Towards defining the tipping point of tolerance to CO_2-induced ocean acidification for the growth, development and metabolism of larval dusky kob *Argyrosomus japonicus* (Pisces: Sciaenidae)

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ABSTRACT

Increased CO_2 production and the consequent ocean acidification (OA) have been identified as one of the greatest threats to both calcifying and non-calcifying marine organisms. Traditionally, marine fishes, as non-calcifying organisms, were considered to have a higher tolerance to near-future OA conditions owing to their well-developed ion regulatory mechanisms. However, recent studies provide evidence to suggest that they may not be as resilient to near-future OA conditions as previously thought. In addition, earlier life stages of marine fishes are thought to be less tolerant than juveniles and adults of the same species as they lack well-developed ion regulatory mechanisms for maintaining homeostasis.

This study follows up on previous studies examining the effects of near-future OA on larval *Argyrosomus japonicus*, an estuarine-dependent marine fish species, in order to identify the tipping point of tolerance for the larvae of this species. These previous studies showed that elevated pCO_2, predicted for the year 2100, had negative effects on growth, development and metabolism and ultimately, survival of larval *A. japonicus* from post-flexion stage. Larval *A. japonicus* in the present study were reared from egg up to 22 DAH (days after hatching) under three treatments. The three treatments, (pCO_2 353 µatm; pH 8.03), (pCO_2 451 µatm; pH 7.93) and (pCO_2 602 µatm; pH 7.83) corresponded to levels predicted to occur in year 2050, 2068 and 2090 respectively under the Intergovernmental Panel on Climate Change (IPCC) Representative Concentration Pathways (IPCC RCP) 8.5 model. Size-at-hatch, growth, development and metabolic responses (standard and active metabolic rates and metabolic scope) were assessed and compared between the three treatments throughout the rearing period.

Five earlier larval life stages (hatchling – flexion/post-flexion) were identified by the end of the experiment. There were no significant differences in size-at-hatch ($P > 0.05$),

development or the active metabolic ($P > 0.05$) or metabolic scope ($P > 0.05$) of fish in the three treatments throughout the study. However, the standard metabolic rate was significantly higher in the year 2068 treatment but only at the flexion/post-flexion stage which could be attributed to differences in developmental rates (including the development of the gills) between the 2068 and the other two treatments. Overall, the metabolic scope was narrowest in the 2090 treatment, but varied according to life stage. Although not significantly different, metabolic scope in the 2090 treatment was noticeably lower at the flexion stage compared to the other two treatments, and the development appeared slower, suggesting that this could be the stage most prone to OA. The study concluded that, in isolation, OA levels predicted to occur between 2050 and 2090 will not negatively affect size-at-hatch, growth, development, and metabolic responses of larval *A. japonicus* up to 22 DAH (flexion/post-flexion stage). Taken together with the previous studies of the same species, the tipping point of tolerance (where negative impacts will begin) in larvae of the species appears to be between the years 2090 and 2100.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

DEDICATION

This thesis is dedicated to my parents and to my little Nthato.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Global Climate Change and Oceans

The Earth's climate has been changing continuously throughout its history at a rate that allowed organisms to evolve with their surrounding environment (Roessig *et al.* 2005). The current rate of change, however, is occurring faster than has been documented in the past and this is attributed largely to the increased emission of greenhouse gases (GHG), such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) into the atmosphere (Raven *et al.* 2005). The general consensus among the scientific community is that the increase in GHG is largely due to anthropogenic activities since the industrial revolution of the 19th century (Roessig *et al.* 2005). The ever-growing human population, coupled with intense use of resources, burning of fossil fuels, cement manufacture and deforestation are some of the processes contributing to increased GHG in the atmosphere (e.g. Rogner *et al.* 2007; Riebesell *et al.* 2010). Since the beginning of the industrial revolution, the atmospheric CO_2 has increased from 280 to 398 parts per million (ppm), an increase of more than 100 ppm in one century (IPCC 2013), with half of this increase occurring in the last 30 years (Feely *et al.* 2009). It is projected that this rate of increase will cause the atmospheric level of CO_2 to reach over 1000 ppm by the end of this century (Raven *et al.* 2005). Although CO_2 is vital for photosynthesis and therefore essential for life on Earth (Beerling 2012; Sunday *et al.* 2014), a substantial increase in atmospheric CO_2 concentrations causes substantial changes in the water chemistry of the oceans, which could affect marine life (Fabry *et al.* 2008; Doney *et al.* 2009; Kroeker *et al.* 2010).

The oceans play a pivotal role in the exchange of CO_2 with the atmosphere by acting as a CO_2 sink. They absorb approximately 30% of the atmospheric CO_2 and this process increases the CO_2 of the ocean and alters seawater chemistry (Sabine *et al.* 2004; Doney *et al.* 2009). Approximately half of the CO_2 produced by burning fossil fuels and cement production alone is predicted to have been absorbed by the oceans in the past two centuries (Raven *et al.* 2005). When CO_2 dissolves in seawater it forms carbonic acid, which dissociates into hydrogen ions (H^+), bicarbonate (HCO_3^-) and carbonate ions (CO_3^{2-}) (Figure 1.1), which cause a drop in the pH of the ocean (Doney *et al.* 2009). The average pH in the ocean has

been estimated to have dropped by 0.1 units from the beginning of the 20th century (Meehl *et al.* 2007; Feely *et al.* 2009) and, if the current CO_2 emissions continue unabated, a further average drop of 0.5 units is expected by the year 2100 (Raven *et al.* 2005). These are significant decreases in the context of pH since it is measured on a log scale (Doney *et al.* 2009). This decline in ocean pH due to increased CO_2 on the sea surface is called ocean acidification (OA).

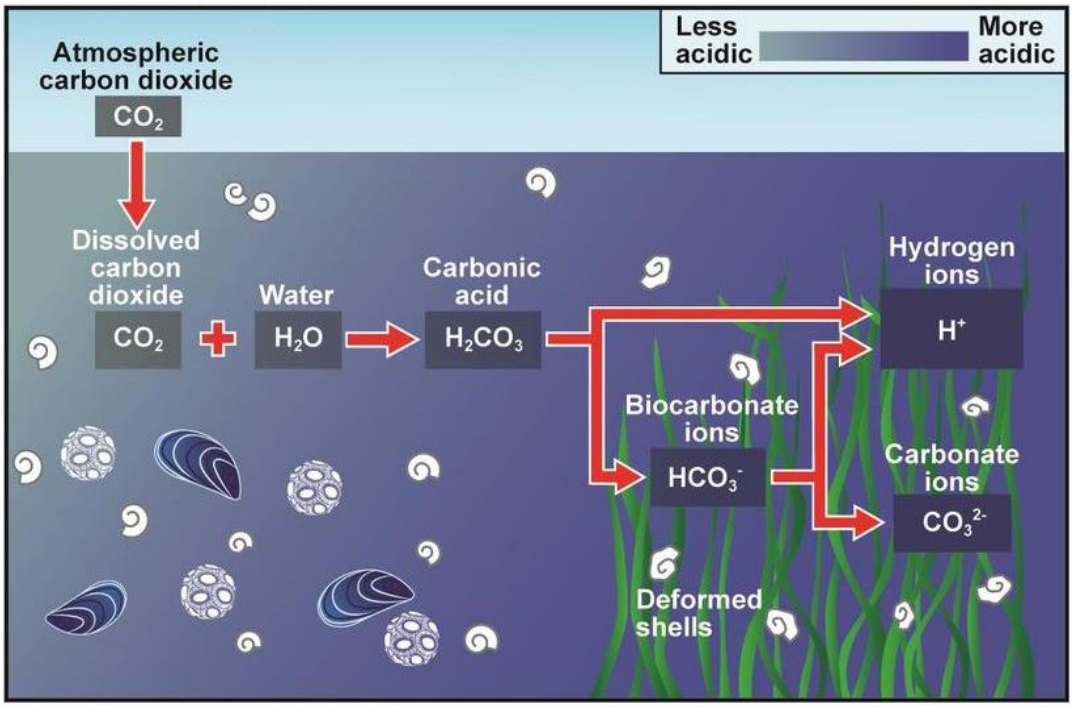


Figure 1. 1: Formation of CO_2-induced ocean acidification. Source: University of Maryland.

1.2 Ocean Acidification and Marine Life

Ocean acidification is predicted to occur across marine environments, which could lead to widespread effects on marine life (Doney *et al.* 2009; Kroeker *et al.* 2013). The effects are predicted to be more severe in coastal waters than in the open ocean because of higher pH variability that could result, in the former, from eutrophication, increased freshwater runoff, and coastal upwelling (Strong *et al.* 2014). The extent of these effects is, however, not fully understood and species' responses to OA appear to vary between marine taxonomic groups (Findlay *et al.* 2009; Kroeker *et al.* 2010). Negative effects of reduced seawater pH have been observed in phytoplankton (Rost *et al.* 2008), polychaetes (Davidson 2013), echinoderms

(Shirimaya and Thorton 2005; Dupont *et al.* 2008; Graham 2014), molluscs (Shirimaya and Thorton 2005; Gazeau *et al.* 2007) cnidarians (Doney *et al.* 2009), crustaceans (Spicer *et al.* 2007; Walther *et al.* 2010) and fishes (Munday *et al.* 2009a; Baumann *et al.* 2012; Chambers *et al.* 2014). Although these studies reveal detrimental effects of hypercapnia, some positive effects have also been observed in algal turfs and kelp (Connell and Russell 2010), seagrass (Connell *et al.* 2017), echinoderms (Wood *et al.* 2008) and fish (Munday *et al.* 2009b; Miller *et al.* 2013). Overall ocean acidification is predicted to impact both vertebrates and invertebrates, calcifying and non-calcifying marine organisms.

The responses of marine invertebrates, especially calcifying organisms, have received most attention. Calcifying organisms (those that form calcium carbonate structures) including foraminifera, molluscs, echinoderms, crustaceans and corals (Findlay *et al.* 2009) are mostly affected through changes in the rate of calcification. Generally, calcification is expected to decrease in response to OA due to changes in seawater chemistry, specifically the decrease in availability of carbonate ions in seawater (Doney *et al.* 2009; Feely *et al.* 2009). Although the response to reduced availability of carbonate ions is thought to be species specific, the general trend is that the rate of calcification is negatively affected (Hofmann *et al.* 2010). In studies where increases in the rate of calcification were observed, this appears to come at costs in terms of health and metabolic rate (Wood *et al.* 2008; Findlay *et al.* 2009). Ocean acidification also has significant negative impacts on survival, growth and abundance in calcifiers (Kroeker *et al.* 2013).

Aside from impacting on calcifiers, increased pCO_2/ reduced pH in seawater has the potential to affect the physiological performance of non-calcifying marine organisms (Pörtner *et al.* 2004; Pörtner 2008), such as marine algae (Connell and Russell 2010), seagrass (Connell *et al.* 2017), annelids (Davidson 2013; Graham 2014), and fish (Heuer and Grosell 2014). For example, it can result in an increase in the CO_2 concentration in the blood and tissue (hypercapnia) of some of these organisms, through diffusion, causing acidosis (Pörtner *et al.* 2004) which, in turn, can impact the physiological function of an organism and affect its growth, development, behaviour, survival, metabolic rate, and abundance (Barry *et al.* 2010; Pörtner *et al.* 2010). Despite this, most studies on the effects of OA on marine life have focused more on calcifying than non-calcifying organisms, possibly due to the notion that they are the most sensitive and, therefore, vulnerable to OA (Findlay *et al.* 2009).

In contrast to sessile, calcifying organisms, non-calcifying, mobile organisms, such as fishes, are believed to have a higher tolerance of future predicted levels of OA (Kroeker *et al.* 2010; 2013). The perceived higher tolerance of OA by fishes is believed to stem from their high metabolic rates and their well-developed acid-base regulatory mechanisms, which enable them to maintain their internal pH under hypercapnic conditions (Pörtner *et al.* 2005; Fabry *et al.* 2008; Brauner and Baker 2009; Heuer and Grossell 2014). During hypercapnic conditions, CO_2 diffuses from the fish's external environment through gill epithelia into blood and tissue, resulting in an increase in blood pCO_2. If this change is not regulated, it can cause acidosis in fishes (Heuer and Grossell 2014; Pörtner *et al.* 2004). The energetic cost associated with this acid-base regulation is, however, likely to result in a decline in energy available for other physiological processes, thereby impacting on the fishes' aerobic performance (Michaelidis *et al.* 2007; Pörtner and Farrell 2008).

Indeed, recent research has suggested that marine fishes may not be as resilient to OA as previously thought (Munday *et al.* 2010; Baumann *et al.* 2012; Forsgren *et al.* 2013). Ocean acidification has been shown to affect growth and development (Bignami 2013; Erasmus 2017), disrupt olfactory and auditory responses (Munday *et al.* 2009c), affect metabolism (Edworthy 2017) and could have an impact on energy partitioning in marine fishes (Pörtner *et al.* 2004). However, as with other taxonomic groups, there appears to be interspecies variation in the response to ocean acidification among marine fishes (Couturier *et al.* 2013; Heuer and Grossell 2014). For example, some species appear to be negatively affected (Munday *et al.* 2009a; Baumann *et al.* 2012; Pimentel *et al.* 2014; Erasmus 2017), while others appear to be positively (Munday *et al.* 2009b; Miller *et al.* 2013) affected by OA. Moderate or no effects of OA were detected in other species (Munday *et al.* 2011; Frommel *et al.* 2012).

The greatest variability seems to be in the responses between different life stages of the same species (Baumann *et al.* 2012; Frommel *et al.* 2013; Chambers *et al.* 2014). Unlike juveniles and adults, the early life stages of fishes lack well-developed ion regulatory mechanisms for maintaining homeostasis and may therefore be less tolerant to near-future levels of OA (Pörtner et al. 2005; Ishimatsu *et al.* 2008; Baumann *et al.* 2013; Llopiz *et al.* 2014). It is thought that OA could impact their survival, growth, fitness, extracellular acid-base balance and metabolism, with ultimate consequences for recruitment (Ishimatsu and Kita 1999; Hurst *et al.* 2013; Llopiz *et al.* 2014). Earlier life stages are expected to have higher rates of oxygen

consumption compared to older life stages of the same species due to their fast growth and because smaller organisms tend to have higher mass-specific O_2 consumption (Peck and Moyano 2006). In addition, metabolic features of organisms could vary with their developmental stages (Pörtner *et al.* 2010). It is therefore important to assess the effects of OA at the early stages of development. Understanding the likely impacts of global climate change stressors, such as OA, on early life history (developmental) stages of fish will enable effective strategic management of the conservation of the species in question (Llopiz *et al.* 2014).

1.3 Physiology

The effects of environmental conditions/stressors on organisms can be examined by assessing physiological responses to these stressors (Pörtner *et al.* 2014; Rodgers *et al.* 2016). One way of achieving this is through the measurement of an individual's aerobic (metabolic) scope (Pörtner and Knust 2007; Chabot *et al.* 2016a). Aerobic scope is defined as the difference between standard and maximum metabolic rate (SMR and MMR, respectively) of an individual measured as O_2 consumption and can be used as an indicator of the whole organism's performance (Fry 1971; Heuer and Grossell 2014). Standard metabolic rate is the minimum metabolic rate that allows for subsistence living in organisms and represents the minimum amount of energy required for supporting essential homeostatic activities only (Chabot *et al.* 2016b). It is therefore the measure of an organism's metabolic rate in the absence of activity, feeding, growth, and reproduction (Chabot *et al.* 2016b). Generally, the physiological functioning is weakened, and life cannot be sustained for long periods in most species when an organism's SMR surpasses its routine metabolic rate (Claireaux and Chabot 2016). In contrast, maximum/active metabolic rate (MMR/AMR) is the estimation of the maximum metabolic rate under which O_2 can be consumed and delivered to body tissues (Clark *et al.* 2013; Claireaux and Chabot 2016). It represents the capacity of the aerobic pathways to use energy under various environmental conditions (Fry 1971; Norin and Clark 2016).

Under periods of stress, an individual responds with a reduced metabolic scope (Clark *et al.* 2013). This reduction in metabolic scope may be a consequence of either i) an increase in SMR coupled with a decrease in or stable MMR, or ii) a decrease in MMR coupled with an increase in or stable SMR (Heuer and Grossell 2014). The general assumption is that OA will

reduce the aerobic scope of marine fishes by increasing SMR as a result of an increment of costs associated with regulating elevated pCO_2 (Heuer and Grossell 2014). On the other hand, increased pCO_2 may also cause acidosis in the blood, which will, in turn, reduce O_2 uptake and delivery, causing reductions in MMR and aerobic scope (Heuer and Grossell 2014). The narrower the aerobic scope, the more susceptible the organism will be to its environmental stressors (Clark *et al.* 2013).

Aside from physiological responses, life history characteristics can be important examiners of environmental stress in fishes. Size-at-hatch, growth and development have been used in other studies to assess the likely impacts of OA on marine fishes (Frommel *et al.* 2013; Hurst *et al.* 2013; Erasmus 2017). For example, whereas no significant effects of increased pCO_2 and reduced seawater pH were detected on the development of Baltic cod, *Gadus morhua* (Frommel *et al.* 2013), and the larval growth of walleye pollock, *Theragra chalcogramma* (Hurst *et al.* 2013), significant effects, slower growth and skeletal development were detected in larval *A. japonicus* reared in high pCO_2 (~ 910 µatm) compared to the control treatment (327.50 µatm) (Erasmus 2017).

1.4 *Argyrosomus japonicus* as an Indicator of Ocean Acidification Stress

To assess the likely impacts of near-future ocean acidification on marine finfish, a marine spawning, estuarine-dependent species, *Argyrosomus japonicus* was used as a model species. It belongs to the family Sciaenidae and is a long-lived species, over 40 years, with delayed maturity (Griffiths 1996; Childs and Fennessy 2013). It is a carnivorous species, feeding on a wide variety of organisms such as crustaceans, cephalopods and other fish species (Marais 1984; Griffiths 1996).

The species occurs in both the northern and southern hemispheres. In the southern hemisphere, it occurs along the south-east coast of southern Africa from the Cape of Good Hope all the way to southern Mozambique, and along the entire southern seaboard of Australia (Griffiths and Heemstra 1995; Griffiths 1996). Adults occur and spawn predominantly in the nearshore marine environment while juveniles, approximately 20–30 mm TL, recruit into estuaries and remain resident in estuaries up to a length of 150 mm TL (Griffiths 1996). Juveniles from 150 to 800 mm TL are found predominantly in estuaries; however, occasional “to and fro” migrations have been observed in juveniles

between estuaries and the inshore marine environment (Griffiths 1996; Childs *et al.* 2015). Throughout its distribution, owing to its palatability and large size, it is highly valued as a source of food and is a sought-after species in commercial and recreational fisheries (Griffiths 1996; Silberschneider and Gray 2008).

In southern Africa, juveniles and adults of this species are highly targeted by recreational estuarine and coastal shore anglers as well as commercial and subsistence fishers (Childs and Fennessy 2013). This species is vulnerable to over-exploitation, with recent data showing declines in its catches (Childs and Fennessy 2013, Mirimin *et al.* 2015). A recent acoustic telemetry study revealed that the species is most vulnerable to over-exploitation during its estuarine phase (Childs *et al.* 2015).

In addition to over-exploitation, climate change poses another great threat to the existence of this species. Since ocean acidification is predicted to be uniform across the oceans (Doney *et al.* 2009), it is therefore expected to occur along the *A. japonicus* distributional range and ultimately impact on the species. Indeed, a review of the likely impacts of climate change on coastal fishes in southern Africa suggests that *A. japonicus,* like most marine fish species in this region, is likely to be affected by ocean acidification, although the responses are not fully understood (Potts *et al.* 2015). Being an apex predator (Griffiths 1997), it is a valuable contributor in shaping food webs in habitats where it occurs and its removal or extinction could therefore have negative impacts on these habitats.

The continual existence of this species, or any other species for that matter, depends on the successful recruitment of early life history stages into juveniles and eventually, spawning adults. Previous studies on the likely impacts of near-future OA (levels predicted for 2050 and 2100) on this species revealed that the species could be affected by OA conditions predicted to occur by the end of the century, the year 2100. The elevated pCO_2 conditions predicted to occur around 2100 (pCO_2 910 µatm; pH 7.78) had negative impacts on the growth, development and metabolism and, ultimately, survival of larval *A. japonicus*. The impacts were only evident after 22 DAH, suggesting that the post-flexion stage is the most vulnerable to OA (Edworthy 2017; Erasmus 2017). In contrast, performance was optimised under levels predicted for the next 50 years (pCO_2 477 µatm; pH 8.01), the year 2050. The authors therefore suggest that these results could signify a "tipping point", (where the effects of OA become negative) in the tolerance of *A. japonicus* larvae to future OA, which needs to be determined for the management of this species (Edworthy 2017, Erasmus 2017).

1.5 Aims and Objectives

The overall aim of the present study was, therefore, to determine the "tipping point" in the tolerance of the early life history stages of *A. japonicus* to near-future ocean acidification by assessing life history traits (size-at-hatch, growth and development) and metabolic responses of larval *A. japonicus* subjected to pH values of 8.03, 7.93 and 7.83 which are predicted to occur in the years 2050, 2068 and 2090, respectively, based on the IPCC RCP 8.5 model. Experimental protocols were kept as similar as possible to those used by Edworthy (2017) and Erasmus (2017).

CHAPTER TWO

GENERAL METHODS

2.1 Husbandry

Argyrosomus japonicus eggs were obtained from a wild-caught brood stock (one male and two females) that were maintained in captivity for over eight years in an 18 000 L tank at Pure Ocean Aquaculture Farm in East London (Eastern Cape, South Africa). They were fed a mixture of sardine, hake and squid three times per week. Spawning was induced by hormone injection and eggs were collected from the egg collector within four hours of spawning. The latter was to ensure synchronised development between the embryos (Mu *et al.* 2015). At the time of egg collection; temperature, salinity and pH read 23 °C, 35 PSU (practical salinity units) and 8.15 respectively. Light regime was kept at 16h: 08h (light: dark).

Collected eggs were transferred into a conical holding tank in order to separate fertilised from unfertilised eggs. Fertilised eggs (hereafter referred to as embryos) floated in the holding tank while the unfertilised eggs sank to the bottom. Embryos from the holding tank were scooped and transferred to fill a 10 L bucket using a 1 L measuring jug. Twenty samples of 2 ml were taken from the bucket, using a pipette, to estimate total number of embryos inside it. Each 2 ml sample was pipetted onto a petri dish and the embryos were counted under a microscope (Leica EZ4 HD). The total number of embryos for all the samples were then averaged (divided by 20) to get the average number of embryos per 2 ml. The obtained average number was then multiplied by 5 000 to scale it up to 10 L. The embryos in the bucket were then gently aerated to ensure uniform distribution before being transferred to the nine experimental tanks (H: 1.3 m, D: 1.1 m, capacity: 1000 L), where each tank was stocked at a density of 60 individuals per litre.

Although *A. japonicus* larvae start feeding exogenously from three days after hatching (DAH), *Brachionus plicatilis* enriched with algal paste *Nannochloropsis spp.* (Nanno 3600, Reed Mariculture), were introduced into the experimental tanks and kept at a density of three individuals per ml from one to 14 DAH (Figure 2.1). A further 5 ml of *Nannochloropsis spp.* was added daily to each experimental tank. The early introduction of *B. plicatilis*, on 1 DAH was to ensure that they were adapted to the culture conditions inside the experimental tanks before the larvae started feeding exogenously. The *Nannochloropsis spp.* served as both the

food for *Brachionus plicatilis* and for maintaining the green-water culture medium in the tank. From 8 DAH, *Artemia spp.* (Ocean Nutrition) were introduced into the experimental tanks until 22 DAH. A 0.2 to 0.3 mm pelleted diet (Gemma Wean, Skretting) was introduced from 19 DAH until the end of the experiment (Figure 2.1).

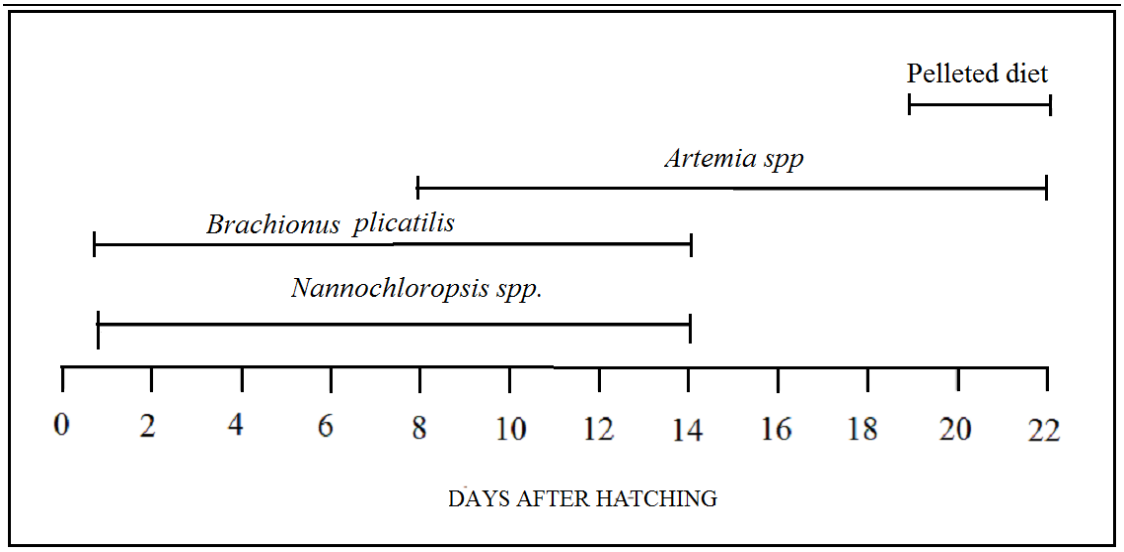


Figure 2. 1: Feeding regime of *Argyrosomus japonicus* larvae during the course of the experiment, from one to 22 days after hatching (DAH).

2.2 Experimental Set Up

2.2.1 System design

A randomised complete block design with three pH treatments (8.03, 7.93 and 7.83) was used in the study (Figure 2.2). The three pH gradients were determined from the predicted pH values for the years 2050 and 2100 using an RCP 8.5 model (Riahi *et al.* 2011). According to the model, pH values of 7.93 and 7.83 fall between these two years, and equate to years 2068 and 2090, respectively (Table 2.1). Nine tanks (H: 1.3 m, D: 1.1 m, capacity: 1000 L) were used in order to have three replicates per treatment. The tanks were independent of each other, forming a non-recirculating system; they were dark green in colour with the inside floor of the tank painted white, making it easy to see the larvae when viewed from the top of the tanks (Figure 2.3).

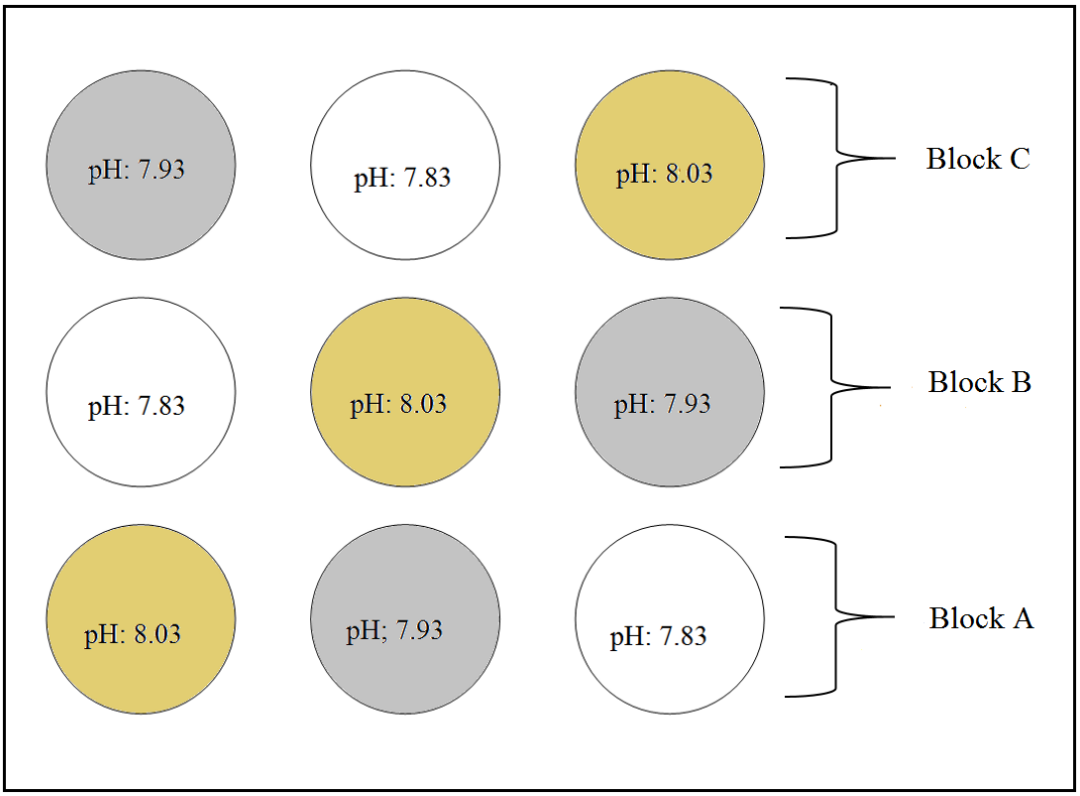


Figure 2. 2: Schematic representation of the independent non-recirculating system used in the rearing of larval *Argyrosomus japonicus* from 0 to 22 days after hatching (DAH).

Table 2. 1: Average predicted pH levels for different years under the RCP 8.5 model. Values marked with asterisks are the ones used in the study.

Years	**pH predicted (in U)**
2050	8.03*
2068	7.93*
2090	7.83*
2100	7.78

Figure 2. 3: Non-recirculating system used in rearing *Argyrosomus japonicus*, a) the nine independent experimental tanks and b) the inside of the tank showing the white painted floor.

Each tank was fitted with an automatic pH-CO_2 controller (Tunze 7074/2, Germany) connected to a CO_2 cylinder to adjust the pH to desired levels, and mimic acidification. This CO_2-injection system regulates pH by bubbling CO_2 into water. The pH meter (part of the pH controller) detects changes in pH and if it increases past the pre-set value, the CO_2 gas from the CO_2 cylinder (connected to the controller) is bubbled into the water until pH returns to the pre-set values.

The light cycle was maintained at 16L: 08D (L = light, D = dark) using a 10W spotlight fitted 70 cm above each tank. Temperature in each tank was maintained by the immersion of two to three submersible aquarium heaters (Eheim Jager, Stuttgart, Germany) connected to a temperature control box (STC 1000). Six of the tanks had two 300W electric heaters each and the other three tanks each had one 300W and two 150W heaters. The heater-control box systems ensured that the heaters switched off once the water temperature reached a pre-set value of 25 °C. Prior to the stocking of embryos, experimental tanks were set to 23 °C (similar to the brood stock), and then gradually increased to 25 °C over period of four hours, once the embryos were stocked. This was done to avoid embryo thermal shock. Each tank was supplied with air by means of an airline connected to a blower.

Seawater for the experimental setup was sourced from the farm where the experiments were carried out. Each tank was filled with seawater to attain a volume of 900 L. Five days before the inoculation of eggs into the tanks, 200 ml of 12.5% sodium hypochlorite (NaClO) was

added to each tank to sterilise the seawater. Twenty-one grams of sodium thiosulfate (Na_2S2O_3) was then added to each tank to neutralise the NaClO.

2.2.2 Physico-chemical parameters

Daily temperature and salinity measurements were recorded using a temperature control box (STC 1000) and a portable refractometer (Hanna Instruments HI 96822, Romania), respectively. Dissolved oxygen was measured daily using a portable oxygen meter (OxyGuard, Denmark), which was air-calibrated prior to each measurement, and was kept at 7 mg/L, above the optimum rearing levels (>6 mg/L) for *A. japonicus* larvae (Fielder and Heasman 2011). Total alkalinity (TA), ammonia (NH_3) and nitrite (NO_2^-) were measured every third day. A mini-titrator (Hannah Instruments HI84531) was used to measure TA, while NH_3 and NO_2 were measured using a photometer (Palintest Photometer 7100). All these water parameters including water temperature were based on standard protocol for optimum rearing of larval *A. japonicus* at Pure Ocean farm.

The bottoms of the tanks were siphoned regularly to maintain the water quality and 20% of the seawater was exchanged at every siphoning session. The siphon was attached to a household broom which was used to gently sweep the bottom of the tanks to re-suspend and remove particles. The frequency of siphoning ranged from every two days while the fish were still feeding on *Brachionus plicatilis* and *Artemia spp.* to every day after the artificial feed was introduced i.e. from 19 DAH onwards.

2.3 Experimental Procedures

Four biological responses, size-at-hatch, growth rate, development and metabolism (O_2 consumption), were measured to determine the potential effects of ocean acidification on the larvae of *A. japonicus.*

2.3.1 Size-at-hatch, growth rate, and development

Larvae were collected on days 0–2, 4–6, 8, 10–12, 14–16 and 22 to ensure that each developmental stage was represented (Table 2.2). The aeration was gently increased just prior to collection to ensure that the larvae were well mixed, after which they were collected using a one-litre measuring jug. Captured larvae were anaesthetised with clove oil, photographed under a stereo microscope (Leica EZ4HD) and their length measured to the nearest 0.01 mm

standard length (mm SL). Photographs were also used to identify developmental stages, based on morphological characteristics.

Table 2. 2: Number and days of sampling for length measurements of *Argyrosomus japonicus* reared from 0 to 22 DAH.

Developmental stage	Days of sampling	Number of sampling days
Hatchling	0–2	3
Early pre-flexion	4–6	3
Late pre-flexion	8, 10–12	4
Flexion	14–16	3
Flexion/Post-flexion	22	1

2.3.2 Metabolism studies (O2 consumption measurements)

Seawater was sterilised with NaClO and neutralised with $Na_2S_2SO_3$ before being disinfected with UV each morning. This sterilised seawater, hereafter referred to as "seawater", was used to bathe and purge the larvae and to fill the respirometry chambers during trial measurements. The use of sterilised seawater was used to reduce micro-organismal respiration, which can confound the readings (Cattano *et al.* 2016).

2.3.2.1 Hatchling to flexion stage larvae

Oxygen consumption rate measurements (MO_2) for each treatment were determined at every developmental stage, from hatchling to flexion stage, using static respirometry (PreSense, SDR SensorDish® Reader, Germany) (Figure 2.4; Table 2.3). The respirometry oxygen sensors were pre-calibrated (factory calibrated). Hatchlings were exposed to the experimental treatments for over 12 hours before they were subjected to metabolic studies. Hatchlings were randomly scooped/sampled out of the experimental tanks and gently pipetted into clean sterilised seawater. To ensure minimal stress during the transfer of hatchlings, the tip of the pipette was cut to create a wider hole for hatchlings/animals to pass through easily. Small microplate chambers (Loligo® Systems) (200 µL each) were used in conjunction with the

respirometer (PreSense, SDR SensorDish® Reader, Germany) to measure the MO_2 of the hatchlings. Eighteen of the 24 chambers were randomly loaded with larvae, which equated to six larvae per treatment. The remaining chambers were filled with clean sterilised seawater and were used as blanks to account for bacterial respiration (Cattano *et al.* 2016).

The chambers were placed inside the mini-water bath where they were kept at a constant temperature by using temperature-controlled water from the large water bath (Figure 2.4). Oxygen consumption rate measurements were then recorded. A total of nine trial days were run for this developmental stage, three trials per day for three consecutive days (Table 2.3). Care was taken that all treatments were represented in every trial by ensuring that three different treatments from one block were sampled at once for every trial.

Oxygen consumption rate measurements for hatchlings and larvae younger than 16 days (hatchling to flexion stage) lasted for 60 minutes. After each measurement trial, hatchlings were immediately dried with a paper towel, placed in a petri dish and photographed under a stereo microscope (Leica EZ4 HD). The digital photographs were later used for length measurements. This was done to account for any variability in MO_2 that may arise because of differences in sizes. Hatchlings that could not be photographed immediately after trial(s) were stored in 10% formalin and photographed within 24 hours.

The same MO_2 methods were used for the larvae. However, the larvae were first purged before each trial measurement. Larvae were randomly sampled from the experimental tanks using 1 L measuring jugs and transferred into glass beakers containing clean sterilised seawater for purging and for reducing micro-organismal respiration. The glass beakers were placed in the water-bath so that the water in the beakers could adjust to the same water temperature as that of the system. Larvae were left in the beakers for one to four hours to purge, depending on age. Once purged, six larvae from each treatment were gently and randomly pipetted into eighteen of the twenty-four chambers that were then filled with fresh sterilised seawater and closed with lids. The chambers were placed in the mini-water bath and oxygen consumption was measured.

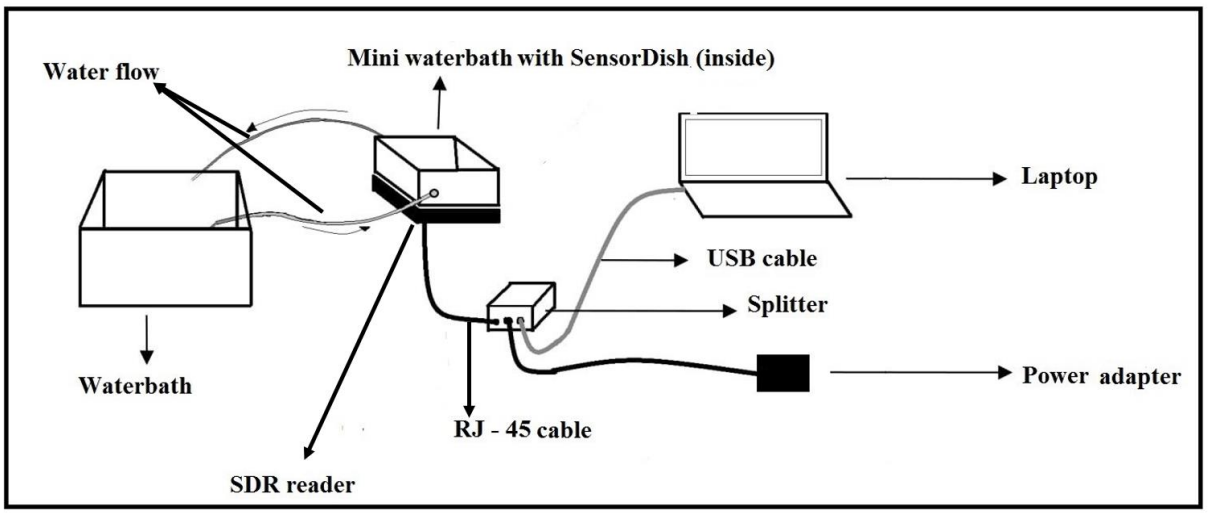


Figure 2. 4: Simplified schematic representation of PreSense, SDR SensorDish® Reader highlighting major components.

Table 2. 3: Early developmental stages of *Argyrosomus japonicus* from 0 to 22 DAH, the sampling days for the metabolic rate studies and the type of respirometer used at each life stage. Numbers in brackets indicate the number of trials run for each developmental stage.

Developmental stage	Stage duration	Days of sampling	Respirometry
Hatchling	0–4	0–2 (9)	PreSense
Early pre-flexion	3–7	4–6 (9)	PreSense
Late pre-flexion	7–13	10–12 (9)	PreSense
Flexion	12–20	14–16 (9)	PreSense
Flexion/Post-flexion	18–22	21–22 (8)	Pyro-Science

2.3.2.2 Post-flexion larvae

Larger respirometer chambers (5 ml) (PyroScience, Germany) were used to measure oxygen consumption rate for post-flexion larvae. The PyroScience respirometry system allowed for only four measurements to be taken per trial. The size and volume of the chambers used were determined by ensuring a decrease of 20% in oxygen saturation in chambers over a period of 60 minutes. Each chamber had an inlet and outlet on its lid that allowed for water circulation

that was maintained by a multi-channel peristaltic pump that drew water from the chamber and passed it through one of the four flow-through cells (PyroScience, Germany) before returning it to the chamber.

Post-flexion larvae were purged for four hours before being transferred into one of the three available chambers using a pipette. Three larvae, one from each treatment, were measured for MO_2 per trial, while the fourth chamber was filled with sterilised seawater and was used as a blank. Dissolved oxygen consumption rates were then measured at an interval of 30 seconds. During each trial, measurements were stopped either after 60 minutes, or once the oxygen saturation dropped below 80%, whichever came first. Four trials were run over consecutive days for the post-flexion stage.

While several studies recommend that larval behaviour be quantified during measurements of oxygen consumption (Chabolt *et al.* 2016; Munday *et al.* 2016) it was not possible in the present study to observe the larvae as they were i) too small and ii) were in the respirometry chambers immersed in water bath.

2.4 Fate of the Animals used in the Study

All fish taken out of the experimental tanks were sacrificed by anaesthetising them with an overdose of clove oil in accordance with the ethical clearance guidelines of Rhodes University.

2.5 Data Analysis

Data for water quality were assessed for normality and homogeneity of variance before analysis, using the Shapiro-Wilk test and Levene's Test for homogeneity of variances, respectively. If data were found to violate the assumptions of analysis of variance (ANOVA), a non-parametric Kruskal-Wallis test was used. Daily pH, temperature and salinity readings and weekly TA readings were used to calculate pCO_2 in CO2SYS. Differences in growth rate and mean body size SL (mm) over time (growth) were tested using analysis of covariance (ANCOVA) and repeated measures ANOVA, respectively. If any significant differences were found, a post-hoc Tukey HSD test was used to identify where the differences occurred.

Oxygen consumption rate measurements were first length standardised to account for variety in size and were expressed as µg O_2/h/mm. Changes in O_2 [ΔO_2] in chambers were determined using a regression model (O_{2max}-O_{2min}) and then incorporated into the equation:

$MO_2 = ([\Delta O_2]*vol/t)/l$

where $[\Delta O_2]$ is the change in oxygen concentration in the chamber measured in µg O_2, *t* is the time in hours (h) it took for the change in oxygen concentration to occur, *vol* is the volume of the respirometer in litres (L) and *l* is the length in millimetres (mm) of a corresponding fish measured for MO_2. Larval volume in respirometry chambers was too small and insignificant and was therefore unaccounted for.

The MO_2 measurements were compared between treatments across sampling days and across life stages using repeated measures ANOVA. Standard (SMR) and active (AMR) metabolic rate were determined from the calculated MO_2 using the 5% and 95% percentile, respectively (Kandjou and Kaiser 2014; Edworthy *et al.* 2018), from all data obtained during each measurement period per treatment. Percentiles of metabolic rate values have been used to estimate the lower and upper indices of metabolic rate to be used in the calculation of aerobic scope (Chabolt and Claireaux 2008; Geist *et al.* 2013; Kandjou and Kaiser 2014; Edworthy *et al.* 2018). Although this method could underestimate aerobic scope, this risk decreases as the number of metabolic rate values used to estimate these indices increases (Chabolt *et al.* 2016).

Metabolic scope was calculated as the difference between AMR and SMR (AMR – SMR). While MMR is required to measure aerobic scope in fish, the present study used AMR, instead of MMR, for the measurement of aerobic scope and for comparability with previous studies. To measure MMR a fish has to be exercised to exhaustion prior to measurement been taken, while for AMR fish is required to be swimming at a constant and sustained speed (Nilsson *et al.* 2007; Peck and Moyano 2016). It was not feasible in the present study to elicit MMR as the larvae were too small, fragile and due to the passive nature of the earliest life stages of *A. japonicus*. Also, marine fish larvae often do not respond well to forced swimming (Peck and Moyano 2016). In reviewing some of the challenges facing researchers in measuring respiration rates in marine fish larvae, Peck and Moyano (2016) reported that only two studies (Killen *et al.* 2007 and Nilsson *et al.* 2007) have been able to elicit and measure MMR in marine fish larvae. However, since AMR could be, but not necessarily, as high as MMR (Peck and Moyano 2016), it was mostly due to this reason and the above mentioned that the present study opted to use AMR in measuring aerobic scope of the earliest life stages of *A. japonicus*. In fact, AMR has been used to measure aerobic scope in fish

although this might not be strictly aerobic scope (Kandjou and Kaiser 2014; Peck and Moyano 2016; Edworthy *et al.* 2018).

Standard metabolic rate, AMR, and metabolic scope were compared between treatments across life stages using a repeated measures ANOVA with replicate incorporated as random factor. All analyses were done using STATISTICA 13 (Dell Inc. 2015).

CHAPTER THREE

RESULTS

3.1 Water Quality Parameters

Mean daily water temperature of all the treatments ranged from 23.6 to 25.0 °C for the experiment, with the lowest temperatures recorded on 13 DAH (Figure 3.1a). A drop in temperature was observed on 13 DAH and 18 DAH. As this effected all three treatments, there were no significant differences in the mean water temperature between treatments during any period of the study (Kruskal-Wallis; $H_{2, 81} = 5.28$; $P = 0.07$) (Figure 3.1b; Table 3.1). Mean total alkalinity ranged between 1789.26 and 1849.31 µmol/kg SW (Table 3.1) and there were no significant differences between the treatments (Kruskal-Wallis; $H_{2, 81} = 3.73$; $P = 0.15$). Both pH and pCO_2 were maintained at the desired level for each treatment, with mean treatment water pH (Kruskal-Wallis; $H_{2, 81} = 71.98$; $P < 0.05$) (Table 3.1; Figure 3.2) and mean pCO_2 (Kruskal-Wallis; $H_{2, 81} = 69.10$; $P < 0.05$) (Table 3.1) significantly different for the three treatments. There were no significant differences in mean dissolved oxygen between the treatments (ANOVA; $F_{2, 26} = 0.16$; $P = 0.85$) (Table 3.1) and salinity remained at 35 ppt for all treatments.

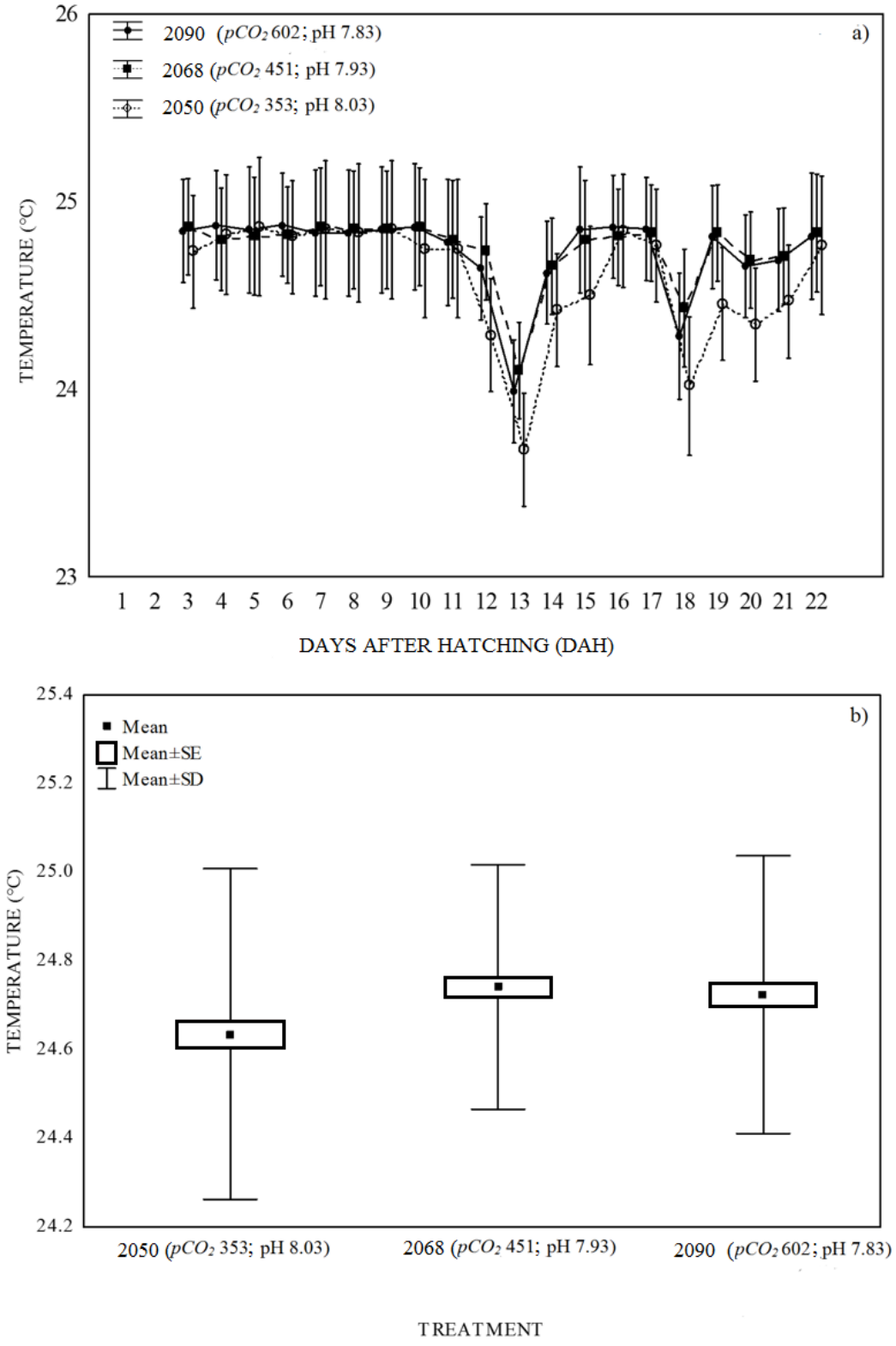


Figure 3. 1: Measurements of a) mean daily temperature ± SD and b) overall mean temperature for the 2050, 2068 and 2090 treatments during the 22-day larval rearing period of *Argyrosomus japonicus*. Measurements were taken three times a day.

Table 3. 1: Mean water quality parameters observed during the 22-day larval rearing period of *Argyrosomus japonicus*. Software CO2SYS (Lewis and Wallace 1998) was used to calculate pCO_2 and all values are means (± SD). TA = total alkalinity; DO = dissolved oxygen ***** signifies significant differences between treatments at $P < 0.05$.

	Temperature	**pH**	**TA**	***p*CO₂**	**DO**
Treatment	°C	Total scale	µmol/kg SW	µatm	mg/L
2090 (pCO_2 602; pH 7.83)	24.73 (± 0.22)	7.83 (± 0.01)	1849.31 (± 197.45)	602.30 (± 67.52)	7.01 ± 0.20
2068 (pCO_2 451; pH 7.93)	24.74 (± 0.17)	7.93 (± 0.02)	1754.68 (± 145.10)	451.82 (± 36.80)	7.03 ± 0.26
2050 (pCO_2 353; pH 8.03)	24.64 (± 0.34)	8.04 (± 0.01)	1789.26 (± 161.18)	353.19 (± 35.41)	7.07 ± 0.14
P-value	> 0.05	< 0.05*	> 0.05	< 0.05*	> 0.05

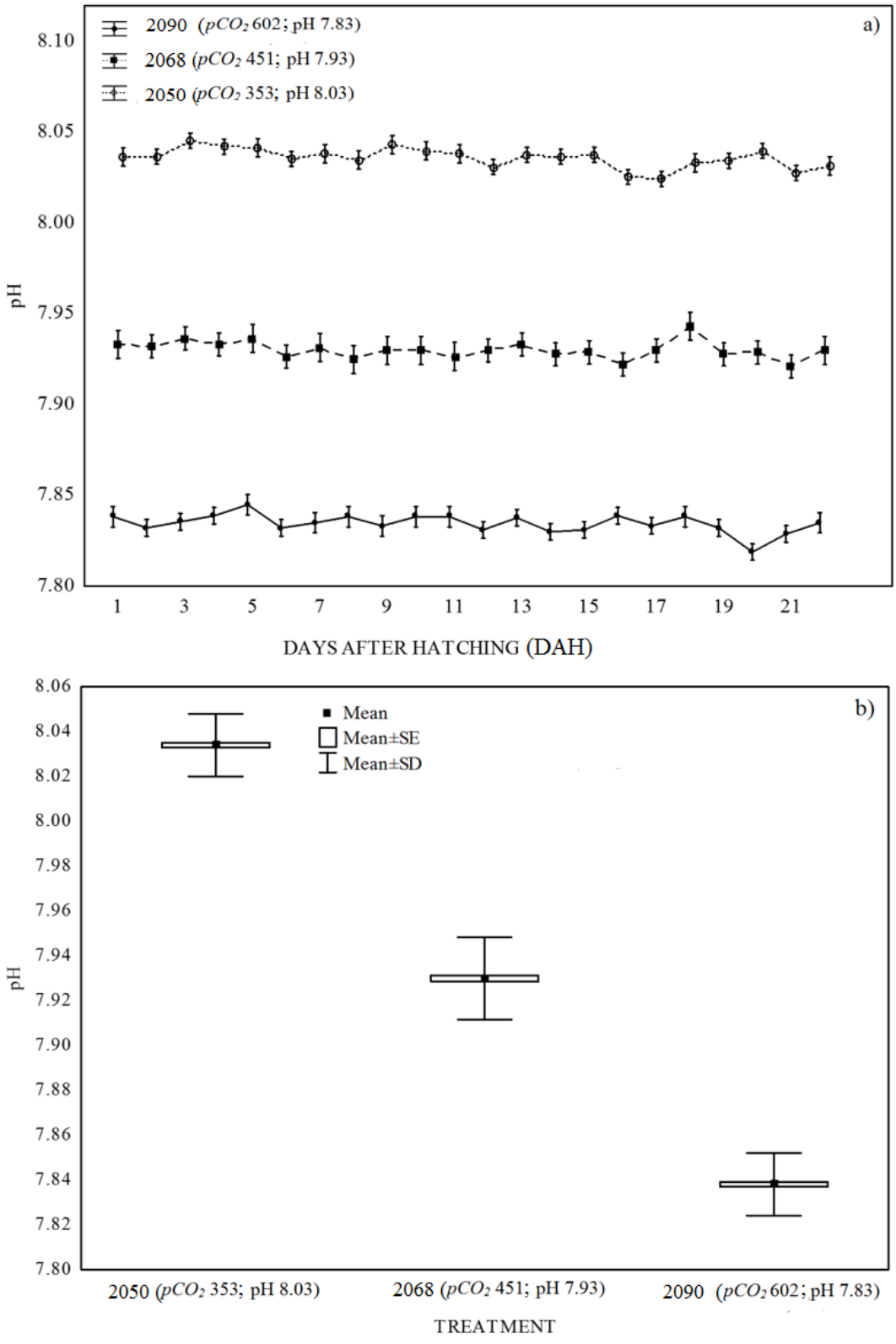


Figure 3. 2: Measurements of a) mean daily pH ± SD and b) overall mean pH (calculated on a log scale) for the three treatments during the 22-day larval rearing period. Measurements were taken three times a day.

3.2 Developmental stages

Five life stages were identified during the study, based on larval behaviour and morphological characteristics (Table 3.2, Figure 3.3). Larval development varied, with individuals belonging to different stages observed through most of the experiment (Figure 3.4). Nevertheless, the rate of development between treatments was similar (Figure 3.4). For the purpose of data analyses, the life stage at any given time was defined as the stage that was the most dominant (Table 3.2). The hatchling stage was present from 1–4 DAH (dominant 1–2 DAH, Table 3.2, Figure 3.4), with hatchlings ranging from 1.08–2.49 mm SL. During this period, the yolk sac was prominent (Figure 3.3a) and larvae relied on the yolk reserve for food. The early pre-flexion stage (Table 3.2) commenced immediately once the yolk sac was absorbed and the larvae started to feed on small prey, *Brachionus plicatilis*. This stage was first present from 2 DAH in the 2090 (pCO_2 602 µatm; pH 7.83) treatment but became dominant in all treatments from 3–6 DAH (Figure 3.4). At this stage, the larvae were starting to form gas bladders and had developed eyes (Figure 3.3b). Larvae sampled at this stage ranged from 1.99 to 4.55 mm SL. From 5 DAH, the stomach began replacing the yolk sac (Figure 3.3c) and larvae started feeding on introduced *Artemia spp*, indicating the start of the late pre-flexion stage. This stage lasted for ten days in the 2090 (pCO_2 602 µatm; pH 7.83) treatment, eight and nine days in the 2068 (pCO_2 451 µatm; pH 7.93) and 2050 (pCO_2 353 µatm; pH 8.03), treatments, respectively (Figure 3.4). However, this late pre-flexion stage was dominant in all treatments from 10–12 DAH (Figure 3.4, Table 3.2). Larvae at this stage ranged in size from 3.08 to 5.92 mm SL. By 10 DAH, some larvae began developing a flexing notochord (Figure 3.3d), suggesting the onset of the flexion stage. This stage was dominant between 14–16 DAH (Table 3.2). The first signs of a developed caudal fin (Figure 3.3e), the post-flexion stage, were visible from 14 DAH onwards but the stage was dominant in all treatments by 22 DAH (Figure 3.4, Table 3.2). However, since the proportions of flexion and post-flexion fish were almost equal at 22 DAH, fish on this day were categorised as flexion/post-flexion stage (Figure 3.4, Table 3.2).

Table 3. 2: Description (including duration) of the developmental stages of larval *Argyrosomus japonicus* identified during the 22-day larval rearing period. Numbers in brackets indicate sample size used for metabolic measurements. "Dominant" refers to days where the life stage was dominant and was when the metabolic data was collected.

Stage (*n* for metabolic data)	**Duration of stage**	**Days when the life stage was dominant**	**Description/Identification**
Hatchling (105)	0–4	1–2	The presence of yolk sac, oil globule and median fin-fold. Start from hatching to absorption of yolk sac.
Early Pre-flexion (103)	2–6	4–6	The start of external feeding on *Brachionus plicatilis.* Presence of gas bladder.
Late Pre-flexion (130)	5–15	10–12	External feeding on *Artemia spp.* Yolk sac replaced with stomach. Myomeres start to form. No oil globule.
Flexion (120)	10–22	14–16	Flexion of the notochord. Formation of caudal fin rays.
Flexion/Post-flexion (22)	14–22	21–22	Caudal fin developed. Larvae start feeding on artificial feed. Increase in swimming efficiency.

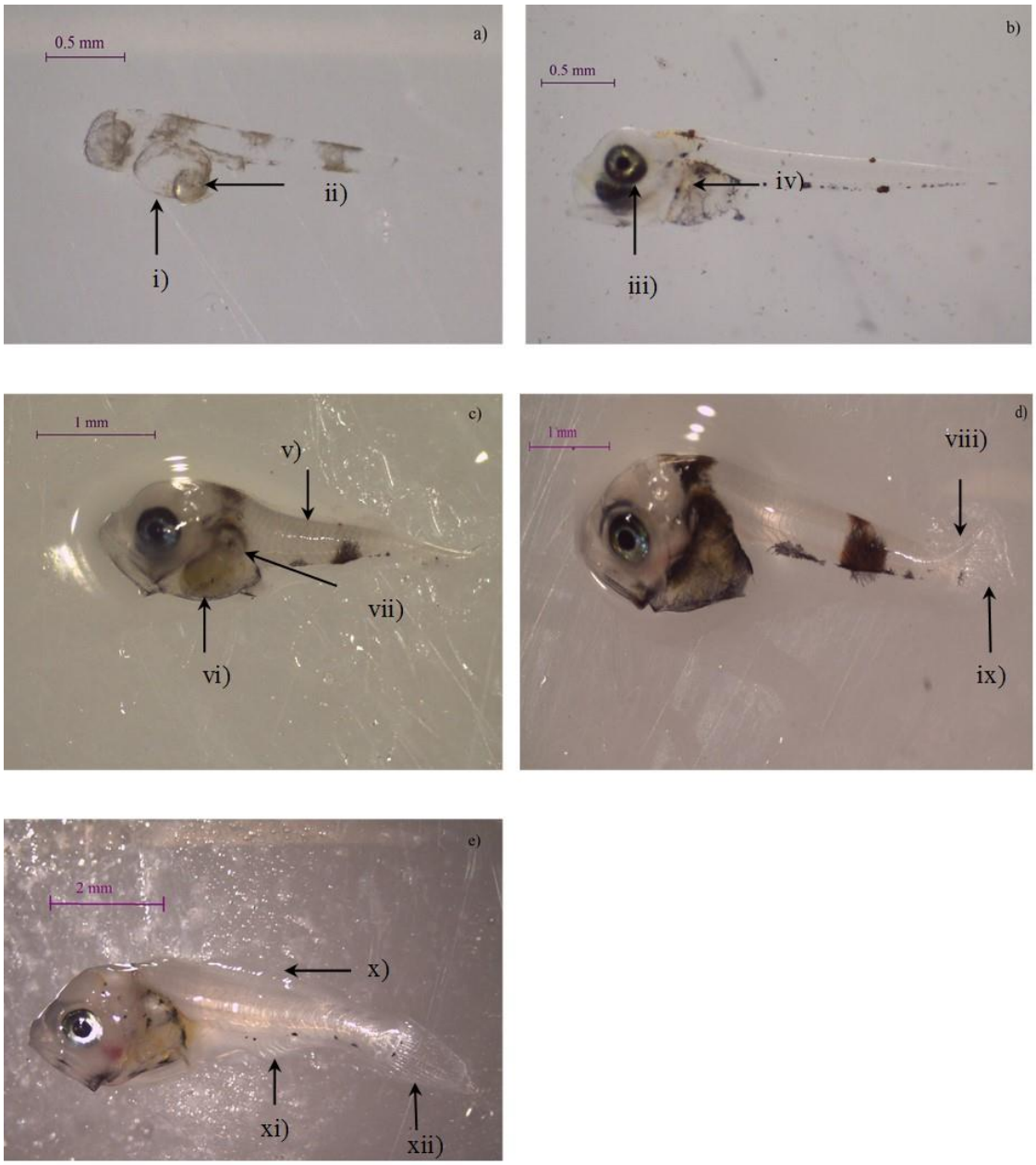


Figure 3. 3: Early life history stages of *Argyrosomus japonicus* from 0 to 22 days after hatching (DAH), highlighting major morphological characteristics distinguishing each stage, a) hatchling b) early pre-flexion c) late pre-flexion d) flexion and e) post-flexion stages. i = yolk sac, ii = oil globule, iii = developed eye, iv = formation of swim bladder, v = myomeres, vi = stomach, vii = developed swim bladder, viii = flexed notochord, ix = formation of caudal fin rays, x = formation of dorsal fin, xi = formation of anal fin and xii = fully developed caudal fin.

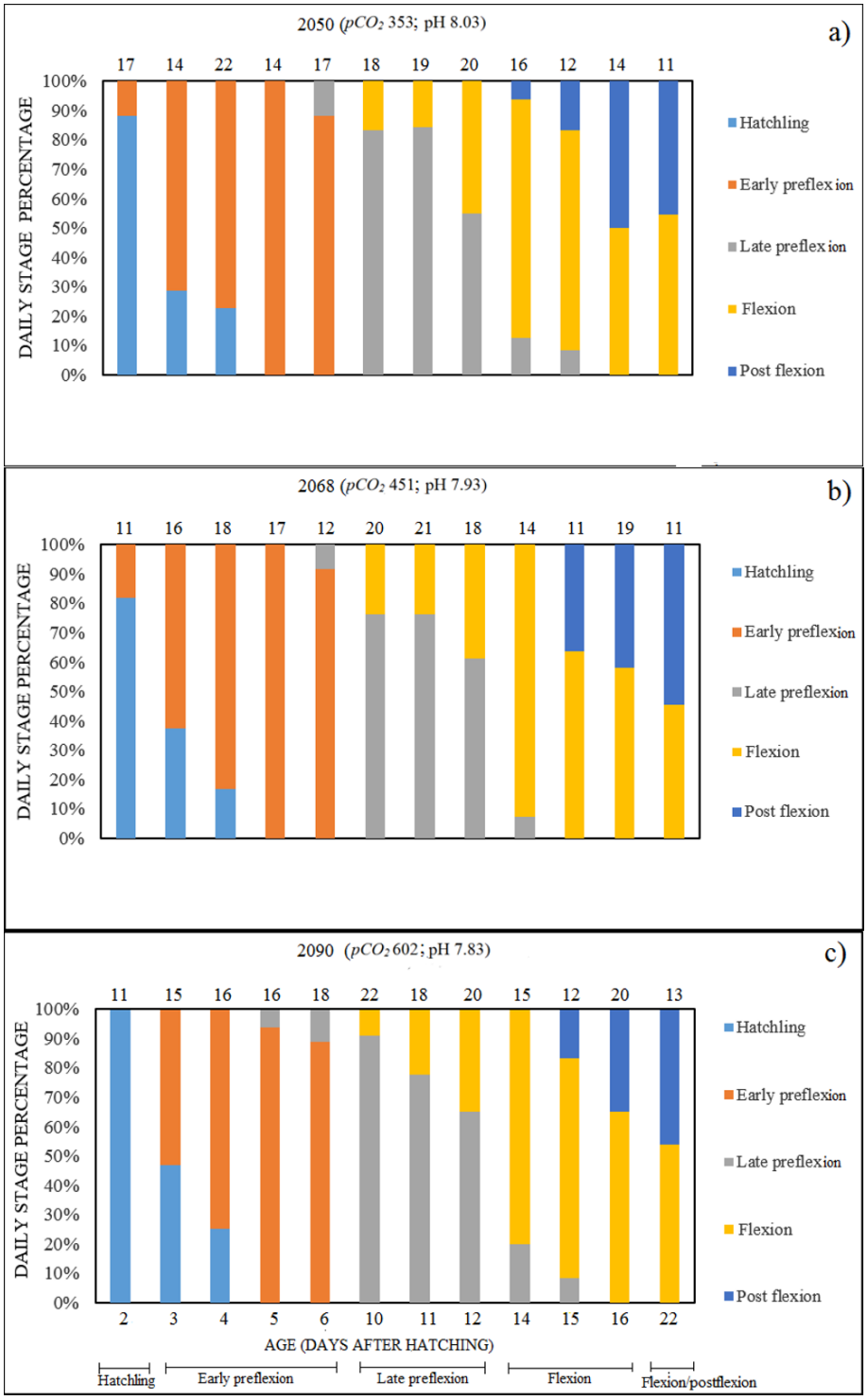


Figure 3. 4: Daily relative frequency of the early life stages (0–22 days after hatching, DAH) of *Argyrosomus japonicus* in pH conditions predicted for 2050 (a), 2068 (b) and 2090 (c). Numeric number above each bar indicates sample size.

3.3 Growth

A total of 984 larvae were measured. Hatching size ranged from 1.67 ± 0.12 mm in 2050 (pCO_2 353 µatm; pH 8.03) to 1.85 ± 0.02 mm SL in the 2090 (pCO_2 602 µatm; pH 7.83) treatment, but was not significantly different among treatments (ANOVA, $F_{(2, 72)} = 1.36$; $P = 0.28$). As expected, there was a significant positive linear relationship (ANCOVA, $F_{(1, 980)} = 2939.55$; $P < 0.01$) between body length and day (Figure 3.5a). However, there were no significant differences in the growth rates (Homogeneity of slopes model, $F_{(2, 978)} = 1.37$; $P = 0.25$) among treatments (Figure 3.5a). There were also no significant differences (repeated measures ANOVA, $F_{(22, 33)} = 0.49$; $P = 0.96$) in mean body length at any sampling day (Figure 3.5b).

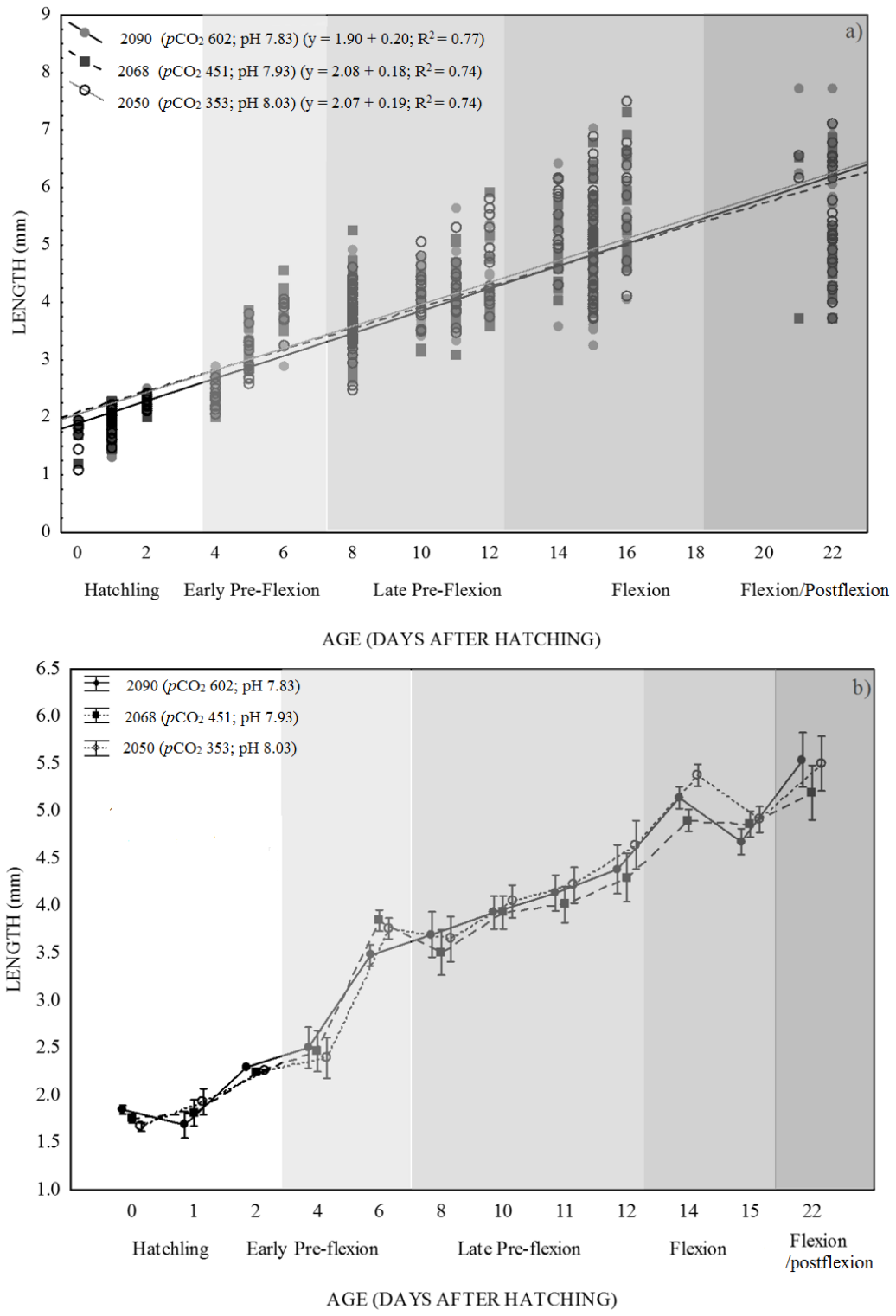


Figure 3. 5: a) Linear growth, based on standard length (SL) measurements and b) mean daily standard length of *Argyrosomus japonicus* individuals reared at pCO_2 and pH levels predicted for 2050, 2068 and 2090, from hatching until 22 days after hatching. Values are given as mean ± standard error (SE).

3.4 Metabolic Rate

A total of 480 rate of oxygen consumption (metabolic rate) measurements from across all five life stages were collected during the study (Table 3.2). Although the mean daily oxygen consumption rate varied with growth, the pattern was similar between the treatments from 0 to 21 DAH (Figure 3.6). Oxygen consumption rate remained relatively stable for the first six days (0.07 – 0.27 µg O_2/mm/h) for all treatments, slightly increasing on 10 to 12 DAH where it remained between 0.24 and 0.46 O_2/mm/h (Figure 3.6). From there on, it increased rapidly and peaked (1.81 – 2.04 µg O_2/mm/h) on 14 DAH (flexion), after which it decreased to between 0.34 and 0.68 µg O_2/mm/h on 21 DAH (Figure 3.6). There were no significant differences in oxygen consumption rate (MO_2) between treatments on any day of sampling (repeated measures ANOVA, $F_{(26, 78)} = 0.71$; $P = 0.83$). By 22 DAH, there was a noticeable difference in the oxygen consumption, with considerably higher consumption by the larvae in the 2068 (pCO_2 451 µatm; pH 7.93) treatment than the 2090 (pCO_2 602 µatm; pH 7.83) treatment and 2050 (pCO_2 353 µatm; pH 8.03) treatment (Figure 3.6). The difference was not, however, significant (ANOVA, $F_{(2, 11)} = 3.38$; $P = 0.07$).

Overall, MO_2 also varied with life stage (Appendix A). It dropped slightly from hatchling (0.10 – 0.16 µg O_2/mm/h) to early pre-flexion (0.05 – 0.06 µg O_2/mm/h) and then increased rapidly until it peaked at the flexion stage (1.04 – 1.33 µg O_2/mm/h) before dropping at the flexion/post-flexion stage (0.55 – 0.92 µg O_2/mm/h) (Appendix A). Although no significant differences were detected between treatments at any life stage (repeated measures ANOVA; $F_{(8, 12)} = 0.21$, $P = 0.98$), on average, MO_2 of fish in treatment 2090 (pCO_2 602 µatm; pH 7.83) was lower at both the flexion and flexion/post-flexion stages than the other two treatments (Appendix A).

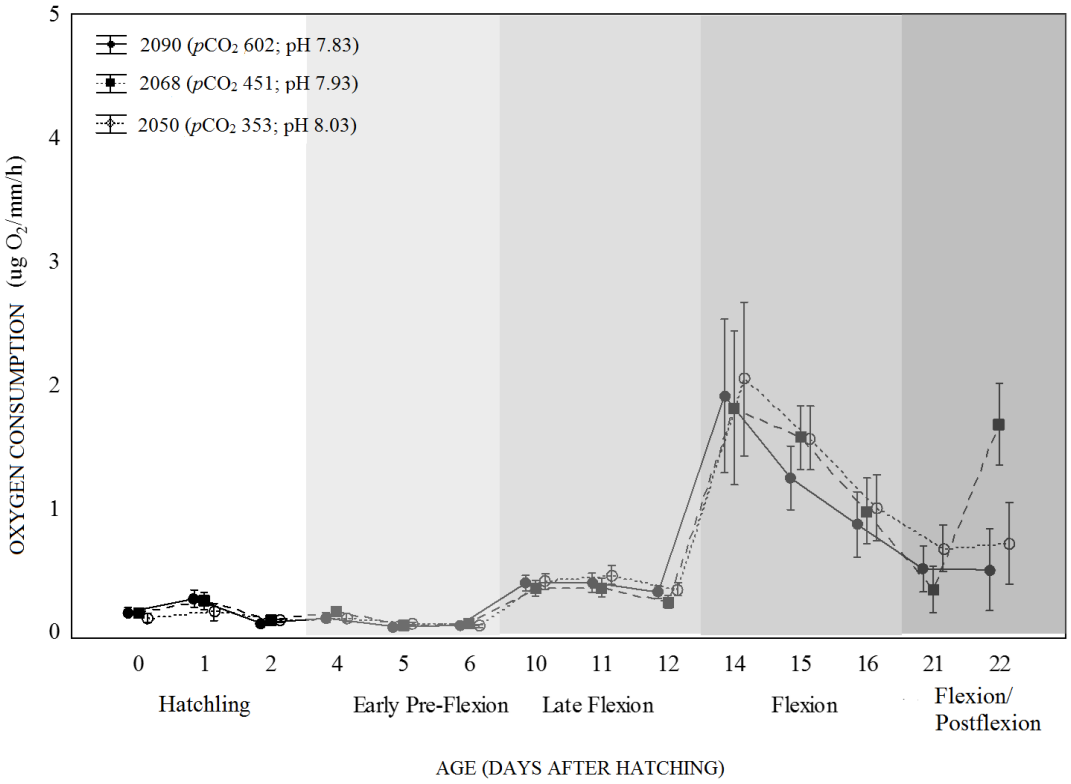


Figure 3. 6: Mean daily oxygen consumption rate (metabolic rate) of early stage (hatchling to flexion/post-flexion) *Argyrosomus japonicus* reared under pCO_2 and pH treatments predicted for the years 2050, 2068 and 2090. Error bars indicate standard error of the mean (SE).

Daily SMR for all treatments remained relatively stable and showed little change from 0 to 22 DAH (Figure 3.7). However, noticeable increases were observed on 15 (flexion) and 22 (flexion/post-flexion) DAH for the 2090 (pCO_2 602 µatm; pH 7.83) and 2068 (pCO_2 451 µatm; pH 7.93) treatments, respectively (Figure 3.7).

Average SMR varied significantly with life stage in all treatments (repeated measures ANOVA, $P < 0.001$, Table 3.3; Appendix B). However, although no significant difference was observed in average SMR between the treatments from hatchling to the flexion stage, there was a significant difference between treatments during the flexion/post-flexion stage ($P = 0.03$, Table 3.3). Here fish in the 2068 (pCO_2 451 µatm; pH 7.93) treatment had a significantly higher (0.48 µg O_2/mm/h) SMR than those in the 2050 (pCO_2 353 µatm;

pH 8.03) (0.28 µg O_2/mm/h) and 2090 (pCO_2 602 µatm; pH 7.83) (0.04 µg O_2/mm/h) treatments (Tukey HSD post hoc test, $P < 0.05$) (Table 3.3; Appendix B).

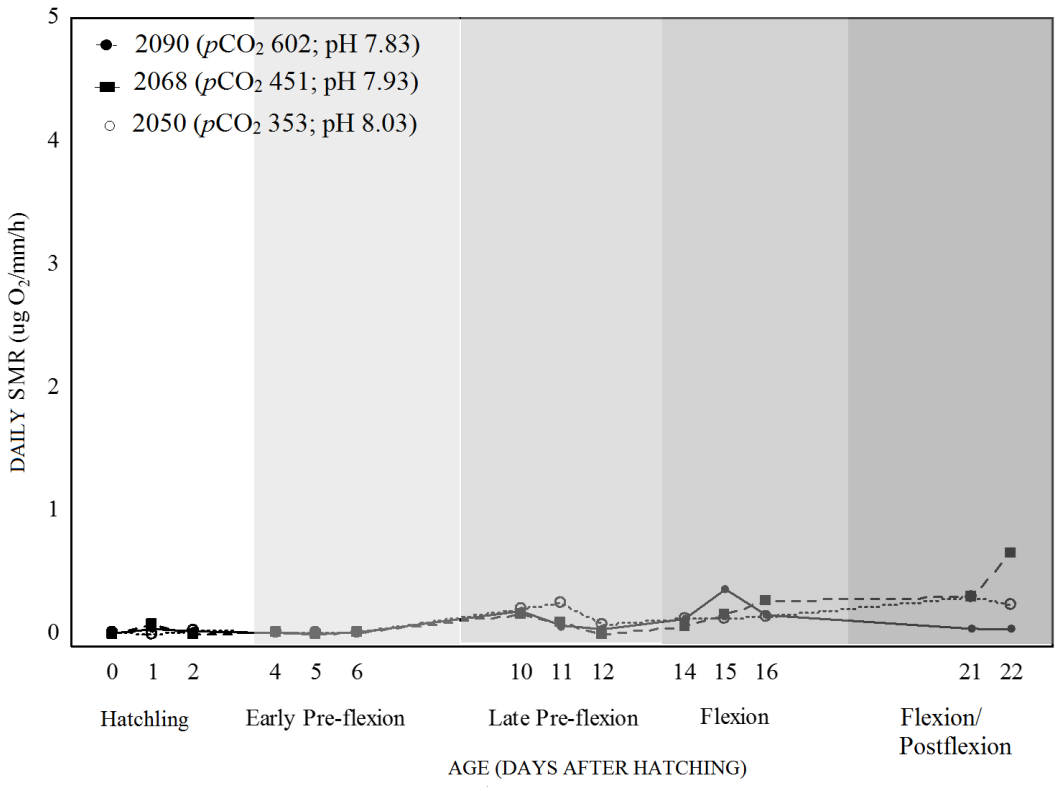


Figure 3. 7: Daily standard metabolic rate (SMR) of early stage (0 to 22 days after hatching) *Argyrosomus japonicus* in pCO_2 and pH conditions predicted for year 2050, 2068 and 2090.

Table 3. 3: Repeated measures ANOVA for standard metabolic rate, active metabolic rate and metabolic scope of early stage (hatchling – flexion/post-flexion) *Argyrosomus japonicus* reared at pCO_2 and pH levels predicted for 2050, 2068 and 2090.

	Df	MS	F	P
SMR				
Treatment	2	0.00	1.46	0.36
Life stage	4	0.00	9.06	0.00
Treatment*Life stage	8	0.00	3.46	0.03
Error	12	0.00		
AMR				
Treatment	2	0.00	0.59	0.63
Life stage	4	0.00	24.10	0.00
Treatment*Life stage	8	0.00	0.90	0.56
Error	12	0.00		
Metabolic scope				
Treatment	2	0.00	0.64	0.61
Life stage	4	0.00	27.22	0.00
Treatment*Life stage	8	0.00	1.57	0.27
Error	8	0.00		

Daily AMR for fish in the 2090 (pCO_2 602 µatm; pH 7.83) treatment was lower than the other two treatments on 0 DAH and then remained low and relatively stable for all treatments from 1 until 6 DAH (Figure 3.8). There were slight increases in AMR for all treatments between 10 and 12 DAH and a rapid increase between 12 and 14 DAH (Figure 3.8). Peaks in AMR for the 2090 (pCO_2 602 µatm; pH 7.83) (3.44 µg O_2/mm/h) and 2050 (pCO_2 353 µatm; pH 8.03) (4.04 µg O_2/mm/h) treatments occurred on 14 DAH, while that of the 2068 (pCO_2 451 µatm; pH 7.93) (4.63 µg O_2/mm/h) treatment occurred on 15 DAH (Figure 3.8). By 21 DAH, the AMR for fish in all treatments had dropped to between 0.38 µg O_2/mm/h (pCO_2 451 µatm; pH 7.93) and 1.05 µg O_2/mm/h (pCO_2 353 µatm; pH 8.03). However, there was an increase in AMR on 22 DAH (0.96 – 2.3 µg O_2/mm/h) and this was most noticeable in the 2068 (pCO_2 451 µatm; pH 7.93) treatment (Figure 3.8).

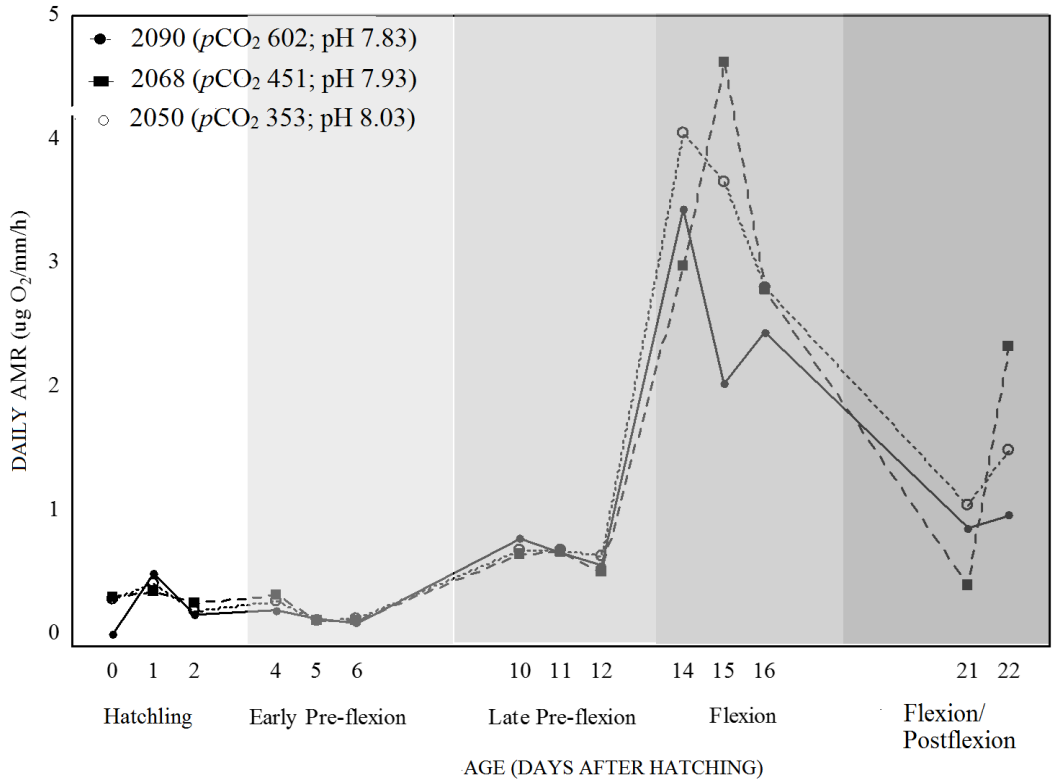


Figure 3. 8: Daily active metabolic rate (AMR) of early stage (from 0 to 22 days after hatching) *Argyrosomus japonicus* reared at pCO_2 and pH levels predicted for 2050, 2068 and 2090.

The average active metabolic rate (AMR) changed significantly with life stage for all treatments (repeated measures ANOVA, $P < 0.001$, Table 3.3; Appendix C). However, there were no significant differences in AMR between the treatments at any life stage (repeated measures ANOVA, $P > 0.05$, Table 3.3), although AMR for fish in treatment 2090 (pCO_2 602 µatm; pH 7.83) (2.02 µg O_2/mm/h) was lower than the 2050 (pCO_2 353 µatm; pH 8.03) treatment (3.84 µg O_2/mm/h) and the 2068 (pCO_2 451 µatm; pH 7.93) treatment (3.8 µg O_2/mm/h) at the flexion stage (Appendix C).

Overall, the metabolic scope was narrowest in the 2090 (pCO_2 602 µatm; pH 7.83) treatment (Figure 3.9a) compared to the 2068 (pCO_2 451 µatm; pH 7.93) (Figure 3.9b) and 2050 (pCO_2 353 µatm; pH 8.03) (Figure 3.9c) treatments, but varied according to life stage. From hatchling to the early pre-flexion stage (0–6 DAH), metabolic scope was narrow in all treatments (Figures 3.9 and 3.10). A slight increase (broader metabolic scope) occurred at the late pre-flexion stage (10–12 DAH). This was followed by a sharp increase in metabolic

scope that occurred at the flexion stage (14–16 DAH) (Figure 3.10). The broadest metabolic scope was observed at 14 DAH in the 2090 (pCO_2 602 µatm; pH 7.83) (Figure 3.9a) and 2050 (pCO_2 353 µatm; pH 8.03) (Figure 3.9c) treatments and at 15 DAH in the 2068 (pCO_2 451 µatm; pH 7.93) treatment (Figure 3.9b). Compared to the other two treatments, fish in the 2090 (pCO_2 602 µatm; pH 7.83) treatment had the narrowest metabolic scope on 15 DAH as a result of a sharp decline in AMR that coincided with an increase in SMR (Figure 3.10). Further reductions in metabolic scope occurred on 21 DAH when fish in the 2068 (pCO_2 451 µatm; pH 7.93) treatment (Figure 3.9b) exhibited the narrowest metabolic scope. Metabolic scope increased sharply in the 2068 (pCO_2 451 µatm; pH 7.93) and 2050 (pCO_2 353 µatm; pH 8.03) treatments and slightly in the 2090 (pCO_2 602 µatm; pH 7.83) treatment on 22 DAH (Figures 3.9 and 3.10).

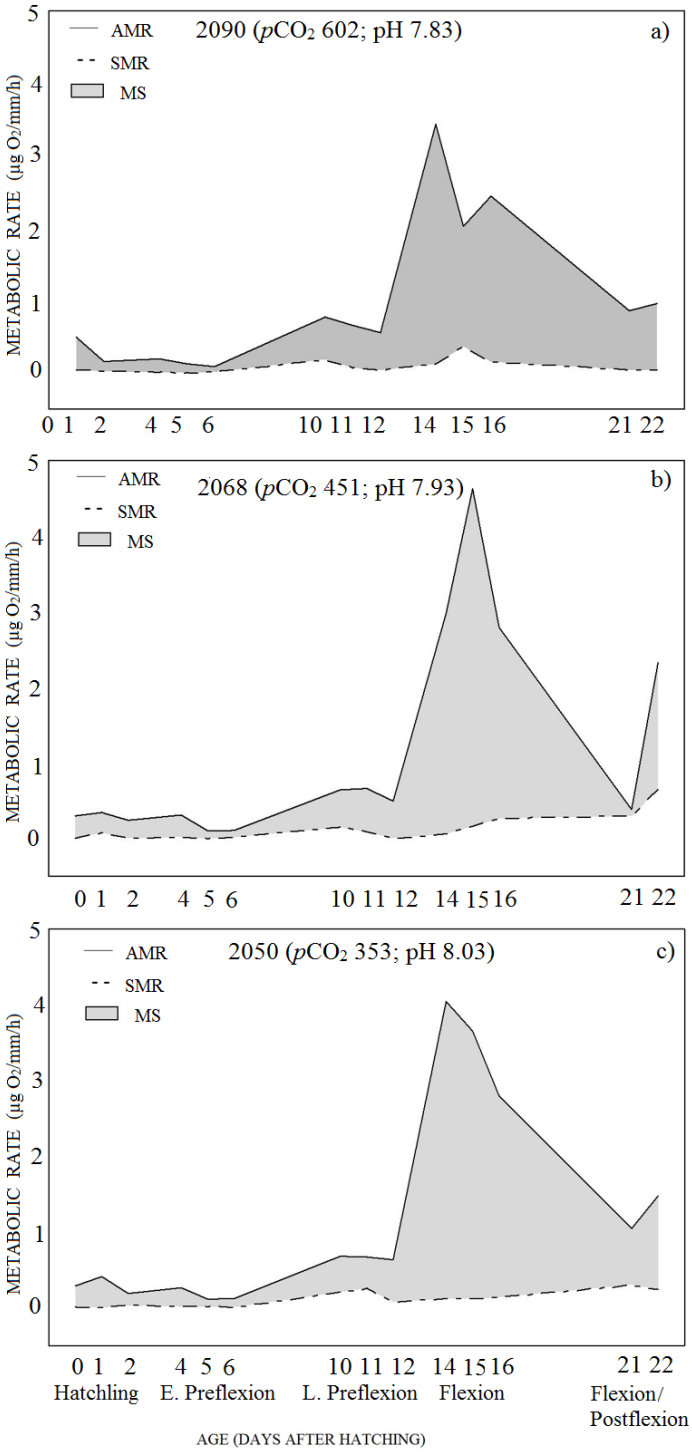


Figure 3. 9: Daily standard metabolic rate (SMR), active metabolic rate (AMR) and metabolic scope (MS) of early stage *Argyrosomus japonicus* (0–22 days) reared at pCO_2 and pH levels predicted for a) 2090 (pCO_2 602 µatm; pH 7.83) b) 2068 (pCO_2 451 µatm; pH 7.93) and c) 2050 (pCO_2 353 µatm; pH 8.03). E. = early, L. = late.

Average metabolic scope also changed significantly with life stages (repeated measures ANOVA, $P < 0.001$, Table 3.3; Appendix D). A rapid increase in metabolic scope occurred from the late pre-flexion to flexion stage, where treatments 2090 (pCO_2 602 µatm; pH 7.83), 2068 (pCO_2 451 µatm; pH 7.93) and 2050 (pCO_2 353 µatm; pH 8.03) increased by 64, 86 and 89%, respectively. Although there were no significant differences in average metabolic scope between treatments at any life stage (repeated measures ANOVA, $P > 0.05$, Table 3.3), the average metabolic scope of fish in treatment 2090 (pCO_2 602 µatm; pH 7.83) was 54% lower than fish in both the 2068 (pCO_2 451 µatm; pH 7.93) and 2050 (pCO_2 353 µatm; pH 8.03) treatments at the flexion stage (Appendix D).

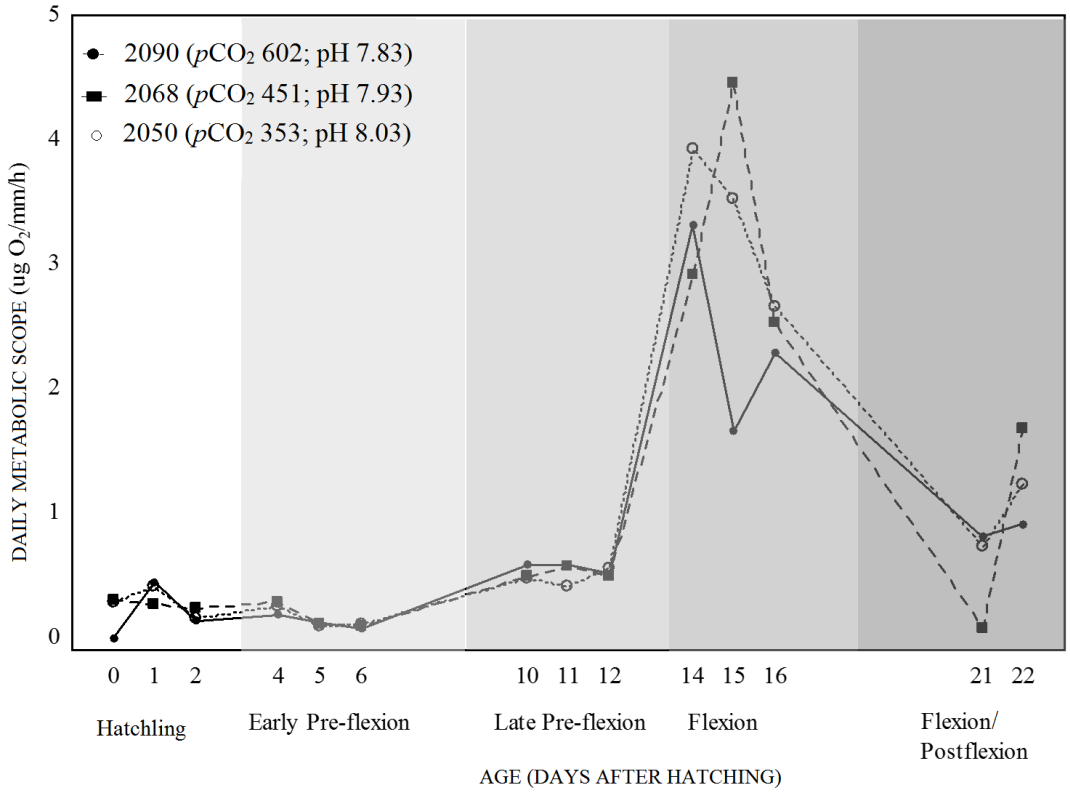


Figure 3. 10: Daily metabolic scope (MS) of early stage *Argyrosomus japonicus* (0–22 days) reared at pCO_2 and pH levels predicted for 2090 (pCO_2 602 µatm; pH 7.83) b) 2068 (pCO_2 451 µatm; pH 7.93) and c) 2050 (pCO_2 353 µatm; pH 8.03).

CHAPTER FOUR

DISCUSSION

The findings of this study contribute to the growing knowledge of the likely impacts of ocean acidification (OA) on marine organisms, and particularly the linefish, *Argyrosomus japonicus*. Results from the present study provide further evidence that size-at-hatch, development, growth and metabolism (SMR, AMR and MS) of the earliest life-stages of *Argyrosomous japonicus*, from egg to the flexion/post-flexion stage will not be significantly affected by the pH and pCO_2 levels predicted to occur before the end of the century. Although not significant, results indicate that OA levels predicted for 2090 (pCO_2 602 µatm; pH 7.83) may negatively affect the metabolic scope from the flexion stage.

While previous studies also looked at the response of the early life-stages of *A. japonicus* to OA treatments, these corresponded to the present day (pCO_2), 2050 (pCO_2 477.40 µatm; pH 8.03) and 2100 (pCO_2 910.20 µatm; pH 7.78) (Edworthy 2017, Erasmus 2017); the current study reared larvae under treatments corresponding to 2050, 2068 (pCO_2 451 µatm; pH 7.93) and 2090 (pCO_2 602 µatm; pH 7.83). This was done to try and identify whether there is a tipping point of tolerance in the larvae of this species. While the present experiment was intended to replicate the methods used in these studies, and hence to be directly comparable, it is necessary to note that the development of the fish in this study, particularly in the pre-flexion (13–14 days long) and flexion (13 days long) stages appeared relatively slow when compared with those (pre-flexion – six days long, flexion – six days long) presented by Erasmus (2017) (Figure 4.1).

These delays may be related to the two drops in temperature on 13 DAH and 18 DAH (Figure 3.1a). The consequence of differential developmental rates is that comparisons in the growth and metabolic patterns between the studies can only be made at the level of life stage, rather than DAH, and it was also not possible to compare the later life-stages (post-flexion and settlement) as ± 50% of the fish were still in the flexion stage by the end of the experiment (Figures 3.4 and 4.1).

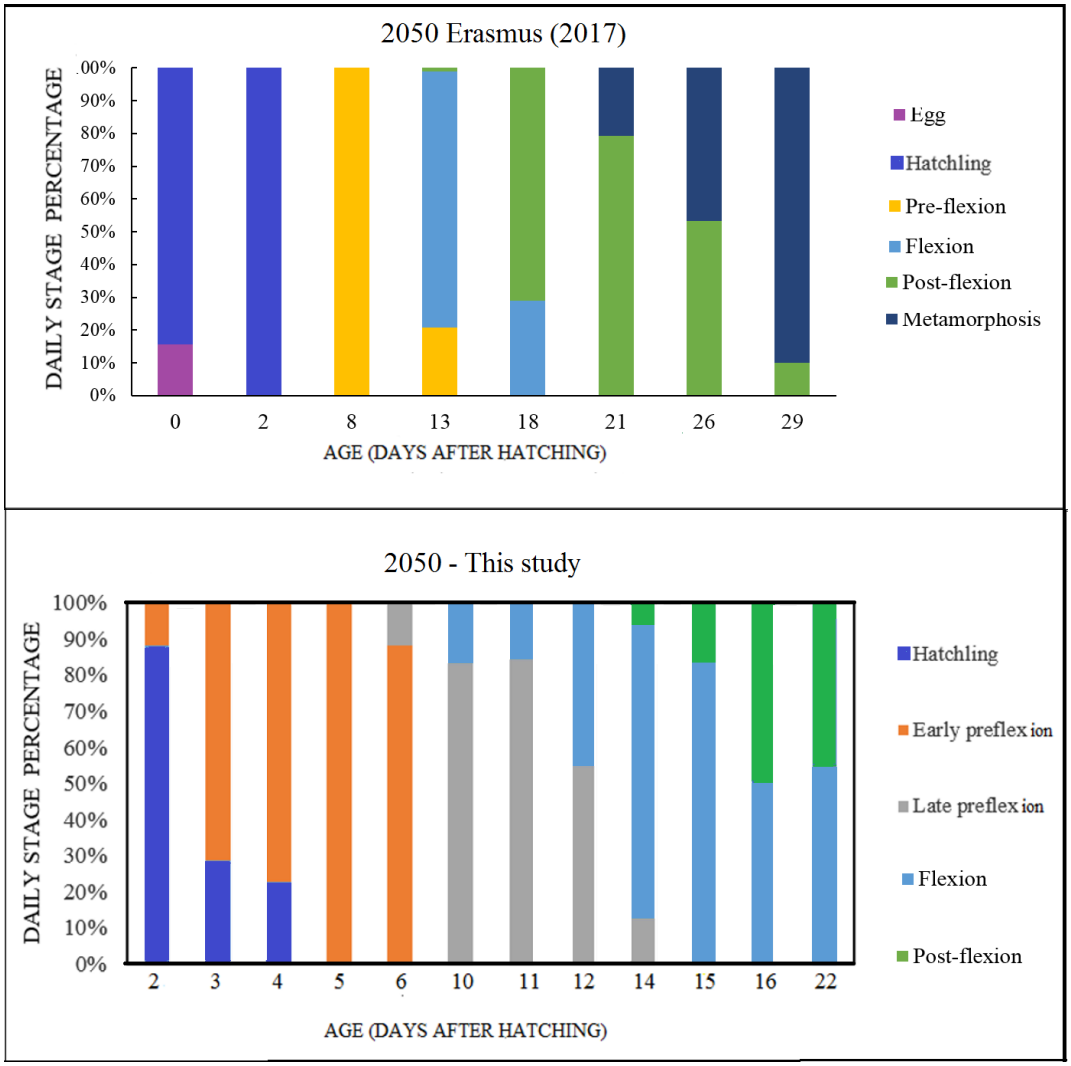


Figure 4. 1: Comparison of *Argyrosomus japonicus* development between Erasmus (2017) and the current study at pCO_2 and pH levels predicted for the year 2050.

When one compares the results by life stage, the results of this study were similar to those of Edworthy (2017) and Erasmus (2017) who also found no negative effects on the development and metabolism of larval *A. japonicus* up to the flexion stage, at OA levels predicted for the years 2050 (pCO_2 477.40 µatm; pH 8.03) and 2100 (pCO_2 910.20 µatm; pH 7.78). Edworthy (2017) and Erasmus (2017) found significantly reduced growth and reduced metabolic scope, respectively, in post-flexion larvae reared in the 2100 (pCO_2 910.20 µatm; pH 7.78). In this study only ± 50% of the larvae were in the post-flexion stage by the end of the experiment (22 DAH) and no significant differences were recorded between treatments for growth. Similar to Edworthy's (2017) findings, metabolic scope was reduced (relative to the other

treatments) in the highest pCO_2 (pCO_2 602 µatm; pH 7.83) treatment at the end of the study and may indicate that, if the experiment had continued into the late post-flexion and settlement stages, then growth and development of these stages may have been affected by OA levels predicted for 2090. However, in the absence of a longer experiment in the current study, together the findings of these studies suggest that there may be a tipping point somewhere between pCO_2 602 µatm (pH 7.83) and pCO_2 910 µatm (pH 7.78) which correspond to the years 2090 and 2100, respectively, when the impacts of OA are expected to negatively affect the larvae from the flexion stage (Figure 4.2).

In the present study, OA levels predicted for the year 2090, pCO_2 602 µatm and pH 7.83, did not have a significant effect on either the size-at-hatch or larval growth of *A. japonicus*, with growth slopes similar between treatments. Erasmus (2017) found negative impacts only on the growth of the late stage (post-flexion) larvae in the extreme 2100 (pCO_2 910 µatm) treatment. Erasmus (2017) proposed that the slower growth in this treatment could be attributed to the additional energy required for metamorphosis and acid-base regulation (which most likely coincided with the development of the gills) at the post-flexion stage. Interestingly, no fish in the present study reached the settlement stage and thus the lack of gill development of fish in this study may account for the lack of differences between treatments. This could suggest that the findings may have been different had the experiment extended beyond 22 DAH (i.e. beyond flexion/post-flexion stage). Another plausible explanation for the lack of variation in growth between treatments may be attributed to the treatment levels used in the present study. It is possible that the pCO_2 and/or pH levels used were not sufficiently different to cause significant impacts on size-at-hatch and growth in larval *A. japonicus.*

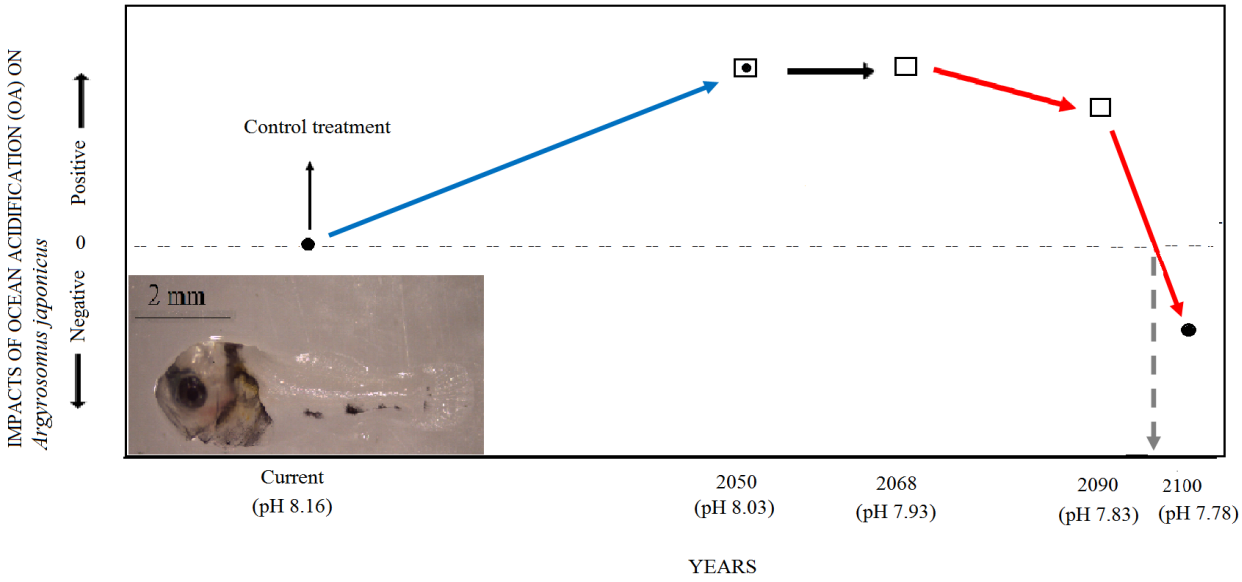


Figure 4. 2: Relative effects of near-future ocean acidification on larval *Argyrosomus japonicus*, up to flexion/post-flexion, highlighting the position of the tipping point defined as where the impact becomes negative (dotted arrow). Dots represent the treatments (in years) assessed from two previous studies (Edworthy 2017; Erasmus 2017), the open squares represent the treatments (in years) assessed in the present study and the arrows represent the positive (blue) and negative (red) trends. The year 2050 was used as a reference point to link the studies.

The early larval stages of several other marine fishes have been found to be tolerant to elevated pCO_2 and reduced pH levels (Munday *et al.* 2011; Frommel *et al.* 2013; Hurst *et al.* 2013, 2016). Hatching and growth of early larval stages of Baltic cod, *Gadus morhua*, were unaffected by pCO_{2air} levels of up to 4000 µatm and pH of 7.16 (Frommel *et al.* 2013). Similarly, pCO_2 levels between 287 and 1933 µatm did not affect the hatching length or growth of larval walleye pollock, *Theragra chalcogramma* (Hurst *et al.* 2013). In contrast, some studies on the early larval stages of marine fish have shown positive (Munday *et al.* 2009b; Kim *et al.* 2015) and others, negative (Baumann *et al.* 2012; Miller *et al.* 2012 and 2015; Pimentel *et al.* 2014 and 2016; Erasmus 2017) effects on larval growth. For example, orange clownfish, *Amphiprion percula*, larvae grew faster and were longer and heavier by the end of the experiment, in more CO_2-acidified water (1030 ppm) than those in the control (390 ppm) (Munday *et al.* 2009b). Similarly, larval olive flounder, *Paralichthys olivaceus*, grew faster in more CO_2-acidified seawater (Kim *et al.* 2015). In contrast, hatchling length

was significantly shorter for anemonefish, *Amphiprion melanopus*, at the elevated pCO_2 treatment (1126 µatm) (Miller *et al.* 2015) while the length of gilthead seabream, *Sparus aurata*, was shorter both at hatching and by the end of the experiment (15 DAH) in the highest pCO_2 treatment (~1400 µatm) (Pimentel *et al.* 2016).

Rates of oxygen consumption differed among developmental stages: later stages, flexion and flexion/post-flexion, had higher oxygen consumption rates. Peaks in oxygen consumption rates for the treatments occurred during the flexion stage on 14 and 15 DAH. This high oxygen consumption indicates high energy usage during this stage, which is not unusual; Ishibashi *et al.* (2005) also found that metabolic rates in larval red sea bream, *Pagrus major*, were highest during the flexion stage. Although oxygen consumption rates differed amongst developmental stages, they were similar between treatments from hatchling to the flexion/post-flexion stage (0–21 DAH), with the only noticeable deviation (although not significant) between treatments observed on the last day (22 DAH which corresponded to the flexion/post-flexion stage). The evidence of treatment effect started to manifest from flexion and continued through to the flexion/post-flexion stage with the highest pCO_2 treatment (pCO_2 602 µatm; pH 7.83) having lower (although not significant) oxygen consumption rates when compared to the other treatments (Appendix A). Edworthy (2017) also found that larvae of *A. japonicus* had the lowest (although not significant) routine metabolic rate (equivalent to oxygen consumption rate) during both the flexion and post-flexion stages in the highest pCO_2 treatment in her study (2100: pCO_2 910 µatm; pH 7.78). The congruence of the findings in this and the Edworthy (2017) study suggest that, despite the lack of significance between treatments, the rate of oxygen consumption could decrease with increasing levels of pCO_2 during both the flexion and post-flexion stages of larval *A. japonicus*. Since dissolved oxygen, along with temperature, is one of the most important determinants of metabolic scope in aquatic organisms (Fry 1971; Lefrançois and Claireaux 2003), the findings of the present study further suggest that flexion and post-flexion *A. japonicus* could be susceptible during periods of hypoxia, when dissolved oxygen is low (Claireaux and Chabot 2016) in future ocean conditions.

Aerobic or metabolic scope is defined as the difference between active metabolic rate (AMR) and standard metabolic rate (SMR) and is thought to reflect the amount of energy available to the organism that can be used to fund different activities. Consequently, several authors have suggested that it can be used as an indicator of the whole organism's performance (Fry 1971;

Norin and Malte 2011; Pörtner 2012; Leferve 2016). A reduction in aerobic scope indicates greater susceptibility of an individual to external stressors (Clark *et al.* 2013). A reduction in metabolic scope will also result in an energy-budgeting conflict between physiological processes such as swimming, feeding, and growth (Lefrançois and Claireaux 2003). Therefore, the peak in aerobic scope at the flexion stage, as observed in the present study, reduces the need for this conflict in energy partitioning. It is therefore expected that, at this period of maximised aerobic scope, physiological processes such as swimming, feeding, and growth are optimised. Unfortunately, the quantification of these parameters was beyond the scope of the present study.

In quantifying baseline metabolic rates in larval *A. japonicus*, Edworthy *et al.* (2018) identified a peak in the metabolic scope in the flexion stage followed by an energy bottleneck (reduced metabolic scope) in the late flexion/post-flexion stage, and a second peak and reduced scope in the post-flexion stage. Although these authors concluded that the late flexion/post-flexion stage may represent an energy bottleneck for this species, treatment effects were only evident after the completion of the flexion stage/second bottleneck (Edworthy 2017). The pattern in this study during the flexion stage was identical, with a peak in the metabolic scope (14–15 DAH) and a bottleneck (21 DAH) in all treatments. While the levels of pCO_2 used in these two studies did not appear to present an insurmountable barrier to the fish at this life stage, it is likely that this may not be the case at higher pCO_2 levels.

Ocean acidification is predicted to negatively affect the aerobic scope of marine organisms and thereby impact their performance (Pörtner *et al.* 2004; Pörtner and Farrell 2008; Munday *et al.* 2009a). Although the lack of significant differences in the metabolic scope of larval *A. japonicus* subjected to different levels of pCO_2 in the present study contrast with these findings, it was apparent that the highest pCO_2 treatment (pCO_2 602 µatm; pH 7.83) had a noticeably lower metabolic scope than the other treatments from towards the end of the flexion stage (Figures 3.9 and 3.10; Appendix D). This may suggest that, although the pCO_2 levels used in the present study were not sufficiently high to initiate a significant impact on the metabolic scope, ocean acidification may have a negative effect on the physiological performance of larval *A. japonicus* from this life stage. Baker and Brauner (2012) also identified poorer physiological performance at elevated pCO_2 levels in white sturgeon (*Acipense transmontanus*). Edworthy (2017) also found a decrease in metabolic scope at 21 DAH in the 2100 treatment; however, by this day in the previous experiment, 90% of the

larvae were in the post-flexion stage (C. Edworthy, pers. Comm. 2018) as opposed to ± 50% in the current study. This makes it difficult to quantify negative effects on the metabolism of later-stage larvae exposed to OA levels predicted for 2068 and 2090.

An individual's metabolic scope could be reduced either through a decrease in AMR or/and an increase in SMR (Heuer and Grossel 2014). In the present study, SMR remained relatively stable throughout the course of the experiment and therefore contributed little in shaping metabolic scopes in all treatments. The low and stable SMR observed suggests a low cost of living in larval *A. japonicus* (Metcalfe 1998), which could be advantageous during periods of food scarcity. However, metabolic plasticity and flexibility in SMR have been shown to occur with food availability in some species (O'Connor *et al.* 2000; Auer *et al.* 2015) and this should be tested in *A. japonicus*. The only noticeable difference occurred at the flexion/post-flexion stage where the 2068 (pCO_2 451 µatm; pH 7.93) treatment had a significantly higher SMR than the other two treatments. These results suggest that minimum energy required for survival, as reflected by SMR (Nelson and Chabot 2011), is similar amongst the life stages, except at the flexion/post-flexion stage, and suggests that metabolic costs of living in high 2090 (pCO_2 602 µatm; pH 7.83) or low 2050 (pCO_2 353 µatm; pH 8.03) treatments are similar for *A. japonicus* between the hatchling and flexion stages and that they are able to meet minimum required metabolic demands (costs) of surviving at acidic environments, up to levels of pCO_2 602 µatm; pH 7.83 (Ishimatsu *et al.* 2005) provided that they do not participate in physical activity. Similarly, Mckenzie *et al.* (2003) found no effects of hypercapnia on the SMR of European eel (*Anguilla Anguilla*).

Edworthy (2017) also found limited change in the SMR from hatchling until flexion, after which there was a rapid increase in the SMR at the post-flexion stage, which is not reflected in this study because of the delayed development of the larvae. Fish have well-developed acid-base regulation, which is primarily facilitated through the gill tissue (Heuer and Grosell 2016). However, since the gills of *A. japonicus* have been shown to develop only from the post-flexion stage (Erasmus 2017), it is likely that the SMR increase in post-flexion larvae can be attributed to the energy apportioned to acid-base regulation. The SMR was significantly lower in post-flexion larvae in the 2100 treatment, suggesting metabolic depression in these larvae was a response to high pCO_2 in order to conserve energy (Edworthy 2017).

The only significant finding in this study was the significantly higher SMR observed in the 2068 (pCO_2 451 µatm; pH 7.93) treatment at the flexion/post-flexion stage. While this may be surprising as the energetic requirements for acid-base regulation should have been higher in the 2090 (pCO_2 602 µatm; pH 7.83) treatment, it is possible that this could have been due to the greater proportion of flexion larvae at flexion/post-flexion stage (22 DAH) in the 2090 treatment (54%) when compared with the 2068 treatment (45.5%, Figure 3.4). Therefore, the higher percentage of post-flexion larvae in the 2068 (pCO_2 451 µatm; pH 7.93) treatment at the flexion/post-flexion stage may explain these findings.

In contrast to the SMR, AMR was mostly responsible for shaping the metabolic structure of larval *A. japonicus* and the lower metabolic scope in the 2090 flexion/post-flexion larvae. Increases in the metabolic scope of all treatments at the flexion stage were as a result of an increase in the AMR, particularly on days 14 and 15 DAH. Active metabolic rate increases on these days coincided with a stable SMR, resulting in broader metabolic scopes. However, the declines in AMR at the flexion/post-flexion stage (21 and 22 DAH) compressed the metabolic scope. Since active metabolic rate represents energetic costs associated with activity which are linked to prey capture, predator avoidance and growth rate (Ohlberger *et al.* 2007), it is clear from these results that activity costs vary with life stage in larval *A. japonicus*.

The AMR was low at both the hatchling and early pre-flexion stages, but increased from late pre-flexion to flexion stage and then reduced slightly at the flexion/post-flexion stage. The relatively low AMR at the first two developmental stages is not unexpected. During this period, larvae rely on yolk reserve or *Branchionus plicatilis* for food, and do not require a great deal of energy to find and capture food. It is possible that the peak in AMR at the flexion stage for all treatments could reflect the greater swimming capabilities of larval *A. japonicus*. Indeed, in the present study, larvae had developed myomeres and caudal fin rays (see figure 3.3), essential for swimming efficiency by this stage. Also, larvae at the flexion stage were feeding exclusively on highly mobile *Artemia spp.* This could mean that larvae were required to use more energy for chasing prey and that this was facilitated by a peak in AMR at this period. Alternatively, since elevated CO_2 could incur energetic costs that are not always reflected in the SMR but in the AMR (maximum oxygen consumption) (Couturier *et al.* 2013; Lefevre 2016), the peak in AMR at the flexion stage could, therefore, be a reflection of the additional O_2 demand (energetic costs) required to maintain

homeostasis, during this period. Interestingly, the rate of oxygen consumption also peaked during this period with no apparent increases in SMR.

The AMR in the 2090 (pCO_2 602 µatm; pH 7.83) treatment was lower (although not significantly) during the flexion and post-flexion stages, causing the observed decline in metabolic scope in this treatment. Edworthy (2017) also found that the decline in metabolic scope in the 2100 treatment post-flexion larvae was caused by a decline in AMR. Higher pCO_2 levels are expected to result in elevated blood and tissue pCO_2 (hypercapnia), which in turn, is thought to cause the impairment of cardio and ventilatory systems responsible for transportation of O_2 (Pörtner *et al.* 2004; Pörtner *et al.* 2005; Munday *et al.* 2009a). Since AMR/MMR is associated with the maximal rate at which oxygen can be transported from the environment to the organism's tissue (Norin and Clark 2016), the impairment of cardio and ventilatory systems will undoubtedly affect oxygen transportation and hence AMR in *A. japonicus*. This could, therefore, explain the observed reduction in AMR for the 2090 (pCO_2 602 µatm; pH 7.83) treatment during the flexion stage. Indeed, reductions in maximum oxygen consumption, equivalent of AMR in the present study, due to hypercapnia have been observed in other studies (Ishimatsu *et al.* 2005; Munday *et al.* 2009a). By contrast, AMR/MMR increased with increasing pCO_2 levels in Ambon damselfish, *Pomacentrus amboinensis*, and spiny damselfish, *Acanthochromis polyacanthus* (Couturier *et al.* 2013; Rummer *et al*. 2013), once again suggesting that the responses may be species-specific.

On account of a lower hatching success than expected, this study was carried out over a short period of time, until flexion/post-flexion stage (22 DAH), and as such, we were unable to assess the effects of elevated pCO_2 beyond the flexion/post-flexion stage. It was therefore impossible to estimate the relative mortality of *A. japonicus* larvae under conditions of elevated pCO_2. This is something that should be considered in future studies.

It is possible that several responses associated with physiology may be affected by OA and these should be examined in future studies. For example, elevated pCO_2 has been shown to affect the senses (such as olfaction) and behaviour of marine fishes (Munday *et al.* 2009c, Munday *et al.* 2014). It is recommended that both physiological and behavioural responses be examined in future studies through the use of choice chambers (e.g. James *et al.* 2008; Munday *et al.* 2009b), and DanioVision systems (Graham *et al.* 2016).

While this study only considered the impacts of OA, it is critical to consider that OA will not occur in isolation. There are other environmental stressors that will co-occur with OA, such as warming of the upper sea surface (Pimentel *et al.* 2016), hypoxic events (Pörtner *et al.* 2008), sea level rise (Mimura 2013) and fishing pressure (Rijnsdorp *et al.* 2009). In addition to OA, temperature is thought to be the most serious threat to marine organisms, including fish (Doney *et al.* 2009). Rising sea surface temperature is expected to have significant impacts on some marine fishes (Pörtner 2001; Potts *et al.* 2015). On the other hand, the interaction of temperature and ocean acidification can have impacts on marine fishes and is believed to cause more impacts in combination than in isolation (Pörtner *et al.* 20005; Pörtner and Farrell 2008; Munday *et al.* 2009a; Ferrari *et al.* 2015; Pimentel *et al.* 2016). Increases in sea surface temperatures are expected to occur in some parts of *A. japonicus* distribution (Potts *et al.* 2015); therefore, the response of *A. japonicus* to hypercapnia could be exacerbated by the expected rise in sea surface temperatures, and this should be taken into account in future studies on this and other coastal fish species.

Besides the potential influence of temperature on the rate of growth and development between this and the studies by Edworthy (2017) and Erasmus (2017), it is possible that parentage may have influenced the findings. The brood stock in these studies were not the same and it is therefore possible that genetics may account for the observed delay in the growth and development between this and the abovementioned studies. Evidence for the effect of parentage on the larval response (size-at-hatch, size and weight at the end of the experiment) has been documented by Munday *et al.* (2009b; 2011) for clutches (larvae) of orange clownfish (*Amphiprion percula*) and spiny damselfish (*Acanthochromis polyacanthus*). If indeed parentage accounted for the observed subtle differences in metabolic structuring of *A. japonicus*, this suggests that the species may have a broad diversity of metabolic phenotypes, which could increase their tolerance to OA.

Evolutionary adaptation and/acclimation could afford some species tolerance to future predicted OA through the selection of individuals that are more tolerant to increases in pCO_2 (Figure 4.2) (Sunday *et al.* 2014). It is suggested that a lack of variation in trait response could occur as a result of a reversible acclimation response to temporary environmental conditions (Sunday *et al.* 2014). It is therefore possible that the lack of significant differences in all physiological responses assessed in the present study could have been as a result of this phenotypic plasticity. In addition, some studies have demonstrated evidence of rapid

adaptation to environmental stressors through epigenetics, where individuals of some species are able to alter their gene expression, and not the DNA of the gene itself (Feil and Fraga 2012; Lighten *et al.* 2016). With an increase in pCO_2 levels (and OA), individuals could change their gene expression, which could lead to changes in the phenotypic expression (Sunday *et al.* 2014), and since this is heritable, could confer tolerance to future hypercapnia levels (Figure 4.3). A study assessing the likely effects of elevated pCO_2 on the epigenetics of *A. japonicus* could therefore provide important information on the likely response of the species to near-future OA.

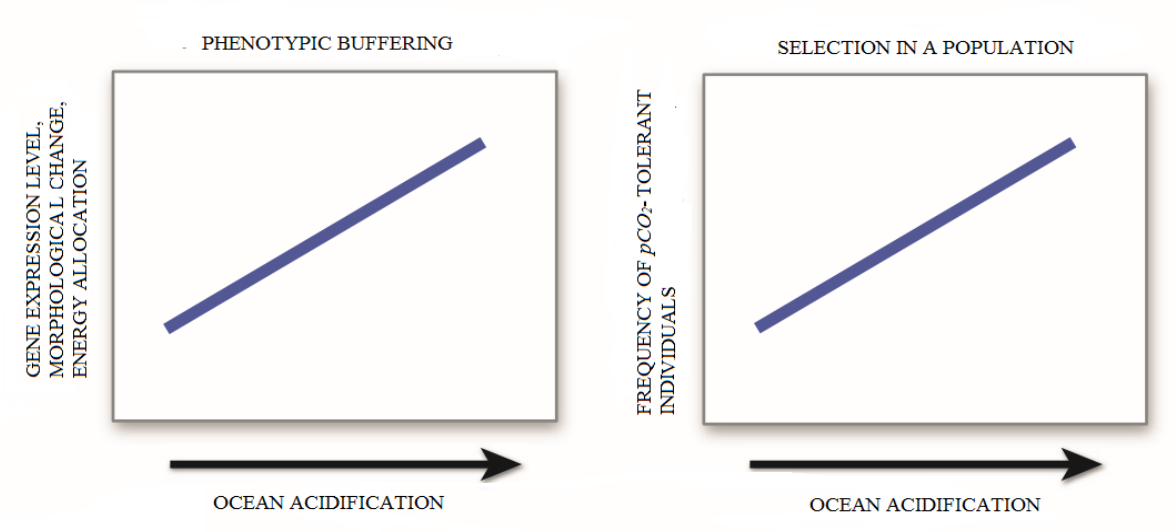


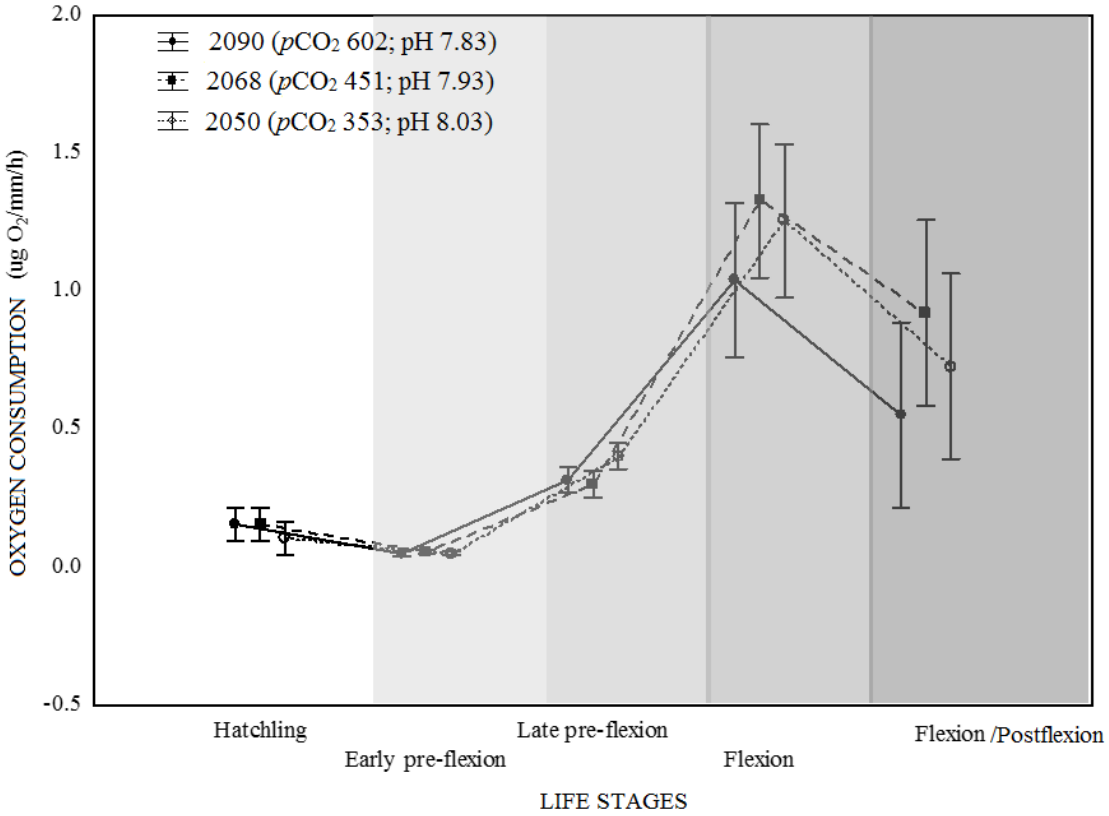
Figure 4. 3: Underlying mechanisms that could afford species tolerance to future OA. See text above. Modified from Sunday *et al.* (2014).

The present study also revealed a reduction in aerobic scope with increasing pCO_2 levels at the flexion stage (as opposed to the post-flexion stage) and suggests that, with increasing hypercapnia, the flexion stage could be more vulnerable to OA. Our study was, however, a single generation experiment and this did not allow for the assessment of responses across generations, and as such, the evolutionary adaptability of the species to future OA (Sunday *et al.* 2014). It is therefore possible that larval *A. japonicus* could tolerate future OA through transgenerational adaptation. Evidence of this has been demonstrated in some fish species, where parental exposure/transgenerational acclimation has been shown to alleviate the

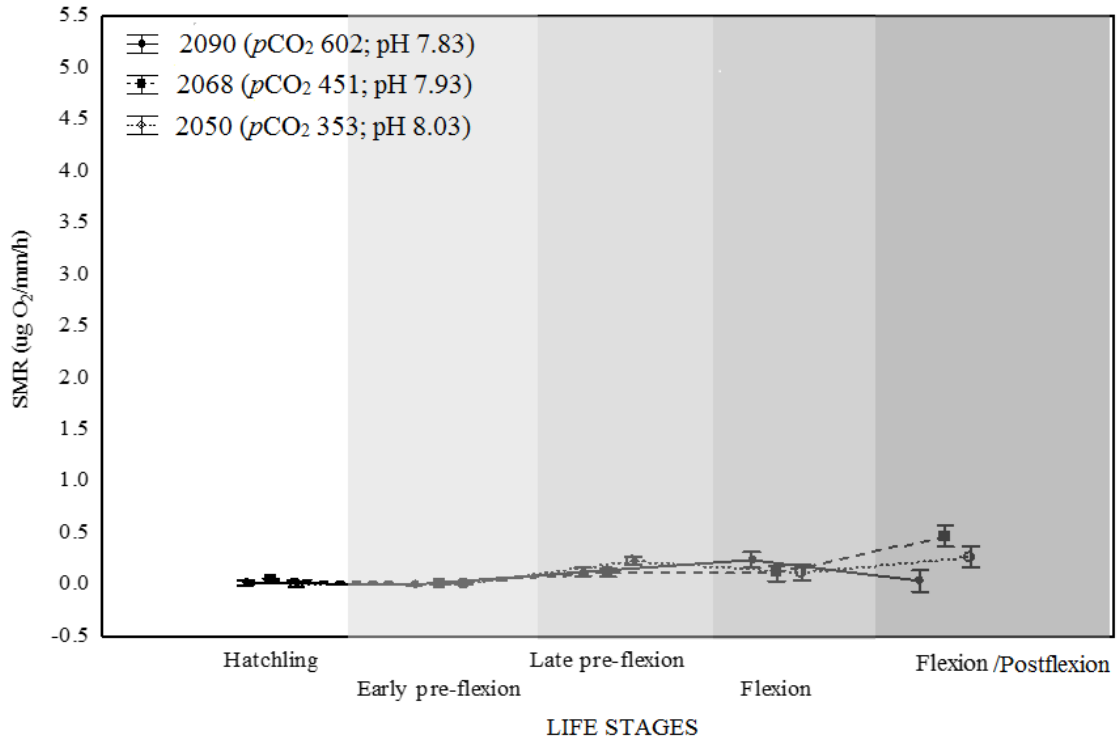
impacts of hypercapnia on offspring (Miller *et al.* 2012; Murray *et al.* 2014), suggesting that it could confer on some species some form of resilience to future OA. Future studies should therefore seek to expose both the parent(s) and offspring of *A. japonicus* to the same environmental conditions (same levels of pCO_2) in order to examine the potential of parental exposure in mediating hypercapnia.

Lastly, food ration has been shown to affect metabolism in some fish species (O'Connor *et al.* 2000; Auer *et al.* 2015). This study ensured that food was always readily available to the larvae. However, it is possible that periods of reduced food density, which is what larvae may encounter in the wild, may alter their behaviour and metabolism. Therefore, studies that examine the response of *A. japonicus* to OA at different food rations should shed light on metabolic plasticity of larval *A. japonicus*.

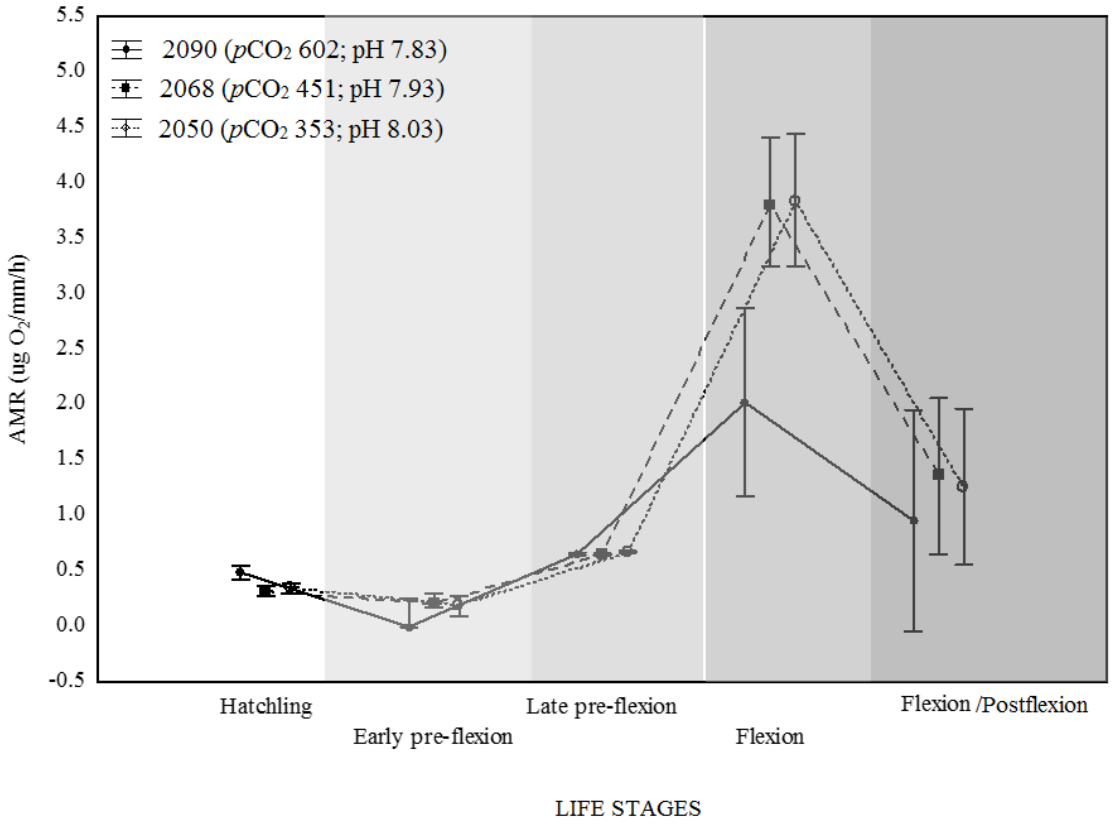
In conclusion, previous studies looking at the responses of larval *A. japonicus* to ocean acidification revealed that pCO_2 levels predicted for the end of the century 2100 (pCO_2 902 µatm; pH 7.78) will have negative impacts on growth, survival, and metabolism of the species (Edworthy 2017; Erasmus 2017). However, the growth and metabolism of later-stage larvae was higher under conditions predicted for the year 2050. The present study followed on these previous findings to try and identify the tipping point at which negative impacts are expected. Using pCO_2 levels predicted between the years 2050 (as a reference point) and 2100, it appears that the positive effects observed for larval *A. japonicus* for the year 2050 in previous studies (Edworthy 2017; Erasmus 2017) will extend until around the year 2090, when metabolic scope was reduced compared to the other treatments, but quickly diminish by the year 2100 (Figure 4.2). However, our knowledge of the adaptation potential of this species is limited, and it is possible that the species may be more resilient to near-future conditions than these studies predict.



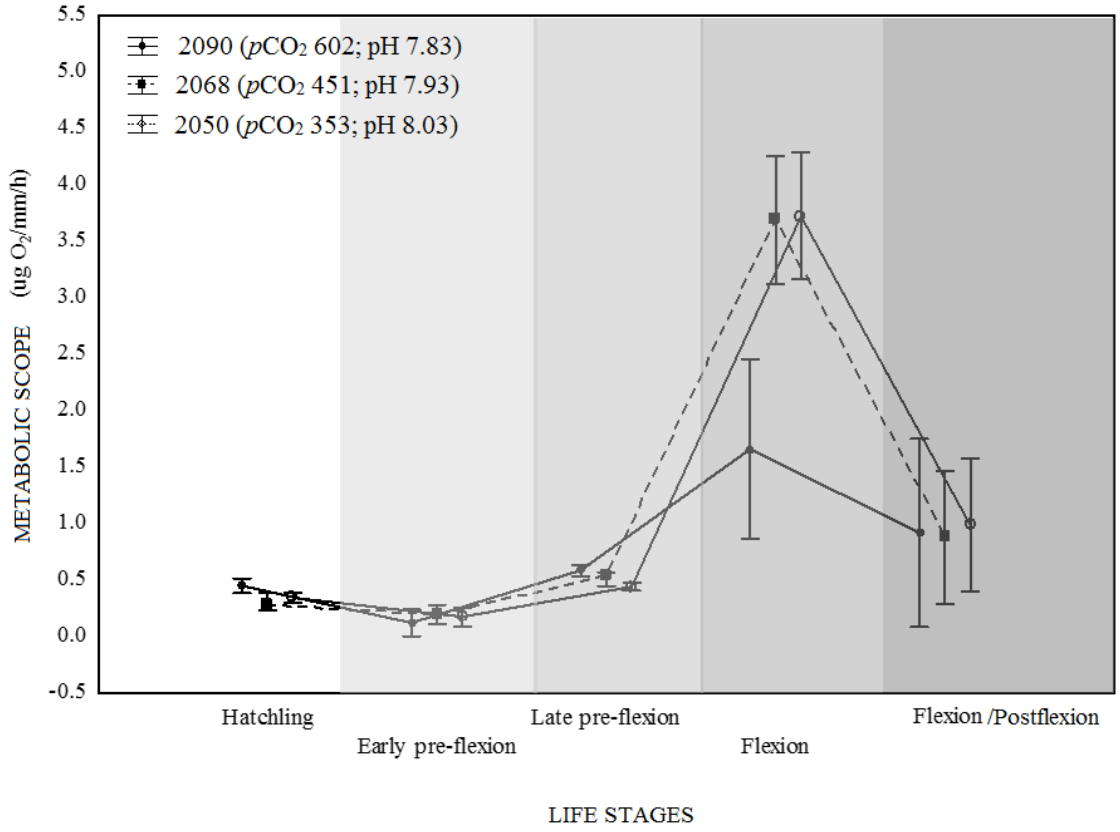
Appendix A: Mean oxygen consumption rate (metabolic rate) of early stage (hatchling to flexion/post-flexion) *Argyrosomus japonicus* reared under three pH conditions predicted for 2050, 2068 and 2100.



Appendix B: Mean standard metabolic rate (SMR) of early stage (hatchling to flexion/post-flexion) *Argyrosomus japonicus* reared under three pH conditions predicted for 2050, 2068 and 2100.



Appendix C: Mean active metabolic rate (AMR) of early stage (hatchling to flexion/post-flexion) *Argyrosomus japonicus* reared under three pH conditions predicted for 2050, 2068 and 2100.



Appendix D: Mean metabolic scope of early stage (hatchling to flexion/post-flexion) *Argyrosomus japonicus* reared under three pH conditions predicted for 2050, 2068 and 2100.

LIST OF REFERENCES

Auer SK, Salin K, Rudolf AM, Anderson GJ, Metcalfe NB. 2015. Flexibility in metabolic rate confers a growth advantage under changing food availability. *Journal of Animal Ecology* **84:** 1405 – 1411. doi: 10.1111/1365-2656.12384.

Baker DW, Brauer CJ. 2012. Metabolic changes associated with acid-base regulation during hypercarbia in CO_2-tolerant chondrostean, white sturgeon (*Acipenser transmontanus*). *Comparative Biochemistry and Physiology* **Part A 161:** 61 – 68.

Baumann H, Talmage SC, Gobler CJ. 2012. Reduced early life growth and survival in a fish in direct response to increased carbon dioxide. *Nature Climate Change* **2:** 38 – 41.

Barry JP, Tyrrell T, Hansson L, Plattner GK, Gattuso JP. 2010. Atmospheric CO_2 targets for ocean acidification perturbation experiments. In: Riebesell U, Fabry VJ, Hansson L, Gattuso JP (Eds), *Guide to best practices for ocean acidification research and data reporting*, European Commission, Luxembourg 53 – 66.

Beerling DJ. 2012. Atmospheric carbon dioxide: a driver of photosynthetic eukaryote evolution for over a billion years?. *Philosophical Transactions of Royal Society B* **367**: 477 – 482.

Bignami SGT. 2013. Effects of Ocean Acidification on the Early Life History of Two Pelagic Tropical Fish Species, Cobia (*Rachycentron canadum*) and Mahi-mahi (*Coryphaena hippurus*). PhD Thesis, University of Miami, Florida, USA, 189pp.

Brauner C J, Baker DW. 2009. Patterns of acid-base regulation during exposure to hypercarbia in fishes. In: Glass ML, Woods SC (Eds), *Cardiorespiratory control in vertebrates*, Springer, Berlin, Germany, 43 – 63.

Cattano C, Giomi F, Milazzo M. 2016. Effects of ocean acidification on embryonic respiration and development of a temperate wrasse living along a natural CO_2 gradient. Conservation Physiology **4:** cov073 doi: 10.1093/conphys/cov073.

Chabot D, McKenzie DJ, Craig JF. 2016a. Metabolic rate in fishes: definitions, methods and significance for conservation physiology (Editorial). *Journal of Fish Biology* **88:**1 – 9. doi.org/10.1111/jfb.12873

Chabot D, Steffensen J F, Farrell AP. 2016b. The determination of standard metabolic rate in fishes. *Journal of Fish Biology* 88: 81 – 121.

Chambers RC, Candelmo AC, Habeck EA, Poach ME, Wieczorek D, Cooper K R, Greenfield CE, Phelan BA. 2014. Effects of elevated CO_2 in the early life stages of summer flounder, *Paralichthys dentatus*, and potential consequences of ocean acidification. *Biogeosciences* **11**: 1613 – 1626.

Childs A-R and Fennessy ST. 2013. Status report of dusky kob *Argyrosomus japonicus*. In: Mann BQ (Ed.), *Southern African Marine Linefish Species Profile*s. Special Publication, Oceanographic Research Institute, Durban **9:** 154 – 156.

Childs A-R, Cowley PD, Naesje TF, Bennett RH. 2015. Habitat connectivity and intra-population structure of an estuary-dependent fishery species. *Marine Ecology Progress Series*, **537:** 233 – 245.

Claireaux G, Chabot D. 2016. Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *Journal of Fish Biology* 88: 232 – 251.

Clark TD, Sandblom E, Jutfelt F. 2013. Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *Journal Experimental Biology* **216:** 2771–2782.

Connell SD, Russell BD. 2010. The direct effects of increasing CO_2 and temperature on non-calcifying organisms: increasing the potential for phase shifts in kelp forests. *Proceedings of the Royal Society* **277:** 1409 – 1415.

Connell SD, Doubleday ZA, Hamlyn SB, Foster NR, Harley CDG, Helmuth B, Kelaher BP, Nagelkerken I, Sarà G, Russell BD. 2017. How ocean acidification can benefit calcifiers. *Current Biology* **27:** 83–102. http://dx.doi.org/10.1016/j.cub.2016.12.004

Couturier CS, Stecyk JAW, Rummer JL, Munday PL, Nilsson GE. 2013. Species-specific effects of near-future CO_2 on the respiratory performance of two tropical prey fish and their predator. *Comparative Biochemistry and Physiology, Part A* **166:** 482 – 489.

Chabot D, Claireaux G. 2008. Quantification of SMR and SDA in aquatic animals using quantiles and non-linear quantile regression. Comparative Biochemistry and *Physiology A-Molecular & Integrative Physiology* **150**: S99.

Davidson L. 2013. Ocean acidification and its effects upon fitness in nereidid polychaetes. PhD Thesis, University of Hull, England, 167pp.

Doney SC, Fabry VJ, Feely RA, Kleypas JA. 2009. Ocean acidification: the other CO_2 problem. *Annual Review of Marine Science* **1**: 169 – 192.

Dupont S, Havenhand J, Thorndyke W, Peck L, Thorndyke M. 2008. Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Marine Ecology Progress Series* 373: 285 – 294.

Edworthy C. 2017. The metabolic physiology of early stage *Argyrosomus japonicus* with insight into the potential effects of pCO2 induced ocean acidification. MSc Thesis, Rhodes University, Grahamstown, South Africa, 79 pp.

Edworthy C, James NC, Erasmus B, Kemp JOG, Kaiser H, Potts WM. 2018. Metabolic activity throughout early development of dusky kob *Argyrosomus japonicus. African Journal of Marine Science* **40(1)**: 67 – 74. doi.org/10.2989/1814232X.2018.1441907

Erasmus B. 2017. Effects of CO_2-induced ocean acidification on the early development, growth, survival and skeletogenesis of the estuarine-dependant sciaenid *Argyrosomus japonicus.* MSc Thesis, Rhodes University, Grahamstown, South Africa, 89pp.

Evans DH, Piermarini PM, Choe KP. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiological Review* **85:** 97 – 177.

Fabry VJ, Seibel BA, Feely RA, Orr JC. 2008. Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science* **65**: 414 – 432.

Feely RA, Doney SC, Cooley SR. 2009. Ocean acidification: present conditions and future changes in a high-CO_2 world. *Oceanography* **22(4):** 37 – 47.

Feil R, Fraga MF. 2012. Epigenetics and the environment: emerging patterns and implications. *Nature Reviews Genetics* **13:** 97 – 109.

Ferrari MCO, Munday PL, Rummer JL, Mccormick MI, Corkill K, Watson S-A, Allan BJM, Meekan MG, Chivers DP. 2015. Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. *Global Change Biology* **21:** 1848 – 1855. doi: 10.1111/gcb.12818.

Forsgren E, Dupont S, Jutfelt F, Amundsen T. 2013. Elevated CO_2 affects embryonic development and larval phototaxis in a temperate marine fish. *Ecology and Evolution* **3(11):** 3637 – 3646.

Fielder DS, Heasman MP. 2011. Hatchery Manual for the production of Australian Bass, Mulloway and Yellowtail Kingfish. Industry & Investment NSW, 176 pp.

Findlay HS, Wood HL, Kendall MA, Spicer JI, Twitchett RJ, Widdicombe S. 2009. Calcification, a physiological process to be considered in the context of the whole organism. *Biogeoscience Discuss* **6:** 2267 – 2284.

Frommel AY, Schubert A, Piatkowski U, Clemmesen C. 2013. Egg and early larval stages of Baltic cod, *Gadus morhua*, are robust to high levels of ocean acidification. *Marine biology* **160 (8):** 1825 – 1834.

Fry FEJ. 1971. The effect of environmental factors on animal activity. In Hoar WS, Randall D J. (Eds), *Fish Physiology*, Academic Press, New York, 1 – 98.

Gazeau F, Quiblier C, Jansen J, Gattuso J, Middelburg J, Heip C. 2007. Impact of elevated CO_2 on shellfish calcification. *Geophysical Research Letters* **34:** L07603.

Geist SJ, Ekau W, Kunzmann A. 2013. Energy demand of larval and juvenile Cape horse mackerels, *Trachurus capensis*, and indications of hypoxia tolerance as benefit in a changing environment. *Marine Biology* 160: 3221 – 3232. doi: 10.1007/s00227-013-2309-2

Graham HE. 2014. Reproduction in a changing ocean: the effects of ocean acidification and other environmental stressors on the sea urchin *Paracentrotus lividus* and the Polychaete *Ficopomatus enigmaticus*. PhD Thesis, Newcastle University, United Kingdom, 176pp.

Graham S, Rogers RP, Alper Rh. 2016.An automated method to assay locomotor activity in third instar *Drosophila melanogaster* larvae. *Journal of Pharmacology and Toxicology Methods* **77:** 76 – 80. doi:10.1016/j.vascn.2015.10.004.

Griffiths MH. 1996. Life history of the dusky kob *Argyrosomus japonicus* (Sciaenidae) off the east coast of South Africa. *South African Journal of Marine Science* **17:** 135 –154.

Griffiths MH. 1997. Feeding ecology of South African *Argyrosomus japonicus* (Pisces: Sciaenidae), with emphasis on the Eastern Cape surf zone. *South African Journal of Marine Science* **18:** 249 – 264.

Griffiths MH, Heemstra PC. 1995. A contribution to the taxonomy of the marine fish genus *Argyrosomus* (Perciformes: Sciaenidae), with descriptions of two new species from southern Africa. *Ichthyological Bulletin of the JLB Smith Institute of Ichthyology* **65**: 1 – 40.

Harley CDG, Hughes AR, Hultgren KM, Miner BG, Sorte CJB, Thornber CS, Rodriguez LF, Tomanek L, Williams SL. 2006. The impacts of climate change in coastal marine systems. *Ecology Letters* **9:** 228 – 241.

Heuer RM, Grosell M. 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **307:** R1061 – R1084.

Heuer RM, Grosell M. 2016. Elevated CO_2 increases energetic cost and ion movement in the marine fish intestine. *Scientific Reports* **6:** 34480.

Hofmann M, Barry JP, Edmunds PJ, Gates RD, Hutchins DA, Klinger T, Sewell MA. 2010. The Effect of Ocean Acidification on Calcifying Organisms in Marine Ecosystems:

An Organism-to-Ecosystem Perspective. *The Annual Review of Ecology, Evolution, and Systematic* **41:**127 – 147.

Hurst TP, Fernandez ER, Mathis JT. 2013. Effects of ocean acidification on hatch size and larval growth of walleye pollock (*Theragra chalcogramma*). *ICES Journal of Marine Science* **70:** 812 – 822.

Hurst TP, Laurel, BJ, Mathis JT, Tobosa LR. 2016. Effects of elevated CO_2 levels on eggs and larvae of a North Pacific flatfish. *ICES Journal of Marine Science* **73:** 981 – 990.

IPCC. 2013. Climate change 2013: *The Physical Science Basis.* In TF Stocker, D Qin, G-K Plattner, M Tignor, SK Allen, J Boschung, A Nauels, Y Xia (Eds), *Contribution of working group I to the fifth assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, 1 – 32.

Ishimatsu A, Kita J. 1999. Effects of environmental hypercapnia on fish. *Japanese Journal Ichthyology* **46**: 1 – 13.

Ishimatsu A, Hayashi M, Lee KS, Kikkawa T, Kita J. 2005. Physiological effects on fishes in a high-CO_2 world. *Journal of Geophysical Research* **110:** CS09S09, doi:10.1029/2004JC002564.

Ishimatsu A, Hayashi M, Kikkawa T. 2008. Fishes in high-CO_2, acidified oceans. *Marine Ecology Progress Series* **373**: 295 – 302.

James NC, Cowley PD, Whitfield AK, Kaiser H. 2008. Choice chamber experiments to test the attraction of postflexion *Rhabdosargus holubi* larvae to water of estuarine and riverine origin. *Estuarine, Coastal and Shelf Science* **77:** 143 – 149. doi: 10.1016/j.ecss.2007.09.010.

Kandjou K, Kaiser H. 2014. Diurnal and water salinity-dependent metabolic activity of juvenile white steenbras *Lithognathus lithognathus*. *African Journal of Aquatic Science* **39:** 263 – 270.

Kim K-S, Shim JH, Kim S. 2015. Effects of CO_2-induced ocean acidification on the growth of the larval olive flounder *Paralichthys olivaceus*. *Ocean Science Jou*rnal **50(2):** 381 – 388.

Kroeker KJ, Kordas RL, Crim RN, Singh GG. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecology Letters* **13:** 1419 – 1434.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo L, Singh G, Duarte CM, Gattusso J-P. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* **19:** 1884 – 1896.

Lefevre S. 2016. Are global warming and ocean acidification conspiring against marine ectotherms? A meta-analysis of the respiratory effects of elevated temperature, high CO_2 and their interaction. *Conservation Physiology* **4:** doi:10.1093/conphys/cow009.

Lefrançois C, Claireaux G. 2003. Influence of ambient oxygenation and temperature on metabolic scope and scope for heart rate in the common sole *Solea solea*. *Marine Ecology Progress Series* **259:** 273 – 284.

Lewis E, Wallace D. 1998. CO2SYS Program. Brookhaven National Laboratory, Upton, NY.

Lighten J, Incarnato D, Ward BJ, van Oosterhout C, Bradbury I, Hanson M, Bentzen P. 2016. Adaptive phenotypic response to climate enabled by epigenetics in a K-strategy species, the fish *Leucoraja ocellata* (Rajidae) Royal Society open science **3:**160299. dx.doi.org/10.1098/rsos.160299.

Llopiz JK, Cowen RK, Hauff MJ, Ji R, Munday PL, Muhling PA, Peck MA, Richardson DE, Sogard S, Sponaugle S. 2014. Early life history and fisheries oceanography: New questions in a changing world. *Oceanography* **27(4):** 26 – 41.

Marais JFK. 1984. Feeding ecology of major carnivorous fish from four Eastern Cape estuaries. *South African Journal of Zoology* **19:** 210 – 223.

McKenzie DJ, Piccolella M, Dalla Valle AZ, Taylor EW, Bolis CL Steffensen JF. 2003. Tolerance of chronic hypercapnia by the European eel *Anguilla anguilla*. *The Journal of Experimental Biology* 206: 1717 – 1726.

Meehl G, Stocker TF, Collins W, Friedlingstein P, Gaye A, Gregory J, Kitoh A, Knutti R, Murphy J, Noda A, Raper S, Watterson I, Weaver A, Zhao ZC. 2007. Global Climate Projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt K,

Tignor M, Miller HL (Eds.), *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, UK, 748 – 845.

Metcalfe NB. 1998. The interaction between behavior and physiology in determining life history patterns in Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Science* **55(Suppl. 1):** 93 – 10.

Metcalfe NB, Taylor AC, Thorpe JE. 1995. Metabolic rate, social status and life - history strategies in Atlantic salmon *Animal Behaviour*. **49:** 431 – 436.

Miller GM, Watson S-A, Donelson JM, McCormick MI, Munday PL. 2012. Parental environment mediates impacts of increased carbon dioxide on a coral reef fish. Nature Climate Change **2(12):** 858 – 861.

Miller GM, Watson S-A, McCormick MI, Munday PL. 2013. Increased CO_2 stimulates reproduction in a coral reef fish. *Global Change Biology* **19:** 3037 – 3045.

Miller GM, Kroon FJ, Metcalfe S, Munday PL. 2015. Temperature is the evil twin: effects of increased temperature and ocean acidification on reproduction in a reef fish. *Ecological Applications* **25(3):** 603 – 620.

Millidine KJ, Armstrong JD, Metcalfe NB. 2009. Juvenile salmon with high standard metabolic rates have higher energy costs but can process meals faster. *Proceedings of the Royal Society* **276(1664):** 2103 – 2108. doi: 10.1098/rspb.2009.0080

Mimura N. 2013. Sea-level rise caused by climate change and its implications for society. *Proceedings of the Japan Academy Series B* **89(7):** 281 – 301.

Mirimin L, Macey B, Kerwath S, Lamberth S, and others. 2015. Genetic analyses reveal declining trends and low effective population size in an overfished South African sciaenid species, the dusky kob (*Argyrosomus japonicus). Marine and Freshwater Research* **66:** 1 – 11.

Mu J, Jin J, Wang J, Zheng N, Cong Y. 2015. Effects of CO_2-driven ocean acidification on early life stages of marine medaka (*Oryzias melastigma*). *Biogeosciences* **12**: 3861 – 3868.

Munday PL, Crawley NE, Nilsson GE. 2009a. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. *Marine Ecology Progress Series* **388:** 235 – 242.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Doving KB. 2009b. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America* **106:** 1848 – 1852.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina GV, Doving KB. 2009c. Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America* 106:1848–1852

Munday PL, Gagliano M, Donelson JM, Dixson DL, Thorrold SR. 2011. Ocean acidification does not affect the early life history development of a tropical marine fish. *Marine Ecology Progress Series* **423:** 211 – 221.

Munday PL, Cheal AJ, Dixson DL, Rummer JL, Fabricius KE. 2014. Behavioural impairment in reef fishes caused by ocean acidification at CO2 seeps. *Nature Climate Change* **4:** 487 – 492.

Murray CS, Malvezzi A, Gobler CJ, Bauman H. 2014. Offspring sensitivity to ocean acidification changes seasonally in a coastal marine fish. *Marine Ecology Progress Series* **504:** 1 – 11.

Nelson JA, Chabot D. 2011. Energy consumption: metabolism (general). In Farrell AP (Ed), *Encyclopaedia of Fish Physiology: From Genome to Environment.* Academic Press, London, 1566 – 1572.

Nilsson GE, Ostlund-Nilsson S, Penfold R, Grutter AS. 2007. From record performance to hypoxia tolerance: respiratory transition in damsel fish larvae settling on a coral reef. *Proceedings of the Royal Society B* 274: 79 – 85. doi: 10.1098/rspb.2006.3706

Norin, T, Malte H. 2011. Repeatability of standard metabolic rate, active metabolic rate and aerobic scope in young brown trout during a period of moderate food availability. *Journal of Experimental Biology* **214:** 1668 – 1675. doi: 10.1242/jeb.054205

Norin T. Clark TD. 2016. Measurements and relevance of maximum metabolic rate in fishes. *Journal of Fish Biology* **88:** 122 – 151.

O'Connor KI, Taylor AC, Metcalfe NB. 2000. The stability of standard metabolic rate during a period of food deprivation in juvenile Atlantic salmon. *Journal of Fish Biology* **57:** 41 – 51.

Ohlberger J, Staaks G, Hölker F. 2007. Estimating the active metabolic rate (AMR) in fish based on tail beat frequency (TBF) and body mass. *Journal of Experimental Zoology* 307A: 296 – 300.

Peck MA, Moyano M. 2016. Measuring respiration rates in marine fish larvae: challenges and advances. *Journal of Fish Biology* **88:** 173 – 205.

Pimentel M, Faleiro F, Dionísio G, Repolho T, Pousão-Ferreira P, Machado J, Rosa R. 2014. Defective skeletogenesis and oversized otoliths in fish early stages in a changing ocean. *Journal of Experimental Biology* **217(12):** 2062 – 2070.

Pimentel MS, Faleiro F, Marques T, Bispo R, Dionísio G, Faria AM, Machado J, Peck MA, Pörtner H, Pousão-Ferreira P, Gonçalves EJ. 2016. Foraging behaviour, swimming performance and malformations of early stages of commercially important fishes under ocean acidification and warming. *Climatic Change* **137(3-4):** 495 – 509.

Potts WM, Götz A, James N. 2015. Review of the projected impacts of climate change on coastal fishes in southern Africa. *Reviews in Fish Biology and Fisheries* **25:** 603 – 630.

Pörtner HO.2001. Climate change and temperature dependent biogeography: oxygen limitation of thermal tolerance in animals. *Naturwissenschaften* **88:** 137 – 146.

Pörtner HO. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Marine Ecology Progress Series **373:** 203 – 217.

Pörtner HO. 2012. Integrating climate-related stressor effects on marine organisms: unifying principles linking molecule to ecosystem-level changes. *Marine Ecology Progress Series* **470:** 273 – 290.

Pörtner HO, Knust R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. *Science* **315:** 95 – 97.

Pörtner H-O, Farrell AP. 2008. Physiology and climate change. *Science* 322:690 – 692.

Pörtner HO, Langenbuch M, Reipschläger A. 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography* **60**: 705 – 718.

Pörtner HO, Langenbuch M, Michaelidis B. 2005. Synergistic effects of temperature extremes, hypoxia, and increases in CO_2 on marine animals: From Earth history to global change. *Journal of Geophysical Research* **110:** 1 – 15.

Pörtner HO, Bock C, Knust R, Lannig G, Lucassen M, Mark F, Sartoris FJ. 2008. Cod and climate in a latitudinal cline: physiological analyses of climate effects in marine fishes. *Climate Research* **37:** 253 – 270.

Pörtner HO, Dupont S, Melzner S, Storch D, Thorndyke M. 2010. Studies of metabolic rate and other characters across life stages. In Riebesell U, Fabry VJ, Hansson L and Gattuso JP (Eds), *Guide to best practices for ocean acidification research and data reporting,* European Commission. Luxembourg, 167 – 177.

Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O, Liss PS, Riebesell U, Sheperd J, Turley C, Watson A. 2005. Ocean Acidification due to Increasing Atmospheric Carbon Dioxide. Royal Society Policy Document.

Riahi K, Rao S, Krey V, Cho , Chirkov V, Fischer G, Kindermann G, Nakicenovic N, Rafaj P. 2011. RCP 8.5 - A scenario of comparatively high greenhouse gas emissions. *Climate Change* **109:** 33 – 57.

Riebesell U, Fabry VJ, Hansson L, Gattuso JP. 2010. Preface. In Riebesell U, Fabry VJ, Hansson L & Gattuso JP (eds). *Guide to best practices for ocean acidification research and data reporting*, European Commission. Luxembourg, pp 5 – 6.

Rijnsdorp AD, Peck MA, Engelhard GH, Möllmann C, Pinnegaret JK. 2009. Resolving the effect of climate change on fish populations. *ICES Journal of Marine Science* **66(7):** 1570 – 1583. doi.org/10.1093/icesjms/fsp056

Rodgers GG, Tenzing P, Clark TD. 2016. Experimental methods in aquatic respirometry: the importance of mixing devices and accounting for background respiration. *Journal of Fish Biology* **88:** 65 – 80.

Roessig JM, Woodley CM, Cech JJ, Hansen LJ. 2004. Effects of global climate change on marine and estuarine fishes and fisheries. *Reviews in Fish Biology and Fisheries* **14:** 251 – 271.

Rogner H, Zhou D, Bradley R, Crabbé P, Edenhofer O, Hare B, Kuijpers L, Yamaguchi M. 2007. Introduction. In: Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA (Eds), *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*, Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, pp 95 – 116.

Rost B, Zondervan I, Wolf-gladrow D. 2008. Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. *Effects of ocean acidification on marine ecosystems* **373:** 227 – 237.

Rummer JL, Stecyk JAW, Couturier CS, Watson S-A, Nilsson GE, Munday PL. 2013. Elevated CO_2 enhances aerobic scope of a coral reef fish. *Conservation Physiology* **1:** 1 – 7. doi:10.1093/conphys/cot023.

Sabine CL, Feely RA, Gruber N, Key RM, Lee K, Bullister JL, Wanninkhof R, Wong C, Wallace DW, Tilbrook B. 2004. The oceanic sink for anthropogenic CO_2. *Science* **305:** 367 – 371.

Shirayama Y, Thornton H. 2005. Effect of increased atmospheric CO_2 on shallow water marine benthos. *Journal of Geophysical Research* **110:** C09S08.

Silberschneider V, Gray CA. 2008. Synopsis of biological, fisheries and aquaculture-related information on mulloway *Argyrosomus japonicus* (Pisces: Sciaenidae), with particular reference to Australia. *Journal of Applied Ichthyology* **24:** 7−17

Spicer J, Raffo A, Widdicombe S. 2007. Influence of CO_2-related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber*. *Marine biology* **151:** 1117 – 1125.

Stiasny MH, Mittermayer FH, Sswat M, et al. 2016. Ocean acidification effects on Atlantic cod larval survival and recruitment to the fished population. PLoS One 11 doi: 10.1371/journal.pone.0155448

Strong AL, Kroeker KJ, Teneva LT, Mease LA, Kelly RP. 2014. Ocean Acidification 2.0: Managing our Changing Coastal Ocean Chemistry. *BioScience* **64(7):** 581 – 592.

Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TBH. 2014. Evolution in an acidifying ocean. *Trends in Ecology and Evolution* **29:** 117 – 120.

Walther K, Anger K, Pörtner HO. 2010. Effects of ocean acidification and warming on the larval development of spider crab *Hyas araneus* from different latitudes (54°vs79° N). *Marine Ecology Progress Series* **417:** 159 – 170.

Wood HL, Spicer JI, Widdicombe S. 2008. Ocean acidification may increase calcification, but at a cost. *Proceedings of the Royal Society B* **275:**1767 – 1773.