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The physiological and behavioural response of juvenile kingfish (*Seriola lalandi*) differs between escapable and inescapable progressive hypoxia

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ABSTRACT

The behavioural and physiological responses of an active fish, the yellowtail kingfish *Seriola lalandi*, were compared under conditions of escapable and inescapable progressive hypoxia. Exposure to a severe inescapable oxygen partial pressure (PO_2) of 4 kPa (~20% air saturation), caused *S. lalandi* to adopt a burst and rest style of swimming but average speed was constant at all levels of PO_2. This behavioural response was associated with anaerobic stress, evident as an increase in plasma lactate ($p<0.01$), glucose ($p<0.05$) and cortisol ($p<0.01$). However, when presented with a choice of progressive hypoxia on one side of the tank and a normoxic PO_2 refuge (90–100% air-saturation) on the other, *S. lalandi* did not avoid any level of reduced PO_2 or show signs of physiological stress. Swimming behaviour (i.e. gait and speed) was also unaffected by all choice presentations. We therefore conclude the following: 1) *S. lalandi* showed a shift in swimming behaviour but, contrary to our expectation of an active pelagic species, they do not increase their average swimming speed in response to inescapable hypoxia. 2) *S. lalandi* do not necessarily avoid severely low O_2 conditions or show any change in swimming behaviour if they are able to mitigate stress as a result of regular forays into well oxygenated areas. The results are discussed with respect to the O_2 lifestyle of this species and compared against the hypoxic response of other species.

1. Introduction

Coastal marine areas, including fjords, reefs and bays, as well as large tracts of open ocean depths are reported to experience low oxygen conditions (hypoxia) (Helly and Levin, 2004; Karlson et al., 2002; Nilsson and Ostlund-Nilsson, 2004). Given that predicted increases in global sea surface temperatures will decrease oxygen solubility within surface layers (Shaffer et al., 2009), whilst additively strengthening oceanic physical boundary layers (halo- and thermoclines), hypoxic regions are predicted to expand and intensify in future years (Bopp et al., 2002; Stramma et al., 2008). Furthermore, land use practices and resulting eutrophication processes are contributing to the increased prevalence of hypoxic zones within coastal areas, exemplified in areas such as the Gulf of Mexico and Baltic Sea (Diaz and Rosenberg, 2008). Because oxygen availability supports aerobic metabolism and efficient energy production, hypoxia is not a desired environmental state for most metabolically active animals. Insufficient oxygen may ultimately hinder a number of important biological processes from cellular to ecological levels (Wu, 2002). With reports of mass mortalities and population decline already being reported (Wu, 2002), an appreciation of how important marine finfish will respond to worsening levels of hypoxia in coastal and pelagic environments is vital.

Hypoxia serves as a major physiological challenge by limiting the O_2 uptake potential of fish (Chabot and Claireaux, 2008). For example, low oxygen availability will at some point be insufficient to sustain maintenance metabolism (defined as the standard metabolic rate, SMR), necessitating metabolic functioning by anaerobic means (Randall, 1982; Richards, 2009) and SMR conformity at even lower external O_2 pressures (PO_2). The point of transition where oxygen availability is no longer able to support SMR is known as the critical oxygen partial pressure (P_{crit}) and life is severely limited by time beyond this level. As aerobic metabolic scope is compressed at non-critical levels of hypoxia (Chabot and Claireaux, 2008), the ability of fish to survive low O_2 conditions is undoubtedly influenced by behavioural strategies too, especially when different levels of energetic expenditure are associated with diverse modes of swimming. Indeed, fish have been observed to respond to inescapable progressive hypoxia either through an increase or decrease in swimming speed. Depending on the ecotype of fish and whether the hypoxic episode is escapable (patchy) or inescapable (extensive), these conflicting modes of swimming are thought to provide different advantages. For example, Atlantic cod *Gadus morhua* often reside in extensive low O_2 areas and it is believed that their marked drop in swimming speed offsets stress through a minimisation of energetic expenditure (Herbert and Steffensen, 2005; Schurmann and Steffensen, 1994). Conversely, active pelagic fish that school, such as tuna and herring,

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Abbreviations: BA, behavioural arena; P_{crit}, critical oxygen partial pressure; PO_2, partial pressure of oxygen.

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generally appear to increase their swimming speed (Brady et al., 2009; Bushnell and Brill, 1991; Dizon, 1977; Domenici et al., 2000; Herbert and Steffensen, 2006), which presumably enables escape from discrete patches of low O_2 and mitigates the physiological stress of hypoxic residence (Herbert and Steffensen, 2006).

Aside from the advantages gained by changes in swimming speed, many fish do also have the capacity to avoid hypoxia when the opportunity to escape is presented (Brady et al., 2009; Herbert et al., 2011; Höglund, 1961; Jones, 1952; Lefevre et al., 2011; Poulsen et al., 2011; Spoor, 1990; Wannamaker and Rice, 2000). The ability to avoid dangerous low O_2 conditions as an effective front-line response to environmental challenge would naturally be considered an adaptive behavioural strategy. However, whilst extreme low O_2 is physiologically dangerous and avoidance is often an option, some fish do still enter dangerously low O_2 on a voluntary basis, typically to feed, over-winter, and/or escape predation (Claireaux et al., 1995; Kaartvedt et al., 2009; Neuenfeldt et al., 2009; Robb and Abrahams, 2003). Current observations therefore lead us to believe that, despite the potential physiological risk of entering low O_2, complete avoidance is not actually a uniform response and, in certain cases, a lack of avoidance may even be adaptive. But a noticeable gap in our understanding of the low O_2 avoidance behaviours of fish still exists. Behavioural and physiological responses to low O_2 have largely been studied within inescapable low O_2 conditions where fish are not provided the opportunity to escape (Bushnell and Brill, 1991; Dalla Via et al., 1998; Herbert and Steffensen, 2005; 2006; Petersen and Gamperl, 2011; Schurmann and Steffensen, 1994; Sloman et al., 2006; Van Raaij et al., 1996). Furthermore, the link between stress and the behavioural response of fish in hypoxia is largely unknown. Understanding and comparing the response of fish to various modes of low O_2 is therefore important, especially since conditions of escapable and inescapable hypoxia are likely to persist in the wild.

Based on key unknowns in our current understanding (above), we set out to compare the response of an active pelagic fish to inescapable and escapable progressive hypoxia with two experimental aims. Firstly, we wanted to establish whether active pelagic species always increase their swimming speed in response to low O_2 conditions, irrespective of hypoxia being escapable or inescapable. Secondly, with a general lack of physiological measures in behavioural hypoxia studies, it was important to resolve whether physiological stress differed between escapable and non-escapable progressively hypoxic conditions and whether stress was associated with observable shifts in swimming speed or avoidance. The yellowtail kingfish (*Seriola lalandi*, Family: Carangidae) was deemed a valuable model species, being a highly active migratory in open ocean and coastal marine environments prone to hypoxia (Diaz and Rosenberg, 2008; Gilbert et al., 2010).

2. Materials and methods

2.1. Experimental fish and holding

Juvenile *S. lalandi* (0+ years) were collected from the National Institute of Water and Atmospheric Sciences (NIWA) Bream Bay Aquaculture Park, Ruakaka, New Zealand, and transferred to the Leigh Marine Laboratory. Fish where held at a stocking density of 5 kg/m^3 within a 1200 l circular tank receiving high quality flow-through water at ambient seasonal temperatures (14–23 °C) and fed a mixed diet of commercial pellets (Nova, Skretting Australia) and frozen pilchards. Fish were maintained under these holding conditions for an 8 month period prior to experimentation, during which fish maintained good health and no unexplained mortalities were observed. All experiments were performed during the Austral winter when holding temperatures were relatively stable for a 3 month period, ranging between 14.2 and 15.3 °C. These temperatures represent the thermal minima of the species.

2.2. Hypoxia test chamber

A hypoxia choice chamber (Fig. 1) similar to that described in Skjæraasen et al. (2008) and Herbert et al. (2011) was used to examine the behavioural and physiological response of individual *S. lalandi* to various low O_2 induction protocols (Fig. 1). Following transfer of individual fish to fully air saturated conditions within the behavioural arena (BA) of the chamber, fish were allowed to recover and explore the new environment for between 16 and 20 h. The BA (80 cm long × 60 cm wide × 30 cm deep) was supplied with two parallel, recti-linear flows of water (each 30 cm wide and 20 cm deep), flowing between 1 and 2 cm s^{-1}. Fish were free to swim between these two discrete bodies of water without any physical hindrance. Water was recirculated through the BA at a total rate 4800 l h^{-1}. Within this recirculation system, the discrete body of water on either side of the BA flowed into a sump bin and was subsequently pumped back to one (of two) tall gassing towers (30.5 cm id, 205 cm height). Water flowing from each of the two gassing towers passed through a series of baffles and honeycomb diffusers, generating micro-turbulent flow. Distinct separation (recti-linearity) of flows were confirmed by the addition of food colouring to the inflowing water whilst fish where absent from the BA. Two oxygen probes were positioned between each set of honeycomb baffles; the PO_2 of the water was therefore determined before entering the behavioural arena. Water PO_2 on each side of the BA was regulated using an Oxyreg control system (Loligo Systems, Tjele, Denmark) which, after comparing water PO_2 against the desired set-point, opened a solenoid valve to provide a supply of compressed air (for oxygenation) or a flow of compressed nitrogen (for deoxygenation) though a fine bubble diffuser. Throughout the design of this system every effort was made to ensure that adequate flow rates and rectilinear flows were apparent within the BA. However, it is likely that the high activity levels of fish may have caused temporary disturbances of flow within the BA. Water temperatures within the choice box were 15.0 ± 0.2 °C (mean ± 95% confidence interval). This temperature range was achieved via a manually operated flow of refrigerated water passing through aluminium heat transfer coils within the mixing towers.

2.3. Behavioural tracking setup

Tracking of fish swimming behaviour was performed in two dimensions using Lolitrack (Loligo Systems, Tjele, Denmark) software, sampling the positional x and y coordinates of the fish's geometric centre of mass at 10 Hz. Video capture was achieved by an analogue CCD camera (Monacor, 720 × 576 pixels, 25 fps) mounted above the BA. The image was streamed to a PC via a USB frame grabber (PCTV USB2, Pinnacle Systems Inc). The BA was enclosed with lightproof block-out material to control light levels but also to prevent any external disturbance. The use of ambient light and an additional 8 W fluorescent light provided low levels of illumination within the BA through a series of light ports. Greater contrast for tracking was achieved by sitting the BA on top of an acrylic sheet (18 mm height) and side-lighting the acrylic sheet with infra-red light. Positional x and y coordinates were used to calculate swimming speed, bursting and location preferences within the BA which were derived as follows: Swimming speed was expressed as the cumulative speed (per second) of the animal within the BA. Swimming direction was not unidirectional within experiments, so swimming speed was not corrected for flow rates. Within the inescapable progressive hypoxia experiment juvenile kingfish were observed to adopt a ‘burst and rest’ style of swimming at low levels of PO_2. In order to quantify this response we tallied the number of bursts observed when speed exceeded 0.5 body lengths (BL) over a 0.1 s period. This threshold was derived on the basis that the equivalent swimming speed of 5BL s^{-1} approximates the escape swimming speed of comparable species (Domenici and Blake, 1997). Our definition of burst activity was therefore used