Report for Friday Harbor Laboratories Fish Swimming Course 2019

Reduced oxygen consumption after critical oxygen level does not immediately impair optimal swimming performance in the Pile Perch, *Rhacochilus vacca*

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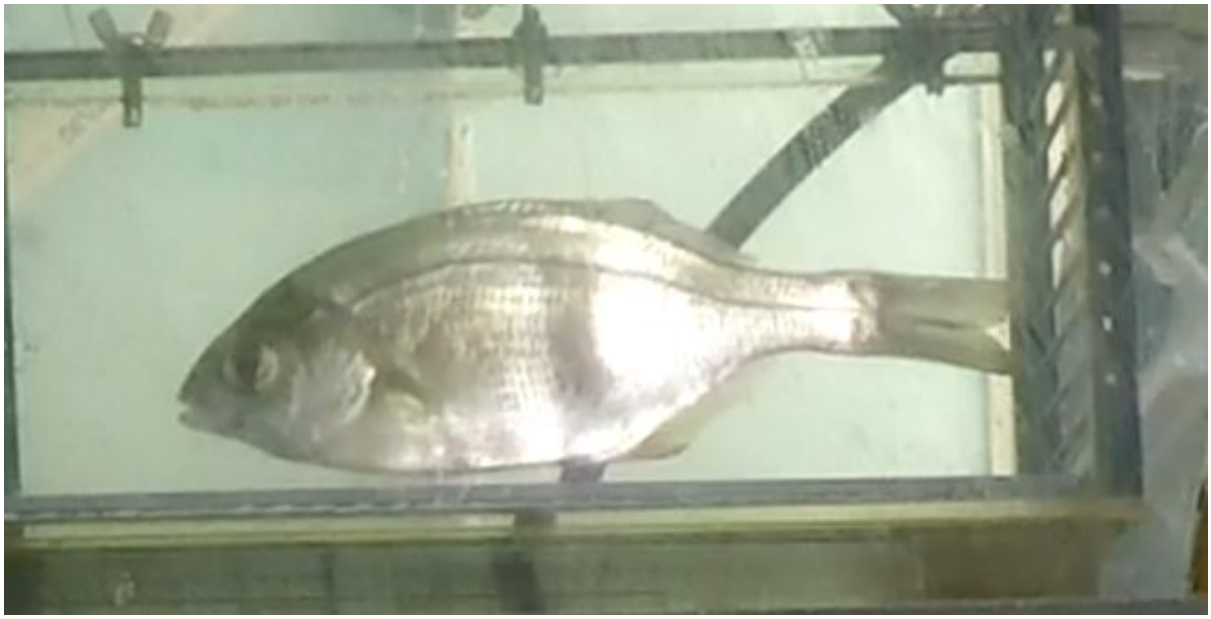

Abstract

Hypoxia is a stressor that occurs naturally in the environment. Hypoxic episodes are increasing globally due to anthropogenic nutrient loading and further exacerbated by global warming. This study examines the effect of progressive hypoxia, i.e. the continuous depletion of oxygen levels, on swimming performance at optimal speed of the pile perch, *Rhacochilus vacca. R. vacca is* a median-paired fin swimmer that exhibits obvious gate transition from pectoral fin swimming to anaerobic burst swimming using the caudal fin.. Oxygen consumption was measured during recovery from hypoxic conditions to determine whether an oxygen debt was associated with exposure to reduced O_2 levels. The optimal swimming speed was estimated to be 1.7 bls^{-1}, using strictly pectoral fin swimming, and gate transition occurred at 2.5 ± 0.13bls^{-1}. When exposed to progressive hypoxia at optimal speed, critical oxygen tension (Pcrit) was determined to be 10.29 ± 4.07 kPa. When approaching Pcrit, burst swimming began at 7.07 ± 0.24 kPa O_2. Pectoral fin beat frequency, significantly increased at 6.3-8.4 kPa oxygen saturation. Surprisingly, swimming speed was maintained beyond Pcrit, and the average oxygen levels at which fish quit swimming was 6.24 ± 0.30 kPa. There was a significant oxygen debt measured during recovery from hypoxia, which lead to a 200% increase in metabolic rate and took approximately two hours) to return to original levels. These unexpected results suggest that *R. vacca* are able to maintain optimal swimming speed in hypoxic conditions by resorting to anaerobic pathways, which leads to a significant oxygen debt.

Introduction

Hypoxia occurs naturally in marine, estuarine and freshwater habitats generally through stratification of the water column with the formation of haloclines and thermoclines which prevent the mixing of oxygen-rich surface water (Rosenberg et al. 1991). As such, fishes often have to contend with large fluctuations in oxygen regimes in their natural environment, which can occur across very short timescales (i.e. minutes or hours). In recent years however, the occurrence of hypoxia has increased due to anthropogenic causes and has become one of the most important issues facing marine organisms (See review, Breitburg et al., 2018). Oxygen minimum zones have increased by 4.5 million km^2 in the past 50 years (Stramma et al., 2010) leading to a 2% (77 billion metric tons) decrease in oxygen in the ocean (Schmidtko et al., 2017). Increased carbon emissions have led to global warming which decreases the solubility of oxygen in the water and weakens ocean circulation, while increasing respiration and therefore oxygen consumption rates (Bopp et al., 2013). In coastal waters, hypoxia is occurring due to increasing anthropogenic causes such as input of nutrients and organic matter which commonly leads to eutrophication (Diaz and Rosenberg, 2008). At severe levels, hypoxia has caused major changes in fish species composition, alteration of food webs, decrease in biodiversity, population declines, and in some cases the extinction of sensitive species (Wu, 1982, Dauer, 1993, Pihl, 1994, Diaz and Rosenberg, 1995, Alexander et al., 2000, Diaz, 2001, Wanink et al., 2001, Wu, 2002).

At a smaller scale, hypoxia lowers the availability of oxygen for metabolic functioning. To counter this, fish can engage in a suite of physiological and behavioural changes. Physiological changes include; altering ventilation rates, ventilation volume, and cardiac function in order to maximise water flow over the gills and enhance oxygen uptake (Holeton & Randall, 1967; Saunders & Sutterlin, 1971; Fritsche & Nilsson, 1989, 1990; Claireaux & Dutil, 1992; Wu, 2002; Lefrançois & Claireaux, 2003). For example, in water that contains only 50% dissolved oxygen (DO), a fish would require twice the amount of water to pass over the gills to maintain basic function than for water at 100% dissolved oxygen. At a behavioural level, fish exposed to hypoxic conditions have been shown to modify swimming activity (Schurmann & Steffensen, 1994; Domenici et al. 2000), reduce feeding rates (Chabot & Dutil 1999) and alter their locomotor strategies (Eriksson and Baden 1997). These physiological and behavioural changes strongly influence fish growth (Forbes & Lopez,1990; Diaz & Rosenburg; 1995, Chabot & Dutil,1999), reproduction (Diez and Davenport, 1990; Zhou, 2001), and consequently fitness and survival (Breitburg, 2002).

There are two main responses to depletion of oxygen in fish: either lower activity levels to lower metabolic rate, the "sit-and-wait" method (Bushnell et al., 1984; Dahlberg et al., 1968), or an increase in activity as an "escape response" to hypoxia (Dizon 1977, Bejda 1987, Pertersen and Petersen 1990; Schurmann and Steffensen 1994, Domenici et al.,2000, and Herbert and Steffensen, 2005). In aquaculture conditions, fish are swum at their optimal speed for optimal growth. Optimal swimming speed is determined in normoxia, but hypoxic conditions are abundant in aquaculture. Given that hypoxia can affect swimming, it is possible that it may also affect swimming mode.

Dutil et al. (2007) found that burst-and-coast swimming was triggered at lower speeds and was more frequent in hypoxia. Energetic biomechanical models have calculated that burst-and-coast swimming has an energetic advantage over continuous swimming (Weihs, 1974; Videler & Weihs, 1982). Burst swimming involves a gait transition from median-paired-fin (MPF) pectoral swimming to combined pectoral and caudal fin oscillations (Svendsen et al. 2010). This transition between pectoral and caudal gaits corresponds to a switch from red muscle to white muscle. (Cannas et al. 2006; Svendsen et al. 2010). In the labriform swimmer *Embiotoca lateralis*, it was found that no EPOC occurred at 1.9 L s^{-1}, where caudal propulsion occurred. This may be due to the axial shift having an aerobic component, the presence of red muscle in the axial muscle (Farrell, 2007) or because paired fin locomotion and axial locomotion may not be completely biochemically decoupled (Svendsen et al., 2010).

Limited information exists regarding the effects of hypoxia on the optimal swimming performance of fishes (but see Vagner et al. 2003). This metric is important because optimal swimming speed is the lowest amount of energy used per unit travelled and for foraging and migration. In their study, Vagner et al. (2003) exposed flathead mullet at optimal swimming speed to acute hypoxia (15% air saturation) and found an increase in oxygen uptake rate during recovery as well as a decrease in stamina. The potential mechanisms behind this increase in oxygen uptake was suggested to be due to fish repaying the oxygen debt associated with swimming anaerobically in hypoxic conditions. This finding also implied that anaerobic metabolism was being used while swimming at optimal speed at low oxygen saturations. However, the body-caudal-fin swimming mode (i.e. mainly using their tail for propulsion), exhibited by flathead grey mullet does not allow easy quantification of red and white muscle use. In MPF (Median-Paired Fin, Webb 1984) swimmers, the gait transition

between pectoral and caudal fin swimming is a useful predictor of change in swimming mode corresponding to aerobic and anaerobic metabolisms.

The pile perch, *Rhacochilus vacca*, is a common recreational fishing species with a distribution across the temperate North-West Pacific Ocean. *Rhacochilus vacca* tends to occur along rocky shorelines, particularly around structures. *R. vacca* are known to occupy habitats that experience hypoxic episodes, such as the Hood Canal – a fjord in close proximity (~100km) to the collection site used in this study. *R. vacca* are median paired fin (MPF) swimmers, that exhibit very obvious gait transition between pectoral and pectoral – caudal swimming, making them a good model species to determine the influence of hypoxia on aerobic vs anaerobic muscle use. Post-experiment dissections showed no evidence of red muscle in the caudal fin, further making *R. vacca* a good model. In this experiment swimming respirometry was used at optimal swimming speed for the *R. vacca* and in decreasing oxygen saturations to answer the following questions;

1. Will *R. vacca* transition to burst swimming when exposed to hypoxia and if they do, at which oxygen saturation?
2. How does $\dot{M}O_2$ respond to decreasing oxygen saturation, and is there a ventilation cost?
3. Is there an oxygen debt associated with exposure to hypoxia, and potentially, associated with anaerobic swimming?

Swimming performance variables such as pectoral fin beat frequency, burst frequency, PO2 at which gate transition occurs (Pburst), the critical oxygen tension (Pcrit), and the oxygen tension in which fish quit swimming (Pquit) were all measured to determine the effects of hypoxia on swimming performance. Under the common assumption that most fishes are oxygen-regulators (Steffensen, 2006; Ultsch et al., 1981), we expect that *R. vacca* will maintain $\dot{M}O_2$ alongside decreasing oxygen levels and continue to swim at optimal swimming speed. As Bushnell (1984) found, oxygen consumption of normoxia-acclimated fish at each swimming speed was independent of environmental PO_2. However, as oxygen levels approach critical levels (Pcrit), we may expect gait transition to occur (i.e. MPF to BCF propulsion) corresponding to the beginning of anaerobic metabolism and a decrease in pectoral fin beat frequency. This allows us to pinpoint the critical oxygen saturation that is limiting to optimal swimming performance.

Materials and methods

Fish collections and husbandry

Adult *Rhacochilus vacca* (n=18; total length=14.84 ± 0.55 cm, min = 14.1 cm, max = 15.7 cm; mass= 50.8 ± 6.96 g, min = 41.03 g, max = 65.25 g; means ± s.d.) were collected using a beach seine net at Jackson's Beach (48.51° N, 123.01° W) on San Juan Island, Washington, USA, in August 2019. Fish were held in flow-through aquaria at the University of Washington's Friday Harbor Laboratories at an ambient light regime (12:12). Tanks were continuously supplied with filtered seawater (salinity; 34 ppt) at a mean temperature of 14°C (± 1°C). Fish were fasted for a minimum of 24 h before the experimental trials to ensure that satiation was standardized across individuals (Niimi and Beamish, 1974; Johansen et al., 2010; Roche et al., 2013). The experimental protocol was approved by the University of Washington in accordance with Institutional Animal Care and Use Committee standards (IACUC permit no. **4238-04**).

Swimming respirometry

An 8.31 litre clear Plexiglas Steffensen-type respirometer (Steffensen et al., 1984; Methling et al., 2011) with a working section of 9.0×26.0×10.0 cm (width×length×depth) was used. Oxygen levels in the respirometer were recorded using a fibre optic oxygen meter (PreSens Fibox 3, Regensburg, Germany) monitored with AutoResp V1 (Loligo Systems, Copenhagen, Denmark). To calibrate the flow, a digital TAD W30 flow meter (Höntzsch, Waiblingen, Germany) was used within the working section of the respirometer to obtain a six-point calibration, ranging from 0 to 75±0.5 cm s–1 (mean ± s.e.m.). The maximum flow calibration is over five times the length of the fish, a more than high speed to ensure calibration exceeds speeds swam during trials. Solid blocking effects of the fish were corrected by the respirometry software (AutoResp V1); the mean fish cross-sectional area was ~3% (n=1) of the swim chamber cross-sectional area.

To reduce bacterial growth in the system, the respirometer was bleached and rinsed with freshwater before and after each trial. This ensured that bacterial respiration rates remained below 15% of the standard metabolic rate of fish. Three $\dot{M}O_2$ determinations were run without fish after each trial to measure bacterial respiration in the test chamber and then averaged. Background respiration rates were then subtracted from each $\dot{M}O_2$ measurement.

This was a more conservative approach as subtracting the average background respiration at the end of the entire trial when background respiration is highest (mean background % i.e. 5-10% of metabolic rate), means that values are a slight underestimation.

Metabolic cost of swimming speeds and Ucrit determination

At the start of a trial, fish were placed in the respirometer and left to acclimate for 6 to 8 h at a swimming speed of 0.5 BL s^{-1} until their oxygen consumption rate stabilized. This speed corresponded to the lowest water flow necessary to ensure constant swimming and minimal spontaneous activity. Eight fish were used to measure $\dot{M}O_2$ as a function of steady swimming speed (*U*) starting at 0.5 BL s^{-1} and increasing flow speed by increments of 0.5 BL s^{-1} every 30 min, following a standard critical swimming speed (*U*crit) protocol (Brett, 1964; Plaut, 2001). When the fish were unable to continue swimming, generally around 3.5 BL $s^{-1,}$ swimming speed was reduced back to 0.5 BL s^{-1} and the fish allowed to recover for 3-4 hours, i.e. until $\dot{M}O_2$ was within 10% of SMR at 0.5 BL s^{-1}. This allowed us to measure the oxygen debt (i.e. amount of oxygen needed to be repaid to tissues and muscles after anaerobic exercise) associated with anaerobic swimming. $\dot{M}$O2 was calculated by the respirometry software (AutoResp V1) as the slope of the linear regression of oxygen concentration decline over time for each determination cycle, using the equation:

$$\dot{M}O_2 = V\ (\Delta PO2/t)\ \alpha\ M.$$

Where V is the volume of the respirometry chamber volume in liters, Δ PO2/t is the change in oxygen partial pressure (kPa) per unit time, α is the solubility coefficient of oxygen in water (salinity of 35, 14.0 °C) in mgO2 kPa^{-1}, and M is the body mass of the fish (kg). Within each level of swimming speed (i.e., 30 minutes), $\dot{M}O_2$ was measured for three 10-minute cycles (flush; 240s, wait; 60s, measure; 300s). Cost of transport (COT), i.e. an estimate of swimming efficiency relative to distance covered, was then calculated for each swimming speed using the equation from Videler (1993):

$$COT = a/U + bU(c-1)$$

Where $\dot{M}O_2$ is the oxygen uptake rate in mg O2 kg^{-1} s^{-1} and U is the swimming speed in cm s^{-1}. The resulting parabola- shaped plot was fit to a second order ($k = 2$) polynomial regression model and the speed with the lowest COT was determined as the optimal swimming speed (U_{opt}). Standard metabolic rate (SMR), maximum metabolic rate (MMR), and aerobic scope (AS) were determined by methodology previously used in Steiglitz et al. (2016).

Hypoxia trials

To measure hypoxia, fish were placed in the respirometer and left to acclimate for 6 to 8 hr at a swimming speed of 0.5 BL s–1. The swimming speed was then increased to an estimate of the optimal swimming speed (2BL s^{-1}) for 30 min. $\dot{M}O_2$ was measured as above using 10-minute cycles (flush; 240s, wait; 60s, measure; 300s). To ensure only aerobic swimming was occurring at 2 BL s^{-1}, swimming speed was reduced back to 0.5 BL s^{-1} for 30 min to see if there was any EPOC. Swimming speed was then increased back to 2 BL s^{-1} for 30 minutes, after which the flush pump was turned off. This allowed the fish to deplete the chamber O_2 to 50% air saturation. During this time, $\dot{M}O_2$ measurements were taken every 600s. At 50% air saturation, the flush pump was turned back on and O_2 levels were replenished back to normoxia (>80% air saturation) within one 240s flush, i.e., using the 10-minute cycles (flush; 240s, wait; 60s, measure; 300s). $\dot{M}O_2$ was again measured for three 10-minute cycles to determine if an oxygen debt was induced by mild hypoxia. The flush pump was turned off again and remained off until the fish was unable to swim (Pquit), critical oxygen saturation (Pcrit) at optimal swimming speed (2BL s^{-1}) using broken stick regression using code provided by Reemeyer and Reyes (2019). The oxygen saturation at the point when the fish first used burst swimming was also recorded (Pburst).

Video recordings

Video recordings were used to analyse fin beat frequency and fin amplitude for each fish during hypoxia exposure and for three fish during Ucrit trials. A GoPro Hero 4 was mounted 27cm above the chamber, and a mirror was placed on a ~45° angle on the side of the tank, allowing the GoPro to capture, both top and side view. The GoPro was plugged into a battery saver cord to sustain the GoPro's life for the entire trial. For hypoxia, 15-minute recordings were taken at 100, 50, and 40-30% air saturation, and for Ucrit, recordings were between 10 – 15 minutes and coincided with each swimming speed measured.

A Logitech webcam and a mirror were setup perpendicular to the swim chamber to provide observation of the fish without disturbance for the duration of the experiment.

Fin analysis

Pectoral fin beats (fp) and caudal bursts (fb) were averaged from three randomly selected one-minute periods within the 15min videos taken at 100, 50, and 40-20% air saturation. The pectoral fin beat was measured as one entire cycle of the fin, i.e. the abduction and adduction movements of the pectoral fin (Drucker and Jensen, 1996a; Mussi et al., 2002). A burst was defined by a full oscillation of the body/caudal fin (Hunter and Zweifel, 1971) that propelled the fish forward by at least 2.5cm and when the pectoral fins were flush against the body of the fish. Videos were uploaded to VLC Media Player (Version 3.0.0 Vetinari; Intel 64bit) and played at half the speed using a tally counter to count the frequency.

Pectoral fin amplitude was obtained by measuring the angle of the pectoral fin to the body at full extension. Individuals frames (60 Hz) from the previously measured one-minute videos were used to measure the angle of the first ten fin beats (approx. first 5 s). The frame was uploaded into ImageJ (Version 1.48, National Institutes of Health, USA) and the angle tool was used to measure the angle between the body of the fish and the trailing edge of the pectoral fin

Fin aspect ratio (Fin AR) was measured by dissecting off the pectoral fin and digitally photographing it. The length of the leading edge and total surface fin area were measured on ImageJ and Fin AR was calculated by dividing the length squared by the total fin area (Wainwright et al. 2002, Binning and Fulton 2011).

Two fish were sacrificed and dissected. A section a third of the fish length from the tail was sampled as this is the point of maximum flexure (Greer-Walker, 1970). The section was then removed of flesh to determine if there was any red myotomal muscle fibres along the lateral line (Greer-Walker and Pull, 1975).

Statistical Analysis

The best linear model for fin beat frequency was determined by step AIC. Differences in fin beat frequency were determined using an ANOVA of the lm (frequency ~ O2 saturation + length) and followed by Tukey post hoc tests for specific pair-wise comparisons.

Results

Determination of optimal swim speed

The relationships between $\dot{M}O_2$ and swimming speed are shown in Figure 1. $\dot{M}O_2$ increased with swimming speed and Ucrit was determined to be 3.11bls^{-1} ± 0.09 (mean ± SEM; Figure 1). The optimal swimming speed for all fish (n=7) was 1.7 bls^{-1} and the cost of transport was calculated as:

$$COT=79.95+33.5\bullet U^{1.9}.$$

Swimming mode under normoxic conditions

There was a positive relationship between pectoral fin beat frequency and swimming speed (R^2= 0.8893; Figure 3) under normoxic conditions. This relationship excluded 0.5 bls^{-1} because fish swimming behaviour was very inconsistent at this low speed (Figure 3).

Hypoxia and swimming mode

The average kPa at which each fish began to burst (Pburst) was determined to be 7.07 ± 0.24 kPa (mean ± SEM) and the kPa at which fish quit swimming (Pquit) was 6.24 ± 0.3 kPa. Pcrit was determined to be 10.29 ± 4.07 (Table 1). The use of pectoral and burst swimming modes varied with a reduction in oxygen saturation. Pectoral fin beat frequencies were 132 ± 1.56 (mean ± SE) beats.min^{-1} in normoxia and there was no difference in fin beat frequency from 100% to 50% oxygen saturation (21 - 10.5 kPa, $P > 0.5$). However, as ambient oxygen levels dropped below 40% (~8.4 kPa) pectoral fin beat frequency increased substantially to 148.09 ± 2.73 (mean ± SE) beats.min^{-1} ($P < 0.001$). This difference suggests that *R. vacca* may show a potential behavioural response to decreased oxygen saturation (Table 2 and Figure 4). For burst swimming; no bursts were observed from any fish swimming during the trials at 100% and 50% O_2 saturation. However, below 40% O_2(~8.4 kPa), burst swimming events increased significantly to 6.67 ± 1.13 (mean ± SEM) bursts.min^{-1} (Table 2 and Figure 4).

Determination of Muscle Myotomal Composition

After the dissection of two fish. No red muscle was seen in the mytomal muscles (Figure 5).

Metabolic data

MMR was determined to be 366.41 ± 11.93 mg O2 kg^{-1} hr^{-1} (mean ± SEM). SMR was determined to be 65.12 mg O2 kg^{-1} hr^{-1}. Aerobic Scope was 284.56 ± 27.55 mg O2 kg^{-1} hr^{-1}.

Oxygen debt

There was no oxygen debt after depletion to 50% oxygen saturation.

Discussion

In this study, we show that *Rhacochilus vacca* was able to maintain optimal swimming speed under hypoxic conditions, even after critical oxygen saturation levels, i.e. the point of no excess activity was reached (Lindroth, 1942). This unexpected result suggests that *R. vacca* are relatively hypoxia tolerant, and are able to tmaintain swimming speed despite the forced transition from being an oxygen regulator to an oxygen conformer as oxygen levels reach their critical minimum. Observations in this study may suggest that *R. vacca* were able to regulate their metabolism under hypoxic conditions through transitioning from pectoral swimming to burst and coast swimming. In general, gait transition from pectoral to burst implies the transition from aerobic red muscle to white muscle aids in the continuation of swimming beyond the Pcrit, although these physiological mechanisms were not explicitly tested for in this study.

When determining Ucrit, i.e. the speed at which the fish stopped swimming, pectoral fin beat frequency increased with speed. Generally, fin beat frequency was consistent to maintain optimal swimming speed. Under normoxic conditions, *R. vacca* used strictly pectoral fin swimming at optimal speed. However, when approaching Pcrit, fin beat frequency significantly increased as well as a transition from strictly pectoral swimming to the use of the caudal fin. This increase in fin beats may be explained by two (not mutually exclusive) reasons; first, an increase in pectoral fin beat swimming may suggest a behavioural response to low oxygen levels. It has been shown that under low oxygen concentrations, fish often increase their fin beat frequency as a potential escape response. Herbert and Steffensen (2006) demonstrated an 18% increase in routine swimming of Atlantic cod when pO_2 was decreased from 19.9 kPa to 13.2 kPa. However, swimming speed declined with decreasing oxygen levels. This may be due to the fact that cod reside in extensive low O_2 areas and therefore, attempt to escape at a higher oxygen saturation and then reduce speed to minimize energy expenditure. On the other hand, active pelagic schooling fish, such as tuna and herring, increase their swimming speed when exposed to hypoxia (Dizon, 1977; Bushnell and Brill, 1991; Domenici et al., 2000; Herbert and Steffensen 2006; Brady et al, 2009). Although *R. vacca* are a schooling, coastal species, they may be exposed to episodic hypoxia events in their environment which could elicit an escape response, although this remains to be tested. Herbert and Steffensen (2006) found that in herring the elevation of plasma cortisol and

osmolarity pre-empted an increase in swimming speed, potentially suggesting a physiological trigger that induces a change in swimming behaviour.

The second reason may be that in low oxygen conditions, there is a switch from aerobic muscles in the pectoral fin to anaerobic muscles in the caudal fin. Anaerobic swimming consumes less oxygen but produces a build-up of lactic acid. Cook and Herbert (2012) found when exposing kingfish to extremely hypoxic conditions, there was a marked transition from steady swimming to burst locomotion. In Cook and Herbert's study, the use of burst locomotion accompanied an increase of lactate in the plasma, suggesting anaerobic swimming. Our study supports this idea, as no red muscle fibres were present in the myotomal fibres, meaning all bursting behaviour was anaerobically performed.

The lack of any oxygen debt after exposure to 50% oxygen saturation suggests that any debt was paid off while swimming or that no anaerobic metabolism was needed to maintain swimming performance at 50% O_2 saturation or higher. Physiological adaptations not measured in this study, such as increased ventilation rates and volume, increased haematocrit, increased blood flow and recruitment to the gills, and altered cardiac function, allow for aerobic swimming to be maintained (Holeton & Randall, 1967; Saunders & Sutterlin, 1971; Booth 1970, 1978; Randall 1982; Fritsche & Nilsson, 1989, 1990; Peterson, 1990; Claireaux & Dutil, 1992; Wu, 2002; Lefrançois & Claireaux, 2003).

Once the fish reached an oxygen level where they were unable to continue swimming (referred to as Pquit), there was a significant oxygen debt, leading to a doubling of oxygen consumption which took approximately two hours to repay. Correspondingly, Lewis et al (2007) found that RMR of the Amazonian cichlid *Astronotus ocellatus* was depressed ~50% when exposed to 10% O_2 saturation, which led to a 270% increase in metabolic rate. This, combined with previous studies that demonstrate the use of anaerobic metabolism to sustain swimming in hypoxic conditions (Muusze et al., 1998; Farrell et al., 1998; Herbert and Steffensen, 2005) suggests that this oxygen debt may be due to the switch to mostly anaerobic swimming. The behaviour exhibited by *R. vacca* suggests that this species are not necessarily well adapted to long term hypoxic conditions but can maintain swimming for short periods of time before escaping to normoxic waters. Fish who are adjusted to long term hypoxia are known to decrease in speed (Chabot and Dutil, 1999; Schurmann and Steffensen, 1994; Herbert and Steffensen; 2005), but further studies are needed on the effects of swimming at one consistent speed since fish spend most time foraging/ migrating at one

speed. The effects of hypoxia on swimming behaviour at optimal speed proved to be costly as a large oxygen debt was observed, and oxygen levels should always be considered while determining the optimal cost of transport whether in ecological or aquaculture conditions.

In conclusion, this study is one of the first to show that a fish species is able to maintain swimming performance beyond their critical oxygen levels. This unexpected and surprising result is contrary to previous studies, and emphasises the need for further research to be conducted on other species to determine whether this relationship is unique to *Rhacochilus vacca* or is common in other fish species.

Tables and Figures

Figures

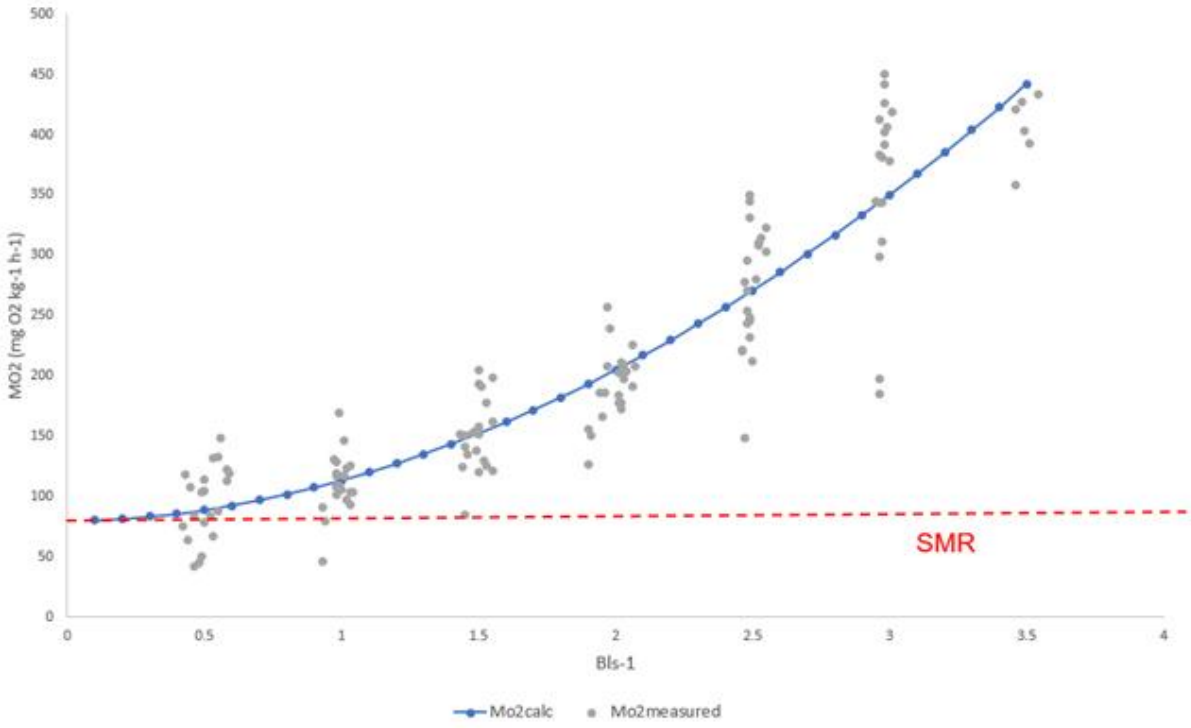

Figure 1: Metabolic costs as a function of swimming speed (U) following Brett 1964 standard critical swimming speed protocol for the Pile Perch, *Rhacochilus vacca*. Blue line represents power curve fitted on MO_2 as a function of swimming speed. Grey points represent individual measurements obtained. SMR, represented by red dashed line, equals 65.12 mg O_2 kg^{-1} h^{-1}.

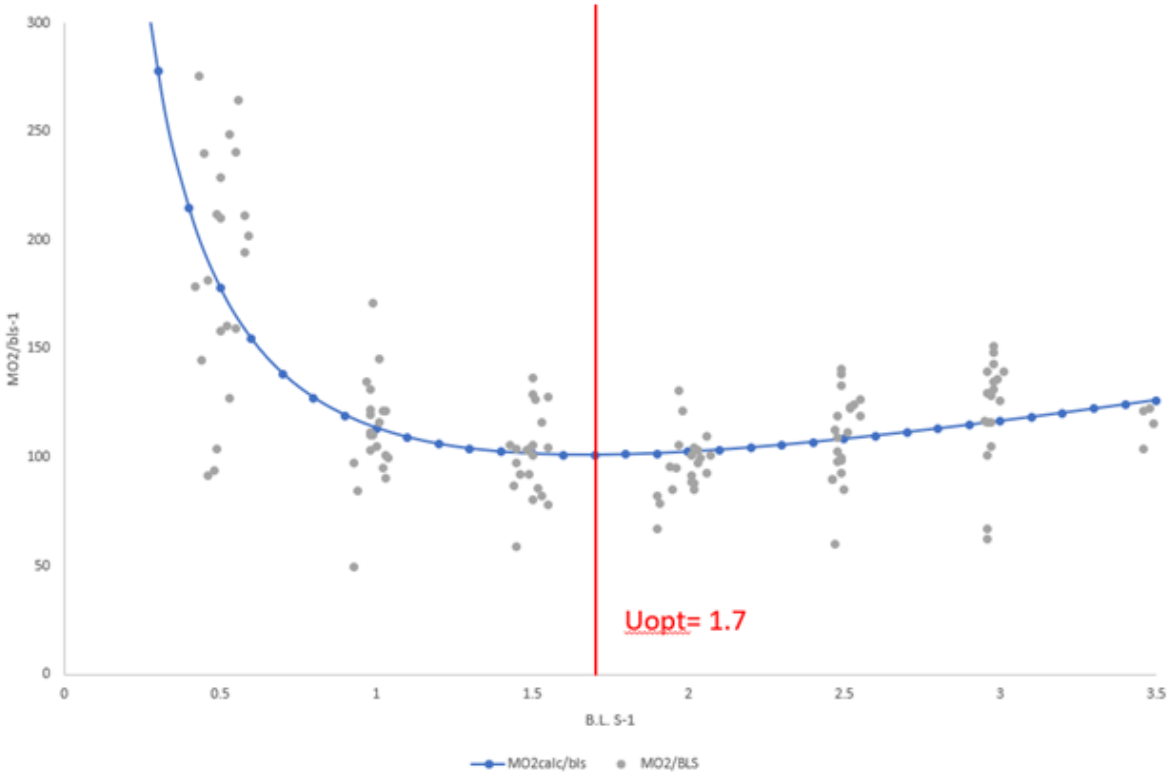

Figure 2: Cost of transport (COT) as a function in relation with increasing swimming speeds (U) in the Pile Perch, *Rhacochilus vacca*. COT was calculated from the derivative of the function of metabolic costs and swimming speed, indicated by the blue line. Optimal swimming speed (i.e., speed with lowest MO_2), as depicted by the red line, equals 1.7 BL s^{-1}.

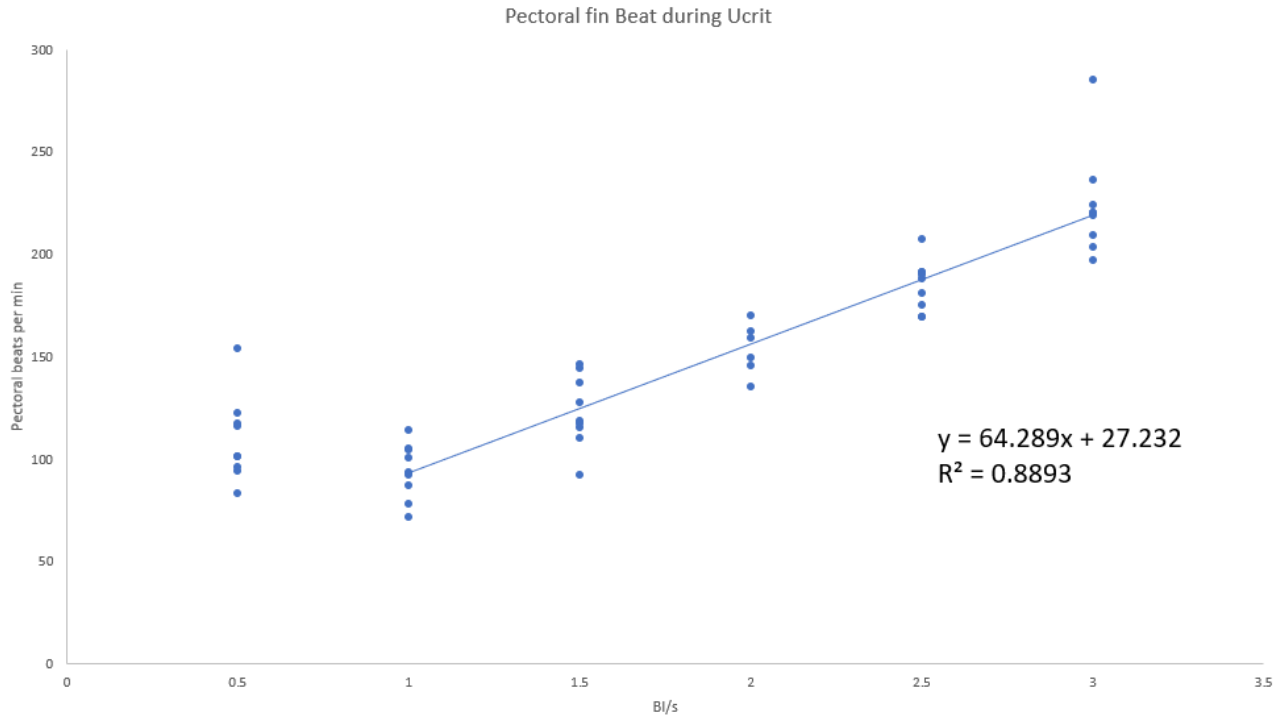

Figure 3: Pectoral fin beat frequency in relation to swimming speed (U) in the Pile Perch, *Rhacochilus vacca*.

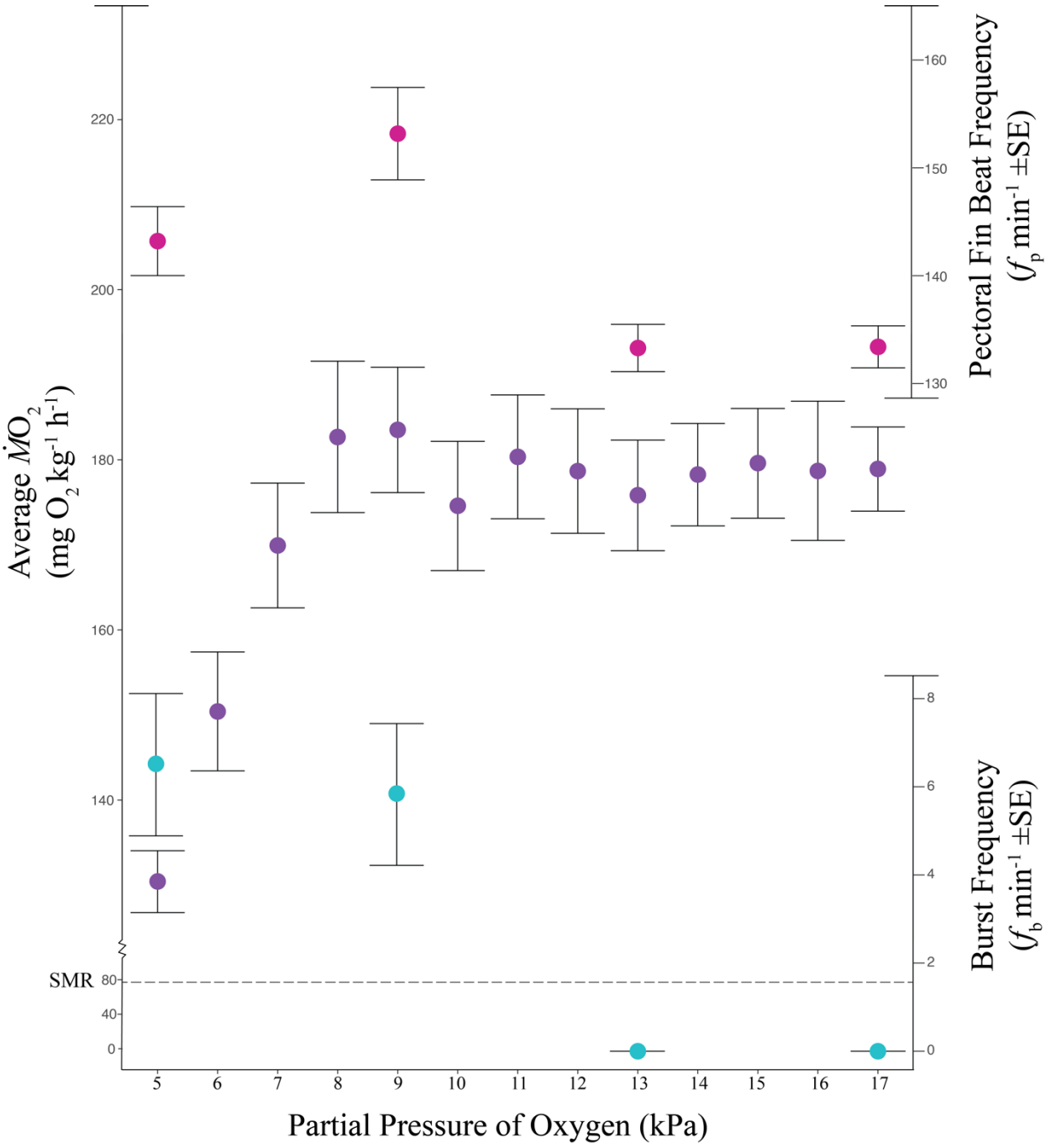

Figure 4: Changes in $\dot{M}O_2$ (purple), pectoral fin beat (f_p; pink), and burst (f_b; blue) frequencies with declining oxygen saturations in the Pile Perch, *Rhacochilus vacca* while maintaining optimal swimming speed (i.e, 2 bl s^{-1}). All points represent mean values ± SE. The horizontal dashed line indicates the standard metabolic rate (SMR; 79.95 mg O_2 kg^{-1} h^{-1}).

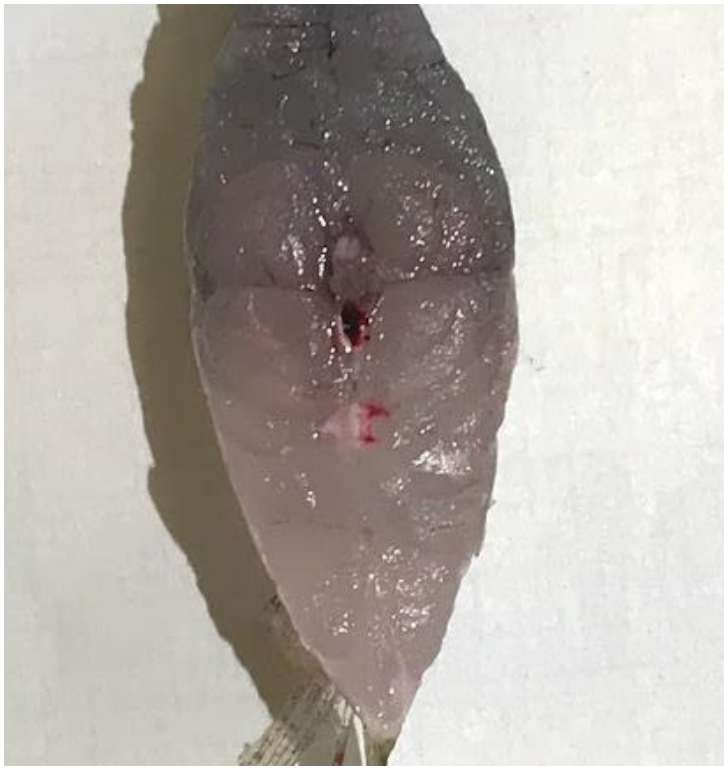

Figure 5: Dissection of *R. vacca* to determine the presence of red myotomal muscle fibres. Any red was the result of cutting the spinal cord.

Table 1: Swim Performance metrics during determination of Ucrit and during hypoxia trials (Mean ± SEM)

Ucrit	3.08 ± 0.08 bl s^{-1}
Uopt (bl s^{-1})	1.7
MMR (mg O_2 kg^{-1} h^{-1})	366.41 ± 11.93
SMR (mg O_2 kg^{-1} h^{-1})	65.12 ± 4.12
Aerobic Scope (mg O_2 kg^{-1} h^{-1})	284.56 ± 27.55
Pquit (kPa)	6.24 ±0.30
Pburst (kPa)	7.07 ± 0.24
Pcrit (kPa)	10.29 ± 4.07

Table 2: Fin Beat Frequency during Hypoxia Trials (Mean ± SEM)

Oxygen Saturation	Fin Beat Frequency (f_p min^{-1})	Burst Frequency (f_b min^{-1})
100% (21 kPa)	132.30 ± 1.96	0
50% (10.5 kPa)	134.41 ± 2.13	0
<40%	148.09 ± 2.73	6.667 ± 1.13

Table 3: Water Quality and Fish info (Mean ± SD)

Mass (g)	50.8 ± 6.96
Length (cm)	14.84 ± 0.55
Acclimation Temperature (◦C)	13.16 ± 1.70
Test Temperature (°C)	14 ± 0.01

References

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