which to study the traits underlying natural hypoxia tolerance. The information garnered from these teleost models may further the understanding of how cells in general can cope with low oxygen and enable the development of therapeutic strategies for mammalian oxygen-related diseases (*e.g.,* heart disease). Fish from the Cottidae Family (sculpins) make an ideal model system on which to study hypoxia tolerance as the different species in this family inhabit a wide range of habitats (intertidal zone to sub-tidal) and as such, have varying degrees of exposure to environmental oxygen. The objective of the current study is to determine the level of hypoxia tolerance of the longhorn sculpin (*Myoxocephalus octodecemspinosus*), which is a sub-tidal species found commonly in Frenchman's Bay, in order to establish it as a potential model species on which to conduct future comparative work on the cellular mechanisms underpinning low oxygen coping mechanisms.

To classify the hypoxia tolerance of longhorn sculpin, behavioral responses to progressive hypoxia were assessed and compared to previously published responses from several species of sculpins[2]. Following the methodology and behavioral criteria outlined in Mandic *et al.*[2], fish were exposed to a gradual decrease in environmental oxygen levels by bubbling nitrogen into the water. Oxygen levels of the water were monitored using an oxygen electrode. Threshold oxygen concentrations were recorded for first sign of agitation, severe agitation, attempt to escape, quiescence and unresponsiveness (which was often accompanied by loss of equilibrium) for five sculpin. Additionally, levels of blood glucose and lactate were analyzed spectrophotometically from blood samples obtained from a second group of sculpin in order to investigate the ability of sculpin to balance metabolic energy demand with oxygen supply during acute hypoxia (PO_2 ~ 7 kPa for 1 h). Six fish were sampled at the following points: control/normoxic conditions (PO_2 = 20 kPa; t=0 h); 1 h post hypoxia (t=4 h), early (t=8 h) and late (t=12 h) normoxic recovery. The PO_2 of 7 kPa oxygen was chosen after initial trials to determine the minimum amount of environmental hypoxia sculpin could withstand and fully recover from without mortality. Various other tissues were harvested for future studies on the cellular mechanisms of hypoxia tolerance.

Table 1. Comparison of the behavioral responses to progressive hypoxia in *M. octodecemspinosus* to the previously published behavioral responses of *M. scorpius* and *O. maculousus*[2]. Data presented as means ± SE. The O_2 thresholds for first sign of agitation (pFAGT), severe agitation (pSAGT), first escape attempt (pESC), quiescence (PQUI), and unresponsiveness (pUNRES) and loss of equilibrium (pLE) are presented in kPa; *n* represents the number of individuals tested for each behavioral trial with numbers individuals performing each behavior in parentheses. N/O means the behavior was not observed.

	M.octodecemspinosus	*M.scorpius*[2]	*O.maculousus*[2]
Mass (g)	165.5 ± 24.2	7.8 ± 1.5	4.8 ± 0.5
Habitat	subtidal	subtidal	tidepool
pFAGT	7.6 ± 1.6 (5)	N/O	7.0 ± 1.7 (6)
pSAGT	4.4 ± 1.0 (5)	N/O	2.6 ± 0.6 (6)
pESC	3.0 ± 0.2 (5)	N/O	1.3 ± 0.4 (7)
pQUI	2.7 ± 0.3 (5)	N/O	0.8 ± 0.2 (7)
pUNRES	2.6 ± 0.3 (5)	N/O	0.3 ± 0.1 (7)
pLE	N/O	1.7 ± 0.2 (5)	N/O
n	5	8	7

Results of the behavioral study place longhorn sculpin in a category of moderate hypoxia tolerance, similar to the closely related shorthorn sculpin (*Myoxocephalus scorpius*)[1,2] that also lives in a sub-tidal environment[3] (Table 1). While the level of environmental oxygen that results in the first signs of agitation are similar in longhorn sculpin and the highly hypoxia tolerant tidepool sculpin, *Oligocottus maculousus,* (7.5 ± 1.6 and 7.0 ± 1.7 kPa, respectively), longhorn sculpin reach a point of unresponsiveness at a much higher level of oxygen saturation (2.6 ± 0.3 kPa) than *O. maculousus* (0.3 ± 0.1 kPa). However, longhorn sculpin are still much more tolerant to environmental hypoxia than the well-known hypoxia sensitive rainbow trout, in which one-hour exposure to oxygen levels of 4.8kPa result in 40% mortality[4].

This classification of hypoxia tolerance at the behavioral level is further supported by results at the biochemical level. Hypoxia sensitive species of fish, such as the rainbow trout (*Oncorhynchus mykiss*), demonstrate a significant elevation in both plasma glucose (1.5 fold increase) and lactate (13 fold increase) in response to environmental hypoxia[4]. This result is a classic depiction of the switch from oxygen dependent to oxygen independent metabolism for energy production. However, in longhorn sculpin, exposure to environmental hypoxia did not induce a significant increase in plasma glucose (0.44 ± 0.2 mM) or lactate (0.07 ± 0.02 mM) levels (Fig 1). These levels of metabolites in longhorn sculpin under normoxic and hypoxic conditions are consistent with previously published values for the closely related shorthorn sculpin[1], which has also been classified as a moderately hypoxia tolerant teleost.

As the behavioral responses of agitation and attempt to escape are displayed at much higher levels of oxygen saturation than any metabolic changes it suggests that longhorn sculpin may use behavioral means as a first line of defense against environmental low oxygen. Additionally, as the sculpin was able to withstand one hour of moderate hypoxia without any increase in plasma glucose and lactate it suggests that these fish possess physiological and/or biochemical mechanisms that enable them to balance their metabolic energy demand with oxygen supply in a hypoxic environment. These results set the stage for future investigation into sculpins' potential coping mechanisms and establish the longhorn sculpin as a moderately hypoxia tolerant teleost on which future studies on the mechanisms of natural hypoxia tolerance can be conducted.

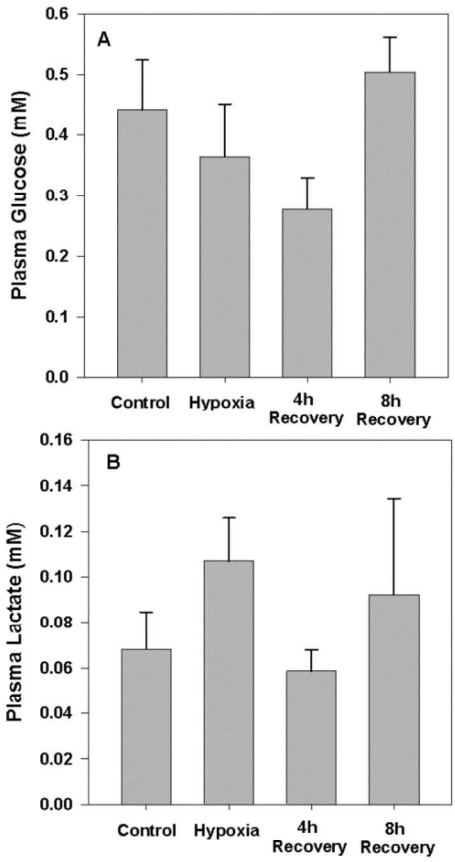

Figure 1. Plasma glucose (A) and lactate (B) concentrations (mM) in *M. octodecemspinosus* under control (PO2 = 20 kPa), 1-hour post hypoxia (PO2 = 7 kPa) and early (4 h) and late (8 h) recovery from hypoxia exposure. Data are presented as mean ± SE (n = 6). Statistical analysis by one-way ANOVA indicated no significant difference between groups ($p > 0.05$).

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