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

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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.

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).

Abbreviations: BA, behavioral arena; BL, body length; f_p, fin beat frequency; f_s, sampling frequency; $\dot{M}O_2$, metabolic oxygen consumption; $MO_{2(max)}$, maximum metabolic rate; MS, metabolic scope; NND, nearest neighbor distance; RMR, routine metabolic rate; S_{crit}, critical oxygen saturation; SMR, standard metabolic rate; SO_2, oxygen saturation of water; U, swimming speed; U_{crit}, critical swimming speed.

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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

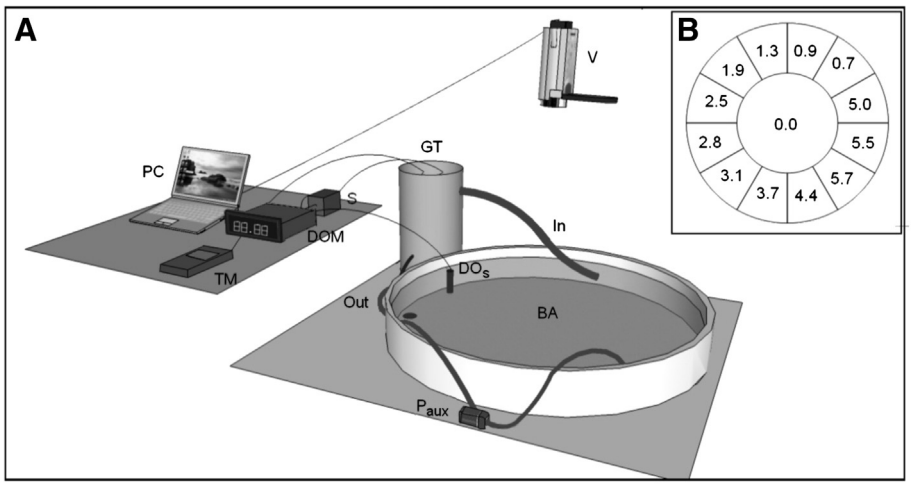

Fig. 1. A. Diagrammatic representation of the experimental setup. Fish occupied the behavioral arena (BA) supplied by a recirculating flow of water draining from the outlet (Out) and passing through a gassing tower (GT). Water passing through the GT could be actively aerated, or deoxygenated with a controlled flow of compressed nitrogen before returning to the BA (In). An auxiliary pump (P_{aux}) supplemented flow rates in the BA, whereby measured flow rates are depicted in the inset (Fig. 1B). Control of O_2 set points was provided by a galvanic dissolved oxygen sensor (DO_S) and DO meter (DOM) which actuated a solenoid valve (S) that controlled the flow of nitrogen into the GT. The maintenance of temperature in the BA was assisted by measurements of water temperature (TM). Fish behavior was observed using a handycam video camera (V) streaming video to a computer (PC).

subject to a circular and unidirectional flow, a disparity between the apparent swimming speed of the fish and their true swimming speed was evident. To account for this disparity, determinations of the fish's polarity (described below), orientation to flow (i.e. whether they were showing positive or negative rheotaxis) and the flow rate experienced in their tracked position were used to adjust values of apparent swimming speed. The 'true' swimming speeds of fish (U, BL s^{-1}) showing positive rheotaxis was calculated by adding the value of experienced water flow to their apparent speed, while the value of water flow was subtracted from the apparent swim speed of fish showing negative rheotaxis. No corrections were performed on fish showing neither positive nor negative rheotaxis (i.e. engaging turning maneuvers).

2.4.2. Spontaneous turning rate and angular correlation

Turning rates ($^\circ$ s^{-1}) were derived from tracked coordinates of the fishes geometric center and the rostral tip of individually identifiable fish (fs = 5 Hz). An arctan transformation of these two coordinates identified the fish's swimming vector (polarity, θ_P). Angular changes in successive swimming vectors (θ_T) identified the spontaneous turning rate. The consistency of each individual's turning rate was investigated using values of angular correlation (r), derived from the cosine of calculated turning angles, $r = \cos(\theta_T)$ (Brady et al., 2009). Individuals that show highly uniform turning behaviors generate high values of angular correlation (i.e. up to 1.0), whereas individuals that show random and erratic turning behaviors produce low values of correlation (i.e. 0.1, for further explanation refer to Fig. 3).

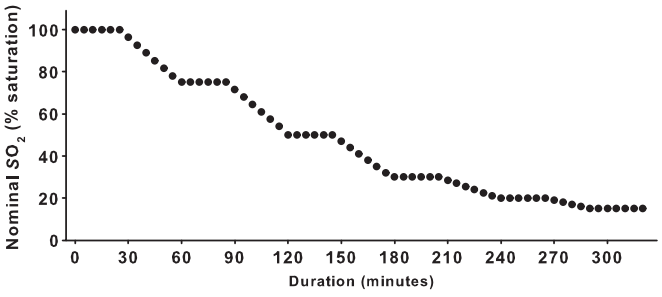

Fig. 2. Progressive hypoxia protocol employed during the experiment. Fish were exposed to an initial control observation period at 100% SO_2, followed by a 30 minute period of unsteady SO_2 in which O_2 levels were decreased to the next exposure level (75% SO_2). This exposure pattern was repeated for 50, 30, 20 and 15% SO_2 exposure levels.

2.4.3. Expected displacement

The total distance traveled in a given period of time (i.e. their net displacement) is strongly influenced by the turning behavior and swimming speeds adopted by a fish. For example, an individual swimming slowly in a straight line could move greater distances than another fish, starting from the same position that swims at greater speeds but turns frequently in a random fashion (Fig. 3A,B). How swimming speeds and turning behavior influence the net displacement of each individually identifiable fish in the shoal was therefore investigated at each SO_2 level according to the following formula, also described in Benhamou (2004) and Brady et al. (2009): $S_{Exp} = 0.886\lambda\ \{[n(1 + r)](1 - r)^{-1}\}^{0.5}$ (Eq. 1), where λ equals swimming speed (m s^{-1}), n = number of seconds (in 30 min), r = angular correlation.

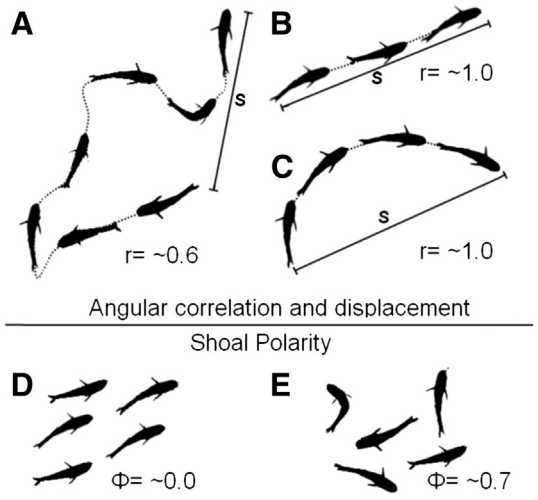

Fig. 3. Diagrammatic representation of behavioral determinations defining the A) Angular correlation and displacement of individuals while swimming, and B) the polarity (circular variance) of the shoal, inside the BA. A-C) depicts the combined movement of fish over an arbitrary unit of time with the displacement(s) and angular correlation of turning behaviors (r) defined, whereby high values of r indicate uniform turning behaviors while low values of r represent random and erratic turning behaviors. D-E) depicts the effect of polarity (or orientation) of each individual within the shoal on the value of circular variance (Φ), with low values of Φ defining a group of fish in schooling formation and high values of Φ identifying a shoal with less polar orientations.

2.4.4. Nearest neighbor distances

NND were determined in two dimensions by calculating the distance between the geometric center of each fish within the shoal (fs = 5 Hz). Average distances at each time point were ranked to identify the first, second and third nearest neighbors and expressed in body lengths (BL) (Delcourt and Poncin, 2012; Partridge and Pitcher, 1980).

2.4.5. Shoal polarity

Aggregations of fish (shoals) can structure themselves in a coordinated or uncoordinated manner, and when a shoal is swimming in the same direction with individuals maintaining polar alignment it is said to be schooling (Pitcher and Parrish, 1993). To quantify the shoaling structure of each experimental group, the circular variance of the swimming vector from each fish in the shoal was determined according to the formula $\Phi = 1 - (\sqrt{[(\sum \sin\theta_{Pi})^2 + (\sum \sin\theta_{Pi})^2]}/N)$ (Eq. 2, Delcourt and Poncin, 2012), whereby values of $\Phi = 1$ indicate high levels of variation (e.g. orientations are uncoordinated/random and there is no polarity of the shoal, Fig. 3E), and values of $\Phi = 0$ indicate low variation (i.e. all orientations are equal and fish are in schooling formation, Fig. 3D).

2.5. Respirometry and estimating the metabolic costs of spontaneous swimming

2.5.1. SMR determination

In order to determine the basal metabolic requirements of the animals (i.e. their standard metabolic rate, SMR) four fish were exposed to a U_{crit} swimming protocol conducted in an automated, intermittent flow, swim flume respirometer (Steffensen et al., 1982). Individual animals (n = 4. Mean ± sd: mass = 113 ± 13 g, FL = 14.7 ± 0.2 cm) were introduced to an 8.31 l 'Steffensen-type' swimming respirometer then left overnight, swimming at 1 BL s^{-1}. Temperature (12.0 °C ± 0.1 °C), flushing and measurement cycles (4 min flushing, 2 min wait and 4 min measurement) were controlled using AutoResp software (Loligo systems, Denmark). Oxygen levels were determined using a PreSens Minisensor (Fibox 3, Presens, Germany) and MO_2 was synchronously calculated through the AutoResp software according to the equation: $MO_2 = V\,(\Delta PO_2/t)\,\boldsymbol{\alpha}\,M_B$ (Eq. 3), where V is the volume of the respirometry chamber less fish volume in liters, $\Delta PO_2/t$ is the change in oxygen partial pressure (kPa) per unit time, $\boldsymbol{\alpha}$ is the solubility coefficient of oxygen in water (salinity of 35, 12.0 °C) in $mgO_2\ kPa^{-1}$, M_B is the body mass of the fish (kg). Fish were swum through a U_{crit} protocol beginning at 1 BL s^{-1} increasing in 0.4 BL s^{-1} increments and terminating when the fish reached exhaustion, defined by the fish undergoing a gait transition to burst swimming followed by a cessation of swimming activity. Fish were held for a total of 20 min at each velocity and speeds adjusted over a 10 s period. SMR was calculated by extrapolating the mean $\overline{M}O_2$ values at measured swimming speeds back to zero activity, using the hydrodynamics based power equation: $MO_2 = a + bU^c$ (Eq. 4), (Korsmeyer et al., 2002; Videler, 1993). Where a, b and c are constants and U = swimming speed. This value of SMR was then utilized to calculate the value of S_{crit} (detailed below) and also the metabolic costs of routine activity (see below, Eq. 6).

2.5.2. S_{crit} determination

The critical oxygen saturation (S_{crit}) defines the minimum SO_2 level required to sustain the fish's SMR. Following the previously described U_{crit} swimming protocol and a subsequent 3 h recovery period, surfperch within the swim flume respirometer were entered into a continuous 'flush' cycle while being maintained at a swimming speed of 1 BL s^{-1} (n = 4). The O_2 level of water circulating through the respirometer was then progressively lowered by purging N_2 gas through a micro-diffuser stone over a one hour period. Upon reaching 40% SO_2, the swim respirometer was entered into a 'closed' measurement phase and the metabolic rate was determined every 4 min while respiratory O_2 extraction provided a progressive decrease in circulating SO_2 levels. As this methodology adopted a prolonged 'closed respirometery' phase to determine S_{crit} fish were potentially subjected to a progressive accumulation of respiratory waste products (e.g. carbon dioxide). By initially equilibrating the chamber to 40% SO_2, we were able to limit waste product accumulation relative to what would have occurred if S_{crit} had been determined using closed respirometry techniques exclusively — whereby O_2 levels are required to deplete from normoxic levels through to the breakpoint of S_{crit}. In fact, by using the technique adopted, the decrease in O_2 levels from 40% to ~15% SO_2 (the identified value of S_{crit}) would have limited carbon dioxide accumulation to less than 1 mm Hg above routine exposure levels. Exposure to these levels of hypercapnia was not considered to have a significant bearing on our determinations. As surfperch approached their S_{crit}, fish were forced into an oxy-conforming state where their metabolic rate is dependent on ambient SO_2. It is likely that the recruitment of anaerobic metabolic pathways would have been required to sustain swimming activity. MO_2 was measured then plotted against SO_2 during this oxy-conforming period, before a linear regression was used to represent this oxy-conformation. The SO_2 value at which the mean value of SMR intercepted this linear regression defined the value of S_{crit}.

2.5.3. Estimating the metabolic costs of spontaneous activity

The pectoral fin-beat frequency ($f_p\ s^{-1}$) of surfperch was used as a determinant of the metabolic costs of spontaneous activity (or routine metabolic rate, RMR) in accordance with the methodology of Tudorache et al. (2009). The pectoral fin-beat frequency of positively identifiable individuals was determined as the number of visible beats per three minute video clip recorded at 25 fps, then scaled to account for differences in body-mass (Drucker and Jensen, 1996; Tudorache et al., 2009): $f_{p\ (0.1kg)} = f_p\ s^{-1}\ (M\ 0.1^{-1})^{(0.88)}$ (Eq. 5).

This value was then utilized to estimate RMR during spontaneous swimming according to the following equation, also described in Tudorache et al. (2009): $RMR = SMR + bf_p^c$ where b = 17.37 and c = 1.08 (eqn. 6).

This methodology for determining RMR was derived for fish occupying normoxic conditions and therefore would not account for the physiological costs of hyper-ventilation, commonly observed when fish are exposed to hypoxia (Randall, 1982). Ventilation is reported to comprise a metabolic cost ranging between 5 and 15% of the SMR of fishes (Farrell and Steffensen, 1987), and these costs may potentially be incurred by surfperch exposed to progressive hypoxia stimuli. As there is no sufficient description of how the metabolic rates of unrestrained marine fishes are affected by progressive hypoxia (at least to the authors' knowledge), the costs of ventilation were cautiously assumed to increase in a linear fashion during hypoxic exposures, requiring 5% of their SMR (or 3.5 mg $O_2\ kg^{-1}\ h^{-1}$) under normoxic conditions then peaking at a maximal value of 15% SMR (or 10.4 mg $O_2\ kg^{-1}\ h^{-1}$) at the lowest O_2 exposure level (e.g. 15% SO_2). The methodology of Tudorache et al. (2009) would have implicitly accounted for the respiratory requirements of individuals under normoxic conditions, so only calculated values of metabolic expenditure during progressively low O_2 conditions were corrected to account for expected metabolic costs of ventilation.

2.6. Statistical analysis

Statistical analyses were performed using Sigmaplot (v11, Systat Software Inc) with significance accepted at $p < 0.05$. One way ANOVA repeated measures were used to investigate behavioral changes during hypoxia exposure, with data log transformed when variances were not equal. Post-hoc tests using the Holm–Sidak method investigated pair-wise comparisons between periods of normoxic 'control' activity and periods of progressively hypoxic activity, so as to identify the SO_2 level at which significant behavioral changes were observed. Changes

in NND were investigated using 2-way repeated measures ANOVA. NND data was log transformed to correct for a non-normal distribution. To avoid pseudo-replication, behavioral and metabolic determinations from each treatment group were individually pooled. All results presented throughout the text, tables and figures are presented as mean values of the eight replicates with 95% confidence intervals, unless otherwise stated.

3. Results

3.1. Behavioral responses to progressive hypoxia

Upon exposure to a progressive hypoxia stimulus surfperch underwent significant changes in swimming speed ($F = 4.331$, $p < 0.01$, Fig. 4A), highlighted primarily by the statistical differences observed when normoxic swimming speeds were compared to those at 15% SO_2 ($t = 3.50$, $p < 0.01$).

Progressive hypoxia also influenced the turning rate of surfperch ($F = 12.57$, $p < 0.01$, Fig. 4B), with post-hoc analysis identifying a 35.9% reduction in turning rate at 20% SO_2 ($t = 3.57$, $p < 0.01$) followed by a 50.9% reduction at 15% SO_2 ($t = 4.86$, $p < 0.01$), when compared to normoxic activity levels. Investigations of angular correlation identify that surf perch not only maintained uniform turning behaviors in normoxia ($r = 0.96 \pm 0.01$, Fig. 4C) but also showed small (yet statistically significant) improvements in the uniformity of their turning behavior throughout progressive hypoxia ($F = 8.27$, $p < 0.01$). Post-hoc analysis identified that this change in angular correlation was most apparent at 20% ($t = 3.99$, $p < 0.01$) and 15% ($t = 2.61$, $p < 0.01$) SO_2. These changes in swimming speed and turning behavior translated into statistically significant changes in expected displacement ($F = 2.87$, $p = 0.03$, Fig. 4D), whereby an 85.1% and 77.4% increase in expected displacement was observed at 20% and 15% SO_2 ($t = 2.80$, $p < 0.01$ and $t = 3.21$, $p < 0.01$), respectively.

NND as a descriptor of group dynamics did not change significantly throughout the duration of hypoxia ($F = 0.972$, $p = 0.448$, Fig. 4E). Although surfperch exhibited moderately cohesive behaviors throughout observation, the circular variance of the shoal never reached values <0.4 (Fig. 4F). Henceforth, the collective behavior of surfperch throughout the investigation is defined as shoaling.

3.2. Metabolic costs of spontaneous activity during progressive hypoxia

During normoxic conditions, the RMR of surfperch was 118.3 ± 15.1 mg O_2 kg^{-1} h^{-1}, utilizing ~29% of the surfperch's normoxic aerobic scope (Fig. 4G). Analysis of the predicted RMR without adjusting for the metabolic costs of ventilation using one way ANOVA identified that significant changes occurred upon exposure to progressive hypoxia ($F = 17.672$, $p < 0.01$), with the most significant reductions apparent at 20% and 15% SO_2 ($t = 6.36$, $p < 0.01$, and $t = 5.88$, $p < 0.01$, respectively). When correcting RMR to account for the metabolic costs associated with ventilation, the changes in RMR remained statistically significant ($F = 9.91$, $p < 0.01$), with significant reductions in RMR observed at both 20% and 15% SO_2 ($t = 3.40$, $p < 0.01$, and $t = 4.05$, $p < 0.01$, respectively).

3.3. Respirometric determinations

The U_{crit} value (i.e. the penultimate swimming speed prior to exhaustion) was 3 BL s^{-1} and $\dot{M}O_{2max}$ was 250.8 ± 21.7 mg O_2 kg^{-1} h^{-1}. Curve

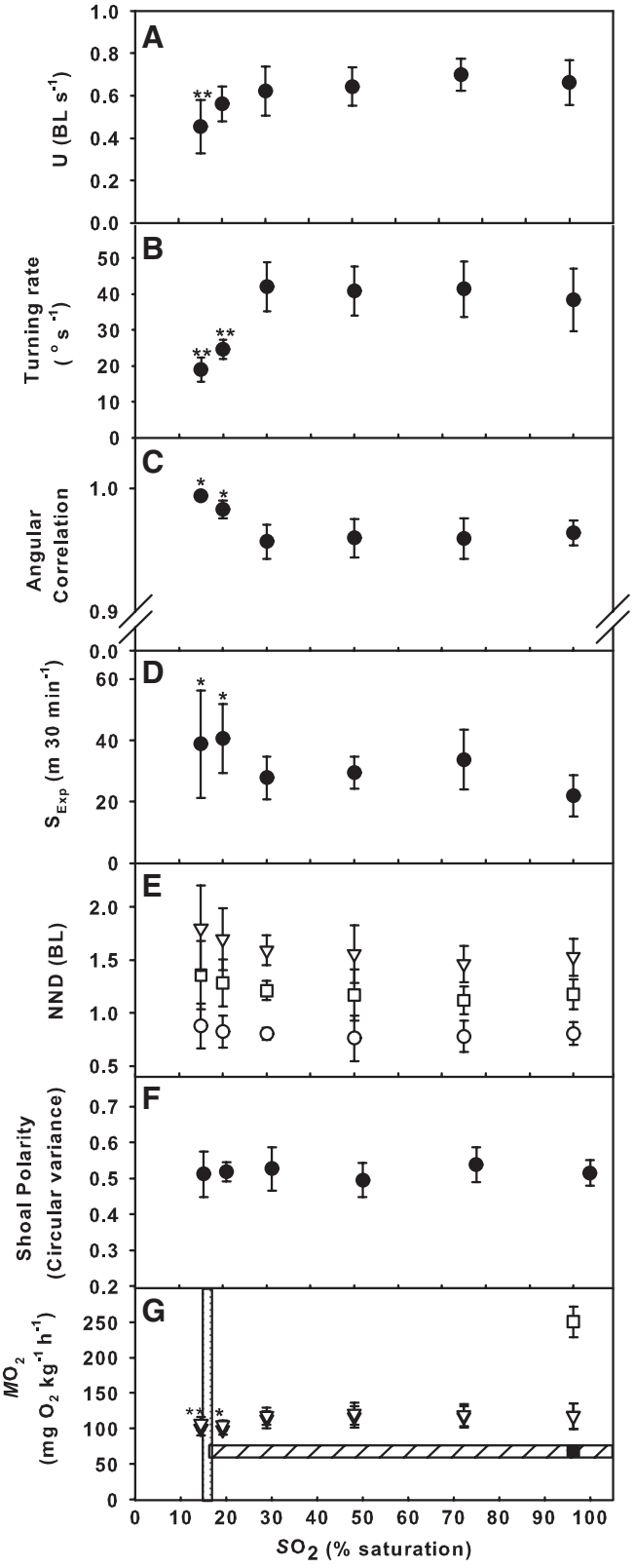

Fig. 4. Behavioral observations of shoaling striped surfperch exposed to a progressive hypoxia protocol. A) Swimming speed, B) spontaneous turning rate, C) angular correlation, D) expected displacement, E) nearest neighbor distances, F) shoal polarity, G) estimated metabolic expenditure. A–D, F) Closed black circles represent the average pooled value for the five individuals comprising a shoal. E) White circles represent the mean spacing between the 1st nearest neighbor, white squares represent spacing between the 2nd nearest neighbor and white triangles represent the spacing of the 3rd nearest neighbor. G) The white square indicates the mean value of $\dot{M}O_{2\ max}$. Black triangles represent the estimated metabolic costs excluding the correction for hyperventilation. White triangles represent the estimated metabolic expenditure including the hypothesized costs of hyperventilation. The vertical shaded bar indicates the calculated value of S_{crit}. The horizontal graded bar indicates the SMR (black square) of surfperch, including 95% confidence intervals. All symbols represent mean values ± 95% confidence intervals. (*) indicates a significant difference of $p < 0.05$, (**) indicates a significant difference of $p < 0.01$.

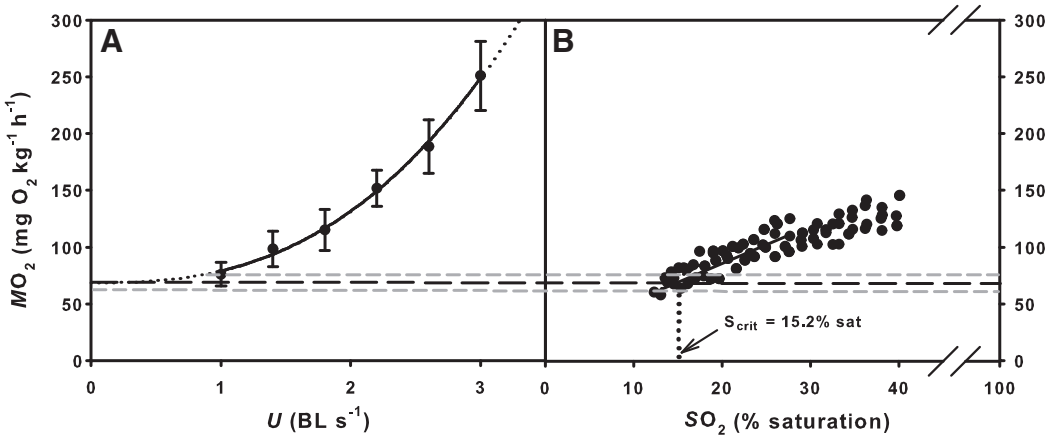

Fig. 5. A) The metabolic costs of swimming during the P_{crit} swim protocol. Curve fitting using the hydrodynamics based power equation was of the form $\dot{M}O_2 = a + bU^c$, where a = 68.56, b = 10.48 and c = 2.59. B) Respiratory determination of S_{crit}. Circular gray symbols indicate the mean values of respiration measured at 1 BL s^{-1}. The horizontal long-dashed line (— — —) denotes the SMR, with 95% confidence intervals denoted by the gray short-dashed line (- - -). A linear regression (solid line) through values where the fish were deemed to be oxy-conforming, is defined by the equation y = 3.23x + 24.46. The intercept of this regression line with the value of SMR identifies the value of S_{crit}.

fitting with Eq. 3 satisfactorily described the relationship between metabolic energy expenditure and swimming speed ($r^2 = 0.99$, Fig. 5A). The y-intercept of this curve, and hence the SMR, was calculated at 68.6 ± 6.7 mg O_2 kg^{-1} h^{-1} (Fig. 3A). During the determination of S_{crit}, a linear regression of the form y = 3.23x + 24.46 (Eq. 7) described oxy-conforming metabolism in the surfperch. This linear regression intercepted the value of SMR at 15.2 ± 2.1% SO_2, defining S_{crit} (Fig. 5B).

4. Discussion

When exposed to moderately hypoxic O_2 conditions surfperch, like many other species of fish, maintain uniform swimming behaviors with no obvious signs of behavioral distress (Behrens and Steffensen, 2007; Cook and Herbert, 2012b). As O_2 conditions become more severe and drop below 30% SO_2, surfperch respond by decreasing their turning rate and swimming speed at 20% and 15% SO_2, respectively. As locomotor activity represents a significant metabolic cost to the animal (Boisclair and Tang, 1993; Webb, 1993), it is not surprising that these reductions in activity enabled surfperch to achieve an estimated 10% reduction in metabolic energy expenditure (Fig. 4G). This finding therefore supports the commonly held notion that decreases in swimming activity enable fish to conserve energy when exposed to inescapable hypoxia (Herbert and Steffensen, 2005; Metcalfe and Butler, 1984; Sloman et al., 2006). Given that reductions in energy expenditure occur as surfperch approach their aerobic limits (S_{crit} = 15.2 ± 2.1% SO_2), the observed changes in behavior are suggested to occur as surfperch become unable to support routine activity by aerobic means and the animal is forced to metabolically conform to what little O_2 is remaining. By attempting to match their metabolic energy expenditure to their capacity for O_2 uptake, surfperch would likely be able to offset some of the physiological and metabolic stresses associated with inhabiting a low O_2 environment (Boutilier et al., 1988; Herbert and Steffensen, 2005; Petersen and Petersen, 1990), thereby decreasing their reliance on the anaerobic metabolic processes that likely support a considerable fraction of their metabolic requirements under hypoxic conditions (van den Thillart et al., 1994; Van Raaij et al., 1996a).

The observed reductions in turning rate then swimming speed could lead one to assume that surfperch show a compliant response to hypoxic challenges, as reductions in activity could conceivably limit their ability to distance themselves from hypoxia forcing them to wait out the hypoxic episode (Domenici et al., 2012). In contrast, by reducing their turning rate and becoming more uniform in their turning behaviors, surfperch were able to swim greater distances despite a reduction in swimming effort. By adopting this activity pattern surfperch may be provided with an enhanced ability to distance themselves from spatially restricted low O_2 conditions, which may in turn form the hypoxic avoidance strategy of the species. Increased observations of coastal hypoxia events has led to revived interest in characterizing the behavioral strategy that fish employ to avoid hypoxia, as the success (or otherwise) of this behavioral strategy will have a significant bearing on whether fish will survive this environmental challenge (Brady et al., 2009; Breitburg, 1994; Diaz and Rosenberg, 2008). Contrary to initial assumptions that increases in swimming activity comprise an avoidance response in many species, recent findings have identified that Australasian snapper (*Pagrus auratus*), rainbow trout (*Oncorhynchus mykiss*) and Atlantic cod (*Gadus morhua*) avoid laboratory exposures to escapable hypoxia with either no change, or in the case of cod a decrease, in swimming speed (Cook and Herbert, 2012a; Herbert et al., 2011; Poulsen et al., 2011). These findings have led some authors to suggest that the increases in swimming speed observed when some species are exposed to inescapable progressive hypoxia may in-fact represent an alarm reaction not functionally involved with low O_2 avoidance (Domenici et al., 2012). Upon evidence that Atlantic cod avoid hypoxia with a decrease in swimming activity, combined with the suggestion that decreases in activity may provide surfperch with a greater ability to distance themselves from zones of hypoxia, it seems plausible that slower swimming speeds may provide a counter-intuitive behavioral strategy that enables fish to avoid low O_2 conditions. Further investigations that expose fish to escapable rather than inescapable hypoxia in either the laboratory or field setting should be performed to corroborate this proposition.

Contrary to the results of other studies investigating the group behavior of fish exposed to hypoxic conditions (Domenici et al., 2000; Moss and McFarland, 1970), striped surfperch do not change their group structure at any low O_2 level investigated. This ability to maintain consistent NND and shoal polarity was observed despite modifications in swimming activity, including the decreases in turning activity and swimming speed observed at severely low O_2 levels. In fact, it is plausible that the observed decreases in activity and improvements in angular correlation may have facilitated group cohesion in severely hypoxic conditions. Given that no changes in NND were observed throughout the current study, it is likely that the somewhat uncoordinated, disorganized movements of shoaling individuals may help to prevent discrete pockets of hypoxia developing as a result of cumulative respiratory O_2 extraction, as is observed in school formations (McFarland and Moss, 1967; Moss and McFarland, 1970). Moreover, it has previously been suggested that changes in group structure may occur as a result of reduced sensory awareness weakening school adherence (Domenici et al., 2007), a notion supported by recent

evidence that the visual acuity of the Australasian snapper (*P. auratus*) is reduced in hypoxia (Robinson et al., 2013). Yet, when shoaling surfperch occupy progressively low O_2 conditions there is no apparent weakening of the sensory abilities required to maintain consistent neighborly distances (i.e. visual and mechano-sensory senses). As gregarious formations are widely considered to exist as a predatory defense mechanism, the ability to maintain a cohesive and stable shoal formation could provide protection against predators that exploit hypoxic environments, as documented elsewhere (Neuenfeldt et al., 2009; Rahel and Nutzman, 1994).

The multifaceted behavioral response of the surfperch to severe hypoxia (including changes in turning rates, angular correlation, and swimming speed) likely enhance the ability of surfperch to conform to the O_2 limited environment, maintain shoal structure, and may even provide surfperch with the opportunity to distance themselves from spatially limited zones of hypoxia. Whether these same individual and collective behaviors are observed in striped surfperch under natural conditions is unknown and further investigations are encouraged. Furthermore, given our finding that turning behaviors form an important component of the hypoxic coping strategy of surfperch, it is cautiously suggested that observations of swimming speed alone, without consideration of the turning behaviors of fish do not sufficiently define whether fish are showing an active, or compliant response to hypoxia.

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