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The effects of temperature and body size on the metabolism and hypoxia tolerance of white abalone (Haliotis sorenseni) and red abalone (H. rufescens)

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The effects of temperature and body size on the metabolism and hypoxia tolerance of white abalone (*Haliotis sorenseni*) and red abalone (*H. rufescens*)

A thesis submitted in partial satisfaction of the requirements for the degree of

Master of Science in Environmental and Ocean Sciences

By

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ABSTRACT

In 2001, white abalone, *Haliotis sorenseni,* became the first marine invertebrate to be listed under the U.S. Endangered Species Act. Allee effects due primarily to overharvesting and disease resulted in individuals being too far apart for successful fertilization. Despite the fishery closure in 1996, *H. sorenseni* still shows no signs of recovery. Today, the White Abalone Recovery Consortium (WARC), a group that includes federal and state agencies, aquaculture organizations, academic institutions, and public aquaria, is working to recover this endangered species back from the brink of extinction.

In order to better understand the metabolic demands and hypoxia tolerance of the endangered white abalone, *H. sorenseni*, this thesis measured aspects of metabolic physiology via closed respirometry techniques, in comparison with red abalone, *Haliotis rufescens*. The relationship of mean oxygen consumption rate of both *H. sorenseni* (n = 29) and *H. rufescens* (n = 27) to environmental dissolved oxygen level showed a logarithmic best-fit function. This curve shape reveals that abalone are slightly oxy-conforming at high dissolved oxygen levels and become increasingly conforming at lower oxygen saturations. Hypoxia tolerance as estimated by determining the P_{90}, P_{75}, P_{50}, and P_{25} (values in which oxygen consumption was 90%, 75%, 50%, and 25% of resting metabolic rate), did not show a significant difference between the species. There were, however, higher levels of variability in P_{90}, P_{75}, P_{50}, and P_{25} for *H. sorenseni* in comparison to *H. rufescens,* suggesting potential fitness differences between *H. sorenseni* cohorts spawned in captivity. Additionally, there was no significant relationship

between abalone length (mm) (a useful metric of abalone size used both in the field and laboratories) and P_{50}. Although no differences between species were detected with hypoxia tolerance, *H. sorenseni* had significantly lower resting metabolic rates (RMR) than *H. rufescens* for most of the size range tested (total mass 7.7 to 85.0 g). Higher temperatures had a significant effect on P_{90}, P_{75}, P_{50}, and P_{25} for *H. sorenseni,* revealing the potential compounding effects of high temperatures and low oxygen on the species.

These metabolic data can help inform outplanting procedures of the endangered *H. sorenseni* such as determining favorable environmental dissolved oxygen and temperatures for outplanting site selection, as well as selecting fit captive bred abalone for outplanting. Higher variation in P_{90}, P_{75}, P_{50}, and P_{25} values for *H. sorenseni* individuals highlights the need to investigate phenotypic and genetic fitness of all cohorts raised in the White Abalone Recovery Program. As the first marine invertebrate listed under the Endangered Species Act, increasing knowledge for successful outplanting of *H. sorenseni* is vital in recovery of the species.

CHAPTER 1: INTRODUCTION

Life history of white abalone

The white abalone, *Haliotis sorenseni,* is a marine gastropod found along the west coast of North America with a historical range from Point Conception, California, USA, south to Punta Abreojos in Baja California, Mexico (Hobday et al., 2000). Of the eight abalone species found along the west coast, *H. sorenseni* is the deepest dwelling species and is found most abundantly in depths from 20-60 m (Butler et al., 2006; Lafferty et al., 2004). This bottom-dwelling gastropod feeds primarily on macroalgae that drifts through its rocky seabed habitat (Tutschulte and Connell, 1988). The kelp forests where *H. sorenseni* reside experience varied temperatures, pH, and environmental dissolved oxygen levels due to upwelling along the coast (Frieder et al., 2012).

H. sorenseni have a flattened oval shaped shell with 3-5 open respiratory pores. Its orange muscular foot is surrounded by an orange-tan epipodium with brown cephalic tentacles protruding out from its shell. *H. sorenseni* has an average adult size of 130-200 mm, but can grow to a maximum size of 250 mm (Leighton, 1972; Hobday et al. 2000). It has a lifespan of around 28-30 years (estimated using bomb radiocarbon dating), and reaches sexual maturity when at 4-6 years (Andrews et al., 2013; Hobday et al., 2000).

H. sorenseni reproduce via broadcast spawning, which requires male and female abalone to be close enough (less than 3m apart) to ensure successful external fertilization (Babcock & Keesing, 1999). Captive spawning of *H.*

sorenseni, shows a pelagic larval duration of approximately 10 days which allows for dispersal along the coast before settling (Leighton, 1972; Rogers-Bennett et al., 2016). *H. sorenseni* have the highest oocyte density and gonad volume in comparison to other California abalone species (Hobday et al., 2000). Despite their benthic and cryptic lifestyle post settlement, juveniles may also disperse by climbing onto drift algae (McCormick et al., 2008). Due to their high fecundity and potential for dispersal, *H. sorenseni* and other abalone were once falsely considered resilient to over-exploitation; however, now the species is considered on the brink of extinction without human intervention.

Abalone fishery

Nearshore abalone species were historically harvested in California by Native Americans for their meat and the decorative qualities of their shells (Hobday et al., 2000). In the 1850s, the commercial fishery for abalone along the North American west coast began with Chinese immigrants collecting abalone along the shore (Rogers-Bennett, 2002). This fishery continued to expand deeper and further from shore and peaked in 1979 with 172 t in abalone landings (Rogers-Bennett, 2002). Because the abalone fishery in California included all abalone species under the same set of regulations, species serial depletion of individual species beginning with red, pink (*Haliotis corrugata*), green (*Haliotis fulgens*), white, and then black abalone (*Haliotis cracherodii*) was masked by seemingly stable landings of the fishery as a whole (Hobday et al., 2000; Karpov et al., 2000). As landings for one species decreased, landings would increase for

another, until eventually all abalone species were substantially depleted. In addition, spatial serial depletion occurred, in which fisherman exhausted populations of abalone, one location at a time. Fishing began on the mainland or nearshore islands, and as the abalone became more difficult to find, efforts extended to more remote areas (Karpov et al., 2000).

Collapse of the white abalone

The fishery for *H. sorenseni* peaked in the 1970s as divers expanded their search with the development of better diving equipment, navigations systems, and depth finders facilitating the search for this deep-dwelling abalone (Hobday et al., 2000). White abalone was particularly targeted due to the tenderness of its meat, and that value only increased with its rarity, as the landings of the species decreased with depletion (Hobday et al., 2000; Karpov et al., 2000). By 1983, the fishery for *H. sorenseni* essentially collapsed after a short time period of about only ten years (Hobday et al., 2000; Karpov et al., 2000). The stock size of *H. sorenseni* was estimated around 360,000 prior to overfishing, and it is now at less than 1% of its original population (Rogers-Bennett, 2002).

In 2001, *H. sorenseni* became the first marine invertebrate to be listed under the US Endangered Species Act (ESA 2001). Once an important part of a commercial fishery, the latest estimate in 2012 from Tanner Bank (an area with a historical abundance of white abalone) estimated only about 3,000 *H. sorenseni* left at that location, a reduction of about 78% (Stierhoff et al., 2012). This large decrease in population size is primarily due to fishery exploitation (Rogers-

Bennett et al., 2016; Stierhoff et al., 2012). Despite the fishery closure in 1996, there has been no evidence of recruitment success, and extinction will be likely without human intervention (Rogers-Bennett et al., 2004; Rogers-Bennett et al., 2016).

With such low population densities, external fertilization is unlikely as adults are too far apart for their gametes to meet during broadcast spawning, a phenomenon known as the Allee effect (Shepherd and Brown, 1993). Data suggest that populations of abalone would decline if their densities are less than 2,000 ha^{-1}, and unfortunately, estimates based off ROV surveys in 2008 and 2010 revealed densities well below that threshold at approximately 6.4 ha^{-1} (Karpov et al., 2000; Stierhoff et al., 2012). Besides the low densities of adults, another indicator of reproductive failure is a lack of juveniles present in wild populations. During abalone population surveys, mostly large adult *H. sorenseni* have been encountered, and the lack of juveniles shows lack of recruitment necessary to rebuild *H. sorenseni* naturally (Rogers-Bennett et al., 2004).

White abalone recovery efforts

The White Abalone Recovery Program was established in 2005 to create a plan to save *H. sorenseni* from extinction. This program includes various groups (White Abalone Recovery Consortium, WARC) committed to abalone restoration, including federal and state agencies, aquaculture organizations, academic institutions, and public aquaria (Rogers-Bennett et al., 2016). Efforts of this group incorporate actions such as assessing wild white abalone populations,

collecting broodstock from the wild, breeding *H. sorenseni* successfully in captivity, planning effective outplanting strategies for captive bred abalone, and educating the public about the importance of this endangered species. After immense work from the White Abalone Recovery Consortium partners since the formation of the program, the first groups of captive bred *H. sorenseni* were successfully outplanted off the coast of southern California in 2019.

Withering syndrome

Withering syndrome is a fatal disease found in abalone species causing extreme pedal atrophy (shrinking of the snail foot) that eventually leads to mortality. This disease, first seen in 1980s in the Channel Islands, is caused by a Rickettsiales-like prokaryote (WS-RLP) that infects the gastrointestinal tissue of the abalone, causing the chronic wasting disease (Moore et al., 2002). Warmer temperatures are correlated with higher mortality of abalone with withering syndrome, with temperatures above 18.5°C resulting in particularly devastating withering syndrome effects (Moore et al., 2002).

This disease occurs regularly in the wild and is also a problem in abalone held in captivity. Fortunately, there are successful treatments for withering syndrome using antibiotics such as oxytetracycline (an antibiotic approved for aquaculture usage), which can reduce mortality and the prevalence of withering syndrome in captive abalone (Friedman et al., 2007; Moore et al., 2002). For captive-held abalone specifically, additions of temperature control, UV sterilization, and ozone filtration of aquarium water has drastically decreased the

prevalence of withering syndrome and increased overall abalone health (Rogers-Bennett et al., 2016). Understanding how to control this disease in captivity has greatly improved spawning and rearing success for captive bred *H. sorenseni* to be used for outplanting.

Outplanting

With *H. sorenseni* being successfully spawned and reared in captivity, best practices for outplanting captive bred abalone to the wild need to be investigated (Rogers-Bennett et al., 2016). Locations for outplanting efforts should be carefully selected based on both abiotic and biotic factors to ensure the highest survival rates of outplanted abalone. Habitat surveys for *H. sorenseni* showed a preference for depths from 20-60 m, and habitat with proper rugosity (Butler et al., 2006). Models have shown that outplanting in areas with suitable habitat can slow decline of the population and increase recruitment in comparison to areas that are unsuitable (Li & Rogers-Bennett, 2017; Li & Jaio, 2015). A good indicator of suitable habitat consists of locations where wild *H. sorenseni* are already present, and stocking models had the best survival rates when resident abalone were already present in the area (Li & Rogers-Bennett, 2017). Outplanting abalone in areas susceptible to high temperatures or poaching had a negative effect on stocking strategy (Li & Rogers-Bennett, 2017).

Another factor to consider in successful outplanting and abalone survival is the condition of the captive bred abalone to be outplanted. In a release and recapture study involving red abalone, *H. rufescens,* the lowest mortality rates

were found in the largest size class of abalone (> 178 mm) compared to the middle (100-178 mm) and small size classes (< 100 mm) (Leaf et al., 2007). Models of stocking strategies determined that outplanting larger abalone may be more effective at promoting recovery than stocking smaller juveniles (Li & Rogers-Bennett, 2017; Li & Jiao, 2015). Another stocking experiment analyzed predators in relation to outplanted juvenile *H. rufescens.* This study showed a surge of octopus predators that coincided with the outplanting event, as well as evidence of predation from the octopuses, crustaceans, and fish predators on the abalone (Hofmeister et al., 2018). This reduction of survival for smaller sized abalone, in addition to evidence of predation in response to outplanting suggests that juvenile abalone may be more susceptible to predation than larger adults once released. Not much is known about the effects of other abiotic factors, such as hypoxia (low levels of dissolved oxygen), and how such effects may vary with size.

Research Objectives

In order to determine how temperature and body size influence metabolism and hypoxia tolerance, metabolic measurements were determined for *H. sorenseni* in comparison to the red abalone, *H. rufescens,* which in southern California inhabits similar depth within the kelp forest. These metabolic measurements of resting metabolic rate and P_{90}, P_{75}, P_{50}, and P_{25} (values in which oxygen consumption was 90%, 75%, 50%, and 25% of resting metabolic rate), were analyzed with different abalone sizes, temperatures (11° and 15°C), and

environmental oxygen saturations for both abalone species. This information is vital to outplanting success of *H. sorenseni* by helping to select locations for outplanting with favorable temperatures and oxygen saturation levels for abalone survival. Understanding hypoxia tolerance of *H. sorenseni* can also help select captive bred cohorts and abalone sizes that are the most resistant to low oxygen stresses that may be encountered in the wild.

CHAPTER 2: METABOLIC STUDY

Introduction

The white abalone, *Haliotis sorenseni*, is a marine gastropod, and one of eight abalone species found off the coast of California. It was the first marine invertebrate to be listed as endangered under the US Endangered Species Act (ESA 2001). Overfishing and poor fishery regulations ultimately led to the collapse of the species through serial depletion (Hobday et al., 2000; Karpov et al., 2000). As a broadcast spawner, abalone need to be in close proximity (less than 3 m apart) for successful fertilization, so even with the fishery closure in 1996, populations of *H. sorenseni* have still shown no signs of recovery (Stierhoff et al., 2012). In order to prevent the extinction of *H. sorenseni*, a White Abalone Recovery Program has been implemented which includes various academic institutions, state and federal government entities, abalone aquaculture groups, and public aquaria (White Abalone Recovery Consortium, WARC) working together to assess wild populations, rear white abalone in captivity, outplant captive-bred abalone back into the wild, and educate the public about this vulnerable species (Rogers-Bennett et al., 2016). Since the start of this recovery program, *H. sorenseni* has been successfully reared in captivity with the first outplanting to the wild occurring in 2019. It is imperative to understand the fitness level of captive bred *H. sorenseni* to have informed stocking strategies and successful introductions to the wild.

H. sorenseni is the deepest dwelling abalone species, being found most abundantly at depths of 20-60 m (Butler et al., 2006). Because this abalone

species is found at greater depths, it is potentially vulnerable to periodic exposure to low levels of dissolved oxygen associated with natural upwelling and currents that can occur in their habitat (Frieder et al., 2012). As a benthic animal with limited mobility, *H. sorenseni* could be especially vulnerable to the adverse effects of low oxygen in comparison to more mobile fauna. Additionally, hypoxic zones in kelp forests could increase with climate change, as studies of environmental dissolved oxygen have shown a shoaling of the oxygen minimum zone (OMZ) in the California Current system and decreased surface mixing due to increased stratification (Bograd et al., 2008) . Low levels of dissolved oxygen have been shown to decrease metabolism, growth, and survival rates in captive juvenile greenlip abalone (*Haliotis laevigata*) and red abalone (*Haliotis rufescens*) (Harris et al., 1999; Kim et al., 2013). Despite being one of the deepest dwelling abalone and most likely to regularly encounter hypoxia, little is known about the physiological response of *H. sorenseni* to low environmental oxygen, and how other factors such as abalone size or environmental temperature may impact this response.

This study examined the metabolic rate and hypoxia tolerance of *H. sorenseni* in comparison to red abalone, *Haliotis rufescens,* another abalone species found along the west coast of North America that inhabits similar temperatures and depths as *H. sorenseni* in southern California. Baseline metabolic measures such as the mass-scaling relationship of oxygen consumption rate and hypoxia tolerance via the P_{90}, P_{75}, P_{50}, and P_{25} (values in which oxygen consumption was 90%, 75%, 50%, and 25% of resting metabolic rate, RMR),

were determined using respirometry techniques. Additionally, the effect of temperature on metabolism (11°C and 15°C) and a Q_{10} was calculated due to the natural variability of temperatures from upwelling encountered in the kelp forests where the abalone are found. These valuable metabolic data on *H. sorenseni* can help identify 1) abalone optimal oxygen saturation levels and temperatures and 2) what abalone sizes or cohorts are more hypoxia tolerant, both of which may inform outplanting decisions and restoration efforts of the White Abalone Recovery Consortium.

Methods

Abalone husbandry

H. sorenseni (n = 29, 4.6 – 103.3 g, 34.82 – 91.55 mm in length) and *H. rufescens* (n = 27, 7.7 – 103.3 g, 39.06 – 94.04 mm) examined in this study were raised at the Southwest Fisheries Science Center (SWFSC) from larvae produced by the Captive White Abalone Breeding Program (*H. sorenseni*) and from The Cultured Abalone Farm (Goleta, CA) and the Abalone Farm (Cayucos, CA) (*H. rufescens*). Abalone were reared in long-term culture tanks with temperature-controlled filtered seawater and fed *ad libitum* a variety of brown algae (mostly giant kelp, *Macrocystis pyrfera*) harvested locally, as well as dulse, *Palmaria mollis,* grown onsite. Abalone were tagged with flexible shellfish tags (FT-LF-97, $^1/_8$ x ¼", FLOY TAG Inc., Seattle, WA) for identification using a cyanoacrylate adhesive (CorAffix, Two Little Fishies Inc., Miami Gardens, FL).

All abalone husbandry and experimentation were performed in accordance with SWFSC Animal Care and Use Committee Protocol #SW1702.

Resting metabolism and hypoxia tolerance trials

Respirometry experiments were performed to examine the effects of environmental oxygen on metabolism in order to better understand the hypoxia tolerance of both *H. sorenseni* and *H. rufescens* over their potential size range for outplanting (~30-100 mm). Prior to experimentation, each abalone was gently dislodged from its home tank by inserting a plastic spatula or credit card under the foot. The abalone was then placed on a 6.7 mm thick PVC disk (57, 77, or 95 mm diameter depending on abalone size) surrounded by fine mesh netting and suspended in the holding tank to fast the animal for a minimum of 48 h prior to respirometry measurements. Following the fasting period, the abalone (attached to the PVC disk) was transferred to the respirometer via a plastic beaker, which allowed the abalone to remain submerged in seawater to minimize handling stress and prevent the introduction of bubbles into the respirometry system.

The respirometer consisted of a cylindrical acrylic holding chamber, that could be changed out to most closely fit the abalone and its PVC disk size (62 x 25 mm, 80 x 30 mm, or 100 x 40 mm; inner diameter x inner height; Loligo Systems; Tjele, Denmark), and a recirculating loop composed of Tygon tubing, a small pump (Brushless DC pump, 12V; ZKSJ; Shenzhen, China), two sensor ports (for dissolved oxygen and temperature sensors), and two 3-way stopcocks (to allow for opening and closing the system to the surrounding buffer tank water)

(Fig. 2.1). Once placed in the respirometer, an abalone was given one hour of acclimation to let the handling stress subside before the 3-way stopcocks were closed to isolate the respirometer from the surrounding buffer tank for oxygen consumption measurements (longer acclimations times (e.g. overnight) were not used as preliminary trials showed that longer acclimation led to an increased bacterial load in the respirometer which increased the background respiration rate). The abalone's respiration caused the system to become progressively hypoxic allowing resting oxygen consumption rate to be monitored until the oxygen level reached approximately 5% saturation. Oxygen saturation (% air saturation) and temperature (°C) were monitored with a fiber optic oxygen probe and a temperature sensor connected to either a Fibox 3 system (PreSens Precision Sensing; Regensburg, Germany), which could monitor one abalone respirometer at a time, or a Witrox 4 oxygen meter system (Loligo Systems; Viborg, Denmark), that could monitor up to four abalone respirometers at once. Following completion of the respirometry trial, the abalone was removed from both the chamber and PVC disk to measure its mass, length, and volume (abalone volume was measured via displacement in a graduated cylinder). The respirometry system was flushed for at least 15 minutes and then resealed with the PVC disk inside, but without the abalone, for a minimum of one hour to obtain measurements of background bacterial respiration.

Respirometry experiments were first conducted for all abalone at 15°C. In order to examine the effects of temperature on metabolism and hypoxia tolerance, respirometry trials were repeated at 11°C on ten abalone of each species from the

middle of the examined body size range. Temperatures of 15°C and 11°C were chosen as these temperatures represent approximate mean seasonal (summer vs winter) temperatures encountered from 20-60 m deep (depths where both species can be found) in the Southern California Bight (Ault et al., 1985; Butler et al., 2006; Frieder et al., 2012; Hickey et al., 2003). Abalone were acclimated to their experimental temperature for a least 30 days prior to respirometry trials.

Calculation of oxygen consumption rate, P_{90}, P_{75}, P_{50}, P_{25}, mass scaling coefficients, and Q_{10}

For each respirometry trial, abalone oxygen consumption rate (M_{O_2} mg O_2 h^{-1}) was determined over 5% oxygen saturation intervals (e.g., the mean M_{O_2} was calculated when the dissolved oxygen level of the respirometer was between 95-100% saturation, 90-95% saturation, down to 5-10% saturation) and graphed versus oxygen saturation (%) (Fig. 2.2). Preliminary analysis and model fitting of the relationship between M_{O_2} and environmental oxygen level revealed outlier points (e.g., abnormally high or low M_{O_2} values, defined as greater than 30% different than the predicted value of the best-fit function) which were removed (such outliers, which were likely associated with abalone activity within the chamber, were rare, comprising less than 3% of the data). This relationship of abalone M_{O_2} versus environmental oxygen level displayed as either a logarithmic, power, or linear function (compare Figs. 2.2A, 2.2B, and 2.2C), and the best-fit model was chosen for each individual using an ordinary least squares analysis via lm() function in base R package (R Core Team, 2020). Resting M_{O_2}, a proxy for

resting metabolic rate (RMR), was estimated using the best-fit function for each individual abalone at the point where oxygen saturation was at 100%. The P_{90}, P_{75}, P_{50}, and P_{25} (dissolved oxygen level in which oxygen consumption was 90%, 75%, 50%, and 25% of RMR) were also estimated using the best-fit functions as shown in Figure 2.2.

The relationship of abalone oxygen consumption with total body mass (including the shell) was determined according to the power-law equation:

$$M_{O_2} = a \times M^b \quad (1)$$

where M_{O_2} is the oxygen consumption rate, a is the intercept at a mass of 1 g, M is mass in grams, and b is the mass scaling coefficient (Schmidt-Nielsen, 1984). Because the shell is not metabolically active, the relationship of M_{O_2} with just the wet tissue mass excluding the shell was also estimated. This was estimated using data on shell and wet tissue mass from 96 *H. sorenseni* (0.5 – 37.6 g, 13.6 – 64.4 mm in length) and 533 *H. rufescens* (42.0 – 237.4 g, 70.0 – 115.0 mm in length) that were measured for unrelated work (J. Moore, California Department of Fish and Wildlife, unpublished data). These data showed the ratio of wet tissue mass to total body mass (including the shell) was 0.683 ± 0.054 for *H. sorenseni* and 0.771 ± 0.049 for *H. rufescens*. This ratio did not change with body size for either species.

The Q_{10}, the factor by which oxygen consumption increases with a 10°C temperature change, was calculated according to the equation:

$$Q_{10} = \left(\frac{M_{O_2 2}}{M_{O_2 1}}\right)^{\left(\frac{10}{T_2 - T_1}\right)} \quad (2)$$

where $M_{O_2 1}$ and $M_{O_2 2}$ represent oxygen consumption rates at T_1 (11°C) and T_2 (15°C) respectively. In order to account for differences in body size between the 11 and 15°C trials (Table 2.2) the M_{O_2} of *H. sorenseni* and *H. rufescens* was scaled to a mass of 40 g using the species-specific mass scaling coefficients calculated in Equation 1.

Statistical analyses

Statistical analyses of the respirometry data were completed using R v3.6.2 (R Core Team, 2020). Means are presented as ± standard deviation unless otherwise indicated. Assumptions of normality and homogeneity for the experimental groups (e.g. species, temperature) were tested using a Shapiro-Wilks test and Bartlett test respectively. A Kruskal-Wallace test was used to test for significant differences between species for measures of P_{90}, P_{75}, P_{50}, and P_{25} and metabolic rate. For examining temperature effects on RMR as well as P_{90}, P_{75}, P_{50}, and P_{25}, a paired t test or paired samples Wilcoxon test was used depending on normality of the groups compared.

For metabolic scaling data, a bootstrap regression analysis (10,000 replicates with replacement) was used to detect significant differences between species for the relationship between both abalone total mass (g) and M_{O_2}(mg O_2 h^{-1}), and tissue mass (g) and M_{O_2}(mg O_2 h^{-1}). Significant difference in M_{O_2} between species was determined if < 5% of the bootstrap regressions intersected at the body mass being compared. Bootstrap analysis was also used to analyze

significant differences between species for the relationship of average M_{O_2} (mg O_2 h^{-1}) and environmental oxygen level (%).

Results

Resting metabolic rate

Representative graphs showing the relationship between abalone M_{O_2} and environmental oxygen (% saturation) are shown in Figure 2.2. For each individual, M_{O_2} at 100% O_2 saturation was used to estimate resting metabolic rate (mg O_2 h^{-1}) which was graphed in relation to whole animal mass (g) (Fig. 2.3) resulting in the best fit power functions of $M_{O_2} = 0.0269M^{0.9531}$ ($R^2 = 0.936$) for *H. sorenseni* and $M_{O_2} = 0.0692M^{0.7794}$ ($R^2 = 0.867$) for *H. rufescens*. Bootstrap analysis revealed that *H. rufescens* had significantly higher (p value < 0.05) metabolic rates (mg O_2 h^{-1}) than *H. sorenseni* for most of the overlapping body mass range (7.7 to 85.0 g). When considering just the metabolically active tissue (e.g., total mass – shell mass), $M_{O_2} = 0.0387M^{0.9531}$ ($R^2 = 0.936$) for *H. sorenseni* and $M_{O_2} = 0.0902M^{0.7794}$ ($R^2 = 0.867$) for *H. rufescens*, and bootstrap analysis indicated that *H. rufescens* also had significantly higher (p value < 0.05) metabolic rates than *H. sorenseni* for most of the tissue mass range (5.5 to 51.9 g).

Hypoxia tolerance

Abalone M_{O_2} typically showed a logarithmic relationship with environmental oxygen, with M_{O_2} decreasing faster at lower oxygen concentrations (Fig. 2.2A; 33/39 white abalone, 30/37 red abalone; ratios include 11°C and 15°C

data). However, the M_{O_2} profiles of some individuals more accurately displayed as a power relation (Fig 2.2B; 2/39 white abalone, 7/37 red abalone) or a linear relationship (Fig 2.3C; 4/39 white abalone, 0/37 red abalone) with M_{O_2} decreasing faster at higher environmental oxygen concentrations than under a logarithmic curve. When data for all individuals were standardized and combined, this relationship presented as a logarithmic curve for both *H. sorenseni* and *H. rufescens* (Fig. 2.4). Bootstrap analysis revealed that *H. rufescens* had significantly higher (p value < 0.05) average standardized metabolic rates (mg O_2 h^{-1}) than *H. sorenseni* from 10.24 - 100% environmental oxygen saturation (Fig. 2.4A).

P_{90}, P_{75}, P_{50}, and P_{25} values were determined based on each individual abalone M_{O_2} vs O_2 saturation curves and means are presented in Table 2.1 and graphically in Figure 2.5. P_{90}, P_{75}, P_{50}, and P_{25} values were not significantly different between *H. sorenseni* and *H. rufescens*; however, *H. sorenseni* showed greater variability, as well as more outliers in comparison to *H. rufescens* (Fig. 2.5). In order to examine how abalone size potentially influences hypoxia tolerance, P_{50} values (percent oxygen saturation) for both abalone species were graphed in relation to abalone shell length (mm), a common metric recorded in both field and laboratory settings (Fig. 2.6). There was no significant relationship between abalone size and P_{50} for either *H. sorenseni* or *H. rufescens*.

Q_{10} calculations and temperature effects

There were significant differences between the resting metabolic rate of *H. sorenseni* at 11°C and 15°C (paired t test; p value < 0.005) resulting in a Q_{10} of 2.90. For *H. rufescens*, resting metabolic rate was not significantly different between 11 and 15 ºC (paired samples Wilcoxon test; p value = 0.084).

When examining how temperature influences hypoxia tolerance, P_{90}, P_{75}, P_{50}, and P_{25} measures were all statistically different between 11°C and 15°C for *H. sorenseni* (paired samples Wilcoxon test; p value < 0.05 for all measures; Fig. 2.7). However, there was no difference in P_{90}, P_{75}, P_{50}, and P_{25} between 11°C and 15°C for *H. rufescens* (paired samples Wilcoxon test; p value > 0.05 for all values).

Discussion

This study establishes important baseline metabolic data for both *H. sorenseni* and *H. rufescens* including mass scaling relationship with metabolism for both species, as well as estimates of resting metabolic rate and P_{90}, P_{75}, P_{50}, and P_{25}, and how these measures are affected by abalone size (length) and temperature (11°C and 15°C).

The resting metabolic rate of *H. sorenseni* was significantly lower than that of *H. rufescens* for most of the overlapping body size range examined. This metabolic difference was apparent both for total animal mass as well as tissue mass (total mass – shell mass). This pattern of *H. rufescens* exhibiting higher resting metabolic rates than *H. sorenseni* also remained true in varying

environmental oxygen levels. The lower metabolic demand of *H. sorenseni* may be an adaption to the deeper depth they inhabit (20-60 m), and the lower, and often varied oxygen levels they may encounter from upwelling in the kelp forests. This natural upwelling causes variable dissolved oxygen levels with an average daily dissolved oxygen range of ~25%, with a maximum daily range of ~85% (measure at depths 7m and 17m) in kelp forests off the coast of southern California, which demonstrates the potential advantage associated with a lower oxygen demand (Frieder et al., 2012). Additionally, the lower metabolism of *H. sorenseni* in comparison with *H. rufescens* would suggest that *H. sorenseni* has lower growth rates. *H. sorenseni* growth rates have been estimated at 0.19 cm y^{-1} shell growth rate while 1.5 cm y^{-1} for *H. rufescens,* however the estimate for *H. sorenseni* is limited and more studies could reveal a stronger growth estimate for the species (Rogers-Bennett et al., 2007; Stierhoff et al., 2012). The calculated mass scaling coefficient, *b*, for both *H. sorenseni* (b = 0.9531) and *H. rufescens* (b = 0.7794) was higher than that of a previous study on pinto abalone *Haliotis kamtshatkana,* which calculated b = 0.62 (Carefoot et al., 1993), suggesting that size has a larger impact on metabolism for the two species in the present study.

The resting metabolic rates estimated for *H. sorenseni* and *H. rufescens* in this study were lower than those of other shallow water abalone species determined previously (Table 2.3), including the Ass's ear abalone (*Haliotis asinina*), a shallow water reef species from the Indo-West Pacific, the South African abalone (*H. midae*), commonly found in about 10 m of water, and the greenlip abalone (*H. laevigata*), an Australian species typically found around 20 m (Baldwin et al.,

2007; Barkai & Griffiths, 1986; Gilroy & Edwards, 1998). These shallow water species likely do not encounter low oxygen as readily as *H. sorenseni* and *H. rufescens* and thus may have higher metabolic rates associated with a more oxygenated habitat. The higher metabolic rates observed for previous abalone studies may also reflect differences in the respirometry methodology. The respiratory trials in the present study were conducted with a one-hour acclimation period and usage of PVC disks for ease of transfer that minimized any metabolic signature associated with handling stress, while also minimizing the time for introduced bacteria to establish and create a problematic background respiration rate. Thus, shorter acclimation times (such as Carefoot et al., 1993 that only had a 10 min acclimation) that could have elevated abalone metabolism from movement stress, or multiday respirometry runs (such as Harris et al., 1999 that had multiple abalone in the respirometers for 3 days) that could introduce a heavy bacterial load could increase a measured metabolic rate and should be considered when making comparisons.

The relationship between mean oxygen consumption rate and percent oxygen saturation was best represented as a logarithmic function for both *H. sorenseni* and *H. rufescens* (Fig. 2.4). For many organisms, metabolic relationships are represented by a broken stick model where an animal is an oxy-regulator at high percent oxygen saturations, and an oxy-conformer at lower oxygen levels, which is common for many fish species (Yeager & Ultsch, 1988). Other invertebrates such as crustaceans typically follow patterns of oxy-regulation, but some may follow a more oxy-conforming pattern with decreases in metabolic rate at higher

oxygen saturations, such as the Hawaiian shrimp, *Halocaridina rubra* (Havird et al., 2014). Juvenile greenlip abalone, *Haliotis laevigata,* followed a broken stick model which indicated oxy-regulation from oxygen concentrations 81-117% (Harris et al., 1999), following which at lower oxygen concentrations metabolism rapidly decreases with decreased environmental oxygen. Infauna benthic invertebrates are typically oxy-conforming and have low survival during hypoxic events, but a polychaete, *Alitta succinea,* demonstrated oxy-regulation to a relatively low oxygen critical point (Kersey Sturdivant et al., 2015) .

In the case of *H. sorenseni* and *H. rufescens*, the logarithmic relationship between oxygen consumption and environmental oxygen (Figs 2.4 A, 2.4B, 2.4C) reveals that these species are slightly oxy-conforming at high oxygen saturations and become more oxy-conforming with lower oxygen saturation levels. This pattern differs from the typical oxy-regulating / conforming relationship with a clear critical breakpoint (critical oxygen concentration, P_{crit}) and reveals that even small reductions in oxygen saturation can affect metabolism and potentially cause stress on the animal.

Hypoxia tolerance was estimated in this study by determining P_{90}, P_{75}, P_{50}, and P_{25}, the percent oxygen saturation when metabolic rate was 90%, 75%, 50%, and 25% of resting metabolic rate. This showed that there were no observed significant differences in P_{90}, P_{75}, P_{50}, and P_{25} between the *H. sorenseni* and *H. rufescens* at the constant temperature of 15°C (Fig. 2.5, Table 2.1). Juvenile greenlip abalone, *H. laevigata*, demonstrated a P_{50} at 68% oxygen saturation (Harris et al., 1999), a much higher and therefore less hypoxia tolerant value than

H. sorenseni (25.3%) and *H. rufescens* (26.8%). *H. laevigata* inhabits shallower waters comparatively to *H. sorenseni* and *H. rufescens*, and thus is less likely to encounter upwelling and hypoxia.

Although *H. sorenseni* and *H. rufescens* demonstrate more hypoxia tolerance than *H. laevigata,* a study that exposed juvenile *H. rufescens* to low oxygen and low pH conditions in the laboratory (simulating upwelling conditions) still found lower growth rates and survival in response to the stressors that occur in their natural habitat (Kim et al., 2013). Another organism that lives in a similar kelp forest habitat with variable dissolved oxygen levels as *H. sorenseni* is the purple sea urchin, *Strongylocentrotus purpuratus.* When exposed to a variety of hypoxia environments simulating upwelling patterns, a reduction of their grazing rates, slower gonad development, and less spine regrowth was observed in response to extended low oxygen levels (Low & Micheli, 2020). When considering the impacts of hypoxia on *H. sorenseni,* besides just the observed lower resting metabolic rates as demonstrated by the P_{90}, P_{75}, P_{50}, and P_{25} values, there could be many other sublethal effects similar to those demonstrated by other invertebrates exposed to hypoxia.

Although *H. sorenseni* and *H. rufescens* were not statistically different in P_{90}, P_{75}, P_{50}, and P_{25}, as visually represented in Figure 2.5, *H. sorenseni* had a larger range in P_{90}, P_{75}, P_{50}, and P_{25} values compared to *H. rufescens.* Such variation in hypoxia tolerance among individual *H. sorenseni* could be due to cohort differences, as expressed by three out of the four highest P_{50} values all coming from abalone in the same cohort (*H. sorenseni* used in this experiment were

spawned in 2014, 2016, and 2019; Fig. S2.1). This finding highlights the potential for fitness differences (i.e. less hypoxia tolerant, slower growth rates, etc.) in some captive-bred white abalone individuals or cohorts. In aquaculture optimization experiments, collection of red abalone from different areas along the California coast exposed to variable amounts of upwelling has shown inherent differences in ocean acidification (OA) tolerance, highlighting natural variability and population level differences to regional environmental stressors (Swezey et al., 2020). For the critically endangered *H. sorenseni* with limited broodstock, the genetic diversity of captive spawned animals is a serious challenge that requires consideration of the phenotypic expression of tolerance to hypoxia, temperature, and other environmental factors to enhance potential outplanting success and ultimate recovery of the species in the wild. Only limited genetic diversity work has been conducted to date (Gruenthal & Burton, 2005; Masonbrink et al., 2019; Purcell et al. pers. comm.) and expanded analysis to quantify the genetic diversity of captive broodstock and offspring and its potential contribution to phenotypic variability in tolerance to environmental stressors is needed.

An increase in temperature (11°C to 15°C) correlated with an increase in the metabolism of *H. sorenseni* with a calculated Q_{10} of 2.90, as well as a decrease in its hypoxia tolerance demonstrated by significant increases of P_{90}, P_{75}, P_{50}, and P_{25} for the species (Fig. 2.7). This suggests that low oxygen and high temperature acting together may add cumulative stress on the animal. Green abalone (*H. fulgens*) show a similar pattern where a stress response was observed when abalone were subjected to both hypoxia and high temperature together (Tripp-

Valdez et al., 2017). Given that *H. sorenseni* live at deeper depths than other abalone species, climate changes may cause simultaneous shoaling of the oxygen minimum zone and an increase in temperature (Bograd et al., 2008; Frieder et al., 2012). In contrast, *H. rufescens,* did not show a significant effect of temperature on these metabolic measures.

P_{50} showed no significant relationship with abalone length for either *H. sorenseni* or *H. rufescens* (Fig. 2.6). This indicates that a larger body size does not positively correlate with a higher tolerance to low oxygen saturation for these species. Although the present study did not show a relationship with body size on hypoxia tolerance, other studies show that smaller sized abalone are less sensitive to environmental stress, such as hypoxia (Aalto et al., 2020; Vosloo & Vosloo, 2013). For the South African Abalone (*H. midae*), larger, adult abalone (mean shell length 65 mm) had less anti-oxidant enzymes than smaller, juveniles (mean shell length 41 mm), and these enzymes are associated with minimizing DNA and protein damage caused by hypoxia and other stressors (Vosloo & Vosloo, 2013). It is thought that juvenile abalone are naturally exposed to variable oxygen levels from the diatom biofilm they feed on, perhaps leading them to have more anti-oxidant enzymes to adapt to such changes (Vosloo et al., 2013). In fish, there are varying relationships between body size and hypoxia tolerance; however, following a similar pattern to the abalone, the red drum, *Sciaenops ocellatus*, demonstrated a decrease in hypoxia tolerance with increasing body size (Pan et al., 2016). While there does not appear to be a size effect on sensitivity to hypoxia in *H. sorenseni* and *H. rufescens*, modeling for *H. sorenseni* outplanting

as well as population monitoring for *H. rufescens* has revealed better stocking success occurs using larger abalone due to their contribution to reproduction and higher survival rates (potentially due to less vulnerability to predation) in comparison to juveniles (Hofmeister et al., 2018; Leaf et al., 2007; Li & Rogers-Bennett, 2017). Additionally, metabolic measurements have been determined for larval *H. sorenseni,* but more information about larval hypoxia tolerance would be useful to understand larval survival in the wild (Moran & Manahan, 2003). Factors such as modeling data, predation, and hypoxia tolerance should all be considered when deciding how to best rear captive bred *H. sorenseni* for outplanting.

The present study examined the acute metabolic response to hypoxia for both *H. sorenseni* and *H. rufescens*; however, little is known about the effects of log-term exposure to hypoxia on *H. sorenseni* metabolism, growth, and hypoxia tolerance. In the South African abalone, the long-term exposure to a mild decrease in environmental oxygen (82.6 – 84.5% oxygen) did not cause a reduction in adult or juvenile abalone oxygen consumption rate in comparison to normoxia (92.4 – 97.8 % oxygen), and this lack of a decrease in rate is likely due to the moderate level of hypoxia employed (Vosloo & Vosloo, 2013). In contrast, for greenlip abalone, both reduction in oxygen consumption rates and growth rates was shown for greenlip abalone kept at lower dissolved oxygen levels (55 – 73% oxygen) (Harris et al., 1999). Long-term exposure and growout experiments on *H. sorenseni*, would allow for understanding of chronic effects of low dissolved oxygen (like juveniles may encounter when outplanted) and contribute optimizing

best rearing practices for producing strong cohorts of captive bred white abalone for outplanting.

Conclusion

This study contributes to valuable knowledge about *H. sorenseni* and *H. rufescens* by determining useful metabolic and hypoxia tolerance data for the species. *H. sorenseni* showed lower resting metabolic rates than *H. rufescens* through most of the size range tested which most likely indicates evolution of a lower metabolism in response to a deeper kelp forest environment that is vulnerable to variable dissolved oxygen levels. This study also revealed that measures of P_{90}, P_{75}, P_{50}, and P_{25} did not differ significantly between *H. sorenseni* and *H. rufescens,* and measure of P_{50} did not have a relationship with abalone length. However, an increase in temperature led to significant increases in P_{90}, P_{75}, P_{50}, and P_{25} for *H. sorenseni*, showing potential compounding effects of low oxygen and high temperature. One notable difference between P_{90}, P_{75}, P_{50}, and P_{25} of *H. sorenseni* and *H. rufescens* was the higher variation in values for *H. sorenseni* that may be due to captive bred cohort differences. Increased understanding of genetic fitness of captive bred white abalone may contribute to further recovery success.

Figure 2.1: Schematic of the abalone respirometry system composed of a cylindrical holding chamber and a recirculating loop for monitoring dissolved oxygen concentration and temperature. Three-way stopcocks within the loop were used to manually open and close the system to the surrounding buffer tank seawater kept at a constant targeted temperature of 11.0 or 15.0°C.

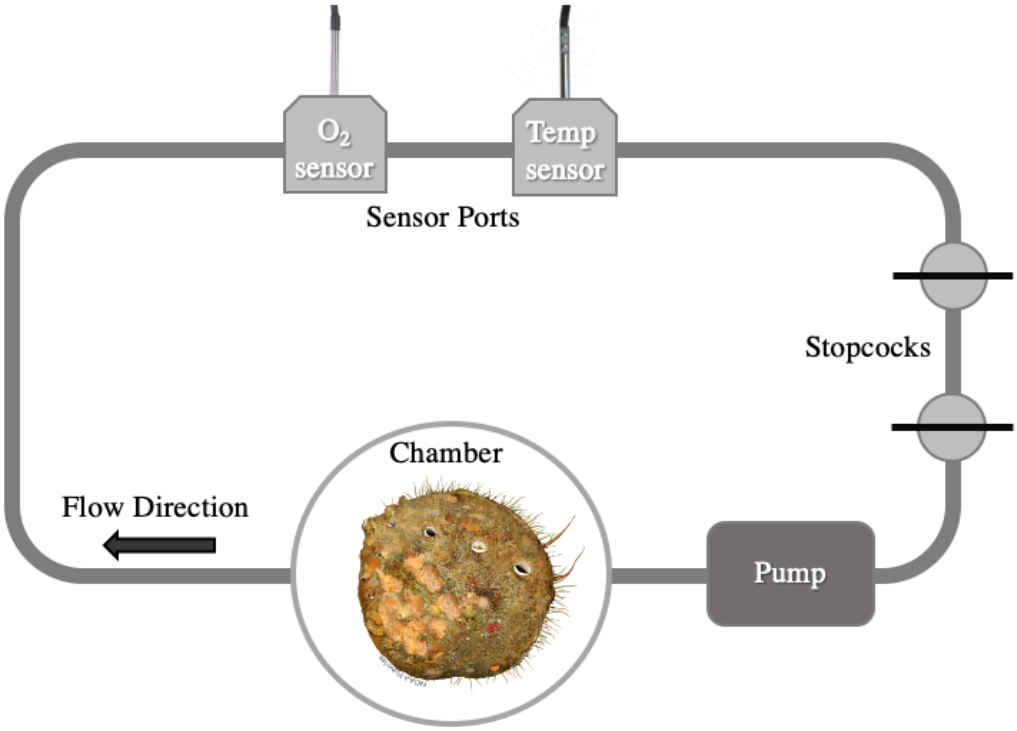

O_2 sensor
Temp sensor
Sensor Ports
Stopcocks
Chamber
Flow Direction
Pump

Figure 2.2: Relationship between oxygen consumption rate (mg O_2 $h^{-1}g^{-1}$) versus environmental dissolved oxygen level (% saturation) showing (A) a logarithmic function for an 81.1 g white abalone (*H. sorenseni*), (B) a power function for a 47.8 g red abalone (*H. rufescens*), and (C) a linear function for a 14.2 g white abalone. These curves were used to determine the P_{90}, P_{75}, P_{50}, and P_{25} (gray dotted lines) representing the environmental dissolved oxygen level at which the oxygen consumption of each individual is 90%, 75%, 50%, 25% of estimated resting metabolic rate (RMR, as estimated by P_{100}).

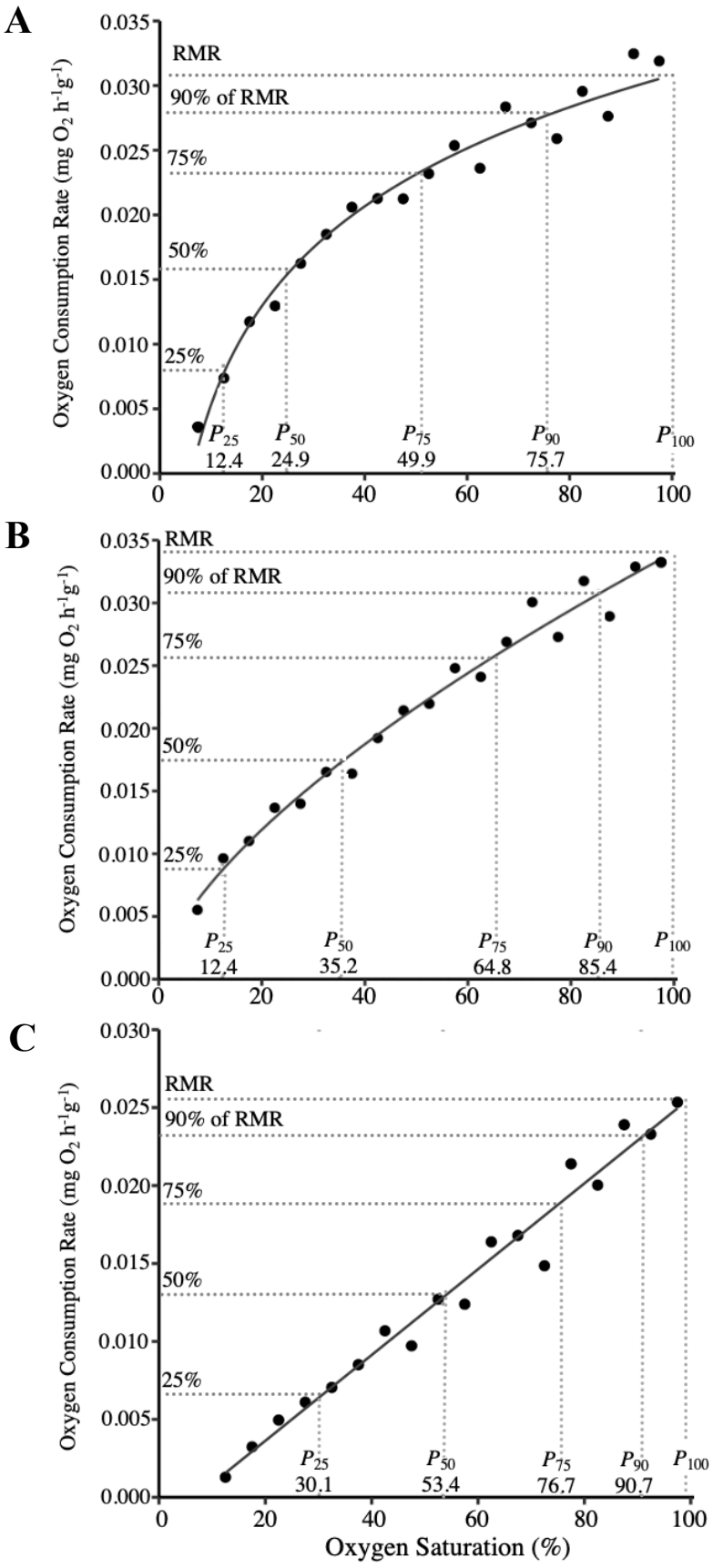

A
RMR
90% of RMR
75%
50%
25%
P_{25} 12.4
P_{50} 24.9
P_{75} 49.9
P_{90} 75.7
P_{100}
B
RMR
90% of RMR
75%
50%
25%
P_{25} 12.4
P_{50} 35.2
P_{75} 64.8
P_{90} 85.4
P_{100}
C
RMR
90% of RMR
75%
50%
25%
P_{25} 30.1
P_{50} 53.4
P_{75} 76.7
P_{90} 90.7
P_{100}
Oxygen Consumption Rate (mg O_2 $h^{-1}g^{-1}$)
Oxygen Saturation (%)

Figure 2.3: The relationship of resting metabolic rate (mg O_2 h^{-1}) versus total body mass (g) for white (*H. sorenseni,* n = 29) and red abalone (*H. rufescens*, n = 27). Lines depict the best-fit allometric scaling equation for each species.

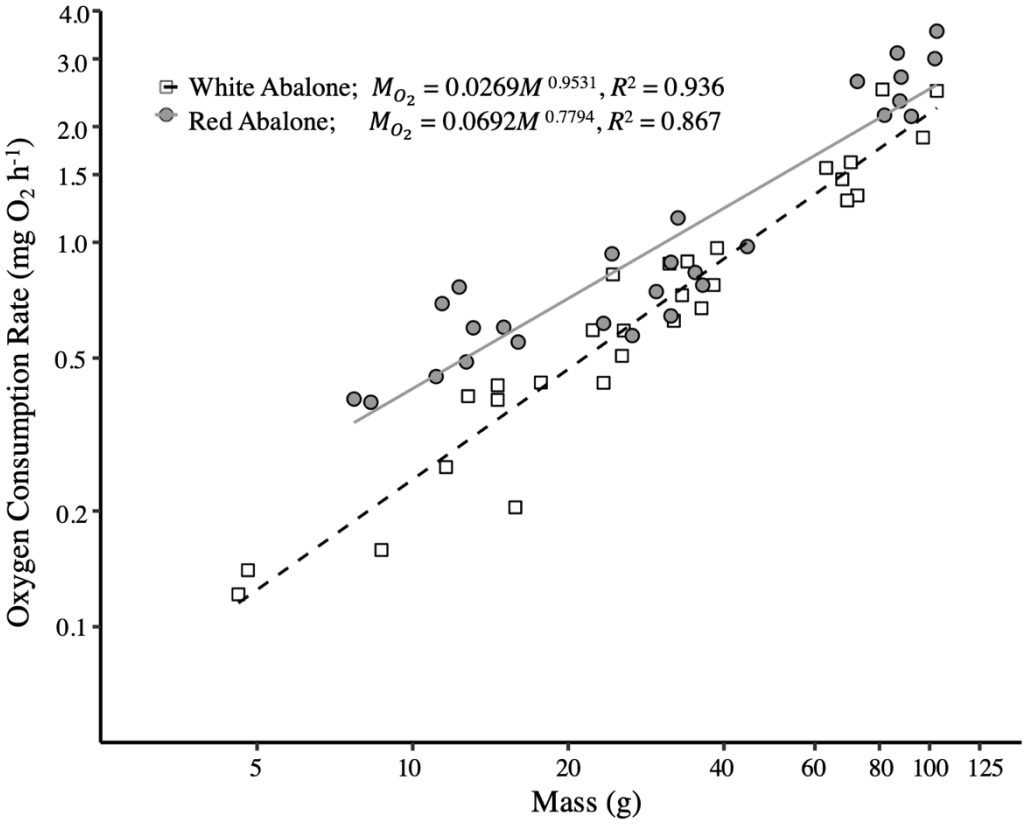

White Abalone; $M_{O_2} = 0.0269M^{0.9531}$, $R^2 = 0.936$
Red Abalone; $M_{O_2} = 0.0692M^{0.7794}$, $R^2 = 0.867$
Oxygen Consumption Rate (mg O_2 h^{-1})
4.0
3.0
2.0
1.5
1.0
0.5
0.2
0.1
5
10
20
40
60
80
100
125
Mass (g)

Figure 2.4: Oxygen consumption rate in relation to environmental dissolved oxygen (% saturation) for *H. sorenseni* (n = 29) and *H. rufescens* (n = 27). (A) Comparison of *H. sorenseni* and *H. rufescens* mean oxygen consumption rates (mg O_2 h^{-1}) standardized to a common total body mass of 40 g (using a scaling exponent of 0.9531 for *H. sorenseni* and 0.7794 for *H.* rufescens as shown in Figure 2.3). (B, C) Mean standardized oxygen consumption rate (as a percent of total resting metabolic rate, stnd M_{O_2}) for *H. sorenseni* and *H. rufescens*, respectively. Lines show best-fit logarithmic functions and error bars show standard error of the mean.

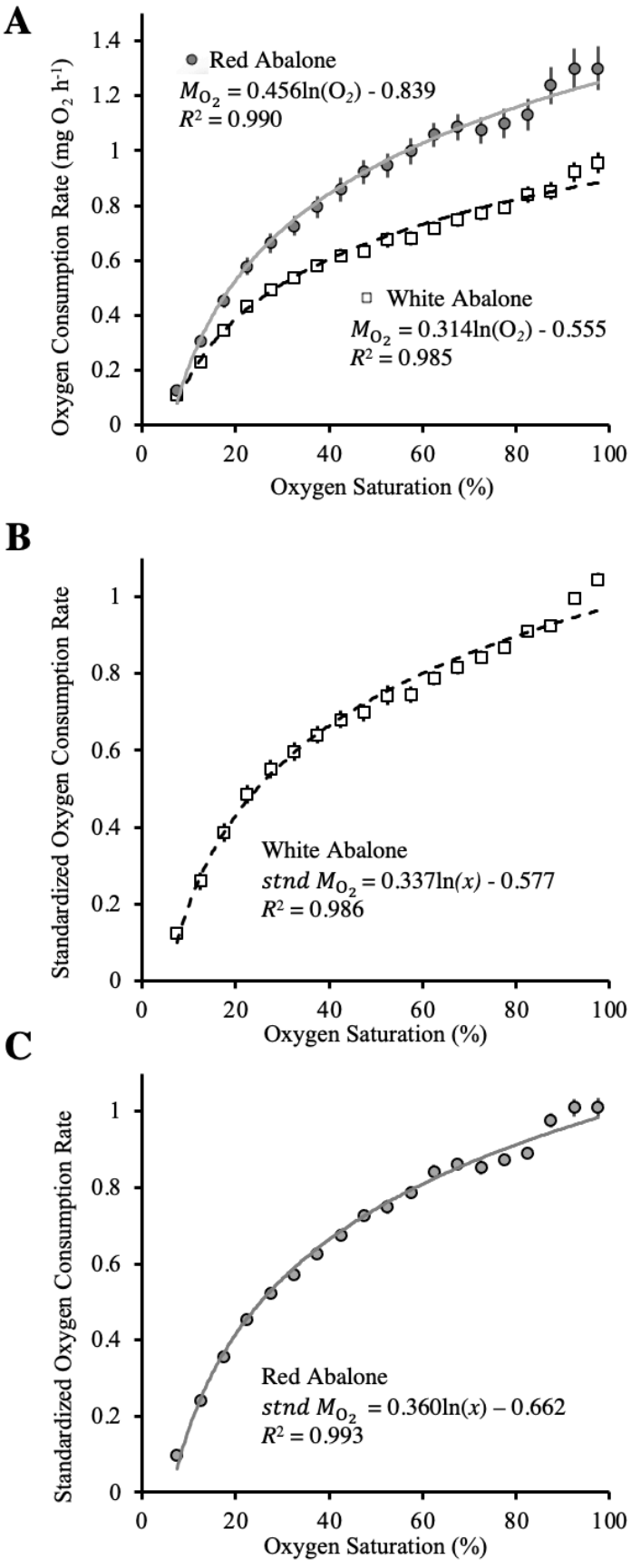

A
Red Abalone
M_{O_2} = 0.456ln(O_2) - 0.839
R^2 = 0.990
White Abalone
M_{O_2} = 0.314ln(O_2) - 0.555
R^2 = 0.985
Oxygen Consumption Rate (mg O_2 h^{-1})
Oxygen Saturation (%)
B
White Abalone
$stnd\ M_{O_2}$ = 0.337ln(x) - 0.577
R^2 = 0.986
Standardized Oxygen Consumption Rate
Oxygen Saturation (%)
C
Red Abalone
$stnd\ M_{O_2}$ = 0.360ln(x) – 0.662
R^2 = 0.993
Standardized Oxygen Consumption Rate
Oxygen Saturation (%)

Figure 2.5: Boxplots showing the hypoxia tolerance measure of P_{90}, P_{75}, P_{50}, and P_{25} measurements (percent oxygen saturation) for white abalone (*H. sorenseni*, n = 29) and red abalone (*H. rufescens*, n = 27).

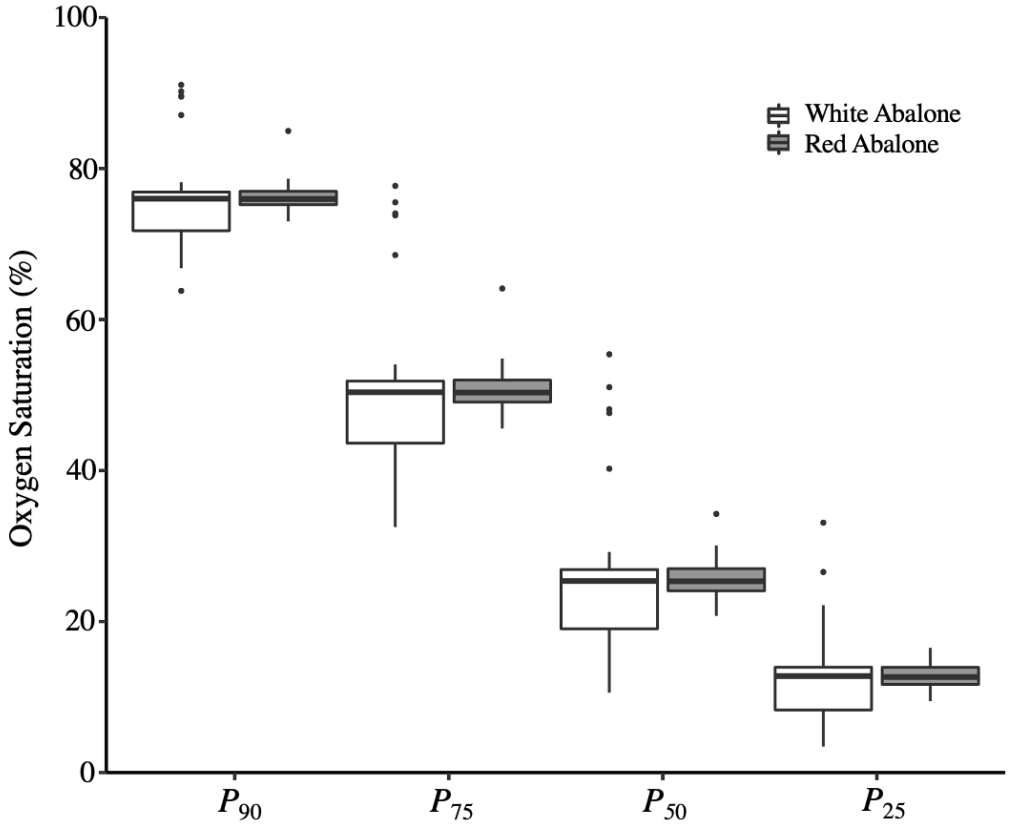

100
80
60
40
20
0
Oxygen Saturation (%)
White Abalone
Red Abalone
P_{90}
P_{75}
P_{50}
P_{25}

Figure 2.6: Relationship between length (mm) and P_{50} (percent oxygen saturation) for white (*H. sorenseni,* n = 29) and red abalone (*H. rufescens,* n = 27).

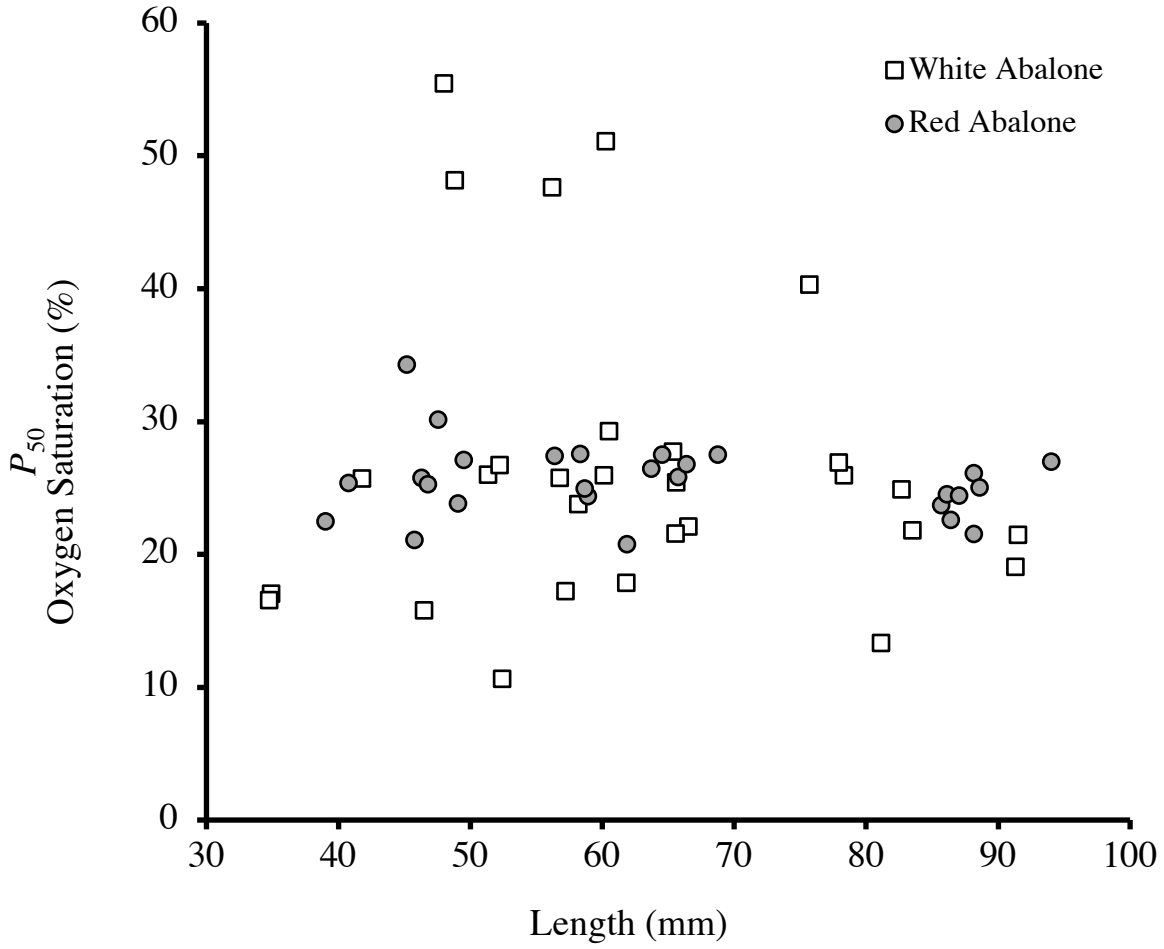

60
50
40
30
20
10
0
30
40
50
60
70
80
90
100
P_{50}
Oxygen Saturation (%)
Length (mm)
White Abalone
Red Abalone

Figure 2.7: Boxplots showing hypoxia tolerance measurements of P_{90}, P_{75}, P_{50}, and P_{25} (percent oxygen saturation) at temperatures of 11°C and 15°C for white abalone (*H. sorenseni*).

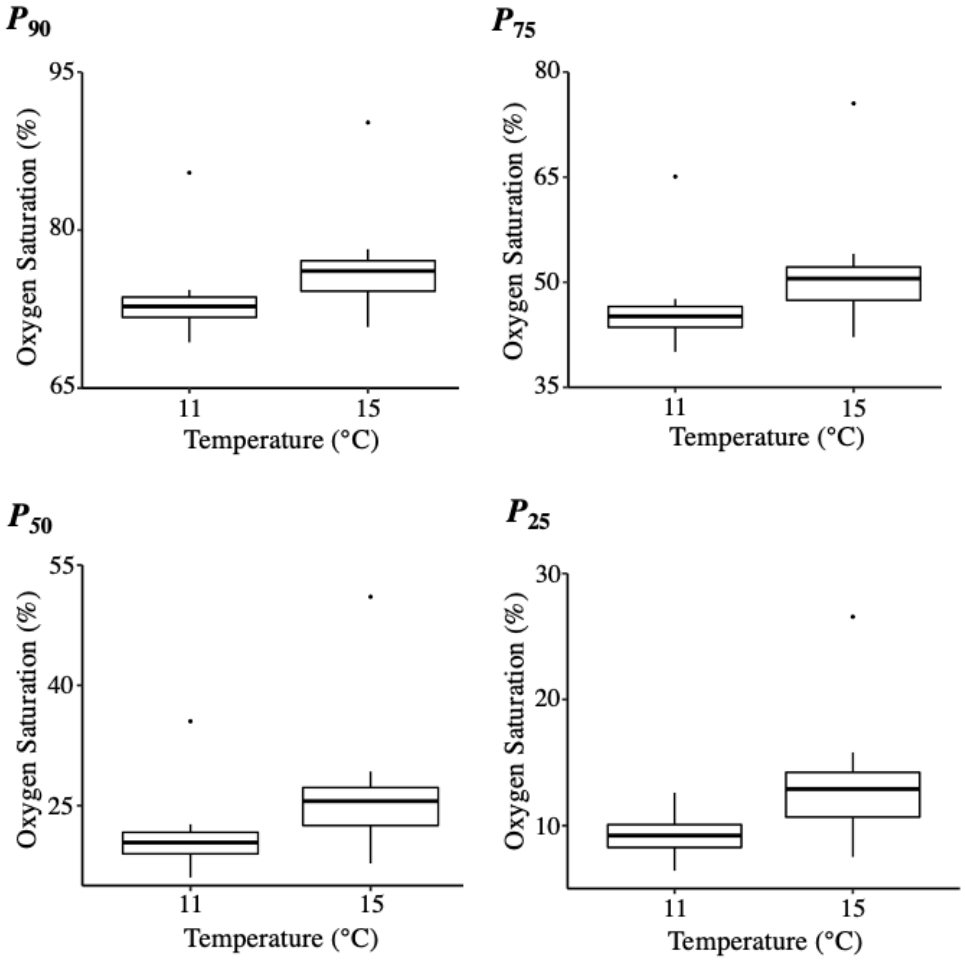

P_{90}
95
80
65
Oxygen Saturation (%)
11
15
Temperature (°C)
P_{75}
80
65
50
35
Oxygen Saturation (%)
11
15
Temperature (°C)
P_{50}
55
40
25
Oxygen Saturation (%)
11
15
Temperature (°C)
P_{25}
30
20
10
Oxygen Saturation (%)
11
15
Temperature (°C)

Table 2.1: Dissolved environmental oxygen level (% saturation) at which white (*H. sorenseni*) and red (*H. rufescens*) abalone oxygen consumption rate is reduced to 90, 75, 50, 25% (P_{90}, P_{75}, P_{50}, and $P_{25} \pm$ standard deviation) of resting metabolic rate (Temperature 15°C).

Species	**n**	**Mass (g)**	**Length (mm)**	P_{90}	P_{75}	P_{50}	P_{25}
White Abalone (*H. sorenseni*)	29	4.6 – 103.3	34.8 – 91.6	75.6 ± 6.5	50.1 ± 10.8	25.3 ± 10.3	11.9 ± 5.8
Red Abalone (*H. rufescens*)	27	7.7 – 103.3	39.1 – 94.0	77.4 ± 4.2	52.6 ± 6.9	26.9 ± 5.7	12.5 ± 2.5

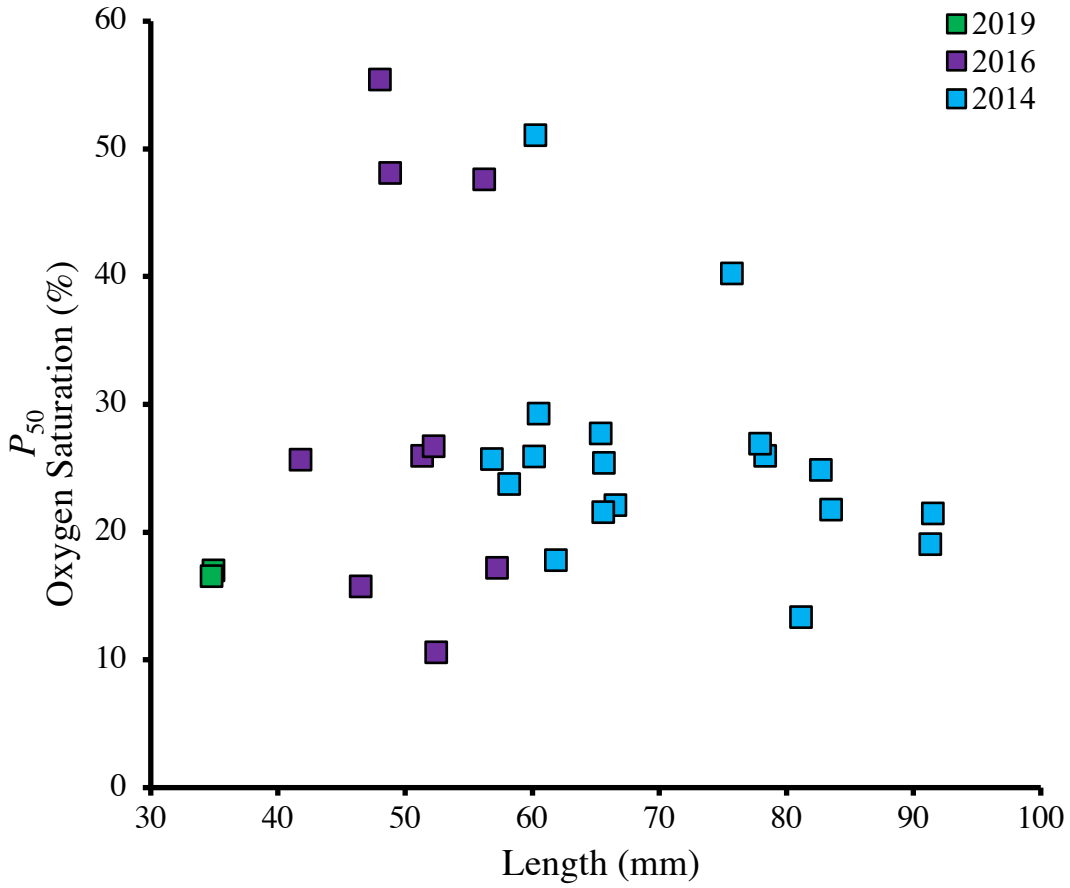
Table 2.2: Dissolved environmental oxygen level (% saturation) at which white (*H. sorenseni*) and red (*H. rufescens*) abalone oxygen consumption rate is reduced to 90, 75, 50, 25% (P_{90}, P_{75}, P_{50}, and P_{25} ± standard deviation) of resting metabolic rate. Data are from individuals in the middle size range of each species (white abalone, 24.4 - 50.5 g; red abalone, 24.3 – 70.0 g; n = 10 for both species) used in respirometry trials at both 11°C and 15°C. Due to the time between the trials, some growth occurred such that the individuals tested at 11°C were slightly larger than when tested at 15°C.

Species	Temperature (°C)	Mass (g)	Length (mm)	P_{90}	P_{75}	P_{50}	P_{25}
White Abalone	11	38.0 ± 7.1	66.0 ± 3.7	73.7 ± 4.4	46.7 ± 6.8	21.5 ± 5.3	9.3 ± 1.7
(*H. sorenseni*)	15	31.9 ± 5.3	62.1 ± 3.5	76.8 ± 5.2	51.8 ± 9.0	27.0 ± 9.1	13.5 ± 5.2
Red Abalone	11	48.4 ± 11.2	70.7 ± 4.8	80.8 ± 6.3	58.0 ± 10.4	30.5 ± 9.2	12.3 ±4.2
(*H. rufescens*)	15	31.6 ± 6.2	62.3 ± 4.1	76.3 ± 1.3	50.8 ± 2.2	25.9 ± 2.1	13.2 ± 1.6

Table 2.3: Resting metabolic rates (mg O_2 h^{-1}) of white abalone (*H. sorenseni*) and red abalone (*H. rufescens*) in comparison to other abalone species. For direct comparison with data from this study, metabolism for each species was scaled to a common mass of 40 g using species-specific scaling exponents (*H. sorenseni, b* = 0.9531*; H. rufescens, b* = 0.7794*; H. kamtshatkana, b* = 0.62) or a mass-scaling exponent of 0.8 if not previously determined, and also adjusted to a temperature of 15°C with a Q_{10} of 2.

Species	RMR ($mg\ O_2\ h^{-1}$)	Study
White abalone *H. sorenseni*	0.891	Present study
Red abalone *H. rufescens*	1.261	Present study
Pinto abalone *H. kamtshatkana*	1.661	Carefoot et al., 1993
Greenlip abalone *H. laevigata*	3.620	Harris et al., 1999
Ass's ear abalone *H. asinina*	1.460	Baldwin et al., 2007
South African abalone *H. midae linnaeus*	2.376	Vosloo et al., 2013

Figure S2.1: Relationship between length (mm) and P_{50} (percent oxygen saturation) for white (*H. sorenseni,* n = 29) separated by spawning year (2014, 2016, 2019).


2019
2016
2014
P_{50} Oxygen Saturation (%)
Length (mm)
0
10
20
30
40
50
60
30
40
50
60
70
80
90
100

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