The response of striped surfperch *Embiotoca lateralis* to progressive hypoxia: Swimming activity, shoal structure, and estimated metabolic expenditure

CrossMark

D.G. Cook [a,b,*], E.J. Brown [b], S. Lefevre [b,c], P. Domenici [b,d], J.F. Steffensen [b,e]

[a] The New Zealand Institute for Plant and Food Research Ltd, Nelson, New Zealand
[b] Friday Harbor Laboratories, University of Washington, WA, USA
[c] Department of Biosciences, University of Oslo, Norway
[d] CNR, IAMC Loc Sa Mardini, Torregrande, Oristano, Italy
[e] Marine Biological Laboratory, University of Copenhagen, Helsingor, Denmark

ABSTRACT

When exposed to moderately low O_2 conditions (75–30% O_2 saturation), shoaling striped surfperch *Embiotoca lateralis* show no changes in swimming activity, uniform levels of metabolic expenditure and an unchanging shoal structure. As O_2 levels become more hypoxic surfperch reduce their locomotor activity, initially with a decrease in turning rate at 20% O_2 saturation, followed by a reduction in swimming speed at 15% O_2 saturation. Reductions in activity occur in combination with individuals adopting more uniform turning behaviors, which translate into an increase in the distance traveled per unit time (i.e. net displacement). This increase in net displacement may enhance the ability of surfperch to distance themselves from discrete zones of hypoxia. Measures of shoal structure, including nearest neighbor distances and shoal polarity, were constant throughout low O_2 exposure despite changes in swimming activity. Estimation of the energetic costs of these behaviors reveals that metabolic expenditure (routine metabolic rate) remained steady at ~120 mg O_2 kg^{-1} h^{-1} between 100 and 30% O_2 saturation, dropping to ~108 mg O_2 kg^{-1} h^{-1} at 20 and 15% O_2 saturation. Given that surfperch possess a critical oxygen saturation (S_{crit}) of 15.2%, reductions in swimming activity and metabolic energy expenditure clearly occur as individuals reach their aerobic metabolic limits. These results identify that surfperch demonstrate a multi-faceted coping strategy when exposed to low oxygen conditions, which may prove advantageous when the species experience hypoxic episodes in their natural habitat.

© 2014 Elsevier B.V. All rights reserved.

1. Introduction

Hypoxic episodes are becoming more frequent in coastal marine environments (Diaz and Rosenberg, 2008), therefore an understanding of how marine fish will respond to these potentially lethal events is of increasing importance. In aerobically respiring organisms, such as fish, environmental hypoxia lowers the availability of oxygen for metabolic functioning. To counter these decreases in ambient oxygen levels fish can engage a suite of physiological changes, among others altering their ventilation rate, ventilation volume, and cardiac function in order to enhance oxygen uptake at the site of gas exchange (the gills), blood oxygen binding properties and to increase oxygen delivery to working tissues (Randall, 1982). These homeostatic adjustments attempt to ensure that physiological O_2 levels remain adequate to support the individual's metabolic O_2 requirements. Despite the vast differences in hypoxia tolerance observed between species, deteriorating oxygen conditions will eventually exceed the physiological capacities of the animal to acquire and deliver sufficient volumes of oxygen to working tissues. The resulting O_2 limitations reduce the maximum metabolic rate and aerobic metabolic scope of the animal, constraining its ability to perform non-essential activities (i.e. swimming and digestion) (Claireaux and Lefrancois, 2007; Claireaux et al., 2000; Fry, 1947). Should environmental O_2 conditions continue to deteriorate, aerobic metabolism can be constrained to such an extent that the fish is only able to support essential metabolic activity (metabolic scope is zero). This point is often referred to as the critical oxygen saturation (S_{crit}) or partial pressure (P_{crit}). Below this O_2 level time limited oxygen independent metabolic pathways (i.e. anaerobic metabolism) are required to sustain essential metabolism, in most cases threatening the individual's survival (Van Raaij et al., 1996b).

As physiological coping mechanisms may not be sufficient to ensure survival in hypoxic conditions, changes in behavior can provide a complementary or alternative response that can enhance survival in low O_2 conditions. Although generalizations are not always consistent, sluggish and benthic fish species show a compliant response when encountering low O_2, decreasing spontaneous activity levels in a likely attempt to offset physiological stress and reduce energetic demands (Dalla Via et al., 1998; Metcalfe and Butler, 1984; Skjaeraasen et al., 2008). Conversely, an active response involving increases in spontaneous activity is observed in athletic schooling and pelagic species (Bejda et al., 1987; Bushnell and Brill, 1991; Dizon, 1977). These increases in spontaneous activity are widely interpreted to represent an avoidance reaction which also proves advantageous when fish are schooling en masse, whereby individuals in rearward positions of the school can be exposed to reduced O_2 conditions as a result of the respiratory O_2 extraction of preceding individuals (Green and McFarland, 1994; McFarland and Moss, 1967). Increases in swimming speed can also complement other behavioral changes including increased shuffling and changes in school structure, which provide hydrodynamic advantages gained by trailing other fish, or increase the probability of finding improved localized O_2 conditions (Domenici et al., 2002; Johansen et al., 2010; Moss and McFarland, 1970). However, increases in swimming activity require increased energetic expenditure (Brett, 1964; McKenzie, 2011), and would likely increase the risk of asphyxiation as has been seen when species such as common sole (*Solea solea*) and rainbow trout (*Oncorhynchus mykiss* L.) increase their activity levels when exposed to severely low O_2 levels (Dalla Via et al., 1998; Van Raaij et al., 1996a).

The current study sought to investigate the behavioral responses of striped surf perch *Embiotoca lateralis* upon exposure to experimental hypoxia. Surfperch are geographically spread across the temperate NW Pacific Ocean and commonly occupy habitats that experience hypoxic episodes, such as the Hood Canal — a fjord in close proximity (~100 km) from the collection site of the individuals used in the present study (Connolly et al., 2010; Eschmeyer et al., 1983; Palsson et al., 2008). As a shoaling species throughout their life history (Eschmeyer et al., 1983), we were particularly interested in how both the collective and individual behavior of surf perch changes during hypoxia, and the energetic consequences of these behavioral adjustments.

2. Methods and materials

2.1. Animal collection and handling

Approximately 100 striped surfperch, *E. lateralis* (mean ± sd: mass = 118.9 ± 24.0 g, fork length = 17.4 ± 1.0 cm) of indiscriminate gender were collected by beach seining (Jackson's Beach, 48°31′ N; 123°01′W, WA, USA). The fish were transported to the Friday Harbor Laboratories and housed indoors in a 1500 L circular tank supplied with flow through, filtered seawater at ambient temperatures of 12.0 ± 1.0 °C (mean ± sd). Of the 100 fish collected, 40 were used for behavioral investigations and a further four were used for swimming respirometry. The remaining surfperch were used in other projects and no mortalities were observed during holding, or experimentation. Fish were maintained under the natural lighting regime and fed ad-libitum throughout the holding period, but food was withheld from fish 48 h prior to experimentation. Upon transfer to the experimental apparatus, five individual surfperch were tagged in different anatomical positions upon the dorsal surface of either flank using flexible polyethylene film affixed with cyanoacrylate adhesive, so to be individually recognizable. Despite best attempts, 18 fish lost tags during transfer and experimentation (typically 2–3 individuals per shoal/experimental replicate). Fish which had lost their tag were excluded from analyses of swimming speed, turning rate, angular correlation, expected displacement, and estimated metabolic expenditure. The fork length and live

mass of each fish were recorded upon completion of the experiment before they were released back into the surrounding harbor.

2.2. Behavioral apparatus

A diagrammatic representation of the experimental apparatus is given in Fig. 1. Behavioral responses to a progressive, step-wise, hypoxia induction protocol were performed in a shallow circular acrylic tank (approximately 1.03 m diameter, volume 270 l) receiving a recirculating flow of water at a flow rate of 10 l min^{-1}. 16 h prior to experimentation, five tagged fish were introduced into the circular tank, henceforth referred to as the behavioral arena (BA). Water draining from the BA was pumped (Eheim 1250, Eheim GmBh & Co., Germany) to the base of a gassing tower before passing through an overflow and gravity-fed back into an inlet line positioned against the tank wall. Normoxic O_2 conditions (>95% O_2 saturation) were achieved by aerating water in the gassing tower with an aquarium air pump and micro-diffuser, and were maintained in the BA prior to experimentation. An additional pump (5 l min^{-1}, Eheim 1046, Eheim GmBh & Co., Germany) served to increase flow rates in the BA. Water velocities in the BA were characterized throughout using a Hönztech handheld HFA flow-meter (Fig. 1).

2.3. Experimental protocol and hypoxia induction apparatus

To investigate the behavioral response to experimental hypoxia, groups of five individuals were transferred into the behavioral arena 20 h prior to the beginning of experimentation. Each experiment began at 0900 whereupon fish were observed for an initial 30 min control measurement period under normoxic conditions before exposure to a progressive, stepwise hypoxia stimulus. Progressively hypoxic conditions were created by ceasing aeration through the gassing tower and actuating a solenoid valve which controlled a flow of compressed nitrogen gas through an additional micro-diffuser. Oxygen saturation (SO_2) levels were controlled via an oxygen control system (Model# 5714, PR Electronics, Denmark), and galvanic O_2 sensitive probe (Oxyguard Handy, Oxyguard, Denmark) which was positioned in the flow of water entering the BA. Steady state SO_2 levels were held for a 30 min period, followed by a 30 min period of unsteady SO_2 in which O_2 levels were decreased. Steady state SO_2 set-points were held at 75, 50, 30, 20 and 15% (Fig. 2). This protocol was repeated on eight different groups (replicates) of naïve fish (i.e. n = 40).

2.4. Fish tracking procedures and behavioral analyses

Video recordings of fish behavior were acquired to analyze individual behavior and shoal structure over the final three minutes of steady state exposure (i.e. between 27 and 30 min). Video capture was performed using a Handycam (DCR-HC51, Sony Corporation, Japan; resolution 720 × 576, 25 fps) mounted 2.0 m above the experimental tank. The video camera was connected to a PC via IEEE1394 cable enabling video sequence capture using Microsoft Movie Maker, (v2.1, Microsoft Corporation). Video files were rendered using VirtualDub (v1.8.8, www.virtualdub.org) where required. Post analysis tracks of the geometric center of mass (COM) for each fish, and its rostrum, were acquired using the software package LoggerPro (v3.5, Vernier Software, OR, USA). Tracked output values enabled investigation of behavioral parameters including the nearest neighbor distances (NND), shoal polarity, swimming speed (U), and turning angle, each described in more detail below.

2.4.1. Spontaneous swimming speed
Swimming speed in two dimensions was derived from tracked x and y coordinates of the geometric center of each individually identifiable fish (fs = 5 Hz). Determination of the vector between successive coordinates defined the 'apparent' swimming speed. As the BA was