Effects of hypoxia on fish: a metabolic approach

Kayla L. Gilmore, Zoe A. Doubleday, Bronwyn M. Gillanders
Southern Seas Ecology Laboratories, School of Biological Sciences and Environment Institute, University of Adelaide, SA 5005, Australia
Email: bronwyn.gillanders@adelaide.edu.au

Summary
Hypoxia has been associated with large scale mortalities in both marine and freshwater systems. However, it can also cause sub-lethal effects, with changes in behaviour, growth and reproduction. Our aim was to identify the sub-lethal effects of reduced dissolved oxygen at several different temperatures on fish with a focus on metabolic performance. Our first experiment investigated the effects of temperature and mild levels of hypoxia on metabolic rate of fish acclimated to experimental conditions for varying lengths of times. Our second experiment assessed the individual and interactive longer term effects of temperature and hypoxia on the survival and metabolic rate on two species of fish. However, one species only survived for 3 months under experimental conditions, and no further analyses were conducted. For the second species, which was exposed to experimental conditions for 8 months, the hypoxia tolerance limit varied depending on exposure time to hypoxic conditions as well as by temperature. Results suggest that the current general tolerance limit of 2 mg L\(^{-1}\) is too low for some more sensitive species, but may be sufficient to ensure most species survive under current guidelines.

Introduction
Hypoxia is rarely the sole source of stress facing aquatic systems, with impacts exacerbated by other stressors such as temperature. Hypoxia and temperature naturally fluctuate within a system as a result of the metabolism and decay of plants and other organisms, and while they may fluctuate independently, they more typically interact to influence the environment (Dean & Richardson, 1999). For example, raised temperatures force fish to increase their metabolism, resulting in higher oxygen requirements. At the same time higher water temperatures reduce the solubility of oxygen in water, compounding the problems facing fish attempting to survive a low oxygen environment (Dean & Richardson, 1999, Vaquer-Sunyer & Duarte, 2008). Furthermore, these events can occur for prolonged periods of time potentially leading to mass mortalities. We examined two species typically found in an environment prone to prolonged and extreme variations in temperature and hypoxia to determine the long term independent and interactive effects on fish tolerance limits.

Materials and Methods
Juvenile golden perch, GP (\emph{Maquaria ambigua}) and silver perch, SP (\emph{Bidyanus bidyanus}), were sourced from Silverwater Native Fish Hatchery, Grong Grong, NSW, Australia. Fish were randomly assigned to 20L treatment tanks (11 fish per tank) after a 10 day acclimation period (250L holding tanks, held at 20\(^{\circ}\)C). Juveniles were treated under all possible combinations of normoxic (6-8mg L\(^{-1}\)) and hypoxic (3-4mg L\(^{-1}\)) oxygen levels, combined with up to three temperature treatments (20, 24 and 28\(^{\circ}\)C, GP n=12 tanks, SP n=8 tanks only run at 20 and 28\(^{\circ}\)C). Oxygen levels were chosen based on a globally accepted standard tolerance limit of 2 mg L\(^{-1}\), enough to cause discomfort over a long term period but not necessarily mortality (Vaquer-Sunyer & Duarte, 2008). Oxygen levels were controlled using nitrogen gas to remove oxygen from the water. A combination of 9L/min of nitrogen gas and 1L/min of air were pumped 24 hrs a day to a 35L loosely sealed tank containing two electric air pumps which were used to transfer mixed gas to individual tanks and maintain a level around 3-4mg L\(^{-1}\) in treatment tanks. Maximum exposure periods for SP were 87 days and 247 days for GP. Resting respirometry was only conducted on GP due to high mortalities of SP during the initial experiment. A 4-chamber system was used to simultaneously test fish (3 chambers for testing fish, 1 chamber for background
respiration). Chambers were 300mL in volume (based on a 1:10 ratio, 1kg animal: 10L water), and submerged in a larger water bath which was used to control treatment conditions. Chambers used a recirculation loop and flush pump to cycle water intermittently on a 2min flushing period and 20min determination period; oxygen levels were recorded using a four channel FireSting Optical Oxygen Meter (Pyroscience, Aachen, Germany). Maximum metabolic rate (MMR) and standard metabolic rate (SMR) were determined using a 3min exhaustive chase/ 1 min air exposure method as described by Roche et al. (2013). Once fish reached a suitable resting state and SMR was recorded, the flush pump was switched off and fish were left to naturally deplete oxygen in chambers. Fish were observed in these conditions until they showed signs of stress (significant location shift, bursting reactions, loss of equilibrium), at which point it was considered that they had reached their tolerance limit; this value was recorded and the time taken to reach it was also noted. Fish were then removed from chambers.

Results and Discussion

Despite being exposed to the same conditions during the initial experimental period silver perch were unable to cope with the hypoxic conditions and suffered high levels of mortality. In hypoxic treatments, there was 50% mortality by week 2 at 28°C and by week 3 at 20°C. Comparatively the golden perch suffered few mortalities over the whole experimental period, showing that the silver perch had a much lower threshold for hypoxic stress.

Golden perch tested for hypoxia tolerance limits showed no significant effect of temperature or combined temperature and hypoxia treatments. However, fish exposed to long term hypoxic pressure had a higher threshold to hypoxia and were able to survive oxygen poor conditions much better than those not exposed to long term hypoxic pressure. Hypoxia, temperature and combined temperature and hypoxic stressors influenced the time taken for fish to reach their tolerance limits. Fish exposed to lower temperatures took much longer to reach tolerance limits than those treated under higher temperatures, which is what we expected to see, as there is less dissolved oxygen available to organisms at higher temperatures. This was only exasperated when combined with hypoxic conditions at higher temperatures, however, golden perch still recorded tolerance limits of around 1 mg L⁻¹ and lower on average across all treatments.

Due to the disparity observed between our two case study species, using a single universal threshold for hypoxia tolerance for aquatic organisms (2mg L⁻¹) may not be adequate to ensure the survival of all species within any one system (Vaquer-Sunyer & Duarte, 2008). The supply of oxygen to aquatic organisms worldwide is going to be affected by projected shifts in climate, with current models predicting substantial warming and deoxygenation throughout much of the ocean (Deutsch et al. 2015). Based on our results some species may be able to develop natural resistance to poor oxygen conditions over time, however, this may be limited to only those species with naturally higher thresholds for hypoxia tolerance.

References


